



Abstracts

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Oral Abstract Presentations

17

CDK4/6 inhibition with Abemaciclib in patients (pts) with previously treated advanced renal carcinoma (RCC)

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Background

Preclinical data suggests rationale for CDK4/6 alone and in combination with HIF-2 inhibitors; single agent activity for CDK4/6 inhibitors in RCC has not been reported. Abemaciclib is an oral CDK4/6 inhibitor approved in combination with hormonal therapy for metastatic breast cancer. In our phase 1b clinical trial ([NCT04627064](#)), we investigated the safety and clinical efficacy of abemaciclib monotherapy in pts with advanced pretreated RCC with clear cell component.

Methods

In this single center trial, adult pts with advanced RCC with a clear cell component and ECOG status of ≤1 progressing after at least one prior regimen including immunotherapy and a VEGF TKI received abemaciclib 200 mg twice daily in 4-week cycles until progression or unacceptable toxicity. Primary objective was to evaluate the objective response rate (ORR) of abemaciclib with secondary endpoint of safety. First imaging was performed after 2 cycles. Response assessed per RECIST 1.1 and toxicity per CTCAE v5.0.

Results

11 pts (10 clear cell RCC and 1 translocation RCC) were enrolled between 12/31/2020 and 10/03/2023. Median age was 62 years (range 54-68) with 18% (n=2) showing sarcomatoid features. 73% (n=8) had IMDC intermediate risk disease and one patient had translocation RCC (tRCC) with a clear cell component. Median number of prior therapies was 4 (range 1-9). Seven patients received 2 cycles and 4 patients received < 2 cycles. ORR was 0% (0/11; 8 progressive disease, 1 stable disease in tRCC stopping for clinical progression, 2 pts not evaluable with clinical progression). 27% (n=3) experienced grade ≥3 treatment-related adverse events (diarrhea n=1, nausea n=1, neutropenia n=1).

Conclusions

In pts with heavily pretreated RCC, abemaciclib had manageable toxicity profile but no clinically meaningful activity as monotherapy. This data will offer important insight into interpretation of results for ongoing trials exploring CDK4/6 inhibition in combination with HIF-2 inhibitors.

Keywords

Renal Cell Carcinoma, CDK4/6

22

Association of a germline single nucleotide polymorphism (SNP) in the interleukin-7 (IL7) gene with immune-related adverse events (irAEs)

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Background

Adverse events (AEs) can limit treatment immune checkpoint inhibitors (ICI) efficacy and worsen patient outcomes. Our group recently identified a germline IL7 SNP as a potential biomarker for prediction of irAEs (Groha, Nat Med 2022). In the current study, we sought to replicate the association between this IL7 SNP (rs16906115) and AEs in two clinical trials of patients with cancer treated with ICI regimens.

Methods

In the CheckMate-025 (CM025) trial ([NCT01668784](#)), involving patients with metastatic renal cell carcinoma (mRCC) randomized to either nivolumab (NIVO) or everolimus (EVE), whole-exome sequencing (WES) data from tumor and peripheral blood samples were analyzed. The Cancer Genome Analysis pipeline was utilized to identify somatic alterations, and the STITCH pipeline was employed to determine SNP carrier status. In the BinTA-0037 (BTA-0037)

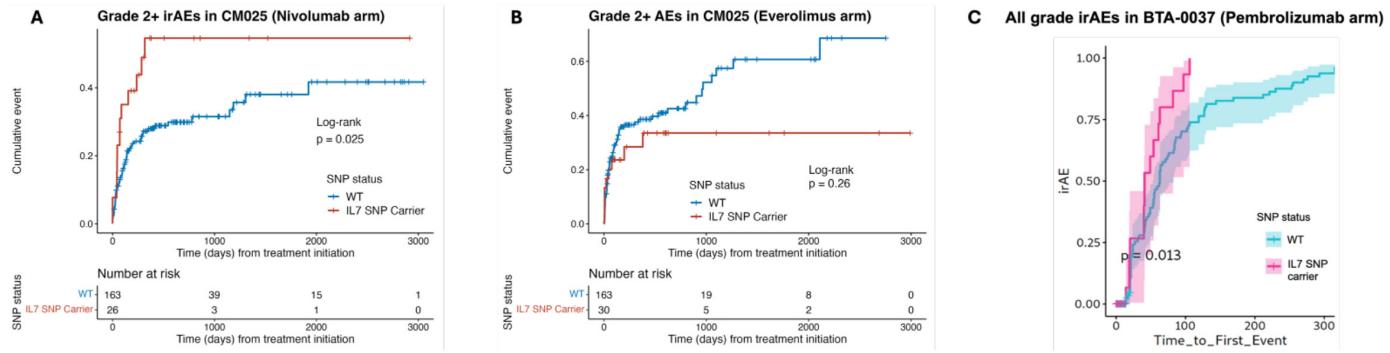


Figure: Kaplan-Meier curves showing the cumulative rate of adverse events in the NIVO (A) and EVE (B) arms of CM025, as well as the PEMBRO arm of BTA0037 (C), in SNP- and SNP+.

trial (NCT03631706), focusing on patients with metastatic non-small cell lung cancer (mNSCLC), the carrier status of a surrogate SNP rs16906062 ($R^2=0.66$) was determined from tumor WES in the pembrolizumab (PEMBRO) arm. Within each treatment arm, time to incident AEs were compared between carriers (SNP+) and non-carriers (SNP-) via multivariable Cox regression, controlling for age, sex, race, ECOG and sample purity. A SNP'treatment interaction term was also included in the entire CM025 cohort. Censoring for AEs occurred at death or last follow-up. A recurrent event analysis for AEs was conducted using the Andersen-Gill model, controlling for the same variables. Additionally, overall survival (OS) and progression-free survival (PFS) were also assessed.

Results

In total, 534 pts were included (NIVO: n=189, PEMBRO: n=152, EVE: n=193), among which 82 (15.4%) were SNP+. There were no differences in clinical and pathological characteristics between SNP+ and SNP-, except for sex (SNP+ 16.1% vs. SNP- 30.1% in females, $P=0.046$). Similarly, no differences in somatic alterations, including single nucleotide and copy number variants were seen between SNP+ and SNP- in CM025. SNP carrier status had no effect on OS nor PFS in all treatment arms (all $P \geq 0.22$). The rate of grade 2+

AEs was significantly higher in SNP+ vs. SNP- in the NIVO arm (**Figure A**, HR=2.91[1.48-5.72]), but not in the EVE (Figure B, control, non-ICI) arm (HR=0.63[0.3-1.29], SNP'treatment Pinteraction=0.002). Similarly, the rate of all grade AEs in the PEMBRO arm of BTA-0037 was higher in SNP+ vs. SNP- (**Figure C**, HR=2.30[1.60-4.60]). The rate of recurrent grade 2+ AEs was also significantly higher in SNP+ vs. SNP- in the NIVO arm (HR=3.43[1.83-6.43]), whereas a trend for fewer recurrent grade 2+ AEs was seen in SNP+ vs. SNP- in the EVE arm (HR=0.46[0.17-1.25], SNP'treatment $P_{\text{interaction}}=0.0005$).

Conclusions

The IL7 SNP (rs16906115) is associated with significantly higher rates of grade 2+ AEs, including recurrent events, in pts with mRCC or mNSCLC treated with single agent PD-1 inhibitors but not with non-ICI regimens, with no effect on survival and efficacy outcomes. These results confirm the SNP's predictive potential as a biomarker for irAEs to guide therapeutic decisions in pts treated with ICIs.

Keywords

immune-related adverse events; immune checkpoint inhibitors; germline variants; biomarkers; immune toxicity

39

Safety profile of belzutifan monotherapy in patients with renal cell carcinoma: A pooled analysis of 4 clinical trials

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Background

The first-in-class hypoxia-inducible factor-2α inhibitor, belzutifan, is indicated in the United States for the treatment of certain patients with von Hippel-Lindau (VHL) disease-associated renal cell carcinoma (RCC), central nervous system hemangioblastomas, or pancreatic neuroendocrine tumors not requiring immediate surgery and for patients with

advanced RCC previously treated with a PD-(L)1 inhibitor and a vascular endothelial growth factor tyrosine kinase inhibitor. Due to its unique mechanism of action, belzutifan has a distinct safety profile. We characterized the safety profile of belzutifan monotherapy in a post hoc pooled analysis of patients with previously treated advanced clear cell RCC in the phase 1 LITESPARK-001 (NCT02974738), phase 3 LITESPARK-005 (NCT04195750), and phase 2 LITESPARK-013 (NCT04489771) studies and patients with RCC associated with VHL disease in the phase 2 LITESPARK-004 study (NCT03401788).

Methods

All patients who received ≥1 dose of belzutifan 120 mg orally once daily across the 4 studies were included. Severity of adverse events (AEs) were graded per the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03 or 5.0, and were descriptively summarized.

Results

A total of 576 patients were included (n = 58 [including 3 patients with advanced solid tumors other than RCC], LITESPARK-001; n = 381, LITESPARK-005; n = 76, LITESPARK-013; and n = 61, LITESPARK-004). Overall, 572 patients (99.3%) had ≥1 all-cause AE and 355 patients (61.6%) had ≥1 grade 3-5 AE. AEs led to dose modification (reduction, interruption, or discontinuation) in 288 patients (50.0%), although treatment discontinuation for AEs occurred in 37 patients (6.4%). The most common all-grade AEs were anemia (including patients with decreased hemoglobin; n = 485 [84.2%]; grade 3 or 4, n = 166 [28.8%]), fatigue (n = 246 [42.7%]; grade 3, n = 16 [2.8%]), nausea (n = 139 [24.1%]; grade 3, n = 5 [0.9%]), and dyspnea (n = 123 [21.4%]; grade 3 or 4, n = 10 [1.7%]). Hypoxia was reported in 94 patients (16.3%); grade 3 or 4 hypoxia occurred in 57 patients (9.9%). Summary and time to first onset of adverse drug reactions (AEs considered associated with belzutifan), including anemia and hypoxia, are reported in the table. Among 485 patients with anemia or decreased hemoglobin, 111 (22.9%) were treated with erythropoiesis-stimulating agent (ESA) only, 85 (17.5%) were treated with blood transfusions only, and 62 (12.8%) were treated with ESA and blood transfusions. Among 94 patients with hypoxia, 66 (70.2%) were treated with oxygen therapy. Treatment-related AEs occurred in 526 patients (91.3%) and 217 (37.7%) experienced a grade 3-5 treatment-related AE. One grade 5 adverse event (multiple organ dysfunction syndrome) was considered related to treatment.

AE	Pooled population						
	N = 576						
	Incidence, n (%)	Led to dose interruption, n (%)	Led to dose reduction, n (%)	Led to treatment discontinuation, n (%)	Time to first onset of AE (any grade), median (range), days		
Anemia ^a	485 (84.2)	41 (7.1)	22 (3.8)	2 (0.3)	29 (1-834)		
Hypoxia	94 (16.3)	31 (5.4)	36 (6.3)	8 (1.4)	31 (1-952)		
Fatigue	246 (42.7)	15 (2.6)	10 (1.7)	1 (0.2)	42 (1-1017)		
Nausea	139 (24.1)	14 (2.4)	2 (0.3)	1 (0.2)	43 (1-1346)		
Dyspnea	123 (21.4)	10 (1.7)	3 (0.5)	1 (0.2)	57 (1-911)		
Dizziness	103 (17.9)	9 (1.6)	0 (0)	1 (0.2)	49 (1-974)		
Weight increased	44 (7.6)	0 (0)	0 (0)	0 (0)	111 (4-671)		

^aIncludes patients with adverse events of anemia and decreased hemoglobin.

Table. Summary and time to first onset of adverse drug reactions

Conclusions

To date, this is the largest pooled safety dataset for belzutifan monotherapy in patients with RCC. The results provide an in-depth characterization of the safety profile for belzutifan and the associated AE management strategies.

Keywords

Belzutifan, HIF-2a, renal cell carcinoma

42

Belzutifan versus everolimus for previously treated advanced clear cell renal cell carcinoma: Subgroup analysis of the phase 3 LITESPARK-005 study

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Background

Belzutifan monotherapy is approved for the treatment of adult patients with advanced renal cell carcinoma (RCC) following a PD-(L)1 inhibitor and a vascular endothelial growth factor tyrosine kinase inhibitor (VEGF-TKI) based on the results of the phase 3 LITESPARK-005 study (NCT04195750). In LITESPARK-005, belzutifan improved progression-free survival (PFS; HR 0.75; 95% CI, 0.63-0.90; $P < 0.001$) and objective response rate (ORR; 21.9% vs 3.5%; $P < 0.00001$) versus everolimus at first interim analysis (IA1); overall survival (OS) did not reach statistical significance at IA2 (HR 0.88; 95% CI, 0.73-1.07; $P = 0.1$). We present efficacy outcomes by prespecified subgroups from IA2.

Methods

Patients with clear cell RCC whose disease progressed after anti-PD-(L)1 and VEGF-targeted therapies and who had 1-3 prior systemic regimens were randomly assigned 1:1 to belzutifan 120 mg by mouth once daily or everolimus 10 mg by mouth once daily until progression or intolerable toxicity. The dual primary end points of PFS by central review per RECIST v1.1 and OS and the key secondary end point of ORR were evaluated by prespecified baseline characteristic subgroups: IMDC risk (favorable vs intermediate/poor), prior VEGF-TKIs (1 vs 2-3), and number of prior lines of therapy (1 vs 2 vs 3). These analyses were not controlled for multiplicity and no formal statistical testing occurred. The database cutoff date was June 13, 2023.

Results

Overall, 746 patients were assigned to belzutifan ($n = 374$) or everolimus ($n = 372$). Baseline characteristics were balanced between groups. Median follow-up was 25.7 months (range, 16.8-39.1). Across analyzed subgroups, PFS and OS results were consistent with the primary analysis (Table). ORR

	IMDC Favorable		IMDC Intermediate/Poor		1 Prior VEGF-TKI		2-3 Prior VEGF-TKIs		1 Prior Line		2 Prior Lines		3 Prior Lines ^a	
	Bel	Eve	Bel	Eve	Bel	Eve	Bel	Eve	Bel	Eve	Bel	Eve	Bel	Eve
n	79	83	295	289	187	190	187	182	46	52	157	166	171	154
PFS, HR	0.74		0.74		0.77		0.73		0.54		0.81		0.77	
(95% CI)	(0.51-1.09)		(0.61-0.89)		(0.61-0.98)		(0.57-0.93)		(0.34-0.87)		(0.62-1.05)		(0.60-1.00)	
OS, HR	0.75		0.90		0.87		0.89		0.83		0.84		0.93	
(95% CI)	(0.47-1.21)		(0.73-1.10)		(0.67-1.13)		(0.68-1.16)		(0.46-1.50)		(0.63-1.10)		(0.70-1.24)	

Bel, belzutifan; Eve, everolimus.

^aIncluded 2 patients in the belzutifan arm and 4 patients in the everolimus arm who had 4 prior lines of therapy and protocol violations.

favored belzutifan over everolimus for all subgroups: IMDC favorable risk (22.8% vs 6.0%), IMDC intermediate/poor risk (22.7% vs 2.8%), 1 prior VEGF-TKI (19.7% vs 3.7%), 2 prior VEGF-TKIs (25.7% vs 3.3%), 1 prior line of therapy (28.3% vs 5.8%), 2 prior lines (19.1% vs 2.4%), and 3 prior lines (24.6% vs 3.9%).

Conclusions

Consistent with the intention-to-treat population of LITESPARK-005, PFS and ORR favored belzutifan over everolimus across prespecified subgroups. These results support belzutifan as a new treatment option for patients with advanced clear cell RCC after prior anti-PD-(L)1 and VEGF-targeted therapies.

Keywords

belzutifan, HIF-2 α , renal cell carcinoma

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Liquid biopsy epigenomic profiling for the detection of sarcomatoid renal cell carcinoma.

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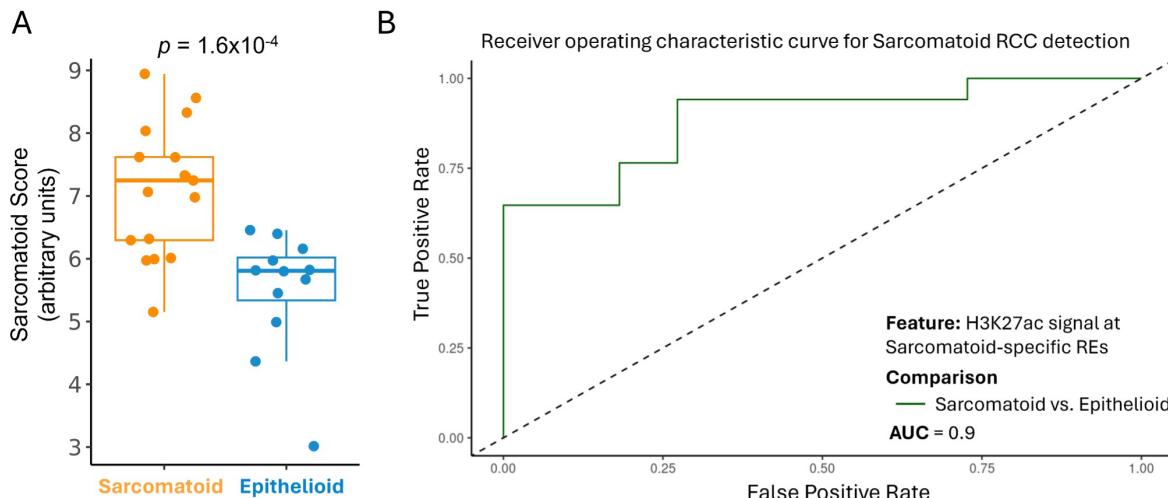


Figure 1. (A) Sarcomatoid Score in plasma from patients with RCC at sarcomatoid-specific REs, comparing sarcomatoid (orange) and epithelioid RCC (blue). (B) ROC curves for distinguishing sarcomatoid from epithelioid RCC plasma samples using H3K27ac cell-free ChIP-seq signal at sarcomatoid-specific REs. 'AUC' indicates area under the ROC curve.

Background

Sarcomatoid differentiation (SD) in renal cell carcinoma (RCC) is associated with poor survival and heightened response to immune checkpoint blockade. Detection of SD can be challenging due to spatial heterogeneity and sampling error. Herein, we introduce a novel tissue-informed epigenomic approach to noninvasively identify sarcomatoid differentiation in patients with RCC from cell-free DNA (cfDNA) using 1mL of plasma.

Methods

Chromatin immunoprecipitation and sequencing (ChIP-seq) for H3K27ac – a histone modification associated with active regulatory elements (REs) – was performed on pathologically reviewed clear cell RCC frozen tissue samples with and without SD (sarcomatoid-RCC and epithelioid-RCC, resp.) collected at the Dana-Farber Cancer Institute. Differentially marked REs between sarcomatoid and epithelioid subtypes were identified using DESeq2 (false discovery rate of $q < 0.01$). After establishing tissue signatures, ChIP-seq was then performed on cell-free chromatin (cfChIP-seq) in plasma from patients with sarc-RCC and epi-RCC. A Sarcomatoid Score was derived for each sample by aggregating the plasma H3K27ac signal at tissue-derived sarcomatoid-specific REs (sarc-REs), while normalizing to signal at epithelioid-specific REs (epi-REs). Scores were compared between the two groups using a Wilcoxon rank-sum test. A classifier was built to distinguish sarc-RCC from epi-RCC based on the Sarcomatoid Score and its performance was evaluated using the area under the receiver operating characteristic (AUROC) curve.

Results

We identified 25,919 differentially marked REs between 8 sarc-RCC and 8 epi-RCC tissue samples at a false discovery rate of $q < 0.01$. We selected 12,868 REs that are enriched

in sarcomatoid vs. epithelioid. We generated cfChIP-seq profiles from plasma of 29 patients, 17 with sarc-RCC and 12 with epi-RCC. The Sarcomatoid Scores were significantly higher in sarc-RCC vs. epi-RCC plasma samples ($p=1.6\times10^{-4}$; Figure 1A). These scores achieved an AUROC curve of 0.9 for classifying patients with sarc-RCC from patients with epi-RCC (Figure 1B).

Conclusions

We present a proof-of-concept study in 1 cc of plasma for the detection of sarcomatoid differentiation in RCC based on the assessment of histone modification signals in cfDNA. This approach could help overcome the challenges of spatial heterogeneity and sampling error from tissue that make identification of sarc-RCC difficult. More generally, it establishes a paradigm for identifying histologic subtypes of cancer based on their epigenomic correlates from cfDNA, with possible therapeutic implications in real-time.

Keywords

Liquid biopsy; epigenomic; cell-free DNA; renal cell carcinoma; sarcomatoid differentiation

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Impact of CBM588 on gut microbiome composition and dysbiosis in patients receiving frontline immune checkpoint inhibitor (ICI) combinations for metastatic renal cell carcinoma (mRCC)

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Background

Two recent randomized phase I clinical trials have provided compelling evidence that CBM588, a Clostridium butyricum-based live biotherapeutic, holds potential to enhance clinical

outcomes in patients with mRCC receiving frontline ICI combinations (Dizman et al Nature Medicine 2022; Ebrahimi et al ASCO 2023). We examined the impact of CBM588 on gut microbiome composition in a combined cohort of these two studies to further investigate its impact on gut microbiome

Methods

We analyzed stool samples from two phase I randomized clinical trials that enrolled patients with mRCC treated with (1) nivolumab/ipilimumab (nivo/ipi) +/- CBM588 and (2) cabozantinib/nivolumab (cabo/nivo) +/- CBM588. We compared gut microbiome diversity and composition at baseline and week 12 between patients in the standard of care (SOC) arms (nivo/ipi or cabo/nivo) and those who received CBM588 in combination with a SOC regimen (SOC/CBM). Taxonomic profiling was performed using MetaPhlan v4, and changes in the abundances of clinically relevant microbial species from baseline to week 12 were assessed using the Wilcoxon matched pairs test. The ratio of Firmicutes/Bacteroidetes, a measure of gut dysbiosis, was computed across time points in the two cohorts.

Results

Among 58 patients included in the analysis, 38 received SOC/CBM588 as first-line treatment. The median age was 60 years (range: 36-90) and the majority of patients were male (71%), had clear cell mRCC (88%), and intermediate/poor risk disease (79%). In both the SOC and SOC/CBM cohorts, there were no statistically significant differences in alpha and beta diversity between baseline and week 12. Among clinically relevant species compared between baseline and week 12, *Alistipes senegalensis* was found to decrease in both the SOC and SOC/CBM cohorts (log fold change [LFC] -0.82 [$P=0.004$] and LFC -0.36 [$P=0.007$], respectively), while *Eubacterium siraeum* decreased only in the SOC cohort (LFC -1.75 [$P=0.005$]). The Firmicutes/Bacteroidetes ratio increased from 89.0% to 96.4% in the SOC cohort, whereas a notable decrease was observed in this ratio from 100.0% to 75.7% in the SOC/CBM cohort.

Conclusions

CBM588 leads to a marked correction of gut dysbiosis and prevents the depletion of species previously associated with ICI response (i.e., *Eubacterium siraeum*). These findings provide a plausible mechanism for the enhanced clinical outcome with CBM588 now seen across two small, randomized trials. A phase III study is planned within the cooperative groups to evaluate the clinical activity and gut microbiome modulation capacity of CBM588 in combination with ICIs in mRCC.

Keywords

Renal cell carcinoma, microbiome, dysbiosis

Rapid Abstract Presentations

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Development of an ex vivo patient-derived tumor model (PDTM) to assess the tumor microenvironment in renal cell carcinoma (RCC)

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Background

With the rise of immune checkpoint inhibitors (ICIs) as the primary treatment option for metastatic RCC, investigating the role of T cells within the tumor microenvironment (TME) is a critical component of understanding both treatment response and resistance. Prior efforts, including single-cell transcriptomic approaches, have provided an important landscape of T cell transcriptional phenotypes. However, these immuno-profiling efforts require validation through functional interrogation of the TME to facilitate the development of novel immunomodulatory therapies. Thus, we established a patient derived tumor model (PDTM) system to directly assess the effect of inhibitory immune interactions on T cell function and anti-tumor activity in the RCC TME. In this initial proof-of-concept study, we evaluated T cell activation in the RCC TME using the PDTM system.

Methods

Fresh tumor samples were obtained from surgical resections of RCC at Yale-New Haven Hospital. The tumor was minced to ~1-3 mm³ pieces and suspended in an air-liquid interface system, consisting of tumor fragments embedded in a collagen matrix on an insert with a semi-permeable membrane, exposed to culture media. The tumor fragment and matrix suspension were carefully pipetted onto the Millicell insert, which served as the top layer. The PDTM setup includes an inner dish

containing the bottom gel layer and the tissue-containing top layer. To complete the assembly, 1.5 ml of DMEM media with or without an anti-CD3 monoclonal antibody (aCD3mAb) and 500 nM of anti-PD-1 monoclonal antibody (aPD1mAb) was added to the outer dish surrounding the insert.

Results

We successfully optimized PDTM experimental workflows for culture, dissociation, and analysis using immunohistochemistry (IHC), flow cytometry (FCM), and enzyme-linked immunosorbent assays (ELISA). Hematoxylin and eosin (H&E) staining and IHC showed that the TME cellular architecture and immune cell composition was broadly preserved during the three day experimental period. Using FCM to analyze the dissociated tumor samples, we identified well-preserved CA9+ tumor cells, CD4+ and CD8+ T cell populations, CD4+CD25+ regulatory T cells, CD56+ natural killer cells, CD20+ B cells, and CD14+CD11b+ myeloid subsets including monocytes and CD163-/+macrophages. Among the T cells, we detected PD1+, LAG3+, TIM3+, and TIGIT+ cells. For dose-response analysis of aCD3mAb, we observed that stimulation with 0.025 ng/mL aCD3mAb for T cells from healthy donor peripheral blood mononuclear cells (PBMCs) yielded significant but non-saturating levels of interferon gamma (IFN-γ) production as quantified by ELISA. We tested the effect of the anti-PD-1 antibody on PDTM under the low-dose of aCD3mAb, and importantly, found that treatment of the PDTM with aPD1mAb resulted in more activated CD8 T cells and higher IFN-γ production than the control samples.

Conclusions

Through optimization of assays evaluating T cell cytokine production, we were able to assess multiple axes of T-cell function in the RCC TME. This study revealed that our PDTM system preserves the RCC TME for functional interrogation. Furthermore, our system for assessing T cell phenotype and cytokine production successfully demonstrated the activity of PD-1 blockade *ex vivo*. Taken together, this novel *ex vivo* PDTM system 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, Tumor microenvironment, Patient-derived tumor model

DOD CDMRP Funding

yes

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A Phase 1 study of fianlimab (anti-LAG-3) in combination with cemiplimab (anti-PD-1) in patients with advanced ccRCC

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Background

Concurrent blockade of lymphocyte-activation gene 3 (LAG-3) may enhance efficacy of anti-programmed cell death-1 (PD-1) therapies. We present safety and clinical activity data from a Phase 1 study (NCT03005782) in patients with clear cell renal cell carcinoma (ccRCC) treated with anti-LAG-3 (fianlimab) + anti-PD-1 (cemiplimab).

Methods

Patients with advanced or metastatic ccRCC who had received no more than two previous regimens of anti-angiogenic therapy who were anti-PD-(ligand[L])1-naïve (cohort 3) or anti-PD-(L)1-experienced with most recent dose within 3 months prior to screening (cohort 4) were eligible. All patients were to receive fianlimab 1600 mg + cemiplimab 350 mg intravenously every 3 weeks for up to 24 months. Tumor measurements were performed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 every 6 weeks for 24 weeks, then every 9 weeks. The study objectives were to assess safety and antitumor activity of fianlimab + cemiplimab combination therapy in patients with ccRCC.

Results

Overall, 15 patients (median age: 64 years) each in cohorts 3 and 4 (total N=30) were enrolled and treated with fianlimab + cemiplimab as of November 1, 2022 data cutoff. For cohorts 3 and 4, 80% and 87% of patients were male, and 40% and 87% were White, respectively. All patients had prior cancer-related systemic therapy. In total, 60% and 93% of patients in cohorts 3 and 4 had ≥2 lines of prior therapies, respectively.

For cohorts 3 and 4, median treatment duration was 27 weeks and 18 weeks, and median follow-up was 13 months and 24 months, respectively. Grade ≥3 treatment-emergent adverse events (TEAEs) occurred in 53% and 33% of patients in cohorts 3 and 4, respectively. Serious TEAEs occurred in 33% and 13% of patients in cohorts 3 and 4, respectively. Treatment-related AEs (TRAEs) were reported in 80% of patients in cohort 3 and 60% of patients in cohort 4. The most common TRAEs (any Grade) were rash (27%) and infusion related reaction (Grade 1 and 2; 27%) in cohort 3, and fatigue (20%) in cohort 4. Grade

≥3 TRAEs occurred in 27% of patients in cohort 3; there were no Grade ≥3 TRAEs in cohort 4. Treatment was discontinued due to any TEAE in three patients in cohort 3 and one patient in cohort 4. There was one death in cohort 3; a 79-year-old woman with a history of antiphospholipid syndrome died from complications of biopsy-proven ischemic colitis, which was attributed to study treatment.

RECIST 1.1-based investigator-assessed objective response rate was 20% (3 partial responses [PRs]) in cohort 3 and 7% (1 PR) in cohort 4. The disease control rate was 60% and 73% in cohorts 3 and 4, respectively. Kaplan-Meier estimation of median progression-free survival was 4 months (95% confidence interval [CI] 1–10) in cohort 3 and 4 months (95% CI 1–7) in cohort 4. Durations of response were 4, 7, and 26 months in three responders in cohort 3, and 6 months in one responder in cohort 4.

Conclusions

Fianlimab + cemiplimab demonstrated promising signs of clinical activity with durable responses among patients who were anti-PD-(L)1-naïve (cohort 3) and anti-PD-(L)1-experienced (cohort 4), with an acceptable safety profile.

Keywords

Fianlimab, cemiplimab, advanced clear cell renal cell carcinoma

11

Tumor evolution of brain-specific tropism in metastatic renal cell carcinoma

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Background

Brain metastases (BMets) pose a clinical challenge in the management of patients (pts) with metastatic renal cell carcinoma (RCC), leading to significant morbidity and mortality. Herein, we sought to comprehensively characterize the molecular landscape of BMets in RCC.

Methods

We performed panel-based DNA (DNaseq) and whole transcriptome (RNAseq) sequencing to analyze BMets, matched non-Brain metastases (nBMets), and matched primary renal tumors (PRT) from pts who underwent surgical resection of BMet(s) from RCC at our institution.

Results

Our cohort consisted of 95 samples from 53 patients (BMets = 54, nBMets = 14, PRT = 27) with clear cell histology in 45 pts (84.9%). DNAseq was available on 85 samples (50 pts). Patient-level mutations revealed recurring mutations in *VHL* (18 pts, 36%), *TP53* (n = 12 pts, 24%), *PBRM1* (12 pts, 24%), *SETD2* (12 pts, 24%), and *BAP1* (4 pts, 8%). Mutations within the MTOR signaling pathway were enriched, with 25 pts (50%) having at least one mutation in *PTEN* (12 pts, 24%), *TSC1* (4 pts, 8%), *TSC2* (5 pts, 10%), or *MTOR* (9 pts, 18%). Copy number analyses revealed frequent deletions in chr14q21 (34 pts, 68%) and chr9p21 (35 pts, 70%) and gains in chr20q13 (n = 31, 62%) and chr7p15 (n = 31, 62%).

At the sample-level, *PTEN* mutations were more common in BMets (11 pts, 22.9%) vs nBMets (0 pts) and PRTs (3 pts, 12%, p = 0.124), as were deletions in chr13q22 (BMets = 13pts [26.5%], nBMet = 1pts [8.3%], PRT = 1 [4%]; p = 0.036) and gains in chr20q13 (BMets = 30pts [61.2%], nBMet = 4pts [33.3%], PRT = 8 [32%]; p = 0.03). Deletions in chr9p21 were enriched in BMets (32, 65.3%) and nBMets (8, 66.7%) relative to PRTs (7, 28%, p = 0.006), whereas other copy number alterations and somatic mutations exhibited similar proportions across specimen sites.

Differential expression analysis performed on 86 samples (51 pts) identified 806 differentially expressed genes (DEGs) between BMets and PRT (adjusted [adj.] p < 0.05) and 399 DEGs between BMet and nBMets (adj. p < 0.05). Gene set enrichment analysis of MSigDB Hallmark gene sets revealed upregulation of MTORC1 signaling, glycolysis, and MYC targets in BMets compared to PRT and nBMETs (adj. p <0.0001). In contrast, immune-related gene sets, such as interferon-alpha, interferon-gamma, and tumor necrosis factor-alpha, were enriched in PRT relative to BMets (adj. p <0.0001), but not nBMETs (adj. p > 0.05). CIBERSORT cellular deconvolution analysis comparing BMets with PRT revealed decreased proportions of M1 macrophages (p = 0.0003) and CD8+ T-cells (p = 0.0106), but an increased proportion of M2 macrophages (p = 0.0009).

Conclusions

RCC with brain metastases are characterized by distinct copy number alterations, enrichment of MTOR pathway mutations, MTOR pathway hyperactivation, and an immunosuppressive tumor milieu. These findings may hold therapeutic implications of MTOR pathway inhibition and immune modulators in treating RCC BMets.

Keywords

Metastasis, MTOR

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Concordance Analysis of Tissue and Circulating Tumor DNA (ctDNA) in Renal Cell Carcinoma (RCC): Insights from a Multimodal Real-World Database

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Background

Next-generation sequencing (NGS) of circulating tumor DNA (ctDNA) has emerged as a powerful complement to tissue NGS, offering a noninvasive and serially conductible test. Its application holds promise in enhancing the assessment of spatial and temporal molecular tumor heterogeneity, thus providing valuable insights into cancer progression and treatment response. In this study, we explore the mutational landscape of renal cell carcinoma (RCC) patients through comprehensive profiling of mutations in ctDNA and matched tissue samples, aiming to elucidate the concordance and clinical significance of molecular alterations detected in both circulating and tissue-derived DNA.

Methods

From the Tempus multimodal database, we retrospectively analyzed de-identified NGS data from patients with RCC that had dual tissue (Tempus xT, 648 genes) and ctDNA testing (Tempus xF, 105 genes). Patients with matched samples (collected +/- 90 days of one another) were included. We evaluated socio-demographic and clinical characteristics and select pathogenic somatic short variants (PSSV) and copy number variants [(amplifications and deletions, two copy number losses (CNL)]. Concordance analyses were restricted to the 105 genes tested on the ctDNA panel and further restricted to short variants, with the exception of amplifications and CNL detected by both xF and xT. Analysis was further stratified by metastatic status (n=260, metastatic, n=120 non-metastatic) prior to collection of both xT and xF.

Results

Among all patients (n=393), the median age was 61 years, and 71% were male. The patient cohort comprised a diverse population based on race, with 75% white, 12% African

American, 4.8% Asian and 8% others. The median time from tissue to blood collection was 21 days (IQR, 7, 39). 67% (n=265) and 68% (n=266) had metastatic disease at the time of tissue and blood collection, respectively. The most common tissue sites were kidney (49%, n=189), bone (11%, n=43), lung (9%, n=34), lymph node (8%, n=29), liver (6%, n=23), and brain/CNS (4%, n=17). Genes harboring the most common PSSV in tissue included *VHL* (59% n=232), *PBRM1* (31%, n=123), *SETD2* (23%, n=91), *TP53* (14%, n=54), *BAP1* (12%, n=46) and *TERT* (11%, n=45). Genes with common PSSV in ctDNA included *TP53* (23%, n=91), *VHL* (18%, n=69), *BAP1* (6%, n=23), *PBRM1* (5%, n=21), *PTEN* (4%, n=15), *KRAS* (4%, n=14) and *NF2* (4%, n=14). The combination of tissue and ctDNA testing increased the detection of mutations (**Table 1**). There was higher concordance between somatic alterations in select genes among patients with metastases vs. non-metastases, including *BAP1* (55.9% vs. 9.1%), *TP53* (36.8% vs. 9.1%), *VHL* (32.3% vs. 12.5%), *ARID1A* (25% vs. 16.7%), and *ATM* (25% vs. 0%).

Conclusions

The analysis conducted in this study highlights the complementary nature of ctDNA profiling alongside tissue-based NGS in RCC, demonstrating an increased detection of mutations. Particularly, we observed a higher concordance between ctDNA and tissue profiling in individuals with metastatic disease, suggesting the potential utility of ctDNA analysis in advanced stages of RCC. Further research is warranted to elucidate how longitudinal ctDNA analysis can delineate biomarkers of response and resistance at both the mutation and ctDNA fraction levels. Understanding these dynamics could offer valuable insights into disease progression and guide personalized treatment strategies for RCC patients.

Keywords

RCC, ctDNA,

DOD CDMRP Funding

no

Gene N (%)	+xT &/or xF All patients	+xT Only All patients	+xF Only All patients	+xT & xF +xT &/or xF	+xT & xF +xT &/or xF (metastatic)	+xT & xF +xT &/or xF (non-metastatic)
VHL	237 (60%)	168 (43%)	5 (1%)	64/237 (27%)	51/158 (32%)	9/72 (13%)
PBRM1	123 (31%)	102 (26%)	0 (0%)	21/123 (17%)	15/88 (17%)	4/30 (13%)
TP53	112 (28%)	21 (5%)	58 (15%)	33/112 (29%)	28/76 (37%)	3/33 (9%)
TERT	49 (12%)	38 (10%)	4 (1%)	7/49 (14%)	6/36 (17%)	1/11 (9%)
BAP1	47 (12%)	24 (6%)	1 <td>22/47 (47%)</td> <td>19/34 (56%)</td> <td>1/11 (9%)</td>	22/47 (47%)	19/34 (56%)	1/11 (9%)
ARID1A	23 (6%)	12 (3%)	4 (1%)	7/23 (30%)	5/20 (25%)	1/6 (17%)
BRCA2	16 (4%)	12 (3%)	3 (1%)	1/16 (6%)	1/12 (8%)	0/3 (0%)
TSC1	14 (4%)	9 (2%)	0 (0%)	5/14 (36%)	3/9 (33%)	1/3 (33%)
MTOR	10 (3%)	8 (2%)	0 (0%)	2/10 (20%)	1/7 (14%)	0/2 (0%)

Table 1: Detection of genetic mutations in tissue and ctDNA testing

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Natural killer cells have impaired cytotoxicity in advanced renal cell carcinoma

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Background

Natural killer (NK) cells are key mediators of anti-tumor activity in multiple cancers, including clear cell renal cell carcinoma (ccRCC). However, the phenotype and function of NK cells across different disease stages of ccRCC is incompletely characterized.

Methods

Single-cell RNA (scRNA)-sequencing (10x Genomics) data from tumor and adjacent normal kidney from ccRCC was analyzed at multiple clinical stages (localized – stage I/II/III; and advanced – stage IV). Graph-based clustering and lineage markers were used to identify distinct NK cell populations. Differential gene expression analysis was performed to further investigate NK cell phenotype and to derive a gene expression signature (GES). Gene signatures from NK cell subclusters of interest were used to interrogate bulk transcriptomic datasets and expression with clinical outcomes. Tumor-infiltrating NK cell function (cytokine production and cytotoxicity) was assessed by isolation of live NK cells from ccRCC tissue, co-culture with K562 target cells, and measurement of cytokine (IFNgamma) and cytotoxicity (CD107a) markers by flow cytometry.

Results

Single-cell transcriptomic data were analyzed from 13 patients with ccRCC (tumor and normal kidney), resulting in 21,139

high-quality NK cells. Patients with advanced/metastatic RCC had a lower proportion of NK cells versus total immune cells in the tumor microenvironment compared to normal kidney ($p=0.036$) and localized ccRCC ($p=0.088$). Clustering analysis revealed 6 distinct NK cell subsets. The C0.Bright-like NK cell cluster was significantly enriched in advanced ccRCC compared to localized ccRCC ($p=0.048$) and normal kidney ($p=0.0059$), expressed markers of tissue residency (ZNF683, ITGA1, CD9, ITGAE), and had decreased expression of cytotoxicity genes (GZMB, PRF1). A GES signature composed of genes upregulated in the dysfunctional C0.Bright-like NK cell cluster was used to interrogate bulk RNA-sequencing data from The Cancer Genome Atlas clear cell cohort, providing validation in a large, independent cohort, of an increase in this dysfunctional NK subset in advanced ccRCC compared to localized ccRCC ($p=0.00039$) and normal tissue ($p=5.3e-05$). To functionally confirm the decreased cytotoxicity of this dysfunctional NK population, CD45⁺CD56⁺CD3⁻ NK cells were isolated from advanced ccRCC, and co-cultured with K562 target cells. Consistent with the transcriptomic phenotype, tumor-resident CD49a⁺CD9⁺ NK cells had cytokine production (IFNgamma) but decreased markers of cytotoxicity (CD107a) compared to CD49a⁻CD9⁻ NK cells. By contrast, CD49a⁺CD9⁺ NK cells isolated from peripheral blood or normal kidney tissue maintained cytotoxic activity.

Conclusions

Among patients with ccRCC, a single-cell transcriptomic analysis revealed heterogeneous NK cell populations. A dysfunctional tumor resident NK cell phenotype was enriched among patients with advanced disease in ccRCC, and functionally confirmed to have diminished cytotoxicity. This study therefore provides insight into a new axis of immune dysfunction – decreased NK-mediated cytotoxicity – in advanced ccRCC. An improved understanding of NK cell dysfunction within ccRCC may provide a foundation for therapeutic restoration of NK-mediated anti-tumor activity in ccRCC.

Keywords

immunology, transcriptomics, natural killer cells, advanced renal cell carcinoma

DOD CDMRP Funding

yes

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Evaluating intermediate endpoints for overall survival in metastatic renal cell carcinoma treated with immune checkpoint inhibitors: an IMDC study

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Background

In Phase 3 trials, the assessment for primary endpoint of overall survival (OS) necessitates extended follow-up periods, a larger pool of events, and higher associated costs. Hence, we sought to determine whether shorter intermediate (IEs) such as Objective Response (OR), Time to Treatment Failure (TTF) and Time to Next Therapy (TTNT) are associated with OS in patients receiving immune checkpoint (ICI)-based therapy.

Methods

We included all International mRCC Database Consortium (IMDC) patients who received contemporary first line(1L) ICI from 2013 to 2023. IEs were defined from ICI start until drug cessation or death for TTF, and initiation of next line or death for TTNT, or censored at date of last follow-up. OR included investigators-assessed complete or partial response per RECIST1.1 criteria. First, we assessed the endpoint correlations across all follow-up times of individual patient data using Kendall's Tau (KT) correlation by a Clayton copula. A KT >0.49 indicates a strong correlation (Wicklin R., 2023). Then we evaluated associations of OS with TTF and TTNT status at the 6-month landmark using Cox regression adjusting for IMDC risk groups, metastatic sites, histology, age, and prior nephrectomy, stratified by ICI type and years of ICI start. The KT correlation was estimated by the R GJRM package (<https://www.r-project.org/>). All other statistical analyses were done with SAS 9.4 software (SAS Institute, Cary, NC).

Results

The cohort consisted of 1667 patients with a median follow-up of 15.4 months (IQR: 7.1-28.6). For the 6-month landmark

Adjusted Hazard ratio (95%CI) for OS*			
	OR (Non-responders vs responders)	TTF (Treatment failure within 6 months: Yes vs. No)	TTNT (Initiating 2nd line within 6 months: Yes vs. No)
Overall	2.77(2.16-3.55)	2.74(2.15-3.49)	2.82(2.22-3.59)
IMDC risk groups			
Favorable	1.72(0.93-3.18)	1.92(1.03-3.60)	3.33(1.66-6.71)
Intermediate	3.54(2.44-5.15)	2.81(1.99-3.96)	2.93(2.09-4.10)
Poor	2.67(1.68-4.24)	3.72(2.39-5.79)	3.00(1.90-4.74)
Treatment			
ICI+ICI	3.35(2.46-4.54)	2.47(1.86-3.26)	2.75(2.09-3.61)
ICI+TKI	1.80(1.17-2.77)	3.41(2.19-5.31)	3.10(1.88-5.11)

ICl: immune-checkpoint inhibitor, IMDC= International Metastatic Renal-Cell Carcinoma Database Consortium, OR: Objective response, OS: Overall survival, TKI: Targeted therapy, TTF: Time to treatment failure, TTNT: Time to next line therapy

Table. Landmark analysis of OS from 6 months of post therapy initiation, according to OR, TTF and TTNT event status at 6 months, in whole cohort and by IMDC and treatment groups.

analysis in OS, patients who died or had less than 6 months of follow-up were excluded, affecting 278 for OR, 373 for TTF, and 380 for TTNT. Median age at 1L start was 63 years (IQR: 56-70), with 73% being male and 65% undergoing nephrectomy before starting 1L. A total of 1132 patients received dual ICI, while 535 received an ICI+TKI combination.

Over the entire follow-up of individual patient data, the Kendall's Tau correlation was 0.49 (95%CI: 0.45-0.52) for TTF with OS and 0.67 (95%CI: 0.64-0.69) for TTNT with OS. The Kendall's Tau correlations between endpoints were also similar in subgroup analyses by IMDC risk group and type of regimen and the strongest in the ICI+TKI subgroup with a Kendall's Tau correlation of 0.73(0.66-0.78).

On the 6-month OS landmark analysis (Table), patients who discontinued their 1L regimen had poor OS (adjusted HR: 2.77 (95%CI: 2.16-3.55)). Additionally, those who transitioned to a 2L therapy within the first 6 months showed worse OS, reflected by an HR of 2.82 (2.22-3.59). Patients who did not have an objective response also had worse OS (adjusted HR: 2.74 (95%CI: 2.15-3.49)). On the other hand, patients with an objective response had an 18-month OS rate (equivalent of 12-month OS post the 6-month landmark) of 91% (95%CI: 88%-93%) vs 74% (95%CI: 70%-78%) for those with no response at 6 months.

Conclusions

Our study explored TTF, TTNT and OR as clinical IEs for OS. TTNT demonstrated the strongest association with OS, particularly in the ICI+TKI subgroup, making it a potentially clinically meaningful intermediate endpoint for evaluating treatment efficacy in ICI-based regimens.

Keywords

intermediate endpoints, immune checkpoint inhibitors, time to next therapy, time to treatment failure

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Enrichment of tertiary lymphoid structures provides novel insight into mediators of anti-tumor immune activity in sarcomatoid renal cell carcinoma

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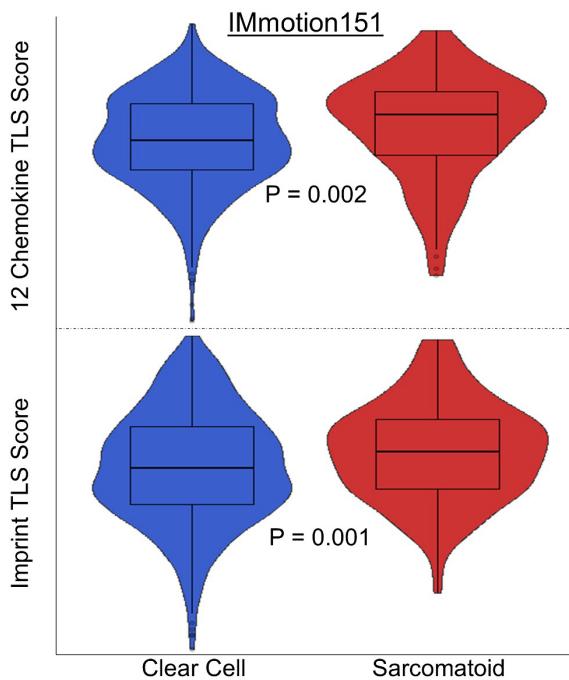
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Background

Renal cell carcinoma (RCC) comprises various histological subtypes, with clear cell RCC (ccRCC) being the most prevalent histotype. RCC with sarcomatoid features (sRCC) is a unique kidney cancer subtype associated with aggressive biological features and poor clinical outcomes that can arise from multiple RCC histologies, most commonly ccRCC. While clinically aggressive, sRCC paradoxically has also demonstrated preferential responsiveness to immune checkpoint blockade (ICB) therapies in subgroup analyses of multiple phase III trials. However, the mediators of this immune sensitivity are largely unknown. We therefore applied transcriptomic techniques to identify orchestrators of immune activity within the sRCC tumor microenvironment (TME).

Methods

Nephrectomy specimens from patients with sRCC and ccRCC were procured for single cell RNA sequencing (scRNASeq). Clustering and dimensionality reduction were performed, and cell populations were annotated based on expression of canonical lineage markers. Immune populations (CD8+ T cells, CD4+ T cells, B/plasma cells, myeloid cells) were computationally extracted and differential gene expression between sRCC- and ccRCC-derived cells within each subpopulation was performed. Gene expression programs enriched in sRCC samples by scRNASeq were validated on publicly available bulk gene expression data comparing sRCC to ccRCC. Spatial transcriptomics were performed on sRCC tumor sections using the 10X Visium platform.



Results

Across 18 RCC specimens (10 sRCC; 8 ccRCC), 73,123 cells were analyzed by scRNAseq. Within the CD8+ T cell compartment, CXCL13 was the most significantly enriched nuclear-encoded gene in sRCC samples (\log_2 -fold change=1.29; $q<0.001$). CXCL13 was also significantly enriched in CD4+ T cells from sRCC ($q<0.001$), suggesting enhanced presence of follicular T cells within the sRCC TME.

As follicular T cells function in support of B cells, we next interrogated the B lymphocyte population. sRCC samples were enriched for mature B cell and plasma cells, with a five-fold increase in the relative abundance of plasma cells compared to ccRCC samples. Immune deconvolution of patient-derived RNA sequencing from the IMmotion 151 trial revealed a significant increase in the predicted proportion of B lymphocytes (including plasma cells) within the sRCC TME relative to ccRCC ($p=0.034$). Further, over 20 B lymphocyte activation and maturation pathways were consistently enriched ($q<0.25$) in sRCC across clinical datasets (Javelin101, CheckMate, and TCGA KIPAN), including Signaling by the B Cell Receptor, Positive Regulation of B Cell Activation, and Immunoglobulin Production Involved in Immunoglobulin Mediated Immune Response, amongst others. B lymphocytes mediate anti-tumor immunity through antibody dependent cellular cytotoxicity (ADCC), and thus the phagocytic effectors of ADCC were interrogated. Myeloid populations differentially expressed FCγR3A in sRCC vs ccRCC ($q<0.001$), which was recapitulated in bulk gene expression populations ($q=0.002, 0.12$, and 1.08×10^{-4} in Javelin101, CheckMate, and TCGA cohorts, respectively).

Given the enrichment of follicular T cell and differentiated B lymphocyte programs, we explored the presence of tertiary lymphoid structures (TLS) in sRCC. Two distinct TLS signatures – the 12 Chemokine Score and the TLS Imprint Signature – were significantly enriched in sRCC vs ccRCC across RCC patient datasets (Figure 1). Furthermore, spatial transcriptomics were applied to H&E slides of sRCC to successfully identify the presence of TLS by expression of TLS-associated genes adjacent to sarcomatoid regions.

Conclusions

TLS, which have previously been associated with response to ICB in RCC (Meylan *et al.* *Immunity*. 2022), are transcriptomically enriched for in sRCC, paralleling an observed increase in CXCL13-expressing T cells and differentiated B lymphocytes. Together, TLS and their constituents offer a previously unexplored mediator of immunosurveillance in the sRCC TME that may underlie the paradoxical responsiveness to ICB seen within this population clinically.

Keywords

Sarcomatoid renal cell carcinoma, Tertiary lymphoid structures, B cells, single cell RNA sequencing, Spatial transcriptomics

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Impact of Latino Ethnicity on the gut microbiome composition of patients with metastatic renal cell cancer (mRCC)

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Background

Latinos with mRCC may have poorer outcomes with frontline immune checkpoint inhibition (ICI) compared to their non-Latino counterparts (Chehrazi-Raffle *et al* *Oncologist* 2023). Recent studies have shown that the composition of the gut microbiome can impact outcomes with ICI (Routy *et al* *Science* 2018). Therefore, we aimed to investigate the differences in gut microbiome composition between Latino and non-Latino patients with mRCC.

Methods

Stool specimens were prospectively collected in treatment-naïve patients with mRCC. We dichotomized patients into Latino vs non-Latino groups. Patients provided a stool sample

(OMNIgene Gut) at baseline. Whole metagenome sequencing was performed on stool specimens collected. Taxonomic profiling was conducted using MetaPhlAn 4. ANCOM-BC analysis was used to identify differences in the relative abundance of bacterial species between groups. Alpha-diversity was evaluated using the Shannon diversity index and Evenness analysis, employing the Kruskal-Wallis test. Beta-diversity was assessed using the Bray-Curtis and Jaccard dissimilarity measures. The ratio of Firmicutes/Bacteroidetes (F/B), a measure of gut dysbiosis, was computed at baseline in the two cohorts.

Results

Among 59 patients assessed, 27 and 32 were Latino and non-Latino, respectively. Median age of the cohort was 60 (range, 36–90). Most were male (71%), had clear cell RCC (88%) and had intermediate/poor risk disease (79%). ANCOM-BC analysis showed an enrichment of 14 bacterial species and a depletion in 3 species at baseline in the Latino group ($p \leq 0.05$). Three *Roseburia* spp. were enriched in the Latino patients, namely *R. faecis* (log-fold change [LFC]: 2.6), *R. hominis* (LFC: 2.0) and *R. inulinivorans* (LFC: 1.8). Additionally, *E. rectale* was also enriched in the Latino group (LFC: 2.0). In contrast, in non-Latino patients *Methylobacterium* spp. was enriched (LFC: 1.3). The F/B ratio was higher in the Latino group as compared to the non-Latino group (1.00 vs 0.92). We did not observe any differences in alpha and beta diversity.

Conclusions

Our examination of the gut microbiota of pts with mRCC revealed significant differences based on ethnicity at baseline. Specifically, the Latino group exhibited an enhancement of *Roseburia* spp. and *E. rectale*, species previously linked to favorable outcomes with ICIs. Our findings suggest that clinical trials related to the microbiome should potentially account for baseline differences in ethnicity.

Keywords

Renal cancer, microbiome, immunotherapy, disparities, Latinos

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Ferroptosis Suppressor Protein 1 is a Potent and Targetable Inhibitor of Ferroptosis in Chromophobe Renal Cell

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Background

There are currently no proven therapies for metastatic or unresectable Chromophobe renal cell carcinoma (ChRCC). Ferroptosis is an iron-dependent form of cell death characterized by the peroxidation of membrane polyunsaturated fatty acids, leading to membrane damage and cell death.

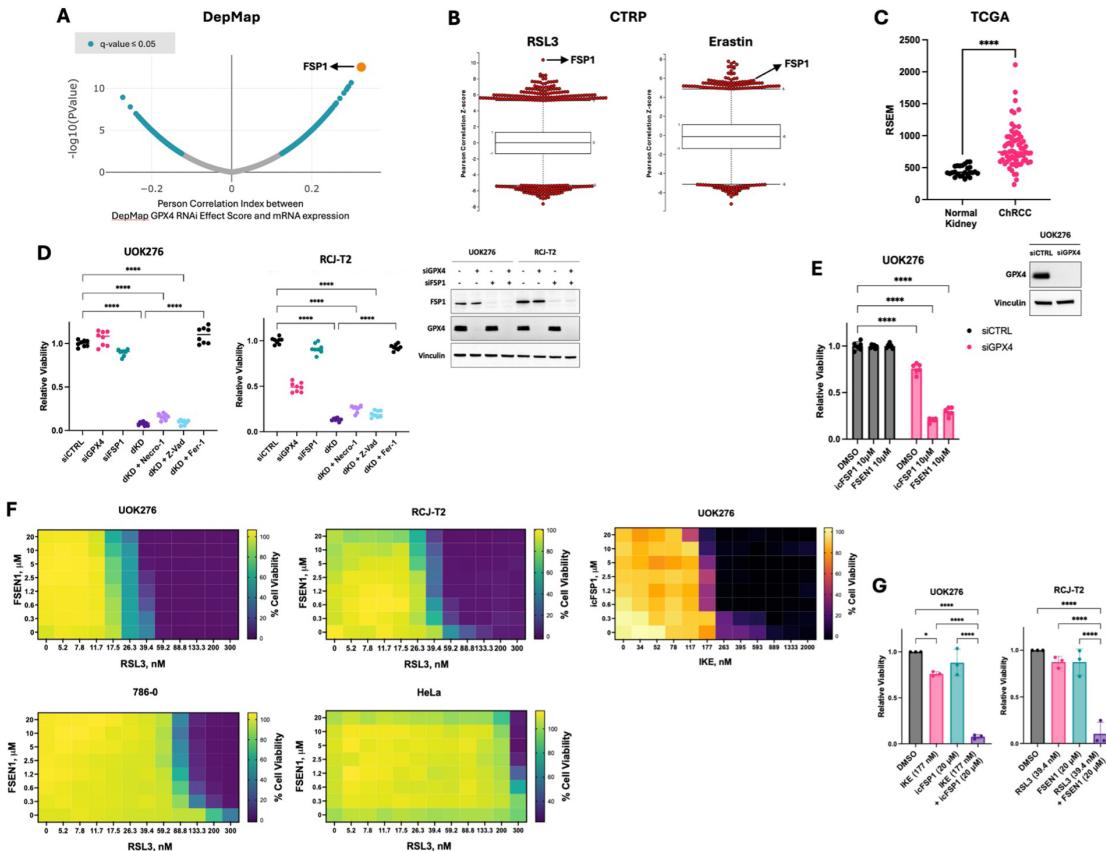
Glutathione, a potent cellular antioxidant, counters lipid peroxidation to prevent ferroptosis. Both reduced and oxidized glutathione are markedly elevated in ChRCC (PNAS, 2018). We have previously discovered that ChRCC is hypersensitive to ferroptotic cell death induced by the pharmacologic disruption of glutathione homeostasis via the cysteine transporter SLC7A11 or glutathione peroxidase (GPX4) inhibition (PNAS, 2022). Ferroptosis suppressor protein (FSP1) is a glutathione-independent suppressor of ferroptosis. FSP1's role in ChRCC is unexplored.

Methods

Transcriptional data from DepMap (484 cell lines) and Cancer Therapeutics Response Portal (CTRP) (860 cell lines) were used to study genetic or pharmacologic inhibition of GPX4 and SLC7A11. Statistical analysis was performed in PRISM 10 using Mann-Whitney U and ANOVA tests. Statistical significance was defined as $p < 0.05$.

Results

DepMap data revealed that FSP1 is the top upregulated gene in cells resistant to RNAi inhibition of GPX4 (Achilles, DEMETER2) (Panel A). In CTRP data, FSP1 was the top upregulated gene in cells resistant to the GPX4 inhibitor RSL3 and the 19th most upregulated gene in cells resistant to the SCL7A11 inhibitor, Erastin (Panel B). In The Cancer Genomic Atlas (TCGA) KICH (ChRCC) dataset, FSP1 was 2-fold higher in ChRCC compared to normal kidney (mean RSEM = 818.9 vs 445.9, p-value <0.0001) (Panel C).



A) DepMap data showing the Pearson correlation index between resistance to GPX4 RNAi and gene expression levels. Each dot represents a gene. FSP1 was the top upregulated gene associated with RNAi inhibition of GPX4 in this dataset.
B) CTRP data showing the Pearson correlation Z-score between resistance to RSL3 (right) and Erastin (left) and gene expression levels. Each dot represents a gene. FSP1 was the top upregulated gene associated with RSL3 resistance and the top 19th gene associated with Erastin resistance.
C) TCGA data showing the expression of FSP1 in ChRCC tumors compared to normal kidney. Each dot represents a patient sample. **** p-value <0.0001.
D) 48 hours crystal violet viability assay in UOK276 and RCJ-T2 cells with siRNA knockdown of GPX4 and FSP1 (n = 8/condition). Western blot shows the level of FSP1 and GPX4 knockdown. **** p-value <0.0001.
E) 48 hours crystal violet viability assay in UOK276 with siRNA knockdown of GPX4 and pharmacologic inhibition of FSP1 (icFSP1 and FSEN1, 10 μ M) (n = 6/condition). Western blot shows the level of FSP1 and GPX4 knockdown. **** p-value <0.0001.
F) 48 hours crystal violet viability assay in UOK276, RCJ-T2, 786-0, HeLa cells. Cells were treated with increasing concentrations of RSL3 or IKE (0-300 nM and 0-2000 nM, respectively) combined with FSEN1 or icFSP1 (0-20 μ M), respectively (n = 3-6).
G) Snapshot of cell viability taken at [IKE] = 177nM and/or [icFSP1] = 20 μ M for UOK276 (left), and [RSL3] = 39.4 nM and/or [FSEN1] = 20 μ M for RCJ-T2 (right) showing synergy between pharmacologic inhibition of SLC7A11 / GPX4 and FSP1. * p-value <0.05, **** p-value <0.0001.

In vitro studies of two ChRCC cell lines, UOK276 and RCJ-T2, revealed that while siRNA-mediated inhibition of GPX4 or FSP1 individually did not induce substantial cell death, their combination was synergistic and resulted in almost complete cell death (UOK276 mean viability 8% of siCTRL, p<0.0001; RCJ-T2 mean viability 13% of siCTRL, p<0.0001). Cell viability was completely rescued by the ferroptosis inhibitor ferrostatin-1 but not by the necroptosis inhibitor necrostatin-1 or the apoptosis inhibitor Z-Vad-FMK (**Panel D**).

siRNA-mediated GPX4 knockdown combined with FSP1 pharmacologic inhibition (icFSP1 or FSEN1) induced extensive cell death (mean viability of siGPX4 + icFSP1 group = 21% of siCTRL + DMSO group, p<0.0001; mean viability

of siGPX4 + FSEN1 group = 29% of siCTRL + DMSO group, p<0.0001) (**Panel E**). Combining RSL3 with FSEN1, or IKE with icFSP1, also demonstrated synergy, with the ChRCC cell lines showing increased overall sensitivity to these drug combinations compared to 786-0 cells (ccRCC-derived) and HeLa cells (**Panel F**). Snapshots from these experiments taken at [IKE] = 177nM and/or [icFSP1] = 20 mM for UOK276, and [RSL3] = 39.4 nM and/or [FSEN1] = 20 mM for RCJ-T2, show almost complete cell death when pharmacologic inhibitors of glutathione-dependent and -independent ferroptosis suppressors are combined, but no substantial cell death when each is used individually (7% and 10% viability in combination group compared to DMSO group for UOK276 and RCJ-T2 respectively, p<0.0001) (**Panel G**).

Conclusions

We show that *in vitro* genetic or pharmacologic inhibition of FSP1, a glutathione-independent inhibitor of ferroptosis, leads to ferroptosis in ChRCC-derived cells when combined with genetic or pharmacologic inhibition of the glutathione-dependent suppressors of ferroptosis GPX4 or SLC7A11.

Keywords

Chromophobe renal cell carcinoma, Ferroptosis, Ferroptosis Suppressor Protein 1 (FSP1)

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Fasting mediated gut microbiome modulation improves response to immune checkpoint therapy in Renal Cell Cancer.

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Background

Gut microbiome and microbial metabolome has been shown to be a predictor and modulator of response to immune checkpoint blocker therapy (ICB) in clear cell renal cancer (ccRCC). Based on preliminary data from a prospective clinical study of 100 ccRCC patients, we showed differences in gut microbiome, microbial metabolome and progression free survival. Specifically higher abundance of indole acetic acid, indole acetaldehyde and indole pyruvic acid (IPyA) , which represent microbial metabolism of tryptophan were significantly associated with immunotherapy resistance and shorter progression free survival.

We hypothesized that time restricted eating would favorably modulate the gut microbiome, microbial metabolome to improve ICB response. We therefore evaluated the efficacy and mechanism of time restricted eating to improve response to immune checkpoint therapy in preclinical kidney cancer models.

Methods

Specific pathogen free (SPF) BL6 mice were injected subcutaneously with 0.5X10⁶ LVRCC67 (Renal adenocarcinoma) cell lines to develop kidney cancer preclinical models followed by randomization into two equal groups when average tumor size reached 100mm³. Both groups were treated with anti-mouse PD1 twice weekly and daily tyrosine kinase inhibitor (TKI) while group 1 was additionally started on 14 hours of daytime fast (corresponding to period of inactivity), with feeding restricted to ten hours at night, 3 days prior to start of systemic treatment. Tumor volumes were measured twice weekly to evaluate progression free survival (defined as 30% increase in tumor volume), and mice were euthanized at end point, defined as completion of eight doses of antiPD1 treatment.

To demonstrate that modulation of gut microbiome is the driving mechanism mediating favorable effect of TRE on ICB response, we conducted fecal microbiota transplant from fasting and non-fasting mice respectively into two groups of germ-free mice. Allowing two weeks for FMT to engraft, mice were injected with RenCa cell lines and subsequently treated with anti-mouse PD1 and TKI, without fasting, once tumors reached an average size of 100 mm³. Tumors were measured twice weekly to assess PFS, until end point, defined similar to above.

Results

TRE was associated with significantly longer PFS via modulation of gut microbiome, which is necessary and sufficient to improve ICB response. Tumor growth was significantly slower and PFS significantly longer in SPF TRE mice as compared to non-TRE mice (median PFS 14 days vs 6 days, TRE vs non-TRE, p=0.02). Further GF mice engrafted with fasting microbiome developed significantly smaller tumors, which regressed after treatment, as opposed to those with non-fasting microbiota [(Mean tumor volume-45 mm³ vs 120 mm³, p=0.001 at day 15 of injection), median PFS- not reached(NR) vs 7 days, P=0.007].

Conclusions

TRE improves response to ICB in renal cell cancer and can be developed into a cost effective, adjunctive therapy to improve PFS and overall survival of ccRCC patients treated with ICB. Deeper understanding of TRE mediated changes in gut microbiome and microbial metabolome can be leveraged to improve ICB response in patients who are unable to fast.

Keywords

kidney cancer, microbiome, microbial metabolites, fasting

Posters

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Genotype-phenotype associations in Von-Hippel Lindau Syndrome: implications for screening

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Background

Von Hippel-Lindau (VHL) is an autosomal dominant genetic disease with an estimated incidence of 1:36,000. The type of germline VHLI alteration has been shown to impact downstream VHL expression and clinical phenotype. However, current screening practices do not differ based on VHL genomic subtype. We aim to assess genotype-phenotype association among an institutional cohort of VHL patients.

Methods

We conducted a retrospective cohort study of 69 patients (27 families) at the University of Utah and Huntsman Cancer Institute from 1998- December 2023. 41 patients had documented germline testing in our system to define VHL type. VHL Type 1 was defined as those with nonsense mutations, deletions, and duplications and Type 2 as those with missense and splice mutations. Mean follow-up was 86- and 59-months for VHL type 1 and 2 respectively. We then evaluated the association of VHL type with risk of pheochromocytoma (Pheo), renal cancer (RCC), and hemangioblastoma (HB).

Results

We identified 24 unique VHL alterations among 41 patients. 83% (29/41) patients had missense mutations, 7.5% (3/41) had nonsense mutations, 17% (6/41) had splice mutations, and 3% (3/41) had a deletion. The median age at VHL diagnosis was 43 years old for type 1 (range 19-76) and 38 for VHL type 2 (range 4-67). VHL Type 2 was more common, 85% (35/41). All VHL Type 1 patients (6/6) developed RCC and HB. No patients with VHL Type 1 developed PheoPara. Of the 35 patients diagnosed with VHL Type 2, 20% (7/35) were diagnosed with PheoPara, 9% (3/35) with RCC, 31% (n = 11) with HB, and 37% (n = 13) were asymptomatic. No VHL Type 1 patients were asymptomatic.

Conclusions

VHL type 2 comprised the majority of VHL diagnoses in our cohort. Individuals diagnosed with VHL type 2 have the highest penetrance for: hemangioblastoma (hb), PheoPara, and renal cell carcinoma (RCC). In our study, 37%

of VHL type 2 participants did not demonstrate any VHL manifestation. VHL type 1 had a high prevalence for RCC and HB, with a notable absence of PheoPara manifestation. However, majority of these patients (75%) were below the age of 20, and thus likely did not develop a manifestation yet. This cohort add to evidence supporting utilizing genetic VHL Types to personalize surveillance guidelines. Validation in larger cohorts with longer follow-up suggest that VHL type 1 patients could forgo pheo/para screening.

Keywords

VHL, retrospective cohort study, cancer

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Durable response to immunotherapy-based regimens for IMDC favorable risk patients with metastatic clear cell renal cell carcinoma: a pooled analysis of four published randomized control phase 3 trials

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Background

An immune checkpoint inhibitor (ICI) backbone of either ICI doublet (ipilimumab-nivolumab [ipi-nivo]) or tyrosine kinase inhibitor (TKI) with ICI (such as axitinib-pembrolizumab [axi-pembro], cabozantinib-nivolumab [cabo-nivo], or lenvatinib-pembrolizumab [len-pembro]) is the current standard of care for previously untreated, metastatic clear cell renal cell carcinoma (mccRCC). The phase 3 trials that evaluated these four regimens were compared to sunitinib, with no direct head-to-head clinical trial data available. With at least 4-year follow up data available, we compared the long-term responders of the IMDC favorable risk patients enrolled in the four phase 3 trials (CheckMate 214, KEYNOTE-426, CheckMate 9ER, and CLEAR).

Methods

Pseudo individual patient data (IPD) was obtained from the Kaplan-Meier (K-M) curves using the graph digitizer software IPDfromKM R package to extract coordinates of points on the curves and applied the numerical algorithm to reconstruct survival results. The hazard ratios (HRs) of the favorable risk group for both progression-free survival (PFS) and overall survival (OS) comparing the experimental arms to sunitinib were extracted. Responses were measured at 24 (durable response [DR]) and 36 months (extreme durable response [EDR]) and long-term OS as OS \geq 48 months. K-M method using the extracted IPD was used to estimate the survival rates at 24-month PFS, 36-month PFS, and 48-month OS. Then, we compared the survival rates at each time point using the generalized linear model for the survival rates at fixed time points obtained from the K-M method.

Results

Len-pembro was associated with the highest DR (57.2%) and EDR (44.5%), while ipi-nivo had the lowest DR (36.0%), and cabو-nivo had the lowest EDR (18.8%). There was no difference in 48 month-OS among regimens ($p=0.11$), ranging between 58.3% (cabو-nivo) and 70.6% (len-pembro).

Comparing the sunitinib control arms among the four studies, a numerically higher PFS at 24 months (59%) and 36 months (40%) was observed in the CheckMate 214 trial compared to the ICI-TKI trials (25-35% and 15-20%, respectively). The 48-month OS for the sunitinib arms ranged from 55-70%, and no differences were observed among trials ($p=0.55$).

Comparisons of survival rates for the immunotherapy arm on fixed time-points for IMDC favorable risk subset of patients

Conclusions

ICI-based regimens provided durable responses to a significant number of patients with favorable risk mccRCC. While TKI-ICI were associated with higher DR and EDR, no differences in OS at 48 months were observed compared to ipi-nivo or sunitinib for favorable risk disease. Longer follow-up is necessary to evaluate long-term outcomes given the favorable prognosis of these patients. We acknowledge the limitations and caveats of cross-trial comparisons.

Keywords

IMDC favorable risk, overall survival, immune checkpoint inhibitor, tyrosine kinase inhibitor

Trial/ Study	24 Months PFS		36 Months PFS		48 Months OS	
	Survival rate (95% CI)	p-value	Survival rate (95% CI)	p-value	Survival rate (95% CI)	p-value
CheckMate 214	0.360 (0.347, 0.373)		0.300 (0.279, 0.321)		0.657 (0.656, 0.658)	
CheckMate 9ER	0.399 (0.383, 0.414)	0.6223 ¹	0.188 (0.123, 0.264)	0.1075 ¹	0.583 (0.579, 0.586)	0.3032 ¹
CLEAR	0.572 (0.569, 0.575)	0.0041 ²	0.445 (0.436, 0.453)	0.0468 ²	0.706 (0.706, 0.707)	0.4517 ²
KEYNOTE-426	0.456 (0.451, 0.461)	0.1475 ³	0.337 (0.324, 0.350)	0.5635 ³	0.601 (0.600, 0.603)	0.3602 ³

¹p-values from CheckMate 214 vs. CheckMate 9ER;

²p-values from CheckMate214 vs. CLEAR;

³p-values from CheckMate 214 vs. KEYNOTE-426

Nivolumab plus ipilimumab vs sunitinib for first-line treatment of advanced renal cell carcinoma: 8-year follow-up with analyses in favorable risk patients from the phase 3 CheckMate 214 trial

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Background

First-line nivolumab plus ipilimumab (NIVO+IPI) has provided substantial long-term survival benefits over sunitinib (SUN) in patients with advanced renal cell carcinoma (aRCC) in CheckMate 214. With a median follow-up of 8 years, the longest follow-up to date for any phase 3 trial of immune checkpoint inhibitor combination therapy in patients with aRCC, we report survival, response per independent radiology review committee (IRRC), and safety in all randomized patients (intent-to-treat [ITT] population) and in patients with International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) favorable risk.

Methods

Patients with clear cell aRCC were randomized 1:1 to NIVO 3 mg/kg plus IPI 1 mg/kg Q3W×4 doses, followed by NIVO (3 mg/kg or 240 mg Q2W or 480 mg Q4W); or SUN 50 mg once daily for 4 weeks on, 2 weeks off. Key study endpoints included overall survival (OS), IRRC-assessed progression-free survival (PFS) and objective response rate (ORR) in intermediate/poor-risk (primary), ITT (secondary), and favorable-risk (exploratory) patients. Cancer-specific survival was evaluated in ITT patients by censoring all causes of death other than RCC. Post hoc exploratory analyses in patients with favorable risk were performed.

Results

With 8 years (99.1 months) median follow-up, OS with NIVO+IPI versus SUN remained superior in ITT patients (hazard ratio [HR], 0.72; Table). The HR for PFS with NIVO+IPI versus SUN was 0.88. ORR was higher with NIVO+IPI versus SUN (Table), with more complete responses (12% vs 3%)

Arm; n	ITT		Favorable risk	
	NIVO+IPI (N=550)	SUN (N=546)	NIVO+IPI (N=125)	SUN (N=124)
OS HR (95% CI)	0.72 (0.62-0.83)		0.82 (0.60-1.13)	
mOS (95% CI), months	53 (46-65)	38 (32-44)	78 (65-92)	67 (56-80)
PFS per IRRC, HR (95% CI)	0.88 (0.75-1.03)		1.76 (1.25-2.48)	
mPFS (95% CI), months	12 (10-17)	12 (10-15)	12 (10-18)	29 (23-43)
ORR per IRRC (95% CI), %	39 (35-44)	33 (29-37)	30 (22-38)	52 (43-61)
Complete response per IRRC, %	12	3	13	6
DOR HR (95% CI)	0.52 (0.38-0.72)		0.70 (0.36-1.34)	
mDOR (95% CI), months	76 (59-NE)	25 (20-33)	61 (28-NE)	33 (25-51)

m, median; NE, not estimable.

Table

and a longer median duration of response (DOR) in the combination arm versus SUN. In patients with favorable risk, OS benefits were similar between arms (HR, 0.82; **Table**). The HR for PFS favored SUN (HR, 1.76). ORR was lower with NIVO+IPI versus SUN, yet more patients achieved complete responses (13% vs 6%, respectively) and median DOR was longer with NIVO+IPI. Median cancer-specific survival (95% CI) in ITT patients was 73.7 (62.8-91.2) months with NIVO+IPI versus 45.1 (37.8-53.3) months with SUN (HR, 0.69; 95% CI 0.59-0.82). In exploratory post hoc analyses of patients with favorable risk, 75/125 (60.0%) patients in the NIVO+IPI arm and 85/124 (68.5%) patients in the SUN arm died over 8 years of follow-up. Of these patients, 31 in the NIVO+IPI arm and 27 in the SUN arm died within 3 years of randomization; the primary reason for death was disease in either arm (71.0% and 85.2%, respectively). Furthermore, among 27 patients in the NIVO+IPI arm with favorable risk who died after disease progression within 3 years, 12 patients did not receive second-line systemic therapy. Beyond 3 years, only 2 of 43 patients who died after disease progression did not receive subsequent systemic therapy. Among all treated patients, incidence of any-grade and grade 3-4 treatment-related adverse events remained largely unchanged. No new drug-related deaths occurred in either arm since the previous database lock.

Conclusions

With a median follow-up up of 8 years, NIVO+IPI continues to demonstrate sustained survival and more durable response benefits versus SUN in the ITT population, including a further reduction in the risk of death with NIVO+IPI as measured by cancer-specific survival. Long-term exploratory data in patients with favorable risk have shown a steady improvement in the HR for OS, and a marked improvement in median DOR and complete response rates with NIVO+IPI versus SUN, thus contributing to the survival and response benefits reported in the ITT population. Furthermore, the disproportionate number of patients with favorable risk who died within 3 years of randomization after documented progression without receiving subsequent therapy may have affected the HR for OS in this patient population early on. NIVO+IPI offers the potential for positive long-term outcomes, regardless of IMDC risk, and with no emergence of new safety signals.

Keywords

Nivolumab; Ipilimumab; CheckMate 214; Advanced RCC; Favorable Risk

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Delving into molecular underpinnings of sarcomatoid/rhabdoid dedifferentiation in renal cancers using spatial profiling

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Background

Renal cell carcinomas (RCC) are characterized by their largely diverse clinical outcomes. Leveraging genomic diversity among individual tumors, our research during the past decade has concentrated on developing personalized medicine approaches for the most common and the most aggressive type of RCC, clear cell renal carcinoma (ccRCC). We generated the largest dataset of ccRCC including somatic genomic and clinical annotations for over 940 ccRCC patients, and developed a genomic classifier, based on mutational status of 12 RCC-relevant genes, which is able to stratify patients according to their risk of relapse after nephrectomy and/or death due to RCC. Furthermore, we noticed the presence of mesenchymal-like cellular phenotypes in tumors of high-risk patients, representing a de-differentiation process, known as sarcomatoic or rhabdoid (S/R) de-differentiation.

Methods

The current knowledge about molecular mechanisms that may drive S/R de-differentiation is primarily generated from molecular profiling of bulk tumors, collecting data from a mixture of cells that co-exist in the tumor milieu. Therefore, high-resolution studies that precisely define molecular characteristics of different cellular phenotypes associated with S/R features are missing. We have used spatial transcriptomic profiling to investigate molecular mechanisms that underline S/R de-differentiation in RCC. While preserving tissue context, we applied spatial whole human transcriptome profiling to areas exhibiting S/R or clear cell phenotypes within the same tumor specimens to generate phenotype-specific transcriptome profiles. In addition, we applied whole-exome sequencing (WES) to independent areas that exhibit different differentiation phenotype within same tumors.

Results

Pathway and network analysis of genesets with upregulation in each area have revealed meaningful differences in cellular pathways which are active in each phenotype. Specifically, we observed that dysregulation of extracellular matrix (ECM) is a hallmark of S/R areas within RCC tumors. Furthermore, WES of phenotypically-distinct areas within each tumor has shed new light on the genome evolution of S/R phenotypes.

Conclusions

S/R dedifferentiation in RCC tumors is characterized by specific genomic evolutionary patterns and substantial dysregulation of ECM components.

Keywords

renal cell carcinoma, sarcomatoid/rhabdoid differentiation, spatial genomics

DOD CDMRP Funding

yes

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Linking VHL and SETD2 in a common oncogenic pathway that converges on the mitotic spindle

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Background

Loss of chromosome 3p is a landmark event in clear cell renal cell carcinoma (ccRCC) that results in mono-allelic loss of VHL (*von Hippel Lindau*) and SETD2 (*Set-domain containing 2*) (and other tumor suppressors co-located on 3p). Second hits in VHL inactivate this key tumor suppressor initiating tumor progression. SETD2, a histone methyltransferase, was previously shown to have a dual function in methylating both histones and microtubules, thereby contributing to both the histone and tubulin codes. Methylation by SETD2 on microtubules occurs at the mitotic spindle and is essential for normal mitosis and cytokinesis, with loss of SETD2 acting as a strong driver of apoptosis. This raises a conundrum of how cancer cells survive early mono-allelic loss of SETD2, escaping cell death.

Methods

Using biochemical kinase assays and mass spectrometry we have identified SETD2 as a substrate for AURKA. In addition, we have used immunoblotting and immunofluorescence assays to probe phosphorylation on SETD2 and the impact of phosphorylation of SETD2 both on its chromatin and cytoskeleton targets.

Results

We have identified the mitotic kinase, Aurora kinase A (AURKA), as a regulator of SETD2. Our data uncover

SETD2 as a unique substrate for phosphorylation by AURKA, with mass spectrometry identifying serine 2080 (S2080) as the site of phosphorylation on SETD2. We found phosphorylation of SETD2 by AURKA at S2080 contributes to its methyltransferase (i.e. enzymatic) activity on microtubules but does not impact chromatin methylation on H3K36 which remains unaltered. We show that VHL regulates SETD2 via AURKA, and loss of phosphorylation on SETD2 results in mitotic defects and genomic instability. Importantly, we demonstrate that inhibition of AURKA is synthetic lethal in the setting of VHL and SETD2 deficiency.

Conclusions

AURKA expression levels are high in VHL-null cells resulting from an inability of VHL to target AURKA for degradation and our data now highlight a direct link between VHL and SETD2, two tumor suppressors believed to independently drive RCC pathogenesis. In summary, our data reveal a tumor-specific vulnerability linked to mitotic fragility that can be precisely targeted to ultimately drive mitotic catastrophe.

Keywords

Aurora Kinase A, SETD2, mitotic fragility, VHL

DOD CDMRP Funding

yes

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Unraveling the Impact of Tumor Microenvironment on Immunotherapy Response/Resistance in Human Sarcomatoid Clear Cell Renal Cell Carcinoma: Insights from a Novel Mouse Model

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Background

Renal cell carcinoma (RCC) with histological sarcomatoid de-differentiation (sRCC) has a historically poor prognosis across all RCC subtypes. Recently, immunotherapy with anti-PD1 + anti-CTLA4 combo has significantly improved disease-specific survival, and complete response are seen in upwards of 20% of patients. However, half of sRCC patients still have no response to this immunotherapy, and the mechanism of response remains unclear.

Methods

Our preliminary bulk RNA sequencing data reveal a distinct tumor microenvironment (TME) in human sRCC tumors, characterized by heightened immune cell infiltration, particularly enriched for regulatory T cells (Tregs). Subsequently, we delineated the spatial gene expression topography within sRCC and clear cell renal cell carcinoma (ccRCC) regions, culminating in the development of the sRCC signature. Concurrently, we established an innovative immunocompetent murine model of sRCC. Leveraging CRISPR technology, we replicated the human sRCC genotype by knocking out Vhl, Bap1, and Cdkn2a/2b genes in a mouse telomerase reverse transcriptase (mTERT) overexpressing renal proximal tubule epithelial cell line. This effort yielded a successfully engineered mouse implantable sRCC cell line bearing $Vhl^{Mut}Bap1^{Del}Cdkn2a^{Del}Cdkn2b^{Del}$ (HJRCC68N).

Results

The syngeneic mouse sRCC model we developed faithfully replicates human sRCC histology and tumor microenvironment and recapitulates the sRCC signature identified in human sRCC regions. Importantly, like in humans, mouse sRCC tumors exhibit a heterogeneous response to anti-PD1 + anti-CTLA4 combination, and anti-CTLA4 monotherapy, reflecting the clinical variability observed in human patients. Furthermore, transcriptomic analysis from our mouse model reveals the involvement of type 1 immunity in responder, providing insights into overcoming immunotherapy resistance.

Conclusions

In conclusion, our study unveiled distinct changes in the TME at the transcriptomic and spatial gene expression levels in human sRCC. Leveraging our novel murine model, which faithfully replicates human sRCC histology, TME characteristics, and mixed response to immunotherapy, we elucidated the involvement of type 1 immunity in treatment response. These insights offer valuable pathways for overcoming immunotherapy resistance and pave the way for the development of tailored treatment strategies aimed at improving outcomes for sRCC patients.

Keywords

sRCC, immunotherapy, mouse model, resistance

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Unconventional mechanisms of action and resistance to rapalogs in renal cancer

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Background

Clear cell renal cell carcinoma (ccRCC) is a prevalent cancer type in the United States driven by inactivation of the von Hippel-Lindau (VHL) gene and with frequent hyperactivation of mammalian target of rapamycin complex 1 (mTORC1). Multiple drugs have been developed to target VEGF/VEGFR2 and mTORC1 for advanced RCC treatment. Two specific mTORC1 inhibitors, temsirolimus and everolimus (known as rapalogs), are FDA-approved for the treatment of RCC but their clinical efficacy is hindered by the emergence of resistance.

Methods

To study the development of rapalog resistance in RCC, we utilized patient-derived tumorgrafts (TGs) implanted in immunocompromised mice and treated the mice with rapamycin for prolonged periods to generate resistance. Resistance was studied by transplanting resistant tumors into additional cohorts of mice and establishing primary cultures. The impact of rapamycin on tumor growth and mTORC1 activity in both tumor and stromal cells was assessed using molecular techniques. To dissect the role of the tumor microenvironment (TME), we generated genetically engineered immunocompromised recipient mice with an mTOR inhibitor resistance mutation (S2035T). Coculture experiments were performed to further explore the interplay.

Results

Prolonged rapamycin treatment resulted in the development of resistance in TGs. Notably, this occurred despite persistent inhibition of mTORC1 in tumor cells. Resistance was transient and lost with subsequent transplantation and in cell culture. Surprisingly, resistance was accompanied by mTORC1 reactivation in the TME, specifically in cancer associated fibroblasts. Introducing an mTOR resistance

mutation (S2035T) into recipient mice was sufficient to cause resistance. Co-culture experiments with fibroblasts further demonstrated that rapamycin-resistant mutant fibroblasts conferred resistance in tumor cells even in the absence of mTORC1 activity.

Conclusions

This study uncovers a critical role of the TME, in mediating the response and resistance to rapalogs in RCC. Our data show that inhibition of mTORC1 in non-tumor cells is essential for the anti-tumor activity of rapalogs. These findings shed light on why mTOR mutations are rarely observed in resistant RCC patients and highlight the significance of the TME in modulating drug response and resistance. Moreover, our study emphasizes the potential of targeting TME cells as a therapeutic strategy in RCC and possibly other cancers.

Keywords

ccRCC, rapamycin, resistance, tumor microenvironment, cancer-associated fibroblasts

DOD CDMRP Funding

yes

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Pre-surgical visceral adipose tissue may be a novel pre-surgical risk factor for acute kidney injury among clear cell renal cell cancer patients undergoing radical nephrectomy

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Background

Patients with renal cancer undergoing partial (PN) or radical nephrectomy (RN) are at risk for acute kidney injury (AKI). Established risk factor for AKI include baseline comorbidities (e.g., obesity, hypertension), normal renal parenchymal loss, and ischemia-reperfusion injury. Pre-surgical body composition may be an overlooked modifiable patient characteristic that influences risk of AKI. We hypothesized that patients with higher visceral adipose tissue quantity or lower skeletal muscle quantity would have a higher risk of AKI.

Methods

The RESOLVE study at Memorial Sloan Kettering Cancer Center is a retrospective study of 1239 patients with stage I-III clear cell renal cell carcinoma (ccRCC) undergoing PN or RN from 2000 to 2020. We excluded from analysis patients with solitary kidney, those without preoperative serum creatinine (sCr) measurements, and those without a sCr measurement within seven days following nephrectomy, yielding a study population of 1,200 ccRCC patients with longitudinal sCr assessment (n=754 PN; n=446 RN). AKI was defined as a binary variable using the Kidney Disease – Improving Global Outcomes (KDIGO) criteria, based on a threshold of either a 1.5x relative or 0.3 mg/dl absolute increase in sCr within seven days. The cross-sectional areas and radiodensities of visceral adipose and skeletal muscle tissues were determined from pre-surgical computed tomography (CT) scans at the third lumbar vertebrae using Automatica software. We used generalized linear models with a binomial family and identity link to estimate seven-day risk differences (RD) and 95% confidence intervals in subgroups defined by surgery type.

Results

AKI was more frequent among patients undergoing RN (66% vs 26% among PN). Male patients, those with higher eGFR prior to surgery, those with lower stage/smaller tumors, and those with a history of hyperlipidemia more frequently experienced AKI in the post-operative period. No associations were observed with other reported comorbidities (diabetes, hypertension). While no association was observed between BMI and risk of AKI, visceral adipose and skeletal muscle variables were significantly associated with risk of AKI in univariate models. After adjustment for age, sex, comorbidities, and other body composition variables, only higher visceral adipose tissue quantity remained significantly associated with increased risk of AKI [RD per 40 unit increase (95% CI): 5.2 (1.3, 9.2)]. Muscle characteristics were not associated with AKI in multivariable models.

Among patients undergoing PN, we observed a higher frequency of AKI among male patients, those with higher stage/larger tumors, and those with longer ischemia times. No significant associations were observed with comorbidity

histories. In univariate models, visceral adipose tissue quantity and quality as well as skeletal muscle quantity were associated with AKI risk; however, after adjustment for age, sex, comorbidities, and other body composition variables, we observed no significant associations between any body composition feature and AKI in patients undergoing PN.

Conclusions

Associations between pre-surgical body composition and risk of AKI vary by surgery type. Visceral adipose tissue quantity was associated with risk of AKI among patients undergoing RN, but not PN. This finding may be relevant for patient counseling, consideration for nephron-sparing surgery, and development of interventions to lower visceral adipose tissue quantity. Future studies should evaluate the impact of both pre-surgical and post-surgical change in body composition in relation on chronic kidney disease after nephrectomy.

Keywords

body composition, acute kidney injury, nephrectomy

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Applying genomic analysis to refine unclassified renal cell carcinoma

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Background

Despite the improvements in genomic and pathological techniques to identify renal cell carcinoma (RCC), 2-6% of all patients with RCC cannot be classified into a particular subgroup, thus called "unclassified" RCC (uRCC). Ascertaining the genomic profile of those patients may help select proper treatment and find novel targets.

Methods

The American Association for Cancer Research (AACR) Project Genomics Evidence Neoplasia Information Exchange (GENIE) database v15.0 was used to select patients with RCC by using the OncoTree codes. All included patients were divided into four groups based on the most frequent subtypes of RCC: clear cell RCC (ccRCC), papillary RCC (pRCC), chromophobe RCC (chRCC), and uRCC. The Cancer Genome Atlas (TCGA) was additionally used to assess corresponding oncogenic signaling pathways. We employed the chi-squared test to compare categorical variables and applied the Benjamini-Hochberg correction to calculate Q-values, thereby controlling the false discovery rate.

Results

Overall, 1,990 tumor samples from 1,888 patients were evaluated. uRCC was observed in 184 patients (9.7%), whereas most had ccRCC (n=1339, 70.9%), followed by pRCC (n=224, 11.9%) and chRCC (n=141, 7.5%). Age distribution at sample sequencing was comparable between uRCC and other RCC subtypes ($P>0.05$). The proportion of female patients with uRCC was higher at 38.4%, compared to 26.5% in ccRCC ($Q=0.002$) and 16.3% in pRCC ($Q<0.001$), yet was comparable to chRCC at 48.6% ($Q=0.210$). The prevalence of uRCC was also greater among black patients, accounting for 8.6% vs. 2.1% in ccRCC ($Q=0.001$). Among patients with uRCC (n=224), the most common genomic alterations (GAs) were detected in *NF2* (15.8%), *SETD2* (15.8%), *TP53* (13.9%), *TERT* (13.4%), and *VHL* (11.8%). *NF2* alterations were also more prevalent in patients with uRCC than in patients with ccRCC (1.8%, $Q<0.001$), chRCC (0.7%, $Q<0.001$), and pRCC (5.8%, $Q=0.058$). Notably, median overall survival (OS) was poorer in uRCC patients with altered *NF2* (n=29) than in those with unaltered *NF2* (n=155, 30.7 vs. 87.1 months, $p=0.058$). Of patients with uRCC, 135 (72.5%) samples were from primary tumors and 39 (20.9%) from metastatic sites, with no difference in GA frequencies between the two. *CDKN2A* and *CDKN2B* were the most frequent co-mutated genes in uRCC ($Q<0.001$), followed by *VHL* and *BAP1* ($Q<0.001$), and *SETD2* and *PBRM1* ($Q=0.023$). GAs in uRCC were primarily observed in pathways related to *TP53* (42.8%), cell cycle (33.3%), *PI3K* (23.5%), and *HIPPO* (7.7%).

Frequent Genomic Alterations in uRCC (n=184,%)	
NF2	29 (15.85%)
SETD2	29 (15.85%)
TP53	26 (13.98%)
TERT	24 (13.48%)
VHL	22 (11.89%)
BAP1	21 (11.48%)
PBRM1	14 (7.82%)
MTOR	13 (7.10%)
FAT1	12 (6.90%)

Conclusions

uRCC exhibited a unique genomic profile distinct from other common RCC subtypes. Notably, NF2 alterations were frequent and correlated with a poorer prognosis.

Keywords

unclassified, kidney, cancer

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Applying single cell RNA sequencing of sarcomatoid renal cell carcinoma for the development of a novel transcriptomic biomarker to predict immunotherapy response

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Background

Renal cell carcinoma with sarcomatoid features (sRCC) is a unique kidney cancer subtype associated with aggressive

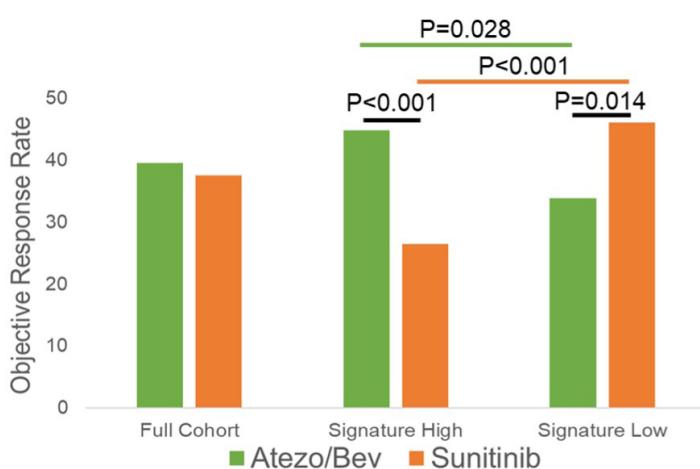
biology and poor clinical outcomes. Intriguingly, sRCC has recently exhibited preferential responsiveness to immune checkpoint blockade (ICB) therapies. Within the RCC population, however, there currently is a paucity of biomarkers predictive for ICB response, representing a critical unmet clinical need for optimal therapy selection. Therefore, we sought to derive a transcriptomic signature from sRCC samples encapsulating this paradoxical hyper-aggression and ICB-responsiveness to identify RCC patients most likely to benefit from ICB irrespective of sarcomatoid feature presence.

Methods

Nephrectomy specimens from patients with RCC were processed for single cell RNA sequencing (scRNASeq). Clustering was performed and annotated tumor cells were computationally isolated. Tumor cell counts were aggregated and differential expression between sRCC and non-sRCC cases was performed. Genes significantly upregulated in sRCC were next filtered against differential gene expression data of sRCC vs non-sRCC from three clinical RCC datasets (TCGA, CheckMate, & Javelin101) to identify genes enriched in sRCC across scRNASeq and bulk RNA sequencing. The prognostic and predictive features of this gene signature were then assessed in cohorts of patients with RCC receiving ICB.

Results

In total, 73,123 unique cells from 18 RCC patients (10 sRCC; 8 clear cell RCC) were analyzed by scRNASeq, including 5,386 tumor cells. Differential gene expression of tumor clones revealed 20 genes significantly enriched in sRCC relative to ccRCC. Filtering against public differential gene expression data resulted in 10 genes included within the final Sarcomatoid Signature (SS). SS expression scores for clinical specimens were calculated by single-sample gene set enrichment analysis. SS scores were significantly enriched in sRCC patient tumors across TCGA KIRC, CheckMate, and IMmotion151 datasets ($p<0.001$ for all cohorts). Within TCGA KIRC, SS scores were significantly increased in patients with nuclear grade 4 ($p<0.001$) and metastatic ($p<0.001$) disease, while stratification by median SS score revealed worsened overall ($HR=2.19$, $p<0.001$) and disease-free ($HR=2.08$, $p<0.001$) survival among SS-high patients. Amongst the CheckMate cohort (including patient samples from the CheckMate-010 & CheckMate-025 trials), SS-high patients experienced improved progression free survival (PFS) when treated with nivolumab relative to everolimus ($HR=0.70$, $p=0.055$), whereas no difference was seen between therapies in the SS-low patient population ($HR=0.98$, $p=0.9$). In the IMmotion151 trial, which was not utilized for gene filtering and signature refinement and thus represents an independent validation cohort, SS-high patients experienced reduced PFS duration ($HR=1.39$, $p<0.001$) irrespective of treatment arm. However, SS-high patients experienced improved objective response



rates with the ICB-containing atezolizumab/bevacizumab regimen compared to sunitinib ($p<0.001$) and relative to SS-low patients receiving atezolizumab/bevacizumab ($p=0.028$) (Figure 1). Within the SS-high population, patients experienced prolonged PFS with atezolizumab/bevacizumab ($HR=0.70$, $p=0.003$) relative to sunitinib whereas no PFS difference between arms was seen in the SS-low population ($HR=1.01$, $p>0.99$).

Conclusions

This novel SS derived from scRNAseq demonstrates negative prognostic yet positive predictive value for ICB response. In a domain currently void of efficacious biomarkers, the SS, upon prospective validation, may assist in optimal therapy selection for patients with RCC.

Keywords

Immune checkpoint blockade, Predictive biomarker, Single cell RNA sequencing, Sarcomatoid renal cell carcinoma

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Spatial Clustering of Immunosuppressive Macrophages in Papillary Renal Cell Carcinoma

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Background

Papillary renal cell carcinoma (pRCC) accounts for up to 15% of all kidney cancer cases, yet our understanding of its tumor immune microenvironment (TIME) remains limited. We utilized multiplex immunofluorescence (mIF) and spatial transcriptomics (ST) to evaluate immune cell spatial architecture in pRCC and compared to that of clear cell RCC (ccRCC).

Methods

Surgical tumor specimens were obtained from localized RCC tumors, followed by mIF using markers for T cells, B cells, and tumor-associated macrophages (TAMs). Spatial data were derived in regions of interest (ROIs) manually selected from spatially distinct tissue compartments of the TIME. Single-cell ST was performed on a subset of patient samples, utilizing probes against 960 transcripts. Cell abundance, cell spatial clustering, and spatially varying gene expression were analyzed to identify unique features of the TIME in pRCC.

Results

Sixteen pRCC and 70 ccRCC patient samples underwent mIF. Compared to ccRCC, global pRCC immune cell abundance was statistically lower amongst functional CD8 T cells, while global cell spatial clustering was higher amongst M2-like macrophages as measured by mIF, including PDL1+ subsets. Using ST, seven genes were significantly associated with spatial clustering of M2-like macrophages in pRCC. Three of seven genes (CCL18, GPNMB, CD9) are known markers of lipid-associated TAMs (LAMs) (adjusted p-value < 0.1).

Conclusions

Compared to ccRCC, pRCC has fewer T cells but greater M2-like macrophage spatial clustering. Using ST, we found that multiple LAM-associated genes are spatially enriched in pRCC. Additional resources should be dedicated to investigating myeloid biomarkers and the impact of myeloid modulating therapeutics in pRCC.

Keywords

multiplex immunofluorescence; spatially resolved transcriptomics; papillary renal cell carcinoma; tumor associated macrophages; tumor immune microenvironment

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Synergistic systematic analysis with fully human tissue models and in silico modeling of “copycats” reveals mechanisms of T cell suppression in clear cell renal cell carcinoma

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Background

Immune checkpoint-inhibitors have become a standard treatment for clear cell renal cell carcinoma (ccRCC). However, responses to checkpoint-inhibition are heterogeneous and many patients are resistant or eventually develop resistance. A better understanding of functional mechanisms governing immune responses in ccRCC is needed to identify biomarkers for therapeutic responses and to unravel novel therapeutic targets to overcome primary or acquired resistance to therapies.

Methods

We developed a fully human explant model derived from freshly resected ccRCC tissue (Fig. 1A). Our tissue explant model preserves all components of the tumor microenvironment including not only tumor cells, but also the immune cell compartment as well as tumor-stroma and -vasculature in culture (Fig. 1B). Following treatment with combined checkpoint-inhibition (Nivolumab plus Ipilimumab) or an anti-CCR5 inhibitor (Maraviroc), the tissue explants were analyzed by immunohistochemistry staining and multiplex cytokine profiling of 50 cytokines. Spatial and functional insights were incorporated in an agent-based *in silico* model (PhysiCell, Ghaffarizadeh *et al.*, *PLoS Comput. Biol.*, 2018) for unlimited exploration of functional cellular dynamics in the tumor microenvironment (Fig. 1D).

Results

Immunohistochemical analyses and cytokine profiling of ccRCC-tissue explants showed heterogeneous immune responses among different patients and indicated an impaired cytotoxic T cell response following checkpoint-inhibition (Fig. 1C). Spatial analyses of immune cell populations revealed clusters of CD8⁺ T cells and CD163⁺ macrophages localized closely to CD31⁺ endothelial cells.

Further characterization of the tumor microenvironment revealed high CCR5-expression in the tumor, particularly on the tumor blood vessels (Fig. 1B). Treatment of the tissue explants with the anti-CCR5 inhibitor Maraviroc led to an increase of CD8⁺ T cells and cytotoxic cytokines (*Granzyme B*, *IFNgamma*, *TNFalpha*, *IFNalpha2*) in the tumor in comparison to checkpoint-inhibition (Fig. 1C).

Spatial and functional information from cultured ccRCC-tissue explants were integrated into an agent-based *in silico* model of the ccRCC tumor microenvironment, which includes tumor cells, T cells, macrophages and endothelial cells (Fig. 1D). *In silico* simulation of different immune cell modulating treatment conditions showed an increase of T cells and cytotoxic cytokines upon blockade of the interaction between T cells with endothelial cells or macrophages (Fig. 1E).

Conclusions

Our combined *ex vivo* and *in silico* analyses provide evidence for immunosuppression in the ccRCC tumor microenvironment mediated by macrophages and endothelial cells. CCR5 was shown to be a potential target to overcome immune resistance to checkpoint-inhibition in ccRCC. Further characterization of immune cell compositions and functional mechanisms in the perivascular area is imperative to enhance therapeutic responses to immunotherapies for ccRCC.

Keywords

clear cell renal cell carcinoma, checkpoint-inhibition, anti-CCR5, human tissue explant model, *in silico* agent-based model

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Efficacy of treatments post-lenvatinib in patients with advanced renal cell carcinoma (aRCC)

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Background

Lenvatinib is a tyrosine kinase inhibitor (TKI) that targets both vascular endothelial growth factor (VEGF) receptor and fibroblast growth factor receptor. It has demonstrated efficacy both in the upfront and refractory disease settings. However, there is a lack of data surrounding the efficacy of TKIs post-lenvatinib exposure. In this study, we investigate the activity of therapies post-lenvatinib in patients with aRCC.

Methods

We conducted a retrospective analysis utilizing the International Metastatic Database Consortium (IMDC). Patients having received treatment post-lenvatinib exposure were eligible and divided into two cohort: patients post-1st line lenvatinib (2nd line cohort) and patients post-2nd line lenvatinib (3rd line cohort). The primary objective was objective response rate (ORR) and time to treatment failure (TTF). ORR was summarized with 95% two-sided exact binomial confidence interval. TTF was defined as time from treatment initiation to drug cessation for any reason censored at the date of last follow-up.

Results

Overall, 84 patients received 1st line lenvatinib of whom 43 (51%) remain on therapy, 20 (24%) received 2nd line treatment, and 21 (25%) received no subsequent treatment. The median duration of prior lenvatinib was 9.7 months. All patients received 1st line pembrolizumab + lenvatinib (ORR 50%, median TTF 19.7 months). Reason for lenvatinib discontinuation was progression (50%), progression + toxicity (20%), toxicity (15%), or other (15%). For the 2nd line cohort, median age was 61 years, most patients were male (85%), had prior nephrectomy (75%), clear cell histology (85%), and were IMDC intermediate/poor risk (55%). 2nd line therapy regimens included TKI monotherapy (80%), TKI-IO (5%), and other (15%). The median follow up from 2nd-line treatment initiation was 4.9 months. The ORR to 2nd line treatment was 5% (95% CI 0.2-25) and median TTF was 5.8 months (95% CI 1.9-14.9).

Of 2nd line lenvatinib-exposed patients (n=84), 24 (29%) remain on treatment, 34 (40%) received 3rd line treatment, and 26 (31%) did not receive additional therapy. The median duration of prior lenvatinib was 5.9 months. Most patients received 2nd line everolimus + lenvatinib (97%) (ORR 31%, median TTF 9.2 months). Reason for lenvatinib discontinuation was progression (59%), progression + toxicity (9%), toxicity (12%), or other (21%). For the 3rd line cohort, median age was 67 years, most patients were male (68%), had prior nephrectomy (88%), clear cell histology (68%), and were IMDC intermediate/poor risk (77%). 3rd line treatments included TKI alone (50%), IO-TKI (38%), and other (12%). The median follow up from 3rd-line treatment initiation was 14.9 months. The ORR to 3rd line treatment was 12% (95% CI 3.3-27) and median TTF was 2.8 months (95% CI 1.9-7.4).

Conclusions

In this analysis, we demonstrate modest activity of TKI-based therapy post-lenvatinib exposure. Our study highlights the need for improved treatment options for patients progressing on lenvatinib-based therapies.

Keywords

Renal cell carcinoma, lenvatinib, targeted therapy, metastatic

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Real-world treatment patterns and clinical outcomes of metastatic renal cell carcinoma patients post immune-oncology (IO) and Vascular Endothelial Growth Factor (VEGF) receptor targeted therapies

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Background

The metastatic renal cell carcinoma (mRCC) treatment landscape has rapidly evolved with approval of newer IO agents and VEGF targeted tyrosine kinase inhibitors (TKIs). However, real-world treatment patterns and clinical outcomes data for mRCC in post IO and TKI setting is limited. The objective of this study is to describe real-world treatment patterns and clinical outcomes of mRCC patients with a prior receipt of IO and TKI therapies in community oncology settings.

Methods

This retrospective cohort study utilized data from The US Oncology Network (iKnowMed) electronic health record database. The study cohort included adult mRCC patients receiving subsequent line of therapy (LOT; index date) post IO and TKI in combination or sequence between January-18'

Individual treatments	Median OS, months (95% CI)	Adjusted HR (95% CI)	Median rwToT, months (95% CI)	Adjusted HR (95% CI)	Median rwTTNT, months (95% CI)	Adjusted HR (95% CI)
Cabozantinib (n = 292)	19.2 (16.1, 23.9)	Reference	2.8 (1.9, 3.5)	Reference	7.7 (6.8, 9.7)	Reference
Everolimus + Lenvatinib (n = 78)	12.6 (7.1, 17.8)	1.32 (0.94-1.86)	1.9 (0.8, 3.4)	1.16 (0.87-1.55)	7.1 (4.7, 10.7)	1.08 (0.81-1.44)
Ipilimumab + Nivolumab (n = 61)	27.6 (12.9, NR)	0.88 (0.55-1.4)	2.1 (1.6, 2.8)	1.72 (1.22-2.42)	3.7 (2.8, 5.1)	1.09 (0.81-1.47)
Axitinib (n = 59)	16.8 (10.7, 22.0)	1.1 (0.76-1.61)	1.4 (0.3, 2.3)	1.33 (0.92-1.93)	5.4 (3.4, 8.5)	1.39 (1.01-1.91)
Cabozantinib + Nivolumab (n = 52)	28.6 (16.1, NR)	0.55 (0.34-0.9)	10.4 (5.7, 16.4)	0.71 (0.5-0.99)	11.3 (7.8, 16.4)	0.44 (0.31-0.61)
Axitinib + Pembrolizumab (n = 47)	22.6 (14.4, NR)	0.75 (0.47-1.2)	11.1 (5.3, 17.0)	0.55 (0.37-0.83)	16.6 (7.4, 33.0)	0.50 (0.36-0.69)
Everolimus (n = 23)	9.7 (3.0, 26.3)	1.48 (0.81-2.7)	0.9 (0.0, 2.0)	1.84 (1.23-2.75)	5.8 (1.8, 8.5)	2.12 (1.29-3.49)
Lenvatinib + Pembrolizumab (n = 19)	NR (10.3, NR)	0.44 (0.14-1.34)	NR (3.0, NR)	0.35 (0.14-0.88)	NR (5.6, NR)	0.27 (0.13-0.54)
Tivozanib (n = 18)	17.8 (5.5, NR)	1.05 (0.47-2.33)	3.4 (0.3, 7.6)	0.79 (0.41-1.52)	7.1 (5.1, NR)	0.89 (0.51-1.55)
P-value	0.030	-	<0.001	-	<0.001	-

Abbreviations: CI, confidence interval; HR, hazards ratio; LOT, line of therapy; NR, not reached; OS, overall survival; rwToT, real-world time on treatment; rwTTNT, real-world time to next treatment

and March-23'. All patients were followed from the index date until June-23' to examine treatment pattern and estimate overall survival (OS), real-world time on treatment (rwToT), and real-world time to next treatment (rwTTNT) by line of therapy (LOT) and individual index regimen (with sample size ≥ 10). LOT was assigned based on patient's absolute order of treatment regimen using start and stop dates. OS was defined as the time between Index date and the date of death due to any cause. Descriptive analyses were used to describe patient characteristics and treatment pattern. Time-to-event outcomes (OS, rwToT, and rwTTNT) from index date were summarized using Kaplan-Meier method with a log-rank test by individual regimens. A multivariate analysis using Cox proportional hazards model was performed to assess the association of index regimens with clinical outcomes after controlling for patient characteristics.

Results

The study cohort included 820 patients. The median age was 66 (range: 58, 72) years, 72.6% were male and 77.1% were Caucasian. Moreover, 60.0% had an Eastern Co-operative Oncology Group (ECOG) performance status of 0-1 and 74.5% had intermediate/poor IMDC risk score. Overall, 253 (30.9%) received index treatment in LOT2, 509 (62.1%) in LOT3 and 58 (7.1%) in LOT4+. The most common treatments post IO and TKI settings were cabozantinib (35.6%), everolimus plus lenvatinib (9.5%), ipilimumab plus nivolumab (7.4%), and axitinib (7.2%). The median OS for LOT2, LOT3, and LOT4 was (18.0, 17.8, and 28.6 months, respectively). The median rwTOT for LOT2 was 2.5 months, LOT3 was 3.5 months, and LOT4 was 6.2 months. The median rwTTNT for LOT2, LOT3, and LOT4 was 6.4, 7.7, and 11.5 months, respectively. Statistically significant difference across individual regimens was observed for OS, rwToT, and rwTTNT (**Table**).

Conclusions

This study presents one of the most comprehensive analysis of treatment patterns and clinical outcomes among mRCC patients in the post IO and TKI settings. TKI-with or without IO-based treatments were the most preferred treatments and associated with improved survival. Overall, clinical outcomes in the post IO and VEGF/TKI setting are limited, and novel treatments are needed in this patient population.

Keywords

Metastatic renal cell carcinoma, immune-oncology, tyrosine kinase inhibitors, treatment patterns, clinical outcomes

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Cost-effectiveness of treatment sequences with front-line nivolumab+ipilimumab therapy vs immuno-oncology+tyrosine kinase inhibitor therapies in intermediate/poor-risk metastatic renal cell carcinoma

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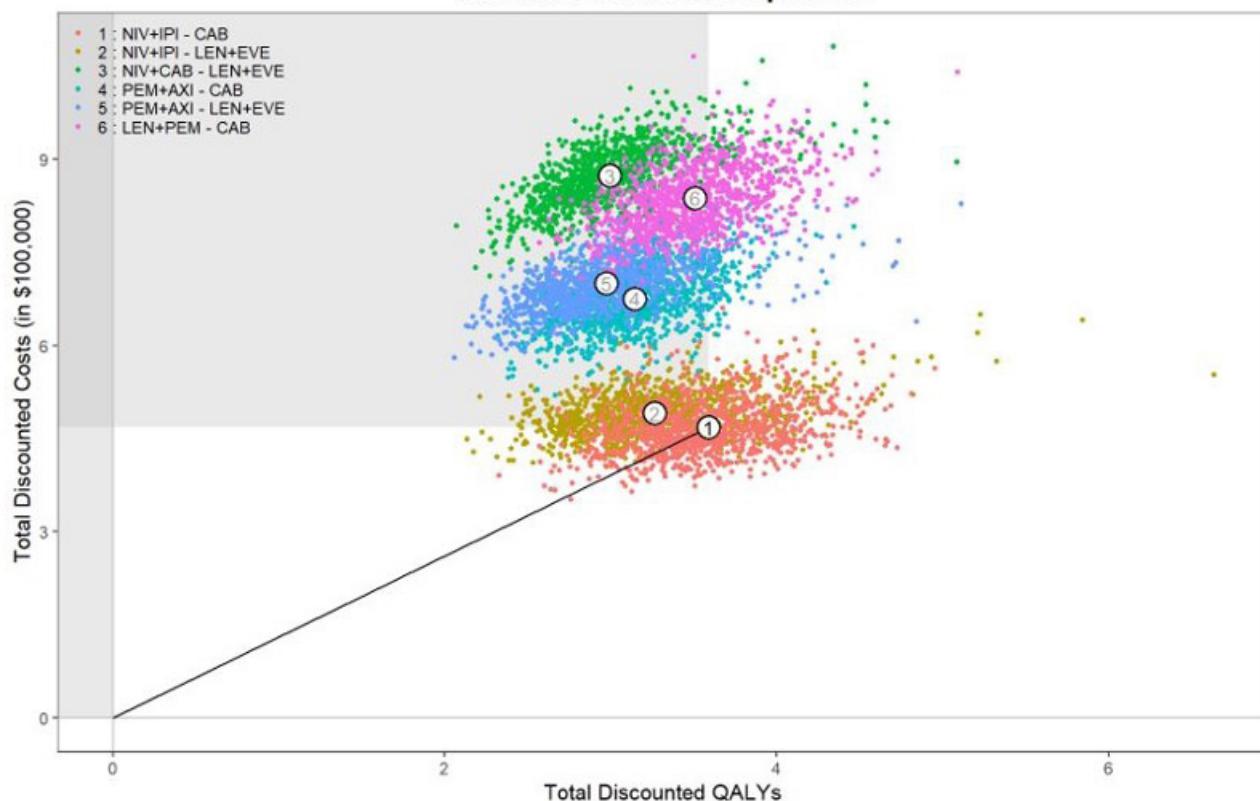
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Background

Immuno-oncology (IO) and vascular endothelial growth factor (VEGF) targeted tyrosine kinase inhibitor (TKI) combinations have transformed the treatment of metastatic renal cell carcinoma (mRCC). However, there is a lack of evidence regarding the most cost-effective sequencing of these systemic therapies. This study aimed to assess the expected health and economic outcomes of various treatment sequences for newly diagnosed patients with intermediate/poor-risk RCC from a US payer perspective.

Methods

We developed a model using a continuous time microsimulation framework. First-line treatment options included: nivolumab+ipilimumab, nivolumab+cabozantinib, pembrolizumab+lenvatinib, and pembrolizumab+axitinib. Second-line treatment options included: cabozantinib and everolimus+lenvatinib. In both lines, the source data for the overall survival (OS) and progression-free survival (PFS) distributions were obtained from the registrational randomized controlled trials (RCTs) of each therapy. The analysis included costs associated with drug acquisition in the US wholesale setting, drug administration and monitoring, management of treatment-related adverse events, disease management, and terminal care. Treatment duration for all first-line IO and TKI therapies was capped at 2 years. Quality-adjusted life-years (QALYs) were estimated by multiplying time in each phase of the disease by phase-specific utility values estimated from the CheckMate 214 trial data using US tariffs. The analysis was run 1000 times for cohorts of 100 patients over a 20-year time horizon. All costs and health outcomes were discounted annually at a 3% rate.

Figure**Efficient Frontier of Sequences**

ABBREVIATIONS: LEN + PEM – CAB, lenvatinib + pembrolizumab followed by cabozantinib; NIV+IPI – CAB, nivolumab + ipilimumab followed by cabozantinib; NIV+IPI – LEN + EVE, nivolumab + ipilimumab followed by lenvatinib + everolimus; PEM + AXI – CAB, pembrolizumab + axitinib followed by cabozantinib; PEM + AXI – LEN + EVE, pembrolizumab + axitinib followed by lenvatinib + everolimus; QALYs, quality-adjusted life-years

NOTE: Each data point represents the result of one simulation for a cohort of 100 patients

Results

Average per-patient QALYs were estimated for each sequence of interest: nivolumab+ipilimumab followed by cabozantinib (3.59), nivolumab+ipilimumab followed by lenvatinib+everolimus (3.26), pembrolizumab+axitinib followed by cabozantinib (3.14), pembrolizumab+axitinib followed by lenvatinib+everolimus (2.97), pembrolizumab+lenvatinib followed by cabozantinib (3.51), and nivolumab+cabozantinib followed by lenvatinib+everolimus (2.99). Average per-patient costs were estimated for each sequence of interest: nivolumab+ipilimumab followed by cabozantinib (\$468,856), nivolumab+ipilimumab followed by lenvatinib+everolimus (\$491,259), pembrolizumab+axitinib followed by cabozantinib (\$675,714), pembrolizumab+axitinib followed by lenvatinib+everolimus (\$700,411), pembrolizumab+lenvatinib followed by cabozantinib

(\$837,888), nivolumab+cabozantinib followed by lenvatinib+everolimus (\$873,377). First-line drug acquisition costs accounted for the majority of costs for all sequences.

Conclusions

Based upon our model using data from registrational RCTs, first-line nivolumab+ipilimumab followed by cabozantinib represents the dominant treatment strategy for intermediate/poor-risk mRCC patients in the US, offering estimated cost savings of up to 45% when compared to IO+TKI options. Additional studies are needed to elucidate whether these cost savings translate to the real-world setting.

Keywords

renal cell carcinoma, IO-IO, IO-TKI, cost-effectiveness, quality-adjusted life-years

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Preclinical evaluation of a novel dendritic cell vaccine for kidney cancer

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Background

Dendritic cell (DC)-based vaccines have been previously shown to promote therapeutic T cell responses in both preclinical tumor models and in cancer patients, where extended patient overall survival has been noted in many cases. The goal of the present study is to develop a novel DC-based vaccine as an effective immuno-oncology (IO) agent for the prevention/treatment of kidney cancer. We previously demonstrated that actin-binding protein profilin1 (Pfn1) is overexpressed in tumor-associated vascular endothelial cells (ECs), where it may serve as a prognostic factor in human clear cell renal cancer (ccRCC). We further established a direct causal relationship between EC Pfn1 dysregulation, immune microenvironment alterations, and tumor progression in mouse models of RCC. The current work specifically explores whether Pfn1-targeted DC-based vaccines are effective in preventing orthotopic RCC in mice.

Methods

To generate Pfn1-targeted DC vaccines, we harvested and matured DCs from Balb/C mouse bone marrow-derived precursor cells in cultures containing rmIL4 and rmGM-CSF. DCs loaded with individual Pfn1 synthetic peptides (these sequences were identified using three MHC class I/II peptide-binding algorithms) were injected into syngeneic mice on days 0, 7 and 14. Spleens were harvested from immunized mice on day 21 to isolate CD4⁺ and CD8⁺ T cells, with T cells then stimulated with unmanipulated DCs or DCs pulsed with individual immunizing peptides to detect specific T cell activation, as measured by IFN-gamma release quantitated by ELISA. Tissue histology was performed to assess immune-related adverse events (iRAE) in vaccinated mice as a safety index. For cancer studies, Balb/c mice were immunized with DCs loaded with pooled Pfn1 peptides a few days prior to establishing either subcutaneous or orthotopic RCC tumors. Tumor-bearing mice were subjected to an identical booster vaccine prior one week later in advance of euthanasia and study end-point analyses.

Results

Our studies show that vaccination of Balb/c mice with DCs pulsed with Pfn1 peptides elicit specific CD4⁺ and/or CD8⁺ T cell responses without promoting irAEs. When applied in the prophylactic setting, DC-Pfn1 peptide vaccines substantially slow the growth of subcutaneous RCC tumors. In support of these findings, we have also developed preliminary data supporting the therapeutic effects of DC/Pfn1-peptide vaccines in a murine orthotopic model of RCC.

Conclusions

In summary, our studies provide first evidence for Pfn1-targeted IO agent in the cancer setting. Given that the survival benefit for dual immune-check point inhibitors (ICI)/anti-angiogenic therapy remains modest and is limited to a small minority of ccRCC patients, Pfn1-targeted vaccines could represent a novel therapeutic agent for improved patient outcomes when applied alone or in combination with in-clinic ICI agents.

Keywords

Profilin, Dendritic Cell Vaccine, Clear Cell Renal Cell Carcinoma

DOD CDMRP Funding

yes

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Epigenomic profiling of translocation renal cell carcinoma via liquid biopsy

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Background

Translocation renal cell carcinoma (tRCC) is an aggressive subtype of kidney cancer usually driven by a fusion involving the *TFE3* gene. Due to histologic overlap with other subtypes of RCC, tRCC is frequently misclassified. Methods for accurate diagnosis and detection of this molecularly distinct entity are therefore a pressing need.

Epigenomic profiling of ctDNA via plasma chromatin immunoprecipitation and sequencing (ChIP-seq) has recently emerged as a powerful tool to detect and molecularly subtype cancers and may offer a more sensitive and specific detection of molecular fusions. We aimed to detect tRCC in plasma and to discriminate tRCC from ccRCC based on epigenomic profiling of cfDNA.

Methods

We first identified differentially expressed gene, methylated regions (DMRs) and regulatory elements (REs) specific to tRCC vs. ccRCC via RNA-seq, methylated DNA immunoprecipitation sequencing (MeDIP-seq) and ChIP-Seq, of 4 tRCC and 5 ccRCC cell lines. We collected 16 plasma samples from metastatic patients with tRCC, 11 with ccRCC and 9 healthy patients (HP). Ultra low pass whole genome sequencing (ulpWGS) was performed to infer ctDNA fraction (TF), and cfMeDIP-seq, H3K4me3/H3K27ac cfChIP-seq for epigenomic profiling. Signal at tRCC-specific regions derived from cell lines profiling was aggregated for each mark and normalized to common active REs and DMRs, then compared between classes using a Wilcoxon rank-sum test. Classification performance was evaluated using the area under the receiver operating characteristic (AUROC) curve.

Results

Overall 8/16 tRCC and 5/12 ccRCC samples had >3% TF by ulpWGS. H3K4me3 and H3K27ac cfChIP-seq signal was significantly higher in all tRCC samples compared to healthy patients ($p < 10^{-6}$, AUROC = 1), at tRCC-specific promoters and tRCC-specific REs respectively. Furthermore, H3K27ac signal in plasma was also significantly higher at tRCC-specific REs in tRCC samples compared to ccRCC ($p < 10^{-4}$, AUROC=0.95). MeDIP-seq could not discriminate between tRCC and ccRCC.

Conclusions

Although a majority of tRCC plasma samples profiled had TF < 3%, all could be distinguished from healthy samples on the basis of cfChIP. H3K27ac cfChIP-Seq was also discriminatory for tRCC vs. ccRCC. Epigenomic profiling of cfDNA appears as a powerful tool for both detection of tRCC and discrimination from ccRCC/healthy plasma, with potential implications for diagnosis and guiding therapy.

Keywords

tRCC, ctDNA, liquid biopsy, epigenomics

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Radiological tumor burden is an independent risk factor for survival in patients with metastatic clear cell renal cell carcinoma (mccRCC) treated with first line immunotherapy (IO)-based regimens.

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Background

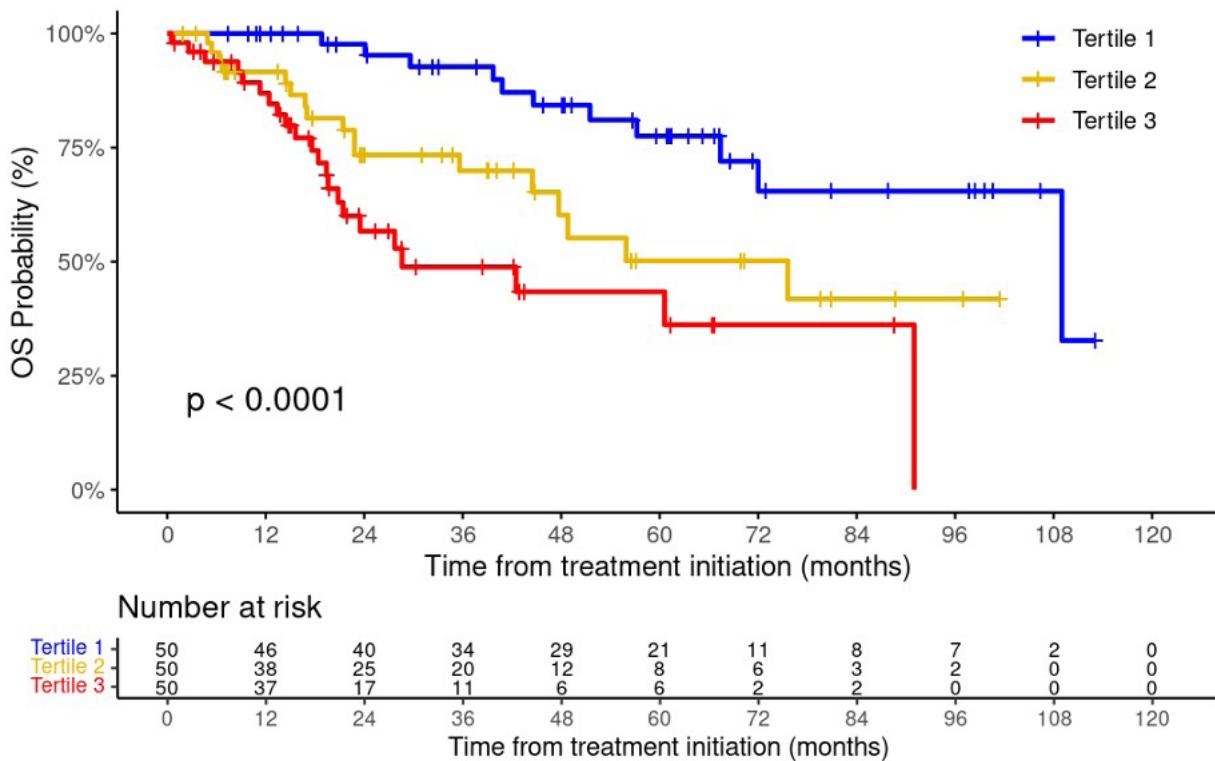
IO-based regimens (IO + IO or IO + VEGF inhibitor) represent current standard of care systemic therapies for the management of patients with mRCC. IMDC is the current standard to assess prognosis in patients with mRCC of clear cell histology. Baseline radiological tumor burden (BRTB) is a readily available measurement to calculate using routine CT-scans. Here we describe the utility of BRTB as prognostic factor in patients with mccRCC treated with first line IO-based regimens.

Methods

We reviewed data of 183 patients with mccRCC treated at Dana-Farber Cancer Institute from August 2014 to October 2023 and who received ≥ 1 dose of an IO-based regimen (IO + IO or IO + VEGF inhibitor). Patients with available CT-scan reports within 1 month prior to first line treatment initiation were included in our analysis. BRTB was assessed by a single operator as the sum of all measurable lesions as listed on the RECIST v1.1 report. The primary outcome was overall survival (OS), and secondary outcomes were time to treatment failure (TTF) and time to next therapy (TTNT). To evaluate the impact of BRTB on OS, TTF and TTNT, we used univariable and multivariable Cox regression models accounting for IMDC risk group, history of nephrectomy, and presence of either bone, liver, or brain metastases prior to first line initiation.

Results

A total of 150 patients satisfied the inclusion criteria and were included in our final analysis, of which 80% of patients were male (120/150). 9% (13/150) had favorable risk, 51%

**Figure:**

Kaplan-Meier curves representing OS based on baseline radiological tumor burden assessed by RECIST v1.1 (Tertile 1: 0 - 7.5 cm; Tertile 2: 7.6 - 15.3 cm; Tertile 3: 15.4 - 47.7 cm). P value is derived from the log-rank test.

(77/150) had intermediate risk and 20% (30/150) had poor risk by IMDC criteria. Overall, 73% (109/150) of patients had nephrectomy prior to first line therapy initiation and 41% (61/150) had either brain, bone, or liver metastasis. Median follow-up was 44.8 months. Every 1 cm increase in BRTB was associated with a 5% increase in risk of death (HR: 1.05; 95% CI: 1.03 - 1.08; $p < 0.0001$). On multivariable analysis, radiological tumor burden was significantly associated with OS (HR: 1.04; 95%CI: 1.00 - 1.08; $p = 0.03$). After categorizing BRTB by tertiles, tertile 1 included 0 - 7.5 cm, tertile 2: 7.6-15.3 cm and tertile 3: 15.4 - 47.7 cm. The 4-year OS estimates were 84% (95%CI: 74 - 97%), 60% (95%CI: 45 - 81%) and 43% (95%CI: 29 - 66%), respectively, for the 1st, 2nd and 3rd tertile (log-rank $P < 0.0001$). BRTB did not show a significant association with TTF on univariable (HR: 1.00; 95%CI: 0.99

- 1.01; $p = 0.783$), or multivariable analysis (HR: 0.99; 95%CI: 0.97 - 1.01; $p = 0.428$). As for TTNT, BRTB also did not show a significant association on univariable (HR: 1.01; 95%CI: 1.0 - 1.02; $p = 0.234$) or multivariable analysis (HR: 1.01; 95%CI: 0.99 - 1.03; $p = 0.453$).

Conclusions

Baseline radiological tumor burden is an independent prognostic factor for OS in patients with mccRCC treated with first line immunotherapy-based regimens. However, tumor burden is not significantly associated with TTF or TTNT.

Keywords

Prognostic tool; Kidney Cancer; Tumor burden; Radiology

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Generation and Characterization of Ex Vivo Expanded Tumor-Infiltrating Lymphocytes from Renal Cell Carcinoma Tumors for Adoptive Cell Therapy

David Einstein

Background

Autologous therapeutic tumor-infiltrating lymphocyte (TIL) therapy is a promising strategy to enhance anti-tumor immunity. Optimization of ex vivo TIL expansion could expand current immunotherapy options. Previous attempts to generate TIL in renal cell carcinoma (RCC) have been technically challenging. We applied a second-generation manufacturing process, currently used to generate the melanoma TIL product lifileucel, in RCC.

Methods

Resected primary and metastatic RCC samples were processed using the Gen 2 manufacturing process comprising of pre-Rapid Expansion Protocol (pre-REP) and REP steps. We assessed REP TILs for viability and performed phenotypic and functional characterization. We correlated the tumor immune microenvironment (TIME) with successful TIL expansion.

Results

Eight of 11 RCC samples underwent successful REP. Three failed cases demonstrated low CD8/FoxP3 ratio and high expression of PD-1 within FoxP3 cells. Expression of exhaustion markers differed between the TIME and expanded TILs; the latter had a TIM3-high/PD-1-low phenotype but retained functional capacity comparable to lifileucel.

Conclusions

The Gen 2 manufacturing process used for lifileucel successfully expanded functional TILs from RCC samples, enabling further study in a clinical trial. TIME features such as low CD8/FoxP3 ratio and high PD-1 expression within FoxP3 cells warrant study as potential biomarkers of successful TIL expansion.

Keywords

tumor infiltrating lymphocytes, tumor immune microenvironment, autologous cellular therapy

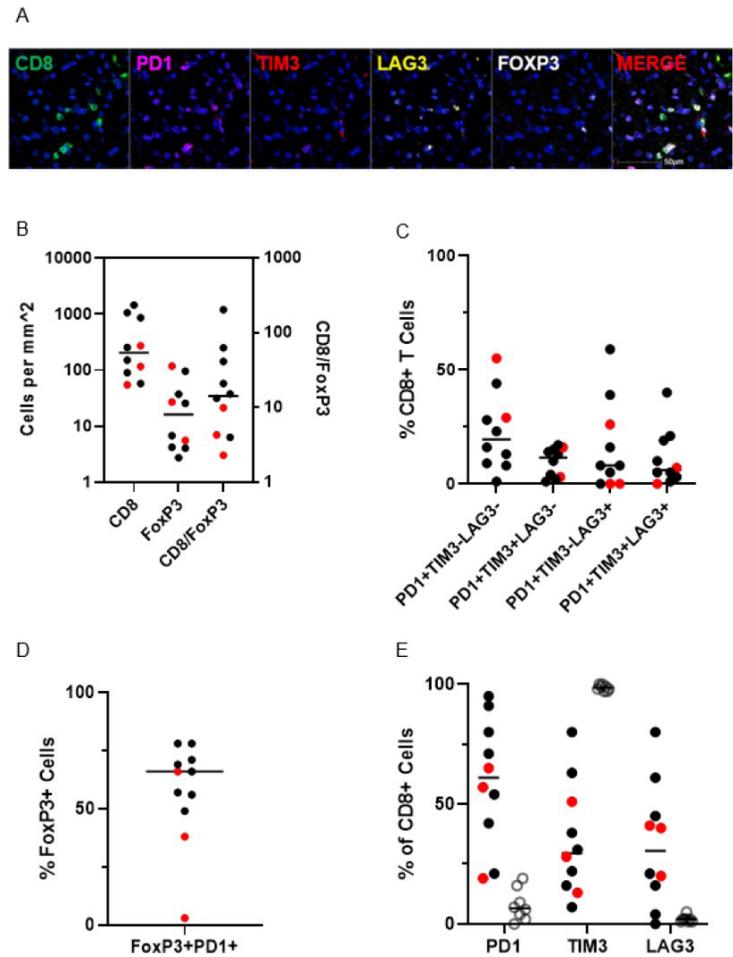


Figure: Assessment of tumor immune microenvironment (TIME) via multiplex immunofluorescence (IF). Cases that failed REP are indicated in red. (A) Representative image at 60x magnification, fluorophore colors as indicated. (B) Density of tumor-infiltrating CD8⁺ and FoxP3⁺ cells. (C) Expression of co-inhibitory exhaustion markers as a percentage of total CD8⁺ cells. (D) Expression of PD-1 in FoxP3⁺ cells. (E) Comparison of tissue TIME (dots) with TIL product post-REP (open circles) exhaustion profiles as a percentage of total CD8⁺ cells.

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Inhibition of AXL along with c-Met potentially halts resistance development in renal cell carcinoma

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Background

c-Met, a receptor tyrosine kinase (RTK), is overexpressed in renal cell carcinoma (RCC) and correlates with a decreased survival rate. Upon binding to its specific ligand, Hepatocyte Growth Factor (HGF), c-Met activates pro-tumorigenic signaling pathways. Cabozantinib (Cabo) inhibits c-Met and a few other RTKs, including AXL, and is approved for the treatment of patients with advanced-stage RCC. AXL and its ligand, GAS6, are overexpressed in RCC and are also markers of poor prognosis. The pro-tumorigenic role of AXL and its potential crosstalk with c-Met in renal cancer need to be thoroughly investigated.

Methods

We generated a Cabo-resistant (CaboR) cell line from wild-type Cabo-sensitive (CaboS) Caki-1 clear-cell RCC cells, and CRISPR/Cas9-mediated AXL knock-out cells (AXL-KO) from 786-O clear-cell RCC cells. Chromatin immunoprecipitation and sequencing (ChIP-seq) was performed to profile H3K27ac, a histone post-translational modification associated with active promoters and enhancers, in CaboR and CaboS cell lines. We also performed RNA sequencing (RNA-seq) on CaboR and CaboS and evaluated differentially expressed genes and enriched pathways using gene set enrichment analysis (GSEA). Transcriptional profiles of control clones and AXL-KO cells were also compared to identify the effect of AXL-KO on pro-tumorigenic signaling. We utilized an AXL-specific small molecule inhibitor, TP-0903, in combination with Cabo to validate our data. Finally, we studied how AXL silencing or inhibition may affect resistance to Cabo.

Results

Our findings revealed that AXL forms a complex with c-Met and may have a significant crosstalk which can be involved in therapeutic resistance. We found that prolonged treatment with c-Met inhibitors Cabo, Crizotinib, and PF-4217903, induced c-Met and AXL overexpression in RCC cells. Interestingly, c-Met inhibitor(s)-induced overexpressed c-Met can also be increasingly phosphorylated (at low

concentrations) in the presence of HGF, which may cause enhanced downstream tumor-promoting signaling. We found that silencing AXL can prevent this c-Met-inhibitors-mediated c-Met overexpression. Moreover, we found marked activation of the promoters and enhancers of several transcription factors, including ETV1, HOXA9, and FOXK1, in CaboR cells compared to CaboS cells. In the CaboR cells, genes involved in oxidative phosphorylation, progression through the division cycle, DNA replication, the NRF2 pathway, and DNA repair were upregulated, whereas genes associated with glycolysis, angiogenesis, hypoxia, and the Met pathway were downregulated (Figure 1). Finally, silencing AXL (using siRNA) or inhibiting AXL (using TP0903) in CaboR cells, induced significant apoptosis.

Conclusions

Together, our data suggest that CaboR cells have a distinct epigenomic and transcriptomic profile, and that targeting AXL along with c-Met inhibition can be beneficial in preventing acquired therapeutic resistance in RCC.

Keywords

Renal cell carcinoma; VEGF-TKI; Resistance; Cabozantinib; c-MET Inhibition

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National trends in the surgical management of renal cell carcinoma in Germany

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Background

The incidence of RCC is increasing worldwide. For most patients with localized RCC, surgery remains the preferred treatment option. We aimed to investigate whether international trends towards minimally invasive procedures and nephron-sparing surgery are reflected in Germany.

Methods

Pseudonymized inpatient billing data from the AOK (*Allgemeine Ortskrankenkassen*), a non-profit oriented health insurance organization in Germany, was used for the conduct of the study. A total of 43,936 RCC cases were identified in which either a partial nephrectomy (PN, n = 20,030) or radical nephrectomy (RN, n = 21,906) was performed between 2012 and 2021. The proportions of different surgical procedures over time, as well as mortality (90 days), transfusion rate, reinterventions, and general postsurgical complications (30 days), were evaluated. Due to the use of pseudonymized data, no ethics vote was required.

Results

During the period of observation, there was a significant shift from RN to PN, with an increase in the proportion of PN among all procedures from 41.0% (1,918/4,684) in 2012 to 57.4% (2,274/3,960) in 2021 ($p < 0.001$). Open surgery decreased for PN and RN but still accounted for the majority of all procedures (2021: 53.2% of PN and 65% of RN). Mortality was higher among patients with RN compared to PN (in hospital: 1.9% vs. 0.6%, 90 days: 4.7% vs. 1.2%). While general complication rates were similar (19.4% after PN versus 20.4% after RN), the reintervention rate was slightly higher after PN at 10.7% than after RN at 7.9%. Transfusion rates decreased significantly in several subgroups, favoring PN and minimally invasive approaches. Open surgical procedures were associated with higher unadjusted complication rates.

Conclusions

Despite shifts in favor of nephron-sparing surgery and minimally invasive surgery during the study period, open surgery remained the dominant surgical option for the treatment of renal cell carcinoma in Germany as of 2021. The adoption of laparoscopic and robotic-assisted techniques appears to reduce overall perioperative morbidity and consequently improve the quality of care in Germany. Further studies are required to examine patient level trends between type of surgical procedure, complication rates, and oncologic outcomes. Optimizing the assessment of the quality of care would enable further improvement of the quality itself.

Keywords

RCC; partial nephrectomy; nephrectomy; robotic-assisted surgery; laparoscopic surgery; open surgery

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Epigenetic regulation of ribosomal RNA transcription and processing by the tumor suppressor SETD2

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Background

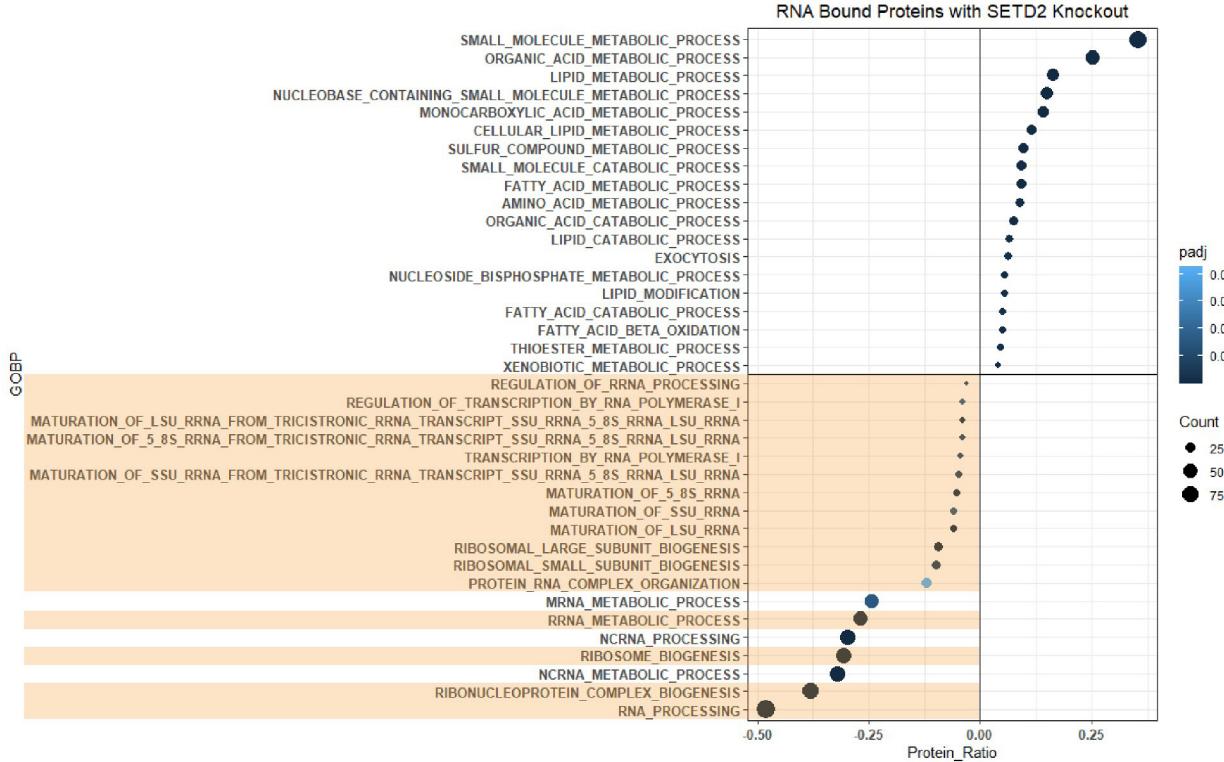
The methyltransferase SETD2 was originally identified as a tumor suppressor in clear cell renal cell carcinoma (ccRCC), and ccRCC with SETD2 mutations have increased metastatic potential and poor progression free survival. A known epigenetic regulator, SETD2 interacts with actively transcribing RNA polymerase II (RNAPII) and tri-methylates Histone 3 at Lysine 36 (H3K36me3), a modification that correlates with active gene transcription and is recognized by mRNA methylation and splicing factors. Despite these known roles in mRNA transcription, loss of SETD2 does not alter genic transcription, raising the question of whether or how SETD2 regulates RNA biology.

Methods

Given that SETD2 influences RNA processing, we sought to identify RNA-protein complexes that are regulated by SETD2. To do this in an unbiased and high-throughput manner, we used orthogonal organic phase separation (OOPS) coupled with mass spectrometry to identify changes in RNA binding proteins and pathways between control and SETD2-knockout cells.

Results

Corroborating previous findings, we identified that RNA binding proteins governing mRNA splicing and cellular metabolism were dysregulated in SETD2 mutant cells. However, the most robust changes in SETD2 knockout cells occurred in pathways regulated ribosome biogenesis (See Figure, orange highlights), resulting in a significant decrease in binding of ribosomal RNA (rRNA) processing factors to RNA. We confirm these alterations lead to defects in rRNA transcription, processing, and ribosome biogenesis. We identify that loss of SETD2 catalytic activity phenocopies these rRNA processing defects, suggesting H3K36me3 is essential for rRNA processing. Disruptions occur to both the large (60S) and small (40S) ribosome subunits, modifying protein translation. Lastly, we identify synthetic lethality between SETD2 deletion or inhibition and small molecules that interfere with rRNA transcription.



Conclusions

While the majority of research emphasizes the role SETD2 in the regulation of mRNA, our data identify that SETD2 and its catalytic activity play a critical role in the transcription and processing of rRNA. Importantly, rRNA comprises 80-90% of cellular RNA yet is typically overlooked and understudied. Next-generation RNA sequencing or transcriptional assays investigate mRNA or genic transcription while ignoring rRNA, demonstrating why SETD2-mediated rRNA processing has been unexplored. This may also have important implications for prospective clinical trials that utilized RNA-sequencing to identify optimal therapeutic regimens. Our work also identified that SETD2-mutated cells are more sensitive to compounds that inhibit rRNA transcription, potentially uncovering a new

therapeutic vulnerability of ccRCC with mono- and bi-allelic loss of SETD2. Alterations in ribosome biogenesis leads to epithelial-to-mesenchymal transition and metastasis in many tumor types, and our future goal will be to investigate the role of ribosomes and rRNA transcription in transformation and metastasis in ccRCC as well as the molecular mechanisms connecting SETD2 to ribosome fidelity.

Keywords

SETD2 Ribosome Transcription Epigenetics

DOD CDMRP Funding

yes

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Associations between body composition, ClearCode34, and clinical outcomes in localized clear cell renal cell carcinoma

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Background

Background: Emerging evidence suggests that pre-surgical body composition is a prognostic factor for cancer outcomes. Lower skeletal muscle quantity and radiodensity have been associated with renal cell carcinoma (RCC) outcomes in other studies, while reports of the relationship between visceral adipose tissue and RCC outcomes have been inconsistent. Mechanisms underlying these associations are unclear but may relate to tumor biology. We leveraged a large molecular epidemiology study of localized clear cell renal cell carcinoma (ccRCC) patients to examine how pre-surgical body composition features were associated with survival post-nephrectomy as well as ClearCode34 status, a previously validated prognostic gene expression signature.

Methods

Methods: The RESOLVE study at Memorial Sloan Kettering Cancer Center is a retrospective study of 1,239 patients with stage I-III ccRCC undergoing nephrectomy without systemic therapy. Pre-surgical CT scans at the third lumbar vertebrae were segmented for the cross-sectional areas and radiodensities of skeletal muscle, subcutaneous adipose and visceral adipose tissues using Automatica software.

Formalin-fixed, paraffin-embedded tumor tissue samples were available for 929 patients, which were sent to the University of North Carolina at Chapel Hill and assayed for gene expression. RNA was extracted from these samples and analyzed using the Nanostring nCounter system on a custom panel of 356 genes, including the 34 genes used in the ClearCode34 assay. Of the 929 patients with tumor tissue available, gene expression data from 837 patients (90.1%) passed RNA extraction and data QC. Samples were classified into either ccA or ccB ClearCode34 classes using a reference data set and PAM (prediction analysis of microarrays) centroid-based classification.

We estimated hazard ratios (HRs) and 95% confidence intervals (CIs) for disease-free survival (DFS) within five years of diagnosis using Cox proportional hazards models for both body composition variables and ClearCode34 subtype. We also estimated odds ratios (ORs) and 95% CIs for associations between ClearCode34 subtype and body composition with logistic regression. All multivariable models were adjusted for age, sex, and all body composition variables.

Results

Results: Of the 837 patients in our analytic sample, 219 (26%) had ccB tumors. Men, patients with high stage or grade tumors, and patients with chronic kidney disease prior to nephrectomy were more likely to present with ccB tumors. Lower skeletal muscle density (SMD) was significantly associated with lower five-year DFS [HR (95% CI) per 10 HU decrease in SMD: 1.7 (1.2, 2.3)]. While no other body composition features were significantly associated, we also observed non-significantly increased HRs for VATI and VATD [HR (95% CIs): 1.3 (0.8, 2.0) and 1.5 (0.9, 2.7), respectively].

ClearCode34 subtype was significantly associated with five-year DFS, where patients with ccB tumors had poorer DFS [HR (95% CI): 1.7 (1.1, 2.5)] compared to patients with ccA tumors. Notably, we observed no significant associations between any body composition feature and ClearCode34 in univariable analyses. However, in multivariable models that accounted for all body composition variables simultaneously as well as age and sex, lower SMD emerged as significantly associated with ccB subtype [OR (95% CI) per 10 HU decrease in SMD: 1.3 (1.0, 1.7)].

Conclusions

Our findings suggest that the associations observed between body composition features and clinical outcomes in ccRCC may be related to tumor biology. Lower pre-surgical SMD, which may represent myosteatosis and metabolic dysregulation in ccRCC patients, was associated with both decreased five-year DFS and an increased likelihood of presenting with a more aggressive ccB tumor. Future studies should investigate body composition features related to ccRCC prognosis and additional markers of tumor biology, such as immune-related gene expression patterns, to extend these findings.

Keywords

Kidney cancer; body composition; ClearCode34; clear cell renal cell carcinoma; renal cell carcinoma survival

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MicroRNA Signatures in Clear Cell Renal Cell Carcinoma: Exploring Potential Implications for Prognosis

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Background

Renal cell carcinoma (RCC), the most common malignancy of the renal epithelium, accounts for over 90% of kidney cancer cases, with clear cell RCC (ccRCC) representing about 75% of these diagnoses. MicroRNAs (miRNAs), short non-coding RNAs of 21 to 23 nucleotides, play significant roles in RNA silencing and post-transcriptional regulation of gene expression. They are also crucial in tumorigenesis. We aimed to identify differentially expressed miRNA between ccRCC tumor and normal-adjacent samples, aiming to find miRNA signatures for ccRCC diagnostics and prognosis.

Methods

We collected tumor and normal-adjacent kidney samples from the Dartmouth Renal Tumor Biobank spanning 1994 to 2009. Samples were mechanically and enzymatically disassociated and preserved at -80 Celsius until processing. The single-stranded sequencing data was processed using the smrnaseq (v2.2.4) Nextflow analysis pipeline to map miRNAs to the reference database. The preprocessed counts data was imported into R (v4.3.3). We employed strict quality control measures excluding samples with low counts with a mean log-transcripts per million of less than -2, a read length below 25% within the range of 20-25 nucleotides, a microRNA fraction less than 20%, non-primate reads exceeding 70%, or a mapping rate greater than 1.5 times the interquartile range. Differential expression analysis was conducted using the Bioconductor DESeq2 package (v1.42.1), comparing tumor and normal samples as well as across different survival time categories. miRNAs with a false discovery rate (FDR) <0.05 and log₂ fold change >2 were considered differentially expressed (DE) after adjustment for batch effects, tumor grade, sex, and tumor stage. An overrepresentation enrichment analysis was performed for the DE microRNAs using the miRNA Enrichment Analysis and AnnotationTool (miEAA). Survival analysis, utilizing stepwise selection in both directions, was conducted using identified DE miRNAs while adjusting for the covariates above plus age at diagnosis. Mature and hairpin miRNAs were analyzed separately for DE and survival.

Results

Among 213 samples with mature microRNA data, 198 samples (excluding one control) passed the quality control. These comprised 153 tumor samples and 45 normal samples, originating from a cohort of 159 patients. Among these patients, 13.8% have survived longer than 5 years, while 51.6% survived between 1 to 5 years, and 34.6% less than 1 year. 35 mature microRNAs were found to be differentially expressed between tumor and normal samples. Three mature miRNAs were under-expressed among samples with longer survival compared to those with short to medium survival. The DE mature miRNAs were overrepresented in vascular diseases (FDR=4.44 E-4), carcinoma (FDR=1.87 E-3) and clear cell renal cell carcinoma (FDR=5.65 E-3). miR-187-3p, miR-224-5p, miR-155-5p, miR-514a-3p, miR-490-5p were significantly associated with survival after adjustment ($p < 0.05$).

Out of the 219 samples analyzed for hairpin microRNA, 200 samples (excluding one control) passed quality control (155 tumor samples and 45 normal samples from 160 patients). Among these patients, 13.7% survived longer than 5 years, 51.9% survived between 1 to 5 years, and 34.4% survived less than 1 year. Differential expression analysis identified 28 hairpin microRNAs showing significant differences between tumor and normal samples. Notably, the differentially expressed hairpin microRNAs were overrepresented in renal cell carcinoma (FDR=5.38e-4) and renal clear cell carcinoma (FDR=0.01), as well as pathways associated with transcription factor TGFB1 (FDR=7.8xe-3), while being underrepresented in pathways associated with transcription factor KDM5B (FDR=9.5xe-3). Furthermore, hsa-mir-21, hsa-mir-155, hsa-mir-1291, and hsa-mir-506 were found to be significantly associated with survival after adjustment ($p < 0.05$).

Conclusions

This exploratory analysis identified distinct miRNA differential expression between ccRCC tumors and normal tissue, suggesting a potential miRNA signature for ccRCC. Our findings will be further explored using miRNA clustering analysis, functional analysis, network analysis, and differential transcript usage analysis.

Keywords

microRNA, renal cell carcinoma, ccRCC, differential expression, gene expression

DOD CDMRP Funding

yes

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Influence of gender on immunosurveillance in a novel mouse model of clear cell renal cell carcinoma

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Background

Renal Cell Carcinoma (RCC) is a cancer that exhibits sex dimorphism. Males have twice the incidence rate of their female counterparts and more than twice the mortality rate. They frequently present with larger, higher-grade tumors, greater metastatic spread, and earlier diagnosis of disease. Many hormonal, genetic, and environmental reasons have been explored, however a considerable amount of uncertainty remains.

Methods

Here, we explored the immunologic differences using a novel syngeneic mouse model of ccRCC. Tumors were orthotopically implanted by sub-renal capsular injection in immunocompetent WT C57Bl/6 and immunodeficient mice NOD/Scid/Gamma (NSG) mice, and tumor growth was longitudinally monitored using ultrasound imaging. At necropsy, liver and lung metastases were quantified and immune populations in the tumor and peripheral blood were analyzed by flow cytometry.

Results

In the immune-competent model, female mice had lower tumor penetrance as well as delayed tumor growth compared to male mice. In contrast, in the immune-deficient model female mice had similar tumor penetrance and growth rates as males suggesting that the female immune system may be protective against tumor development. No metastases were seen in either sex in the immune-competent model, whereas both lung and liver masses were found in the immune-deficient model suggesting the immune system is critical for restricting metastatic development. Interestingly, increased lung metastases were observed in females compared with male counterparts in NSG mice, suggesting that perhaps hormonal or other non-adaptive immunity-mediated mechanisms can promote metastatic seeding into the lungs.

Flow cytometry characterization revealed higher peripheral B- and T-cells in females, with a higher CD8/CD4 T-cell ratio. Tumors from females had more activated and cytotoxic CD8 T cells and were more frequently metastatic to the lung compared to male mice.

Conclusions

Our findings suggest sex-related immunologic differences in immunosurveillance and metastatic tropism that warrants further interrogation.

Keywords

Gender, immune surveillance, metastatic development

DOD CDMRP Funding

yes

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Clinical Pharmacokinetic/Pharmacodynamic (PK/PD) Relationship Confirms Best-in-class Potential of Casdatifan (AB521), a Small Molecule Inhibitor of HIF-2 α Being Developed in Renal Cancer

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Arcus Biosciences, Inc.

Background

Casdatifan, an orally bioavailable small molecule inhibitor of HIF-2 α , potently inhibits transcription of HIF-2 α -dependent genes in cell lines and preclinical species. The objective of this analysis was to develop an understanding of the relationship between clinical dose, casdatifan PK, erythropoietin (EPO), a PD biomarker for peripheral (non-tumor) HIF-2 α inhibition, and hemoglobin and to use this information to guide dose selection in future clinical trials.

Methods

Casdatifan plasma concentrations, serum EPO concentration, and hemoglobin data were obtained from 79 healthy participants in two Phase 1 studies, ARC-14 (NCT05117554) and ARC-28 (NCT05999513), and from 71 patients with clear cell renal cell carcinoma (ccRCC) and other solid tumors in an ongoing Phase 1 study, ARC-20 (NCT05536141). The available PK and PD data were collected following single oral doses of casdatifan ranging from 3 mg to 100 mg in healthy participants, and multiple oral doses of casdatifan ranging from 15 mg to 150 mg once daily (QD) in healthy participants and cancer patients. Serial PK, EPO, and Hb data were gathered in all study participants pre-dose till end of treatment. The

population PKPD model was developed using mixed effects methodology with NONMEM software to relate dose, PK, and PD (EPO and Hb) data.

Results

Casdatifan showed dose-proportional increases in plasma exposure over the 3-150 mg dose range after single and multiple doses. Casdatifan PK was also invariant over time in patients with an approximately 2.0-fold accumulation at steady-state compared to first dose. The mean terminal half-life of casdatifan was approximately 24 h. The PK was similar in healthy participants and cancer patients. Dose-dependent reduction in EPO was observed after single and multiple doses in healthy participants and patients.

A two-compartment model with first-order absorption adequately described the casdatifan plasma PK across the dose range tested. The effect of casdatifan plasma concentrations on EPO production rate was modeled using an inhibitory function.

Analysis of the casdatifan dose-PD (EPO suppression) relationship indicated that casdatifan 20 mg once daily provided a similar level of EPO suppression in patients as belzutifan 120 mg daily (benchmark peripheral PD). Furthermore, due to the dose-proportional PK of casdatifan, the selected dose (100 mg daily) for further development results in plasma levels approximately 5 times higher than those associated with the benchmark peripheral PD.

Conclusions

Casdatifan exhibits dose-linear and time-invariant PK in the dose range 15-150 mg. Dose-dependent reduction in EPO levels, consistent with the mechanism of action of HIF-2 α inhibition, was seen after casdatifan administration. A PKPD analysis of available data indicated that 20 mg daily dose of casdatifan would result in similar EPO effect as the registered dose of belzutifan. Available PKPD data and analyses indicated best-in-class properties of casdatifan.

Keywords

HIF-2 α , clear cell renal cell carcinoma, pharmacokinetic/pharmacodynamic (PK/PD) relationship

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Tumor immune microenvironment determinants of response to Lenvatinib and aPD-1 blockade in clear cell renal cell carcinoma

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Background

Combinations of immune checkpoint blockade (ICB) and anti-angiogenesis tyrosine kinase inhibitors (TKI) are the mainstay of metastatic ccRCC front-line treatment, but heterogenous responses and lack of biomarkers strongly emphasizes the need to understand RCC-specific mechanisms of effective treatment-related anti-tumor immunity. Although prior work consistently highlights tumor associated macrophages (TAMs) in RCC prognosis and treatment response, TAM targeting has yet to experimentally assessed as a viable treatment sensitizer.

Methods

We therefore assessed the impact of TAM depletion using a clinical-grade colony stimulating factor receptor-1 inhibitor (CSF1Ri) on ICB or TKI sensitivity in a spontaneous mouse model of ccRCC with inducible kidney-specific Vhl, p53 and Rb1 deletions (KVpR). Mice were randomized to receive either no treatment, single agent Lenvatinib (TKI), aPD-1, or combinations with CSF1Ri. We longitudinally monitored a total of 307 tumors from 96 mice using magnetic resonance imaging (MRI) and collected responding and non-responding tumors for single cell RNA sequencing (scRNASeq) to elucidate the underlying mechanisms of response.

Results

Surprisingly we found that TAM depletion abolished aPD-1 response, with no effect on Lenvatinib response. ScRNASeq analyses suggest that CSF1R selectively depletes antigen presenting TAMs, leading to a striking loss of T cell infiltration. This suggests that TAMs, rather than dendritic cells as shown in other cancer types, may play a dominant role in eliciting T cells during aPD-1 therapy in RCC. On the other hand, Lenvatinib, which elicited a stronger T cell response than aPD-1, was associated with significant increases in cDC1 and plasma cells in responders, together suggesting that these two treatment modalities may operate through distinct antigen-presenting cells. Also unexpectedly, we found that both Lenvatinib and CSF1Ri induced severe intratumor hypoxia, which is typically associated with poor outcomes in most cancers. We identify a hypoxic niche-specific TAM subset that when abundant at baseline is predictive for poor response to ICB/TKI combos

across multiple RCC trials, but post-treatment seems to capture hypoxia-mediated tumor cell death in responders in mouse and humans. We therefore wondered whether high levels of existing tumor hypoxia would exacerbate TKI-induced hypoxia leading to worst outcomes and found that in two novel RCC syngeneic models with high-baseline hypoxia, TKI indeed augments metastatic disease.

Conclusions

Our results offer mechanistic insight into the failure of CSF1R-based TAM depletion approaches in clinical trials and presents cautionary evidence against the use of hypoxia-inducing agents in tumors with high-baseline hypoxia. We show that TKI and ICB-induce distinct TME and hypoxia-related changes which require further study. More generally our study strongly emphasizes the importance of developing mechanism-guided clinical biomarkers.

Keywords

Immunotherapy, angiogenesis, hypoxia, macrophages, lenvatinib

DOD CDMRP Funding

yes

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Risk Factors of Immune Related Adverse Events in Patients with Metastatic Renal Cell Carcinoma Treated with Immune Checkpoint Inhibitors

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Background

Immune checkpoint inhibitor (ICI) based combinations have become the standard of care for first-line treatment in patients with metastatic renal cell carcinoma (mRCC). However, ICI can be responsible for immune-related adverse events (irAEs). Herein, we aimed to identify risk factors of immune related adverse events (irAEs) in patients treated with current first line (1L) combination of ICIs (ICI+ICI) or ICI with vascular endothelial growth factor (VEGF) targeted therapy (ICI+VEGF).

Methods

Data was collected retrospectively from patients treated for mRCC at Dana-Farber Cancer Institute, either receiving dual ICI or ICI+VEGF as 1L treatment regimen. Patients were categorized into two groups: those who developed grade ≥ 2 irAEs and those with grade 1 or no AEs. Time to toxicity (TT) was defined as the primary outcome. Univariate Cox regression analysis was initially conducted to identify potential predictive factors associated with irAEs. Subsequently, significant variables related to treatment adverse events were included in a multivariate Cox regression analysis with adjustments for multiple baseline factors.

Results

Among the 158 patients included, 122 (77.2%) were male, and 36 (22.8%) were female. Median age of patients at the treatment start date of therapy was 61 years (Q1-Q3: 62-69). A total of 57 (36.1%) patients developed grade ≥ 2 irAEs. The median follow-up of patients was 25.7 months.

Univariate analysis showed that diabetes, male sex, and ccRCC may indeed influence the development of irAEs with a trend towards significance. However, thrombocytosis could be a protective factor against developing irAEs ($p < 0.05$, Table).

Upon multivariate analysis, only ccRCC remained significant as a risk factor for irAEs compared to other subtypes, with a HR of 2.93 (95% CI :1.05-8.18, p = 0.04) (**Table**).

Conclusions

In this real-world study, we investigated potential clinical risk factors at baseline related to irAEs in patients with mRCC undergoing ICI-combination therapy. ccRCC histology was significantly associated with a higher risk of developing irAEs compared to other RCC subtypes. The retrospective design and the number of patients could be potential limitations of our findings.

Keywords

metastatic renal cell carcinoma, immune-related adverse events, risk factors, immunotherapy

	Univariate analysis		Multivariate analysis	
	HR	P-value	HR (95%CI)	P-value
Age at TT start	1	0.51		
Sex (Male vs Female)	2.36	0.03*	2.05 (0.92-4.56)	0.07
Regimen type				
(IO+VEGF vs IO+IO)	0.84	0.51		
Subtypes of RCC				
(ccRCC vs nccRCC)	3.37	0.02*	2.93 (1.05-8.18)	0.04*
IMDC risk group (poor vs intermediate)	0.6	0.18		
IMDC risk group (favorable vs intermediate)	1.42	0.26		
BMI	1	0.8		
Nephrectomy	0.8	0.47		
KPS<80	0.8	0.77		
Multiple metastatic sites	1.29	0.5		
Anemia	1	0.12		
Neutrophile count	0.9	0.73		
Thrombocytosis	0.37	0.03*	0.42 (0.16-1.06)	0.07
Hyponatremia	0.7	0.38		
Hypercalcemia	0.54	0.24		
Hyperlipidemia	1.32	0.29		
Diabetes	2.12	0.01*	1.61 (0.89-2.91)	0.11

Table: Cox regression analysis for assessment of risk factors associated with irAEs

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Dissecting the Cellular and Epigenetic Landscape of Clear Cell Renal Cell Carcinoma Through Paired snRNA/ATAC-Seq Analysis

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Dartmouth College

Background

Clear cell renal cell carcinoma (ccRCC) stands as one of the most prevalent and aggressive forms of renal malignancies, accounting for a substantial portion of kidney cancer-related mortality worldwide. The heterogeneity within ccRCC poses a significant challenge to understanding its molecular mechanisms and devising effective therapeutic strategies.¹

Previous studies utilizing single nucleus RNA sequencing (snRNA-seq) have revealed transcriptional contributions to cell-type specificity in both mature human and mouse kidneys. Moreover, recent advancements have expanded this approach to single-cell profiling of chromatin accessibility. Single-nuclei assay for transposase-accessible chromatin using sequencing (snATAC-seq) has enabled the measurement of chromatin accessibility in thousands of individual cells, providing insights into the dynamic process that drives nephron development and differentiation. Integration and analysis of multimodal single-cell datasets, such as snRNA-seq and snATAC-seq, allows for the prediction of cell-type-specific cis-regulatory DNA interactions and transcription factor activity, complementing the transcriptional information obtained by snRNA-seq.^{2,3}

Methods

This study leveraged paired snRNA/ATAC-seq in tumor and normal-adjacent samples from the Renal Tumor Biobank at Dartmouth (n= 63) to explore the ccRCC landscape at single-cell resolution using 10x multiome technology. The snRNA-seq dataset was processed with Seurat v5.0 to remove low-quality nuclei. Doublets were removed with DoubletFinder v2.02, and ambient DNA was removed using decontX from celda. Normalization was conducted via SCTransform, and missing data was imputed with ALRA. Clustering was performed by constructing a KNN graph and running the Louvain algorithm. Cell type assignments were predicted using Azimuth with the human kidney reference dataset. The snATAC-seq data was processed with ArchR v1.0.1, which performed dimensionality reduction and clustering. Cluster identities defined in the Seurat workflow were cross-annotated to the ATAC-seq data, and open peaks were identified within these cell-type clusters using MACS3 v3.0.1. Identification of marker peaks, motif enrichment, and motif enrichment deviations were inferred utilizing ArchR's *getmarkerFeatures*, *peakAnnoEnrichment*, and *addMotifAnnotations* functions, respectively.

Results

Motif enrichment analysis revealed canonical cancer-associated transcription factor (TF) dysregulation in tumor cells (nephron loop-like), including enrichment of HNF1B, SMARCC1, FOSL1, and JUND. Interestingly, HNF and SMARCC were also enriched in the lymphoid compartment, indicating possible crosstalk between these two cell types. Previous work has investigated the involvement of the HNF1 in ccRCC and in chromophobe RCC for prognosis, and we plan on investigating the relationship of these enrichments to tumor grade, stage and outcome.⁴ Additional TF enrichments included BCL families and ELF within the myeloid compartment, and vascularization related TF families in endothelial cell types including enrichment of various ETS TFs. Investigation of cell-cell interactions between immune and endothelial cell populations as well as between tumor and normal adjacent tissues will be performed to further disentangle the role differentially accessible chromatin plays in ccRCC.

Conclusions

Our multi-omic approach has several advantages. First, it enables the characterization of cellular heterogeneity within the tumor microenvironment, identifying cell populations and their contributions to disease progression. Secondly, by integrating transcriptomic and epigenomic data, we gain insights into the dynamic interplay between gene expression and chromatin accessibility, uncovering potential therapeutic vulnerabilities and biomarkers for personalized treatment strategies.

Keywords

ccRCC, Single-nucleus RNA sequencing (snRNA-seq), Single-nucleus ATAC sequencing (snATAC-seq), Tumor microenvironment, Cellular heterogeneity, Gene expression profiling, Multi-omic-analysis, Biomarkers, Personalized treatment

DOD CDMRP Funding

yes

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Prognostic biomarker development using deep learning and spatial proteomics in papillary renal cell carcinoma

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Background

Papillary renal cell carcinoma (pRCC) is a prevalent but understudied kidney cancer pathology, constituting 15-20% of cases. While many patients achieve curative therapy using nephrectomy, others go on to develop progressive, metastatic disease with poor overall prognosis. In order to improve the current standards of care for pRCC patients, it is critical to identify new methods to identify high-risk patients likely to require more intense and risk-modulated follow-up. Our goals in this project are two-fold: (1) to identify prognostic patient clusters based on spatial architecture of tumor tissue, and (2) to identify a minimal subset of markers to accurately predict the defined clusters for clinical translation.

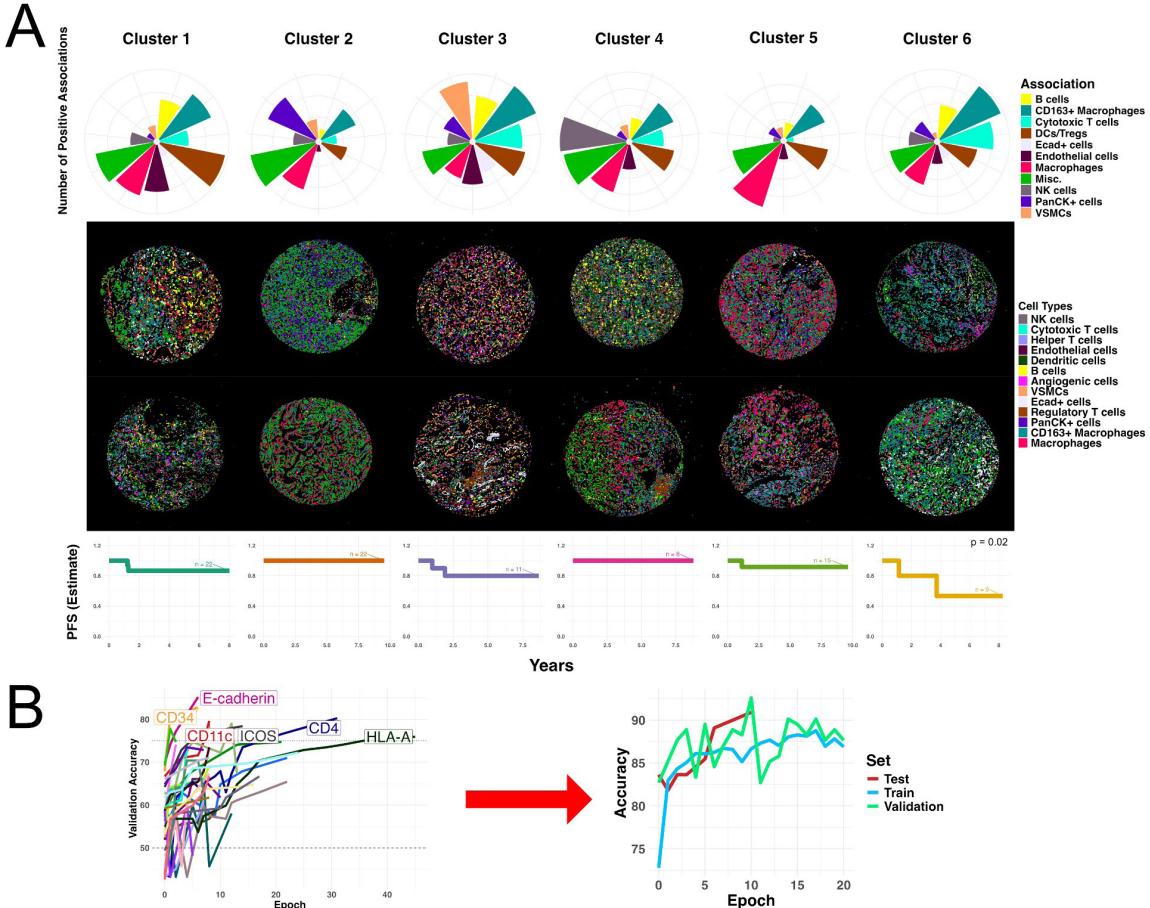
Methods

A tissue microarray of 100 pRCC patients was assayed using multiplex immunofluorescence with a 31-antibody panel with immune- and cancer-related proteins. We have developed novel algorithms to identify spatial neighborhoods and features. These features are then used to identify unsupervised clusters of patients. This new approach to classifying patients allows for a more specific and comprehensive description of each patient tumor's spatial composition and association with diverse clinical outcomes.

Next, we developed attention-based deep learning models capable of predicting these spatially-defined clusters directly from the underlying immunofluorescence images and subsequently profiled these for interpretability by retraining based on individual channels. We assessed each model iteratively on a validation set and a held-out test set for final model selection. We focussed on developing an accurate model with the minimal number of channels to enable development of a cost-efficient biomarker assay for the clinic.

Results

We performed a systematic analysis of the cellular and spatial phenotypes for each patient in our cohort and identified six (6) major spatial clusters. These clusters are based on the



spatial neighborhood composition of the tumors (**Fig 1A**) and describe the composite effects of cell-cell interactions within a patient tumor. We identified one cluster in particular (cluster #6, mainly associated with spatial interaction of CD163+ macrophages) with a significantly worse prognosis than the other clusters.

Using our advanced deep learning models, we have been able to achieve excellent validation accuracy in predicting a variety of clinical phenotypes and spatial features, including patient clusters and histological grade. We wished to then reduce the set of required features and began our efforts in predicting tumor vs. normal based on individual immunofluorescence channels and identified 6 channels with >75% individual predictive accuracy (**Fig. 1B**). Compositing all 6 markers further improves our accuracy to 90%. This success paves the way for exciting future prospects as we work to extend these efforts to identify a minimal clinically-applicable subset of protein markers.

Conclusions

The combination of computational spatial proteomics and deep learning models has the potential to identify new biomarkers specific to the spatial organization of patient tumors. We have identified a particular patient cluster with multiple associations with CD163+ macrophages demonstrating poor prognosis; selecting these patients may allow for tailored and more informed patient care. We are also working to enhance our deep learning models to circumvent the need for stepwise spatial analysis and, instead, allow for direct-from-image assessment of patients for particular spatial features indicative of various prognostic indicators. Our identified set of 6 markers serves as a basis for further refinements as we work to identify a minimal set of relevant markers able to predict various clinical outcomes from immunofluorescence imaging, offering a promising new avenue in the field of cancer pathology and biomarker development.

Keywords

Immunology, histology, spatial biology, proteomics, artificial intelligence

DOD CDMRP Funding

yes

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Preclinical evaluation of PSMA-based ^{68}Ga -/ ^{225}Ac -labeled radiotheranostic pair in a syngeneic mouse model of renal cell carcinoma

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Background

Despite the significant progress achieved with PD-1/PD-L1 inhibitors in the treatment of metastatic renal cell carcinoma (mRCC), most patients ultimately progress. Targeted radiotheranostics offer a new potential approach. Although a radiotheranostic platform targeting the prostate-specific membrane antigen (PSMA) has been tested in mRCC in patients preliminarily, thorough preclinical optimization has not been reported. Prostate-specific membrane antigen (PSMA) is overexpressed in tumor-associated neovascular endothelial cells of many solid tumors, including metastatic RCC. Furthermore, radiopharmaceutical therapy using β - and α -particle emitting agents causes immunomodulation by inducing immunogenic cell death, and release of tumor-associated antigens, potentially enhancing inflammatory phenotype. Here, we investigated whether PSMA-based radiotheranostics can be utilized to improve the efficacy of PD-1 therapy.

Methods

The PSMA+ RENCA model was developed by lentiviral transduction of RENCA (wt) cells and then characterized for basal and IFN-g induced PD-L1 expression. ^{68}Ga -L1 and ^{225}Ac -L1 were synthesized in high radiochemical yield and purity following our reported methods. Male and female BALB/c mice were used for tumor inoculation. ^{68}Ga -L1 was evaluated in small animal PET/CT imaging in flank and PET/MR imaging in orthotopic implantation. A treatment study was conducted to assess the effect of combination therapy in seven groups in the flank tumors at 2 weeks post-inoculation: Control (saline); PD-1 (10 mg/kg); Anti-PD-1 (10 mg/kg)+axitinib (tyrosine kinase inhibitor, 5 mg/kg, oral gavage, for five days/wk); ^{225}Ac -L1 (37 kBq); ^{225}Ac -L1 (2×37 kBq, 1 wk apart); [^{225}Ac -L1 (37 kBq)+PD-1 (10 mg/kg) and [^{225}Ac -L1 2×37 kBq]+PD-1 (10 mg/kg)]. Therapeutic efficacy was assessed by tumor weight and time to progression of tumor volume doubling (TVD).

To determine the mechanism of action of the $^{225}\text{Ac-L1}$ /anti-PD-1 combination treatment, tumor-infiltrating lymphoid populations from tumor samples were collected at day 30, and Fluorescence-Activated Cell Sorting analysis was done with fluorochrome-labeled antibodies against CD45, CD3, CD8, IL10, and IL12.

Results

$^{68}\text{Ga-L1}$ and $^{225}\text{Ac-L1}$ confirmed 10-fold and 2-fold higher uptake, respectively, in the PSMA+ RENCA cells compared to RENCA (wt) cells. PET imaging in the flank model displayed ~7-fold higher accumulation of $^{68}\text{Ga-L1}$ in PSMA+ RENCA than the RENCA (wt). Two-fold higher accumulation of $^{68}\text{Ga-L1}$ was observed in orthotopic tumors than the normal kidneys during 1-3 h post-injection. Significant lung metastases were detected with $^{68}\text{Ga-L1}$ PET at 3 weeks in mice with orthotopic tumors. A combination therapy study was conducted using anti-PD-1 and anti-PD-1+axitinib and compared the efficacy with $^{225}\text{Ac-L1}$ as a single agent (37 kBq and 2×37 kBq, 1 wk apart) and in combination with PD-1. Median TVD increased from 12 d (control) to 16 d (anti-PD-1), 16 d (anti-PD-1+axitinib), 24 d [$^{225}\text{Ac-L1}$ (37 kBq) $P<0.001$], 24 d [$^{225}\text{Ac-L1}$ (2×37 kBq) $P<0.0001$], 30 d [anti-PD-1+ $^{225}\text{Ac-L1}$ (1×37 kBq) $P<0.0001$], and undefined, [anti-PD-1+ $^{225}\text{Ac-L1}$ 2×37 kBq], $P<0.0001$, respectively. Furthermore, treatment with $^{225}\text{Ac-L1}$ (2×37 kBq, 1 wk) resulted in a significant lowering of tumor growth ($P<0.01$) compared to control, whereas anti-PD-1 (vs. control) alone moderately reduced tumor growth. The combination of the treatment of [PD-1+ $^{225}\text{Ac-L1}$ (2×37 kBq), $P=0.0001$] was the most effective in enhancing tumor growth inhibition compared with the PD-1+axitinib group. Flow cytometry of tumor-infiltrating immune cells of the treatment groups PD-1+ $^{225}\text{Ac-L1}$ (37 kBq) and PD-1+ $^{225}\text{Ac-L1}$ (2×37 kBq) revealed a higher proportion of effector CD3+CD8+ T cells, accompanied by a significant decline in the proportion of immunosuppressive and pro-tumoral CD8+IL10+ T cells and increase in antitumor CD8+IL12+ T cells compared to untreated and treatment control groups (PD-1 or PD-1+axitinib groups).

Conclusions

Combining radiotheranostic platform, $^{68}\text{Ga-L1}/^{225}\text{Ac-L1}$ with PD-1 therapy reduces tumor burden and improves TVD in a syngeneic model of RCC. This is a promising option for metastatic RCC patients with low and heterogeneous PSMA expression. Translation of this method will be pursued actively.

Keywords

PD-1/PD-L1 inhibitors, metastatic renal cell carcinoma (mRCC), Targeted radiotheranostics, Prostate-specific membrane antigen (PSMA)

DOD CDMRP Funding

yes

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Use of artificial intelligence to derive International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) elements from unstructured medical records

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Background

Artificial intelligence technologies invoking large language models (LLMs) may be able to automate data collection for the IMDC registry, an otherwise labor-intensive process. We evaluate a proprietary tool (HopeLLM) in expediting data abstraction.

Methods

We utilized a City of Hope-managed IMDC data set that includes patients with metastatic renal cell carcinoma. From this data set, we randomly selected patients who initiated care after 2018 and had sufficient treatment-related information within the Epic electronic health record system to determine HopeLLM performance. Patient identification numbers were manually extracted to prompt HopeLLM for data abstraction. We compared treatment start and end dates between manually registered information and HopeLLM estimations. The difference ratio in months was compared between the two sources. Lin's concordance correlation coefficient was performed to quantify the rate of agreement between both sources.

Results

Among 513 records, 91 patients were randomly selected. A total of 164 lines of treatment were recorded manually in the registry, and 146 were recorded by HopeLLM. A total of 75 lines of treatment were unique to the registry, while 42 were unique to HopeLLM. There were 117 unmatched lines of treatment between the registry and HopeLLM, which were not considered for concordance analysis. We recorded a difference (median (IQR)) of 0 mos (0-0.09 mos) for start dates between the registry and HopeLLM, with a concordance

correlation coefficient of 0.99 (95%CI 0.99-0.99). We recorded a difference of 2.15 mos (0.46-5.54 mos) for end dates between the registry and HopeLLM, with a concordance correlation coefficient of 0.92 (95%CI 0.88-0.95).

Conclusions

HopeLLM can streamline data abstraction from unstructured medical records onto the IMDC database, as it accurately predicts treatment start and end dates for patients with metastatic renal cell carcinoma.

Keywords

Kidney cancer, artificial intelligence, IMDC.

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Adherence to the ASCO Language of Respect guidelines in renal cell carcinoma abstracts in an international oncology meeting

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Background

The American Society of Clinical Oncology (ASCO) *Language of Respect* (LoR) guidelines were developed to promote the use of patient-centered language in all communications in 2020. The *Language of Respect* guidelines provide a directive to encourage the highest level of respect in addressing patients with cancer. In this study, we aimed to analyze the adherence to these guidelines in renal cell carcinoma (RCC) abstracts presented at the 2023 ASCO Annual Meeting, the largest international meeting of oncologists.

Methods

All RCC abstracts published in the 2023 ASCO Annual Meeting were evaluated. Statements from the abstracts were collected and stratified into the three categories of the LoR

guidelines: (1) "Do not blame patients", (2) "Respect the role of patients", and (3) "Do not dehumanize patients". Abstract and author data were summarized using descriptive statistics, and univariable and multivariable analyses were utilized to identify factors associated with odds of noncompliance with the guidelines.

Results

In total, 101 RCC abstracts were assessed. Most abstracts were published as poster presentations (51.5%) followed by online publication only (44.6%) and oral abstracts (4.0%). First authors affiliated with institutions in native English-speaking countries constituted 69.3% of the abstracts. Authors affiliated with institutions from a single country comprised 67.3% of the abstracts, whereas 32.7% of the abstracts were affiliated with authors from multiple countries. 40.6% of abstracts received no funding, 36.6% of abstracts received funding from a pharmaceutical company, and 22.8% received funding from non-profit organizations, institutions, or grants. There were 34 (33.7%) abstracts associated with clinical trials versus 67 (66.3%) associated with non-clinical trials. 51.5% of abstracts remained within 5% of the character count limit. Overall, 60.4% of the abstracts contained at least one statement that violated the guidelines. Abstracts with at least one statement violating "Do not dehumanize patients", "Do not blame patients", and "Respect the role of patients" directives were found in 46.5%, 21.8%, and 1.0% of abstracts, respectively. Abstracts within 5% of the character count limit were associated with increased odds of guideline noncompliance in the univariable analysis (OR 0.33 [95% CI 0.14-0.75], p=0.008). By multivariable analysis, abstracts within 5% of the character count limit were also associated with higher odds of violating the guidelines (OR 0.31 [95% CI 0.13-0.71], p=0.006).

Conclusions

A significant portion of RCC abstracts were found to violate the LoR guidelines. Our results highlight the importance of considering the expansion of the character count limit for abstract submissions to increase adherence to the LoR guidelines. With the incorporation of LoR guidelines in all forms of communication, the scientific community can promote increased use of respectful language in addressing patients, families, and colleagues.

Keywords

language of respect; communication guidelines

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Circulating leptin and immune-related transcriptomic patterns in clear cell renal cell carcinoma

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Background

Obesity has been associated with better outcomes in localized and metastatic clear cell renal cell carcinoma (ccRCC), as well as improved survival on immunotherapy treatment. However, the mechanisms underlying this association remain unclear. Leptin is an adipokine secreted by adipocytes, and circulating levels of leptin are higher in individuals with obesity. Additionally, leptin promotes inflammation and angiogenesis and has been proposed as pro-tumorigenic in colorectal and breast cancer. In this study, we examined how circulating leptin levels relate to both tumor and perinephric fat transcriptomic patterns in a cohort of ccRCC patients by evaluating the associations with body mass index (BMI), sex, and immune-related gene expression patterns.

Methods

We conducted a retrospective cohort study of 92 treatment-naïve ccRCC patients undergoing nephrectomy at Memorial Sloan Kettering Cancer Center. Available data included circulating leptin from fasting blood samples, clinical characteristics from medical records, and, in a subset of patients, RNA sequencing from tumor and perinephric fat specimens. We analyzed differentially expressed genes (DEG) according to circulating leptin levels using Gene Set Enrichment Analysis (GSEA) to describe pathways that were enriched in patients with high levels of leptin. We performed analysis for the whole cohort and stratified by sex based on differences in leptin levels.

Results

From the 92 patients with available peripheral leptin measurements, 51 and 44 had available tumor and perinephric fat RNA sequencing data, respectively. Of these, 64 (70%) were male, the median age was 60 years old, and most tumors were low grade and stage. Leptin distribution was significantly different in males than females, with circulating leptin levels

of 7 ng/ml (IQR 4-14) and 22 ng/ml (IQR 9-52), respectively. Higher BMI was associated with higher leptin levels, with a correlation coefficient of 0.63 ($p<0.001$) in males and 0.77 ($p<0.001$) in females. GSEA of tumor DEGs by circulating leptin, showed upregulation of pathways related to adaptive immune activity in patients with higher leptin levels across sexes, although this association was attenuated in females. Strikingly, in the perinephric fat there were stark differences in opposite directions in female and male specimens, with females showing significantly enriched transcription of genes associated with B cell signaling, humoral and adaptive immune responses.

Conclusions

Higher leptin levels were associated with a modest increase of immune-related gene expression in the tumors in both males and females, but significantly different directional changes were observed in the perinephric fat. Circulating leptin may be involved in peritumoral immune responses which may link host factors to sex related tumor outcomes. Further studies should aim to address the relationship between leptin activity and the tumor and fat microenvironment.

Keywords

leptin; obesity paradox; clear cell RCC; perinephric fat; immune pathways

DOD CDMRP Funding

yes

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Exploring DNA-methylation and DNA-hydroxymethylation in clear cell renal cell carcinoma using Dirichlet multinomial regression

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Background

DNA methylation alterations have been found related to clear cell renal cell carcinoma (ccRCC). In addition, DNA methylation has been used to identify cell heterogeneity at the tumor level using cell deconvolution methods. Other cytosine modifications are less studied in the field. In this study, we aim to explore the use of compositional method, Dirichlet multinomial regression¹, to simultaneously explore DNA methylation and DNA hydroxymethylation in ccRCC.

Methods

A total of 243 clear cell renal cell carcinoma (ccRCC) samples were collected from the Dartmouth Renal Tumor Biobank. DNA methylation analysis was conducted using the Infinium MethylationEPIC BeadChip, using tandem bisulfite (BS) and oxidative bisulfite (oxBS) treatments to differentiate between 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) states. Beta values were calculated for all the arrayed samples. Quality control was performed using the ENmix pipeline to ensure the inclusion of high-quality samples using a stringent pOOBHA <0.05. The oxBS maximum likelihood estimate (oxBS-MLE) method was applied to assess 5mC and 5hmC levels based on paired BS and oxBS datasets.

To determine the inclusion threshold for 5hmC beta values in the Dirichlet analysis, the R package oxBSCut was used to mask values close to zero². Missing 5hmC values and those values masked below the detection limit were imputed using a compositional approach and a Tobit regression model to retain the model properties. The Dirichlet model was applied to analyze three methylation states: unmethylated, methylated, and hydroxymethylated, providing comprehensive insights into the methylation landscape of ccRCC samples.

Results

Here, we analyzed the top 1% most variable methylated sites (n=3020). When comparing tumor vs normal adjacent samples we tested the cytosine modifications in the same model adjusting for sex, age at diagnosis, tumor stage, and grade. For this exploratory analysis, we selected those results that passed an FDR<0.01 and an absolute difference >0.2 for further exploration. Four sites were hyperhydroxymethylated related to MYO5A, DLG2, ANKRD33B, and one open sea site. A total of 23 sites were significantly hypermethylated. Using eForge-TF, these sites tracked to transcription factors targeting estrogen receptors, retinoic acid receptors alpha and gamma, and FOXC1 among others³.

Conclusions

In this preliminary analysis, using a new modeling approach to interrogate cytosine modifications simultaneously, we found some intriguing results consistent with previous literature. We will expand this analysis to evaluate the relation between extreme changes in hydroxymethylation in particular in relation to alternative splicing alterations.

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Keywords

DNA-methylation, DNA-hydroxymethylation, Dirichlet multinomial regression

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Impact of microbiome modulation on serum cytokine profile in a randomized, phase I trial of cabozantinib/nivolumab (cabo/nivo) with or without CBM588 in pts with metastatic renal cell carcinoma (mRCC)

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Background

We have previously demonstrated that the orally administered, live bacterial product CBM588 can augment clinical outcomes with cabo/nivo in pts with mRCC (Ebrahimi

et al ASCO 2023). As it is postulated this effect may be mediated through changes in systemic immunity, we sought to characterize changes in serum cytokine profile induced by CBM588.

Methods

Pts with previously untreated mRCC with clear cell, papillary or sarcomatoid histology were enrolled. Key eligibility criteria included Karnofsky performance status $\geq 70\%$ and measurable disease. Pts were randomized to receive cabo/nivo on a standard schedule alone or the same regimen with CBM588 at 80 mg bid. Pts had blood collection performed at baseline and at weeks 9, 13, 17 and 25. Samples underwent processing within 4-6 hrs of collection, and a total of 30 cytokines and growth factors were assessed using the Luminex Flexmap 3D system (Biotechne). Changes in circulating cytokine levels between baseline and week 13 (± 7 days) were examined across the treatment arms to investigate the impact of CBM588 on the immune system. The Wilcoxon matched-pairs test was used to compare the levels of cytokines at the two timepoints.

Results

A total of 30 pts were enrolled; the majority were male (67%) and had intermediate- or poor-risk disease (60%). Most pts had clear cell histology (87%); 5 pts (17%) had sarcomatoid features. As previously reported, pts receiving cabo/nivo/CBM588 had a significantly higher response rate as compared to pts receiving cabo/nivo alone (74% vs 20%; $P=0.01$). Across baseline and week 13, a total of 53 samples were available for assessment. In pts receiving cabo/nivo/CBM588, there was a significant change in the level of IL-12, IL-13, eotaxin, interferon- γ , and granulocyte-macrophage colony stimulating factor (GM-CSF); this was not observed in the cabo/nivo alone arm. Additional flow cytometric analyses characterizing relevant CD4- and CD8-positive subsets will be presented.

Conclusions

Taken together with our previous study evaluating nivolumab/Ipilimumab with CBM588 (Dizman et al Nat Med 2022), these results suggest that the augmented clinical outcome seen with the addition of CBM588 to cabo/nivo may be related to systemic immunomodulation. Combined analyses of the cohorts across studies are planned.

Keywords

combination therapy; microbiome; probiotic

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Unraveling the Complexities of Obesity in Clear Cell Renal Cell Carcinoma Carcinogenesis

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Background

Obesity has been identified as an established risk factor in the development of clear cell renal cell carcinoma (ccRCC). ccRCC arises from the proximal convoluted tubule (PCT) of the kidney which is also the site of obesity-associated chronic kidney disease. Obesity can lead to chronic cellular insults which may lead to DNA injury and whether these mechanisms are critical to cancer initiation in the kidney is unclear. One potential way that obesity can lead to cancer is by altering lipid metabolism, including increasing circulating saturated fatty acids. In particular, palmitic acid is known to be lipotoxic to proximal tubules of the kidney. Understanding how free fatty acids initiate cancer is critical to understanding the role of obesity in cancer initiation and development. We hypothesize that chronic injury and repair due to excess FFA exposure could lead to ccRCC carcinogenesis and aim to define the metabolic phenotype of obesity associated ccRCC.

Methods

Using publicly available data from The KIRC Cancer Genome Atlas Program (TCGA) and clinicopathologic data, we compared the RNAseq profiles of obese ($BMI \geq 30$) to normal weight ($BMI = < 25$) sample origins. Of the 337, a total of 333 had BMI available for analysis. We evaluated differences in Hallmark and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Using DESeq, we adjusted for age and sex. *In vitro*, HK PCT cells were cultured in DMEM+10%FBS treated with bovine serum albumin (BSA)-palmitic acid complex for 48 hrs. We measured cell viability using Alamar Blue. Results were normalized to controls with BSA only. We extracted protein and performed western blots to identify DNA damage (pATM, H2AX) and endoplasmic reticulum stress (ATF4, eIF2alpha).

Results

Most of the patients in our clinical data cohort are male (64.4%), while 45.4% of patients are categorized by BMI as obese and 33.2% are categorized as normal. We utilized 333 individuals from the Kyoto Encyclopedia of Genes and Genomes (KEGG) to link genomic information to pathways

associated with fatty acid metabolism as well as other cancer-associated hallmark metabolic pathways. We confirmed that excess free fatty acid supplementation leads to decreased cell viability in renal proximal tubule cells and renal embryonal cells and aim to assess differences in gene expression and metabolomic variation with fatty acid supplementation.

Conclusions

Through the investigation of these questions, we hope to improve understanding of obesity associated ccRCC and ultimately impact oncological outcomes for patients with

this cancer. Understanding changes in lipid metabolism around ccRCC initiation will help identify novel pathways for pharmacologic intervention at earlier stages of the disease.

Keywords

ccRCC, obesity, fatty acid metabolism, RNAseq,

DOD CDMRP Funding

yes

Trials in Progress

2

Advanced Renal Cell Cancer Combination Immunotherapy Clinical Trial (ARCITECT; HCRN GU 22-587)

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Dana Farber Cancer Institute

Background

First-line treatment for patients with metastatic clear cell renal cell carcinoma (mccRCC) often includes an anti-PD1 inhibitor in combination with either an anti-CTLA inhibitor (IO/IO) or a VEGF receptor tyrosine kinase inhibitor (TKI) (IO/TKI). Although some patients treated with nivolumab/ipilimumab (Nivo/Ipi) (IO/IO) experience durable responses leading to treatment free intervals, over two-thirds experience disease progression. Resistance to Nivo (anti-PD1) monotherapy has been associated with increased presence of a subpopulation of Tregs in the tumor microenvironment. Botensilimab (Bot) is an IO agent that leverages novel FcγR-associated mechanisms of action to enhance T cell priming, deplete intratumoral Tregs and enhance myeloid activation. Combination botensilimab/balstilimab (Bot/Bal) (anti-CTLA/anti-PD1) has shown impressive anti-tumor activity in diseases where Nivo/Ipi has shown little to no efficacy.

Methods

ARCITECT is a phase II, multicenter study evaluating the efficacy and safety of Bot/Bal relative to Nivo/Ipi. Patients

with mccRCC (favorable, intermediate, or poor risk), no prior systemic therapy (including adjuvant or neoadjuvant), and at least one measurable lesion as defined by RECIST 1.1 are eligible for enrollment. 120 patients will be randomized in a 2:1 fashion to Arm A (Bot/Bal induction followed by Bal maintenance) or Arm B (Nivo/Ipi induction followed by Nivo maintenance) each for a maximum of 2 years. Stratification factors include IMDC risk groups and sarcomatoid histology. The primary endpoint is overall response rate (ORR) per RECIST 1.1. We hypothesize that Bot/Bal will lead to a superior ORR (55%) relative to Nivo/Ipi (40%). This trial has > 90% power to detect the alternative hypothesis while maintaining a one-sided significance level of not more than 0.10 using the exact binomial. The study will be monitored for early stopping in favor of the null hypothesis based on a Simon's two stage design. In the first stage, 69 patients will be enrolled (Arm A:46 and Arm B:23). If at the end of the first stage, Arm A has either at least 18/42 (42.8%) of eligible patients responding or an ORR at least numerically equivalent to that for eligible patients in Arm B, then the trial will progress to the second stage. The primary endpoint will be met if there are 38/80 responders (ORR > 47.5%) in Arm A. Key secondary endpoints include landmark progression-free survival, treatment-free survival and rates of immune-related adverse events. Correlative studies will explore immune and molecular predictors of response and resistance to IO/IO in tumor and blood. [Clinical trial information: NCT05928806](#).

Keywords

immunotherapy, checkpoint inhibitor, renal cell carcinoma

Advanced Renal Cell Cancer Combination ImmunoThErapy Clinical Trial (ARCITECT)

hoosier.
CANCER RESEARCH NETWORK

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Background and Rationale

- First-line treatment for metastatic clear cell renal cell carcinoma (ccRCC) often includes a PD1 inhibitor in combination with either a CTLA inhibitor (IO/IO) or a VEGF receptor tyrosine kinase inhibitor (TKI) (IO/TKI).¹
- Although some patients treated with IO/IO experience durable responses leading to treatment free intervals, over two-thirds experience disease progression.²
- Resistance to Nivolumab (anti-PD1) monotherapy has been associated with increased presence of a subpopulation of Tregs in the tumor microenvironment.³
- Botensilimab/balstilimab (Bot/Bal) has shown anti-tumor activity in diseases where Nivolumab/Ipilimumab has shown little to no efficacy.^{4,5}

Botensilimab & Balstilimab Structure

Study Design

120 patients randomized 2:1 to Bot/Bal (n = 80) or Ipi/Nivo (n = 40).

- The sample size will provide 90% power at a one-sided alpha level of 0.10 to detect Bot/Bal ORR 55% (H_a) against the null hypothesis ORR 40% (H₀).
- The study will be monitored for early stopping in favor of the null hypothesis based on a Simon's two stage design.

Stratified by:

- IMDC Risk Group (favorable, intermediate, poor risk)
- Mesothelial tumor tissue with 5% available for biomarker testing

Randomize 2:1

Arm A: 80

Induction: Cycle 1-6 weeks

- Cycle 1: Nivolumab 3 mg/kg D1 until D22
- Balstilimab 450 mg D1 until D22
- Cycle 2: Balstilimab 450 mg D1 until D22
- Maintenance: Cycle 1-12 weeks
- Cycle 3: Botensilimab 450 mg D1 until D22
- Cycle 4: Botensilimab 450 mg D1 until D22
- Cycle 5: Botensilimab 450 mg D1, D22, D45, D54

Arm B: 40

Induction: Cycle 1-6 weeks

- Cycle 1 and Cycle 3: Nivolumab 3 mg/kg D1 and D22
- Ipilimumab 1 mg/kg D1 and D22
- Maintenance: Cycle 1-12 weeks
- Cycles 4-5: Nivolumab 400 mg D1, D22, D45, D54

Study Objectives

Primary

- To determine the objective response rate (ORR) per RECIST 1.1

Secondary

- Duration of response (DOR) per RECIST 1.1
- 12- & 24-month landmark progression free survival (PFS)
- Treatment free survival (TFS)
- Safety and tolerability

Exploratory

- To evaluate the relationship between percentage of regulatory T cell (Treg) and efficacy outcomes (ORR, landmark PFS, TFS).

Study Population

Key Inclusion Criteria

- Age ≥ 18
- ECOG ≤ 2
- Histologically confirmed metastatic or unresectable ccRCC
- All IMDC Risk Group (fav, int, poor)
- No prior adjuvant or systemic therapy
- Measurable disease per RECIST 1.1

Key Exclusion Criteria

- Prior adjuvant or systemic therapy
- Untreated Brain Metastases
- Known or suspected autoimmune disease requiring treatment within past 2 years
- Condition requiring >10mg daily prednisone or equivalent

Study Status

- ARCITECT is a multicenter investigator-initiated study planning enrollment at 13 HCRN sites.
- Funding Provided by Agenus Inc.
- [www.clinicaltrials.gov](#) Identifier: [NCT05928806](#)

References

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Results

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A Phase 1b, Open-Label, Safety, Tolerability, and Efficacy Study of HC-7366 in Combination with Belzutifan (WELIREGTM) in Patients with Advanced or Metastatic Renal Cell Carcinoma, NCT06234605

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Background

HC-7366 is a novel, orally administered, highly selective and potent activator of general control nondepressible 2 (GCN2) kinase, a core regulator of metabolic stress through activation of the integrated stress response (ISR). Activation of GCN2 promotes cell survival, whereas prolonged activation induces apoptosis. GCN2 activation also suppresses general protein synthesis and induces cell cycle arrest, thereby

preventing cell growth during nutrient scarcity. Additionally, HC-7366 decreases HIF expression and inhibits glycolysis, oxidative phosphorylation, and TCA cycle function. HC-7366 also inhibits HIF expression in immunosuppressive myeloid cells, including macrophages. These effects of HC-7366 on metabolism, HIF signaling, and immune suppression suggest therapeutic benefit in ccRCC with clear rationale for combinations with HIF2a antagonists and immune checkpoint inhibitors.

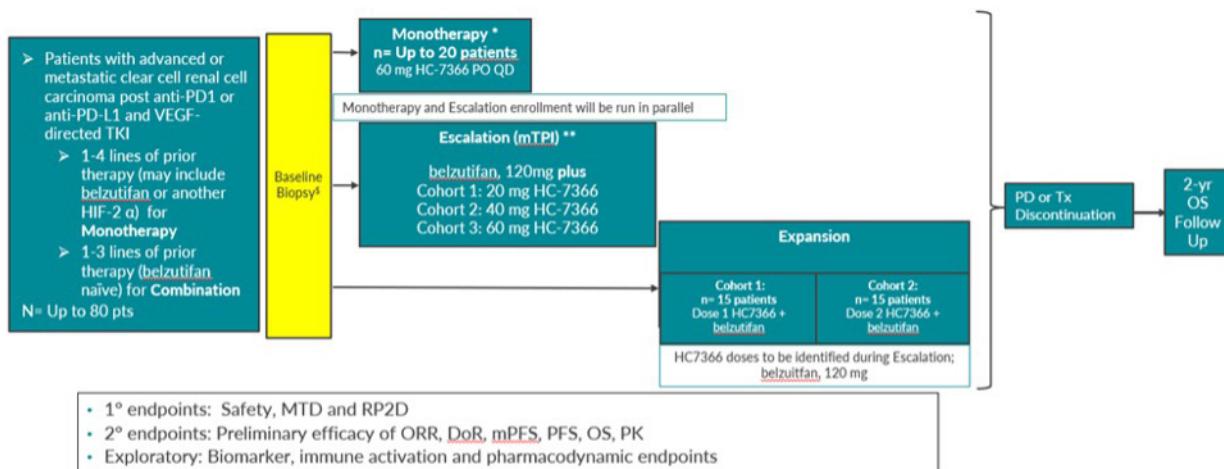
HC-7366 (0.5-1 mg/kg), combined with belzutifan (1 mg/kg), exhibited combination benefit in HIF-2 dependent A-498 and 786-O RCC xenografts, yielding 90% tumor growth inhibition and a three-fold increase in complete responses, respectively. Additionally, HC-7366 drives significant monotherapy antitumor activity in PDX models that demonstrated belzutifan resistance. Mechanism of action studies have identified several pathway engagement and potential efficacy biomarkers (Stokes, et al., 2024).

Trial design/schema: This multicenter, open-label phase 1b study will identify the Maximum Tolerated Dose (MTD) and/or the Recommended Phase 2 Dose (RP2D) of HC-7366 in combination with fixed-dose belzutifan, 120 mg po qd, in patients with advanced or metastatic RCC with renal cell histology irrespective of VHL gene mutation. Monotherapy HC-7366 will be evaluated in parallel.

The monotherapy arm, HC-7366, 60 mg p.o. qd, includes patients who have relapsed after 1 to 4 prior lines of standard of care that may include belzutifan or another HIF-2α inhibitor. The combination arm includes patients who have received 1 to 3 prior lines of standard of care and are belzutifan or HIF-2α naïve. HC-7366 dose escalation arm evaluates combination fixed dose belzutifan, 120 mg po qd plus HC-7366 at 20, 40, or 60 mg po qd.

Phase 1B Renal Cell Carcinoma Study

Monotherapy, Dose Escalation and Dose Expansion Design



Enrollment will start with the HC-7366 monotherapy arm. Subsequently, patients will begin to enroll in the combination therapy HC-7366 dose escalation arm, while the monotherapy arm will continue to enroll. Expansion will evaluate the combination of fixed dose belzutifan with two doses of HC-7366 selected from escalation.

Assessments include safety, PK, and anti-tumor activity. The study will enroll up to 80 patients at US study sites.

Significance and vision: Combination HC-7366 plus belzutifan, supported by preclinical evidence, is being studied to assess antitumor activity in the RCC relapsed setting. Determining safety and evaluation of dose are foundational to this study.

Reference

Stokes M, Tameire F, Wojnarowicz P, et al. HC-7366, a potent GCN2 activator, complements belzutifan, a HIF-2 α antagonist, by providing combination benefit in belzutifan-sensitive models and monotherapy activity in belzutifan-resistant models. Meeting of the American Association for Cancer Research; 2024 Apr 5-10; San Diego (CA); AACR; 2024. Abstract 4615.

This study is in collaboration with Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA

Keywords

Renal Cell Carcinoma; Clear Cell Carcinoma

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Phase II Trial of Ubamatamab in MUC16-Expressing SMARCB1-Deficient Renal Medullary Carcinoma and Epithelioid Sarcoma

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Background

Mucin 16 (MUC16) is a large integral membrane glycoprotein that is highly expressed in malignancies such as ovarian cancer but only at low abundance in epithelial cells of normal tissues. Proteolytic cleavage of cell surface MUC16 results in the shedding of its extracellular portion, known as cancer antigen 125 (CA-125), into the bloodstream and a short, membrane-associated C-terminal MUC16 domain that remains on the cell surface. Renal medullary carcinoma (RMC) and epithelioid sarcoma (ES) are aggressive SMARCB1-deficient

malignancies found to have elevated serum CA-125 levels in 70-80% of cases. Our preclinical studies suggest that upregulation of MUC16 upon SMARCB1 loss is a viable and attractive tumor target for SMARCB1-deficient malignancies such as ES and RMC.

Ubamatamab is a human IgG4-based anti-MUC16 x anti-CD3 bispecific antibody that specifically targets the cell surface bound C-terminal MUC16 domain and can thus induce T cell-directed killing of tumor cells even in the presence of high concentrations of CA-125. It would thus be a rationale therapy to use in SMARCB1-deficient malignancies expressing MUC16, including those such as RMC known to downregulate MHC Class I as a resistance mechanism to conventional immunotherapy. This is because CD3-targeting bispecific antibodies such as ubamatamab replace conventional signal 1 by engaging T cells regardless of MHC class I expression. As proof of concept, a 20-year-old patient with metastatic SMARCB1-negative ES expressing high levels of serum CA-125 achieved a durable (12+ months) partial response to ubamatamab after progressing on multiple prior therapies, including EZH2 inhibition with tazemetostat as well as anti-PD1/CTLA4 immune checkpoint inhibition with nivolumab plus ipilimumab. The patient reported Grade 2 CRS, pleural effusion, and pericardial effusion, all of which resolved without intervention¹. This serves as proof-of-concept for the ability of CD3-targeted bispecifics to overcome resistance to immune checkpoint inhibition.

Given this strong preclinical and clinical evidence, we have developed a phase II clinical trial of ubamatamab alone or in combination with the anti-PD1 immune-checkpoint inhibitor cemiplimab in patients with MUC16-expressing RMC and ES who have progressed on at least one line of prior therapy. Up to 20 patients will be enrolled from each disease cohort (ES and RMC) for a total of up to 40 patients. Patients enrolled in Stage I of the trial will receive ubamatamab monotherapy. Patients with disease progression on ubamatamab monotherapy during Stage I can proceed to stage II, which will evaluate the combination of ubamatamab with cemiplimab combination. The co-primary endpoints will be objective response rate (ORR) at any time during the trial and disease control rate (DCR) through 18 weeks. Secondary endpoints will include overall survival, progression-free survival, duration of response, and safety. All endpoints will be analyzed and reported separately for each disease cohort (RMC and ES), and for each Stage (ubamatamab monotherapy and combination ubamatamab with cemiplimab).

The trial will utilize the time-to-event Bayesian Optimal Phase II (TOP) design, which maximizes statistical power with well-controlled type I errors. For patients with ES, the joint null hypotheses are that the objective response rate (ORR) is 15% and the disease control rate (DCR) is 26%. The regimen will be promising if either ORR is greater than 15% or DCR is greater than 26% with 69.6% power to declare this

an interesting regimen if ORR is 30% and the DCR is 43%. For patients with RMC, the joint null hypotheses are that the objective response rate (ORR) is 15% and the disease control rate (DCR) is 15%. The regimen will be promising if either ORR is greater than 15% or DCR is greater than 15% with 71.4% power to declare this an interesting regimen if ORR is 30% and the DCR is 30%. Pre- and post-treatment correlative analyses will be performed in blood and tumor biopsy tissues to identify changes in specific immune cell subsets and elucidate the dynamic evolution of tumor and immune cell compartments as well as their spatial relationships following ubamatamab alone or in combination with cemiplimab.

References:

¹Revon-Riviere G, Chami R, Mills D, et al. Mucin 16 (cancer antigen 125) Expression in Epithelioid Sarcoma leads to Single-Patient Study with Bispecific T-Cell Engager Ubamatamab (Mucin16xCD3): A Bench-To-Bedside Experience. Connective Tissue Oncology Society, Nov 16–19, 2022, Vancouver, Canada.

Keywords

renal medullary carcinoma, epithelioid sarcoma, CA-125, MUC16, ubamatamab, bispecific agents

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Pilot study of an implantable microdevice for in vivo evaluation of drug response in renal cell carcinoma (NCT05700461)

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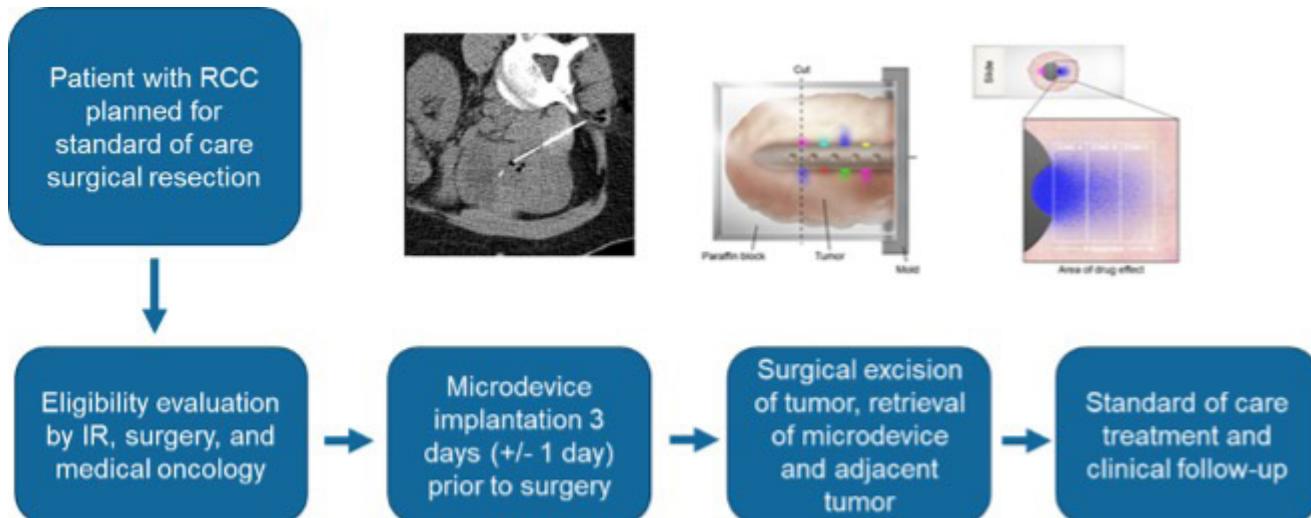
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Background

There are multiple therapies for renal cell carcinoma (RCC), yet no personalized biomarkers exist that can guide optimal treatment for individual patients. We developed implantable microdevices (IMD) that can be placed within a target tumor using a standard small-gauge interventional needle under imaging guidance. The IMDs deliver spatially segregated microdoses of up to 20 different drugs and/or combinations over multiple days, and then are retrieved surgically with surrounding tissue. This allows for the assessment of tumor response to multiple drugs *in vivo* within the native tumor microenvironment, without exposing the patient to systemic drug toxicity. We are conducting a trial to evaluate the safety and feasibility of IMD implantation in RCC tumors and subsequent collection of drug response data on targeted, cytotoxic, and immune-modulatory agents.

Methods

Eligible patients have suspected or confirmed RCC and are planned for standard surgical resection of either their primary



tumor or metastasis. Three days prior to surgery, one or more IMDs are implanted percutaneously into the tumor under CT guidance. Following nephrectomy or excision of a metastasis, the microdevices with surrounding tumor are removed *en bloc*; subsequently, the tissue is fixed, embedded and sectioned. The tumor/IMD cross-sections are analyzed for drug effect for each of the spatially separate treatment effects using a wide range of techniques, including immunohistochemistry for proliferation and pathway markers, spatial immune profiling using a panel of 25 tumor microenvironment markers, and spatial transcriptomics. Patients receive subsequent standard of care systemic treatment as per their oncologist's discretion.

Five patients have been enrolled and a total of 16 IMDs have been implanted to date. Enrollment is ongoing for a planned total of 20 patients. Success is defined as meeting both safety and feasibility endpoints. Safety is defined as two or fewer safety failures out of 20 enrolled patients. Feasibility is defined as 16 or more patients with successful microdevice implantation and retrieval of interpretable data.

IMDs will be analyzed to explore spatially segregated drug effects on the tumor microenvironment including tumor cell death and immune cell activation, as well as evidence of differential tumor drug sensitivity across treatments and patients.

Conclusions

Implantation of microdevices in RCC tumors may be a useful approach to improve treatment selection and accelerate drug development. Trial enrollment is ongoing.

Keywords

biomarkers, RCC, microdevice, tumor microenvironment, drug response

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INTerpath-004: A phase 2, randomized, double-blind study of pembrolizumab with V940 (mRNA-4157) or placebo in the adjuvant treatment of renal cell carcinoma

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Background

The PD-1 inhibitor pembrolizumab is approved as monotherapy for the adjuvant treatment of patients with renal cell carcinoma (RCC) who are at increased risk of recurrence following nephrectomy or nephrectomy and resection of metastatic lesions based on results from the phase 3 KEYNOTE-564 trial. Novel combination strategies could provide further clinical benefit in the adjuvant setting. V940 (mRNA-4157) is an individualized neoantigen therapy that is hypothesized to increase the antitumor activity of T cells and enhance the activity of pembrolizumab. V940 plus pembrolizumab showed improved clinical outcomes for stage III/IV melanoma compared with pembrolizumab alone in the phase 2b KEYNOTE-942 study. INTerpath-004 is a global, multicenter, randomized, double-blind, phase 2 trial (NCT06307431) designed to evaluate the efficacy and safety of adjuvant pembrolizumab with V940 or placebo in patients with RCC who have undergone nephrectomy.

Methods

Eligible patients are adults with histologically or cytologically confirmed RCC with clear cell or papillary histology (intermediate-high risk [pT2 Gr4, N0, M0 or pT3 Gr3/4, N0, M0], high-risk [pT4, N0, M0 or pT any stage, N1, M0], or M1 NED [solid, isolated, soft tissue metastases that can be completely resected at the time of nephrectomy or ≤2 years from nephrectomy]) with or without sarcomatoid features. Patients must have undergone nephrectomy and/or metastasectomy ≤12 weeks prior to randomization and be tumor-free as assessed by investigator. Patients must not have received prior systemic therapy ≤4 weeks or radiotherapy ≤2

weeks prior to randomization. Approximately 272 patients will be randomly assigned 1:1 to receive pembrolizumab 400 mg intravenously every 6 weeks for up to 9 cycles in combination with either V940 1 mg or placebo intramuscularly every 3 weeks for up to 9 doses or treatment discontinuation due to unacceptable toxicity, disease recurrence, patient withdrawal, or investigator decision. Randomization will be stratified by histology (clear cell vs papillary) and disease risk (intermediate-high vs high vs M1 NED). Imaging assessments (computed tomography or magnetic resonance imagery) will be performed every 12 weeks through year 2, every 16 weeks in years 3-5, and every 24 weeks in year 6 and beyond. The primary endpoint is disease-free survival by investigator assessment. Secondary endpoints include distant metastasis-free survival, overall survival, and safety parameters including adverse events (AEs), laboratory test results, and vital signs. AEs will be monitored throughout the study and for 30 days after the last dose of treatment (90 for serious AEs) and graded per National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0. Efficacy will be assessed in all randomly assigned patients, and safety will be assessed in all patients who received ≥ 1 dose of study intervention. Recruitment is ongoing.

Keywords

pembrolizumab, adjuvant, renal cell carcinoma, neoantigen therapy

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WIRE: Window of Opportunity Clinical Trials Platform for Evaluation of Novel Treatments Strategies in Renal Cell Cancer

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Background

Despite the success of immunotherapy and VEGFR directed tyrosine kinase inhibitors (TKIs) for the treatment of renal cell carcinoma (RCC), a significant proportion of patients display primary or secondary resistance to systemic therapy. New

treatment approaches are needed for these patients. Pre-surgical clinical trials allow us to study the effects of cancer treatment on the tumour microenvironment, comparing baseline biopsy with post-treatment nephrectomy in a 'window of opportunity' study. The results may give us insight into the mechanisms of new cancer therapies, that may be advanced into further clinical trials.

Methods

The WIRE trial (NCT03741426) is a phase II, multi-centre, multi-arm, non-randomised, neoadjuvant clinical trial platform. The overall aim of the trial is proof of mechanism for new RCC therapies. There are five investigational arms: 1. Cediranib (a VEGFR directed TKI), 2. Cediranib + Olaparib (a PARP inhibitor), 3. Olaparib, 4. Volrustomig (anti PD-1/CTLA-4 bispecific) and 5. Rilvegostomig (anti PD-1/TIGIT bispecific). The study uses a Bayesian adaptive design to optimise recruitment, with pre-specified criteria assessed at interim analyses to stop for futility, continue recruitment to that arm, or move to the next investigational arm. Eligible patients have cT1b+, cN0/1, cM0/1 clear cell RCC, planning to undergo surgery, without medical contraindication to trial therapy.

Patients undergo baseline biopsy to confirm clear cell RCC and multiparametric dynamic contrast enhanced (DCE) MRI scan of the primary tumour. For tablet arms 1-3, patients receive at least 14 days of investigational medicinal product (IMP). For immunotherapy arms 4 & 5, patients receive one infusion of IMP. The tumour is re-evaluated by DCE-MRI immediately before surgery. At nephrectomy, multi-region tissue sampling from the tumour is performed both pre-arterial ligation and post resection. Serial translational blood and urine samples are collected before, during and after treatment.

The primary endpoint for arms 1-3 is a $\geq 30\%$ reduction in DCE-MRI assessed capillary permeability (K_{trans}) compared to baseline. The primary endpoint for arms 4 & 5 is a $\geq 30\%$ increase in IHC assessed CD8+ T cell density comparing the resected tumour to baseline biopsy. Secondary endpoints include RECIST v1.1 primary tumour response and adverse events. A comprehensive translational science programme accompanies the trial, which will assess the tumour microenvironment and peripheral blood response to therapy, using techniques including imaging mass cytometry and single cell RNA sequencing. This will allow us to gain a deep understanding of mechanisms of response in each investigational arm.

To date, arms 1 & 2 have completed recruitment, and arm 3 is actively recruiting.

Conclusions

WIRE offers a unique opportunity to investigate the mechanisms of new therapies for RCC. Successful completion of these peri-surgical studies depends on co-ordination

between many specialties, including surgery, oncology, radiology, and pathology. The adaptive design allows for efficient allocation of patients to treatment arms to generate optimal data for each IMP. WIRE may identify novel biomarkers of response and toxicity, to inform treatment selection for patients. The data generated will be a foundation for further trials of these IMPs in advanced disease.

Keywords

Platform trials

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RCC Clinico-Genomics Consortium: A database of linked clinical data and molecular alterations in RCC patients receiving with immunotherapy-based treatments

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Background

Immunotherapy-based regimens with PD-1 or PD-L1 checkpoint inhibitors (ICI) have emerged as the standard of care for patients with advanced renal cell carcinoma (RCC) with clear cell component. While clinical benefit is frequently observed, most patients develop acquired ICI resistance and a subset have intrinsic resistance to treatment. While many biomarkers have been explored, no biomarker to guide selection for therapy is used clinically. Identifying positive

and negative biomarkers to guide selection for IO based treatments remains an unmet clinical need.

Methods

The RCC Clinico-Genomics Consortium is a multi-institutional collaboration of academic institutions formed in September 2023. This consortium aims to gather de-identified clinical, molecular, and outcomes data of patients with unresectable and metastatic RCC treated with an ICI-based regimens. Inclusion criteria include a diagnosis of locally advanced/irresectable or metastatic RCC, treatment with an ICI-based approach in the front-line setting, and tissue-based molecular sequencing data including whole exosome sequencing, whole transcriptome sequencing, and immunohistochemistry performed by commercially available CLIA-certified lab (CARIS). All patient data will be stored in a secure Redcap database. Data collection began in September 2023. As of May 2024, a total of 78 patients have been captured in the database. Data entry is proceeding as planned with goal to have 150 records by the end of 2024 and 250 by the end of 2025.

Conclusions

Significance and Vision: This collaborative effort will provide a useful clinical-genomic tool in exploring putative predictive biomarkers in metastatic RCC and provide valuable information from the real-world setting including data from patients not included or ineligible for clinical trials. The primary objective is to assess the presence of genomic signatures and their association with clinical and disease characteristics of patients and with clinical outcomes such as progression-free survival, response rate, and overall survival in ICI-based treatments.

Keywords

Renal cell carcinoma, immunotherapy, cancer genetic testing

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Molecular Residual Disease (MRD) Guided Adjuvant Therapy in Renal Cell Carcinoma (RCC) -MRD Gate RCC

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¹University of Alabama at Birmingham. ²University of California at Davis. ³Case Western Reserve University. ⁴University of California, San Francisco

Background

Molecular Residual Disease (MRD) assays are high sensitivity and specificity circulating tumor DNA assays that are designed to identify patients at high risk of recurrence after surgical intervention. Emerging data suggests that these assays can be used as a prognostic marker in various tumor types including in renal cell carcinoma (RCC) where serially ctDNA negative patients appear to have a favorable prognosis. Pembrolizumab is currently the only approved adjuvant immunotherapy for high risk resected RCC patients. Several adjuvant immunotherapy trials have not proven any clinical benefit. We designed a study to incorporate a MRD-guided approach to the assignment of adjuvant immunotherapy to obtain early estimates of clinical outcomes as a initial step to evaluate the viability of larger biomarker integral clinical trials in adjuvant RCC.

Methods

Key inclusion criteria include age ≥ 18 years, histologically confirmed clear cell RCC of intermediate-high risk (pT2, grade 4 or sarcomatoid, N0 M0; or pT3, any grade, N0 M0), high risk (pT4, any grade, N0 M0; or pT any stage, any grade, N+ M0); ; no prior systemic therapy for advanced RCC; positive surgical margins are allowed; Eastern Cooperative Oncology Group (ECOG) performance status 0 - 2; and tumor sample available for development of a tumor informed ctDNA MRD probe. M1 NED patients are excluded from this study. Patients will be assigned to receive pembrolizumab 400 mg every 6 weeks by intravenous infusion only if found to be ctDNA positive within a MRD surveillance window (<120 days from surgery). Treatment will continue until disease recurrence, treatment discontinuation, or completion. Imaging will be performed based on NCCN guidelines. The primary end point is disease-free survival (DFS) per investigator assessment at 1 year . The secondary end point is overall survival (OS) at 1 year . An

anticipated 100 patients will be accrued across an estimated 5-6 centers. [Funding : National Comprehensive Cancer Center Network]

Keywords

RCC , MRD

28

A multi-center, open-label phase II study of lenvatinib plus pembrolizumab (LEAP) in renal cell carcinoma patients with brain metastasis previously treated with immune checkpoint blockade

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Background

Brain metastasis incidence has historically ranged from 2%-15%, whereas modern series post-immunotherapy (IO) have found incidences as high as 29%. Despite the revolution of the RCC treatment landscape with the discovery of

targeted therapy and IO agents, treating RCC patients with brain metastasis remains challenging. Our snRNA-seq studies showed that brain metastases have a unique immunosuppressive environment with a layer of neuronal regulation, which is targetable by inhibiting FGFR2. Moreover, neuronal cells have proliferative signaling on tumor cells through FGFR4 signaling, which is targetable by a multi-target tyrosine kinase inhibitor of VEGFR, FGFR1-4, PDGFR and other receptors. Additionally, Keynote-146 showed efficacy of pembrolizumab+lenvatinib on extracranial metastasis sites in patients who progressed on immunotherapy alone. Based on these findings we hypothesize that pembrolizumab+lenvatinib can modulate the immunosuppressive brain metastasis microenvironment and is safe and effective in patients with renal cell carcinoma (RCC) and brain metastasis who were previously treated with immune checkpoint blockade.

Methods

This is a multi-center, open-label phase II study evaluating the efficacy and safety of pembrolizumab+Lenvatinib in patients with RCC and untreated brain metastasis who were previously treated with immune checkpoint blockade. The study will implement a Bayesian design with 40 patients, with futility monitoring based on a null hypothesis median intracranial progression free survival (icPFS) of 4.8 months with a target improvement of median PFS equal to 8.7 months. Pembrolizumab (200 mg IV Q3W) and lenvatinib (20 mg PO QD) will be administered in 21-day cycles for a maximum of 24 months (or 35 cycles) in the absence of disease progression or until unacceptable toxicity, death, withdrawal of consent, or discontinuation from the study treatment for any other reason. The primary endpoint is icPFS as assessed by Response Assessment in Neuro-Oncology-Brain Metastases (RANO-BM) criteria. The key secondary endpoints are intracranial objective response rate (ORR) of non-irradiated measurable (tumor diameter 0.5-3.0 cm on magnetic resonance imaging (MRI)) brain metastases and distant brain failure rate defined by the recurrence of new brain metastases outside of the radiation field, as assessed by RANO-BM, and overall survival (OS). Other secondary endpoints are safety, extracranial ORR, extracranial PFS, as assessed by the RECIST 1.1 and iRECIST. Exploratory analyses will include evaluation of tissue, blood-based and cerebrospinal fluid immune-related correlates, identification of imaging characteristics of treatment, evaluation of the neurological and cognitive function, seizure reduction, steroid, and opiate pain medication.

Keywords

renal cell carcinoma, brain metastasis, CNS metastasis, lenvatinib

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Multiplexed Quantification of Urinary Biomarkers for Non-invasive Classification of Imaged Kidney Tumors

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²Siteman Cancer Center. ³Washington University in St. Louis, St. Louis, Missouri USA. ⁴Urologic Surgery. ⁵Interventional Radiology

Background

This study seeks to find urinary biomarkers of renal cell carcinoma (RCC) that can be developed into a reliable low-cost screening for RCC in at risk populations as imaging techniques are expensive and less apt to cover large groups of individuals. Additionally, most imaging technology cannot reliably identify malignant from benign tumors. Estimates indicate that benign tumors occur 12-20% of time. Our proposed study is simple and minimally invasive. The unmet need is identification of suitable biomarkers to reliably detect occult kidney cancer early enough to find pre-metastatic tumors and reliably diagnose benign tumors to inform next steps in clinical care. It is hypothesized that these biomarkers are an early indicator of the cancer, can be detected in the urine with our enhancing plasmonic-fluor technology when the tumor is at a treatable stage confined within the capsule of the kidney, and serve as a non-invasive molecular diagnosis of the imaged kidney mass in point of care settings.

Methods

There is no active intervention or treatment associated with this protocol. Urine is collected prior to surgery/ablation and during the first post-surgical/ablation follow-up visit. Radiologic images will be evaluated from the medical record for patients scheduled for surgery/ablation to remove an imaged kidney mass of 4cm and under. Additionally, we will collect one urine sample from self-described healthy individuals age-, sex-, and racially/ethnicity-matched to the surgical/ablation patients.

This is a prospective study with planned enrollment of 450 individuals in three cohorts over a three-year period. The protocol combines the ability to assemble a collection of patient-matched clinical material with state-of-the-art quantitative multiplexed enzyme-linked immunosorbent assay (ELISA) and lateral flow assay (LFA) enhanced by our proprietary plasmonic-fluor (PF) technology that

adds orders of magnitude sensitivity to evaluate several urinary biomarkers of kidney cancer. Based on our previous findings and recent findings in the literature, we expect the concentrations of AQP1, PLIN2, Mxi-2 and KIM-1 to be higher in urine samples from patients with a malignant tumor while the concentration of Vim-3 to be higher in urine samples from benign oncocyotoma tumors. Achieving our goals will enable pre-surgical/ablation differentiation of malignant from benign incidentally discovered imaged renal masses. Cohorts (n=150 each) include a) patients undergoing surgery with presumed diagnosis of RCC, b) patients undergoing ablation with a presumed diagnosis of RCC, and c) normal, self-described healthy controls. Our proposed study is simple and minimally invasive. A urine will be collected from patients undergoing surgery/ablation prior to removal of a renal mass 4cm or under and from matched healthy volunteers. Additional collections of urine will occur at the first surgical/ablation post-procedure follow up visits. Radiology image and post procedure pathologic reports will be assessed for tumor size, the patient's clinical course, stage, grade, and discoverable metastases will be gathered. The overall objective of our study is to find and evaluate novel biomarkers, predictive of disease, that are present and measurable in whole urine of patients undergoing surgery or ablation for renal cancer based on discovery of an imaged kidney mass. These biomarkers would be absent or severely decreased in the urine of these same patients following removal/ablation of the affected kidney or tumor. It is further hypothesized that the urine content of these novel biomarker proteins is measurable by plasmonic-fluor technology and will serve as a non-invasive liquid biopsy of the kidney tumor by molecular diagnosis. Both ELISA and LFA assays will be developed during the study.

Keywords

Urine biomarkers. Malignant tumor, Benign tumor, Point of service

33

Combination nivolumab and ipilimumab with and without camu camu in first-line treatment of metastatic renal cell carcinoma (mRCC).

Dr Regina Barragan-Carrillo MD

City of Hope Comprehensive Cancer Center

Background

Combination immune checkpoint inhibition (ICI) with ipilimumab and nivolumab (ipi/nivo) is an established first-line

treatment for patients (pts) with intermediate and poor-risk mRCC. CheckMate214 demonstrated a survival advantage with ipi/nivo over sunitinib; unfortunately, 20% of cases developed primary progression to immunotherapy. Recent data suggest that the gut microbiome is key in modulating clinical responses and immune-related toxicities. Therefore, modulating the gut microbiome is a novel adjunct strategy to dual ICI in mRCC. Our group has previously proven that the addition of a live bacterial product (CBM588) enhances clinical responses in pts with mRCC treated with ICI, and the combination of ICI (Dizman *et al.*, 2022) or with a tyrosine kinase inhibitor (Ebrahimi *et al.*, 2023). Camu camu (*Myrciaria dubia*) is a comestible berry characterized by a polyphenol-rich nutritional profile. In extract form, it is rich in castalagin, which appears to have probiotic properties. In mouse tumor models, camu camu increased the abundance of fecal *Ruminococcus* spp. when combined with ICI (Messaoudene *et al.*, 2022). This shift in the gut microbiome composition was associated with stronger CD8+ T-cell and CD4+ Th1-dependent antitumor responses. Camu camu and ICI reestablished the efficacy of anti-PD1 therapy, reducing tumor size compared to ICI alone. This pilot study aims to identify the biological effect of camu camu with ipi/nivo in pts with mRCC.

Methods

This is an investigator-initiated, randomized, open-label, single-center trial comparing camu camu with ipi/nivo versus ipi/nivo alone in pts with treatment-naïve mRCC. Eligibility criteria include pts \geq 18 year old, PS 0-1, histological confirmation of clear-cell RCC with or without a sarcomatoid component, intermediate or poor risk per IMDC, no prior systemic treatment and measurable disease,. 30 pts will be enrolled and randomized in a 2:1 fashion, favoring the study arm. Pts will be treated with camu camu at 1500 mg PO daily, in with ipi/nivo at standard dosing. Pts will be followed monthly. Treatment will be continued until progression (RECIST v1.1) or toxicity. The primary endpoint is change in the abundance of *Ruminococcus* spp. in the stool from baseline to week 12 of therapy. We have 80% power to detect a 1 SD difference between the mean change detected in the two groups using a two-group T-test with a one-sided type I error of 0.05. Secondary endpoints include overall response rate, progression-free survival, safety, effect on gut microbiome diversity and function, comparison of the proportion of circulating cytokines and chemokines from baseline to week 12, and changes in the abundance of metabolic pathways and fungal microbiome profile. Response will be assessed by CT after the first 12 weeks of therapy and every 12 weeks, thereafter. The study is currently open to enrollment. [Clinical trial information: NCT06049576](#).

Keywords

Renal cancer, microbiome, integrative oncology

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Phase II Study of Bevacizumab, Erlotinib and Atezolizumab in Patients with Advanced Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) Associated or Sporadic Papillary Renal Cell Cancer

Dr Gabriela Bravo Montenero MD^{1,2}, Dr Ramaprasad Srinivasan MD, PhD^{1,2}

¹National Institutes of Health/National Cancer Institute. ²Urologic Oncology Branch

Background

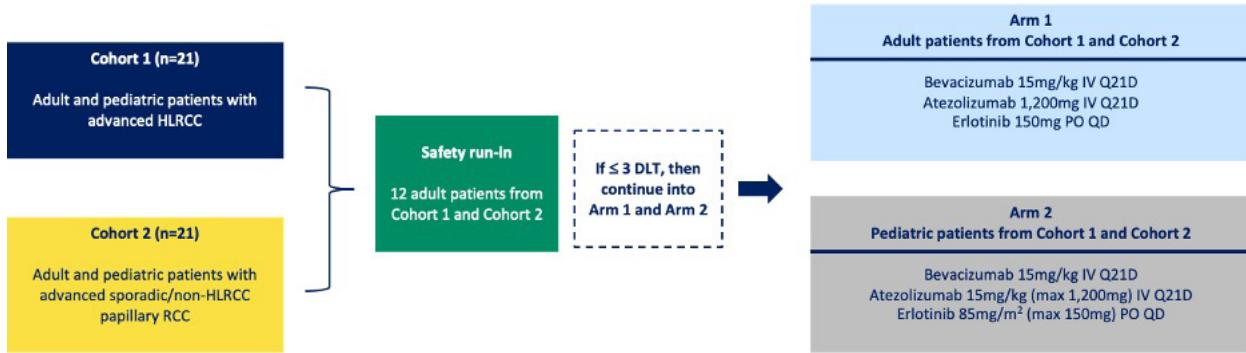
This is an open-label, multicenter, phase 2 study evaluating bevacizumab, erlotinib and atezolizumab in adult and pediatric patients with advanced HLRCC-associated RCC or sporadic papillary RCC.

NCT04981509

Trial Schema

Keywords

HLRCC, papillary, bevacizumab, erlotinib, atezolizumab



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Phase I/II Study of Palbociclib and Sasanlimab for the Treatment of Advanced Clear Cell Renal Cell Carcinoma (ccRCC) or Papillary Renal Cell Carcinoma (pRCC)

Dr Gabriela Bravo Montenegro MD^{1,2}, Dr Ramaprasad Srinivasan MD, PhD^{1,2}

¹National Institutes of Health/National Cancer Institute. ²Urologic Oncology Branch

Background

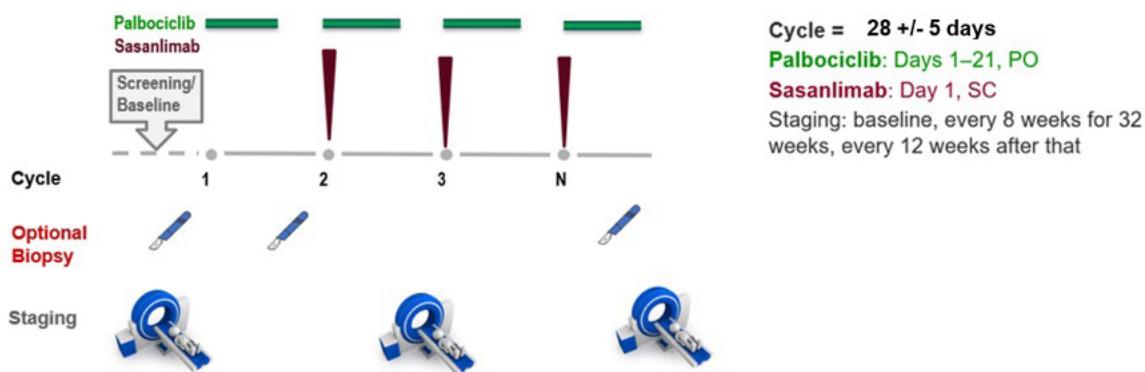
This is an open-label, phase I/II study evaluating Palbociclib and Sasanlimab in adult patients with advanced clear cell RCC or papillary RCC.

NCT05665361

Trial Schema

Keywords

clear cell, papillary, palbociclib, sasanlimab



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SWOG S2200 (PAPMET2): A phase II randomized trial of cabozantinib (cabo) with or without atezolizumab (atezo) in patients with advanced papillary renal cell carcinoma (PRCC).

Benjamin Maughan MD, PharmD

Huntsman Cancer Institute, University of Utah

Background

The role of immune therapy is not fully established in PRCC. The S1500 (PAPMET) clinical trial established single agent cabo as the standard of care for PRCC (PMID 33592176) with a median progression free survival (PFS) of 9.0 months compared to 5.6 months with sunitinib. Trials have shown activity of PD-(L)1 antagonists as monotherapy (PMID 33529058) or in combination with targeted therapy (PMID 34491815). In a single arm study of cabo/nivolumab the median PFS was 12.5 months (PMID 35298296). There are no prior randomized studies of immune therapy in PRCC. Single arm trials often overestimate the true effect size (PMID 31218346). Real world data suggests that combination therapy is not more effective than single-agent treatment (PMID 36610815). This underscores the importance of conducting a prospective, randomized trial in PRCC testing combination versus single-agent therapy. We hypothesize that the combination will have higher clinical activity than single agent cabo.

This is a prospective randomized phase II clinical trial conducted through the NCTN and led by SWOG. The primary endpoint is a comparison of PFS between cabo versus cabo/atezo. Secondary endpoints include objective response rate, overall survival and safety. Patients are treated with cabo 60mg/day versus cabo 60mg/day + atezo 1200 mg q3 weeks. 200 patients will be enrolled and randomized 1:1. Key inclusion criteria include a pathologic confirmation of PRCC; presence of metastasis; 0-1 prior systemic lines of therapy for metastatic disease; and measurable disease as defined by RECIST 1.1 criteria. Prior treatment with adjuvant pembrolizumab is allowed if completed more than 6 months before enrollment. Key exclusion criteria include clinically significant autoimmune disease; ongoing use of strong CYP3A4 inhibitors or strong CYP3A4 inducers. Planned correlates include stool microbiome testing and genomic/transcriptomic analysis from blood and baseline tissue assays. Updated enrollment and site activation status will also be presented.

Clinical trial information: NCT05411081. Research Sponsor: NIH/NCI grant awards: U10CA180888 and U10CA180819; Genentech, Inc (a member of the Roche Group), and Exelixis Inc.

Keywords

Cabozantinib atezolizumab papillary kidney cancer

DoD CDMRP/KCRP Awards (FY2022)

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PET Imaging of HIF-2a in Renal Cancer

Xianka Sun

University of Southwestern Medical Center at Dallas, Texas

Technical Abstract

Background: Despite extensive advances in targeted new therapies, 5-year survival rates for patients with metastatic renal cell carcinoma (RCC) are approximately 11% nationally. Compared to the existing targeted therapies, the development of inhibitors targeting hypoxia inducible factor 2 (HIF2) has several potential advantages: (i) HIF2 inhibitors target clear cell RCC (ccRCC) tumor cells offering a more direct and potentially more selective approach than angiogenesis inhibitors or even immunotherapy; (ii) HIF2-targeting drugs exert broader effects inhibiting not only angiogenesis but also tumor cell survival, stemness, and cell proliferation; and (iii) while HIF2 is required for tumor cells, it is largely dispensable for most physiological processes including angiogenesis in general. Thus, targeting HIF2 represents a promising therapeutic strategy given its activity and tolerability. Indeed, the HIF2alpha inhibitor belzutifan was evaluated in patients with familial kidney cancer, von Hippel-Lindau (VHL) syndrome, where it showed remarkable activity and tolerability leading to U.S. Food and Drug Administration (FDA) approval on August 13, 2021. However, we have shown that the efficacy of HIF2 inhibitors is limited by the development of resistance, which is a well-known phenomenon for targeted therapies and is often accompanied by mutation of the drug target. Importantly, the development of drug target mutations reveals an intrinsic dependency on the target for tumor growth, which is often exploited through second-generation inhibitors. Our data have shown that HIF2alpha is a core dependency in ccRCC. To query HIF2alpha expression in ccRCC, we have developed a theranostic method by radiolabeling PT2385, a first-generation HIF2alpha inhibitor, with fluorine-18 for positron emission tomography (PET). Indeed, to date we have obtained an FDA's investigational new drug (IND156933) approval for human dosimetry assessment and Phase I trial in patients with ccRCC (NCT04989959). Whereas our first seven patient scans showed net unidirectional radiotracer uptake rates in 2/3 of HIF2alpha positive subjects and exquisite specificity, its rapid metabolism (likely due to in vivo glucuronidation) has prompted us to evaluate a second-generation HIF2alpha inhibitor, PT2977, the FDA-approved belzutifan, which was developed, precisely, to escape glucuronidation. PT2977

differs from PT2385 in that it consists of a vicinal difluoro group instead of a germinal one in PT2385. Such a structural modification improved in vivo pharmacokinetics by blocking glucuronidation, decreased lipophilicity, and enhanced potency.

Objective/Hypothesis: With the goal to improve our previously developed theranostic method using [18F]PT2385, we propose to develop a second-generation radiotracer, [18F]PT2977, for PET imaging of HIF2alpha in patients with ccRCC. The central hypothesis is that the chemical modification presented in PT2977 would significantly suppress the glucuronidation rate of [18F]PT2977 in humans as has been reported. A higher plasma level of [18F]PT2977 would facilitate its binding to the intracellular HIF2alpha. Our rationale is based on the chemically identical structure of [18F]PT2977 to PT2977.

Specific Aims: (1) To design and develop synthetic routes to the precursor of [18F]PT2977 and accomplish automated radiosynthesis of [18F]PT2977; (2) To validate PET imaging of HIF2alpha with [18F]PT2977 in RCC tumorgrafts (TGs); and (3) To complete the required pre-IND experiments for an FDA IND application and set everything in place to launch a clinical trial.

Study Design: We have proposed a practical synthetic route to [18F]PT2977 based on our established experience in developing [18F]PT2385. To evaluate the proposed PET imaging of HIF2alpha, we will perform a set of experiments with [18F]PT2977 in mouse models using 10 RCC tumorgraft lines expressing high and low HIF2alpha levels to validate the anticipated correlation between PET signal readout and expression of HIF2alpha. In addition, the in vivo stability of [18F]PT2977 will be assessed by our established LC/MS procedures or through the Metabolism Core services. Radiation dosimetry will be assessed by using biodistribution along with the clearance data. Later, we will follow the same procedures as we did with [18F]PT2385 to validate the production reliability and reproducibility for an FDA IND application towards the translational studies of [18F]PT2977.

Impact: The development of a highly specific radiotracer that informs on HIF2alpha expression in tumors would have broad application not only to sporadic renal cancer, but also to identify other tumor types that may similarly be HIF2-dependent. If successful, the theranostic method developed in this project may become transformative not only in oncology, but also in other diseases such as cardiac ischemia or strokes.

FY22 KCRP Focus Area Addressed: Identify and develop new strategies for screening, early-stage detection, and accurate diagnosis and prognosis prediction of kidney cancers, with examples including biomarkers and imaging

Principal Investigator: SUN, XIANKAI

Institution Receiving Award: UNIVERSITY OF TEXAS SOUTHWESTERN

Program: KCRP

Proposal Number: KC220060

Award Number: HT9425-23-1-0903

Funding Mechanism: Translational Research Partnership Award

Award Amount: \$834,798

Tumor cells with mutated or down-regulated SETD2 were found to be therapeutic targets for viral mimicry activation by treatments with an HMA or WEE1 inhibitor including our preliminary data for HMA.

Area of Emphasis: (1) Chromatin and Gene Regulation; (2) Targeted Therapies

Hypothesis: Test whether kidney cancer cells with impaired H3K36 methylation caused by mutated or down-regulated SETD2 can be effectively targeted by HMAs and WEE1 inhibitors due to defects in DNA remethylation and inhibition by dNTP starvation-induced apoptosis as potential mechanisms for therapeutic targeting.

Specific Aims: We will test our hypothesis by pursuing two specific aims: (1) Test whether cancer cells with impaired H3K36 methylation can be effectively targeted by HMAs and determine how sustained down-regulation of oncogenes and up-regulation of endogenous retroviruses (ERVs) to stimulate immune responses due to DNA demethylation are potential mechanisms for HMA or WEE1 inhibitor sensitivity; (2) Evaluate the antitumor effects and epigenetic changes caused by treatment of HMAs or WEE1 inhibitor +/- immune checkpoint inhibitor (PD-L1) in immunocompetent mouse model with or without impaired H3K36 methylation (Setd2) using preclinical in vivo methodologies.

Study Design: For Aim 1, determine the antitumor effects and global epigenetic changes after HMA treatment of kidney cancer cell lines with the SETD2 aberrancies and uncover the molecular mechanism of HMA or WEE1 inhibitor action. We will manipulate SETD2 so as to impair H3K36 methylation and then measure phenotypic, epigenetic changes (DNA remethylation) in kidney cancer cells as a function of HMA or WEE1 inhibitor treatment using cell culture assays to measure cytotoxicity, cell growth inhibition and/or apoptosis, stimulation of immune response, re-sensitization of drug-resistant cells, the subsequent chromatin structure alterations, and ultimately achieve the desired therapeutic effect. For Aim 2, evaluate the effect of altered Setd2 expression in kidney cells to HMA or WEE1 inhibitor treatment and/or immune therapy in orthotopic allograft models. These experiments will be performed in immune competent mice with Setd2-knockout kidney cancer cells in syngeneic orthotopic kidney cancer allografts. We will comprehensively characterize the change in oncogene/tumor suppressor expression and immune infiltrate for its response to HMA alone and in combination with PD-L1 inhibition. The studies will provide biomarkers for therapeutic efficacy and preclinical parameters for tumor response in support of translating this approach for ccRCC patients.

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SETD2 Deficiency is a Therapeutic Target to Upregulate Viral Mimicry in Treating Aggressive Clear Cell Renal Cell Carcinoma Patients

Gangning Liang

University of Southern California

Technical Abstract

Background: Decreased or depleted histone H3K36 trimethylation (H3K36me3, respectively) has been shown to play an important role in diverse forms of cancer due to somatic mutations in or down-regulation of regulatory proteins such as SETD2 (H3K36me3 methylase), most notably in clear cell renal cell carcinoma (ccRCC). Recent studies have demonstrated that H3K36me3 is associated with actively transcribed genes as well as DNA double-strand break (DSB) repair, de novo DNA methylation and DNA remethylation. Altered chromatin remodelers related to impaired H3K36me3 occupancy are linked to cancer aggressiveness in renal cancer. The relative 5-year survival rate of localized ccRCC is 91%, however, survival rates drop to 11% once the ccRCC has metastasized (advanced ccRCC). Thus, there is an urgent need to develop novel treatments to target H3K36me3-compromised cancer cells, as these alterations represent attractive targets for diagnostic and treatment purposes. It is well recognized that aberrant DNA methylation (5mC) is a key feature in human cancers. Most important is that 5mC is dynamic and pharmacologically reversible, which makes it an attractive therapeutic target. Interestingly, WEE1 was found to be inversely proportional to the expression of SETD2. As such, SETD2 deficient cells were found to be vulnerable to WEE1 inhibition by dNTP starvation-induced apoptosis.

Impact: The success of this project will immediately lead to clinical trials, especially for patients with aggressive ccRCC, those with mutations or down-regulated expression of SETD2 or impaired H3K36 methylation.

Innovation: This study will provide new opportunities for clinical therapeutic intervention to increase the efficacy of precision medicine approaches. We expect that this work will serve as a guiding force for future clinical applications, since currently there is no efficient therapy that shows any impact on survival times for patients with aggressive ccRCC.

Principal Investigator: LIANG, GANGNING

Institution Receiving Award: UNIVERSITY OF SOUTHERN CALIFORNIA

Program: KCRP

Proposal Number: KC220052

Award Number: HT9425-23-1-1069

Funding Mechanism: Idea Development Award - Established Investigator

Partnering Awards:

Award Amount: \$915,936

clinical trial in which 10 patients with ICI-refractory ccRCC will be treated with CIML-NK cell therapy. In this translational proposal, we seek to characterize the activity, immunogenicity, and host response of patients with ccRCC treated with CIML-NK cellular therapy and to better understand determinants of clinical response and resistance. We hypothesize that (1) changes in NK cell phenotype in peripheral blood and the surrounding tumor microenvironment after CIML-NK cell therapy will identify cell populations associated with response, and (2) CIML-NK cell therapy causes secondary changes in immune cell populations in the host and tumor microenvironment. Understanding the mechanisms of response and resistance to CIML-NK therapy in ccRCC will enable us to identify patients most likely to respond to CIML-NK cellular therapy, and identify novel therapies that can reverse resistance to therapy.

Specific Aims: (1) Deep phenotypic and transcriptomic characterization of naive NK cells and adoptively transferred CIML-NK cells in advanced ccRCC. (2) Determine predictive biomarkers for CIML-NK therapy in ccRCC.

Study Design: In Aim 1, we will characterize the persistence and longevity CIML-NK cells and interrogate NK cell phenotype, function, and antitumor activity. This aim will include analysis of pre- and on-treatment blood and tumor biopsy samples from patients enrolled on the clinical trial. Differential gene expression and clustering analysis between (a) naive NK cells, (b) CIML NK cells prior to infusion, and (c) persistent adoptively transferred NK cells will be conducted to better understand the underlying biologic pathways that underlie response to CIML-NK cell-based therapy in RCC. Previously established CyTOF assays will be used to interrogate (a) persistence and longevity of CIML-NK cells; (b) the differences in memory-like NK cell phenotypes among responders vs non-responders; and (c) effect on TME. We will interrogate the functional activity of NK cells collected before and after adoptive CIML-NK cell therapy against ccRCC cell lines. In Aim 2, we will use flow cytometry to identify changes in both tumor biopsies and peripheral NK cell populations pre- and post-therapy. We will use circulating tumor DNA (ctDNA) analysis to assess whether changes in peripheral NK cell populations correlate with ctDNA burden, and with clinical outcomes including response and survival. We will assess whether peripheral cytokine production, NK cell expansion and persistence, and decreased suppressive Tregs and MDSCs are associated with successful CIML-NK therapy. Non-responders will be analyzed to identify potential mechanisms of resistance.

Impact: This project will study how memory-like NK cells function and persist in patients with ccRCC, which will lead to better understanding of why NK immune effector cell dysfunction occurs, and how this may be reversed using this new class of therapeutics with the goal of improving patient outcomes. This proposal addresses the FY22 Kidney

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Mechanisms and Predictors of Adoptive Cytokine-Induced Memorylike NK Cell Therapy in Renal Cell Carcinoma

Rizwan Romee

Dana-Farber Cancer Institute

Technical Abstract

Background: Despite recent advances, metastatic ccRCC remains fatal for most patients and new treatment approaches are needed. NK cells play a role in the immunologic response to ccRCC but the therapeutic use of NK cells is limited by their short half life. We have previously demonstrated that in vitro cytokine (IL-12, IL-15, and IL-18) activation of patient derived NK cells causes differentiation into cytokine induced memory-like NK cells (CIML-NK), which have prolonged survival, enhanced proliferation, and enhanced antibody-dependent cellular cytotoxicity. This is a promising new treatment strategy for ccRCC but the biology of CIML-NK cells in RCC patients has not previously been described.

Objective/Hypothesis: Recent and ongoing trials in both AML and head and neck squamous cell carcinoma show that CIML-NK therapy can lead to robust peripheral CIML-NK cell expansion and tumor response. We are conducting a phase 1

Cancer Research Program Focus Areas by exploring a novel therapeutic strategy for ccRCC, and enhancing understanding of the biology of NK cells in ccRCC

Principal Investigator: ROMEE, RIZWAN

Institution Receiving Award: DANA-FARBER CANCER INSTITUTE

Program: KCRP

Proposal Number: KC220072P1

Award Number: HT9425-23-1-0478

Funding Mechanism: Translational Research Partnership Award - Correlative Studies Option

Partnering Awards: KC220072

Award Amount: \$576,031

imaged kidney tumors (benign vs. malignant) and (ii) molecular detection of oncocytoma to avoid surgical or ablative procedures, which represent over treatment of these kidney tumors, to preserve future kidney function.

Specific Aims: Aim 1: Develop an ultrasensitive and multiplexed assay for simultaneous quantification of five urinary protein markers using plasmonic-fluors as ultrabright fluorescent nanolabels. Aim 2: Determine the optimal cut-off values of the multiplexed biomarkers to achieve the highest sensitivity and specificity in differentiating benign vs. malignant kidney tumors using a training cohort with known pathology. Aim 3: Corroborate the accuracy of the multiplexed biomarker assay to noninvasively molecularly classify imaged kidney tumors (benign vs. malignant) in a blinded fashion compared to eventual biopsy results using a validation cohort.

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Multiplexed Quantification of Urinary Biomarkers for Noninvasive Classification of Imaged Kidney Tumors

Srikanth Singamaneni, Jeremiah Morrissey

Washington University in St Louis

Technical Abstract

Background and Significance: Currently, diagnosis of imaged small renal masses in military or civilian populations requires an invasive biopsy procedure. However, there are hurdles to the widespread adoption of a biopsy-based treatment algorithm. Factors such as suitability for surgery may preclude biopsy and that 70% to 80% of patients with small renal masses have malignant disease anyway and require some form of further treatment (ablation or surgery) after their biopsy. Biopsy of these masses increases health care costs and exposes these patients to some degree of risk with little or no benefit. The most nephron-sparing approach to managing benign small renal masses is to diagnose the lesion as benign and avoid unnecessary treatment. Identifying suitable biomarkers of kidney cancer and developing efficient technology to rapidly and noninvasively detect these biomarkers are important to disease diagnosis and documenting response to therapy. Therefore, a critical unmet need is sensitive and specific biomarkers and methodologies to quantify them for possible early detection of renal cell carcinoma (RCC) and the differential diagnosis of imaged kidney masses as benign or malignant, informed enabling specific intervention.

Objective: The objective of the proposed effort is to develop a highly sensitive and specific biomarker-based urinalysis method for (i) noninvasive and accurate classification of

Study Design: We will design and develop a multiplexed assay to simultaneously quantify five urinary proteins: aquaporin-1 (AQP1), perilipin-2 (PLIN2), kidney injury molecule-1 (KIM-1), vimentin 3 (Vim3), and MAPKp38 (Mxi-2). Collectively, the quantification of these five biomarkers enables (i) noninvasive and accurate classification of imaged kidney tumors (benign vs. malignant) and (ii) molecular detection of oncocytoma to avoid surgical or ablative procedures. Specifically, we will harness plasmonic-fluor, an ultrabright fluorescent nanoconstruct recently introduced by our lab, as a nanolabel for achieving ultrasensitive detection and quantification of the target biomarkers. We will optimize various bioanalytical parameters (e.g., sensitivity, specificity, limit-of-detection, dynamic range, resolution of molecular concentration, and spike recovery from urine) of the multiplexed assay. We will assay urine samples obtained from patients with imaged renal mass and known pathology (i.e., training cohort) for the five protein biomarkers. We will perform a univariate receiver operating characteristic (ROC) analysis for each of the protein biomarkers and multivariate ROC analysis for the combination of the protein biomarkers. We expect the five biomarker panel to provide higher sensitivity and specificity than any individual or pair of biomarkers. Finally, we will validate the accuracy (positive predictive value (PPV), negative predictive value (NPV)) of the multiplexed assay in differentiating benign vs. malignant tumors using a different cohort of samples than that used in the training phase. In addition to differentiating benign vs. malignant tumors, the panel of biomarkers is expected to accurately identify patients with oncocytoma and determine the biomarker signature of benign angiomyolipomas.

Innovation: We propose to harness our recently introduced ultrabright nanolabel, plasmonic-fluor, for realizing an ultrasensitive multiplexed assay for the simultaneous detection and quantification of five urinary protein biomarkers. The novel multi-marker assay can noninvasively and accurately differentiate benign vs. malignant tumors and detect oncocytoma in patients with imaged renal mass. This

is in stark contrast with the existing methods that rely on an invasive biopsy procedure and histology.

Impact: By using a noninvasive biomarker test as a first-line diagnostic tool to molecularly diagnose an imaged renal mass, we may avoid overtreatment of small renal masses and overextending health care budgets. Avoiding resection or ablation of benign small renal masses could significantly decrease patient morbidity, loss of productivity, and risk of developing or exacerbating chronic kidney disease from nephron loss, all of benefit to the military and civilian populations.

Principal Investigator: SINGAMANENI, SRIKANTH
Institution Receiving Award: WASHINGTON UNIVERSITY IN ST LOUIS
Program: KCRP
Proposal Number: KC220085
Award Number: HT9425-23-1-0996
Funding Mechanism: Idea Development Award - Established Investigator
Partnering Awards:
Award Amount: \$1,049,625

and integrating this information in an optimal way is a tedious, bias-sensitive, experience-driven, complex task, leading to a wide range of clinical practice. Artificial intelligence (AI) has demonstrated that it can be a reliable assistant to clinical practice with the ability to extract appropriate information from multimodal data to predict patient outcomes. AI thus has the potential to change clinical practice by accurately and reliably predicting tumor outcomes, postoperative kidney function, best surgical approach, and perioperative complications. This will help increase the diagnostic certainty and objectify patient counseling.

Hypothesis: We hypothesize that fully automated AI point-of-care tools that incorporate radiology, pathology, clinical, and genomic data through a multimodal fusion approach will more accurately, faster, and with less bias stratify patient oncologic risk and predict postoperative kidney function and survival after nephrectomy, relative to current standard-of-care clinical stratification and prediction.

Specific Aims: (1) Develop and determine the effectiveness of multimodal AI risk stratification models by leveraging the Multimodal Kidney Cancer (MMKC) dataset and comparing them to existing clinical prediction models of patient oncologic risk. (2) Develop and determine the effectiveness of the AI kidney function prediction models by leveraging the MMKC cohort and comparing them to existing clinical prediction models of postoperative kidney function.

Study Design: We will utilize the MMKC cohort of circa 1,700 patients who underwent PN or RN from 2010-2022 at the University of Minnesota Medical Center or Cleveland Clinic Foundation. All patients have pre-operative CT with clinical information, pathology images, and genomic data (in a subset of patients). We have developed technologies for analyzing and fusing the various modalities and a clinical equation to predict kidney function in a population of VA patients undergoing treatment for suspected RCC. These clinical models have provided a baseline for an AI multimodal model that we believe will be more serviceable in clinical practice. We will compare the clinical to the AI models independently in their predictive abilities for oncologic risk and perioperative outcomes, testing all models for (i) accuracy through AUC and p-values, (ii) speed by timing the average inference time, and (iii) robustness and bias-free prediction by performing the Brier score and calibration tests. We will compare different models' AUC through the DeLong test.

Impact: Our work will address several fiscal year 2022 Kidney Cancer Research Program Focus Areas. The addition of pathology and genomic variables through explainable AI has the potential to deepen our understanding of disease biology, pathogenesis, and progression and lead to more informed conclusions and prevention strategies. The goal is to develop more accurate risk assessment strategies and to select more appropriate treatments that improve the quality of life and

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Multimodal AI-Based Renal Cancer Patient Care

Christopher Weight

Cleveland Clinic Foundation

Technical Abstract

Background: Small renal masses (SRM) are tumors less than 4 cm and account for almost half of newly diagnosed renal masses. Recommendations for patient treatment of SRM depend on estimates of the (1) oncologic risk of the tumor and (2) post-treatment kidney function. However, there are few non-invasive or accurate tools to help clinicians make these estimates, leading to significant variability in care. In recent studies of surgical management of renal masses, benign pathology was found in 30% of SRM, pointing to overdiagnosis. An additional 30% to 50% of SRM are found to be indolent renal cell carcinomas (RCC) unlikely to develop metastases, resulting in potential overtreatment in tens of thousands of patients with SRM. This has an economic impact on the health care system; it was shown that as of 2014, the estimated national inpatient cost of management for benign renal tumors was \$153 million dollars (\$55,573/individual). At the same time, identifying the most effective patient care requires the analysis of multiple individual factors, and both collecting

survivorship of patients with SRM by avoiding overtreatment and the long-term sequelae of kidney function loss associated with RCC treatment, as well as a significant lowering of medical costs. Moreover, using AI methods reduces evaluation time and removes the intra and inter-observer assessment bias, reducing health disparities. By blending clinical and AI expertise, we widen the field's reach.

Principal Investigator: WEIGHT, CHRISTOPHER
Institution Receiving Award: CLEVELAND CLINIC FOUNDATION
Program: KCRP
Proposal Number: KC220086
Award Number: HT9425-23-1-0918
Funding Mechanism: Translational Research Partnership Award
Partnering Awards: KC220086P1
Award Amount: \$893,768

mechanisms underlying the hypersensitivity to ferroptosis induction in ChRCC, to identify genes and drugs that synergize with ferroptosis induction in ChRCC, and to test these ferroptosis-inducing therapies in preclinical mouse models of ChRCC.

Study Design: Aim 1. To determine the metabolic mechanisms underlying hypersensitivity to ferroptosis induction in ChRCC. Hypothesis: xCT-dependent mechanisms impact glutathione homeostasis, ROS levels and membrane lipid composition in ChRCC. Our approaches will include: (1) isotope tracing for serine, cystine, and glutamine *in vivo* as well as metabolomic and lipidomic analyses in two established cellular models of ChRCC (UOK276 and ChromoA cells) versus controls; and (2) lipidomics of ChRCC patient specimens to determine the saturated vs. unsaturated fatty acid content of membrane lipids. ChRCC and matched normal kidney tissue specimens are available through our institutional tissue repository.

Aim 2. To identify genetic perturbations and compounds that synergize with ferroptosis induction in ChRCC. Hypothesis: Genetic perturbations and therapeutic combinations including ferroptosis inducers will provide an effective treatment for ChRCC. Our approaches will include: (1) a CRISPR knockout screen to identify genes involved in the xCT-dependent viability of ChRCC cells; (2) a high-throughput drug screen to identify compounds that synergize with the ferroptosis inducer IKE; (3) integration of results from the CRISPR and drug screens to identify gene networks underlying responses; and (4) primary cultures from ChRCC for drug validation.

Aim 3. To test ferroptosis-inducing therapies in preclinical models of ChRCC. Hypothesis: Drug combinations including the xCT inhibitor IKE will effectively induce cell death in clinically relevant models of ChRCC. Our *in vivo* approaches will include: (1) combined treatment with IKE and selected compounds from the integration of drug and CRISPR screens (Aim 2); and (2) CITE-seq (Cellular Indexing of Transcriptomes and Epitopes by Sequencing) of tumors generated in humanized mice treated with the ferroptosis inducer IKE, to determine the role of the tumor immune microenvironment in the therapeutic response at a single-cell level.

Impact: Currently, there are no proven targeted therapies for ChRCC. We have identified a fundamental metabolic vulnerability of ChRCC: these tumors are sensitive to inhibition of the xCT cystine/glutamate antiporter, resulting in ferroptotic cell death. Our CRISPR screen in Aim 2 will allow us to identify genes that participate in the xCT-dependent regulation of ChRCC cell viability. Our drug screen in Aim 2 will begin with agents that are U.S. Food and Drug Administration-approved or in clinical development, to enhance the opportunities for rapid translation to clinical trials. We expect this project to have high clinical impact by identifying critical genes and effective therapeutic combinations to induce

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Identification of Novel Therapeutic Strategies for Chromophobe RCC

Elizabeth P Henske

Brigham and Women's Hospital, Inc

Technical Abstract

Background: Chromophobe renal cell carcinoma (ChRCC) is the third most frequent type of RCC, representing 5% of all kidney cancers. We and others have found strikingly increased levels (50-fold higher) of GSH and GSSG in ChRCC compared with matched normal kidney. Using preclinical models, we have identified a distinct, targetable metabolic vulnerability of ChRCC: a dependency on cystine uptake via the cystine/glutamate exchange transporter system XC-(xCT) for glutathione synthesis and viability (*in vitro* and *in vivo*). Enhanced activity of xCT contributes to resistance to ferroptosis, a distinct cell death mechanism characterized by accumulation of membrane lipid peroxidation, which is impacted by levels of glutathione, reactive oxygen species (ROS), and polyunsaturated fatty acid chains. Ferroptosis has been implicated in the pathogenesis of multiple disorders, including neurodegenerative diseases, kidney injury, and cancer. There are also emerging links between ferroptosis and the tumor microenvironment.

Hypothesis and Objectives: Our central hypothesis is that ChRCC are hypersensitive to induction of ferroptotic cell death. Our objectives are to elucidate the metabolic

ferroptotic cell death in ChRCC, and high scientific impact by investigating glutathione and lipid homeostasis in ChRCC. We note that this project has a high degree of innovation, including state-of-the-art lipidomics and in vivo metabolic tracing, a high-throughput drug combination screen focused on induction of ferroptosis, and the innovative hypothesis that ferroptosis induction can be used to selectively target ChRCC.

Principal Investigator: HENSKE, ELIZABETH P

Institution Receiving Award: BRIGHAM AND WOMEN'S HOSPITAL, INC.

Program: KCRP

Proposal Number: KC220094

Award Number: HT9425-23-1-0974

Funding Mechanism: Translational Research Partnership Award

Partnering Awards: KC220094P1

Award Amount: \$653,343

druggable RCC dependency in this analysis was the BCL2L1 gene, which encodes the anti-apoptotic BCL-XL protein. In rigorous studies, using cell-based and in vivo models, we found that RCCs were specifically dependent on BCL-XL, but not its closely related paralogs BCL2 and MCL1. Moreover, acquisition of mesenchymal features, which were evident in approximately 30% -- typically aggressive -- human renal tumors promoted BCL-XL dependence. Pharmacological agents that block BCL-XL with high specificity and potency (e.g., BH3 mimetics and PROTACs) are presently in clinical development. Pursuing BCL-XL, therefore, decreases the risk associated with developing drugs against uncharacterized targets and speeds up clinical transition. Finally, BH3 mimetics are experiencing a clinical resurgence because next-generation molecules not only have lower toxicities but can also augment other anti-cancer therapies, thus improving clinical response in several cancers. In summary, our findings are not only novel, but also timely and actionable, making these studies significant.

Focus Area: Develop novel therapeutic strategies for treatment.

Hypothesis and Objectives: We hypothesize that BCL-XL dependency is a hallmark of RCC tumors that present with more aggressive, dedifferentiated, clinical features. Through a complementary set of mechanistic studies (e.g., apoptosis characterization, kill curves, and CRISPR/Cas9 screens), and using physiologically relevant models (e.g., RCC cell lines, in vivo models, and patient-derived specimens), our objectives are to establish the determinants of BCL-XL dependency in RCC and thereby provide justification for the use of apoptotic modulators as a novel class of therapeutics in the management of kidney cancer.

Specific Aims: (1) Address the hypothesis that interrogating BCL-XL dependency in human tumor models can identify biomarkers and mechanistic drivers of BCL-XL dependence in RCCs. (2) Address the hypothesis that BCL-XL blockade can augment therapeutic response in kidney cancer. (3) Address the hypothesis that unbiased CRISPR/Cas9 screens can identify candidate "targets" whose loss can promote BCL-XL dependence.

Study Design: Aim 1 will annotate human RCC tumors (n=24, age- and sex-matched, all disease subtypes) based on their BCL-XL dependence (e.g., using shRNAs and BCLXL inhibitors) (1A), characterize their apoptotic response (e.g., BH3 profiling, etc.) (1B), and ultimately use transcriptomic signatures to identify biomarkers and functional drivers of BCL-XL dependence (1C). Using a panel of patient-derived organoids, Aim 2 will address how BCL-XL blockade, using BCL-XL inhibitors and PROTACs, promotes sensitivity to existing therapeutic strategies for advanced (often treatment refractory) kidney tumors, including mTOR inhibition (2A) and immunotherapy (2B). Using cell viability and apoptotic

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Interrogating the Therapeutic Relevance of Targeting the Antiapoptotic BCL-XL Protein in Kidney Cancer

Abhishek Chakraborty

Cleveland Clinic Foundation

Technical Abstract

Background and Significance: Kidney cancer [or Renal Cell Carcinoma (RCC)] is among the most common forms of human cancer and accounts for approximately 14,000 deaths annually in the United States. Moreover, exposure to chemical and environmental toxins at military bases, and lifestyle risks, including obesity and smoking, elevate the risk of developing kidney cancer in defense personnel. Major treatment milestones, including combinations of checkpoint inhibitors (CKIs) with other CKIs or with Tyrosine Kinase Inhibitors (TKIs) have improved disease outcomes. However, several challenges remain. Despite robust initial responses, durable remissions are seen only in a small subset of patients. Additionally, undesirable adverse effects remain a concern. Altogether, advanced RCC remains incurable, and identifying novel drug targets represents an unmet clinical need. To address this need, using publicly available genetic dependency maps (e.g., DepMap), we sought to identify genes whose loss led to fitness defects preferentially in kidneylineage cancer cells and shortlisted approximately 20 genes. The topmost

response (e.g. BH3 profiling, membrane depolarization, etc.) upon BCL-XL blockade as a readout, Aim 3 will interrogate the importance of five candidate genes identified from our preliminary CRISPR/Cas9 screens: metabolic regulators (IDO1 and IDO2) and chromatin modifiers (SMARCAD1, KANSL1, and NAPIL2) in conferring BCLXL dependence in ccRCC.

Innovation and Impact: Our proposal is innovative because it (a) repurposes an existing drug target for use in a novel indication, thereby potentially expediting translation; (b) demonstrates how cell state impacts BCL-XL dependency; (c) finds novel modulators of BCL-XL dependence; and, (d) addresses how BCL-XL blockade could complement existing clinical practice. Unlike earlier studies, which focused on BCL-2, we find that ccRCCs are instead hyper-dependent on BCL-XL. Altogether, given its clinical significance and, perhaps more importantly, its direct path to clinical translation using molecules that are already in clinical development, our proposal has the potential to make an important impact to clinical care in RCC patients.

Principal Investigator: CHAKRABORTY, ABHISHEK

Institution Receiving Award: CLEVELAND CLINIC

FOUNDATION

Program: KCRP

Proposal Number: KC220095

Award Number: HT9425-23-1-0771

Funding Mechanism: Idea Development Award - Established Investigator

Partnering Awards:

Award Amount: \$1,023,225

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The Effect of Clonal Hematopoiesis on Cardiovascular-Related Outcomes in Patients Diagnosed with Kidney Cancer

Maxine Sun, Valisha Shah

Dana-Farber Cancer Institute

Technical Abstract

Career Development and Sustainment Plan: The proposed research project is unique and amongst one of the few to assess an increasingly important topic in oncology, which is that of survivorship care. As reported for many cancers, patients diagnosed with kidney cancer are at risk of worse cardiovascular health outcomes than the general population. This represents an understudied research area that merits all the attention it needs as the treatment paradigm of the disease has changed drastically over the last 20 years. The current research would allow the PI to have access to the resources and environment that is required to increase awareness to this issue, and simultaneously perform high-level science that can help patients. The funding that will be dedicated to exploring the mechanistics and inflammatory biology of what was observed in the Principal Investigator's (PI) preliminary analyses. This will give the PI the opportunity to be one of the first independent kidney cancer researchers to properly address this problematic, thereby putting her front and center of the survivorship space.

Background: Kidney cancer figures amongst the top 10 cancers with the highest percentage of cardiovascular disease (CVD)-related deaths. In this setting, the use of cardiac biomarkers for the purpose of assessing individual sub-clinical CVD risk at cancer diagnosis is highly anticipated. Lately, clonal hematopoiesis (CH) has recently been described as a novel driver for cancer and CVD. The PI's preliminary analyses suggest that individuals specifically diagnosed with kidney cancer with CH as captured by mosaic chromosomal alterations (mCAs) had a higher risk of CVD- and coronary artery disease (CAD)-related death compared to mCA non-carriers within the UK Biobank ($n=1,910$).

Hypothesis and Objective: To better understand the underlying mechanisms of CH and kidney cancer, and its relationship with CVD, we propose to study the genetic correlations of the disease across previously identified hematopoietic phenotypes, to measure the effect of CH on CVD-related outcomes following a kidney cancer diagnosis, and to characterize the immunologic profiles of CH carriers. In the genetic analyses, we expect to find that certain germline variants affecting kidney cancer risk may also increase the risk of clonal expansions and thereby influencing CVD.

Subsequently, we postulate that for carriers of CH diagnosed with kidney cancer have an increased risk of CVD outcomes compared to non-CH carriers diagnosed with kidney cancer. In the transcriptomic analyses, we speculate that patients carrying CHIP mutations will have increased expression of inflammatory genes compared to patients lacking those mutations.

Specific Aims:

Aim 1. To examine genetic correlations between kidney cancer and hematopoietic-related phenotypes. We will estimate the genetic correlations between renal cell carcinoma and hematopoietic phenotypes using GWAS summary statistics. A linkage disequilibrium score reference panel based on European ancestry individuals combined from the 1000 Genomes Phase 3 and UK10K cohorts, totaling 17,478,437 available variants, will be used.

Aim 2. To assess the effect of CH on cardiovascular outcomes in patients diagnosed with kidney cancer. We will evaluate the risk of CAD in CH+ patients with kidney cancer, defined as CHIP (measured through whole-exome sequencing) or mCA (measured via phasing algorithm tool called Eagle2) and compare them to CH- patients with kidney cancer within the Dana-Farber/Harvard Cancer Center (DF/HCC) biobank. The combined landscape of both CHIP and mCA on CVD outcomes will also be compared to those with CHIP or mCA alone.

Aim 3. To compare inflammatory gene expressions from carriers of common CHIP mutations diagnosed with kidney cancer compared to CHIP non-carriers via bulk RNAseq. We will assess the transcriptomic profiles of peripheral blood mononuclear cells in patients with kidney cancer who harbor DNMT3A and TET2 mutations vs. those without any CHIP mutations via bulk RNAseq. Subset analyses will consider patients who also experienced a cardiovascular event.

Impact: The current proposal contributes toward impacting the quality of life and long-term survivorship care of patients with kidney cancer. Furthermore, the current work, if funded, will allow the PI to situate herself at the center of collaborative, multi-disciplinary kidney cancer research effectively involving applied clinicians and/or clinical scientists in a growing movement combining medical oncology and cardiology focused on survivorship.

Principal Investigator: SUN, MAXINE

Institution Receiving Award: DANA-FARBER CANCER INSTITUTE

Program: KCRP

Proposal Number: KC220227

Award Number: HT9425-23-1-1002

Funding Mechanism: Academy of Kidney Cancer Investigators - Early Career Scholar Award

Partnering Awards:

Award Amount: \$1,290,499

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Purinergic Receptor Antagonist Therapeutics in Treating Metastatic Renal Cancer

Jean X Jiang

University of Health Science Center at San Antonio, Texas

Technical Abstract

Background: Kidney cancer (renal cell carcinoma, RCC) is the 8th most frequently diagnosed malignancy. Clear cell RCC (ccRCC) is the most common subtype (80%) and advanced ccRCC has a poor prognosis. The biggest challenges facing renal cancer patients are late diagnosis, cancer recurrence, metastasis, and a lack of effective therapies. Thus, there is an urgent need to develop new and effective therapies with improved efficacies and fewer side effects. The adenosine A2B receptor (A2BR) is overexpressed in ccRCC and an increased expression level is correlated with poor prognosis, supporting the hypothesis that A2BR is a potential drug target for ccRCC. More importantly, we have developed novel compounds that target A2BR and shown that several of these compounds significantly inhibit triple negative breast cancer growth and metastasis. Our preliminary data also shows that four compounds inhibit ccRCC cell proliferation and migration. In this application, we propose to assess the efficacies, using both in vitro models and in vivo mouse models, and drug properties to identify a drug candidate that will represent a first-in-class therapy for kidney cancer, which remains an unmet medical need.

Focus Areas: This proposal will address one of the specific areas listed in the FY22 KCRP Focus Areas: "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." We have generated novel compounds with demonstrated effects on ccRCC growth and migration. We propose to conduct comprehensive studies to identify an IND drug candidate with strong efficacy, favorable drug characteristics, and minimal toxicity.

Hypothesis: The adenosine A2B receptor is a promising new therapeutic target for treating primary and metastatic ccRCC and inhibiting this receptor with antagonists has a great potential for an effective treatment.

Objectives: We propose to identify a drug candidate and evaluate its suitability as a drug to inhibit ccRCC growth and metastasis and increase antitumor immunity by increasing antigen presenting cells and anti-tumor infiltrating T lymphocytes. We will advance the development of this unique therapeutic with three Specific Aims:

Specific Aims: (1) Characterize the efficacy of the four lead compounds to reduce ccRCC cell growth and migration in vitro via alteration of A2B purinergic pathways. (2) Determine the efficacy and antitumor immunity effects of the four lead compounds on ccRCC in in vivo mouse models. (3) Select a drug candidate and determine its PK properties and efficacy in a PDX model.

Study Design: (1) We will study the effects of the four novel lead compounds on cell viability, cell proliferation, and anchorage-independent growth, cell migration, and invasion on various subtypes of ccRCC in vitro. (2) We will assess the maximum tolerated dose (MTD) and dose-dependent in vivo efficacy (PD) using a human xenograft ccRCC mouse model and an immunocompetent murine syngeneic model, and determine changes in T cell subtypes (Tc, Th and Treg in tumor and lymph nodes) with the four lead compounds. The tumor-bearing mice will be orally administered at various single doses. The primary ccRCC tumor growth and metastasis will be monitored. (3) We will select a drug candidate based on all previous studies and investigate its PK profile and tumor distribution and validate its efficacy using a PDX ccRCC model.

Impact: The successful completion of the proposed studies will (1) Identify a drug candidate that reduces ccRCC growth and metastasis via antagonizing A2BR pathways; and (2) Provide preclinical data related to the drug efficacies, PK/PD, and drug properties to further preclinical and IND-enabling studies. Our team includes experts in critical aspects of the project and a ccRCC survivor, serving as a patient advocate. In partnership with an industrial partner, we are well positioned to develop this new therapy for treating primary and metastatic ccRCC. The support from this DoD Kidney Cancer Research Program Idea Development Award will enable us to translate this de novo discovery from the lab to the bedside.

Principal Investigator: JIANG, JEAN X

Institution Receiving Award: TEXAS, UNIVERSITY OF, HEALTH SCIENCE CENTER AT SAN ANTONIO

Program: KCRP

Proposal Number: KC220116

Award Number: HT9425-23-1-0495

Funding Mechanism: Idea Development Award - Established Investigator

Partnering Awards:

Award Amount: \$978,159

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Delineate Tumor - Immune Contexture That Shapes ccRCC Metastatic Progression and Response to Immunotherapy

Srinivas Malladi

University of Southwestern Medical Center at Dallas, Texas

Technical Abstract

Background: Despite increased early detection of renal adenocarcinoma, approximately 25% of patients are presented with metastatic disease and more than one-third of the patients who undergo nephrectomy with curative intent experience local or distal relapse. Around 78% of metastases occur within 5 years and 11% occur after more than 10 years of initial diagnosis. Molecular understanding of how tumor microenvironment aids metastatic competence is needed to improve early detection, predict aggressive disease, enhance treatment options for patients, and ultimately prevent metastatic relapse.

Immune checkpoint inhibitors (ICI), especially PD-1 blockade has been transformative for management of advanced ccRCC. However, the observed benefit of immunotherapy in ccRCC patients is not universal and is independent of mutation burden, PD-1/PDL1 expression and antigen load. Moreover, not all patients even with defined clinical biomarkers respond to immunotherapies. Understanding of how tumor microenvironment modulates immunotherapy response is needed to improve clinical benefit and overall survival of metastatic ccRCC patients.

The application aligns well with the FY22 KCRP focus area on conducting 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. The proposal aims to address two key clinical challenges with broad implications affecting clinical decision making and patient care. In spite of having similar oncogenomic status why some invasive ccRCC tumors metastasize and some don't -- and why immunotherapies are effective in some patients and not all. The objective of our proposal is to delineate immune contexture associated with metastatic progression and immunotherapy response/resistance using humanized mouse models and dissect the role of immune contexture in determining metastatic incidence and immunotherapy response.

Specific Aims and Study Design:

Aim 1: Delineate and dissect role of tumor microenvironment in promoting metastasis. Employing ccRCC patient-derived xenografts from invasive ccRCC tumors resected from metastatic (XP166, XP26, and XP454) and non-metastatic patients (XP659, XP243, and XP470) in humanized mice, we will identify changes in stromal composition during metastatic progression. Through longitudinal sampling and single cell sequencing analysis we will identify immune contexture associated with metastatic progression, associated changes in infiltrating immune cells in metastatic and non-metastatic tumors, and define tumor cell traits promoting metastasis. To dissect the role of identified molecular and cellular determinants on metastasis, we will perform genetic loss and gain of function experiments.

Aim 2: Delineate and dissect role of tumor microenvironment in immunotherapy response/resistance. Treatment naive (XP166, XP26, and XP454) or treatment experienced (XP373(sunitinib), XP871(sunitinib) and XP213(11-2)) ccRCC PDXs in humanized mice will be exposed to immunotherapy to delineate traits associated with immunotherapy response and resistance in tumor and immune cells. Furthermore, we will define if immunotherapy response is primary or adaptive in these tumor and also evaluate the role of identified traits associated with promoting immunotherapy response and resistance.

Impact: The proposed studies will aid investigators in clinical decision making and optimize clinical outcomes. These studies will also provide novel insights to improve survival and therapy response rates in metastatic ccRCC patients and reduce potential drug and financial toxicity by identifying patients likely to have metastatic disease and therapy response.

Innovation: (1) Identification and validation of metastasis and immunotherapy response determinants. (2) Establishment of novel humanized PDXs models to investigate ccRCC disease progression and therapy response. (3) Based on tumor and immune contexture of primary tumors, successful completion of these studies, we will aid investigators in identifying ccRCC patients that are at risk to develop metastasis and also determine the likelihood of clinical response to immunotherapies in metastatic ccRCC patients.

Principal Investigator: MALLADI, SRINIVAS

Institution Receiving Award: TEXAS, UNIVERSITY OF, SOUTHWESTERN MEDICAL CENTER AT DALLAS

Program: KCRP

Proposal Number: KC220003

Award Number: HT9425-23-1-0867

Funding Mechanism: Idea Development Award - Established Investigator

Partnering Awards:

Award Amount: \$1,106,902

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Uncovering the Tumor-Immune Microenvironmental Determinants of Immunotherapy Response in Renal Cell Carcinoma Through Ex Vivo Patient-Derived Models

David Braun

Yale University

Technical Abstract

Background: Although immune checkpoint inhibitors (ICIs) have transformed the management of advanced clear cell renal cell carcinoma (ccRCC), the majority of patients do not receive durable benefit, and novel therapies are needed to overcome resistance. Work from our group (Braun, Cancer Cell, 2022) and others demonstrated that the tumor microenvironment (TME) plays a fundamental role in disease progression, with immunosuppressive macrophages and terminally exhausted T cells forming an inhibitory immune circuit. However, the design of novel therapeutics to target this inhibitory immune circuit will require functional interrogation of the effects of individual immune inhibitory interactions on T cell function and antitumor activity.

Hypothesis/Objectives: Building on our prior work isolating and interrogating individual cellular populations from ccRCC tumors, our research group's recent optimization of an ex vivo patient-derived tumor model (PDTM) platform (where tumor fragments are embedded in a collagen matrix in an air-liquid interface culture system to preserve the 3D architecture of the TME), and our extensive access to fresh ccRCC surgical specimens for modeling, our laboratory is uniquely poised to functionally interrogate cell-cell interactions within the ccRCC TME and identify clinically relevant therapeutic targets. We hypothesize that candidate immune cell interactions in the ccRCC inhibitory immune circuit can be pharmacologically perturbed to restore T cell function and antitumor immune activity. We will then utilize transcriptomic analysis to identify the subsets of ccRCC patients most likely to benefit from the blockade of additional inhibitory immune activity.

Specific Aims and Study Design:

Aim 1: Determine the impact of inhibitory immune interactions on tumor-infiltrating T cell function. We hypothesize that pharmacologic blockade of the T cell -- M2-like macrophage immune-inhibitory circuit will improve T cell function. Utilizing fresh RCC tumor biospecimens from surgical resections, we will flow cytometrically isolate tumor-infiltrating terminally exhausted PD-1+ TIM-3+ CD8+ T cells and CD163+ M2-like macrophages, and co-culture them ex vivo in the presence or absence of monoclonal antibodies that pharmacologically

block the candidate inhibitory interactions between these two populations (identified in Braun, Cancer Cell, 2021, for example, TIGIT-PVR, CSF-1R-CSF-1, etc.). Following inhibition of these interactions, we will use flow cytometry-based assays to assess the impact on T cell phenotype and function, including cytokine production, proliferation, and degranulation.

Aim 2: Define the effect of perturbing inhibitory immune interactions on the TME and anti-tumor activity. We hypothesize that pharmacologic inhibition of the candidate immune cell interactions will lead to increased tumor cell death. Using our PDTM platform for RCC, we will assess the overall effect of pharmacologically perturbing inhibitory immune interactions on all cell types within the TME and on anti-tumor cell activity. Following inhibition of candidate immune interactions, we will assess the impact on T cell function (by flow cytometry) and anti-tumor activity (by immunohistochemistry for quantifying the number of tumor cells, and by flow cytometry for quantifying tumor cell apoptosis). We will further investigate the overall impact of perturbing specific inhibitor immune interactions on the TME composition with single-cell RNA-sequencing and high-dimensional spatial phenotyping for quantifying cellular populations and spatial proximity between putatively interacting cells.

Aim 3: Elucidate the connection between functional inhibitory immune interactions and response to ICI in RCC patients. We hypothesize that specific inhibitory immune interactions are associated with resistance to anti-PD-1-based therapy in ccRCC. Utilizing pre-treatment tumor RNA-sequencing data from RCC patients treated with PD-1 blockade (or a control therapy, as part of phase I, II, and III clinical trials, from Braun, Nature Medicine, 2020), we will assess the expression of gene signatures for candidate inhibitory immune interactions, and connect this to response to and survival with ICI. This transcriptomic analysis will aim to identify the subsets of RCC patients most likely to benefit from blockade of additional inhibitory immune interactions.

Impact: The functional validation of immune inhibitory interactions in RCC will provide novel targets for immune modulation. Once targetable immune interactions are validated, I will be well positioned to rapidly translate these findings into clinical trials as I have already done with an investigator-initiated, phase I study of neoantigen-targeting vaccination in RCC (NCT02950766; Co-PI: Braun). Therefore, this project directly addresses the Focus Area on developing novel therapeutic strategies for the treatment of kidney cancer.

Innovation: Our proposed study moves well beyond the important initial step of immune profiling, utilizing a novel PDTM platform to uncovering functional immune interactions, with the goal of identifying novel therapeutic targets for patients with ccRCC.

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