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Are the IMmotion151-molecular signatures predictive of treatment outcomes in the JAVELIN Renal 101 trial?

Dr Renee Maria Saliby MD, MSc1, Tejas Jammiah2, Dr Chris Labaki MD3, Wanling Xie3, Talal El Zarif1, Robert Motzer4,5, Thomas Powles6, Brian Rini7, Laurence Albignes8, Sumanta Pal9, Rana McKay10, Sabina Signoretti11, Sachet Shukla12, Eliezer Van Allen13, Toni Choueiri13, David Braun13,14

1Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA. 2Department of Hematopoietic Biology and Malignancy, The University of Texas MD Anderson Cancer Center, Texas, Houston, TX, USA. 3Department of Data Sciences, Dana-Farber Cancer Institute, Boston, MA, USA. 4Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 5Department of Medicine, Weill Cornell Medical Center, New York, NY, USA. 6Barts Cancer Institute and the Royal Free Hospital, Queen Mary University of London, London, UK. 7Division of Hematology and Oncology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA. 8Department of Cancer Medicine, Gustave Roussy, Villejuif, France. 9Department of Medical Oncology, City of Hope Comprehensive Cancer Center, Duarte, CA, USA. 10Moores Cancer Center, University of California San Diego Health, La Jolla, CA, USA. 11Department of Pathology, Brigham and Women’s Hospital, Boston, Massachusetts, USA. 12Department of Hematopoietic Biology and Malignancy, The University of Texas MD Anderson Cancer Center, Texas, Houston, TX, USA. 13Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA. 14Center of Molecular and Cellular Oncology (CMCO), Yale University, New Haven, CT, USA.

Background

Biomarkers that can predict response to contemporary RCC regimens are needed. IMmotion151 (IM151) was a phase-III trial that compared atezolizumab (an anti–PD-L1 inhibitory antibody, ICI) in combination with bevacizumab (anti-angiogenic antibody) to sunitinib. Molecular subsets based on transcriptomic data were defined and found to be predictive of clinical outcome to specific treatments in IM151. We applied the same methodology to the JAVELIN Renal 101 (JR101), a phase III trial of VEGF TKI axitinib in combination with PD-L1 inhibitor avelumab vs. sunitinib, to see whether the previously described IM151 signatures predict outcome with these classes of therapy (ICI + VEGF TKI) more broadly.

Methods

Bulk RNA-sequencing from tissue samples and clinical data (data cutoff: 28 January 2019) from JR101 were obtained. First, we trained a machine-learning algorithm (random forest model) on the IM151 dataset and evaluated the accuracy metrics of the model. We then used this model to classify patients from JR101 into the previously defined molecular clusters. Finally, we tried to correlate molecular subgroups with treatment outcomes: progression-free survival (PFS), objective response rate (ORR), and overall survival (OS).

Results

Our model was highly accurate (82%) at assigning samples to the correct molecular subset in our training set (IM151). After applying the model to the JR101 dataset, the proportion of patients in each molecular subtype and across MSKCC risk groups was comparable between the two trials, with no significant differences. Avelumab combined with axitinib was generally superior to sunitinib for PFS and ORR (table 1) regardless of molecular subsets, including angiogenic and immune-based clusters. The T-effector/proliferative and proliferative groups exhibited an improved PFS (HR:0.65; 95% CI: 0.44-0.97) under the avelumab/axitinib combination vs. sunitinib.

Table 1: ORR from the JR101 trial in relation to previously defined molecular clusters. Cluster 7 separately was excluded as it contains 1 patient per treatment arm.

<table>
<thead>
<tr>
<th>Molecular Clusters</th>
<th>ORR % AA/Sun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>53/27</td>
</tr>
<tr>
<td>1-Angiogenic/Stromal</td>
<td>60/29</td>
</tr>
<tr>
<td>2-Angiogenic</td>
<td>60/34</td>
</tr>
<tr>
<td>3-Complement/Oxidation</td>
<td>58/32</td>
</tr>
<tr>
<td>4-T-eff/Prolif</td>
<td>53/24</td>
</tr>
<tr>
<td>5-Proliferative</td>
<td>40/15</td>
</tr>
<tr>
<td>6-Stromal/Prolif</td>
<td>44/17</td>
</tr>
<tr>
<td>Clusters 1+2</td>
<td>60/33</td>
</tr>
<tr>
<td>4+5</td>
<td>48/22</td>
</tr>
</tbody>
</table>

Conclusions

Patterns of gene expression across molecular clusters were similar between JR101 and IM151. However, we could not validate all the correlations between molecular clusters and treatment outcomes.

Keywords: renal cell carcinoma, immune-checkpoint inhibitors, biomarkers
Molecular Characterization of the Tumor Microenvironment in Chromophobe Renal Cell Carcinoma (ChRCC) and Renal Oncocytic Neoplasms

Dr Chris Labaki MD1, Dr Long Zhang PhD2, Dr Yue Hou PhD1, Dr Kevin Bi PhD1, Dr Charbel Hobeika MD2, Dr Ziad Bakouny MD, MSc2, Sabrina Camp BS1, Dr Carmen Priolo MD3, Damir Khabibullin MSc2, Nicholas Schindler MSc1, Dr Michel Alchoueiry MD, MSc2, Dr Thomas Denize MD2, Dr Renee Maria Saliby MD, MSc1, Dr Sayed Matar MD2, Dr Sabina Signoretti MD2, Dr Eliezer M. Van Allen MD1, Dr Sachet Shukla PhD4, Dr Elizabeth P. Henske MD2, Dr T oni K. Choueiri MD1, Dr David A. Braun MD, PhD3

1Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA. 2Brigham and Women's Hospital, Boston, MA, USA. 3Yale University, New Haven, CT, USA. 4The University of Texas MD Anderson Cancer Center,  Texas, Houston, TX, USA

Background

ChRCC is a rare form of kidney cancer and presents with a poor prognosis in the metastatic setting, with limited response to immune checkpoint inhibitors (ICI) and targeted therapy. We thought to evaluate the molecular characteristics of ChRCC and other related oncocytic neoplasms to better characterize the tumor immune microenvironment and identify potential therapeutic strategies.

Methods

ChRCC, renal oncocytoma (RO) and low-grade oncocytic tumor (LOT) samples with matched normal kidney specimens were evaluated using single-cell RNA sequencing (scRNA-seq) and single-cell T-cell receptor sequencing (scTCR-seq). T-cell antigenic specificities from scTCR-seq were inferred using a comprehensive database of annotated T-cell receptor sequences (VDJdb). The infiltration of CD45+ immune cells in renal oncocytic tumors and clear-cell RCC samples was quantified using immunohistochemistry (IHC). Bulk RNA-sequencing (RNA-seq) data of ccRCC, papillary RCC (pRCC) and ChRCC were further analyzed using The Cancer Genome Atlas (TCGA) KIRC, KIRP and KICH cohorts, respectively. Clinical data was obtained from the International Metastatic RCC Database Consortium (IMDC) to evaluate the survival outcomes (i.e. progression-free survival [PFS] and overall survival [OS]) of patients with metastatic ChRCC (versus mccRCC) across regimen types in the first-line setting.

Results

Following quality-control, 46,817 cells from 5 tumors (ChRCC: n=3, RO: n=1 and LOT: n=1) and 4 normal samples were isolated for scRNA-seq and IHC analysis. Renal oncocytic tumors (ChRCC, RO, and LOT) exhibited a low density of CD45+ cells (mean: 739 ± 114 cells/mm2; n=5) compared to ccRCC (mean: 3,420 ± 1,979 cells/mm2; n=5) (p<0.05). Across all tumors, CD8+ T-cell clusters displayed a low expression of immune exhaustion markers (i.e. PDCD1 [PD-1], CTLA4, LAG3, HAVCR2 [TIM-3], and TIGIT). Analysis of TCGA bulk RNA-seq data showed a significantly lower expression of all immune exhaustion markers (i.e. PDCD1, CTLA4, LAG3, HAVCR2, and TIGIT) in ChRCC compared to both ccRCC (p<0.01) and pRCC (p<0.01). Analysis of the T-cell repertoire (scTCR-seq) of ChRCC, RO and LOT samples did not identify a pattern of clonal expansion, and a higher proportion of T-cells in ChRCC were inferred to have a viral specificity, as compared to ccRCC (0.43 vs. 0.15%, respectively). Analysis of the clinical data of 9,160 patients with mRCC (ccRCC: n=8,931; ChRCC: n=229) from the IMDC showed that patients with metastatic ChRCC treated with ICI dual therapy in the first-line setting presented worse survival outcomes as compared to those with mccRCC (mPFS [95%CI]: 2.5 mos [2.06-NR] vs. 8.2 mos [6.7-9.8], respectively; p(log-rank) = 0.004; and mOS [95%CI]: 17.6 mos [5.4-NR] vs. 48.0 mos [41.3-NR], respectively; p(log-rank) = 0.03). Similar results were also seen for OS in relation to IO monotherapy in the first-line setting in metastatic ChRCC versus ccRCC (mOS [95%CI]: 35.3 mos [6.3-NR] vs. 76.9 mos [53.6-NR], respectively; p(log-rank) = 0.01), contrasting with no difference in outcomes for VEGF TKI monotherapy (mPFS [95%CI]: 6.8 mos [5.0-8.4] vs. 8.1 mos [7.8-8.3], respectively; p(log-rank) = 0.09; and mOS [95%CI]: 25.8 mos [21.2-32.5] vs. 26.0 mos [25.2-27.2], respectively; p(log-rank) = 0.84).

Conclusions

Renal oncocytic tumors, of which ChRCC, present a low infiltration of immune cells, along with a non-exhausted immune phenotype and a lack of clonally expanded tumor-specific T-cells. These findings may help to understand the molecular basis for the lack of response to immunotherapy identified in the clinical analysis of patients with advanced ChRCC and show the unique exhaustion phenotype of renal oncocytic tumors.

Keywords: Chromophobe RCC; immunotherapy; renal oncocytoma; non-clear cell RCC

CDMRP DOD Funding

yes
Genomic and Epigenomic Profiling for Target Discovery in Translocation Renal Cell Carcinoma

Jiao Li1, Daniel Gallant1, Ananthan Sadagopan1, Shatha AbuHammad1, Bingchen Li2, Ziad Bakouny1, Toni Choueiri1, Cheng-Zhong Zhang1, Srinivas Viswanathan MD, PhD1

1Dana-Farber Cancer Institute. 2

Background

Translocation renal cell carcinoma (tRCC) is a rare and aggressive type of non-clear cell renal cell carcinoma (RCC) that represents 1-5% of sporadic RCC in adults and 20-75% of kidney cancers in children. Biologically, tRCC is driven by rearrangements involving a member of the MiT/TFE transcription factor family, most commonly TFE3. There are currently no molecularly-targeted therapies specific to translocation renal cell carcinoma (tRCC) and effective treatments for this aggressive cancer remain a major unmet medical need. A barrier to effective therapies in tRCC is an incomplete mechanistic understanding of precisely how MiT/TFE gene fusions exert their oncogenic function.

We have previously leveraged “histologic overlap” between tRCC and other RCC subtypes in order to identify tRCC cases from across multiple genomic, clinical trial, and retrospective datasets and to define the molecular landscape of this disease. In this study, we performed functional epigenomic profiling across an array of tRCC cellular models and intersected with our prior genomic data to nominate key pathways involved in driving tRCC.

Methods

From previously published datasets, we re-analyzed data from DNA-sequencing of 74 tRCC cases (profiled by either Whole Exome Sequencing, Whole Genome Sequencing, or gene panel sequencing) and RNA-sequencing of 46 tRCC cases. We also performed whole genome and transcriptome sequencing on a cohort of institutional tRCC cases. We performed chromatin immunoprecipitation and sequencing (ChIP-Seq) on a panel of tRCC and clear-cell RCC (ccRCC) cell lines, using antibodies against TFE3 as well as the active enhancer mark H3K27ac. Functional studies in cell lines were used to validate our epigenomic profiling results and to study the role of MiT/TFE gene fusions in driving a tRCC-specific transcriptional program.

Results

Transcriptional profiling of tRCC tumors revealed overexpression of genes implicated in the antioxidant stress response and NRF2 signaling compared to other forms of RCC. This was confirmed by epigenomic profiling and functional studies, which suggest multilevel transcriptional and post-transcriptional regulation of NRF2 signaling by TFE3 fusions. In addition, tRCC cell lines demonstrated variable levels of NFE2L2 dependence in vitro. Signatures of NRF2 activation, which are enriched in tRCC, correlate with resistance to many targeted therapies, including agents used in the treatment of kidney cancer.

Conclusions

tRCC tumors and cell line models share an epigenetic and transcriptional profile characterized by activation of the antioxidant stress response and heightened NRF2 signaling. This program appears driven directly by TFE3 fusions, and may be responsible for poor responses to existing targeted therapies. Modulation of this pathway may a potential strategy for overcoming drug resistance in tRCC.

Keywords

Translocation renal cell carcinoma; genomics; TFE3; TFEB; MITF; NRF2; VEGFR; immune checkpoint inhibition; immunotherapy; oxidative stress

CDMRP DOD Funding

Yes

Impact of race and payor status on patterns of utilization of partial and radical nephrectomy in patients with localized renal cell carcinoma (RCC)

Dr Regina Barragan-Carrillo MD1, Dr Errol J Philip MD, PhD2, Dr Ameish Govindarajan MD3, Dr Neal Chawla MD3, Daniela Castro MS3, Dr Alex Chehrazi-Raffle MD3, Dr Nazli Dizman MD4, JoAnn Hsu BS5, Dr Cristiane Bergerot MD, PhD6, Dr Karyn Eilber MD6, Dr Sumanta Pal MD7, Dr Kai Dallas MD7

1Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico. 2University of California-San Francisco, San Francisco, California, USA. 3Department of Medical Oncology & Experimental Therapeutics, City of Hope Comprehensive Cancer Center, Duarte, CA, USA. 4Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA. 5Centro de Cancer de Brasilia, Instituto Unity de Ensino e Pesquisa, Brasilia, DF, Brazil. 6Division of Urology, Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA. 7Division of Urology, City of Hope Comprehensive Cancer Center, Duarte, CA, USA.
Background

Although prospective trials have shown similar outcomes with partial and radical nephrectomy in patients with localized RCC (Van Poppel et al Eur Urol 2011), multiple studies suggest increasing use of the former technique (Breau et al Can J Urol 2020). We hypothesize that patients that stem from minority groups as well as non-private insurance have less access to this specialized procedure and therefore have a higher rate of radical nephrectomy.

Methods

We interrogated the California Office of Statewide Health Planning and Development (OSHPD) database, which collects information from all inpatient admissions, emergency room visits and inpatient/outpatient procedures in the state. All patients undergoing nephrectomy (both partial and radical) were identified from Jan 1, 2012 to Dec 31, 2018 using CPT and ICD-9/10 codes to identify patients with RCC undergoing radical nephrectomy and partial nephrectomy. Demographic data was collected with specific attention to race and payor status. Univariate and multivariate analyses were conducted to determine the association between demographic data and procedure type.

Results

In total, 31,093 patients were identified; 57% were males, with a mean age of 58 years. Among these, 16,142 (51.9%), 8,645 (27.8%), 2,795 (9.0%), 2,032 (6.5%) and 1,479 (4.8%) were characterized as White, Hispanic, Asian, Black and other, respectively. Partial nephrectomy and radical nephrectomy were performed in 15,840 (50.9%) and 15,253 (49.1%) of patients. By race, partial nephrectomy was performed in 8,576 (53.1%), 4,107 (47.5%), 1,286 (46.0%), 1,124 (55.3%) and 747 (50.5%) of White, Hispanic, Asian, Black and other patients, respectively (p<0.001). Use of partial nephrectomy also differed among patients based on payor status, with rates of 6,800 (56.4%), 5,036 (43.9%), 1,817 (38.3%) and 2,187 (77.7%) among patients with private, Medicare, indigent coverage (e.g., MediCal or Medicaid) and other insurance, respectively (p<0.001). On multivariate analysis controlling for age, gender, comorbidities and frailty, race was independently associated with type of nephrectomy procedure (partial versus radical).

Conclusions

Our study is the first to show that race and payor status may have an influence on utilization of partial versus radical nephrectomy, with the highest rate of partial nephrectomies among whites and patients with private insurance. Although there are multiple potential confounders (e.g., latency of diagnosis and resulting tumor size/complexity), it is possible access to care is a driver of this phenomenon.

Keywords: Kidney Cancer, Nephrectomy, Healthcare Disparities

77

Phase 2 study of neoadjuvant cabozantinib in patients with locally advanced non-metastatic clear cell renal cell carcinoma

Dr Mehmet Bilen MD, Dr Yuan Liu, Dr Bassel Nazha, Dr Jacqueline Brown, Dr Adeboya Osunkoya, Sierra Williams, Wilena Session, Lauren Yantorni, Greta Russler, Sarah Caulfield, Dr Shreyas Joshi, Dr Vikram Narayan, Dr Christopher Filson, Dr Kenneth Ogan, Dr Omer Kucuk, Dr Bradley Carthon, Dr Haydn Kissick, Dr Viraj Master

Winship Cancer Institute of Emory University

Background

Cabozantinib is a small molecule inhibitor of the tyrosine kinases c-Met, AXL and VEGFR2 that has been shown to reduce tumor growth, metastasis, and angiogenesis. After the promising results from the METEOR, CABOSUN and Checkmate-9ER trials, cabozantinib was approved for use in patients with advanced renal cell carcinoma (RCC). The increased response rates with cabozantinib in metastatic RCC, along with the other neoadjuvant TKI data, support an expanded role for cabozantinib in the neoadjuvant setting.

Methods

Patients with clinical stage ≥ T3Nx or TanyN+ or deemed unresectable by the surgeon with biopsy-proven clear cell RCC were eligible for this study, and received cabozantinib at a starting dose of 60 mg daily for 12 weeks. The primary outcome was objective response rate per RECIST v1.1 (complete and partial responses) at week 12 after the administration of cabozantinib as determined by independent radiologist review. Secondary outcomes included safety, tolerability, clinical outcome (DFS, OS), surgical outcome and quality of life.

Results

As of 27 July 2022, 17 biopsy-proven clear cell RCC patients were treated with neoadjuvant cabozantinib. The median age was 58 years (range: 42-86 years) and 82.4% male. All patients completed 12 weeks of treatment, and 16 of them underwent surgery as planned without any delay after completion of 4 weeks wash-out. One patient refused to undergo surgery due to personal reasons and received further systemic treatment. All patients had tumor reduction. Five patients (29.4%) experienced partial
response [ORR = 0.29, 95% CI = (0.10, 0.56)], and 12 patients had stable disease. We reject the initial hypothesis of H0: ORR < 5% vs. HA: OR > 24%. There was no progression of disease while on cabozantinib. Median reduction of primary renal tumor size was 23% (range: 6-45%). The one patient who was deemed to be unresectable became resectable at the end of treatment. Two patients were converted from radical to partial nephrectomy. The most common AEs were diarrhea, anorexia, fatigue, hypertension, nausea. One patient had treatment related SAE due to pulmonary embolism. Dose reduction due to treatment related AEs listed in table 2. No treatment related grade 4 or 5 AEs related to cabozantinib or surgery occurred. One patient had disease recurrence after nephrectomy. Three patients have died since start of clinical trial (1 due to COVID, 1 cancer-related, and 1 unknown cause).

Conclusions
Cabozantinib was clinically active and safe in the neoadjuvant setting in patients with locally advanced non-metastatic clear cell RCC. Additional data will be reported including long term outcomes, correlative studies, and others (NCT04022343).

Keywords: Renal cell carcinoma, clear cell, neoadjuvant treatment, cabozantinib

Background
IO, either as frontline combination therapy or second-line monotherapy, has improved outcomes for patients with advanced RCC. With movement away from upfront CN, limited data are available on the outcomes of patients who receive IO with delayed CN. In this study, we characterized the pathologic and survival outcomes for patients who received IO followed by CN.

Methods
We conducted a multi-center, retrospective analysis of patients with advanced/metastatic RCC having received IO combination or monotherapy followed by CN. An IRB-approved and HIPAA-compliant registry was used to collect data from the electronic medical record. A binomial logistic regression was performed to ascertain the effects of renal mass size, M stage at diagnosis, presence of rhabdoid or sarcomatoid differentiation, clear cell histology, use of IO immediately preceding surgery and number of prior lines of therapy on the likelihood that patients experience pathological T downstaging. Our primary endpoint was the rate of pathologic downstaging comparing baseline clinical T stage to pathologic T stage. Secondary endpoints included investigator assessed response using RECIST principals, progression-free survival (PFS), and overall survival (OS).

Results
52 patients were included across 9 institutions. The majority of patients were male (65%), 81% had clear cell histology, 11% had sarcomatoid differentiation, and 75% presented with de novo metastatic disease. At the start of therapy immediately preceding CN 6% had favorable (n=3) risk disease, 60% intermediate (n=31), and 25% poor (n=13) risk. Most patients (65%) were treatment naïve at the time of IO therapy initiation prior to CN. 44% of patients received nivolumab/ipilimumab (n=23), 25% PD-1/PD-L1 monotherapy (n=13), 23% PD-1/PD-L1 + VEGF TKI (n=12), and VEGF TKI monotherapy (8%, n=4). The median duration of systemic therapy treatment prior to CN was 8.1 months [MR1] [JP2] (95% CI, 5.97-11.18). For pathologic outcomes at time of CN, 44% of patients experienced pathologic downstaging. Downstaging occurred in 20% of patients with cT1 (n=5), 29% of cT2 (n=14), 37% of cT3 (n=19), and 85% of cT4 patients (n=13) tumors at baseline. It was also notable that seven (13%) patients had no residual disease (ypT0) in their renal primary at CN. Pathologic outcomes at the time of CN, 44% of patients experienced pathologic downstaging. Downstaging occurred in 20% of patients with cT1 (n=5), 29% of cT2 (n=14), 37% of cT3 (n=19), and 85% of cT4 patients (n=13) tumors at baseline. It was also notable that seven (13%) patients had no residual disease (ypT0) in their renal primary at CN. Pathologic outcomes at the time of CN demonstrated median tumor size 6.5 cm with 85% (n=42) of patients having negative margins and 75% (n=39) with necrosis. Median follow-up for the entire cohort was 25.3 months and median PFS was 21.97 months (95% CI [18.26, 28.2]). Median OS was 22.29 months (95% CI [17.84, 26.38]). The 3-year overall survival for patients with versus without ypT downstaging

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Pathologic Outcomes at Cytoreductive Nephrectomy (CN) Following Immunotherapy (IO) for Patients with Advanced Renal Cell Carcinoma (RCC)

Justine Panian BS1, Dr Ava Saidian MD1, Kevin Hakimi BS1, Archana Ajmera NP1, Dr Pedro Barata MD2, Dr Stephanie Berg DO3, Dr Steven Lee Chang MD4, Dr Toni K Choueiri MD4, Dr Vincent D’Andrea MD4, Dr Hannah Dzimitrowicz MD3, Dr Hamid Emamekhoo MD4, Evan Gross BS7, Dr Deepak Kilari MD8, Dr Elaine Lam MD9, Isabel Lashgari10, Dr Sarah Psutka MD7, Grant Rauterkus BS2, Dr Bicky Thapa MD6, Nicole Weise BS3, Dr Kendrick Yim MD6, Dr Tian Zhang MD11, Dr Ithaar Derweesh MD1, Dr Rana R. McKay MD1

1University of California, San Diego. 2Tulane University. 3Loyola University Chicago. 4Dana-Farber Cancer Institute. 5Duke University. 6University of Wisconsin. 7University of Washington. 8Medical College of Wisconsin. 9University of Colorado. 10San Diego State University. 11UT Southwestern
at time of CN was 94.4% versus 94.4%, respectively (p=0.685). The 3-year PFS for patients with versus without ypT downstaging at time of CN was 86.1% versus 84.2%, respectively (p=0.749). M stage at diagnosis, renal mass size at diagnosis and presence of clear cell histology on surgical pathology were predictive of pathologic downstaging.

**Conclusions**

IO-based interventions prior to CN in patients with advanced or metastatic RCC demonstrates efficacy, with a small fraction of patients showing a complete response. Additional prospective studies are warranted to investigate the role of CN in the modern IO-era.

**Keywords**: renal cell carcinoma, immunotherapy, nephrectomy, metastatic

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**Figure 1**: Degree of pathological downstaging
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**Taxonomic and functional assessment of gut microbiome in patients with metastatic renal cell carcinoma (mRCC) regarding response to immune checkpoint inhibitors (ICI)**

Luis Meza MD, Nazli Dizman MD, Keehoon Lee PhD, Regina Barragan-Carrilo MD, Alex Chehrazi-Raffle MD, Nicholas Salgia BSc, Paulo Bergerot MD, JoAnn Hsu BSc, Zeynep Zengin MD, Daniela Castro MS, Tanya Dorff MD, Sabrina Salgia BSc, Neal Chawla MD, Jeffrey Trent PhD, Greg Caporaso PhD, Sumanta Pal MD

1Department of Medical Oncology, City of Hope Comprehensive Cancer Center, Duarte, CA. 2Yale University School of Medicine, New Haven, CT, USA. 3The Translational Genomics Research Institute (TGen), Phoenix, AZ, USA. 4Cettro Oncologia, Brasilia, Brazil

**Background**

Several investigations have shown an association between gut microbial species and outcomes with ICIs in mRCC (Derosa et al Cancer Discov 2021). However, the mechanisms by which the gut microbiome impacts the activity of ICIs has not been clearly identified. Herein, we examine the taxonomic and functional profile of the gut microbiome in patients with mRCC and investigate its role in predicting outcomes with ICIs.

**Methods**

In this prospective observational study, patients with mRCC were enrolled prior to the initiation of ICIs. Data pertaining to disease characteristics, treatments and clinical outcomes were prospectively collected. Treatment response was assessed using RECIST v1.1 criteria, and patients were categorized as responders (partial response or complete response) or non-responders (stable disease or progressive disease). Stool samples were collected at baseline and week 12 of ICI treatment. HUMAnN 3 was used to functionally profile the genes and pathways from metagenomes and assess species’ contributions to community function. The differentially abundant functional pathways between responders and non-responders at baseline were identified by the Linear discriminant analysis (LDA) Effect Size (LEfSe) algorithm.

**Results**

A total of 31 patients were enrolled. The median age was 61 (40-77), 71% of the patients were male, 90% had clear cell histology, and 61% had IMDC intermediate/poor risk disease. The most frequently used ICIs were nivolumab (77%) and nivolumab/ipilimumab (23%). Eleven (35%) patients demonstrated a response to ICI therapy. Patients with response to treatment had a statistically significantly higher relative abundance of *Bacteroides ovatus* (LDA 3.8), *Ruminococcus torques* (LDA 3.7) and *Eubacterium ramulus* (LDA 2.8) while patients with no response to treatment had higher relative abundance of *Clostridium bolteae* (LDA 3.5) and *Clostridium lavalense* (LDA 2.8). With regard to functional profiling of stool samples, differentially abundant pathways included sucrose degradation IV pathway (LDA 2.6) and glycogen degradation II pathway (LDA 2.9) in responders and 4-deoxy-L-threo-hex-4-enopyranuronate degradation pathway (LDA 2.7) and D-galacturonate degradation I pathway (LDA 2.5) in non-responders.

**Conclusions**

Taxonomic and functional profiling of the gut microbiome could serve as a factor distinguishing responders from non-responders with ICIs in mRCC. The fecal abundance of the species known to produce short chain fatty acids, i.e. *Bacteroides ovatus*, was greater in responders, whereas the stool samples of non-responders were more abundant in species known to produce carcinogenic compounds, i.e. *Clostridium lavalense* (Min lee et al. Front Microbiol. 2018 and Horvath et al. iScience 2022). Incorporation of taxonomic and functional profiling of the gut microbiome in larger studies investigating biomarkers of ICI outcomes in mRCC is warranted.

**Keywords:** Kidney cancer, immunotherapy, microbiome
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Genomic and Transcriptomic Predictors of Response to First-Line Treatment with Ipilimumab with Nivolumab (Ipi+Nivo) in patients with Metastatic Clear Cell Renal Cell Carcinoma (ccRCC).

Dr Nishita Tripathi MBBS1, Dr Nicolas Sayegh MD1, Dr Luis Meza MD2, Dr Sara Byron PhD3, Jaming Zhang3, Dr Zeynep Zengin MD2, Beverly Chigarira1, Dr Haoran Li MD1, Dr Benjamin Maughan MD1, Dr Umang Swami MD1, Dr Sumanta Pal MD2, Dr Neeraj Agarwal MD1

1University of Utah, Huntsman cancer institute, Salt Lake City, Utah, USA. 2City of Hope Comprehensive Cancer Center, Duarte, CA, USA. 3Integrated Cancer Genomics Division, Translational Genomics Research Institute, Phoenix, AZ, USA

Background

Ipi+nivo is currently approved as a front-line treatment for patients with intermediate and poor risk metastatic ccRCC due to improvement in overall survival compared to sunitinib (PMID: 29562145). However, ~35% of these patients experience disease progression within six months (PMID: 29562145). Currently, there are no biomarkers to identify these early progressors. We hypothesize that metastatic ccRCC with early progression on the first-line ipi+nivo will have a distinct genomic and transcriptomic profile compared to those who do not.

Methods

This was an IRB-approved, multi-institutional, retrospective study. Eligibility criteria included a diagnosis of metastatic ccRCC with intermediate and poor risk and availability of tumor DNA and RNAseq profiling by a CLIA-certified laboratory before systemic therapy. Disease control for this study was defined as the absence of radiographic or clinical disease progression until six months after starting treatment with Ipi/Nivo. The frequency of genomic alterations was assessed using a univariate chi-square and Fischer exact test. Multivariate regression was used to assess the baseline demographic and IMDC prognostic variables for disease control. Differential gene expression analysis between the two groups was performed using DESeq2 with a p-adjusted value of less than 0.1 considered significant after multiple testing corrections. These results were subjected to Gene Set Enrichment software analysis (GSEA) to identify pathways enriched in each cohort. All bioinformatic analysis was undertaken using R v4.2.

Results

Patients were categorized into two cohorts: disease control until six months (DC) or progression within six months of initiation of Ipi/Nivo (NDC). 49 patients were eligible and included in the genomic analysis, with 33 pts achieving DC and 16 pts with NDC. Among the baseline characteristics, the presence of thrombocytosis prior to starting ipi+nivo was favorable for DC (p=0.005). Although VHL (20% vs 10%) and PBRM1 (17% vs 8%) were more frequently altered genes in DC vs NDC cohorts respectively; these differences were not statistically significant. Baseline tumor transcriptomic data were available for 14 patients:

![Figure 1: Gene set enrichment scores between disease control vs. no disease control group.](image-url)
10 in DC vs. 4 in NDC. 51 genes were differentially expressed between DC and NDC (q<0.1). 40 genes were overexpressed in the DC group, while 11 genes were overexpressed in the NDC group. Gene set enrichment analysis showed the upregulation of cell-cycle signaling pathways (E2F, G2M checkpoint, MYC targets v2) in DC vs. upregulation of TGF-Beta signaling pathway in NDC (q<0.1) [Figure 1].

Conclusions
Our hypothesis-generating data suggest that transcriptomic analysis may identify patients with metastatic ccRCC who experience early disease progression on first-line ipi+nivo. After external validation, these results may help with treatment selection, prognostication, and patient counseling.

Keywords: Ipilimumab, Immunotherapy, Nivolumab, Renal cell carcinoma

Clonal neoantigen load and tertiary lymphoid structure formation are associated with exceptional response to immune checkpoint inhibition in clear cell renal cell carcinoma

Tejas Jammihal MS, M Tech1, Renee Maria Saliby2, Chris Labaki2, Bradley McGregor2, Talal El Zarif2, David Braun3, Toni Choueiri2, Sachet Shukla1

1Department of Hematopoietic Biology and Malignancy, The University of Texas MD Anderson Cancer Center, Texas, Houston, TX, USA. 2Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA. 3Center of Molecular and Cellular Oncology (CMCO), Yale University, New Haven, CT, USA.

Background
Checkpoint inhibition (CPI) in clear cell renal cell carcinoma (ccRCC) has yielded benefit in a subset of patients. Recent multi-omic and spatial phenotyping studies have yielded insights about tumor-intrinsic features and microenvironment immune states that are associated with response. However, little is known about correlates and underlying drivers of ‘exceptional’ response to CPI in ccRCC.

Methods
We analyzed genomic and transcriptomic data in previously untreated patients with advanced ccRCC treated with either dual CTLA-4 and PD-1 inhibition (IO/IO; n=224 with whole exome sequencing (WES)) or the combination of PD1/L1 and VEGF inhibition (IO/VEGF; n=357 with both WES and RNA-seq). We defined three groups of patients in each cohort: (a) extreme responders (ER): patients with complete response (CR) and progression free survival (PFS) 12 months or partial response (PR) with tumor shrinkage 50% and PFS 24 months or PR with PFS 36 months; (b) patients with CR and PR who were not extreme responders (nonER-CRPR); (c) patients with progressive disease (PD). We additionally obtained clinical outcome data from the International Metastatic RCC Database Consortium (IMDC) for patients treated with first-line CPI-based therapies to compare the survival outcomes of the three patient groups in our cohorts and in IMDC. Analysis of WES data from matched tumor/normal pairs comprised somatic variant calling, copy number analysis, cancer cell fraction estimation HLA typing and mutational detection, followed by neoantigen binding prediction for each patient. Gene set enrichment analysis was performed for multiple previously described molecular signature sets. A multivariate cox proportional hazards analysis was performed for identifying significant correlates of PFS.

Results
The clonal neoantigen load was significantly higher in patients treated with dual IO/IO who had ER vs PD (p=0.035). Furthermore, the number of clonal neoantigens were also more abundant in ER vs nonER-CRPR patients (p=0.036), while there was no detectable difference between nonER-CRPR vs PD groups (p=0.19). Extreme response was also associated with a statistically significant reduction in intra-tumoral heterogeneity compared to PD (p=0.017). However, in a multivariate Cox proportional hazards model comprising age, sex, race, IMDC risk score, clonal neoantigen load and intra-tumoral heterogeneity, only clonal neoantigens had a statistically significant association with progression-free survival (p=0.021).

In patients treated with IO/VEGF, we observed that a tertiary lymphoid structure (TLS) signature was significantly elevated in ER compared to nonER-CRPR (p=0.002), ER compared to PD (p=0.00012) as well as nonER-CRPR compared to PD (p=0.077) patients. ER with a low TLS score were alternatively enriched in clonal neoantigen load (p=0.03). This negative association between TLS score and number of clonal neoantigens was not observed in the nonER-CRPR and PD groups. A gene set enrichment analysis also revealed strong enrichment of B-cell receptor pathways in the ER group compared to the PD (q=0.08, p=7.03e-04).

Conclusions
Our results suggest that exceptional response to CPI in ccRCC may be driven in part by two potentially independent
mechanisms. The first mechanism is a direct cytotoxic T-cell response elicited by the presence of clonal neoantigens in the tumor. The second mechanism comprises the formation of tertiary lymphoid structures in the tumor microenvironment and may involve a strong B-cell driven antibody response. While these mechanisms likely co-exist and function synergistically, our data suggests that either one may be sufficient to achieve an exceptional response. Our findings suggest that novel therapeutic combinations aimed at eliciting both T- and B-cell directed anti-tumor immunity may be important to achieve exceptional benefit in ccRCC.

Keywords: exceptional response, clonal neoantigens, tertiary lymphoid structures, immune checkpoint inhibition

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Circulating biomarkers associated with resistance to Nivolumab and Ipilimumab based regimens indicate persistent immunosuppression and activation of STAT3 signaling

Dr Marice Alcantara PhD, Dr Nazli Dizman MD, Dr Alexander Chehrazi-Raffle MD, Wilson Tang BS, Dr Luis Meza MD, Dr Dongfeng Wang PhD, Dr Dayson Moreira PhD, JoAnn Hsu BS, Dr Sumanta Pal MD, Professor Marcin Kortylewski PhD

Department of Immuno-Oncology, Beckman Research Institute, City of Hope National Medical Centre. Yale University School of Medicine, New Haven, CT. Department of Immuno-Oncology, Beckman Research Institute, City of Hope National Medical Centre. Department of Pharmacology, Sutro Biopharma

Background

Combination anti-PD-1 (Nivolumab) and anti-CTLA-4 (Ipilimumab) have improved objective response rates and overall survival in patients with renal cell carcinoma (RCC) over Sunitinib. However, few patients with metastatic disease will have durable responses, suggesting further research is required to improve patient responses. We investigated whether serum cytokine dynamics while on treatment with immune checkpoint inhibitors can demonstrate and uncover mechanisms of resistance to immunotherapy and investigated myeloid cell specific STAT3 silencing combined anti-PD-1 in preclinical models of RCC.

Methods

Patients who received nivo/ipi as first line treatment of RCC were identified using an institutional database. Baseline and week 12 (+/- 4 weeks) samples from previously conducted prospective clinical studies were included in this analysis and response to treatment was assessed using RECIST 1.1 criteria, with patients stratified into responders and non-responders to therapy. Patient peripheral blood was stained with 22 fluorochrome panel and spectral cytometry analysis undertaken to investigate alterations in immune populations including MDSCs, PMN-MDSCs, M-MDSCs, CD8+ T cells, CD4+ Foxp3+ Tregs and CD8/Foxp3+ Treg ratios. viSNE projection of peripheral blood analyzed using spectral cytometry was investigated for expression of PD-L1 and STAT3 activation. Luminex analysis was then used to measure 30 circulating cytokines including IL-6, IL-8 and IL-10 in patient plasma. For studies utilizing syngeneic mouse models of RCC, 6-8 week female Balb/C mice were injected subcutaneously with 500,000 RENCA cells resuspended in 1:1 ratio of 1x PBS and Matrigel and treated with either PBS, IgG, anti-PD-1, CpG-STAT3ASO or a combination of anti-PD-1 and CpG-STAT3ASO after establishment of tumors and tumor growth kinetics, survival were assessed via caliper measurements every other day. Immune alterations in tumor were assessed via flow cytometric analysis of tumor and tumor draining lymph nodes. Statistical significance was assessed using Wilcoxon signed ranked test with SEM.

Results

A total of 37 patients with metastatic RCC receiving combination nivolumab and ipilimumab in 2 clinical trials at City of Hope National Medical Centre were included in our study. A significant increase in plasma concentrations of cytokines IL-6 (p=0.0046), IL-8 (p=0.0174), IP-10 (p=0.0067), IL-2R (p=0.0174), IL-1RA (p=0.0079) and high STAT3 activation were observed in patients who did not respond to combination Nivo/Ipi based treatment strategies. In addition, our preliminary data in syngeneic models of RCC indicate that novel immunotherapeutic strategy utilizing anti-PD-1 in combination with oligonucleotide-based STAT3 inhibitor (CpG-STAT3ASO) targeting can achieve significant tumor growth inhibition vs PBS treated (p=0.0006), IgG (p=<0.0001) and anti-PD-1 (p=0.0285) groups, mediated through the activation of myeloid cells, particularly M1 macrophages.

Conclusions

Our results indicate immune alterations specific to patients unresponsive to nivolumab and ipilimumab based treatment strategies, including high IL-6, IL-8, IL-10 and elevated STAT3 (pSTAT3) within myeloid cells. These changes may play a role in resistance to ICIs in RCC and indicate further improvement of patient clinical responses may require targeting of myeloid cells. In fact, our preliminary data
indicate that novel immunotherapeutic strategy utilizing oligonucleotide-based STAT3 inhibitor (CpG-STAT3ASO) targeting myeloid cells with anti-PD-1 can achieve significant tumor growth inhibition and warrant further investigation into myeloid cell targeting with anti-PD-1 therapy in RCC.

**Keywords:** Nivolumab, Ipilimumab, RCC, STAT3, myeloid cells, immunosuppression

**CDMRP DOD Funding**
yes

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**Tumor Cell State Regulates Dependency on the Anti-apoptotic BCL-XL Protein in Kidney Cancer**

Abhishek Chakraborty PhD

*Cleveland Clinic Lerner College of Medicine of CWRU*

**Background**

Background: clear cell RCC (ccRCCs) make up roughly 75% of all kidney cancers. Although, early stage disease can be managed effectively, metastatic/advanced disease presents a clinical challenge. Recent strides in therapy, including combination regimens of immunotherapy with tyrosine kinase inhibitors, have vastly improved outcomes. However, these strategies are effective only in a subset of patients and can have undesired adverse effects. Consequently, the discovery of novel therapeutic targets to complement existing therapies remains a critical clinical need.

**Methods**

Cell-based assays included treating cultured cells with genetic (shRNA and CRISPR/Cas9) and pharmacological agents, followed by measurements of apoptosis, using cell viability (IC50 measurements), flow-cytometric apoptosis assays, and BH3 profiling assays. Dependency maps were generated using algorithms developed by the Broad Institute and used for lineage-specific comparisons. Transcriptomics analysis were done using RNA-Seq and pathway-level differences were identified using Gene Set Enrichment Analysis. Mouse models were used to establish the relevance of BCL-XL blockade in kidney cancer. Clinical relevance was interrogated using human renal tumor data retrieved from TCGA.

**Results**

Using recent genetic dependency maps (e.g. the Broad Institute’s Achilles and Novartis’ DRIVE datasets, which relied on genome-wide shRNA genetic screens), we identified several cellular dependencies that were specifically enriched in kidney cancers versus cells of other lineages. We reasoned that some of these dependencies might represent targetable vulnerabilities in kidney cancer. We focused our attention on the top druggable target in this list, BCL2L1, which encodes the anti-apoptotic protein, BCL-XL. Employing cell-based assays in a large panel of ccRCC cell lines, we found that a subset (~40%) of ccRCCs were highly-sensitive to both genetic and pharmacologic inactivation of BCL-XL. Moreover, BCL-XL blockade sensitized ccRCCs to standard chemotherapeutics.

Interestingly, neither expression of the BCL-2 family proteins nor p53 mutational status was sufficient to predict dependence on BCL-XL. Instead, transcriptomics analysis determined that tumor cell state changes were associated with response to BCL-XL blockers. Manipulating cell state using pharmacological and genetic tools demonstrated that promoting mesenchymal features were both necessary and sufficient to confer BCL-XL dependence. Finally, using mRNA expression data mined from TCGA, we demonstrated that the BCL-XL dependency signature, identified from ccRCC cell lines, was present in nearly a third – typically the more aggressive – human ccRCC tumors.

**Conclusions**

Our study demonstrates the utility of combining functional studies with publicly available genetic dependency datasets to identify lineage-specific actionable dependencies. Our mechanistic studies reveal an unexpected link between cell state changes and BCL-XL dependence in kidney cancer. The BCL-2 family has been targeted in many pharmacological campaigns, yielding many highly-potent and specific inhibitors. Our findings justify exploration of anti-BCL-XL agents as potential therapeutics against aggressive ccRCCs.

**Keywords:** Apoptosis; Epithelial-Mesenchymal Transition; Genetic Dependency Maps
Tumor Cell State Impacts BCL-X_L Dependence

Schema representing the protective apoptotic mechanisms that lead to cell death in normal cells that are shed from an organized epithelium (left). Upon oncogenic transformation, mesenchymal renal tumor cells rely on BCL-X_L’s anti-apoptotic activity to counteract this protective mechanism (right).
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Targeting HIF2α inhibitor resistance in clear cell renal cell carcinoma

Dr Qing Zhang PhD
UT Southwestern Medical Center

Background
ccRCC is the most common form of kidney cancer. Most ccRCC cases are associated with the inactivation of Von Hippel-Lindau (pVHL) protein, which leads to the stabilization of HIF-2α protein and drives ccRCC. Very recently, a HIF-2α inhibitor MK-6482 was developed and approved by FDA to treat certain patients with VHL disease-associated RCC. However, in preclinical studies, both intrinsic and adaptive resistance to this drug was reported. These findings highlight the necessity of uncovering the mechanisms underlying drug resistance and developing alternative treatments for HIF-2α inhibitor-resistant patients.

Methods
We have treated ccRCC cell line 786-O with PT2399, a structure analog of MK-6482, for an extended period of time. By combining the drug treatment and 3-D soft agar growth assay, we have isolated several clones of 786-O cells that develop drug resistance. We have performed RNA sequencing and Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq). In addition, we also performed ChIP sequencing for different histone marks, including H3K4me3, H3K4me1 and H3K27ac. We aimed to identify dysregulated gene expression that may account for HIF2α inhibitor therapeutic resistance in ccRCC.

Results
By performing integrated analysis of ChIP-seq, RNA-seq and ATAC-seq, we have identified the interferon signaling pathway is among the most significantly upregulated pathways in resistant clones. We identified several genes in the interferon signaling pathway exhibiting both upregulation in RNA level and increased chromatin accessibility in the promoter region. We hypothesize that these genes involved in interferon signaling promote the adaptive resistance to HIF-2α inhibitor in ccRCC. We are currently performing functional validation experiments to examine the potential role of these genes mediating HIF2α inhibitor resistance in ccRCC.

Conclusions
In conclusion, we have established the experimental system to examine molecular mechanism on the adaptive resistance to HIF-2α inhibitor and to explore the potential pathways as drug targets, which may provide alternative treatment strategies for HIF-2α inhibitor-resistant patients.

Keywords: HIF2α, therapeutic resistance, ccRCC

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Tumoral immunometabolic coevolution defines unique molecular niches in ccRCC

Cerise Tang1,2, Minwei Liu1, Fengshen Kuo3, Robert Motzer4, Paul Russo3, Jonathan Coleman2, Maria I Carlo6, Martin Voss4, Nikoala Schultz1, A Ari Hakimi5, Ed Reznik1,5

1Computational Oncology, Memorial Sloan Kettering Cancer Center, New York, NY. 2Physiology, Biophysics and Systems Biology Graduate Program, Weill Cornell Medicine, New York, NY. 3Urology Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY. 4Genitourinary Oncology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY. 5Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, NY

Background
Intratumoral heterogeneity (ITH) in clear cell renal cell carcinoma (ccRCC) reflects both the past genetic evolution of the disease and the present local metabolic and cellular microenvironment. Importantly, tumor and immune cells directly compete for the availability of key biosynthetic metabolites (glucose, glutamine) and secrete immunomodulatory signaling molecules (kynurenine, adenosine), raising the possibility that ccRCC evolution produces unique immunometabolic niches, defined jointly by specific patterns of metabolite availability and immune composition. However, analysis of ITH in ccRCC has historically focused on its genetic manifestation in the form of clonal and subclonal driver alterations, overlooking how metabolic and immunologic phenotypes vary spatially across the tumor.

Methods
A combination of liquid and gas chromatography coupled to mass spectrometry was used to metabolically profile approximately 600 metabolites from 123 tumor and 75 adjacent normal regions from 30 ccRCC patients. A median of 5 tumor regions and 3 normal regions were profiled from each patient. Whole exome DNA sequencing and RNA sequencing were completed on the majority of the profiled tumor regions. Intratumoral metabolic heterogeneity was defined as the expected log2 fold-change of a metabolite
between two randomly selected regions from the same patient. Immune infiltration was assessed using RNA signatures validated with matched immunofluorescence of cell-population-specific markers. Mixed effects models, with patient as a random effect, were used to assess covariation between immune signatures and metabolite levels.

Results
Tumors with high metabolic intratumor heterogeneity had unique genetic, metabolic, and immune profiles, showing an elevated rate of loss-of-heterozygosity of HLA (chi-squared p-value = 0.01) (Figure a), higher abundance of sphingomyelins and phosphatidylethanolamines, and lower levels of macrophage infiltration (FDR-corrected p-value = 0.01). Subsequent analysis revealed that all patients showed a common pattern of intratumoral heterogeneity across their tumor regions associated with compensation against ferroptosis. Every tumor featured regions defined by elevated levels of glutathione (FDR-adjusted p-value = 0.03), the glutathione precursor cysteine (FDR-adjusted p-value = 1x10^{-7}), polyunsaturated fatty acids, and antioxidants, and elevated expression of OXPHOS (FDR-adjusted p-value = 6x10^{-10}) and ROS-associated genes (FDR-adjusted p-value = 2x10^{-4}). By investigating the covariation between gene expression and metabolite levels, we discovered that a significant amount of the metabolome was associated with the expression of immune pathways and genes. A mixed effects model corroborated that approximately 8% of the measured variance in the tumor metabolome could be explained by the Immune Score signature, a measure of the abundance of immune cell populations. Pathway analysis revealed that several metabolites associated with NAD+ metabolism including quinolinate and nicotinamide mononucleotide, were significantly enriched both across patients in tumors with elevated immune infiltration and within individual patients in specific regions with elevated immune infiltration (Figure b). While the Immune Score signature represents a crude estimation of the total immune infiltration, RNA signatures capturing specific microenvironmental features, including angiogenesis and myeloid/T-effector abundance, were associated with response to checkpoint blockade therapy in ccRCC. Using mixed effects models, we found downstream metabolites of IDO1, a drug target under clinical investigation, were associated with the angiogenic signature. The myeloid cell signature was highly associated with n-acetylated amino acids and TCA cycle metabolites. In contrast to the angiogenic and myeloid signatures, the T-effector signature showed fewer metabolic associations, though many of the highly associated metabolites were involved in NAD+ metabolism.

Conclusions
Together, these analyses indicate that the immune microenvironment and metabolism coevolve in ccRCC to produce spatially-restricted niches with defined nutrient supplies and immune profiles.

Keywords: intratumoral heterogeneity, tumor microenvironment, cancer metabolism

CDMRP DOD Funding
yes

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Interim Analysis of PADRES (Prior Axitinib as a Determinant of Outcome of Renal Surgery NCT03438708) Clinical Trial
Mr Kevin Hakimi BS1,2, Dr Ithaar Derweesh MD1

1University of California, San Diego. 2

Background
In renal cell carcinoma (RCC), partial nephrectomy (PN) is imperatively indicated for individuals with solitary kidney, chronic kidney disease, or bilateral tumors. Neoadjuvant Tyrosine Kinase Inhibitor therapy can potentially cytoreduce renal tumors and may therefore permit PN in circumstances not otherwise feasible. We report interim analysis of the PADRES (Prior Axitinib as a Determinant of Outcome of Renal Surgery NCT03438708).

Methods
This was a single arm phase II clinical trial of neoadjuvant axitinib in patients with complex renal mass (RENAL nephrometry score 10-12 and cT1b-cT3M0) biopsy-proven clear cell RCC with strong indications for partial nephrectomy (PN), and in whom radical nephrectomy may result in dialysis dependence. Axitinib 5 mg was administered orally twice daily for 8 weeks prior to surgery. Primary outcome was partial nephrectomy following axitinib. Secondary objectives included change in tumor diameter, RENAL Nephrometry score, renal function in estimated glomerular filtration rate (DeGFR), tumor response based on RECIST v1.1 criteria, and surgical complications.

Results
26 patients consented for study of which 25 proceeded with protocol (median age 69 years; median follow-up 12
months). Prior to therapy, 20 (80%) patients had ≥ clinical T3a staged tumors. Post therapy, 17 (65.4%) patients had ≥ T3a staged tumors, and 8/25 (32%) of patients were downstaged on imaging. Axitinib resulted in reductions in median tumor size (19%, 7.7 vs. 6.3 cm, \(p<0.001\)) and RENAL score (11 vs. 10, \(p <0.001\)). Partial nephrectomy was performed in 19/25 (76%) with median ischemia time of 34 minutes and with 24/25 (96%) achieving negative margins. All radical nephrectomy patients had ≥T3a tumors on final pathology. Six (23.1%) had Clavien III-IV post-surgical complications. At last follow up, median DeGFR was 4.5 mL/min/1.73m², with only two patients having a DeGFR≥50%, both of which received radical nephrectomy.

**Table:** Interim Analysis of PADRES (Prior Axitinib as a Determinant of Outcome of Renal Surgery NCT03438708) Clinical Trial

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumor size</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Tumor Size (cm; IQR)</td>
<td>6.3; 3.8</td>
<td>18.80%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percentage Change Tumor Size</td>
<td>7.7; 3.8</td>
<td>18.80%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Median RENAL score (IQR)</strong></td>
<td>11 (1)</td>
<td>10 (2)</td>
<td></td>
</tr>
<tr>
<td><strong>RENAL Score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 to 9</td>
<td>0 (0%)</td>
<td>9 (34.6%)</td>
<td></td>
</tr>
<tr>
<td>10 to 12</td>
<td>26 (100%)</td>
<td>17 (65.4%)</td>
<td>0.017</td>
</tr>
<tr>
<td><strong>Primary Tumor Stage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1a</td>
<td>0 (0%)</td>
<td>5 (19.2%)</td>
<td></td>
</tr>
<tr>
<td>T1b</td>
<td>4 (15.4%)</td>
<td>3 (11.5%)</td>
<td></td>
</tr>
<tr>
<td>T2a</td>
<td>2 (7.7%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>T2b</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>T3a</td>
<td>17 (65.4%)</td>
<td>17 (65.4%)</td>
<td></td>
</tr>
<tr>
<td>T3b</td>
<td>3 (11.5%)</td>
<td>1 (3.8%)</td>
<td></td>
</tr>
<tr>
<td>T3c</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0.022</td>
</tr>
<tr>
<td><strong>N Stage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>24 (92.3%)</td>
<td>25 (96.2%)</td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>2 (7.7%)</td>
<td>1 (3.8%)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>M Stage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>26 (100%)</td>
<td>25 (96.2%)</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>0 (0%)</td>
<td>1 (3.8%)</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>RECIST v1.1 Criteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete Response (n, %)</td>
<td>0 (0.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial Response (n, %)</td>
<td>9 (34.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable Disease (n, %)</td>
<td>17 (65.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progressive Disease (n, %)</td>
<td>0 (0.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Preoperative adverse events (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>9 (34.6%); 1 (3.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>10 (36.5%); 3 (11.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transaminitis</td>
<td>1 (3.8%); 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>5 (19.2%); 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand Foot Syndrome</td>
<td>6 (23.1%); 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td>1 (3.8%); 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucositis</td>
<td>3 (11.5%); 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taste Change</td>
<td>3 (11.5%); 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td>0; 1 (3.8%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusions
In this interim analysis, neoadjuvant Axitnib resulted in significant reductions in tumor size and complexity, enabling partial nephrectomy in a cohort of highly complex renal masses, and with acceptable safety and functional preservation. Accrual is ongoing to reach a target of 50.

Keywords: Renal Cell Carcinoma; Partial Nephrectomy

CDMRP DOD Funding
no

Machine learning modeling to assign patients to biologically driven RNA sequencing-based subtypes for the OPTIC prospective phase II clinical trial in renal cell carcinoma

Dr Anupama Reddy PhD¹, Dr Scott Haake MD, PhD², Dr Kathryn Beckermann MD, PhD², Dr Adnan Derti PhD¹, Dr Kimryn Rathmell MD, PhD², Dr Brian Rini MD²

¹Vindhya Data Science, Morrisville, NC, USA. ²Vanderbilt University Medical Center, Nashville, TN, USA

Background
RNA sequencing has shown promise in defining individual renal cell carcinoma patient’s tumor biology. However, these biomarkers have not yet been translated to the clinic for prospectively assigning optimal treatments to patients. Challenges for RNA-seq biomarker development include translating classifiers across different assays/platforms, normalization of data collected from single patients in the clinic and establishing robust thresholds for assigning prediction groups. We have designed a prospective phase II clinical trial to test the utility of an RNA-seq based biomarker in predicting treatment based on biologic drivers relevant to angiogenesis, immune microenvironment, and other biological features. This RNA-seq biomarker is based on clusters previously described in the IMmotion 151 dataset. Here, we will describe the development and optimization of a machine learning model for assigning individual patients to biologically driven clusters in real time to facilitate RNA-seq based biomarker trials.

Methods
We have utilized RNA-seq data from IMmotion 151 (N=823) to develop a machine learning model. IMmotion 151 clusters were grouped into three based on their association to treatment: (1) Cluster 1+2 (anti-angiogenesis), (2) Cluster 4+5 (immunotherapy), (3) Cluster 3+6 (other biologic drivers). Random forest classifier was used to train a multi-class model to predict the three groups. We have
addressed the challenge of data normalization to perform predictions on single patients by transforming the gene expression space to ratios of genes. By computing ratios of genes, we are comparing relative levels of a patient’s gene expression, which enables translation of the classifier to individual patients. Performance metrics such as accuracy, sensitivity and positive predictive value are computed for the predictions. The model is evaluated using bootstrapped cross-validation performed 50 times. Hyper-parameters were tuned using nested cross-validation to optimize the performance of the model.

Results

Our machine learning classifier was built using 188 genes (11,772 gene expression ratios) and has a cross-validation accuracy of 85% and sensitivity of >90% in predicting patients into one of the three biological clusters groupings from our training data. Predictions of the classifier are significantly associated with progression-free survival across different treatments (atezolizumab/bevacizumab vs. sunitinib) within each of the predicted groups. We also observed significant odds ratios when comparing responders (CR/PR/SD) to non-responders (PD) across the treatment groups. The model was then applied to an independent test set of 54 clear cell renal cell carcinoma (ccRCC) patients treated with anti-angiogenesis+immunotherapy (Angio+IO) or immunotherapy (IO). The heatmap shows gene expression data across the 54 ccRCC patients with the top bars indicating the predicted clusters from our machine learning model, treatment arm and response to therapy. We observed that gene expression patterns in each of the clusters are consistent with model predictions. Additionally, there is a significant enrichment of responders to Angio+IO treatment for the predicted Cluster_Angio patients compared to IO treatment (p=0.05), supporting the hypothesis that cluster-directed therapy can improve clinical outcome.

Conclusions

We have developed an accurate machine learning model to assign individual patients to IMmotion 151 RNA-seq clusters in real time. This classifier will facilitate the prospective OPTIC RCC trial (NCT NCT05361720). If successful, our biomarker strategy will serve as a proof of concept for selecting biologic-based treatment for RCC patients.

Keywords: Biomarkers, RNA-sequencing, machine learning, ccRCC

CDMRP DOD Funding

yes
Evaluation of Growth Rates for Small Renal Masses in Elderly Patients Undergoing Active Surveillance

Dr Ridwan Alam MD, MPH, Ms Emelia Watts, Mr Ayman Alam MSc, Dr Sunil Patel MD, MSc, Dr Mohamad Allaf MD, MBA, Dr Phillip Pierorazio MD, Dr Nirmish Singla MD, MSCS

1Johns Hopkins University School of Medicine, 2The Dr Kiran C. Patel College of Allopathic Medicine at Nova Southeastern University, 3Oakland University William Beaumont School of Medicine, 4Perelman School of Medicine at the University of Pennsylvania

Background
Emerging data suggests that tumor microenvironments may differ among patients based on age, which can be of concern to patients who are enrolled in active surveillance (AS) for a prolonged period. We sought to examine if particular age cutoffs were associated with an increased growth rate (GR) for patients enrolled in AS for small renal masses (SRMs).

Methods
The Delayed Intervention and Surveillance for Small Renal Masses registry is a prospective, multi-institutional study examining outcomes in patients undergoing AS for SRMs. Two definitions of GR were examined: growth rate from the initial image (GRi) and growth rate from the prior image (GRp). Image measurements were dichotomized based on patient age at the time of imaging. Multiple age cutoffs were examined: 65, 70, 75, and 80 years. Mixed effects linear regression examined associations between age and GR, with controlling to account for multiple measurements from the same individual.

Results
We examined 2542 measurements from 571 patients. The median age of patients at enrollment was 70.9 years (IQR 63.2-77.4) with a median tumor diameter of 1.8 cm (IQR 1.4-2.5). When examined as a continuous variable, age was not associated with GRi (-0.0001 cm/year, 95% CI -0.007 – 0.007, P=0.97) or GRp (0.008 cm/year, 95% CI -0.004 – 0.020, P=0.17) after adjustment. The only age thresholds associated with an increased GR were 65 years for GRi and 70 years for GRp [Table]. Patients who underwent delayed intervention had increased GRi (0.282 cm/year, 95% CI 0.078 – 0.487, P=0.007) but no difference in GRp (0.319 cm/year, 95% CI -0.032 – 0.670, P=0.07) compared to those who remained on AS.

Conclusions
Patients with SRMs on AS do not exhibit a clear association between tumor GR and patient age, suggesting that AS is a safe and durable management option for aging patients with SRMs.

Keywords: active surveillance, small renal mass, growth rate, elderly age
Optimal Treatment by Invoking biologic Clusters in Renal Cell Carcinoma (OPTIC RCC): A prospective two arm phase II trial utilizing a novel RNA sequencing-based biomarker to assign treatments

Dr Scott Haake MD, Dr Katy Beckermann MD/PhD, Dr Yu-Wei Chen MD, Dr Yu Shyr PhD, Dr Anupama Reddy PhD, Dr Adnan Derti PhD, Dr Jennifer Gordetsky MD, Dr W Kimryn Rathmell MD/PhD, Dr Brian Rini MD

1Vanderbilt University Medical Center. ²Vindhya Data Science

Background

There are several approved and/or emerging therapies in renal cell carcinoma. However, the field lacks molecular biomarkers to optimally match therapies to patients, thus preventing true precision medicine for kidney cancer patients. As part of sustained efforts to identify such biomarkers, our group participated in a multi-omics evaluation of tumors from patients enrolled in the phase III IMmotion 151 trial of the immuno-oncology (IO)/anti-angiogenesis combination of atezolizumab plus bevacizumab versus the anti-angiogenesis tyrosine kinase inhibitor (TKI) sunitinib. This study used RNA sequencing(seq)-based gene expression technology to identify seven tumor clusters with unique biology and differential response to treatment, including those driven predominantly by angiogenesis (clusters 1 and 2), others showing increased expression of inflammatory and/or proliferative pathways (clusters 4, 5 and 7), and poor prognosis subgroups with high myeloid and/or low T-effector (Teff) gene expression patterns (clusters 3 and 6). Clinical outcome varied according to cluster designation, with cluster 1/2 tumors with improved outcomes overall (as both arms contained an anti-angiogenic agent) and cluster 4/5/7 tumors having significantly superior outcomes with the IO-containing regimen. Clusters 3 and 6 had the worst clinical outcomes, likely because neither the IO nor the anti-angiogenesis agents targeted underlying biological drivers. Based on this data, we hypothesize that RNA seq analyses can be used to identify biologic drivers of ccRCC in individual patients and improve clinical outcomes by matching therapy to the biology of an individual patient’s tumor. The current proposal will test this hypothesis by prospectively evaluating gene expression in ccRCC tumors, assigning tumors to IMmotion 151 clusters, and using this cluster assignment to choose an FDA-approved first line therapy for patients with metastatic ccRCC.

Methods

This phase II multicenter trial contains two arms, each based on a Simon’s MinMax two-stage design. We will enroll patients with the following inclusion criteria: advanced RCC with clear cell component, no prior systemic therapy for RCC, KPS >/= 70%, measurable disease, and tissue (excluding bone) available for RNA seq. Metastatic and primary tumors will be submitted for RNA seq at a central site and assigned to IMmotion 151 clusters based on gene expression analyses (preference given to the metastatic tumor). Patients whose tumors are assigned to the angiogenesis-high clusters 1 or 2 will receive the TKI-containing regimen of cabozantinib plus nivolumab (n=28). Patients whose tumors are assigned to the IO-responsive clusters 4 or 5 will receive the pure IO strategy of ipilimumab plus nivolumab (n=26). Cluster 3/6/7 tumors will not be included. Those patients will be treated per the discretion of their medical oncologist and clinical outcome
data collected on a separate IRB-approved protocol for later analysis. Patient outcomes will be compared to unselected historical controls from the registration phase 3 trials. The primary endpoint will be overall response rate per RECIST 1.1. Biological correlates include RNA seq on both primary and metastatic tissue so as to evaluate tumor heterogeneity and IMmotion 151-cluster concordance rates between the two tissue types. In addition, peripheral blood will be collected for eventual analysis of multiple biological correlates including peripheral blood mononuclear cells and cell free DNA at baseline, after 1 cycle of therapy, after three months of therapy, and at end of therapy. Enrollment is expect to begin in Fall 2022. This study is funded by the DOD/CDMRP Clinical Trial Award.

Results
N/A; Clinical TIPS

Conclusions
N/A; Clinical TIPS

Keywords: renal cell carcinoma, biomarker, RNA sequencing, gene expression

CDMRP DOD Funding
yes
32
VHL-mediated ubiquitination of the kinase Mps1 regulates the mitotic checkpoint in clear cell renal cell carcinoma

Dr Mark Woodford PhD1,2,3, Dr Sarah Backe PhD1, Dr Rebecca Sager MD/PhD1, Dr Oleg Shapiro MD1, Dr Imad Nsouli MD1, Laura Wengert BS1, Dr Gennady Bratslavsky MD1,2,3, Dr Dimitra Bourboulia PhD1,2,3, Professor Mehdi Mollapour PhD1,2,3

1Department of Urology, SUNY Upstate Medical University, Syracuse, NY, USA. 2Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY, USA. 3Upstate Cancer Center, SUNY Upstate Medical University, Syracuse, NY, USA

Background
Abnormal chromosome segregation during mitosis causes aneuploidy. This hallmark of cancers is associated with a high risk for tumorigenesis, which is normally regulated by the mitotic checkpoint. Mps1 kinase activity is essential for spindle checkpoint signaling. Mps1 is over-expressed in clear cell renal cell carcinoma (ccRCC) and requires the molecular chaperone Hsp90 for its activity. Mps1 phosphorylates Hsp90, regulating chaperone function of numerous oncogenic client proteins, including Mps1, and conferring tumor selectivity of Hsp90 inhibitors in ccRCC. The most frequent alteration leading to ccRCC is loss of von Hippel Lindau (VHL), the recognition subunit of an E3-ubiquitin ligase complex that targets proteins for degradation. As Mps1 is over-expressed in ccRCC, the objective of the current study was to determine whether Mps1 is targeted for degradation by VHL and whether this regulates the mitotic checkpoint.

Methods
VHL-mediated ubiquitination of Mps1 was examined in vitro and VHL-dependent degradation of Mps1 was examined in ccRCC cell lines by Western blot. Site-directed mutagenesis was utilized to determine Mps1 ubiquitination sites and the effect of increased Mps1 stability on mitotic checkpoint was determined using flow cytometry.

Results
Mps1 kinase is ubiquitinated by a VHL containing E3-ubiquitin ligase complex and Mps1 activity is essential for its ubiquitination. Re-expression of VHL in VHL-null ccRCC cell lines leads to proteasomal degradation of Mps1. VHL degrades Mps1 in an oxygen independent manner by ubiquitination of Mps1-K86, K827, and K848. Mps1 ubiquitination regulates cell cycle progression via exit from the mitotic checkpoint.

Conclusions
Mps1 is targeted for degradation by the tumor suppressor VHL in a hypoxia-independent manner and Mps1 is over-expressed in VHL-null ccRCC. VHL-mediated ubiquitination of Mps1 regulates mitotic checkpoint progression. Mps1 stability additionally mediates Hsp90 post-translational modification and tumor selectivity of Hsp90 inhibitors.

Keywords: ccRCC, VHL, Hsp90, cell cycle, aneuploidy, phosphorylation, chaperone, ubiquitination

33
No Difference in Renal Function Outcomes for Patients with Oncocytoma Managed with Active Surveillance vs. Partial Nephrectomy

Spencer Bell MD, Kevin Ginsburg MD, MS, Alexander Kutikov MD

Department of Surgical Oncology, Division of Urologic Oncology Fox Chase Cancer Center, Temple University Health System, Philadelphia, PA, USA

Background
A recent publication investigating the association of partial nephrectomy (PN) and active surveillance (AS) with renal function in patients with oncocytomas suggested patients’ longitudinal renal function may benefit from management with resection over surveillance, presumably by negating potential future effects of a growing renal mass on renal parenchymal loss. This finding is incongruent with existing literature which suggests AS and PN have similar renal function outcomes when considering malignant and other benign lesions. As such, we evaluated our institutional experience with patients harboring oncocytoma managed with AS or PN for potential differences in longitudinal renal function between these management strategies.

Methods
We reviewed our institutional database for patients with biopsy/surgically confirmed oncocytoma from 2000-2020. The primary outcome was to assess for differences in renal function outcomes in patients undergoing AS vs. PN. We fit a multivariable generalized estimating equation (GEE) with an interaction term between follow up time and management strategy to predict mean estimated Glomerular Filtration Rate (eGFR) for patients managed with AS or PN for potential differences in longitudinal renal function between these management strategies.
Disease (CKD) stage III or greater for patients managed with AS vs. PN for patients with a baseline eGFR >60 mL/min/1.73m2.

Results
We identified 114 patients with biopsy or surgically confirmed oncocytoma, of which 32 were managed with AS. Median follow up was 21 months vs. 44 months for PN vs. AS patients. AS patients tended to be older (median: 72 years vs. 65 years, p<0.001) and have lower baseline renal function (median: eGFR: 71 mL/min/1.73m2 vs. 82 mL/min/1.73m2, p<0.001) compared with PN patients. Renal mass size from baseline imaging was similar between groups (2.8 cm vs. 2.9 cm, p=0.634). For patients undergoing PN vs. AS, there was not a significant difference in predicted longitudinal eGFR (interaction term: -0.079, 95% CI -0.18-0.023, p=0.129). Additionally, the predicted probability of progression to CKD stage III or greater was not significantly different for patients managed with PN or AS (interaction term: OR: 0.61, 95% CI: 0.16-2.33, p=0.47).

Conclusions
AS and PN for patients with an oncocytoma in our cohort was associated with a change in renal function and probability of progression to CKD stage III or greater. Management with partial nephrectomy or active surveillance should be considered equivalent when considering long term effect on renal function in patients with oncocytomas and surgery should be reserved for selected cases of known oncocytoma.

Keywords: Active Surveillance; Partial Nephrectomy; Oncocytoma; Renal Function

34 Monitoring Disease Burden and Biology Using Tumor Cell Free DNA in Metastatic Kidney Cancer

Dr Scott Haake MD, PhD1, Dr Katy Beckermann MD/PhD1, Rebecca Prather1, Dr Naomi Haas MD2, Dr Ulka Vaishampayan MD3, Dr Hans Hammers MD/PhD4, Madelyn Landis1, Dr Jacob Berchuck MD5, Dr Matthew Freedman MD5, Dr Daniel George MD4, Dr David McDermott MD7, Dr Ben Ho Park MD/PhD3, Dr Kimryn Rathmell MD/PhD1, Dr Brian Rini MD1, Dr Eric Johnasch MD8

1Vanderbilt University Medical Center. 2University of Pennsylvania. 3University of Michigan. 4UT-Southwestern Medical Center. 5Dana-Farber Cancer Institute. 6Duke University. 7Beth Israel Deaconess Medical Center. 8MD Anderson Cancer Center

Background
While metastatic kidney cancer is nearly universally fatal, immuno-oncology (IO) strategies utilizing immune checkpoint inhibitors have revolutionized the care of these patients. However, significant challenges persist in caring for these patients. For example, the only validated method for assessing IO tumor response is imaging to measure the tumor size, typically computerized tomography (CT) scans every 2-3 months. However, tumors infiltrated with immune cells can “grow” despite a potent anti-tumor response (i.e., pseudo-progression). In addition, there may be a significant lag time between when tumors develop IO-resistance and tumor growth is observed on imaging. This “lag” is a lost opportunity to intensify and/or change therapies. Other problems with disease monitoring are rapidly emerging. For example, the field needs sensitive assays that can detect active cancer that is below the threshold of detection for conventional imaging. Such assays could help clinical decision making regarding the use of potential IO-based adjuvant treatments as well as determining when it is safe to withdraw IO therapy in patients with complete or near-complete radiographic response. Therefore, a clear need exists for new methods to measure tumor burden in kidney cancer patients.

One approach to garnering “real time” information regarding the burden and biology of metastatic tumors is the use of “liquid biopsies”. Blood is an easily accessible source of tumor cell free (cf) DNA. Both normal and malignant cells shed DNA into the circulation, and advanced genomics technologies can analyze tumor cfDNA, making blood a source of real-time genomic tumor profiling. The goal of the current project is utilize Next Generation Sequencing
(NGS) technologies to evaluate tumor cfDNA in clear cell renal cell carcinoma patients receiving immunotherapy for metastatic disease as a method to monitor disease burden overtime.

Methods
To achieve this goal, we are collaborating with the DOD-funded Kidney Cancer Research Consortium (KCRC, PI = Eric Jonasch, MD) to accrue patients to this observational clinical trial. We are currently accruing patients at six sites including Vanderbilt University Medical Center, the Nashville VA Hospital, MD Anderson Cancer Center, UT-Southwestern, University of Pennsylvania and University of Michigan with plans to add Beth Israel Deaconess soon. Whole blood samples are collected in Streck tubes at baseline as well as at three months, six months, and time of progression or two years (whichever comes first) with goal accrual of 150 patients. Samples are shipped to Vanderbilt for central processing. This study is funded by the CDMRP Kidney Cancer Research Program.

Results
To date, we have enrolled and collected baseline samples for 101 patients. In addition, we have collected a three month sample from 65 patients, a six month sample from 33 patients, and a time of progression sample from 4 patients. We have collected a mean whole blood volume of 23.67 mL (+/- SD 10.01 mL) and a mean plasma volume of 9.87 mL (+/- SD 3.36 mL). From that plasma, we have extracted a mean cfDNA quantity of 124.1 ng (+/- SD 162.9) and a median of 74.2 ng (25th percentile 50.6, 75th percentile 127.3). In terms of assays, we have performed 25X whole genome sequencing on 52 samples from 25 patients as well as two healthy controls as we develop novel techniques to measure tumor fraction in cfDNA extracted from patient plasma. In addition, we continue to work to develop collaborations with investigators with expertise in 1) cell free methylated DNA immunoprecipitation and sequencing as well as 2) personalized and tumor-informed somatic sequencing of tumor cfDNA as we seek out leading technologies to measure tumor fraction in these samples. Pilot studies with these techniques are on-going as we continue to accrue patients and collect samples.

Conclusions
N/A; Clinical TIPS

Keywords: renal cell carcinoma, cell free DNA, immunotherapy, next generation sequencing

CDMRP DOD Funding
yes

Role of Histology in Influencing Outcomes after Cytoreductive Nephrectomy for Metastatic Renal Cell Carcinoma

Pranjal Agrawal MD, Ridwan Alam, Joseph Cheaib, Lisa Young, Gaurish Agrawal, Sunil Patel, Anirudh Yerrapragada, Maximilian Pallauf, Philip Pierorazio, Mohamad Allaf, Yasser Ged, Nirmish Singla

1Johns Hopkins University School of Medicine, Baltimore, Maryland. 2The James Buchanan Brady Urological Institute at Johns Hopkins, Baltimore, Maryland. 3Silver Creek High School, San Jose, California. 4Department of Urology, Penn Presbyterian Medical Center, Philadelphia, Pennsylvania.

Background
Systemic therapy for metastatic RCC (mRCC) has evolved considerably, together with the role and timing of cytoreductive nephrectomy (CN), which has been a moving target. Though RCC is characterized by several histological subtypes, most studies evaluating the role of CN have been conducted primarily in patients with clear cell RCC (ccRCC), while evidence for CN in patients with non-clear cell RCC (nccRCC) remains scarce. Thus, we sought to characterize perioperative and survival outcomes among patients who underwent CN for mRCC based on their histologic subtype.

Methods
We identified patients with mRCC who received CN at our institution between 1996-2021. Patients undergoing CN were stratified by histologic subtype (ccRCC or nccRCC). Baseline clinicopathologic characteristics were compared between groups using independent-sample Mann-Whitney U and chi-squared tests. Differences in overall survival (OS) between groups were assessed using the Kaplan-Meier method. Independent predictors of OS were identified using univariable and multivariable Cox regression analyses.

Results
Of 155 patients identified, 126 (81.3%) had ccRCC, and 29 (18.7%) had nccRCC. Compared to ccRCC, patients with nccRCC were more likely to be Black (27.6 vs 3.2%, p<0.001), present with preoperative symptoms (51.7% vs 21.4%, p=0.020), and have lower primary tumor stage (p = 0.046). There were no observed differences in baseline comorbidities (p=0.68), cN stage (p=0.06), or number of metastatic sites (p=0.55) between the cohorts. Patients with nccRCC were less likely to receive systemic therapy than patients with ccRCC (48.3% vs 73.8%; p=0.006), though the timing of therapy with respect to CN did not differ between groups (p=0.16). Perioperative and postoperative...
outcomes were similar between groups, with no differences in estimated blood loss (p=0.20), intraoperative complications (p=0.51), hospital length of stay (p=0.59), or 90-day readmission rates (p=0.30). After a median follow-up of 20.9 months, 91 deaths were observed overall with no significant differences in OS between ccRCC and nccRCC patients (median OS 2.7 years vs. 1.4 years, respectively, p=0.102; Figure 1). On Cox-regression analysis, histologic subtype was not associated with OS, while sarcomatoid features and higher metastatic burden were the strongest predictors for worse OS on multivariable analysis.

Conclusions

nccRCC histology does not negatively impact perioperative or survival outcomes after CN for metastatic RCC compared to those with ccRCC. While patient selection remains paramount to determining eligibility for CN, our results suggest that histologic subtype alone should not be an exclusionary factor to offer CN to otherwise appropriately selected patients at high-volume, experienced centers.

Keywords: clear-cell renal cell carcinoma; non-clear cell renal cell carcinoma; histology; cytoreductive nephrectomy

CDMRP DOD Funding

no
elucidated, given the paramount role of systemic therapy in controlling disease. Patients with IVCTT in the setting of mRCC pose an even greater challenge, as effectively treating the IVCTT while avoiding the progression of metastases must be counterbalanced. Herein, we sought to characterize perioperative and survival outcomes in patients with mRCC undergoing CN in presence of IVCTT.

Methods
We identified patients with mRCC who underwent CN at our institution between 1996-2021. Patients undergoing CN were stratified by the presence or absence of IVCTT. Baseline clinicopathologic characteristics were compared between groups using independent-sample Mann-Whitney U and chi-squared tests. Differences in overall survival (OS) between groups were assessed using the Kaplan-Meier method. Independent predictors of OS were identified using univariable and multivariable Cox regression analyses.

Results
Of 152 patients who underwent CN, 26 (17.10%) exhibited IVCTT. Compared to patients without IVCTT, those with IVCTT were more likely to have more baseline comorbidities (CCI score of 9 vs 8, p<0.001), larger primary tumor size (11.5 cm vs 8.5 cm; p<0.001), and sarcomatoid features (30.8% vs 8.7%, p<0.001), while the presence of rhabdoid features was less prevalent (0% vs 10.3%, p=0.035). Rates of systemic therapy administration, the timing of systematic therapy with respect to CN, and the number of metastatic sites did not differ between the two cohorts. All patients with IVCTT underwent an open approach, whereas 69% of patients without IVCTT underwent a minimally-invasive CN. Patients with IVCTT had increased estimated blood loss (1000 mL vs 250 mL, p<0.001) and length of hospital stay (5.5 days vs 3 days, p=0.022) compared to patients without IVCTT, while rates of intraoperative complications (p=0.085) and 90-day readmission (p=0.97) were similar between groups. After a median follow-up of 22.7 months, 87 deaths were observed with no significant differences in OS between patients with and without IVCTT (median OS 2.1 years vs. 2.8 years, respectively, p=0.110; Figure 1). On Cox-regression analysis, the presence of IVCTT was not significantly associated with OS.

Conclusions
Although the need to perform IVC tumor thrombectomy at the time of CN is inherently associated with increased surgical morbidity, we did not observe increased perioperative complication rates or worse OS in patients undergoing CN in the presence of IVCTT compared to those without IVCTT. Patient selection and the timing of surgery with respect to systemic therapy administration must be carefully weighed in a multidisciplinary fashion at high-volume, experienced centers in order to effectively treat the IVCTT while avoiding the progression of metastases.

Keywords: cytoreductive nephrectomy; inferior vena cava tumor thrombus; metastatic renal cell carcinoma; systemic therapy
Vascular endothelial profilin-1 Inhibition suppresses tumor progression in renal cancer

David Gau PhD¹, Abigail Allen¹, Andrew Daoud¹, Jessica Kunkel¹, Anurag Paranjape PhD², Flordeliza Villanueva MD², Partha Roy PhD¹

¹University of Pittsburgh. ²UPMC

Background
Clear-cell renal cell carcinoma (ccRCC) is the most common subtype of renal cancer with poor patient prognosis. A distinguishing hallmark of ccRCC is the highly vascularized tumor microenvironment caused by loss of the Von-Hippel Lindau (VHL) gene. Current anti-angiogenic therapies, while initially effective, do not display long-term therapeutic benefit with almost all patients developing drug-resistant disease. We previously discovered dramatic transcriptional upregulation of actin-binding protein profilin-1 (Pfn1) in tumor-associated vascular endothelial cells (VEC) and higher expression of Pfn1 correlated with poor patient outcome in human ccRCC. The goal of the present study was to further investigate whether vascular endothelial Pfn1 promotes tumor progression in renal cancer.

Methods
To induce wide-spread loss of Pfn1 function selectively in VEC, we engineered mice with tamoxifen-inducible excision of the Pfn1 gene by Cre driven by CDH5-promoter. For restricting loss of vascular endothelial Pfn1 to kidney only, we performed sub-capsular injection of adenovirus encoding Cre (Ad-cre) driven by CDH5 promoter, with Ad-GFP administration serving as control. Orthotopic tumor was established by sub-capsular injection of RCC cells in the kidney of syngeneic immunocompetent mice. Tumor angiogenesis and apoptosis of cancer cells were assessed by CD31- and TUNEL-staining of tumor histosections. Novel inhibitor of the Pfn1-actin interaction (Pfn1i) was discovered by computationally guided biochemical screen of small molecules followed by structure-activity relationship assays to identify improved analogs.

Figure 1. Loss of endothelial Pfn1 inhibits tumor progression in orthotopic model of renal cancer. A) Representative H&E staining of renal tumors (panel A) harvested on day 28 from Pfn1+++/+ (wild-type) vs Pfn1---/- (VEC) animals - Tumors were allowed to form by subcapsular injection of RCC cells in mouse kidney for 7-10 days before initiating tamoxifen-mediated excision of the Pfn1 gene in VEC. Pfn1---/- (VEC) mice exhibit dramatic defect in tumor angiogenesis and tumor growth relative to Pfn1+++/+ animals. B) Quantification of tumor burden data based on the assessment of weight of tumor-bearing relative to contralateral kidneys between the two group of animals (* p < 0.05, N = 8 animals/group). C) Representative H&E staining of lung histosections show dramatic difference in overall metastatic burden between the two groups of animals.
Results
We found that triggering endothelial Pfn1 deletion, either globally or restricted to kidney only, dramatically inhibits tumor formation and metastatic dissemination from syngeneic transplants of RCC cells. Loss of endothelial Pfn1 led to a major suppression of tumor angiogenesis ensuing massive tumor cell death. In a delayed induction setting, loss of endothelial Pfn1 also retarded progression of pre-established tumors (Figure 1). Consistent with these genetic proof-of-concept findings, we further demonstrated capability of Pfn1i to inhibit aggressiveness of RCC cells in vitro, reduce tumor angiogenesis and tumor growth in vivo. Furthermore, toward the goal of targeted delivery of Pfn1i in tumor microenvironment, we have successfully encapsulated Pfn1i into lipid microbubbles, and demonstrated proof-of-concept for prominent anti-angiogenic action by Pfn1i in cell culture setting released by ultrasound-mediated disruption of microbubbles. These findings lay the groundwork for our ongoing effort focused on localized ultrasound-guided delivery of Pfn1i in tumor microenvironment as a therapeutic strategy in mouse model of RCC.

Conclusions
Collectively, these findings establish endothelial Pfn1 as a driver of tumor progression as well as a potentially novel therapeutic target in RCC.

Keywords: Profilin1, renal cancer, clear cell renal cell carcinoma

Outcomes of patients with advanced variant histology renal cell carcinoma (RCC) treated with systemic therapy

Danielle Urman, Leah Deshler, Nicole Weise, Ithaar Derweesh, Aditya Bagrodia, Brent Rose, Rana R. McKay

University of California San Diego

Background
Patients with variant histology RCC and RCC with sarcomatoid dedifferentiation (SRCC) have historically demonstrated inferior outcomes to standard systemic therapy. While immune checkpoint inhibitors have demonstrated impressive activity for patients with clear cell RCC (ccRCC), data of the use of such therapies for patients with variant histology RCC are limited. The goal of this study was to evaluate the outcomes of patients with variant histology RCC and sRCC compared to those with ccRCC receiving systemic therapy.

Methods
We conducted a retrospective analysis of patients with histologically confirmed RCC with advanced or metastatic disease receiving systemic therapy at the University of California San Diego. Patients were divided into groups based on histology (non-clear cell RCC [nccRCC] vs ccRCC) and sRCC (sRCC vs variant RCC). The primary endpoint was overall survival for each group calculated from the time of systemic therapy initiation to last follow-up or death. Secondary endpoints included time to treatment failure defined as the time from treatment initiation to discontinuation for any reason censored at the date of last follow-up.

Results
Overall, 251 patients were included in the analysis of whom: 58 had variant histology RCC (14 had papillary RCC, 8 chromophobe, 29 unclassified, and 7 Xp translocation) and 193 had ccRCC. Overall 25 had sarcomatoid differentiation of whom 5 were variant histology and 20 were ccRCC. First line therapies included VEGF monotherapy (69.3%), IO monotherapy (7.6%), IO combination therapy (19.1%) or other (4.0%). Overall survival for nccRCC was 34.3 months (95% CI [24.8, 75.0]) compared to ccRCC of 81.1 months (95% CI [50.2, 107.2]) (Table 1). Time to first-line treatment failure for nccRCC was 4.8 months (95% CI, [3.8, 9.9]) compared to ccRCC of 8.2 months (95% CI [5.9, 10.2]). Overall survival for patients with sarcomatoid differentiation was 43.4 months (95% CI [18.6, INF]) compared to patients without sarcomatoid differentiation 75.0 months (95% CI [50.0, 100.5]). Time to first-line treatment failure for patients with sarcomatoid differentiation was 4.0 months (95% CI [2.2, 5.0]) compared to 8.6 months (95% CI [6.6, 10.2]) for patients without sarcomatoid differentiation.

Conclusions
In this analysis, consistent with prior studies, we demonstrate inferior outcomes among patients with variant histology RCC and those with sarcomatoid differentiation. Further prospective studies are warranted testing novel treatments and combination therapy in these patients who continue to represent an unmet need in the treatment of RCC.

Keywords: non-clear cell renal cell carcinoma, sarcomatoid differentiation, overall survival
Table 1. Treatment outcomes of patients with advanced variant histology RCC and sRCC receiving systemic therapy.

<table>
<thead>
<tr>
<th>Treatment outcomes</th>
<th>Time to first treatment failure</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of events/Total n=250*</td>
<td>Median (95% CI), months</td>
</tr>
<tr>
<td></td>
<td>Median (95% CI), months</td>
<td>No. of events/Total</td>
</tr>
<tr>
<td>Overall</td>
<td>199</td>
<td>7.1 (5.2, 9.0)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ccRCC</td>
<td>155</td>
<td>8.2 (5.9, 10.2)</td>
</tr>
<tr>
<td>nccRCC</td>
<td>44</td>
<td>4.8 (3.8, 9.9)</td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>differentiation in any histology type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>24</td>
<td>4.0 (2.2, 5.0)</td>
</tr>
<tr>
<td>Not present</td>
<td>175</td>
<td>8.6 (6.6, 10.2)</td>
</tr>
</tbody>
</table>

*1 observation with missing drug stop date or last follow up date was removed from time to first treatment failure analysis

+9 observations with missing metastasis diagnosis date were removed from overall survival analysis

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

Dr Toni Choueiri, MD

Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA

Background

Papillary renal cell carcinoma (PRCC) is the most common subtype of non-clear cell renal cell carcinoma (RCC) and accounts for 10–15% of RCCs. Many PRCC cases are MET-driven, characterized by genomic abnormalities resulting in dysregulation of the MET signaling pathway, making these abnormalities a potential therapeutic target for treatment. Savolitinib is an oral, potent and highly selective MET tyrosine-kinase inhibitor (TKI) demonstrating preliminary clinical activity in advanced solid tumors, including in MET-driven PRCC, defined as presence of any of the following molecular alterations, in the absence of co-occurring fumarate hydratase mutations: chromosome 7 gain, MET amplification, MET kinase domain variations, or hepatocyte growth factor amplification. In the Phase III SAVOIR study, in PRCC, savolitinib monotherapy showed encouraging efficacy vs the multi-targeted TKI, sunitinib. In addition, non-clinical studies suggest a possible synergistic anti-tumor effect of MET-inhibitors and programmed cell death-ligand (PD-L1) inhibitors, such as durvalumab; emerging data from the Phase I/II CALYPSO study investigating savolitinib plus durvalumab shows a notable efficacy signal in patients with MET-driven PRCC. The estimated prevalence of MET-driven status is 35–40% in PRCC. Following these findings, the SAMETA study (NCT05043090) is designed to evaluate the efficacy and safety of savolitinib in combination with durvalumab vs sunitinib and durvalumab monotherapy in PRCC.

Trial Schema

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

Current status
The first patient was enrolled onto the study on 28 October 2021. At the early enrollment stage, during the biomarker pre-screening, approximately 28% of patients with PRCC who submitted a sample had eligible MET-driven status and of these patients with MET-driven PRCC, approximately 70% also met other eligibility criteria and were randomized.

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

CDMRP DOD Funding
no

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Characterization of FH variants in their role in HLRCC-associated kidney cancer

Dr Blake R Wilde PhD¹, Nishma Chakraborty¹, Nedas Matulionis¹, Daiki Ueno MD², Stephanie Hernandez³, Dr Edward D. Esplin MD, PhD³, Dr Karen Ouyang PhD¹, Dr Keith Nykamp PhD³, Dr Brian Shuch MD², Dr Heather R Christofk PhD¹

¹Department of Biological Chemistry, University of California Los Angeles, Los Angeles, California. ²Department of Urologic Oncology, University of California Los Angeles, Los Angeles, California. ³Invitae, San Francisco, CA

Background
Loss-of-function mutations in fumarate hydratase (FH) result in accumulation of fumarate, a bona fide oncometabolite, which drives oncogenic signaling and transformation. Germline FH alterations lead to an autosomal dominant condition known as hereditary leiomyomatosis and renal cell cancer (HLRCC) which predisposes patients to an aggressive form of kidney cancer. FH alterations are more common than previously thought, with 0.6% of patients having a variant of unknown significance (VUS) and potentially increased risk of kidney cancer; thus, there is an unmet need to classify FH variants by their cancer-associated risk, advising screening to enable early diagnosis and treatment.

Methods
We sought to predict the pathogenicity of FH variants by quantifying catalytic efficiency in cell-free assays. We produced and purified patient-derived recombinant FH variants and used enzymatic assays to quantify their catalytic efficiencies. Since FH forms homotetramers with 4 active sites for fumarate/malate-binding and catalysis, we analyzed multimerization status of each VUS as well.

These VUS also serve as a catalogue for interrogating fumarate-dependent changes in HLRCC cells. To gain insight into the metabolic consequences of fumarate accumulation, we generated a panel of HLRCC cell lines expressing FH variants with a range of catalytic activities. We then used liquid chromatography-mass spectrometry (LC-MS) to investigate interactions between fumarate accumulation and other metabolic pathways.

Results
Of the 74 VUS analyzed in the cell-free assays, nearly half were enzymatically inactive, indicating that these variants are likely pathogenic. The abundance of tetramers correlated, although weakly, with catalytic efficiencies, which is consistent with the notion that tetramerization is a prerequisite for enzymatic efficiency. While disrupting tetramerization appears to be sufficient to restrict catalytic activity, multimerization status alone has limited value for predicting catalytic activity and associated pathogenicity.

Our panel of cell lines expressing FH variants with a range of catalytic activities presented an opportunity to investigate how minor alterations in FH activity and fumarate levels affect HLRCC metabolism. We found that fumarate is an inhibitor of adenylosuccinate lyase (ADSL), a lyase that catalyzes multiple steps of de novo purine biosynthesis. Our data indicate that FH-deficient cells, which accumulate fumarate, rely on purine salvage for nucleotide biosynthesis, thus suggesting a potential targetable metabolic vulnerability in HLRCC-associated kidney cancer. Consistent with this idea, FH-deficient cells are more sensitive to 6-mercaptopurine (6-MP), an inhibitor of de novo purine biosynthesis and the purine salvage pathway.

Conclusions
Our investigation of 74 FH VUS will inform clinical decision making for patients harboring these VUS. Further, we find that HLRCC cells are reliant on the purine salvage pathway and have increased sensitivity to 6-mercaptopurine, suggesting a new rapidly translatable HLRCC treatment strategy.

Keywords: HLRCC, fumarate hydratase, metabolism, nucleotide biosynthesis
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Pro-survival role of protein phosphatase-5 (PP5) in clear cell renal cell carcinoma through its post translational regulation

Rebecca A Sager MD, PhD, Elham Ahanin, Sarah J Backe, Natela Dushukyan, Michael Daneshvar, Gennady Bratslavsky, Mark R Woodford, Dimitra Bourboulia, Mehdi Mollapour

1Department of Urology, Upstate Medical University, Syracuse, NY, USA. 2Department of Biochemistry and Molecular Biology, Upstate Medical University, Syracuse, NY, USA. 3Department of Urology, University of California Irvine, Orange, CA, USA

Background

The serine/threonine protein phosphatase-5 (PP5) plays a key role in the regulation of both hormone- and stress-induced signaling networks that allow cells to respond appropriately to stress. The majority of PP5 substrates are in complex with the molecular chaperone heat shock protein-90 (Hsp90) and include the glucocorticoid receptor (GR), tumor suppressor p53, and the co-chaperone Cdc37. Classically, PP5 interaction with Hsp90 is necessary for the phosphatase to be active through conformational release of its autoinhibition. Hsp90 and its co-chaperones are also, however, subject to post-translational modifications (PTMs) that regulate their activity. Through dephosphorylation of Cdc37, PP5 regulates the chaperoning of many oncogenic kinases, and a role for PP5 in survival in breast and colorectal cancer has been implicated previously. We have shown increased levels of PP5 and increased PP5 activity in clear cell renal cell carcinoma (ccRCC) in both cell lines and primary tumor samples. The majority of ccRCC exhibits loss of the tumor suppressor von Hippel Lindau (VHL), the recognition subunit of an E3 ubiquitin ligase complex. We hypothesized that PP5 hyperactivity plays an important role in RCC.

Methods

A phosphorylation site that regulates PP5 activity and VHL-mediated ubiquitination sites on PP5 were identified by site-directed mutagenesis followed by in vitro and in vivo functional assays. The effect of PP5 on survival of ccRCC cell lines was analyzed by Western blot, flow cytometry, and proliferation and colony formation assays.

Results

Mechanistically, we demonstrate PP5 undergoes ubiquitination on PP5-K185/K199 and proteasomal degradation in a VHL-dependent manner as shown through biochemical and cell-based assays. Interestingly, unlike classic VHL targets this occurs in a hypoxia- and prolyl hydroxylation-independent manner. PP5 is further activated in ccRCC by phosphorylation of PP5-T362 by casein kinase-1 δ (CK1δ). While interaction with the molecular chaperone Hsp90 is needed for PP5 interaction with its substrates we additionally show that, unlike previously suggested, PP5 activity and conformation are not directly correlated. Lastly, down-regulation of PP5 by siRNA mediated silencing or blocking PP5 phosphorylation and activity via inhibition of CK1δ causes apoptosis in VHL deficient cells.

Conclusions

This pro-survival role of PP5 in VHL-deficient ccRCC suggests PP5 may serve as a potential therapeutic target in the treatment of this disease.

Keywords: PP5, ccRCC, VHL, post-translational regulation

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yes

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Investigating germline susceptibility to renal cell carcinoma within the Canadian population

Kate I Glennon BSc, Mikiko Endo BSc, Yoshiaki Usui MD, PhD, Yusuke Iwasaki MEng, Rodney Breau MSc, MD, FRCSc, Anil Kapoor MD, FRCSc, Simon Tanguay MD, FRCSc, Yukihide Momozawa DVM, PhD, Yasser Riazalhosseini PhD

1Department of Human Genetics, McGill University, Montreal, Canada. 2McGill Genome Centre, Montreal, Canada. 3Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan. 4University of Ottawa, Ottawa, Canada. 5McMaster University, Hamilton, Canada. 6Division of Urology, Department of Surgery, McGill University, Montreal, Canada

Background

There are large, unexplained, variations in the incidence of renal cell carcinoma (RCC) across the globe. Germline genetic variation contributes strongly to individual differences in susceptibility to cancer, however genetic risk factors for RCC are still poorly understood, indicating the need for large-scale studies investigating susceptibility to RCC among different populations. Large-scale studies investigating pathogenic germline variations in RCC have mostly been limited to individual populations (European, Japanese, etc.), which have shown contrasting results in
which genes harbor the most germline pathogenic variants associated to RCC. Additionally, many datasets are limited for studying differences between RCC subtypes due to sample size. This highlights the need to investigate genetic susceptibility to RCC among additional populations to further understand which risk-factors may be driving differences in RCC rates, and which pathogenic variants are associated to each subtype. We conducted an investigation into the genetic susceptibility to RCC within the Canadian population, and compared potential risk-genes to those identified within other populations.

**Methods**

We conducted targeted sequencing of 19 RCC-related and 27 cancer-predisposition genes in a cohort of 960 Canadian patients with RCC recruited through the Ontario Tumor Biobank. We called germline variants and identified pathogenic/likely pathogenic variants based on ClinVar classification and those predicted to be loss-of-function (LOF) mutations. Gene-based association tests were conducted between patients with RCC and non-cancer control data from the gnomAD public database (European, non-Finnish population). We compared the frequency of germline pathogenic variants between RCC patients and the control population, and patients were stratified into clear cell (ccRCC) and non-clear cell (nccRCC) groupings for the identification of risk genes.

**Results**

We identified germline pathogenic variants in 7.1% of patients (68/960), with no significant difference in the overall number of germline mutations between ccRCC (8.8%, 52/579) and nccRCC (8.0%, 16/201) patients. The most frequently mutated gene was CHEK2, with 69% of CHEK2 mutations being the c.1100delC variant (11/16 patients), which is an established breast cancer susceptibility allele, and has also been suggested to increase the risk of developing other cancers. Within ccRCC, germline pathogenic variants were most commonly found in CHEK2 (14), ATM (7), BRCA2 (6), and MITF (5). In patients with nccRCC, the most commonly mutated genes were FH (4), CHEK2 (2) and MSH6 (2). When investigating association to disease compared to the control population, CHEK2, ATM and MITF genes showed significant association to ccRCC. Additionally, FH and MSH6 showed significant association to nccRCC (Table 1).

We also conducted a burden analysis comparing genes showing significant disease risk to other large studies investigating susceptibility to RCC. We saw no significant difference in germline burden between Canadians and the European population (Yngvadottir et al.), which reflects the largely European-ancestry of the Canadian population. However, when compared to the Japanese population (Sekine et al.), Canadians had significantly more carriers of germline pathogenic variants in MITF and CHEK2, whereas the Japanese population had significantly higher burden in TP53.

**Conclusions**

This study serves as the first investigation into renal cancer susceptibility within the Canadian population. We demonstrate that germline pathogenic variants in CHEK2, MSH6, ATM, MITF and FH genes may be associated to risk of RCC within Canadians. Additionally, the prevalence of germline pathogenic variants in the Canadian population appears similar to that of the European population, indicating that studies of RCC-susceptibility within the European population may be able to apply for Canadians as well. These findings also provide further insight into differences in RCC-susceptibility around the world, and that investigation of germline risk genes in additional populations are needed to fully understand the heterogeneous susceptibility to RCC.

**Keywords:** Renal cell carcinoma, Germline susceptibility, Targeted sequencing

<table>
<thead>
<tr>
<th>Gene</th>
<th>ccRCC patients (%)</th>
<th>Control</th>
<th>Adjusted P-Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MITF</td>
<td>5 (0.66)</td>
<td>3 (0.003)</td>
<td>2.39 x 10-8</td>
<td>224.4 (43.7 - 1454.2)</td>
</tr>
<tr>
<td>CHEK2</td>
<td>14 (1.84)</td>
<td>474 (0.40)</td>
<td>8.59 x 10-5</td>
<td>4.7 (2.5 - 7.9)</td>
</tr>
<tr>
<td>ATM</td>
<td>7 (0.92)</td>
<td>259 (0.22)</td>
<td>0.035</td>
<td>4.2 (1.7 - 8.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>nccRCC patients (%)</th>
<th>Control</th>
<th>Adjusted P-Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH</td>
<td>4 (1.99)</td>
<td>12 (0.01)</td>
<td>1.60 x 10-7</td>
<td>199.1 (46.4 - 661.4)</td>
</tr>
<tr>
<td>MSH6</td>
<td>2 (1.00)</td>
<td>20 (0.02)</td>
<td>7.14 x 10-3</td>
<td>59.3 (6.7 - 246.3)</td>
</tr>
</tbody>
</table>

Table 1. Gene-association tests between ccRCC and nccRCC and gnomAD non-cancer control populations.
Evaluating the clinical utility of Circulating Tumor Cells (CTC) profiling to predict selection of preferred therapeutic regimens in Newly Diagnosed or Pretreated Refractory Renal Cell Carcinoma (RCC)

Dr Ulka Vaishampayan MD¹, Dr Darshana Patil MD², Prof Jeremy Taylor PhD², Mr Joe Dilb BS², Dr Ajjai Alva MD¹, Mrs Archana Adhav MS², Ms Anuja Mhalsekar MS², Dr Dadasaheb Akolkar PhD²

¹University of Michigan, Ann Arbor, USA. ²Datar Cancer Genetics Private Limited, Nasik, India

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

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

Results
44 patients have been enrolled; 10 females, 34 males. The study cohort included 2 African American, 1 Asian, 1 American Indian and 36 Caucasian patients. Details about race were not available for four patients. Median age was 64 years (range 40-85 years). 17 patients were untreated, 21 were pretreated and 6 were under adjuvant therapy. IMDC Risk Category revealed 15 patients with Intermediate, 11 patients with poor, 8 patients with Favorable risk category and data was not available for remaining patients. 42 of the 44 (95.5%) patients had detectable CTCs in the baseline sample. 21 on treatment samples have been collected to date, with detectable CTCs in all except one sample. 53 of 65 samples (81.5%) demonstrated detection of at least one biomarker by ICC. VEGFA was the most commonly detected biomarker in ICC (detected in 28 of 63 samples).

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

Keywords: Renal Cell Carcinomas, Circulating Tumor Cells, Non-invasive, Biomarkers

The study was supported by Datar Cancer Genetics Private Limited and Rogel Cancer Center Grant National Cancer Institutes of Health (Award Number P30CA046592).

Biomarkers of Disease Burden and Treatment Response in Renal Medullary Carcinoma

Dr Kyle Blum MD, MS

Division of Urology, University of Texas Houston McGovern Medical School, Houston, TX, USA. Department of Urology, MD Anderson Cancer Center, Houston, TX, USA

Background
Renal medullary carcinoma (RMC) is a devastating renal malignancy that predominately affects young persons of African descent with sickle hemoglobinopathies. RMC is
aggressive and resistant to therapies used routinely in other renal cell carcinomas. More than 90% of patients will be diagnosed with advanced disease (stage III or IV) and have an objective response rate of 29% to established therapies with a median survival of only 13 months. Therefore, there is a critical need to develop new ways to screen, diagnose, and treat RMC in an effort to raise this dreadful survival curve. To meet this need we established a uniquely large RMC patient cohort and assessed the relationship between known serum tumor markers and RMC disease severity (e.g. extent of metastatic burden) and correlate serum marker levels to response to novel systemic therapeutic agents. In this study we hypothesize that the magnitude of tumor marker CA-125 circulating within a RMC patient’s body correlates to disease severity and can be used as a therapeutic target. Our aims are to evaluate CA-125 levels, along with other known serum tumor markers (CA19.9, CA15.3, CEA, LDH, AFP) in patients with RMC and correlate these levels with overall disease extent/burden and treatment response.

Methods
Using a prospective, IRB-approved collection protocol, serum markers were captured from the medical record in patients with primary RMC. All patients were pooled into a de-identified HIPAA compliant password-protected database with restricted access. A database search of all RMC patients that were treated at MD Anderson within the past 10 years was conducted. Those without serum tumor markers available for review were excluded. Inclusion criteria required a diagnosis of RMC to be confirmed with tissue either from biopsy or final nephrectomy specimen. Using routine serum tests, known tumor biomarkers in other malignancies were assessed to determine differences in magnitude for a given patient with metastatic RMC. These tumor markers included CA-125, CEA, AFP, CA19.9, CA15.3 and LDH and trended over time with respect to key clinical events within the cohort including treatment regimens, responses, relapses, and progression of disease.

Results
A total of 18 patients met inclusion criteria. The cohort had a median age was 30 years, comprised of 72% male, 94% Black race, with 78% carrying hemoglobinopathy (e.g. sickle cell trait). Approximately 89% were metastatic at presentation, with an average follow-up time of 5.8 months. A general trend of higher overall levels of CA-125 in RMC patients was observed. However, in metastatic patients, CA-125 was often 20-100x above normal ranges, and during metastatic convalescence through treatment, these values recessed back to substantially lower levels. Similarly, we observed that throughout the course of systemic treatment, responses correlated with CA-125 serum levels. For instance, positive treatment response resulted in a significantly less circulating CA-125 serum level and that this response could be measured over time (Figure 1A). Indeed, we observed that on a consistent basis, LDH and CA-125 were significantly elevated above upper limit normal ranges, and other tumor markers such as AFP, CA19.9, CA15.3, and CEA were not (Figure 1B). Intriguingly, that magnitude of LDH and CA-125 elevation correlated to amount of metastatic burden (brain, bone, liver vs bone only), with CA-125 levels in widely metastatic patients sometimes 200+% higher than upper limit of normal ranges.
Conclusions
Trending levels of serum biomarkers such as CA-125 in RMC may assist in (1) predicting development or location of metastatic disease, (2) speed the development of biomarkers for treatment response and resistance, (3) correlate to treatment response or efficacy, (4) identify a new therapeutic target. Further work to evaluate the expression of such markers on the cell surface of RMC cells is currently ongoing.

Keywords: Renal Medullary Carcinoma, Biomarker

CDMRP DOD Funding
no

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Induction of mitochondrial metabolic activity and repression of antigen presentation underly progression of localized clear cell renal cell carcinoma.

Behrouz Shamsaei PhD 1, Bhargav Vemuri1, Juechen Yang1,2, Shuchi Gulati3, Megan E. Bischoff2, Julio A Landero Figueroa4, Tom Cunningham2, Jarek Meller1, Maria F Czyzyk-Krzeska2,5

1Environmental and Public Health Sciences, Division of Biostatistics and Bioinformatics, University of Cincinnati, College Of Medicine. 2Departments of Cancer Biology, University of Cincinnati, College of Medicine. 3Division of Hematology/Oncology, University of Cincinnati, College of Medicine. 4Trace Elements Group, Icahn School of Medicine at Mount Sinai, NY. 5Cincinnati Veteran Affairs Medical Center, Department of Veterans Affairs, Cincinnati OH

Background
Clear cell renal cell carcinoma (ccRCC) is frequent and malignant renal cancer characterized by the loss of tumor suppressors on short arm of chromosome 3. First line treatment of localized disease is surgical removal of tumor. However, up to 50% of patients relapse after surgery, and there are no biomarkers for risk of recurrence. Therefore, there is an urgent scientific and clinical need to understand the molecular mechanisms leading to the relapse, to establish prognostic biomarkers in primary tumors, and to develop adjuvant treatment strategies.

Methods
TCGA Firehose Legacy cohort was used to determine differential gene expression between stage 3 ccRCCs from patients who remained disease free (S3DF) vs. those who relapsed (S3RL). Single cell RNA-seq (scRNAseq) analysis was performed on primary ccRCCs from 15 patients (Young et al. Science, 2018, Obradovic et al., Cell, 2021, Narayanan et al., PNAS, 2021) including seven stage 3 (S3), two stage 2 (S2), and 6 stage 1 (S1) tumors. Cancer cells were selected based on ccRCC gene markers and inferred transcriptional Copy Number Variations (CNVs) to determine 3p chromosome deletion. Seurat V4 package and module score were used to analyze patterns of gene expression associated with tumor progression at the single cell level. Single cell pseudo-time analysis in Monocle V3 was used to reveal dynamics of tumor progression. Transcriptional data in Clinical Proteomic Tumor Analysis Consortium (CPTAC) ccRCC cohort was used to obtain further mechanistic insights explaining the observed expression patterns. These analyses were complemented by metallomics analysis of a separate cohort of tumors from S3RL and S3DF patients using size exclusion chromatography-coupled to inductively coupled plasma mass spectrometry (SEC-ICP-MS) to experimentally validate the presence of functional mitochondrial electron transfer chain copper-binding proteins.

Results
Transcriptomic comparison of ccRCCs from TCGA Firehose Legacy cohort revealed induction of genes encoding mitochondrial respiratory complexes and mitochondrial ribosomal proteins (MRPs) and decrease in expression of immune genes, particularly from the MHC-II group, in S3RL as compared to S3DF. Using a set of 23 differentially regulated mitochondrial and immune genes we established a transcriptomic signature that stratifies patients according to survival status and therefore may serve as prognostic biomarker. The signature contains genes encoding components of respiratory Complex I and Complex IV, a Copper (Cu) containing cytochrome c oxidase (Cu-COX). Using independent cohort of S3RL and S3DF ccRCCs, we found that total Cu content and the Cu-COX complex was significantly higher in tumors from S3RL, implicating increased activity of respiratory chain. ScRNA-seq data analysis determined increased expression of respiratory complexes’ gene-sets, and decreased levels of glycolytic genes and HIF targets across many cancer cells during tumor progression from S1 to S3. This was particularly pronounced in distinct subpopulations of cells. The expression of MHC-II genes was also diminished during ccRCC progression and was associated with hypermethylation of immune response genes in more advanced tumors as found in CPTAC dataset.

Conclusions
The study identifies increased mitochondrial activity and loss of cancer cell immunity as mechanisms associated with progression of localized ccRCC. Mitochondrial activity
represents a targetable vulnerability and pharmacologic approaches targeting complex I activity as well as Cu homeostasis disruptors like Cu-chelators that are used in clinical trials. Moreover, we determined transcriptomic signature and metallomic measurement of Cu-COX complex as potential prognostic and predictive biomarkers. Finally, the analysis will provide mechanistic insights into identification of cancer cell subpopulation involved in tumor relapse.

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Keywords: ccRCC, mitochondria, MHC-II, scRNAseq, tumor progression

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The tumor suppressor folliculin inhibits lactate dehydrogenase A and regulates the Warburg effect in renal cancer

Jennifer Heritz

Department of Urology, SUNY Upstate Medical University, Syracuse, NY, 13210, USA. Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY, 13210, USA. Upstate Cancer Center, SUNY Upstate Medical University, Syracuse, NY, 13210, USA

Background

Mutation of the tumor suppressor folliculin (FLCN) causes Birt-Hogg-Dubé (BHD) syndrome, a condition characterized by fibrofolliculomas, spontaneous pneumothorax, and chromophobe kidney cancer. However, the molecular mechanism of the tumor suppressive function of FLCN underlying these pathologies remains unknown. Lactate dehydrogenase-A (LDHA) catalyzes the interconversion of pyruvate and lactate and drives the observed 'Warburg' shift from oxidative phosphorylation to aerobic glycolysis in cancers. Previously published data demonstrates that the loss of FLCN leads to increased LDHA activity. We therefore hypothesized that FLCN is involved in the endogenous regulation of LDHA and investigated the role of FLCN-mediated regulation of LDHA in the suppression of kidney cancer.

Methods

Interaction between wild-type (WT) FLCN and LDHA was confirmed via mass spectrometry, immunoprecipitation of LDHA-FLAG and coimmunoprecipitation of endogenous FLCN, and immunofluorescence microscopy. The interacting domain of FLCN was determined using truncated mutants coimmunoprecipitated with LDHA. Within this domain, a series of synthetic peptides were synthesized and screened for LDHA inhibitory activity via fluorescence polarization anisotropy.

FLCN peptide uptake and subsequent LDHA inhibition in UOK257 cells were confirmed by fluorescence microscopy and Seahorse metabolic assay. These were explored in BHD patient-derived tumor and normal tissue samples via western blotting and immunostaining. Apoptosis induction with and without treatment of both the linear FLCN peptide and an optimized, cyclic version of the peptide were examined in HEK293 cells and multiple kidney cancer cell lines via flow cytometry and western blotting.

Results

We found that FLCN is an uncompetitive inhibitor of LDHA and that interaction with the FLCN peptide displaces and prevents the closure of the LDHA catalytic loop. Pre-incubation of FLCN with LDHA lead to a dose-dependent decrease in LDHA activity. We found that the amino acids 220-230 of FLCN contain a region that is essential for binding and inhibiting LDHA. The 'AQRMNTAFTP' peptide synthesized from this region demonstrated high binding and inhibitory activity towards LDHA.

The FLCN peptide was able to permeate cells and decrease glycolytic activity through LDHA in FLCN-null patient-derived UOK257 cell mode line. In the BHD patient-derived tissues, the FLCN peptide preferentially accumulated in and decreased the amount of activated LDHA in tumor cells, demonstrating the peptide's ability to inhibit LDHA activity ex vivo. Treatment with the linear peptide induced apoptosis in a dose- and time-dependent manner in multiple kidney cancer cell lines, but not in normal control cells. The cyclic FLCN peptide demonstrated higher cellular accumulation while maintaining cancer-cell specific LDHA inhibition and apoptosis induction.

Conclusions

Here, we show that FLCN interacts with and suppresses the activity of LDHA by binding to a dimer of LDHA to prevent assembly of the active tetramer. Further, we show that the glycolytic shift observed in cancer cells including clear cell renal cell carcinoma is a result of the dissociation of FLCN from LDHA. By creating a series of mutations within FLCN, we have identified a decameric peptide of
FLCN (AQRMNTAFTP) that is capable of inhibiting LDHA activity and inducing apoptosis in multiple cancer cell lines, suggesting a potential therapeutic intervention for Warburg-shifted tumors.

Keywords: BHD, FLCN, LDHA, cancer, Warburg effect

Belzutifan plus lenvatinib versus cabozantinib after anti–PD-1/PD-L1 treatment in patients with advanced renal cell carcinoma: the randomized, phase 3 LITESPARK-011 study

Daniel YC Heng MD, MPH1, Manuela Schmidinger2, Masatoshi Eto3, Cristina Suarez4, Robert Figlin5, Kenneth Grossmann6, Rodolfo Perini6, Ananya Roy6, Robert J Motzer7

1Tom Baker Cancer Centre, Calgary, AB, Canada. 2Medical University of Vienna, Vienna, Austria. 3Kyushu University Hospital, Fukuoka, Japan. 4Medical Oncology, Vall d’Hebron Institute of Oncology (VHIO), Hospital Universitari Vall d’Hebron, Vall d’Hebron Barcelona Hospital Campus, Barcelona, Spain. 5Cedars Sinai Medical Center, Los Angeles, CA, USA. 6Merck & Co., Inc., Rahway, NJ, USA. 7Memorial Sloan Kettering Cancer Center, New York, NY, USA

Background
PD-1/PD-L1 therapy is the standard of care for renal cell carcinoma (RCC), but patients who experience disease progression after first-line therapy have limited treatment options. One promising target is hypoxia-inducible factor (HIF)-2α, which was recently identified as a key oncogenic driver in clear cell RCC (ccRCC) and is involved in resistance to anti–vascular endothelial growth factor (VEGF) therapy. The first-in-class HIF-2α inhibitor belzutifan has shown antitumor activity both as monotherapy and in combination with the tyrosine kinase inhibitor (TKI) cabozantinib in ccRCC. Combining belzutifan with the TKI lenvatinib is hypothesized to enhance the antitumor activity of each agent by inhibiting the production of VEGF by different mechanisms. LITESPARK-011 (NCT04586231) is a randomized, open-label, active-controlled trial to evaluate the efficacy and safety of belzutifan plus lenvatinib combination therapy versus cabozantinib in patients with heavily pretreated advanced ccRCC who experienced disease progression after anti–PD-1/PD-L1 therapy.

Methods
LITESPARK-011 will enroll approximately 708 patients.
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In vitro, in vivo and molecular effects of triptolide and minnelide in renal cell carcinoma

Dr Valery Chavez PhD

University of Miami. Sylvester Comprehensive Cancer Center

Background

The worldwide incidence of Renal Cell Carcinoma (RCC) is increasing. Although new therapies have improved outcomes in advanced RCC, most patients eventually fail treatment and succumb to this devastating disease. Systemic therapy strategies have focused on TKI and immunotherapy, alone or in combination. Triptolide, a diterpenoid epoxide extracted from Tripterygium wilfordii Hook f (TWHf), has potent antitumor activity in multiple cancer models. Few studies have focused on the potential of triptolide as a novel therapeutic option in RCC.

Methods

The in vitro effects of triptolide (T) on human and murine RCC (786-0, A498, Caki-1, ACHN, and RENCA) cell proliferation was assessed using cell count and xCelligence assays at 24, 48, 72 and 96h. Molecular and mechanistic characterization of triptolide’s effects in 786-0 cells were analyzed by the (Reverse Phase Protein Array) and validated by western blot analysis. The in vivo effects (tumor progression and survival) of M were assessed in nude mice bearing 786-0 tumors (8x10⁶ cells per mouse). M was administered to tumor-bearing mice at two different doses (0.21mg/kg and 0.42mg/kg daily), intraperitoneally, for 21 days. Correlative studies to explain the in vivo effect of M and the correlation with the molecular changes in vitro seen are being performed and will be shown in the poster.

Results

T significantly inhibited, in a dose-response manner, cell proliferation in all human and murine renal cancer cell lines, with IC50 ranging between 12.5 and 25 nM). T was associated with significant negative modulation of proliferation, cell cycle, survival, increased apoptosis, and ER stress pathways in 786-0 cells, as demonstrated by RPPA analysis and validated by western Blot of selected pathway proteins, such as apoptosis (PARP cleavage and Caspase 3 activation), ER stress induction (pEIF2a, CHOP), and down-regulation of survival and proliferation pathways (pAKT). In vivo, M was associated with significant antitumor effects in 786-0 xenografts, with complete responses in the majority of mice while on treatment. These effects were associated with significant prolongation in overall survival in M treated vs. control mice. No significant toxicity or treatment-related deaths were observed.

Conclusions

Our results have shown for the first time the potent in vitro and in vivo antitumor effects of T in RCC and the molecular changes associated with these effects. The profound antitumor effects in the aggressive 786-0 RCC xenograft model are highly encouraging and warrant further preclinical studies and potential clinical trials of M this devastating disease. Correlative tumor studies to understand the mechanisms of M in vivo antitumor effects are underway and will be presented at the meeting.

Keywords: Renal Cell Carcinoma, Triptolide, Minnelide

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Correlation of serum iron level and duration of response to ipilimumab and nivolumab in metastatic clear cell renal carcinoma (mccRCC)

James Brundage MS

University of Utah School of Medicine

Background

Combination ipilimumab + nivolumab (ipi+nivo) is one recommended combination immune therapy for treatment of intermediate-poor risk patients. Durable responses are observed in some patients. However approximately 20% of patients do not respond to this treatment. There are no predictive biomarkers for this treatment representing an unmet clinical need. Iron is involved in many inflammatory pathways and it may represent an important biomarker for immunotherapy response. We hypothesize that low serum iron may be predictive of poor response to ipi+nivo therapy.

Methods

Patients with mccRCC treated with ipi/nivo were retrospectively identified from databases at the Huntsman Cancer Institute and the University of Iowa. Patients must have had serum iron labs drawn prior to the start of ipi/nivo or up to 1 month after initiation. Patients could have received ipi/nivo in any line. The primary endpoint is the duration of response correlated with serum iron levels. Patients were categorized as either having low serum iron levels or normal serum iron levels. Secondary endpoints included response correlated to other iron indices: total
iron binding capacity (TIBC) and ferritin. Duration of response was defined as the time from initiation of ipi/nivo to the time of discontinuation or death. Responders were considered those who had a duration of treatment >4 months, while non-responders were those with a treatment <4 months. Statistical significance was determined by chi-squared test.

Results
89 patients in total were included between both centers. 102 (89.5%) treated at HCI and 12 (10.5%) treated at Iowa. Patients were treated between December 2013 and June 2021. 44 (38.6%) of patients were treated in the first-line. In total 14 (12.4%), 79 (69.3%), 20 (17.7%) of patients had MSKCC favorable, intermediate, and poor risk disease respectively. 33 (37.1%) of patients had normal serum iron and 56 (62.9%) of patients had low serum iron.

Patients with serum iron below lower level normal (LLN) had a response rate of 69.6% while patients with serum iron above LLN had a response rate of 87.9% (n=89, p=0.05). Patients with ferritin below (37) and above (45) LLN had a response rate of 71.1% and 81.1% respectively (n=82, p=0.30). Patients with TIBC below (41) and above (35) LLN had a response rate of 70.1% and 82.9% respectively (n=76, p=0.22).

Conclusions
In this cohort of patients a low serum iron was associated with improved likelihood of a clinical benefit from ipi/nivo compared to patients with a low serum iron. Ferritin and TIBC were not predictive of a clinical response. This is a hypothesis generating, retrospective study. Therefore these results need to be verified in future prospective studies or validated in banked samples of already conducted phase III clinical trials.

Keywords: mccRCC Iron Immune-Therapy

Clinical and Decision-Making Drivers of Renal Mass Biopsy for Clinical T1 Kidney Tumors

Kathryn Gessner MD, PhD1, Pranav Akella1, Amir Feinberg1, Shannon Myers1, Hillary Heiling2, Allison Deal2, Sara Wobker3, Allison Lazard1,4, Marc Bjurlin1, Mathew Raynor1, Eric Wallen1, David Johnson1, William Kim5,2, Hung-Jui Tan1,2

1Department of Urology, University of North Carolina at Chapel Hill, Chapel Hill, NC. 2Lineberger Comprehensive Cancer Center; 3Department of Pathology, University of North Carolina at Chapel Hill, Chapel Hill, NC. 4Hussman School of Media and Journalism, University of North Carolina at Chapel Hill, Chapel Hill, NC. 5Division of Oncology, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

Background
Over the past two decades, the incidence of kidney cancer has more than doubled, driven mostly by the incidental detection of clinical T1 kidney tumors ≤7 cm in size. Historically, the standard treatment for clinical T1 kidney tumors has been surgical removal, either through a partial or radical nephrectomy. However, for small renal masses (SRM) ≤4 cm in size, approximately 30% are benign while only 20% contain aggressive disease and a very low percentage metastasize. Renal mass biopsy (RMB) can yield accurate pathologic information that can help guide clinical decision-making. However, past studies suggest low and variable usage in the population at large. In this study, we seek to understand the drivers of RMB use in the management of clinical T1 kidney tumors.

Methods
From October 2018–June 2022, we enrolled patients with new clinical T1 kidney tumors onto GRADE-SRM (Genomic Risk Assessment and Decisional Evaluation for Small Renal Masses), a comparative, non-randomized hybrid trial that assesses the decision-making experience and cancer genomics. In addition to clinical information, patients completed a battery of surveys on decision-making preferences/traits (e.g., maximizer-minimizer tendency, autonomy preference, decisional control preference) as well as patient-reported decisional conflict, anxiety/worry, uncertainty, communication, and health-related quality of life. For this analysis, we performed bivariable analysis by receipt of RMB using parametric and non-parametric testing. Additionally, we fitted a multivariable regression model adjusting for age, gender, Charlson comorbidity index, performance status, and other key variables.

Results
Among 261 patients, 29.5% underwent RMB. Mean age was 60.7 years old (SD 14.7), and 61% of patients were male. The sample was relatively healthy with ECOG 0 in 68%, eGFR ≥60 in 75%, and no anticoagulation in 90% of patients (Table). On bivariable analysis, patients who underwent RMB versus those who did not were more likely to have a tumor >4 cm vs. 0-2 cm (OR 1.82, 95% CI 1.01, 3.28), high nephrometry score vs. low (OR 1.89, 95% CI 1.07, 3.37), and ECOG ≥2 vs 0 (OR 2.18, 95% CI 1.09, 4.33). Compared to patients who did not, patients who underwent RMB also had higher maximizer-minimizer scores (48.5 vs. 44.8, p=0.0094), higher uncertainty of illness (11.1 vs.
10.3, p=0.0130), and higher decisional conflict (20.4 vs. 15.2, p=0.0069). On multivariable analysis, RMB remained significantly associated with nephrometry, bilateral tumors, ECOG ≥2, uncertainty of illness, and greater maximizer tendencies.

Conclusions

Patient health (ECOG status), tumor burden (bilateral tumors, high complexity), and decision-making traits (uncertainty, maximizer-minimizer tendency) predict the use of RMB. While health status and tumor burden likely reflect the medical/surgical aspects of decision-making, the association with maximizer-minimizer score and uncertainty of illness highlight the patient-specific concerns that impact evaluation and management for SRMs. Future research will evaluate how RMB impacts the decision-making experience (e.g., decisional conflict, anxiety, worry, uncertainty) among patients with these specific traits.

Keywords: Small renal mass, renal mass biopsy, decisional conflict
Impact of Age on Functional Decline Following Radical Nephrectomy: Analysis of the International Marker Consortium for Renal Cancer (INMARC)

Ms Mimi Nguyen BS1, Dr Arman Walia MD1, Dr Ava Saidian MD1, Dr Margaret Meagher MD1, Dr Luke Wang MD1, Dr Juan Javier-DesLoges MD1, Dr Yosuke Yasuda MD2, Dr Kazutaka Saito MD2, Dr Hajime Tanaka MD2, Dr Viraj Master MD3, Dr Yasuhisa Fujii MD2, Dr Ithaar Derweesh MD1

1UC San Diego. 2Tokyo Medical and Dental University. 3Emory University School of Medicine

Background

Radical Nephrectomy (RN) is a mainstay of management of localized renal cancer >4 cm. RN is associated with renal functional decline, however the solitary impact of age on functional decline is unclear. We investigated impact of age on renal function post RN, focusing on decline to moderate and severe chronic kidney disease (CKD).

Methods

This was a retrospective analysis of the INMARC registry of patients who underwent RN. Primary outcome was development of de novo CKD stage 3b [estimated glomerular filtration rate (eGFR)<45 mL/min/1.73m2]. Secondary outcomes included de novo CKD stage 3a (eGFR<60) and de novo CKD 4 (eGFR>30). Patient clinical characteristics were stratified by age groups (<50, 50-70 and >70 years old). Multivariable logistic regression analysis (MVA) was utilized to identify risk factors for renal functional decline to lower CKD stage. We utilized linear regression analysis to identify risk factors that significantly predicted change in eGFR pre- and post- operatively. Kaplan-Meier analysis (KMA) was utilized to evaluate functional outcomes with respect to the different age groups.

Results

Overall, 2436 patients were analyzed (≤50 years, n=513; 50-70 years, n=1344; >70, n=579; median follow up 31.9 months). On MVA, increasing age was independently associated with increased risk of development of CKD Stage 3b [compared to ≤50 years (referent), 50-70 years, HR 3.4, p<0.001 and >70 years HR 7.7, p<0.001]. Increasing BMI (OR 1.03, p=0.002), coronary artery disease (OR 1.70, p=0.01), diabetes mellitus (OR 1.40, p=0.029) and African American race (OR 1.6, p=0.01) were independent risk factors for CKD stage 3b. Increasing age [50-70 years, OR 3.4 p=0.001 and >70 years OR 9.4, p<0.001] in addition to increasing BMI (OR 1.03, p=0.002) and coronary artery disease (OR 1.9, p=0.015) were risk factors for de novo CKD 3a. Age >70 years [OR 1.96, p=0.027], male (OR 1.5, p= 0.036), increasing BMI (OR 1.03, p=0.003), diabetes mellitus (OR 2.69, p <0.001), and African American race (OR 2.02, p=0.002) were risk factors for de novo CKD 4. On linear regression analysis, increasing age was shown to be significantly correlated with increased delta eGFR (β = -0.212, p<0.001) while increasing BMI and tumor size did not significantly predict change in eGFR (p=0.069, p=0.965, respectively). KMA demonstrated age associated declines in 5-year freedom from de novo CKD 3a (≤50 years 73.9%, 50-70 years 53.7%, and >70 years 37.0%, p<0.001), de novo CKD 3b (age ≤50 years 92.7%, 50-70 years 71.8%, and >70 years 55.5%, p<0.001) and de novo CKD 4 (age ≤50 years 93.7%, 50-70 years 89.8%, and >70 years 81.2%, p<0.001).

Table. Multivariable analysis for predictors of de novo eGFR<45

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR (95% CI)</th>
<th>95% C.I.</th>
<th>P-Value</th>
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<tbody>
<tr>
<td>Age (≤50, referent)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;50 and ≤70</td>
<td>3.35</td>
<td>2.14 – 5.25</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>&gt;70</td>
<td>7.70</td>
<td>4.77-12.43</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ethnicity (AA vs. other)</td>
<td>1.60</td>
<td>1.12-2.28</td>
<td>0.01</td>
</tr>
<tr>
<td>Increasing BMI (continuous)</td>
<td>1.03</td>
<td>1.01-1.05</td>
<td>0.002</td>
</tr>
<tr>
<td>DM (Yes vs. No)</td>
<td>1.37</td>
<td>1.03-1.83</td>
<td>0.03</td>
</tr>
<tr>
<td>CAD (Yes vs. No)</td>
<td>1.70</td>
<td>1.13-2.55</td>
<td>0.01</td>
</tr>
<tr>
<td>Tumor Size (&lt;4cm, referent)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-7cm</td>
<td>0.82</td>
<td>0.60-1.13</td>
<td>0.23</td>
</tr>
<tr>
<td>&gt;7cm</td>
<td>0.76</td>
<td>0.55-1.04</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Conclusions
Increasing age is a risk factor for progressive and clinically significant renal functional decline after RN. Prioritization for nephron sparing management should be considered whenever safe and feasible in elderly patients to reduce potential risk of sequelae of functional decline.

Keywords: Renal cell carcinoma, radical nephrectomy, age, renal functional decline

Comparison of Nephrectomy and Ablation for cT1b Renal Masses

Ms. Kerith Wang BA1,2, Rishabh K Simhal1, Yash B Shah1, Cassra B Clark1, Andrew Denisenko1, Andrew Shumaker2, Andrea Quinn1, Connor McPartland1, Adam Schneider1, Samuel Alfonso1, Shreya Swaminathan1, Jason Hyman1, Jacky Reny1, Kelly McGuigan1, Peyton Stauffer1, Charles Nagel1, Patrick Lee1, Colette Shaw1, Kevin Antol1, Thenappan Chandrasekar1, Leonard Gomella1, Costas Lallas1, Joseph Izes1, James Mark1, Edouard Trabuls1, Mark Mann1

1Thomas Jefferson University. 2

Background
Ablative techniques are outlined by current AUA guidelines to treat small renal masses measuring less than 3 cm (cT1a). Patients with high comorbidity indexes have been successfully treated with ablative techniques, and such techniques are preferential for poor surgical candidates. NCCN and AUA guidelines currently only indicate partial and radical nephrectomy as procedural interventions cT1b renal tumors. We aim to compare peri-operative and survival outcomes for patients who have undergone partial or radical nephrectomy to ablation for cT1b renal masses at our institution.

Methods
We performed a retrospective chart review of all patients who were treated for renal masses at our institution from 2008-2020. Patient demographics, treatment modality, peri-operative outcomes, and survival data were assessed.

Results
112 patients were included. 75 patients underwent nephrectomy and 37 patients received ablation for cT1b renal masses. There was a statistically significant difference in age between patients undergoing nephrectomy to those receiving ablation (60.13 vs 71.54, p-value = 0.014). There were no statistically significant differences in BMI, pre- and post-operative creatinine, and complications. Kaplan-Meier

Figure 1. Log–rank Kaplan-Meier Survival Curve, P=0.0603
survival curve analysis was performed and showed no statistically significant difference between treatment groups (p = 0.0603) with survival in patients who received nephrectomies trending higher than survival in patients who received ablation (Figure 1).

Conclusions
This study did not find a significant difference in survival rate between patients receiving partial or radical nephrectomies and patients receiving ablation for cT1b renal masses. While partial and radical nephrectomy remain the standard of care for these, some data suggests that ablation may be appropriate for patients where surgery is contraindicated.

Keywords: cT1b renal mass, nephrectomy, ablation, survival

Clinical and genomic characteristics of patients with metastatic renal cell carcinoma who developed venous thromboembolism

Nicolas Sayegh MD, Haoran Li MD, PhD, Kamal Kant Sahu MD, Shruti Adidam Kumar MD, Nishita Tripathi MD, Umang Swami MD, MS, Benjamin L Maughan MD, Neeraj Agarwal MD

Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA

Background
Venous thromboembolism (VTE) is a common complication in patients with metastatic renal cell carcinoma (mRCC). It can be caused by inherited and/or acquired risk factors such as hypercoagulability, and treatment-related factors. Anti-cancer therapies such as tyrosine kinase inhibitors (TKIs) and immunotherapies (IOs) have also been reported to be associated with higher VTE events. Identifying patients with mRCC at the highest risk of developing VTE may provide the rationale for initiating prophylactic anticoagulation.

Methods
In this IRB-approved retrospective study, patients who were diagnosed with mRCC between July 1st, 2012, and June 30th, 2022 were included. Variables included were patients' clinical characteristics, treatment, and anticoagulation therapy. Patients with mRCC who were diagnosed during the same period were used as the control arm. Pearson's Chi-square test was used for comparison between categorical variables. The multivariable logistic model was used to assess predictors of VTE.

Results
Among 346 patients who were diagnosed with mRCC, 44 patients developed venous thrombotic events within 12 months after their cancer diagnosis (incidence rate: 12.7%). Patients of the two groups (with or without VTE) did not differ significantly when compared for the median age, gender, race, cytoreductive nephrectomy rate, clear cell histology, grade of tumor, IMDC risk factors (Table 1). Patients who developed VTE had a higher BMI at diagnosis. In the univariate analysis, patients with immunotherapy (not with TKI) [31.8% vs. 12.3%, p=0.008], and high BMI (31.7 vs. 29.1 kg/m2, p=0.03) were at significantly higher risk of VTE development. In multivariate analysis, only BMI (OR=1.06, [1.01-1.12], p=0.028) was associated with increased VTE risk. Correlation of transcriptomic and genomic data with occurrence of VTE will be presented in the meeting.

Conclusions
In this real-world population of patients with mRCC, VTE is more commonly associated with mRCC than what was reported in previous clinical trials. These hypothesis generating data, upon external validation, may provide guidance for clinical use of prophylactic anticoagulation in patient with mRCC.

Keywords: metastatic renal cell carcinoma; venous thromboembolism; body mass index; immunotherapy; genomic
**Table 1:** Patients’ characteristics, tumor grade, IMDC parameters, genomic characters, treatment details, and their association with VTE risk

<table>
<thead>
<tr>
<th>Characteristics, No (%)</th>
<th>VTE (Total=44)</th>
<th>No VTE (Total=302)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (95%CI), y</td>
<td>63.7 (60.8-66.7)</td>
<td>61 (60.4-62.9)</td>
<td>0.29</td>
</tr>
<tr>
<td>Male</td>
<td>34 (79.1)</td>
<td>207 (68.7)</td>
<td>0.23</td>
</tr>
<tr>
<td>BMI, mean (95%CI)</td>
<td>31.7 (29.2-34.1)</td>
<td>29.1 (28.3-29.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>0.89</td>
</tr>
<tr>
<td>White</td>
<td>39 (92.9)</td>
<td>260 (89)</td>
<td></td>
</tr>
<tr>
<td>Black/African American</td>
<td>0 (0)</td>
<td>1 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>2 (4.8)</td>
<td>16 (5.4)</td>
<td></td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
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Short-Term Outcomes of Active Surveillance for Small Renal Masses in Patients With End-Stage Renal Disease and Immunosuppression

Dr Zoe Gan MD¹, Dr Behdad Besharatian MD², Dr Ali Naji MD³, Dr Phillip Pierorazio MD¹

¹Division of Urology, Department of Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. ²Division of Renal Electrolyte and Hypertension, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. ³Division of Transplant Surgery, Department of Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Background
Renal transplant candidates are often referred to urology for treatment of an asymptomatic small renal mass (SRM) suspicious for a cT1a renal cell carcinoma. While surgical treatment of the mass may improve transplant candidacy, it may not impact oncologic or transplant outcomes. Active surveillance (AS) for SRMs may minimize morbidity of treatment, but outcomes of AS in renal transplant candidates and immunocompromised patients have not been established.

Methods
The multi-institutional Delayed Intervention and Surveillance for Small Renal Masses (DISSRM) prospective registry, which includes patients with SRMs less than equal to 4 cm from 2009 onwards, was reviewed up to December 2021. Patients with end-stage renal disease (ESRD) or immunocompromised status were included. Immunocompromised status was defined as having any of the following characteristics: prior organ transplant, on prednisone or any combination of immunosuppressive medications such as tacrolimus, leukemia or lymphoma, human immunodeficiency virus, or acquired immunodeficiency syndrome. For included patients, the following variables were extracted: follow up period, mass size at diagnosis, mass growth rate, timing and type of intervention if applicable, and development of metastases.

Results
A total of 15 patients with ESRD were identified, 8 of whom were designated as transplant candidates. Of the 15 patients, the mean size of the SRM at diagnosis was 2.3 cm, and over a mean follow up period of 2.4 years, the mean SRM growth rate was 0.1 cm/year. Six patients (40%) underwent either intermediate (4 patients) or delayed intervention (2 patients; both for transplant purposes). Of the 11 patients (60%) remaining on surveillance, none developed metastases. Similar findings were noted in 44 immunosuppressed patients. The mean size of the SRM at initial diagnosis was 1.9 cm, and over a mean follow up period of 3.5 years, the mean SRM growth rate was 0.2 cm/year. Fourteen patients (32%) underwent either immediate (9 patients) or delayed intervention (5 patients; 2 for progression, 3 for patient preference). Of the 30 patients (68%) remaining on surveillance, none developed metastases.

Conclusions
Limited prospective data suggests that ESRD and immunosuppressed patients on AS for SRMs have similar outcomes to those of immunocompetent controls with SRMs of the same size. These findings demonstrate promise for the safety of AS in renal transplant recipient candidates, and greater follow-up may provide definitive conclusions.

Keywords: small renal mass, active surveillance, renal transplant, immunosuppression, ESRD
contribution of event accumulation and data maturation on the stability of KM survival estimates.

Methods

Intent-to-treat analyses of Cox proportional hazards and log-rank statistics were used to estimate the HR and 95% CI for OS in the TIVO-3 trial at prespecified (2-years after last-patient-in [LPI]; ≥251 events) and exploratory extended follow-up timepoints (≥270 events; database closure). Patients were followed for survival until death, consent withdrawal, or loss to follow-up.

Results

350 patients were randomized 1:1 to TIVO (n=175) or SOR (n=175). 2-years post LPI and a mean follow-up of 17.9 months (data cut-off August 2019), 65% of patients experienced an event (HR: 0.99, 95% CI, 0.76-1.29). Subsequent analyses are reported at 20.3 (May 2020), 21.9 (January 2021), and 22.8 (May 2021) months follow-up. Accumulation of events and HR over time is shown in the Table. After almost 23 months of follow-up and realization of 80% of events, OS HR has decreased to below 0.90, in favor of TIVO.

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<td>0.97 (0.75–1.24)</td>
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<td>May 2021</td>
<td>22.8 (20.9–24.6)</td>
<td>280</td>
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Conclusions

Serial OS analyses using KM estimates are subject to increased curve reliability with decreased censoring and limited residual patients at risk for death. Long-term follow-up of TIVO-3 suggests early and consistent PFS benefit with TIVO over SOR is associated with an OS HR decline over-time with more events.

Keywords: tivozanib, overall survival, TKI

CDMRP DOD Funding

yes

Gut microbiome metagenomic analysis identifies key functional pathways associated with sarcopenia development in patients with metastatic renal cell carcinoma

Zeynep Zengin MD¹, Neal Chawla MD¹, Keehoon Lee PhD², Nazli Dizman MD³, Daniela Castro MS¹, Benjamin Merceier BSc¹, Jasnoor Malhotra BSc¹, Sabrina Salgia MS¹, Ameish Govindarajan MD¹, Joann Hsu BSc¹, Luis Meza MD¹, Regina Barragan-Carillo MD¹, Alex Chehrazi-Raffle MD¹, Gregory Caporaso PhD², David Engelthalser PhD², Sumanta Pal MD¹

¹Department of Medical Oncology & Experimental Therapeutics, City of Hope Comprehensive Cancer Center, Duarte, CA, USA.
²Pathogen and Microbiome Division, Translational Genomics Research Institute North, Flagstaff, AZ, USA

Background

Sarcopenia, or the loss of skeletal muscle mass, is associated with poorer clinical outcomes among patients with various cancers (Shachar et al., Eur J Cancer, 2016). In this study, we explored the relationship between the gut microbiome and metabolome signatures and sarcopenia among individuals diagnosed with metastatic renal cell carcinoma (mRCC).

Methods

mRCC patients who had stool whole metagenome sequencing as part of institutional research studies were retrospectively identified. Those with computerized tomography prior to the stool sample collection were identified in order to compute L3 axial segment muscle mass area (MMA; sliceOmatic, TomoVision, Canada). Sarcopenia status was determined by using previously published gender and body mass index based skeletal muscle index (MMA/height²) cutoffs (Mourtzakis et al., Appl Physiol Nutr Metab. 2008). Following Taxonomy profile was generated with MetaPhlAn 3.0. Differentially abundant species and expressed metabolic pathways were identified with LDA effect size analysis and HUMAnN 3.0, respectively.

Results

A total of 62 (45:17, M:F) mRCC patients were included in the study. The median age was 69 (range 33-93), with the majority being White (64.6%) and possessing clear cell histology (88.7%). Species that were differentially abundant with an LDA score three and above in sarcopenic patients (n=27) were *Parabacteriodes distasonis* and *Dialister sp* CAG 357, whereas in non-sarcopenic patients (n=35), *Bacteriodes vulgatus, Colinsella aerofaciens, and Streptococcus*
Parasanguinis were more abundant. Sarcopenic patients were enriched in pathways involved in gluconeogenesis I, methanogenesis from acetate and TCA cycle VII pathways. Whereas, in non-sarcopenic patients, colonic acid or M antigen synthesis pathway expression were enriched.

Conclusions
This is the first study examining the association between sarcopenia and metabolic expression of the gut microbiome in patients with mRCC. We observed an increased expression in the gluconeogenesis related pathways in patients with sarcopenia suggesting a potential catabolic state of the host.

Keywords: Sarcopenia, microbiome, metabolomics, renal cell carcinoma

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Immune dysfunction revealed by digital spatial profiling of immuno-oncology markers in progressively advanced stages of renal cell carcinoma, including brain metastases

Dr David Schoenfeld MD, PhD

Section of Medical Oncology, Yale School of Medicine, New Haven, Connecticut, USA.

Background
The tumor immune microenvironment (TME) is an important contributor to cancer progression and response to therapy, including in renal cell carcinoma (RCC). Prior studies have investigated the TME in RCC, including using single-cell transcriptomics. However, these studies often include limited sample sizes and lack spatial orientation. One method to overcome some of these limitations involves digital spatial profiling (DSP), which allows for the quantitative assessment of multiplexed proteins or RNAs using oligonucleotide tags while preserving spatial orientation. DSP can be performed on formalin-fixed, paraffin-embedded tissue sections, enabling a high-throughput workflow and the use of archived samples. Specific cellular compartments, such as certain tissue or immune populations, can be interrogated separately using fluorescent markers. In this study, we aim to further characterize the TME of progressively advanced stages of RCC, including brain metastases, using DSP.

Methods
We performed DSP on a GeoMx DSP instrument (NanoString Technologies) using a panel of 52 validated immuno-oncology markers, as well as three housekeeping proteins and three negative controls. We divided each tumor specimen into cellular-molecular compartments based on fluorescence patterns with the following collection hierarchy: macrophage compartment (CD68+); leukocyte compartment (CD68−CD45+); and tumor compartment (pan-cytokeratin+). We profiled three different RCC tissue microarrays (TMAs), consisting of: 1) 25 matched adjacent normal kidney and primary RCC cases; 2) 14 matched primary and metastatic RCC cases; and 3) 95 tumor specimens from 59 unique patients with brain metastases, with 24 matched primary tumor and metastases pairs, with 25% of the TMA spots consisting of brain metastases specimens. More than one tumor core or “replicate” was profiled for >70% of the tumor specimens from the second and third TMAs. Data were analyzed using a mixed-effects model with false-discovery rate correction for multiple comparisons.

Results
Compared to adjacent normal kidney, primary tumor samples were more infiltrated with macrophages and had higher levels of B7-H3, a B7 ligand family member with protumorigenic effects (in the macrophage and leukocyte compartments). In the tumor compartment, adjacent normal kidney had higher levels of p53 and the apoptotic proteins BAD and BIM. In both TMAs with matched primary and metastatic tumor specimens, expression of the immune checkpoints TIM-3, CTLA-4, and LAG3, as well as markers of T cell activation, GMZA, GZMB, and CD25, was lower in the leukocyte compartment of metastatic samples. In the macrophage compartment, expression of M1-like macrophage markers HLA-DR and CD127 was also lower in metastatic samples compared to primary tumors. Comparison of brain metastases to metastases from other anatomic locations revealed higher levels of the anti-apoptotic, BCL-2-family protein BCL-XL in all tissue compartments in brain metastases, and lower levels of STING.

Conclusions
DSP of progressively advanced stages of RCC, including brain metastases, revealed reduced levels of multiple immune checkpoints and T cell activation markers in metastases versus primary tumor samples, and higher inflammatory macrophage activation markers in primary samples. As predictive biomarkers are developed for immunotherapy in RCC, care should be taken to sample tissue from the site requiring systemic therapy. Brain metastases also had features unique from metastases to other sites. These distinct TME features may have important implications for the design of future biomarker and treatment studies.

Keywords: RCC, DSP, TME, brain metastases
Decisional conflict among patients newly diagnosed with clinical T1 renal masses

Amir Feinberg1, Kathryn Gessner1, Shannon Myers1, Hillary Heiling2, Allison Deal2, Sara Wobker3, Allison Lazard1, Marc Bjurlin1, Matt Nielsen1, Matthew Raynor1, Angela Smith1, Eric Wallen1, David Johnson1, William Kim2, Hung-Jui Tan1,2

1Department of Urology, University of North Carolina at Chapel Hill, Chapel Hill, NC. 2Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC. 3Department of Pathology, University of North Carolina at Chapel Hill, Chapel Hill, NC. 4Hussman School of Media and Journalism, University of North Carolina at Chapel Hill, Chapel Hill, NC. 5Division of Oncology, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

Background

Increasingly, the management of clinical T1 renal masses has become a complex, preference-sensitive decision for newly diagnosed patients. Partial nephrectomy achieves high cure rates (98%) on par with the historic standard of radical nephrectomy with better preservation of renal function though more technical complications. Thermal ablation can be performed percutaneously under image-guidance and be curative in 92-94% of cases. For small renal masses (SRMs) ≤4 cm in size, active surveillance has been embraced by some given the favorable prognosis, particularly for patients with significant competing risks. While having multiple options has the potential for yielding better decisions, it can also generate significant decisional conflict and ultimately regret; in a previous study, 30% of patients with SRMs reported high levels of decisional conflict. Accordingly, we sought to evaluate clinical, tumor, and decision-making factors that drive decisional conflict among patients with clinical T1 renal masses suspicious for kidney cancer.

Methods

From October 2018–June 2022, we enrolled patients with new clinical T1 kidney tumors onto GRADE-SRM (Genomic Risk Assessment and Decisional Evaluation for Small Renal Masses), a comparative, non-randomized hybrid trial that investigates the decision-making experience and cancer genomics. Upon study entry, participants completed a baseline survey to characterize decision-making (i.e., self-efficacy, numeracy, maximizer-minimizer tendency) and communication (i.e., information-seeking behavior, patient-centered communication) in addition to demographic, health status, and tumor burden. For this analysis, decisional conflict served as the primary outcome as captured by the decisional conflict scale (DCS), a validated instrument that measures patient perceptions of uncertainty and effective decision-making. We compared total DCS score (0 being best and 100 being worst) by patient and tumor characteristics using bivariable analyses. We then fitted multivariable regression models with selected health status (e.g., overall health, smoking status), tumor burden (e.g., nephrometry score, bilaterality), decision-making (e.g., uncertainty in illness, patient-centered communication) characteristics.

Results

Overall, 236 of 265 enrollees completed a baseline DCS survey. Mean age was 62.4 years (SD 11.2), 61.4% were male, and 31.3% were non-White. While 74.9% had an SRM, 10.7% had high complexity tumors, 5.9% had bilateral masses, and 6.4% had multifocal masses. Overall, the mean DCS score was 16.8 (SD 14.1) though 38.1% demonstrated high levels of decisional conflict (score >25). DCS scores increased significantly with age (p=0.015) but not with performance status or comorbidity. Patients with high complexity (p=0.025), bilateral (p=0.043), or multifocal masses (p=0.027) had significantly higher DCS scores compared to counterparts. With respect to decision-making traits, DCS scores increased with lower self-efficacy (p=0.005) but did not differ based on maximizer-minimizer tendency or numeracy. Patients with more uncertainty, less information-seeking behavior, and who received less patient-centered communication reported greater DCS (p<0.001). On multivariable analysis, nephrometry score, uncertainty, and patient-centered communication remained significantly associated with DCS score (p<0.05).

Conclusions

Over a third of patients with newly diagnosed clinical T1 renal masses suspicious for kidney cancer experienced significant decisional conflict. High scores appeared to be driven by tumor complexity (e.g., nephrometry score, bilaterality, multifocality), perhaps relating to the potential risks of treatment. Additionally, high decisional conflict relates to lower self-efficacy, less information-seeking behavior, greater uncertainty, and less patient-centered communication, highlighting the importance of patient decision-making needs and subsequent patient-provider interactions. Efforts to improve the decision-making experience for patients with clinical T1 renal masses will need to focus on the collaborative aspects of shared-decision making, including how patients process information and can work best with their provider to make decisions.

Keywords: early-stage kidney cancer, small renal masses, medical decision-making
A Comparison of Radiographic and Morphometric Characteristics and Outcomes in T3A Pathologically Upstaged and Non-Upstaged Renal Cell Carcinoma

Saeed Ghassemzadeh, Aastha Shah, Luke Wang, Franklin Liu, Sohail Dhanji, Kevin Hakimi, Mimi Nguyen, Ryan Nasseri, Margaret Meagher, Clara Cerrato, Juan Javier-Desloges, Ithaar Derweesh

University of California San Diego, Department of Urology, La Jolla, CA, USA

Background
A significant portion of patients presenting with clinically localized Renal Cell Carcinoma (Stage 1, 2) are pathologically upstaged to Stage 3 following surgical intervention. This is due to previously undetected extracortical extension into the renal venous system, perirenal or renal sinus fat, or collecting system. Improved detection of potential T3 upstaging may prompt changes in disease management, such as more aggressive surgical interventions which may impact patient survival or decrease risk of progression. Previous studies have identified factors associated with upstaging from cT1/cT2 to pT3a, but focused comparisons between pathologically upstaged T3a masses and non-upstaged masses are seldom found. We sought to compare pathologically upstaged and non-upstaged T3a masses to identify characteristics of upstaged masses and predictors of T3a tumors and impact on oncological outcomes.

Methods
We conducted a single center retrospective analysis of patients with pathologic T3a RCC who underwent surgical intervention. The cohort was divided into a cohort of patients whose tumors were not preoperatively identified as cT3a (upstaged, cT1-cT2/pT3a RCC) and a cohort of patients whose masses were preoperatively identified as cT3a RCC (non-upstaged, cT3a/pT3a RCC) for descriptive and outcomes analyses. We sought to delineate proportion of under-diagnosed pT3a RCC, location of upstaged disease, and predictors for upstaging. Primary outcome was overall survival (OS), with secondary outcome being recurrence-free survival (RFS). Multivariate analyses (MVA) were performed to identify predictors of T3a tumor invasion and outcomes. Kaplan Meier survival analyses (KMA) were performed to compare survival outcomes between upstaged and non-upstaged groups.

Results
We analyzed 185 patients, of which 120 (64.9%) were cT1/cT2 and subsequently upstaged and 65 (35.1%) were cT3a and non-upstaged. When compared to non-upstaged masses, upstaged masses were significant for smaller size (6.8 vs 8.2 cm, p=0.008), lower R.E.N.A.L score (8.7 vs 9.9, p<0.001), less hilar involvement (29.2% vs 86.2%, p<0.001), and increased exophyticity (41.7% vs 23.1%, p=0.011). On pathology, upstaged masses had significantly greater proportions of perirenal fat invasion (53.3% vs 33.8%, p=0.011), but significantly less renal venous system (44.2% vs 78.5%, p<0.001) and renal sinus fat invasion (35.8% vs 63.1%, p<0.001). R.E.N.A.L domains R (OR=1.8-2.0, p=0.020-0.048), E (OR=0.39-2.32, p=0.003-0.008), and h (OR=0.53-3.42, p<0.001-0.031) were independent predictors for pT3a disease. MVA did not demonstrate association between non-upstaged status and recurrence (HR=1.166, p=0.660) or all-cause mortality (HR=1.174, p=0.682). KMA noted no significant differences for 5-year RFS (82.5% vs 75.4%, p=0.248) or 5-year OS (83.1% vs 79.2%, p=0.586) between upstaged and non-upstaged cohorts.

Conclusions
While pathologically upstaged T3a RCC had similar outcomes to non-upstaged T3a RCC, pathologically upstaged T3a RCC nonetheless was associated with different morphology and invasion patterns when compared to non-upstaged T3a masses. R.E.N.A.L nephrometry score domains can be useful in identifying masses with upstaging potential and predicting their location of invasion and may aid in preoperative planning and risk stratification.

Keywords: Urology, Radiology, Renal Cell Carcinoma, Upstaging

Multiome single-cell genomics profiling identifies epigenetic, transcriptional, and cellular compositional shifts in clear cell renal cell carcinomas

Dr Lucas Salas MD, PhD, MPH, Mr Ze Zhang MS, Dr Brock Christensen PhD

Geisel School of Medicine at Dartmouth

Background
Sporadic clear cell renal cell carcinoma (cCRCC) is a group of tumors characterized by alterations in the short
arm of chromosome 3, 90% of them showing deletions, hypermethylation, or mutations in the VHL gene with several alterations upstream or downstream (PBRM1, SETD2, and BAP1) conferring unique (and non-overlapping) characteristics for prognosis. Here we aim to evaluate historical samples from the Dartmouth Renal Tumor Biobank to characterize epigenetic, transcriptional, and compositional shifts in patients with different stages and survival.

Methods
This study analyzed 57 tumor samples and six normal-adjacent kidney samples from patients in the Dartmouth Renal Tumors Biobank collected between 1994 and 2009. Tumors were split between pathology and the biobank at the time of collection. The biobank fractions were mechanically and enzymatically disassociated and preserved at -80 Celsius until processing. Cells were revived with rapid thawing, debris was cleared, and cells were processed using the 10X multiome protocol to measure single-cell transcriptomes and chromatin accessibility (simultaneously). Samples were processed in the Dartmouth Cancer Center Genomics Core. Fastaq files were preprocessed and aggregated using cellranger. RNA counts and Chromatin accessibility peaks were extracted using the Seurat/Signac algorithm. Cell identity was annotated using Azimuth. Copy number alterations were estimated using CopyKat, and compositional and differential expression analyses were analyzed using CODA and Deseq2 through Cacoa.

Results
The mean age at diagnosis was 61.7 yrs (SD: 12.5), 67% of the samples were stages I and II. After quality control, 85,771 cells were analyzed from 57 tumors and six normal tissues. All the samples in this analysis showed deletions in the short arm of chromosome 3 for cells marked as aneuploid and assigned kidney epithelial cells. The cluster of aneuploid cells also overlapped with the cells overexpressing CA9. Relative cellular compositional shifts were observed with an increased proportion in the tumors vs. normal adjacent in immune cells (22 vs. 15%), descending and ascending thin limbs (36 vs. 23%, and 2 vs. ~0%), fibroblasts (22 vs. 15%) pericytes (13 vs. 5%) and T cells (7 vs. 1.8%).

Similarly, the normal-adjacent samples had more endothelial cells (15 vs. 20 %), principal, proximal tubule, and thick ascending limb. 37% of all the cells captured in the tumor were aneuploid, most of them from descending thin limb (19,172 or 24% of all the cells captured). A subpopulation of aneuploid descending thin limb cells (14%) and ascending thick limb (9.8%) was present in the normal-adjacent tumor that requires further investigation. There were non-epithelial aneuploid cells captured in immune, endothelial and other stromal cells in less than 4% of all the cells captured. The CODA analysis revealed a statistically significant shift in the tumor’s proportion of ascending thin limb cells and an increase in the cortical thick ascending limb cells in the normal-adjacent samples. Coordinated alterations in gene expression and chromatin accessibility programs were observed in all the kidney epithelial, endothelial, and fibroblast components. After adjusting for sample cell compositions, we calculated gene programs. Among them, the top program showed 125 genes altered related to chromatin binding and organization, 2-oxoglutarate, and dioxygenase activity that require further exploration. Additional analyses of ligand-receptor and survival are pending.

Conclusions
In this single cell genomics analysis of historical samples, we recapitulated distributional shifts with alterations in the cell composition of the samples. Here the predominant tumor cells were the descending thin limb, and aneuploid descending thin limb cells were captured in both the tumor and the normal-adjacent samples. Altered programs were observed related to aneuploid cells.

Keywords: Clear cell renal cell carcinoma, single-cell RNA-seq, single-cell ATAC-seq, cell compositional analysis, cell heterogenity.

CDMRP DOD Funding
yes

85
Pulmonary and cutaneous manifestations of Birt-Hogg-Dube Syndrome in a large clinical cohort
Brian Cortese BS
Division of Urology, Department of Surgery, University of Pennsylvania Health System, Philadelphia, PA, USA

Background
Birt-Hogg-Dube (BHD) syndrome is a rare autosomal dominant condition caused by a mutation in the folliculin (FLCN) gene and is characterized by fibrofolliculomas, pulmonary cysts, and renal masses. The cutaneous and pulmonary manifestations associated with BHD are often the impetus for patients to seek treatment and thus are of importance for urologists to recognize. Urologic oncologists would also like to counsel patients regarding the risk of renal masses in the context of a patient’s other BHD phenotypes. The purpose of this study is to characterize the presenting

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<td>Unknown</td>
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<td>1 [1.3%]</td>
</tr>
<tr>
<td><strong>First Phenotype of Presentation [N]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>26 [32.1%]</td>
<td>26 [38.8%]</td>
</tr>
<tr>
<td>Retinal Tumors</td>
<td>5 [6.2%]</td>
<td>4 [6.0%]</td>
</tr>
<tr>
<td>Lung Cysts</td>
<td>8 [9.9%]</td>
<td>7 [9.9%]</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>28 [34.6%]</td>
<td>26 [34.6%]</td>
</tr>
<tr>
<td>None</td>
<td>16 [19.8%]</td>
<td>6 [19.8%]</td>
</tr>
<tr>
<td>Other</td>
<td>1 [1.2%]</td>
<td>1 [1.2%]</td>
</tr>
<tr>
<td><strong>Method of Fibrofollliculoma Diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin Exam by Dermatologist</td>
<td>4 [4.9%]</td>
<td>5 [6.0%]</td>
</tr>
<tr>
<td>Skin Exam by Medical Geneteticist</td>
<td>17 [21.0%]</td>
<td>17 [25.4%]</td>
</tr>
<tr>
<td>Confirmed with Biopsy</td>
<td>17 [21.0%]</td>
<td>17 [25.4%]</td>
</tr>
<tr>
<td>Unknown</td>
<td>7 [8.6%]</td>
<td>7 [10.4%]</td>
</tr>
<tr>
<td><strong>Fibrofollliculoma Findings by Age [N]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49</td>
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<tr>
<td>50-59</td>
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<tr>
<td>60-69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70-79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80+</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>History of Pathology [%]</strong></td>
<td></td>
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</tr>
<tr>
<td>Lung Cysts</td>
<td>51 [63.0%]</td>
<td></td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>34 [42.0%]</td>
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</tr>
<tr>
<td>Abnormal PFTs</td>
<td>14 [17.5%]</td>
<td></td>
</tr>
<tr>
<td><strong>Age [Median, [IQR]]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung Cysts</td>
<td>45 [35-59]</td>
<td></td>
</tr>
<tr>
<td>Abnormal PFTs</td>
<td>49 [39-53]</td>
<td></td>
</tr>
<tr>
<td>First Pneumothorax</td>
<td>36 [27-50]</td>
<td></td>
</tr>
<tr>
<td>Second Pneumothorax</td>
<td>40 [28-50]</td>
<td></td>
</tr>
<tr>
<td>Third Pneumothorax</td>
<td>34 [28-45]</td>
<td></td>
</tr>
<tr>
<td>Fourth Pneumothorax</td>
<td>28.5 [22-34.5]</td>
<td></td>
</tr>
<tr>
<td><strong>Pneumothorax History [Median [IQR]]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Pneumothorax Events</td>
<td>2 [1-2]</td>
<td></td>
</tr>
<tr>
<td>Range of Pneumothorax Events</td>
<td>1-8</td>
<td></td>
</tr>
<tr>
<td>Age at First Pneumothorax (years)</td>
<td>36 [27-50]</td>
<td></td>
</tr>
<tr>
<td>Age at Second Pneumothorax</td>
<td>40 [28-50]</td>
<td></td>
</tr>
<tr>
<td>Age at Third Pneumothorax</td>
<td>34 [28-45]</td>
<td></td>
</tr>
<tr>
<td>Age at Fourth Pneumothorax</td>
<td>28.5 [22-34.5]</td>
<td></td>
</tr>
<tr>
<td><strong>Treatments for Pneumothorax Patients [%]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>4 [12%]</td>
<td></td>
</tr>
<tr>
<td>Chest Tube</td>
<td>22 [65%]</td>
<td></td>
</tr>
<tr>
<td>Blebectomy</td>
<td>12 [35%]</td>
<td></td>
</tr>
<tr>
<td>Pleurodesis</td>
<td>23 [68%]</td>
<td></td>
</tr>
<tr>
<td><strong>Germline Mutations in Patients with Pneumothorax History [N]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.1285delC</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>c.1117C&gt;T</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>c.1285dupC</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>c.1021delC</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Partial Deletion including exons 11-14</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>c.1036.1043del</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>c.1177-3- 5delCTC</td>
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<td></td>
</tr>
<tr>
<td>Del. Exon 1</td>
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<td></td>
</tr>
<tr>
<td>c.499C&gt;T</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>c.494dupC</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Partial Deletion (Exons 13-14)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>c.1062+2T&gt;G</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>c.1219delA</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>c.927 954dup28</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>c.1062+2&gt;T=C</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5TUTRdel</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>c.1523delO</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>c.381dupT</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No Germline Testing</td>
<td>10</td>
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</tr>
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</table>
cutaneous and pulmonary phenotypes from our center’s cohort of BHD patients to provide additional insights into the range of clinical phenotypes with which BHD patients may present and the correlations of these phenotypes with renal mass development. Furthermore, we seek to report on FLCN mutational status for these patients.

Methods
A retrospective single center study of a cohort of patients with BHD syndrome was performed. We identified patients via clinical care or through the Penn Medicine BioBank (PMBB) by querying for folliculin gene defects. The PMBB enrolls consenting adults and stores tissue specimen as well as records of genetic testing. The medical record was queried for data corresponding to classic BHD phenotypes including cutaneous, pulmonary, and renal pathologies. For patients with a history of fibrofolliculomas, age of first identification and biopsy as well as method of diagnosis were recorded. For patients with a history of pneumothorax, age, treatment methods, history of and corresponding age for lung cysts and abnormal PFTs were noted. Quantitative results were reported as median and inter-quartile range (IQR). Associated germline mutations were obtained.

Results
Eighty-one BHD patients were identified, 67 (80.2%) through clinical care and 14 (19.8%) via PMBB. At the time of BHD diagnosis, patients were 38 (28-57) years old. Twenty-six (32.1%) patients had characteristic skin findings at BHD diagnosis with fibrofolliculomas being documented in 47 (58%) total patients at a median age of 46.5 (33-58) years. Twenty-eight (34.6%) patients had a history of pneumothorax at BHD diagnosis with pneumothorax affecting 34 (42%) total patients. The first pneumothorax episode occurred at median age of 36 (27-50) years with patients experiencing a median 2 (1-2) episodes in their lifetime. Of patients with pneumothorax history, 10 (29%) had non-unique germline mutations with the remainder being unique or missing testing. Ten (12.3%) patients had a total of 15 renal masses. Notably, 9/47 (19.1%) patients with fibrofolliculomas and 4/34 (11.8%) patients with pneumothorax history had a renal mass. Four (40%) renal tumor patients had pneumothorax history.

Conclusions
Skin findings or pulmonary manifestations were present at time of BHD diagnosis and the earliest presenting phenotypes in over two-thirds of this BHD cohort. Only 19% patients with fibrofolliculomas and 12% of patients with pneumothorax history developed a renal mass, but of those with a renal mass, 40% had experienced a pneumothorax in their lifetime. Limitations of this study include the small sample size, limited follow up time, and retrospective nature of the cohort. These results reinforce contemporary understanding of presenting phenotypes for BHD syndrome and potentially suggest that screening for BHD in patients with fibrofolliculomas or spontaneous pneumothorax may elucidate a higher incidence of BHD in the population. This study also provides helpful correlations between non-renal BHD phenotypes and renal mass development to help guide urologists in management and risk counseling for BHD patients.

Keywords: Birt-Hogg-Dube syndrome; renal tumors; oncology

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Financial Burden of Kidney Cancer Care: CDC National Health Survey Analysis
Dr Spencer Bell MD
Fox Chase Cancer Center, Philadelphia, PA

Background
Financial toxicity (FT) describes the overall mental and economic burden that results from cancer diagnosis and treatment. Due to rising costs of direct and out of pocket patient responsibility, novel diagnostics, and therapeutics in kidney cancer (KC), many of the survivors are dealing with FT implications as part of their cancer journey. Existing data have identified FT to impede delivery of the highest quality of care and long-lasting psychological impact. We aimed to analyze the subjective financial distress experienced by survivors with KC utilizing large national Center for Disease Control (CDC) health surveys over the last decade.

Methods
We used 2008-2016 CDC National Health Interview Surveys (NHIS) to identify adults with history of KC. We measured the following financial stressors 1) delaying or foregoing medical care 2) delay in dental care 3) worry about medical bills 4) delay in seeking mental health 5) difficulties paying for prescription medication. We used both sampling and design variables to account for the complex survey design of the NHIS and participant nonresponse, as well as to make the estimates nationally representative. Multivariable logistic regression modeling using the predictive margins methods was used to estimate the association between covariates and the KC related problems.
Results

We identified adults aged 18-64 years (n=217,043) and ≥65 years (n=54,235) from the 2008-2018 NHIS. Compared to those without cancer, KC survivors (18-64 years) were more likely to report a) inability to afford prescription medication (29.6% vs 17.5%, p<0.001) and b) inability to afford mental health care (4.7 % vs 2.71%, p<0.001). After adjusting for covariates, younger patients with history of KC were more likely to experience problems affording prescription medication (OR 2.03(1.49-2.78), p<0.001) as compared to healthy adults. Similarly, older KC survivors were more likely to report financial distress secondary to prescription medication (OR 1.47(1.05-1.99), p=0.02), however, they were less likely to report worrying about paying for medical costs (OR 0.66 (0.52-0.89), p<0.01).

Conclusions

Our study underscores the importance of individual assessment of FT by age. Younger KC survivors are particularly vulnerable to toxicities associated with cancer treatment, including rising cost of prescription medication and inability to afford mental healthcare.

Keywords: kidney cancer, financial toxicity, mental health, age

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The Mutational Landscape of Resected Clear Cell Renal Cell Carcinoma with Cystic components

Dr Laura Bukavina MD, MPH

Fox Chase Cancer Center, Philadelphia, PA. Urology Institute, University Hospitals Cleveland Medical Center, Cleveland, OH

Background

Clear cell renal cell carcinoma (ccRCC) with cystic components poses a clinical and diagnostic challenge. Although cystic RCC (defined as <25% solid) are known to be predominantly low-stage and low-grade tumors, there exists significant variability in morphology and oncologic outcomes. Herein, we aimed to characterize the mutational landscape and long-term oncological outcomes of cystic masses that underwent resection within the cancer genome atlas (TCGA) database.

Methods

Tumor samples from kidney renal clear cell carcinoma (KIRC) within TCGA were stratified into cystic vs. solid based on pathology reports and DICOM images available. In total, 251 solid masses and 63 ccRCC with cystic components were eligible for analysis. Initial comparison involved the transcriptome profiles (HTSeq-Count), with mutational data annotated in somatic mutation format (maftools R pkg). Clinical data for the corresponding patients were collected including age, sex, tumor stage, and survival information. KM survival and log-rank test were performed to evaluate differences in overall survival (OS) between resected cystic and solid ccRCC. This was repeated in a sub-cohort of patients with radiographically confirmed cystic components. Pairwise differential gene expression (DESeq) and principal component analysis was performed (Costalab R). Functional analysis performed via gprofiler2.

Results

Global pattern of somatic alteration is shown in Figure 1. We identified a median of 44 mutations per sample across
Figure 1: A) Mutation events per sample and top 10 mutations present in solid vs. cystic ccRCC. KM curve representing OS between tumors identified as large cystic components vs. none. Differentially expressed genes (DEGs) within cystic ccRCC compared to solid ccRCC with p<0.001 (FDR adjusted). B) Distribution of mutation within a radiographically confirmed ccRCC with cystic features, including high prevalence of LRP1 mutation. Lollipop plot illustrating LRP1 variants in our cohort, and correlation analysis among cystic and solid RCC. * FDR, False discovery rate; LRP1, Low-density lipoprotein receptor; DEGs, Differentially expressed genes; ccRCC, clear cell renal cell carcinoma.
Renal mass manifestations of the Birt-Hoge-Dube syndrome in a large clinical cohort

David Ostrowski BS¹, Dr Raju Chelluri MD, MS¹, Brian Cortese BS¹, Dr Daniel Roberson MD¹, Dr Phillip Pierorazio MD¹, Dr Katherine Nathanson MD²

¹Division of Urology, Department of Surgery, University of Pennsylvania Health System. ²Abramson Cancer Center, University of Pennsylvania Health System

Background

Birt-Hoge-Dube (BHD) syndrome is an underdiagnosed autosomal dominant inherited condition caused by mutations in the folliculin (FLCN) gene. Renal masses associated with BHD have been described to have an indolent course with the common pathologies being chromophobe renal cell carcinoma (chRCC), oncocytoma, or hybrid oncocytic tumor with features of both. The purpose of this study is to expand the literature on this rare disorder by reporting our center’s experience of the renal mass manifestations of BHD.

Methods

A retrospective single center study of a cohort of patients with BHD was conducted. Patients were identified through either clinical care via clinical cancer geneticist or through the Penn Medicine BioBank (PMBB). The PMBB has been enrolling any patient in the Penn Medicine system older than 18 years who consent to storage and analysis of tissue samples. The medical record was evaluated for medical and imaging histories corresponding to phenotypes associated with BHD renal masses. For patients with history of at least one renal mass the age of onset, imaging modality at diagnosis, imaging indication at diagnosis, number of tumors, size of tumors, growth rate, pathology, and any interventions were noted. Genetic mutations from germline testing are reported. Quantitative results reported as median with inter-quartile range (IQR).

Results

Eighty-one BHD patients were identified, 67 (80.2%) through clinical care and 14 (19.8%) via the PMBB. Median age at BHD diagnosis was 38 [28-57] years. Ten (12.3%) patients developed a total of 15 masses. Age at first tumor diagnosis was 58 [50.8-60.2] years. Median lesion size was 1.5 [1-2.1] cm at diagnosis. Indications for imaging include abdominal pain, non-renal malignancy BHD surveillance, flank pain, and abnormal liver function tests. 6 (60%) of tumor patients were managed non-operatively and 4 (40%) underwent resection. Per tumor, 9 (60%) were not resected, 3 were chRCC (20%), and 1 (6.7%) each were clear cell RCC, papillary RCC, or angiomyolipoma. Surveilled tumor growth rate was 0 [-0.02 – 0] cm/yr during a median 2.3 [2.3-5.6] year follow-up. A partial deletion in FLCN exon 1 occurred in 2 [20%] patients with both requiring surgery. The other tumor patients had unique mutations.

Conclusions

Renal tumors were found to affect 12.3% of BHD patients in this clinical cohort, consistent with prior reports of 12-34% of BHD patients developing renal tumors. These BHD-associated tumors were mostly small and indolent with a static growth rate. chRCC was the most common pathologic type although clear cell RCC and papillary RCC were also seen. The age of onset of the tumors was older than as compared to other hereditary renal cancer syndromes. Forty percent of our cohort required operative management. Limitations of this study include its retrospective nature, small sample size of BHD patients with renal lesions, and low follow-up time. Overall, these results support the current understanding of BHD-associated renal tumors as most often small, indolent lesions for which a conservative approach may be appropriate. Additionally, we report genetic mutation status to bring a precision medicine approach to this cohort of patients.

Keywords: Birt-Hoge-Dube; Renal tumors; Germline mutations
Table 1. Renal Tumors and Treatments of the BHD Cohort.

<table>
<thead>
<tr>
<th>Variables</th>
<th>BHD Cohort [n= 81]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient Characteristics [Median [IQR]]</strong></td>
<td></td>
</tr>
<tr>
<td>Age at BHD Diagnosis [years]</td>
<td>38 [28-57]</td>
</tr>
<tr>
<td>Patients with Known Renal Mass [N [%]]</td>
<td>10 [12.3%]</td>
</tr>
<tr>
<td>Age at First Tumor Diagnosis [years]</td>
<td>58 [50.8-60.2]</td>
</tr>
<tr>
<td>Number of Lesions</td>
<td>1 [1-5]</td>
</tr>
<tr>
<td>Size of Tumor at Diagnosis [cm]</td>
<td>1.5 [1-2.1]</td>
</tr>
<tr>
<td>Range of Tumor Size at Diagnosis [cm]</td>
<td>0.8-12</td>
</tr>
<tr>
<td><strong>Diagnosis of First Tumor [N [%]]</strong></td>
<td></td>
</tr>
<tr>
<td>Imaging Modality</td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>8 [80%]</td>
</tr>
<tr>
<td>CT</td>
<td>1 [10%]</td>
</tr>
<tr>
<td>PET/CT</td>
<td>1 [10%]</td>
</tr>
<tr>
<td><strong>Indication for Imaging [N [%]]</strong></td>
<td></td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>3 [30%]</td>
</tr>
<tr>
<td>Flank Pain</td>
<td>1 [10%]</td>
</tr>
<tr>
<td>Abnormal Liver Function Tests</td>
<td>1 [10%]</td>
</tr>
<tr>
<td>Imaging for Non-Renal Malignancy</td>
<td>3 [30%]</td>
</tr>
<tr>
<td>Surveillance of Known BHD</td>
<td>2 [20%]</td>
</tr>
<tr>
<td><strong>Treatment by Patient [N [%]]</strong></td>
<td></td>
</tr>
<tr>
<td>None/Surveillance</td>
<td>6 [60%]</td>
</tr>
<tr>
<td>Partial Nephrectomy</td>
<td>2 [20%]</td>
</tr>
<tr>
<td>Bilateral Partial Nephrectomies</td>
<td>1 [10%]</td>
</tr>
<tr>
<td>Radical Nephrectomy</td>
<td>1 [10%]</td>
</tr>
<tr>
<td><strong>Treatment by Tumor</strong></td>
<td></td>
</tr>
<tr>
<td>None/Surveillance</td>
<td>10 [66.7%]</td>
</tr>
<tr>
<td>Partial Nephrectomy</td>
<td>4 [26.7%]</td>
</tr>
<tr>
<td>Radical Nephrectomy</td>
<td>1 [6.7%]</td>
</tr>
<tr>
<td><strong>Pathology</strong></td>
<td></td>
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<tr>
<td>Unknown/Surveillance</td>
<td>9 [60%]</td>
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<tr>
<td>Chromophobe RCC</td>
<td>3 [20%]</td>
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<tr>
<td>Clear Cell RCC</td>
<td>1 [6.7%]</td>
</tr>
<tr>
<td>Papillary RCC</td>
<td>1 [6.7%]</td>
</tr>
<tr>
<td>Angiomyolipoma [MRI diagnosis]</td>
<td>1 [6.7%]</td>
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<tr>
<td><strong>Growth of Surveilled Tumors [Median [IQR]]</strong></td>
<td></td>
</tr>
<tr>
<td>Interval of Follow Up [years]</td>
<td>2.3 [2.3-5.6]</td>
</tr>
<tr>
<td>Growth Rate [cm/yr]</td>
<td>0 [-0.02 - 0]</td>
</tr>
<tr>
<td>Range of Growth Rate [cm/yr]</td>
<td>-0.04 - 0.43</td>
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<tr>
<td><strong>Germ line Mutations in Patients with Renal Mass†</strong></td>
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</tr>
<tr>
<td>Partial deletion including exon 1</td>
<td>2*</td>
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<tr>
<td>c.1474_1475delAAinsG</td>
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</tr>
<tr>
<td>c.1219delA</td>
<td>1</td>
</tr>
<tr>
<td>c.1021delC</td>
<td>1</td>
</tr>
<tr>
<td>c.927_954dup28</td>
<td>1</td>
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<tr>
<td>c.1062+T&gt;C</td>
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<tr>
<td>c.1285dupC</td>
<td>1**</td>
</tr>
<tr>
<td>c.1117C&gt;T</td>
<td>1</td>
</tr>
</tbody>
</table>

† One patient requiring partial nephrectomy did not have germline genetic testing.
* Both patients required surgical intervention [one radical nephrectomy, one bilateral partial nephrectomy].
** Required partial nephrectomy.
The role of exercise on the physical and mental health of kidney cancer patients and survivors

Dr Khalid Alkhatib MD, MMSc1,2, Dr Daniel Roberson MD2, Dr Phillip Pierorazio MD2

1Urology Division and Center for Surgery and Public Health, Brigham and Women’s hospital, Harvard Medical School, Boston, MA, USA. 2Division of Urology, University of Pennsylvania, Philadelphia, PA, USA

Background

The burden of kidney cancer diagnosis and treatment takes its toll on the patient’s physical and mental health. While the impact pertains to survivors as well, it is crucial to consider behavioral factors in improving the overall quality of life. In the efforts to identify modifiable behavioral factors to improve the outcomes for kidney cancer patients and survivors, we investigated the role of self-reported exercise and physical activity among a cohort of kidney cancer patients and survivors using the national Behavioral Risk Factor Surveillance System (BRFSS) between the years of 2016 and 2020. We hypothesized that the lack of activity and exercise is associated with worse mental and physical health.

Methods

A cross-sectional retrospective study of BRFSS survey participants between the years 2016 and 2020 of those reported ever being diagnosed with kidney cancer. Descriptive characteristics of the identified cohort were calculated (See Table1). To test our hypothesis, we used multivariable logistic regression modeling analyses (MVA) to assess the outcomes of (a) 14+ days when mental health was not good and (b) 14+ days when physical health was not good. In both models, we adjusted for gender, age, treatment status, income, marital status, smoking status, and BMI. (See Table 2) An institutional review board waiver was obtained according to the use of de-identified data. Two-sided statistical significance was defined as P<0.05. All statistical analyses were performed using Stata v.17.0 (StataCorp, College Station, TX, USA).

Results

Out of 2,193,981 survey participants, we identified 576 individuals reporting ever being diagnosed with kidney cancer. Of those, 217(37.7%) reported no physical activity or exercise in the last 30 days, whereas 358(62.3%) reported physical activity or exercise. (Table1)

In our MVA modeling, we found that those who reported physical activity or exercise were significantly less likely to report worse mental status compared to those who had no physical activity or exercise in the last 30 days (OR 0.41, 95% CI 0.20 – 0.85, p=0.02). Moreover, physical activity and exercise had similar results for the outcomes of worse physical status (OR 0.44, 95% CI 0.27 – 0.72, p<0.01). Both MVA models, with other significant predictors are available in Table2. Standard C-statistics assessment for our MVA models yielded a Hosmer-Lemeshow test of p=0.59 for the mental status model and p=0.16 for the physical status model. Receiver operating characteristics (ROC) with an area under the curve (AUC) were 0.80 and 0.75, respectively.
Table 2 Multivariable logistic regression models for the outcomes of having worse mental health >14 days per month and having poor physical health >14 days per month in bladder cancer patients

<table>
<thead>
<tr>
<th>Outcome of worse mental status</th>
<th>Outcome of worse physical status</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR 95% CI p-value</td>
<td>OR 95% CI p-value</td>
</tr>
<tr>
<td><strong>Exercise and Physical Activity</strong></td>
<td></td>
</tr>
<tr>
<td>No physical activity or exercise in last 30 days</td>
<td>1 Ref - 1 Ref -</td>
</tr>
<tr>
<td>Had physical activity or exercise</td>
<td>0.41 (0.20-0.85) 0.02 0.44 (0.27-0.72) &lt;0.01</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1 Ref - 1 Ref -</td>
</tr>
<tr>
<td>Male</td>
<td>0.75 (0.38-1.48) 0.4 0.88 (0.54-1.44) 0.62</td>
</tr>
<tr>
<td>Age (continuous)</td>
<td>0.96 (0.93-1.00) 0.03 0.99 (0.97-1.02) 0.56</td>
</tr>
<tr>
<td><strong>Receiving treatment for cancer?</strong></td>
<td></td>
</tr>
<tr>
<td>No, I haven't started treatment</td>
<td>0.39 (0.08-1.91) 0.25 1.07 (0.42-2.73) 0.89</td>
</tr>
<tr>
<td>Yes</td>
<td>0.75 (0.28-2.05) 0.58 2.68 (1.35-5.30) 0.01</td>
</tr>
<tr>
<td>No, I've completed treatment</td>
<td>Ref - Ref -</td>
</tr>
<tr>
<td>No, I've refused treatment</td>
<td>1.16 (0.17-8.09) 0.88 2.11 (0.39-11.5) 0.39</td>
</tr>
<tr>
<td>Don't know/Not Sure</td>
<td>0.38 (0.04-3.81) 0.41 0.54 (0.08-3.46) 0.52</td>
</tr>
<tr>
<td>Treatment was not necessary</td>
<td>0.53 (0.21-1.36) 0.19 1.22 (0.67-2.23) 0.51</td>
</tr>
<tr>
<td><strong>Income</strong></td>
<td></td>
</tr>
<tr>
<td>less-$15,000</td>
<td>1 Ref - 1 Ref -</td>
</tr>
<tr>
<td>$15,000 to &lt; $25,000</td>
<td>1.02 (0.38-2.75) 0.97 0.75 (0.34-1.67) 0.49</td>
</tr>
<tr>
<td>$25,000 to &lt; $35,000</td>
<td>1.48 (0.48-4.54) 0.49 0.52 (0.22-1.27) 0.15</td>
</tr>
<tr>
<td>$35,000 to &lt; $50,000</td>
<td>0.67 (0.20-2.25) 0.51 0.3 (0.12-0.73) 0.01</td>
</tr>
<tr>
<td>$50,000 or more</td>
<td>0.49 (0.16-1.49) 0.21 0.32 (0.14-0.71) 0.01</td>
</tr>
<tr>
<td><strong>Marital Status</strong></td>
<td></td>
</tr>
<tr>
<td>Never Married</td>
<td>1 Ref - 1 Ref -</td>
</tr>
<tr>
<td>Married/couple</td>
<td>0.7 (0.21-2.26) 0.55 0.89 (0.33-2.42) 0.82</td>
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<tr>
<td>Other/Divorced/Widowed/separated</td>
<td>1.44 (0.43-4.85) 0.55 0.85 (0.30-2.40) 0.76</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
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</tr>
<tr>
<td>Never smoked</td>
<td>1 Ref - 1 Ref -</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0.81 (0.33-1.97) 0.64 0.96 (0.49-1.88) 0.9</td>
</tr>
<tr>
<td>Former smoker</td>
<td>0.78 (0.37-1.68) 0.53 1.4 (0.83-2.37) 0.21</td>
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<tr>
<td><strong>BMI</strong></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>Normal Weight</td>
<td>1 Ref - 1 Ref -</td>
</tr>
<tr>
<td>Overweight</td>
<td>3.43 (1.05-11.16) 0.04 1.15 (0.58-2.27) 0.69</td>
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<tr>
<td>Obese</td>
<td>2.12 (0.64-6.96) 0.22 1.33 (0.69-2.56) 0.39</td>
</tr>
<tr>
<td><strong>Physical status</strong></td>
<td></td>
</tr>
<tr>
<td>0 or less than 14 days bad physical status</td>
<td>1 Ref - - - -</td>
</tr>
<tr>
<td>14+ days when physical health not good</td>
<td>3.13 (1.58-6.19) &lt;0.01 - - -</td>
</tr>
<tr>
<td><strong>Mental status</strong></td>
<td></td>
</tr>
<tr>
<td>0 or less than 14 days bad mental health</td>
<td>- - - 1 Ref -</td>
</tr>
<tr>
<td>14+ days when mental health not good</td>
<td>- - - 3.15 (1.61-6.18) &lt;0.01</td>
</tr>
</tbody>
</table>
Conclusions

In our kidney cancer cohort, those who reported physical activity and exercise were significantly less likely to report poor mental and physical health status. Our results highlight the importance of exercise and physical activity as a lifestyle modifiable behavioral risk factor in the improvement of the quality of life of kidney cancer patients and survivors, and perhaps in mitigating or preventing some of the well-known side effects associated with cancer diagnosis and treatment.

While we acknowledge the limitations of not controlling for comorbidities, the lack of granularity in terms of disease staging and treatment modality, and the range of biases associated with retrospective study designs. Yet, we think our data and results are significant particularly for research and patient care programs interested in the implementation of physical activity and exercise in the management of kidney cancer.

**Keywords:** Kidney cancer, exercise, physical health status, mental health status

**Table 1** Table A: Characteristics of kidney cancer participants in BRFSS datasets between 2016 and 2020

<table>
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<tr>
<th>Factor</th>
<th>Value</th>
<th>0 or less than 14 days bad mental health</th>
<th>14+ days when mental health not good</th>
<th>p-value</th>
<th>0 or less than 14 days bad physical status</th>
<th>14+ days when physical health not good</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>576</td>
<td>495</td>
<td>73</td>
<td>0.003</td>
<td>66.8 (10.7)</td>
<td>65.4 (10.9)</td>
<td>0.17</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>66.6 (10.8)</td>
<td>67.0 (10.6)</td>
<td>63.0 (11.2)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Physical health status</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 or less than 14 days bad physical status</td>
<td>388 (69.8%)</td>
<td>-</td>
<td>-</td>
<td>388 (100%)</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>14+ days when physical health not good</td>
<td>168 (30.2%)</td>
<td>-</td>
<td>-</td>
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<td>168 (100%)</td>
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<td></td>
</tr>
<tr>
<td><strong>Mental health status</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0 or less than 14 days bad mental health</td>
<td>495 (87.1%)</td>
<td>495 (100%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>14+ days when mental health not good</td>
<td>73 (12.9%)</td>
<td>73 (100%)</td>
<td>-</td>
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<tr>
<td><strong>Exercise and Physical Activity</strong></td>
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<td></td>
<td>&lt;0.001</td>
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<td>&lt;0.001</td>
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<tr>
<td>No physical activity or exercise in last 30 days</td>
<td>217 (37.7%)</td>
<td>166 (33.6%)</td>
<td>50 (68.5%)</td>
<td>114 (29.4%)</td>
<td>93 (55.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had physical activity or exercise</td>
<td>358 (62.3%)</td>
<td>328 (66.4%)</td>
<td>23 (31.5%)</td>
<td>274 (70.6%)</td>
<td>74 (44.3%)</td>
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<tr>
<td><strong>Gender</strong></td>
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<td>0.008</td>
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<td>0.094</td>
</tr>
<tr>
<td>Female</td>
<td>265 (46.0%)</td>
<td>216 (43.6%)</td>
<td>44 (60.3%)</td>
<td>171 (44.1%)</td>
<td>87 (51.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>311 (54.0%)</td>
<td>279 (56.4%)</td>
<td>29 (39.7%)</td>
<td>217 (55.9%)</td>
<td>81 (48.2%)</td>
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<tr>
<td><strong>Smoking status</strong></td>
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<td></td>
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<td>Never smoked</td>
<td>267 (46.7%)</td>
<td>232 (47.3%)</td>
<td>31 (42.5%)</td>
<td>194 (50.4%)</td>
<td>65 (38.9%)</td>
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</tr>
<tr>
<td>Current smoker</td>
<td>90 (15.7%)</td>
<td>72 (14.7%)</td>
<td>16 (21.9%)</td>
<td>57 (14.8%)</td>
<td>31 (18.6%)</td>
<td></td>
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</tr>
<tr>
<td>Former smoker</td>
<td>215 (37.6%)</td>
<td>187 (38.1%)</td>
<td>26 (35.6%)</td>
<td>134 (34.8%)</td>
<td>71 (42.5%)</td>
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<tr>
<td><strong>Receiving treatment for cancer?</strong></td>
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<td></td>
<td>0.45</td>
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<td></td>
<td>0.001</td>
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<tr>
<td>No, I haven't started treatment</td>
<td>36 (6.8%)</td>
<td>32 (7.0%)</td>
<td>4 (6.2%)</td>
<td>23 (6.5%)</td>
<td>10 (6.3%)</td>
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<tr>
<td>Yes</td>
<td>64 (12.1%)</td>
<td>54 (11.8%)</td>
<td>9 (13.8%)</td>
<td>29 (8.2%)</td>
<td>32 (20.3%)</td>
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</tr>
<tr>
<td>No, I've completed treatment</td>
<td>299 (56.5%)</td>
<td>257 (56.4%)</td>
<td>38 (58.5%)</td>
<td>215 (61.1%)</td>
<td>75 (47.5%)</td>
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<tr>
<td>No, I've refused treatment</td>
<td>11 (2.1%)</td>
<td>7 (1.5%)</td>
<td>3 (4.6%)</td>
<td>5 (1.4%)</td>
<td>6 (3.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don't know/Not Sure</td>
<td>13 (2.5%)</td>
<td>10 (2.2%)</td>
<td>2 (3.1%)</td>
<td>8 (2.3%)</td>
<td>3 (1.9%)</td>
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</tr>
<tr>
<td>Treatment was not necessary</td>
<td>106 (20.0%)</td>
<td>96 (21.1%)</td>
<td>9 (13.8%)</td>
<td>72 (20.5%)</td>
<td>32 (20.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------</td>
<td>------------</td>
<td>-----------</td>
<td>------------</td>
<td>------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Income</td>
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<td>&lt;0.001</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>less-$15,000</td>
<td>58 (11.5%)</td>
<td>43 (9.9%)</td>
<td>13 (20.0%)</td>
<td>26 (7.5%)</td>
<td>31 (21.4%)</td>
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</tr>
<tr>
<td>$15,000 to &lt; $25,000</td>
<td>90 (17.8%)</td>
<td>69 (15.9%)</td>
<td>19 (29.2%)</td>
<td>47 (13.6%)</td>
<td>40 (27.6%)</td>
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<tr>
<td>$25,000 to &lt; $35,000</td>
<td>70 (13.9%)</td>
<td>58 (13.4%)</td>
<td>12 (18.5%)</td>
<td>47 (13.6%)</td>
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<tr>
<td>$35,000 to &lt; $50,000</td>
<td>83 (16.4%)</td>
<td>74 (17.1%)</td>
<td>8 (12.3%)</td>
<td>62 (18.0%)</td>
<td>19 (13.1%)</td>
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<tr>
<td>$50,000 or more</td>
<td>204 (40.4%)</td>
<td>190 (43.8%)</td>
<td>13 (20.0%)</td>
<td>163 (47.2%)</td>
<td>36 (24.8%)</td>
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<td>Never Married</td>
<td>33 (5.7%)</td>
<td>27 (5.5%)</td>
<td>6 (8.2%)</td>
<td>19 (4.9%)</td>
<td>14 (8.4%)</td>
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</tr>
<tr>
<td>Married/couple</td>
<td>325 (56.6%)</td>
<td>290 (58.8%)</td>
<td>31 (42.5%)</td>
<td>231 (59.7%)</td>
<td>83 (49.7%)</td>
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</tr>
<tr>
<td>Other/Divorced/Widowed/separated</td>
<td>216 (37.6%)</td>
<td>176 (35.7%)</td>
<td>36 (49.3%)</td>
<td>137 (35.4%)</td>
<td>70 (41.9%)</td>
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<tr>
<td>BMI</td>
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<td>0.008</td>
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<tr>
<td>Underweight</td>
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<td>3 (0.6%)</td>
<td>0 (0.0%)</td>
<td>4 (1.1%)</td>
<td>0 (0.0%)</td>
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</tr>
<tr>
<td>Normal Weight</td>
<td>123 (22.4%)</td>
<td>113 (23.9%)</td>
<td>8 (11.8%)</td>
<td>91 (24.5%)</td>
<td>26 (16.2%)</td>
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<tr>
<td>Overweight</td>
<td>180 (32.8%)</td>
<td>153 (32.3%)</td>
<td>25 (36.8%)</td>
<td>129 (34.8%)</td>
<td>47 (29.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>242 (44.1%)</td>
<td>204 (43.1%)</td>
<td>35 (51.5%)</td>
<td>147 (39.6%)</td>
<td>87 (54.4%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Characteristics and Outcomes of T1a Renal Cell Carcinoma Presenting with Metastasis

Luke Wang MD1, Dhruv Puri1, Franklin Liu1, Sohail Dhanji2, Margaret Meagher1, Aastha Shah1, Saeed Ghassemzadeh1, Juan Javier-Desloges1, Aditya Bagrodia1, Brent Rose1, James Murphy1, Ithaar Derweesh1, Rana McKay1

1University of California San Diego. 2

Background

T1a renal cell carcinoma is associated with excellent cure rates. However, a small fraction present with metastatic disease or develop metastatic disease after definitive treatment. We sought to determine the clinical characteristics, survival outcomes, and variables associated with synchronous and metachronous metastasis in patients with pathologic pT1a renal cell carcinoma using the National Cancer Database (NCDB).

Methods

From 2004 to 2019, all cases of renal malignancy were extracted from NCDB. Patients with pT1a tumors were characterized as those 1) never developing metastasis, 2) developing synchronous metastasis [pT1aNxM1 at diagnosis] or 3) developing metachronous metastasis [pT1aNxM0 at diagnosis with systemic therapy initiation >= 6 months from diagnosis]. Multivariable logistic regression and multivariable Cox Proportional-Hazards regression were used to determine the impact of clinical and pathologic characteristics on overall survival. Patients with metachronous metastasis were not included in analyses given limited sample size.

Results

440,230 cases of renal malignancy were identified. 131,433 (29.9%) cases were >18 years of age at diagnosis and had pT1a. Of patients with pT1a, 130,830 (99.5%) presented with no metastasis and had no recurrence, 603 (0.46%) presented with synchronous metastasis, 17 (0.013%) experienced metachronous metastasis (Table 1). Synchronous metastasis was associated with increasing age (Odds ratio [OR] 1.02, 95% Confidence Interval [CI] 1.01-1.04), increasing tumor size (OR 1.19, 95% CI 1.11-1.26), increasing tumor grade (OR 2.28, 95% CI 1.83-2.84), sarcomatoid differentiation (OR 3.76, 95% 2.05-6.90), tumor necrosis (OR 3.82, 95% CI 2.66-5.48), and lymphovascular invasion (OR 4.00, 95% CI 2.50-6.40), and was inversely associated with papillary histology (OR 0.57, 95% CI 0.33-0.97) (p<0.05 for all estimates). It was not associated with sex, Charlson score, race, chromophobe and collecting duct histology, and year of diagnosis. In patients with pT1a presenting with synchronous metastasis, all-cause mortality on followup was associated with increasing age (OR 1.03, 95% CI 1.01-1.06), increasing Charlson score (OR 1.53, 95% CI 1.08-2.18), metastasis to bone (OR 2.78, 95% CI 1.47-5.26) and liver (OR 3.64, 95% CI 1.47-9.06). In patients with pT1a presenting with synchronous metastasis, 5-year and 10-year overall survival is 40.1% and 26.5%, respectively, with median followup of 31.8 months.

Conclusions

In this analysis of pT1a tumors with metastatic disease, we demonstrate that known prognostic histologic features were associated with metastasis on presentation. In particular, tumor grade and presence of sarcomatoid features were associated with metastasis development. Future studies investigating the underlying biology driving metastasis development in small renal masses are warranted.

Keywords: T1a Renal Mass Metastasis
<table>
<thead>
<tr>
<th></th>
<th>pT1a without Metastasis (n=130830, 99.5%)</th>
<th>pT1a with Synchronous Metastasis (n=603, 0.46%)</th>
<th>pT1a with Metachronous Metastasis (n=17, 0.013%)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, yr</strong></td>
<td>Median 69.0</td>
<td>Median 55.7</td>
<td>Median 64.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Followup time, mo</td>
<td>Median 38.3</td>
<td>Median 31.8</td>
<td>Median 34.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>No.</strong></td>
<td>% 67.6</td>
<td>% 34.3</td>
<td>% 18.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Years of Diagnosis</strong></td>
<td>2004-2009</td>
<td>2010-2014</td>
<td>2015-2019</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Male Sex</strong></td>
<td>No. 59979</td>
<td>No. 5553</td>
<td>No. 5705</td>
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</tr>
<tr>
<td><strong>Race</strong></td>
<td>White 107634</td>
<td>Black 8611</td>
<td>Native American 711</td>
<td>&lt;0.05</td>
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<td></td>
<td>Asian/Pacific Islander 3159</td>
<td>Other/Unknown 3315</td>
<td>Hispanic 9946</td>
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</tr>
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<td>1 26909</td>
<td>2 8187</td>
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<td>3 5162</td>
<td>Clinical N1 213</td>
<td>Clear Cell 74254</td>
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<tr>
<td></td>
<td>Papillary 19030</td>
<td>Chromophobe 7438</td>
<td>Collecting Duct 70</td>
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<tr>
<td></td>
<td>Unspecified /Other 30038</td>
<td>Sarcomatoid Features 610</td>
<td>Lymphovascular Invasion 1676</td>
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<tr>
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<td>Tumor Necrosis 3640</td>
<td>Tumor Grade 1 20391</td>
<td>Metastasis at diagnosis to** 1 20391</td>
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</tr>
<tr>
<td></td>
<td>Bone 212</td>
<td>Brain 29</td>
<td>Liver 29</td>
<td>&lt;0.05</td>
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<td></td>
<td>Lung 88</td>
<td>Timing of systemic therapy*** 0 no surgery or systemic therapy 121473</td>
<td>Before surgery of primary site 28</td>
<td>&lt;0.05</td>
</tr>
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<td></td>
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<td>After surgery of primary site 90</td>
<td>Before &amp; after surgery of primary 6</td>
<td>&lt;0.05</td>
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<tr>
<td></td>
<td>Dead at last followup 14910</td>
<td>12.5 381</td>
<td>10.0 29</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*p values pertain only to patients with pT1a without cM1 at diagnosis and pT1a with cM1 at diagnosis, due to low sample size of patients with pT1a with recurrence after definitive surgery.

**Only 2010-2019 data available for organ-specific metastasis at diagnosis.


mo = months; yr = years; IQR = interquartile range; No. = number.
Trials in Progress: Oral

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

Dr Katy Beckermann MD, PhD
Vanderbilt University Medical Center

Background

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

Methods

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

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

Results

n/a

Conclusions

n/a

Keywords: adenosine, metabolism, first-line, immunotherapy

CDMRP DOD Funding

yes
APART – A Phase 2 trial of Axitinib, Palbociclib and Avelumab as Renal Cell Carcinoma Therapy

Dr Praful Ravi MD
Dana-Farber Cancer Institute

Background
Despite recent advances with approval of immunotherapy (IO) and IO-tyrosine kinase (TKI) combinations, there is a need to develop new therapeutic targets for the treatment of advanced clear cell renal cell carcinoma (ccRCC). The cell cycle pathway is dysregulated in a significant proportion of ccRCC and pre-clinical studies have suggested that CDK4/6 inhibitors (i) have single-agent activity in ccRCC as well as synergism with IO. The combination of avelumab and axitinib is approved as first-line therapy for advanced ccRCC, while palbociclib is a CDK4/6i that is approved in treatment of breast cancer. The combination of axitinib, avelumab and palbociclib has been evaluated in a phase 1/2 trial in non-small cell lung cancer and shown to be safe without major dose-limiting toxicities. We hypothesize that this triplet will have efficacy in untreated advanced ccRCC and demonstrate additive activity compared to the axitinib/avelumab doublet.

Trial Schema
NCT05176288 is a multi-center single-arm phase 2 trial involving Dana-Farber Cancer Institute, Boston Medical Center, New England Cancer Specialists and Beth Israel Deaconess Medical Center. The primary objective is to evaluate the overall response rate (ORR) per RECIST 1.1 of axitinib, avelumab and palbociclib in untreated advanced ccRCC. Key secondary objectives are to evaluate the safety of the triplet in ccRCC, the rate of complete response (CR) and deep partial response (PR, ≥80% reduction in target lesions) and determine progression-free and overall survival (PFS, OS). Exploratory objectives include immunologic and biologic correlates of response, resistance and survival with the triplet. Optional research biopsies will be performed prior to cycle 1 and cycle 3 and at time of progression, with the expectation that 10 patients will consent to these. Bulk whole-exome sequencing (seq), RNA-seq, and single-cell RNA-seq will be performed to evaluate changes in somatic alterations, gene signatures, endogenous retrovirus expression, and immune cell composition, during therapy. Alterations in candidate genes such as CCND1 (cyclin D), CDK4 and CDK6 will be correlated with response to therapy. Blood will be collected every 3 months in all patients to evaluate serum thymidine kinase 1 (sTK1) levels, a functional biomarker of cell cycle activity. Key eligibility criteria include untreated advanced ccRCC (sarcomatoid histology allowed), measurable disease per RECIST 1.1, adequate organ and marrow function, and ECOG performance status ≤2. Patients who have received prior systemic therapy for metastatic ccRCC, those with untreated brain metastases, active autoimmune disease or a history of interstitial lung disease will be excluded. Patients who have received adjuvant or neoadjuvant IO therapy are eligible provided more than 12 months have elapsed since treatment completion. All International Metastatic RCC Database Consortium risk groups are permitted. Avelumab will be administered at 800mg intravenously every 14 days, axitinib at 5mg orally twice daily (po bid) and palbociclib at 75mg po daily on days 8-28 of a 28 day cycle. Dose reductions of axitinib to 3 mg and subsequently 2mg po bid are permitted; dose escalations are not allowed. Radiographic assessments will be performed every 8 weeks for the first 4 months and then every 12 weeks thereafter. The planned sample size of 25 patients will provide 85% power to detect an improvement in ORR from 50% (seen with axitinib/avelumab) to 75% with the triplet under the exact binomial test at a one-sided alpha of 0.05. This would provide a clinically meaningful signal to merit further study of this triplet in advanced ccRCC.

Current Status
The trial is due to activate in July 2022.

Methods
N/A

Results
N/A

Conclusions
N/A

Keywords: CDK4/6 inhibition; triplet; clear cell RCC; immunotherapy
Cabozantinib (C) in combination with Nivolumab (N) and Ipilimumab (I) (CaNI) for advanced Renal Cell Carcinoma with Variant Histology (aRCCVH)

Bradley McGregor MD
Dana Farber Cancer Institute

Background
Despite advances in therapy of clear cell renal cell carcinoma, outcomes for patients with aRCCVH remain poor and these patients have typically been excluded from pivotal phase III studies. COSMIC-313, which (NCT03937219) investigated C/N/I vs N/I and showed an improvement in PFS with C/N/I vs N/I, excluded those with aRCCVH. Given responses seen with C, C+N and N/I in aRCCVH, there is reason to explore the triplet combination in this population.

Methods
NCT04413123 is single arm phase 2 trial multi-institutional trial involving Dana-Farber Cancer Institute, Beth Israel Deaconess Medical Center, Winship Cancer Institute, University of California in San Diego and University of Texas Southwestern. The primary objective is to assess the objective response rate (ORR) by investigator-assessed Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1 of C in combination with N/I in aRCCVH. Key secondary endpoints are progression-free survival (PFS), overall survival (OS) and toxicity by Common Terminology Criteria for Adverse Events (CTCAE) version 5. Mandatory pretreatment biopsy (unless medically infeasible) is required for correlative analysis to define the composition and transcriptional states of tumor and immune cells within the aRCCVH microenvironment in addition to determining the number and state of tumor-infiltrating T cell clones in aRCCVH and relation to response. Any variant histology is allowed, including clear-cell RCC with over 80% sarcomatoid features. Patients may be treatment naïve or have received prior therapy limited to one anti-vascular endothelial growth factor agent not including C; prior therapy with immune checkpoint inhibitors is exclusionary. All International Metastatic RCC Database Consortium risk classifications are allowed; patients should have adequate organ function with ECOG performance status 0-1. C will be administered at a starting dose of 40 mg daily. N will be dosed at 3 mg/kg with I 1 mg/kg every 3 weeks for four cycles followed by maintenance N 480 mg IV every 4 weeks until progressive disease or unacceptable toxicity. C can be reduced to 20 mg daily or 20 mg every other day as needed for toxicity. Dose reductions of N or I are not permitted but delays up to 12 weeks are allowed; N may be continued without I if toxicity can be directly attributed to I. Radiographic imaging is performed at baseline with first scheduled assessment at 12 weeks then every 8 weeks thereafter extending to every 12 weeks after 6 months of therapy. A one-stage design is employed to enroll 60 eligible patients, which would provide 93% power to distinguish an ORR of 40% versus 20% with the exact binomial test at one-sided alpha of 0.025. Exploratory analysis of efficacy by histology will be performed. Thirty of the planned 60 patients have been enrolled.

Results
N/A

Conclusions
N/A

Keywords: Immunotherapy, cabozantinib, Variant histology RCC
A randomized trial of radium-223 (Ra-223) dichloride and cabozantinib in patients (pts) with advanced renal cell carcinoma (RCC) with bone metastases (RADICAL / Alliance A031801)

Dr Rana McKay MD1, Pamela Atherton2, Heather Jacene MD3, Veronique Marcotte4, Archana Ajmera1, Shiva Baghaie4, Janet Koball5, Tyler Zemla2, Ronald Chen6, Atish Choudhury4, Joshua Lang7, Elizabeth Wulff-Burchfield8, Mamta Parikh9, Tareq Al Baghdadi10, Young Kwok11, Alan Tan12, Himisha Beltran9, Daniel George13, Michael Morris14, Toni Choueiri6

1University of California San Diego. 2Mayo Clinic. 3Dana-Farber/Partners CancerCare, Boston. 4University of Chicago. 5University of Kansas Medical Center. 6Dana-Farber/Partners CancerCare. 7University of Wisconsin. 8University of Kansas Hospital-Westwood Cancer Center. 9UC Davis Comprehensive Cancer Center. 10IHA Hematology Oncology at St. Joe’s Ann Arbor. 11University of Maryland. 12Rush University. 13Duke University. 14Memorial Sloan Kettering Cancer Center

Background
Bone metastases are prevalent in approximately 30% of pts with advanced RCC. Pts with bone metastases have a worse prognosis compared to pts without bone metastases and are at risk of symptomatic skeletal events (SSEs). Cabozantinib, a multitypered inhibitor of multiple kinases, including vascular endothelial growth factor (VEGF) receptor and MET, has improved survival in pts with metastatic RCC and has enhanced activity in bone. Ra-223, an alpha-emitting radioisotope with natural bone-seeking proclivity, has been shown to prolong survival in men with metastatic castration-resistant prostate cancer. We previously conducted a pilot study of Ra-223 with VEGF inhibition in pts with RCC and bone metastases and demonstrated safety and declines in markers of bone formation and resorption with the combination (McKay et al, CCR 2018). Given that decreasing rates of SSEs and improving outcomes are unmet needs in pts with RCC and bone metastases, we designed a randomized phase 2 study through the National Clinical Trials Network (NCTN) investigating cabozantinib with or without Ra-223 in patients with RCC with bone metastases.

Methods
This is an open-label multicenter study. Eligible pts have metastatic RCC of any histology with ≥1 metastatic bone lesions untreated with prior radiation therapy and any number of lines of prior systemic therapy. Pts with non-clear cell RCC are eligible and will be capped at 20% of the total accrual goal. Pts must have a Karnofsky performance status of ≥60% and be on osteoclast-targeted therapy unless otherwise contraindicated. Pts are randomized 1:1 to cabozantinib with (Arm A) or without (Arm B) Ra-223. Starting dose of cabozantinib for Arm A is 40 mg by mouth daily to be escalated to 60 mg daily after cycle 1 (1 cycle=28 days) if there is no persistent grade 2 or grade ≥3 toxicity. Ra-223 is administered at a fixed dose of 55 kB/kg IV every 28 days x 6 doses. The primary endpoint is SSE-free survival. Secondary endpoints include safety, progression-free survival, overall survival, quality of life measures, and correlative analyses including liquid biopsy studies and tumor tissue analysis. The study has 90% power to detect an improvement in 6-month SSE-free survival rate from 65% to 78% with one-sided α=0.025 significance. To ensure 191 evaluable patients, target accrual is 210 pts. This design includes a safety run-in and an interim analysis for futility when 50% of the expected number of events (72 SSE events) have been observed. Final data analysis will occur when 143 events have been observed. The study was activated in December 2019 and accrual is currently ongoing throughout the NCTN.

Results
N/A

Conclusions
N/A

Keywords: Bone metastases, radium-223, cabozantinib, radiopharmaceutical.
Trials in Progress: Poster

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Phase II Trial of Alternative Cabozantinib Dosing Schedule in Metastatic Renal Cell Carcinoma

Dr Kevin Zarrabi MD MS, Dr Daniel Geynisman MD, Dr Elizabeth Plimack MD, Dr Pooja Ghatalia MD, Dr Fern Anari MD, Ms Katherine Ansel BS, Dr Mahvish Tafsee MD, Mr Ryan Romasko BS MBA, Dr Elizabeth Handorf MD, Dr Matthew Zibelman MD

1Department of Medical Oncology, Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA. 2Department of Medical Oncology, Fox Chase Cancer Center, Temple University Health System, Philadelphia, PA, USA. 3Office of Clinical Research, Fox Chase Cancer Center, Temple University Health System, Philadelphia, PA, USA. 4Biostatistics and Bioinformatics Facility, Fox Chase Cancer Center, Philadelphia, PA, USA

Background

Cabozantinib is an oral, small-molecule tyrosine kinase inhibitor targeting multiple kinases implicated in renal cell carcinoma (RCC) tumorigenesis including VEGF1-3, MET, and AXL. Cabozantinib is approved for advanced RCC as front-line monotherapy or in combination with immune checkpoint blockade, and as monotherapy in any line after prior anti-angiogenesis therapy. Despite its clinical efficacy, tolerability to cabozantinib has been a challenge in clinical practice, with high frequency of dose reductions and discontinuations due to toxicity. Most adverse events (AE) can be mitigated with supportive measures and when needed dose reductions and treatment breaks. Thus, optimal usage of cabozantinib is dependent upon balancing the AEs and tolerability to maximize efficacy. The FDA approved fixed starting dose is 60mg daily when used as monotherapy based on the METEOR trial, and 40mg daily when used in combination with nivolumab based on the CheckMate 9ER trial. Due to formulation and insurance coverage constraints, the only other dose level available is 20 mg daily. We sought to test if an alternative dosing strategy could increase average daily drug exposure and ultimately improve efficacy and minimize toxicity.

Methods

This is a multi-site, single arm phase II trial. The primary objective of this study is to show that alternative cabozantinib dosing can improve average daily dose compared to historical controls. Secondary objectives include: (1) to demonstrate that alternative dosing can decrease overall toxicity compared to historical controls, (2) show that alternative scheduling can improve median duration of time on drug compared to historical controls, and (3) to determine the ORR per RECIST 1.1 criteria of all patients treated. The study includes two cohorts: Cohort A) mRCC patients in any line of therapy who have not previously been treated with cabozantinib. Both clear cell and non-clear histology are allowed. In this cohort we plan to accrue 49 patients to start at 40 mg of cabozantinib daily and dose escalate or deescalate based on pre-specified criteria (see figure), with dosing schedules to allow for smaller median dose changes between adjustments. Adjustments are made in 10 mg average daily dosing increments by utilizing alternate day dosing schedules (e.g 60 mg/40 mg every 2 days or 80 mg/20 mg every 2 days).

Figure 1
other day) rather than titrating by 20 mg. The maximum dose of cabozantinib is 60 mg daily, minimum is 20 mg every other day. The mean daily dose in the METEOR trial, 42.8 mg, will serve as the historical control. Exploratory cohort B) IMDC All-Risk patients with clear cell histology receiving front-line cabozantinib + nivolumab combination therapy. Cohort B will accrue 37 patients. Primary objective is progression free survival and landmark 12-month survival. Secondary objectives are the same as Cohort A. The mean daily dose in the CheckMate 9ER trial, 29.4 mg, will serve as the historical control.

Correlative Studies

We aim to explore soluble serum biomarker profiles as predictive tools for treatment response. Blood will be collected at baseline, C2D1, C4D1, and at the time of progression/end of treatment due to toxicity or any other reason. We will perform assays to assess dynamic changes in soluble markers of angiogenesis (MET, AXL, Tie-2, VEGFR1/sFlt-1, and VEGFR2) and will correlate markers to treatment response and survival to predict treatment sensitivity and resistance.

The study is currently enrolling.
Clinical Trial Information: NCT05263050

Results

N/A

Conclusions

N/A

Keywords: Renal cell carcinoma, targeted therapy, alternative dosing

41

TiNivo-2: A Phase 3 Study to Compare Tivozanib Plus Nivolumab to Tivozanib Monotherapy in Patients with RCC Who Have Progressed Following ≤2 Lines of Therapy Including an Immune Checkpoint Inhibitor

Toni Choueiri MD, Laurence Albiges MD, PhD, Rana McKay MD, Sumanta Pal FASCO, MD, Hans Hammers PhD, MD, Daniel Heng MD, MPH, Katy Beckermann MD, PhD, Vijay Kasturi MD, Robert Motzer MD

1Dana-Farber Cancer Institute, Boston, MA. 2Institut Gustave Roussy, Villejuif, France. 3UC San Diego Health, San Diego, CA. 4Department of Medical Oncology & Therapeutics, City of Hope Comprehensive Cancer Center, Duarte, CA. 5UT Southwestern Kidney Cancer Program, Dallas, TX. 6Tom Baker Cancer Centre, Calgary, AB, Canada. 7Vanderbilt-Ingram Cancer Center, Nashville, TN. 8AVEO Oncology, Boston, MA. 9Memorial Sloan Kettering Cancer Center, New York, NY

Background

Tivozanib, a highly selective and potent vascular endothelial growth factor tyrosine kinase inhibitor, has demonstrated single-agent efficacy in advanced renal cell carcinoma (aRCC) along with minimal off-target toxicities and a favorable adverse event profile (Rini et al Lancet Oncol 2020). Tivozanib was approved by the FDA on March 10, 2021, for the treatment of patients with aRCC who had progressed on 2 or more prior systemic therapies. Tivozanib was combined with nivolumab in the phase 1b/2 TiNivo trial (NCT03136627), showing an objective response rate of 56%, disease control rate of 96%, median PFS of 18.9 months and a tolerable safety profile (Albiges et al Ann Oncol 2021).

Methods

TiNivo-2 (NCT04987203) is a phase 3, randomized, controlled, multicenter, open-label study to compare tivozanib in combination with nivolumab to tivozanib monotherapy in patients with RCC who have progressed following 1-2 lines of therapy including an immune checkpoint inhibitor (ICI). Eligibility criteria include age ≥18 years, clear cell RCC, ECOG PS 0-1, and disease progression during or following at least 6 weeks of treatment with ICI for RCC. Patients will be stratified by IMDC risk category and whether ICI was received in most recent line of treatment. Patients will receive tivozanib 1.34 mg orally once daily for 21 consecutive days followed by 7 days off on the monotherapy arm, and tivozanib 0.89 mg at the same schedule in addition to nivolumab 480 mg intravenously every 4 weeks on the combination arm. Study assessments include CT scan or MRI of the chest, abdomen, and pelvis every 8 weeks following Cycle 1 Day 1 for 2 years and every 12 weeks thereafter until disease progression is confirmed by independent radiology review (IRR). The primary objective is to compare the progression-free survival (PFS) of tivozanib in combination with nivolumab to tivozanib. A sample size of 326 patients, with 191 events will provide at least 80% power to detect a 50% improvement in PFS, 12 months vs. 8 months, as assessed by an IRR. Secondary endpoints include assessment of overall survival, objective response rate, and duration of response, as well as safety and tolerability. Exploratory endpoints are to assess the quality of life (FKSI-DRS and EORTC QLQ C-30) and to investigate the pharmacokinetics of tivozanib. TiNivo-2 is actively enrolling and planning to open at 190 sites in the United States and the European Union.
Methods
n/a

Results
n/a

Conclusions
n/a

Keywords: tivozanib, nivolumab, VEGF, TKI

49

A Phase 1/2, Open Label Dose-Escalation and Expansion Trial of NKT2152, an orally administered HIF2α inhibitor, to investigate safety. PK, PD and clinical activity in patients with advanced ccRCC

Dr Eric Jonasch MD, Dr Ralph Hauke MD, Dr Gerald Falchook MD, Dr Theodore Logan MD, Dr Michael Gordon MD, Dr Evan Hall MD MPhil, Brian Shuch MD, Dr Mehmet A Bilen MD, Dr Abishek Tripathi MD, Dr Yousef Zakharia MD, Satwant Lally, Dr Jim Xiao PhD, Dr Zachary Zimmerman MD, PhD, Dr Ramaprasad Srinvasan MD, PhD, Dr Toni K Choueiri MD

1MD Anderson Cancer Center. 2Oncology Hematology West PC. dba Nebraska Cancer Specialists. 3Sarah Cannon Research Institute at HealthONE. 4Indiana University Melvin and Bren Simon Cancer Center. 5HonorHealth Research Institute. 6Seattle Cancer Care Alliance. 7University of California Los Angeles. 8Emory University Hospital. 9University of Oklahoma - Health Sciences Center. 10University of Iowa Hospital and Clinics. 11NiKang Therapeutics, Inc. 12US National Cancer Institute. 14Dana-Farber Cancer Institute

Background

Inactivation of the VHL gene leading to aberrant HIF2α activity is nearly universal in clear cell renal cell carcinoma (ccRCC). NKT2152 is a novel, potent, selective orally available HIF2α inhibitor optimized for enhanced PK exposure and sustained target inhibition which has demonstrated robust activity in both ccRCC cell line-derived and patient-derived xenograft RCC and other solid tumor models.

This is a Phase 1/2 open label, multicenter, first in human study of NKT2152 in adults with advanced clear cell renal cell carcinoma (ccRCC) (NCT05119335). In Phase 1, up to 48 patients will be enrolled according to a 3+3 design with backfill as permitted by the Safety Review Committee. The primary objective of phase 1 is to determine the MTD and/or RP2D based on the totality of clinical data.

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

Adults who have advanced ccRCC without available standard therapy (Phase 1) or who have received at least 1 prior PD/L-1 and/or VEGF targeting agent with ≤ 4 prior lines of therapy (Phase 2), ECOG PS 0-2, with measurable disease per RECIST 1.1 are eligible. Patients who have had prior HIF2a inhibitors, require supplemental oxygen, and with significant cardiac disease are excluded. Tumor assessments by CT or MRI are conducted every 8 weeks until 48 weeks, then every 12 weeks thereafter. Adverse events will be monitored and graded in severity using CTCAE v5.0. The study is currently actively accruing in the United States.
Pembrolizumab plus belzutifan as adjuvant treatment of clear cell renal cell carcinoma: phase 3 LITESPARK-022 study

Toni K Choueiri1, Jens Bedke2, Jose A. Karam3, Rana R McKay4, Robert J. Motzer5, Sumanta K Pal6, Cristina Suárez7, Robert Uzzo8, Hong Liu9, Joseph Burgents9, Manish Sharma9, Thomas Powles10

1Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA. 2Eberhard Karls University of Tübingen, Tübingen, Germany. 3The University of Texas MD Anderson Cancer Center, Houston, TX, USA. 4UC San Diego, La Jolla, CA, USA. 5Memorial Sloan Kettering Cancer Center, New York, NY, USA. 6City of Hope, Duarte, CA, USA. 7Vall d’Hebron Institute of Oncology (VHIO), Vall d’Hebron University Hospital, Barcelona, Spain. 8Fox Chase Cancer Center, Temple Health, Philadelphia, PA, USA. 9Merck & Co., Inc., Rahway, NJ, USA. 10Barts Health NHS Trust and the Royal Free NHS Foundation Trust, Barts Cancer Institute, Queen Mary University of London, London, United Kingdom

Abstract

In the phase 3 KEYNOTE-564 trial, the PD-1 inhibitor pembrolizumab produced significant improvement in disease-free survival after surgery in patients with clear cell renal cell carcinoma (ccRCC). As a result, pembrolizumab was granted approval by the US Food and Drug Administration (November 17, 2021) and the European Union (January 27, 2022) for adjuvant treatment of patients with ccRCC at intermediate-high or high risk of recurrence following nephrectomy or following nephrectomy and resection of metastatic lesions. Despite advances in the treatment landscape for RCC, more effective adjuvant treatment strategies are needed for patients at risk of recurrence after surgery. Hypoxia-inducible factor 2α (HIF-2α) has been established as an oncogenic driver in ccRCC. The HIF-2α inhibitor belzutifan (MK-6482) has shown promising activity and tolerability in patients with advanced ccRCC and von Hippel-Lindau disease–associated RCC. In patients with heavily pretreated advanced ccRCC, treatment with belzutifan resulted in an objective response rate of 25% and a median progression-free survival of 14.5 months (Choueiri TK, et al. Nat Med. 2021;27[5]:802–805). The multicenter, randomized, double-blind, phase 3 LITESPARK-022 study (NCT05239728) will compare the efficacy and safety of belzutifan plus pembrolizumab with placebo plus pembrolizumab as adjuvant treatment of patients with ccRCC who underwent nephrectomy.

Methods

Eligible patients have histologically or cytologically confirmed intermediate-high risk, high risk, or M1 NED RCC with a clear cell component and have not previously received systemic therapy. Patients must have undergone nephrectomy and/or metastasectomy within 12 weeks before randomization and be tumor free, per computed tomography/magnetic resonance imaging. Approximately 1600 patients will be randomly assigned 1:1 to receive belzutifan 120 mg orally every day plus pembrolizumab 400 mg intravenously every 6 weeks or oral placebo plus pembrolizumab 400 mg intravenously every 6 weeks until verified disease recurrence by blinded independent central review, start of new anticancer treatment, unacceptable toxicity, or decision to withdraw. Randomization is stratified by tumor grade (1 or 2 vs 3 or 4) and risk type (intermediate-high risk versus high risk versus M1 NED). Patients will be radiologically evaluated every 12 weeks from randomization through year 2, every 16 weeks in years 3 to 5, and every 24 weeks in years 6 and beyond. Adverse events will be monitored throughout the study and for 30 days following cessation of study treatment (90 days for serious adverse events). The primary end point is disease-free survival. The key secondary end point is overall survival; other secondary end points are safety, disease recurrence-specific survival, and patient-reported outcomes. Enrollment is ongoing globally.

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Results

N/A

Conclusions

N/A

Keywords: Pembrolizumab; belzutifan; HIF-2α; adjuvant; renal cell carcinoma

CDMRP DOD Funding

yes
Abdominal Adiposity in Early-Onset and Aggressive Clear Cell Renal Cell Carcinoma and Health Disparities

Ken Batai PhD
University of Arizona, Tucson

Background
Hispanic Americans (HAs) and Native Americans (NAs) have heavy kidney cancer burden with a younger age of diagnosis and higher mortality rates than European Americans (EAs). Kidney cancer incidence rates rose until the 2000s, especially among younger age groups. The overall incidence rate for renal cell carcinoma (RCC) stabilized after 2010, but the incidence rates of early-onset (age < 50) cases and clear cell RCC (ccRCC) continue to rise. This trend coincides with increase in obesity, particularly in younger generations. Obesity, especially during adolescence and early adulthood, increases RCC risk. Obesity is common among HA and NA RCC patients, and race/ethnicity and obesity are associated with early-onset. However, to date there are no studies investigating the relationship between abdominal adiposity, particularly visceral adipose tissue (VAT), and early-onset, genomic and metabolomic signatures, or relationship of all these factors.

Area of Emphasis: This is a Basic Science research project using genomic and metabolomic approaches.

Hypothesis/Objective
The objectives of this study are to characterize early-onset and aggressive ccRCC and investigate association between VAT and early-onset ccRCC metabolomic and genomic profile, including patients from high-risk populations. We hypothesize that the VAT influences age of onset, gene expression, and metabolomic characteristics of early-onset ccRCC, and aggressiveness and these obesity-related factors disproportionately affect HAs compared to EAs. By focusing on a previously underrepresented racial/ethnic group with a high burden of kidney cancer and obesity-related comorbid conditions, we aim to understand biological basis of early-onset ccRCC.

Specific Aims
This project first will determine the relationship between VAT and subcutaneous adipose tissue (SAT), age of onset, and ccRCC aggressiveness (Specific Aim 1). Variation in VAT and SAT among HA, NA, and EA ccRCC patients will be examined, and then, associations (1) between abdominal adiposity and early-onset and (2) between abdominal adiposity and aggressiveness will be examined. We hypothesize that HA patients have higher VAT than EA patients, and VAT significantly influences the age of onset and aggressiveness. Second, this project will identify transcriptomic signatures in tumors and metabolomic signatures in the blood that are associated with VAT and SAT, age of onset, and aggressiveness (Specific Aim 2). Genes and metabolites associated with abdominal adiposity and early-onset ccRCC will be compared between HAs and EAs. Then, we will identify commonly altered pathways of early-onset ccRCC from molecular and metabolomic profiling and assess identified genes and metabolites that are associated with aggressiveness. We hypothesize that genes and metabolites involved in abdominal adiposity or pathways involved in glucose metabolism are more likely dysregulated in HA early-onset ccRCC compared to later-onset ccRCC or EA ccRCC.

Study Design
Pre-operative CT and MRI scans and tissue and serum samples from ccRCC patients who underwent nephrectomy will be obtained. For Specific Aim 1, VAT and SAT at the third lumber vertebrae will be measured to test the associations with age of onset, race/ethnicity, and aggressiveness (n=200). For Specific Aim 2, whole transcriptome sequencing will be performed during the discovery phase (n=180), and findings will be validated with rt-qPCR, in-situ hybridization, or immunohistochemistry in an independent samples (n=30). Metabolomic profiling will be performed using 90 serum samples in discovery phase, and targeted metabolomic assay will be performed for validation analysis (n=40). Multi-omics bioinformatic analysis will be performed to identify altered pathways and driver genes for early-onset ccRCC. Finally, associations between identified genes and metabolites and ccRCC aggressiveness will be tested.

Impact/Innovation
The long-term goal of our research is to develop clinically useful diagnostic and prognostic biomarkers that can be used in racially/ethnically diverse patient populations. Precise measurement of abdominal adiposity using pre-operative imaging data will allow us to examine relationships between abdominal adiposity and early-onset and aggressive ccRCC more accurately than use of body mass index.
Recently developed efficient transcriptomic and metabolomic profiling techniques allow us to utilize a novel multi-omics method to identify altered pathways or potential driver genes for early onset ccRCC, along with a single-omic approach. A multi-omic approach will help us to understand the underlying biologic mechanism of early-onset ccRCC that would be missed using a single-omic approach. Understanding the molecular and metabolomic profile of early-onset and aggressive ccCC in HAs and EA will help us develop robust biomarkers that can be used in diverse populations, and then identified biomarkers may help urologists diagnose patients in early-stage, assess patients’ prognosis, and lead to the development of more individually tailored treatments that will improve patient outcomes with ccRCC.

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The Genomic Landscape of Papillary Renal Cell Carcinoma Arising in African American Patients

Adam Metwalli MD

Howard University, Washington

Background

Papillary RCC (pRCC) is the second most common histology representing between 10%-15% of renal cortical neoplasms. pRCC is almost three times more common in African Americans than in Caucasians. Yet, at this time, genomic data from tumors explaining the fact that the incidence of pRCC is higher among African American (AA) patients has not been discovered. Finally, despite the disproportionate incidence of pRCC in the AA community, subjects of African descent are markedly underrepresented. In a study of the National Cancer Database, only 10% of subjects were AA despite higher rates of RCC among AA in the United States. However, in a recent study of SEER evaluating patients with metastatic RCC, AA subjects represented 25% of patients with metastatic non-clear cell RCC. Consequently, the conclusions drawn from current studies regarding genomic factors that contribute to the development of pRCC may not be as applicable to AA patients with pRCC. Papillary RCC is a rare tumor that disproportionately affects patients of African descent. The genomic landscape of pRCC in AA subjects has not been thoroughly characterized and current literature on genomics of pRCC does not reflect the ethnic incidence of this disease.

We aim to comprehensively examine the genomic landscape of pRCC in African Americans. We will (1) Perform whole genome sequencing to characterize patterns of somatic variation in tumors and normal tissue exclusively from African American subjects. (2) Quantify RNA expression and correlate somatic mutations, including non-coding alleles, with changes in RNA abundance.

Study Design

Tumor samples from AA will be collected and papillary RCC type 1 will be confirmed by central pathologic review. Samples will be selected from tumor specimens from which several different areas can be sampled; normal renal parenchyma is available for sampling as well. Whole genome sequencing will be performed on each sample from each subject, and the resulting genomic data will be analyzed to identify somatic genomic alterations in both tumor specimens and normal renal tissue. From these same samples, RNA extraction will be performed and RNA expression profiles for each specimen created. Non-coding alleles will also be evaluated and these data will be analyzed for RNA expression differences and abundance variations.

Expectations and Implications

This proposal seeks to identify novel somatic mutations and patterns of gene expression that contribute to oncogenesis of papillary RCC in African Americans. The novelty of this work is that no previous analyses have evaluated both somatic genomic alterations concurrently with RNA expression changes in the same cells in a cohort that is exclusively African American. Funding for this research effort will allow us to generate preliminary data, which will support a broader grant application to fully characterize genomic differences and RNA expression.

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Identifying the Molecular Mechanisms Underlying HIF2-Independent Tumorigenesis in RCC

Faeze Saatchi PhD

University of Texas, Southwestern Medical Center at Dallas

Background

VHL is the key tumor suppressor in ccRCC, and more than 90% of ccRCC tumors are VHL-deficient. HIF-1alpha and HIF-2alpha are key VHL substrates targeted for degradation by VHL-mediated ubiquitination. Several lines of evidence have unequivocally demonstrated an oncogenic role for HIF-2alpha in ccRCC, which has led to the development and use of highly efficient and specific inhibitors of HIF-2alpha. As a therapeutic approach currently being tested in clinical
trials. HIF-2alpha inhibition has proven quite effective, but only a subset of tumors respond (both in tumor grafts and in patients). This indicates that there are other VHL-regulated HIF2-independent drivers in ccRCC. Our goal is to identify the HIF2-independent drivers and molecular pathways in ccRCC. The first potential candidate is HIF-1alpha. Although some evidence supports a tumor suppressor function for HIF-1alpha, studies of early renal tubule lesions as well as mouse models of ccRCC suggest a role for HIF-1alpha in early tumor development. In Aim 1, we will determine the role of HIF-1alpha in tumorigenesis in HIF2-independent tumors. We will use HIF2-independent patient-derived xenografts with high levels of HIF-1alpha to evaluate the role of HIF-1alpha in ccRCC. Primary cells derived from these tumor grafts will be used in conditional knockdown experiments using HIF1A-specific shRNA constructs (or scrambled shRNA as control). Tumor growth and the differentially expressed genes will be analyzed. In addition to HIF-1alpha and HIF-2alpha, other VHL substrates as well as E3 ligase-independent functions for pVHL have been described. In Aim 2, non-HIF-related roles of pVHL in ccRCC tumor development will be evaluated. To achieve this, we will use VHL-deficient PDX lines with undetectable HIF-1alpha and HIF-2alpha levels. ZHX2 and SFMBT1 are two VHL substrates that are targeted for ubiquitination and degradation by pVHL. In Sub-Aim 1, we will test the role of ZHX2 and SFMBT1 using similar strategies and approaches as used for HIF-1alpha in Aim 1. In Sub-Aim 2, we will identify novel pathways regulated by pVHL in tumors independent of canonical and non-canonical targets. To achieve this, we will reconstitute pVHL in VHL-deficient PDX lines and use RNA-Seq to identify differentially expressed genes and novel molecular pathways.

The PI is a postdoctoral fellow with a strong background in genetics, molecular biology, and biochemistry. The PI’s career goal is to become an independent investigator at a research-intensive institute focusing on basic/discovery research in kidney cancer in areas with translational potential. Completing the proposed project, with consistent guidance and feedback from the mentor, will help the PI gain broad and in-depth knowledge and acquire essential technical skills to design and perform an impactful research project in kidney cancer. The mentor is an established leader in kidney cancer research who is recognized for his work in kidney cancer research. The mentor and the PI have regular weekly meetings to discuss experimental design and review data. Additionally, the PI has the opportunity to interact with other experienced researchers in the kidney cancer program at UT Southwestern. The kidney cancer program provides an optimal training environment to advance the PI’s career.

The impact of the proposed research project is: (1) identifying novel functions for VHL, the most important tumor suppressor in ccRCC; (2) examining the role of HIF-1alpha in HIF2-independent tumors, a type of ccRCC tumors that are poorly characterized; (3) increase our knowledge of tumor suppressors, oncogenes, and their biology; and, (4) identifying novel targets for kidney cancer therapy.

Loss of VHL Activates SFMBT1-SPHK1 Oncogenic Signaling in Kidney Cancer

Xijuan Liu PhD

University of North Carolina at Chapel Hill

Background

Inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene is tightly associated with renal cell carcinoma (RCC). It is well-established that the VHL-associated complex has E3 ubiquitin ligase activity and VHL loss leads to accumulation of hypoxia inducible factor alpha (HIFalpha, including HIF1alpha and HIF2alpha), which contributes substantially to the transforming phenotype of renal cancer. Recent reports showed that the specific HIF2alpha inhibitor PT2399 inhibits primary tumor growth and invasion of a subset of kidney cancer. However, a significant portion of kidney cancer remains resistant to HIF2alpha inhibitor treatment, highlighting the importance of identifying additional therapeutic vulnerabilities of VHL-deficient kidney cancer. Recently, my published work identified the SCM Like With Four Mbt Domain (SFMBT1) protein as a novel VHL substrate by using a newly developed genome-wide screen. I showed that knockdown of SFMBT1 blocks cell proliferation and soft agar growth of VHL-deficient renal cancer cells and blocks xenograft tumor growth. Importantly, SFMBT1 expression levels are strongly elevated in ccRCC tumors compared to normal patients. Mechanistically, integrated analyses of ChIP-Seq and RNA-seq reveal that SFMBT1 positively regulates a subset of genes in a HIF-independent manner by co-occupying with H3K4me3 and H3K27ac chromatin marks. The gene encoding the sphingosine kinase SFHK1 was identified to be a direct target of SFMBT1. A new SFHK1 inhibitor blocked soft-agar growth of ccRCC cells, suggesting that SFHK1 is an important downstream effector of the oncogenic action of SFMBT1 in renal cancer cells with VHL loss.

Hypothesis/Objectives

I hypothesize that SFMBT1 promotes VHL-deficient ccRCC through SFHK1-dependent signaling. Our objectives are
to broadly analyze the oncogenic role of SFMBT1 in renal cancer, focusing on its ability control SPHK1 expression. Further, I hypothesize that SPHK1 controls critical NF-kappaB transcription factor activity in VHL-deficient renal cancer, as NF-kappaB activity is suggested to be critical in this cancer, although how it is activated is unclear. Finally, I hypothesize that pharmacologic inhibition of SPHK1 will suppress renal cancer growth, focused on xenografts as well as a PDX tumor bank that is available to me at our center. It is important to note that this is the first study directed at a pro-oncogenic function for SFMBT1, with a focus on its role in renal cancer with loss of VHL.

**Specific Aims**

**Aim 1:** Determine the functional significance of deregulated SFMBT1-SPHK1 signaling in VHL-deficient renal cancer.

**Aim 2:** Determine if SPHK1 controls critical NF-kappaB signaling in ccRCC with VHL loss.

**Aim 3:** Determine the therapeutic potential of targeting SFMBT1-SPHK1 signaling axis in renal tumorigenesis upon VHL loss.

**Study Design**

In Aim 1, I will address whether upregulation of SFMBT1 is necessary to promote renal cancer phenotypes. Subsequently, I will examine the role of SFMBT1 in VHL-deficient renal tumorigenesis by using a syngeneic ccRCC tumor model. Lastly, I will determine the functional significance of SPHK1 as an SFMBT1 direct target gene in ccRCC.

In Aim 2, based on interesting preliminary data, I will determine if SPHK1 promotes NF-kappaB activity, which is known to be activated downstream of VHL-loss and to be important in the ccRCC oncogenic phenotype.

In Aim 3, I will examine the effect of SPHK1 inhibitor (PF-543) on kidney tumorigenesis by using orthotopic xenografts and patient-derived xenografts (PDXs).

**Personnel/Researcher Development Plan**

My career goal is to establish my own independent lab within the next three years with a focus on kidney cancer research. I will also be guided by my collaborator, Dr William Kim, at UNC, a kidney cancer researcher and clinician, who will provide me with important PDX models and with research guidance as well as clinical insight.

**Impact**

If successful, the work proposed in this application will elucidate novel oncogenic mechanisms associated with SFMBT1 in VHL-deficient renal cancer, with a particular emphasis on its ability to upregulate the expression of the sphingosine kinase SPHK1. Also, if successful, our work will provide new therapeutic avenues in kidney cancer by targeting the SFMBT1-SPHK1 oncogenic signaling axis. Personally, this research project will provide a strong foundation for me to develop a career at the forefront of kidney cancer research.

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**24 Analyzing the Therapeutic Impact of di-ABZI on PBRM1-Deficient ccRCC Tumors**

**Haifeng Yang PhD**

**Thomas Jefferson University**

**Background**

Kidney cancer is among the top ten most frequent cancers, both in incidence and mortality, in both men and women. Inactivation of the VHL tumor suppressor gene is a causal event in the pathogenesis of clear cell Renal Cell Carcinoma (ccRCC), the most frequent subtype of kidney cancer. PBRM1 is a major secondary mutated driver in ccRCC, and its mutation rate is the second highest and is only lower than that of VHL. Currently, there is no drug to target PBRM1 deficiency to treat ccRCC patients.

We recently discovered that after loss of VHL, the activated HIF up-regulated the interferon-stimulated gene factor 3 (ISGF3), a heterotrimeric transcription factor that induces a subset of interferon-responsive genes. Strikingly, loss of PBRM1 led to the inactivation of ISGF3. Similarly, acute deletion of Pbrm1 in mouse kidney cells led to reduced expression of ISGF3 target genes. In addition, we discovered that ISGF3 was potently tumor-suppressive, as suppression of its subunits strongly enhanced tumor growth in a xenograft model. Consistent with this observation, overexpression of ISGF3 subunits significantly suppressed tumor growth. This suggests an ISGF3-activating compound, mimicking the overexpression of ISGF3, could also have antitumor activity. To this end,
we identified a drug candidate compound developed by GSK that can activate ISGF3 and elicit potentially tumor-suppressing biological effects in kidney tumors in mice. We will examine its antitumor activity in various PBRM1-deficient tumor models.

Our proposal addresses "Therapeutic Development" and "Chromatin and Gene Regulation" Areas of Emphasis.

We hypothesize that di-ABZI will have a strong antitumor effect on PBRM1-deficient ccRCC tumors in various tumor models.

This hypothesis will be tested with the following set of Specific Aims: (1) Investigate how PBRM1 mechanistically regulates ISGF3. (2) Examine the antitumor effect of di-ABZI in PBRM1-deficient xenograft models and PDX models. (3) Investigate the antitumor effect of di-ABZI in a syngeneic mouse ccRCC model.

We will examine how PBRM1 activates ISGF3 pathway, either through inducing the expression of interferon or directly binding the promoters and regulating the expression of ISGF3 target genes, or both. We will investigate whether di-ABZI, which can potently activate ISGF3 in xenograft tumor and elicit multiple biological effects, has strong antitumor effect in PBRM1-deficient xenograft models. We will also test its efficacy with using patient-derived xenograft models. In addition, we will test the antitumor efficacy of di-ABZI in a PBRM1-deficient mouse syngeneic kidney cancer model that will be supplied to us by Dr Kaelin's laboratory. We will further test whether the re-expression of mouse Pbrm1 in the cancer cells will dampen the tumors' response to di-ABZI.

This research proposal potentially can impact kidney cancer research in two ways: (1) In the area of chromatin and gene regulation, it could provide a detailed understanding of how a cancers driver, being a chromatin regulator, regulates the expression and function of a key tumor suppressive factor; (2) In the area of therapeutic development, it could establish ISGF3 as a novel key target that needs to be activated to treat patients. Since a compound from the same chemical group (GSK-3745417) is under phase 1 clinical trials in hundreds of cancer patients with solid tumors, if di-ABZI is proven to have potent antitumor activity, it will lay the foundation for clinical trial of the most effective compound in kidney cancer patients. There could be a clinical impact in the not-so-distant future.

This proposal is novel as it reveals a key negative feedback loop that is activated by HIF and maintained by many secondary tumor suppressors including PBRM1. Once confirmed, this will establish a new paradigm.

With the combined power of two kidney cancer researchers from two renowned institutions, we will make fundamental advances in both the understanding of kidney cancer and the potential treatment of the patients.

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Therapeutic Targeting of the HHLA2/KIR3DL3 Axis as a Novel Immune Checkpoint Pathway in Renal Cancer

Rupal S Bhatt, Kathleen Mahoney MD, PhD
Beth Israel Deaconess Medical Center, Boston

Background

Immune checkpoint blockade-based therapy has revolutionized the field of cancer therapy. Modulation of the immune inhibitory vs immune stimulatory functions of T cells is effective in multiple cancer types including melanoma, lung cancer, bladder cancer, and renal cancer but a significant percentage of patients does not respond to this treatment, and the many patients that do respond eventually develop resistance. There is a critical, unmet need to find additional immune pathways that are non-redundant with the PD-1 pathway. HERV-H LTR-associating 2 (HHLA2) is a B7 gene family member that is highly expressed in several solid and hematologic cancers including primary human RCC. Different groups have reported a co-stimulatory or co-inhibitory effect of HHLA2 on T cell activation. TMIGD2, a CD28 gene family member, was shown to be a co-stimulatory receptor for T and NK cell activation, but the co-inhibitory receptor was unknown. In this study we identify KIR3DL3 as a co-inhibitory receptor for HHLA2 and confirm the co-stimulatory activity of TMIGD2. KIR3DL3 is a largely uncharacterized member of the KIR family of inhibitory receptors that contain an ITIM domain.

We show that KIR3DL3 is expressed on a subpopulation of activated T cells and some NK cells and inhibits their function. Until now, no ligand or function has been known for KIR3DL3. We have generated monoclonal antibodies (mAbs) against HHLA2 and KIR3DL3 that specifically block the KIR3DL3 inhibitory activity while preserving the TMIGD2 immune stimulatory effects of HHLA2. Additionally, we show that HHLA2 is upregulated in a variety of tumor types including ccRCC, where its expression and induction are distinct from PD-L1.
Hypothesis
Antibodies that selectively block the inhibitory signal of HHLA2:KIR3DL3 represent a new means to enhance antitumor immune responses in RCC.

Objective
We will identify the optimal agents to block the immune inhibitory effects of the HHLA2 pathway in vitro and in vivo. We will also study the regulation and expression of HHLA2 and KIR3DL3. The main objective is to generate the data needed to launch a phase 1 clinical trial in RCC patients.

Specific Aims
Aim 1. Mechanisms of KIR3DL3 immune inhibition
*What factors upregulate KIR3DL3 expression in T cells and NK cells?
*How does KIR3DL3 suppress T and NK cell cytokine production and cytolysis?
*Does the ITIM motif mediate the inhibitory function of KIR3DL3?
*What pathways induce expression of HHLA2, the KIR3DL3 ligand?

Aim 2. Blocking HHLA2:KIR3DL3 immune suppression in humanized tumor models
*To determine whether the HHLA2:KIR3DL3 pathway is an actionable checkpoint in HHLA2-expressing tumors, we will use humanized models of RCC to study this immune pathway in vivo. A humanized model will be necessary as there is no rodent homolog of HHLA or KIR3DL3.

*Does inhibition of the HHLA2:KIR3DL3 pathway improve response to PD-1 blockade in vivo?

Innovation
Our findings suggest that the HHLA2 immune checkpoint has parallels to the B7 - CD28/CTLA4 pathway, where the B7:CD28 interaction is stimulatory and the B7:CTLA-4 interaction is inhibitory. Thus, HHLA2 could have immune inhibitory effects or immune stimulatory effects, depending on its receptor interaction. Together these data suggest that targeting this new inhibitory checkpoint pathway alone or in combination with PD-1 blockade could have a great impact on the treatment of patients with metastatic cancer.

Impact
We have identified a new immune inhibitory pathway in RCC. The HHLA2:KIR3DL3 axis is a new target in RCC that is distinct from PD-1 and may represent an immune therapy alone or in combination with PD-1 pathway inhibitors.

The FY20 Kidney Cancer Research Program area(s) of emphasis include development of novel therapeutics for kidney cancer and the study of kidney cancer microenvironment and immunology.

25 Targeting the DNA Damage Repair Network to Promote an Innate Immune Response in ccRCC
Guang Peng MD, PhD
MD Anderson Cancer Center, University of Texas

Background
An urgent clinical need for patients with clear cell renal cell carcinoma (ccRCC) is to identify patients who can benefit from immunotherapy using immune checkpoint blockade (ICB) and to develop a mechanism-based combination approach to enhance the therapeutic efficacy of ICB in patients with ICB-insensitive tumors. The long-term goal of our research is to combine the research expertise of both Principal Investigators to determine if defects in the network of DNA damage response (DDR), a fundamental mechanism to maintain genomic stability and prevent endogenous DNA damage, (1) may occur in ccRCC tumors as a molecular consequence resulting from loss of major tumor suppressors during ccRCC progression; (2) may activate antitumor innate immune responses by accumulation of endogenous DNA; (3) may be used to guide the development of rational genetic-based combination therapeutic strategies that are more effective, less toxic, and that will impact survival of patients with ccRCC tumors.

Genetically, ccRCC is characterized by the inactivation of the VHL tumor suppressor gene. However, studies have demonstrated that VHL loss alone, though necessary, is not sufficient for ccRCC initiation. Molecularly, loss of VHL induces replication stress and increases replication fork instability, which are major endogenous sources for genomic DNA damage. We took both candidate-gene and unbiased genome-wide genetic screen approaches to identify molecular determinants for counteracting replication stress-induced DNA damage, which is required for the survival of VHL-deficient cells and thus the initiation/progression of ccRCC tumors driven by VHL loss. Our preliminary studies identified that two mutually exclusive genetic alterations in ccRCC, namely loss of PBRM1, a gene...
mutated in more than 30% ccRCC, and loss of NPRL2, a gene located in the 3p genome region and deleted in more than 10% of ccRCC, play distinct roles in regulating DDR, accumulation of endogenous DNA damage, and innate immune response.

Hypothesis/Study Design

Based on our preliminary data, we hypothesize that molecular changes caused by VHL deficiency and PBRM1/NPRL1 deficiency alter the DDR network and trigger antitumor innate immune responses mediated by the DNA-sensing STING pathway during ccRCC progression, which may create unique therapeutic vulnerabilities in ccRCC tumors to specific DDR/DNA repair inhibitors in combination with immunotherapy based on the genetic makeup in tumors (VHL-PBRM1 or the VHL-NPRL2 genetic axis) and/or the stages of tumor development. We will employ both in vitro cell models, preclinical mouse models, and patient specimens to test this hypothesis in three Specific Aims: (1) Determine molecular mechanisms and molecular requirements for DDR/DNA repair inhibitors to modulate the accumulation of endogenous DNA damage and initiate DNA-sensing signals of innate immune response in ccRCC cells with VHL deficiency and PBRM1/NPRL2 deficiency. (2) Characterize molecular changes in DDR and innate immune responses mediated by the DNA-sensing STING pathway in human ccRCC tumor samples. (3) Develop and optimize combination regimens using specific DDR/DNA repair inhibitors targeting VHL deficiency and PBRM1/NPRL2 deficiency to enhance the efficacy of immunotherapy in preclinical ccRCC mouse models.

Impact

The proposed project under this Translational Research Partnership Award represents an important step forward in applying our understanding of the DDR network from laboratory and preclinical studies to develop clinically testable approaches and improve the efficacy of immunotherapy in ccRCC patients in a mutation-specific manner. We will generate key preclinical data for developing optimized, personalized combination regimens, which can be translated into clinical trials in near future. All inhibitors used in this study are currently being tested in clinical studies. We anticipate the data we generate will permit us to enter into discussion with pharmaceutical companies who are developing various DDR inhibitors well before this grant is completed, thus achieving substantial impact on the renal cell carcinoma clinical trials landscape within three years.

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Novel cellular therapies to achieve cures in clear cell renal cell carcinoma

Wayne A Marasco MD, PhD

Dana-Farber Cancer Institute

Background

Curative treatment for advanced renal cell carcinoma (RCC) remains rare. To translate Chimeric Antigen Receptor (CAR) T cell therapy to cure clear cell renal cell carcinoma (ccRCC), several major hurdles common to solid tumors need to be overcome. They include heterogeneity of tumor associated antigens (TAAs) expression, on-target, off-tumor toxicity due to sharing of the TAAs that are overexpressed on tumor cells while also present on healthy cells, and the immunosuppressive tumor microenvironment (TME) that commandeers the tumor infiltrating lymphocytes (TILs), myeloid and stromal cells to become exhausted and/or dysfunctional. This Idea Development Award (IDA) proposal will build on the success of our first-generation lead anti-CD70-B7/anti-carbonic anhydrase IX (CAIX)-G9 Dual-targeted Fine-tuned Immune-Restoring CAR (DFIR-CAR) that will secrete anti-PD1/CTLA4 bispecific antibody (BsAB) at the tumor site. In this IDA project we plan to engineer further improvements in second-generation DFIR-CAR T cells by conducting a systematic evaluation of the optimal binding affinities of both the anti-CD70 and anti-CAIX scFvs. To achieve that, we will profile ccRCC heterogeneity and TME to ensure the secreted CBI payload will represent the optimal "public" targets to restore antitumor immunity.

Objective/Hypothesis

We anticipate the second-generation DFIR-CAR-T cells will achieve superior therapeutic index compared to the first-generation DFIR-CAR-T cells. We can achieve greater tumor-killing efficacy and safety profiling with further optimization of both the anti-CD70 and anti-CAIX scFvs. Increased efficacy will be achieved through anti-CD70/CAIX dual-targeting, which allows the CAR-T activation and tumor cell-killing with the presence of either or both antigens. Elevated safety is addressed though Fine-tuned CARs, which have the affinities of the scFv targeting moieties tailored to only recognize high-density tumor TAAs but not the same antigens expressed as physiologic levels on normal tissues. We will mine scRNAseq data from patients with advanced ccRCC to identify the key checkpoint blockage molecules that represent "public signatures" in exhausted TILs. Our antibody discovery pipeline will complete efforts to test CBIs against newly identified potential targets. Our
Objective is to obtain a greater therapeutic index (increased safety and efficacy) of the second-generation DF CAR-T cells that will be compared to results that we separately achieve with our first-generation anti-CD70 B7/anti-CAIX G9 DFIR CAR-T cells.

Specific Aims

Aim 1. Profile tumor heterogeneity and tumor microenvironment in ccRCC patients with advanced disease.

Subaim 1.1 Quantify CAIX and CD70 in ccRCC patient samples using immunohistochemistry and dSTORM.

Subaim 1.2 Profile immune exhausted ccRCC tumor microenvironment by using 10X genomics and GeoMx digital spatial profiling.

Aim 2. Optimize CAIX/CD70 dual-targeting and CBI payload of DFIR-CAR T cells to improve therapeutic index.

Subaim 2.1 Use Tet-On/Hsp70 inducible expression of CAIX/CD70 in ccRCC skrc-59 cell line model to optimize dual-targeted CAIX/CD70 CAR-T cells in vitro.

Subaim 2.2 Assess checkpoint blockade inhibitor (CBI) payloads in the powerful 3D ex vivo culture method using patient-derived organotypic tumor spheroids (PDOTS).

Subaim 2.3 Assemble CBIs to the optimized CAR-T cells and demonstrate CAR-T cell efficacy in humanized ccRCC orthotopic mouse model.

Study Design

Dr Marasco’s laboratory will continue to lead the CAR-T cell effort, as he has expertise in antibody engineering and CAR-T cell therapy. Tumor samples from ccRCC patients with advanced disease will be obtained through the support of DF/HCC Kidney Cancer SPORE Program (Dr Choueiri) and Dr Wee. Dr Barbie’s laboratory developed PDOTS to study kidney cancer. Dr Sabina Signoretti will provide her expertise in pathology to evaluate IHC stains. Antitumor immunity and tumor-killing efficacy of DFIR-CAR T cells will be restored on these advanced ccRCC PDOTS that conserve the patient tumor microenvironment and safety using control cholangiocyte-derived organotypic spheroids (CDOS). Humanized ccRCC orthotopic mouse model (ccRCC-hNSG-SGM3) with reconstructed human immune system (HLA 5/6 matched to ccRCC skrc-59 cells) will be used to assess DFIR-CAR T tumor-killing activity in vivo with the support of the Lurie Family Image Center (LFIC). We will utilize a variety of technologies, funds permitting, including spatial immunohistochemistry and dSTORM, single-cell RNA sequencing, and GeoMx digital spatial profiling.

Impact and Innovation: Building on our long-term research of developing ccRCC cellular therapy, we anticipate filing an Investigational New Drug application and advancing second-generation DFIR-CAR T to clinic after completion of these studies. Significant scientific advances will be made to overcome the barriers that have prevented this promising “living drug” therapy to be developed for patients with advanced ccRCC and to deliver safe and effective therapy.

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Pilot Development of New Forms of Chimeric Antigen Receptor Therapies for Kidney Cancer

Sidi Chen PhD

Yale University

Background

Background and Significance: Major hurdles in kidney cancer treatment: Kidney cancer at its late stage is highly lethal, currently with little success in treatments, where the distant type has a 12% 5-year survival rate. Recently, immunotherapy has shown success in treating kidney cancer, however, the majority of patients still do not benefit from it. There is an unmet medical need in kidney cancer that calls for innovative and potentially better treatments. CAR-T based cell therapy provides new options for kidney cancer treatment: Cell therapies such as chimeric antigen receptor T cells (CAR-Ts) have been proven to be powerful cancer therapeutics. CAR-T cell therapy has demonstrated remarkable efficacy in certain hematological cancers and is approved by FDA. Challenges of CAR-T therapy in solid tumors: However, despite current success, there remain major challenges for CAR-T therapy in solid tumors including kidney cancer, with no FDA-approved products. Thus, engineering better CAR-Ts is critical to achieving the full potential of cell therapy against kidney cancer.

Addressing FY20 KCRP Areas of Emphasis: (1) Therapeutic Development and (2) Genetics.

Objective

The overall objective of this project is to harness our enabling technologies such as KIKO and CLASH to generate, test, and further develop new forms of potentially better CAR-T therapies against kidney cancers.

Methods

Preliminary Data and Innovation: One of the most efficient ways to engineer better persistent CAR-T is to engineer
thousands of different CAR-T variants and efficiently select the best ones. Harnessing the Cas12a/Cpf1 systems with AAV, we have built a novel system that enables stable CAR-T with HDR knockin and immune checkpoint knockout (KIKO) generation at high efficiency in one step (Dai et al., 2019, Nature Methods). The modularity of AAV-Cpf1 KIKO enables flexible and efficient generation of multiple CARs elements in the same T cell. We recently developed CLASH (Cas12a-based Library-scale AAV-perturbation with Simultaneous HDR knockin), a novel and highly enabling platform for advanced massively parallel CAR-T engineering (Dai et al., in revision at Cell 2020). These versatile systems open new capabilities of therapeutic cellular engineering with simplicity and precision. CLASH allows us to achieve massively parallel engineering of CAR-Ts and identify the best candidates for potential therapeutic applications by exhausting many possible candidates and thereby providing lead therapies with enhanced efficacy or other desirable features against kidney cancer.

Why This Project is Highly Innovative and Potentially Transformative for Kidney Cancer (Highlights)

– Develops transformative T-cell engineering technologies invented by the investigators

– Harnesses highly versatile systems to enable massive CAR-T generation at an unprecedented scale

– Can bring multiple novel, potent, and specific lead CAR-T candidates against kidney cancer

Results

Specific Aims and Study Design:

Aim 1. Adapting KIKO and CLASH systems to generate advanced kidney cancer CAR-T candidates. We recently analyzed tumor surface proteome and identified a number of CAR targets for kidney cancer. We will generate kidney cancer CARs using the KIKO system and validate their expression, function, and antigen recognition. We will adapt the CLASH system to generate a massive number of CAR-T candidates and identify the promising ones.

Aim 2. Improving CAR-T efficacy by loss-of-function immune engineering for kidney cancer. To improve CAR-T efficacy against solid tumors, we will first adopt loss-of-function (LOF) engineering strategies. We identified multiple novel immunotherapy targets, where their LOF leads to enhanced antitumor functions (Dong et al., 2019, Cell; Ye et al., 2019, NBT). We will perform targeted LOF engineering to enhance kidney cancer CAR-Ts and immune profile them on effector function, proliferation, apoptosis, antigen-specific killing, memory, persistence, and exhaustion; and validate their efficacy against kidney cancer in tumor models.

Aim 3. Improving CAR-T efficacy by gain-of-function metabolic engineering for kidney cancer. We identified several metabolic boosters that enhanced antitumor efficacy of CAR-Ts (Ye et al., in revision, Nature Biotechnology). With these novel targets we will generate gain-of-function (GOF) engineered CAR-Ts and similarly immune profile them. We will then validate their efficacy against kidney cancer in vivo.

Conclusions

Outcome, Future Directions, and Impact: This project will establish new forms of promising CAR-T candidates with enhanced CAR-T antitumor efficacy against kidney cancer. Success of this project will enable us to advance the best version into investigational new drug (IND)-enabling activities, which if promising can transition into an early phase clinical trial. This, if it continues to succeed, can provide brand-new and promising CAR-T cell therapies for the treatment of kidney cancer patients, especially those at late stage, who are often out of options.

Overcoming Resistance to Immunotherapy in Renal Cancer by Targeting Telomerase

Esra Akbay PhD

University of Texas, Southwestern Medical

Background

Tumors exhibit features that differentiate them from the normal cells, alert the immune cells, and allow them to be recognized and eliminated by them under normal conditions. In cancer patients, tumors are able to bypass these mechanisms, suppress the immune system and outgrow. Recent efforts have resulted in better understanding of particular interactions between tumor cells and immune cells called immune checkpoint pathways. Treatments that block the immune checkpoint pathways, i.e., immunotherapies, and release the break on the immune cells have provided durable responses in a subset of cancer patients. These findings suggest that targeting the mechanisms tumor cells utilize to evade the host immune system can be efficacious, and more effective immune modulating treatments are needed for therapy-resistant tumors. A subset of clear cell renal cell carcinoma (ccRCC) patients show response to immune checkpoint blockade combinations. However, a majority of the tumors do not respond to these existing immunotherapies, highlighting the importance of developing novel immune-modulating treatments.
Methods
One of the hallmarks of cancer is to bypass telomere shortening induced replicative senescence. The very ends of chromosomes (telomeres) cannot be replicated as the rest of the genomic DNA and thus get progressively shorter with each cell division as the cells age. Normal cells are equipped with the proper machinery to detect the short telomeres to stop cell division when these structures become very short. This likely evolved as an initial anti-cancer protection mechanism. Unlike normal cells, the majority of cancer cells activate an enzyme called telomerase to replicate the ends of chromosomes to bypass these normal defense mechanisms to continue to grow. Telomerase is essential for the survival of almost all cancer cells. We are proposing to utilize a molecule that interferes with the function of telomerase to rapidly stop cancer cell growth with minimal or no cytotoxic side-effects in normal telomerase silent tissues. This molecule not only can kill cancer cells in culture dishes, it can potentially activate antitumor immunity. We propose to (1) discover the mechanism of activation of antitumor immunity by this molecule to (2) determine whether it can overcome resistance to existing immunotherapy treatments. For this goal we will utilize a fully immunocompetent renal cancer mouse model. Therapies that activate the immune system, i.e., immunotherapies, have been less toxic compared to other treatments and often provide more durable responses in patients. Our goal is to also determine whether targeting telomerase can synergize with current immunotherapy in renal cancer. Thus, we are addressing the "developing novel treatments" area of emphasis of the Kidney Cancer Research Program.

A Functional Genomics Approach to Identify Renal Cell Carcinoma Antigens Targeted by T Cells in the Tumor Microenvironment

Edus H Warren MD, PhD
Fred Hutchinson Cancer Research Center

Background
Systemic immunotherapy with immune checkpoint inhibitors is the initial therapy for most patients with renal cell carcinoma (RCC). The sustained tumor regression in some patients suggests the restoration of an immunologically active tumor microenvironment favoring antitumor activity. In contrast, other patients exhibit primary refractory disease, suggesting that tumor-reactive T cells are rare or absent within the tumor microenvironment. One approach to augmenting T-cell immunotherapy is with autologous T cells engineered to express a T-cell receptor (TCR) targeting a tumor-specific antigen and infused to the patient in large numbers. However, the tumor antigen targets of T cells in patients responding to systemic immunotherapy remain largely unknown. The identification of RCC-reactive TCRs and their cognate tumor-specific antigens would facilitate the development of tumor vaccines and TCR-engineered cellular immunotherapy. Our studies will directly address the 2020 KCRP Area of Emphasis to understand the basic and translational science of the tumor microenvironment and immunology in RCC.

While our laboratory and others have developed robust methods to assess the TCR repertoire and phenotype of tumor-infiltrating T cells, a major limitation is the lack of ability to link TCRs to their cognate antigens. Current methods for tumor antigen discovery are low throughput, often limited to certain HLA alleles, and are not suitable to unbiased screening of peptide antigens at a genome-wide scale. While computational methods may converge on an accurate model for peptide epitope prediction based on TCR sequence in the next decade, they are currently limited by the scale of data on actual TCR-peptide interactions. Therefore, we propose to leverage our unique experience in CRISPR/Cas9-based whole genome screening to develop an innovative platform for tumor antigen discovery in RCC. The objective of this study is to develop a robust platform for identification of the antigen targets recognized by TCRs isolated from T cells with cytotoxic activity against RCC tumor cells.

Methods
Specific Aim 1: To develop a novel CRISPR/Cas9-based whole genome platform to identify the genes expressed in RCC cells that are essential for the recognition of known antigens by CD8+ T cells. We will use TCR-engineered CD8+ T cells with redirected cytotoxicity against known RCC antigens encoded by TPBG and C19ORF48. RCC cell lines will be lentiviral-transduced with a library consisting of 121,756 unique single-guide RNAs (sgRNAs) targeting 19,050 protein coding genes. We have designed an innovative and improved genome-wide CRISPR-Cas9 library containing sgRNAs targeting highly conserved protein domains and exonic regions. The library-transduced RCC cells will be co-cultured with TCR-engineered CD8+ T cells and sgRNA representation before and after co-culture will be assessed by deep sequencing. We will optimize conditions including effector:target ratio and length of co-culture. We expect to not only enrich for sgRNAs targeting the known antigen-encoding genes, but also to comprehensively discover novel genes involved in antigen processing and presentation as well as genes in RCC cells that modulate effector T cell functions.
Specific Aim 2: To deploy a CRISPR/Cas9-based whole genome platform to identify the genes encoding unknown antigens recognized by TCRs derived from RCC-reactive CD8+ T cells. We will leverage our laboratory's unique collection of TCR sequences with unknown antigen specificity isolated from T cells with known cytotoxicity against primary RCC cultures and RCC cell lines. Using the optimized CRISPR/Cas9 screening approach, we will identify candidate genes encoding novel RCC antigens. After deconvoluting the specific epitopes using minigene and peptide-pulsing approaches, we will confirm the specific peptide antigens using targeted CRISPR/Cas9 deletion with peptide rescue, investigate mRNA and protein expression in RCC versus normal-adjacent tissue, and validate the functional capacity of TCR-transduced CD8+ T cells to kill autologous primary RCC tumor cells matched for HLA and antigenic protein expression.

Results

If successful, we will establish a platform that can be applied to discover the tumor-antigen specificity of tumor-infiltrating T cells and inform a new generation of rationally designed vaccines and engineered T cell reagents creating new strategies to significantly impact outcomes for patients with RCC.