Next Generation Sequencing in RCC: Towards Precision Medicine

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New Directions in Combination IO Therapy: Case Report on Overcoming Resistance
Comparing First-Line Therapies: A ‘Real World’ Analysis
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The purpose of Kidney Cancer Journal is to serve as a comprehensive resource of information for physicians regarding advances in the diagnosis and treatment of renal cell carcinoma. Content of the journal focuses on the impact of translational research in oncology and urology and also provides a forum for cancer patient advocacy. Kidney Cancer Journal is circulated to medical oncologists, hematologist-onscologists, and urologists.

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A Retrospective Look at Progress to Date and New Directions in 2020 and Beyond

Ulka Vaishampayan, MD

If we could locate points on a compass serving as a metaphore for future directions in kidney cancer management, the directions most likely would turn toward combination therapies, biomarkers, precision medicine, and innovative treatment approaches. There are numerous points in between arising from the unmet need to explore a myriad of different directions—all of which provided touchstones for discussion at the recently held 18th International Kidney Cancer Symposium in Miami, November 15-16, attracting more than 400 attendees. This was one of the largest crowds to attend the IKCS, sponsored by the Kidney Cancer Association. Two international symposia are held each year, the second scheduled for Antwerp, Belgium in 2020.

I had the pleasure and honor to serve as one of 80 speakers at the scientific sessions as key opinion leaders and investigators presented, analyzed, debated, and looked toward some consensus on numerous issues in a program ranging across the broadest possible spectrum of topics on renal cell carcinoma offered at any meeting worldwide. And yet, as broad as that spectrum was, there are a handful of overarching concerns that still dominate the narrative—among them the four mentioned at the beginning of my message.

During the last year, for example, two major approvals of combination therapies have provided compelling evidence for changing the paradigm of first-line therapy. The FDA approval of axitinib and pembrolizumab and of axitinib and avelumab expand our options to optimize combinatorial approaches, in addition to the approval of ipilimumab and nivolumab in 2018. And what about the other “points on the compass” identified earlier? Verification of biomarkers to help guide these choices remains arguably the most elusive goal and a formidable challenge we face. Innovative and novel approaches are emerging as more of the tumor microenvironment is revealed.

Successful prognostication of patients is critical to both the clinical practice of oncology and research, as it influences treatment approaches and may be used to stratify patients in clinical trials. Classically, the most robust prognostic variables were related to histology (subtype, grade, tumor size), clinical features (performance status and pace of disease), or laboratory parameters. As nomograms utilizing these variables have come under closer scrutiny, we are seeing a dramatic evolution in how these models can be improved with underlying genomic information. The article in this issue of the Kidney Cancer Journal on precision medicine offers a striking perspective on how BAP1 and PBRM1 expression, for example, can potentially enhance these prognostic models. Yes, we are making progress on extending the frontier of biomarker applications but the limitations are still formidable to achieve truly personalized medicine.

(continued on page 114)
Kidney Cancer Journal Author Guidelines

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- Reviews that summarize and synthesize peer-reviewed literature to date on relevant topics in a scholarly fashion and format.
- Original contributions based on original, basic, clinical, translational, epidemiological, or prevention studies relating to kidney cancer that are well documented, novel, and significant.
- Letters to the Editor on timely and relevant subjects pertaining to the diagnosis and treatment of renal cell carcinoma.
- Clinical case studies.

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Essential Peer-Reviewed Reading in Kidney Cancer

The peer-reviewed articles summarized in this section were selected by the Editor-in-Chief, Robert A. Figlin, MD, for their timeliness, importance, relevance, and potential impact on clinical practice or translational research.


Summary: Among 314 patients, 276 (87.9%) and 38 (12.1%) were treated with VEGFR-TKI and mTORI therapy, respectively. The most common tyrosine kinase inhibitor treatments were axitinib, cabozantinib, and sunitinib following IO therapy. In adjusted models, patients treated with VEGFR-TKI versus mTORI therapy had lower hazard of TTD after IO treatment (aHR=0.46; 95% CI: 0.30-0.71; P < 0.01). One-year OS probability (65% vs 47%, P < 0.01) and proportion of ORR (29.8% vs 3.6%, P < 0.01) were significantly greater for patients treated with VEGFR-TKIs versus those treated with mTORIs.

Conclusion: Targeted therapy has clinical activity following IO treatment. Patients who received VEGFR-TKIs versus mTORI therapy following IO therapy had improved clinical outcomes. These findings may help inform treatment guidelines and clinical practice for patients post-IO therapy. Patients may continue to experience clinical benefits from targeted therapies after progression on immuno-oncology treatment.


Summary: The authors reviewed the institutional nephrectomy registry of 1,990 adults who underwent radical or partial nephrectomy for unilateral, sporadic, nonmetastatic ccRCC between 1995 and 2011. Baseline anxiety and depression were identified using ICD-9 codes. Associations of anxiety or depression with 30-day complications and oncologic outcomes were evaluated. A total of 197 (10%) patients were identified with a diagnosis of anxiety or depression. Median follow-up among survivors was 10.0 (IQR 7.3-13.6) years, during which time 864 patients died, including 363 from RCC. After PS adjustment, clinical and pathologic features were well balanced between groups. Patients with anxiety or depression had increased overall 30-day complications compared to those without (17% vs. 11%, P = 0.011). No significant differences were noted in time to local ipsilateral recurrence (P = 0.54), distant metastases (P = 0.96), or death from RCC (P = 0.42) between patients with vs. without anxiety or depression, while patients with anxiety or depression trended toward worse overall survival (hazard ratio 1.29, 95%CI 0.98-1.69, P = 0.065).

Conclusion: Neither anxiety nor depression were significantly associated with oncologic outcomes among patients who underwent surgery for localized ccRCC. The trend toward worse overall survival among patients with anxiety or depression warrants further investigation.


Summary: This study calculated race- and sex-specific population attributable risk percentages (PAR%) and their 95% confidence intervals (CI) for hypertension and chronic kidney disease (CKD) among black and white subjects ≥ 50 years of age from the US Kidney Cancer Study (USKC; 965 cases, 953 controls), a case-control study in the Kaiser Permanente Northern California health care network (KPNC; 2,162 cases, 21,484 controls). The authors also estimated PAR% for other modifiable RCC risk factors (cigarette smoking, obesity) in USKC. In USKC, the PAR% for hypertension was 50% (95% CI 24-77%) and 44% (95% CI 25-64%) among black women and men, respectively, and 29% (95% CI 13-44%) and 27% (95% CI 14-39%) for white women and men, respectively. In KPNC, the hypertension PAR% was 40% (95% CI 18-62%) and 23% (95% CI 2-44%) among black women and men, and 27% (95% CI 20-35%) and 19% (95% CI 14-24%) among white women and men, respectively. The PAR% for CKD in both studies ranged from 7 to 10% for black women and men but was negligible (<1%) for white subjects. In USKC, the PAR% for current smoking was 20% and 8% among black and white men, respectively, and negligible and 8.6% for black and white women, respectively. The obesity PAR% ranged from 12 to 24% across all race/sex strata.

Conclusion: If the associations found are causal, interventions that prevent hypertension and CKD among black Americans could potentially eliminate the racial disparity in RCC incidence (hypothetical black:white RCC incidence ratio of 0.5).
Newsworthy, late-breaking information from Web-based sources, professional societies, and government agencies

Highlights from the IKCS, Miami, November 14-16: New Data on Combination Therapy, Potential Strategy in Refractory RCC, and Brain Mets

More than 400 attendees gathered in Miami for the 18th International Kidney Cancer Symposium, as key opinion leaders presented emerging and pivotal data from an agenda representing the most comprehensive information available on kidney cancer. Selected highlights are covered here. Detailed results and perspectives on the scientific symposium are available on the Kidney Cancer Association website.

To view slides of all presenters, please visit:
http://www.euikcs.com/kca/miami2019

To view videos of all presentations, please visit:
https://www.oncologytube.com/channel/kidneycancer

Lenvatinib and Pembrolizumab Combination Demonstrates Clinical Activity in Metastatic Renal Cell Carcinoma Patients Who Received Prior Immunotherapy

MIAMI—The combination of lenvatinib and pembrolizumab demonstrates clinical activity with a promising objective response rate (ORR) in metastatic renal cell carcinoma (mRCC) patients who progressed on prior immunotherapy. An interim analysis of the ongoing phase II clinical trial (NCT02501096) was presented by Dr. Chung-Han Lee of Memorial Sloan Kettering Cancer Center.

A total of 33 mRCC patients who received ≤2 prior systemic therapies, including those who progressed on a prior anti–PD-1/PD-L1 inhibitor, were included in the analysis. Patients were treated with the combination of the oral multikinase inhibitor lenvatinib 20 mg daily and the intravenous PD-1 inhibitor pembrolizumab 200 mg every 3 weeks. The primary endpoint was ORR by week 24 of treatment. Secondary endpoints included ORR, progression-free survival (PFS), duration of response, and safety and tolerability. A majority of the patients (58%) were previously treated with nivolumab/ipilimumab (21%) or VEGF-targeted therapy plus PD-1/PD-L1 inhibitor combinations. Per IMDC risk criteria, 29%, 55%, and 6% of the patients were in the favorable-, intermediate-, and poor-risk categories, respectively.

ORR as of week 24 was 61%. The best ORR was partial response in 64% and stable disease in 30% of the population. PFS was 11.3 months (95% CI, 7.3–NE) per investigator assessment using the irRECIST v1.1 definition. From a safety and toxicity standpoint, all patients experienced at least one treatment-related adverse event (TRAE). Grade 3 or 4 TRAEs were seen in 55% of the cohort. Most frequently seen TRAEs were fatigue (49%), diarrhea (39%), dysphonia (36%), stomatitis (33%), and nausea (27%). TRAEs led to the discontinuation of lenvatinib in 21% of patients and pembrolizumab in 18% of patients. One patient died due to potentially treatment-related upper gastrointestinal bleeding.

Dr Lee highlighted the promising activity of the lenvatinib and pembrolizumab combination in mRCC patients previously treated with immunotherapy. The adverse event profile of the combination was considered manageable. Dr Lee underscored that the clinical trial (NCT02501096) is still recruiting.

Updated OS Data on Tivozanib Suggest Potential Role in Refractory Setting, But FDA Still Unconvinced Pending More Results

MIAMI—Updated results from the Phase 3 TIVO-3 trial point toward a potential role for this TKI, but so far the FDA has withheld approval pending additional results on the use of tivozanib. Based on the data emerging from studies of tivozanib, there is speculation that the drug may have a role in patients previously treated with checkpoint inhibitors as well as two VEGFR-TKIs.

As previously presented, results for the intent to treat (ITT) population showed that tivozanib significantly improved progression free survival (PFS), the study’s primary endpoint, and overall response rate (ORR) compared to sorafenib, with responses to tivozanib more durable than sorafenib. Newly presented data include the recently announced interim overall survival (OS) hazard ratio (HR) of 0.99 within the ITT population, as well as results from two prespecified subgroup analyses of patients previously treated with a checkpoint inhibitor and a VEGF-TKI, or two VEGFR-TKIs. Superior PFS and ORR, as well as OS HRs below 1, favoring tivozanib, were observed in the prespecified subgroups. Tivozanib was shown to have lower overall rates of adverse events and fewer dose interruptions and reductions versus sorafenib, indicating better patient tolerability.

“Until the TIVO-3 trial results, limited prospective data existed to inform sequencing of treatment after checkpoint inhibitor therapy, the emergent standard of care in earlier-line treatment,” said Sumanta Pal, MD, in his presentation at IKCS. “Tivozanib’s outcomes within this population, as well as in those receiving two prior VEGF-TKIs, suggest an important potential role for tivozanib in the evolving refractory advanced RCC setting. Furthermore, tivozanib’s unique tolerability profile is potentially well suited to an advanced setting, where many are reluctant to accept higher rates of adverse events following multiple courses of therapy.” AVEO plans on completing a final OS analysis of TIVO-3 in June 2020 after a planned submission of a new drug application to the FDA in the first quarter of 2020.

Treatment-Free Survival With and Without Toxicity With Nivolumab and Ipilimumab in Metastatic Renal Cell Carcinoma

MIAMI—Treatment-free survival (TFS) without toxicity, a (continued on page 115)
Next Generation Sequencing in Renal Cell Carcinoma: Towards Precision Medicine

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Introduction
Worldwide, there were over 400,000 new cases and 175,000 deaths attributable to renal cell carcinoma (RCC) in 2018.1 In the United States alone, there are predicted to be over 73,000 new cases of RCC, accounting for nearly 15,000 deaths.2 17% of patients present with metastatic disease with only 12% of patients surviving 5 years.3 Fortunately, outcomes are improving. The number of efficacious systemic therapies for RCC has increased over the past decade and there are now over a dozen FDA approved agents and combinations for use in metastatic RCC.4-6 The treatment landscape has changed from one comprised exclusively of recombinant cytokines to one which includes angiogenesis inhibitors (mostly tyrosine kinase inhibitors, TKI), mammalian target of rapamycin (mTOR) inhibitors, and most recently, the immune checkpoint inhibitors (ICI).4,7 Despite this progress, precision medicine has advanced little and there are no biomarker tests approved by the FDA to guide treatment selection.

Classically, RCC is subdivided histologically into clear cell RCC (ccRCC) accounting for 75% of cases, type I and II papillary RCC (pRCC) accounting for 10% of cases, chromophobe RCC (chRCC) accounting for 5% of cases, and other less frequent subtypes.8 RCC is now recognized to be a diverse group of diseases with updated society guidelines incorporating molecular and genomic data along with histologic information when defining RCC subtypes.9,10

Discovery of the von Hippel-Lindau Tumor Suppressor Gene
The first tumor suppressor discovered in ccRCC was the von Hippel-Lindau (VHL) gene. VHL syndrome is an autosomal dominant disease caused by germline mutations in the VHL gene. This syndrome results in development of numerous ccRCCs among other manifestations.11 Genetic linkage analysis of affected kindreds located the responsible gene to the chromosome region 3p25-26 in the late 1980s.12,13 Latif and colleagues were the first to identify the VHL gene,14 and the following year VHL was also shown to be mutated in sporadic ccRCC.15 Nearly two decades later, Nickerson and colleagues evaluated a cohort of 205 ccRCC samples, the largest cohort at that time, for aberrations in the VHL gene through targeted sequencing. They identified non-silent somatic mutations with a prevalence of 82% and VHL promoter hypermethylation in an additional 8.3% of tumors, for a total of 90% of tumors.16 In sporadic ccRCC, the initiating event is thought to be loss of 3p through a chromothripsis event.17 This is likely followed by mutation or epigenetic silencing of the second allele. While VHL loss is nearly universal in ccRCC, VHL inactivation has also been shown in preneoplastic cysts, and mice with VHL disruption in the kidneys do not develop ccRCC.18,20 Thus, while VHL loss is an important initiating step in the development of ccRCC, additional driver mutations were suspected.

The VHL protein forms a complex with Elongin B, Elongin C, and RBX1 that serves as an E3 ubiquitin ligase which, at normal oxygen tension, acts on the alpha unit of hypoxia inducible factor (HIF) transcription factors, leading to their degradation.21 Loss of VHL in ccRCC results in constitutive activation of HIF and expression of its target genes.21,22 Interestingly, mutations in TCEB1 (encoding Elongin C) have been reported in up to 5% of ccRCC cases and are associated with loss of heterozygosity of chromosome 8, which contains TCEB1.23 In addition, mutations in CUL2 were found in up to 1% of ccRCCs.24 Mutations in TCEB1 and CUL2 tend to be mutually exclusive with VHL mutations and likely represent other mechanisms to disrupt VHL function.25

Identification of the PBRM1 and BAP1 Tumor Suppressor Genes
The first large scale sequencing reports in ccRCC came out in 2009, and demonstrated frequent mutations in the chromatin remodeling genes SETD2, KDM6A (also known as UTX), KDM5C (also known as JARID1C), and MLL2.26,27 These reports were limited to sequencing a panel of ~3,500 genes, a small subset of the entire human exome, and the genes identified were mutated in up to 15% of tumors. In the subsequent two years, whole exome sequencing (WES) allowed the discovery of two major drivers of ccRCC; polybromo 1 (PBRM1) and the BRCA1 associated protein-1 (BAP1). Varela and colleagues performed WES of seven

Keywords: Kidney cancer, molecularly targeted therapies, immune checkpoint inhibitor, next generation sequencing, genomics, renal cell carcinoma, BAP1, PBRM1

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ccRCC and matching normal samples and identified truncating mutations in PBRM1 in four tumors.\(^{28}\) Non-silent mutations in PBRM1 were subsequently identified in 88 of 221 (39.8\%) ccRCC cases, making it the second most common mutation in ccRCC.\(^{28}\) The following year, WES of paired tumor and patient-derived xenografts (PDX, also called tumorgrafts) identified BAP1 as an important driver of ccRCC.\(^{29}\) By incorporating tumorgrafts, our studies allowed for accurate calls of mutant allele frequencies (MAF) thereby confidently nominating putative two-hit tumor suppressor genes. In tumorgraft models, BAP1 was the only gene in addition to VHL and PBRM1 to demonstrate a MAF of \(~1\) (signifying absence of the wild-type allele). Subsequent BAP1 targeted sequencing studies identified mutations in 24 out of 176 (14\%) largely primary ccRCC samples.\(^{29}\)

PBRM1 and BAP1 are both involved in chromatin modification and epigenetic regulation of gene expression. PBRM1 encodes BAF180, a component of the switching defective/sucrose nonfermenting (SWI/SNF) family of nucleosome remodeling complexes, which is thought to mediate recruitment of the complex to specific nucleosomes through recognition of acetylation patterns.\(^{25,30}\) The BAP1 protein is a nuclear-localized deubiquitinase which acts to deubiquitylate H2AK119ub1 to reverse polycomb-mediated gene repression.\(^{30}\) These processes likely regulate different gene subsets, and we discovered that BAP1 and PBRM1-deficient tumors can be distinguished by their gene expression signature.\(^{31}\)

Interestingly, both BAP1 and PBRM1 are located on chromosome 3p, within a 43 Mb region that is frequently lost in ccRCC, which also includes VHL and SETD2.\(^{17,32}\) Notably, BAP1 and PBRM1 mutations were found to anticorrelate in ccRCC and the prevalence of combined BAP1/ PBRM1 deficient ccRCC is \(~1\%-2\%)\, less than the \(~5\%) rate expected given the rates of PBRM1 \((~45\%)\) and BAP1 \((~12\%)\) mutations in ccRCC.\(^{29,31-34}\) While mutual exclusivity often indicates that the encoded proteins are in the same pathway (and reflects the low selective pressure to have them simultaneously mutated), the finding that loss of BAP1 and PBRM1 results in distinct gene expression signatures, suggested that this was not the case for these genes.\(^{29,31}\) Furthermore, BAP1 mutant tumors exhibited higher nucleolar grade, more aggressive histology, and demonstrated worsened RCC-specific survival.\(^{31,35-38}\) This finding was subsequently confirmed in metastatic RCC, as BAP1 mutations were independently associated with worsened overall survival.\(^{39,40}\) Together these findings led us to propose a model where following inactivation of VHL and 3p loss, inactivation of the remaining copy of BAP1 caused aggressive ccRCC and inactivation of PBRM1, less aggressive ccRCC.\(^{25}\) Using methods we developed,\(^{29}\) this model was subsequently revised by the TRACERx consortium to show that the first event in sporadic tumors is likely the loss of 3p.\(^{17}\)

To determine the role of BAP1 and PBRM1 in ccRCC development, we inactivated them in nephron progenitor cells in the mouse and assessed their impact on RCC development. By simultaneously targeting Bap1 and Vhl, we developed the first mouse model of ccRCC thereby overcoming a decade-long struggle.\(^{19}\) We showed that ccRCC development required not only Vhl inactivation, but also the inactivation of Bap1 (or Pbrm1). As for Bap1, Pbrm1 loss was not sufficient to induce RCC. However, the simultaneous inactivation of Vhl and Pbrm1 caused ccRCC.\(^{37}\) Similar observations were made by others.\(^{41,42}\) We also found, that as in humans, Bap1-deficient tumors were of high grade, whereas Pbrm1-deficient tumors were of low grade. In addition, Pbrm1-deficient tumors developed after a longer latency period.\(^{37}\) Interestingly, targeting one allele of Tsc1, which encodes a negative regulator of mTOR complex 1, in a Vhl/Pbrm1-deficient background reduced the latency period and increased the frequency of higher grade tumors.\(^{37}\) (Figure 1).

These discoveries explain why germline VHL mutations cause kidney cancer in humans, but not in mice. As it turns out, we found that in mice the Bap1 and Pbrm1 genes are on a different chromosome than Vhl and as such, loss of the chromosome arm containing the Vhl gene in the mouse.

Figure 1. PBRM1, BAP1 and TSC1 are drivers of ccRCC development and tumor grade. According to PBRM1 and BAP1, ccRCC can be subclassified into 4 different subtypes. Double mutant tumors are under-represented suggesting that simultaneous mutations are mutually exclusive in tumors. PBRM1- and BAP1-mutant tumors are associated with different biology (gene expression), pathological features, and outcomes in patients. Modeling studies in the mice show that (i) Vhl inactivation is insufficient for ccRCC development; (ii) the combination of Vhl and Pbrm1 mutations results (as in humans) in low grade tumors; (iii) the combination of Vhl and Bap1 mutations results (as in humans) in higher grade tumors; and (iv) Tsc1 mutations increase the grade of Vhl/Pbrm1-mutant tumors. 
would have no effect on Bap1 or Pbrm1 genes. In contrast, loss of 3p in humans results not only in the loss of a VHL allele, but also one allele of both BAP1 and PBRM1. Therefore, the arrangement of tumor suppressor genes in the genome likely accounts for differences in tumor predisposition across species.

In summary, somatic mutations in either PBRM1 or BAP1 tend to be mutually exclusive and activate distinct gene expression programs in tumors leading to differentiated pathological features, ultimately causing divergent clinical outcomes. As such, these discoveries resulted in the first molecular classification of ccRCC. Several unanswered questions remain, however. For instance, what other events cooperate with VHL in tumors that are seemingly wild-type for BAP1 and PBRM1? Or how do mutations in TCEB1 and CUL2, which are located on chromosome 8 and 10 respectively, lead to ccRCC formation, and what are the specific cooperating events?

**Next Generation Sequencing Supports Comprehensive Integrated Analysis of RCC**

The development of NGS and improved bioinformatics tools allowed the collection and integration of data from WES, copy-number analyses, DNA-methylation analyses, and messenger RNA and microRNA sequencing, from individual samples at a large scale. Through integrated analyses of ~400 ccRCC samples, The Cancer Genome Atlas (TCGA) consortium not only confirmed previous findings, but significantly expanded on them through the identification of altered subnetworks. Initial genome-wide copy number analysis, using next-generation sequencing technologies, identified 3p and 14q loss, as well as 5q gain as the three most common somatic copy number alterations (SCNA) in ccRCC (43, 44). These were verified in a larger TCGA cohort - 3p loss (91%), 14q loss (45%) and 5q gain (67%). The most frequently mutated genes involved chromatin remodeling pathways. These included genes encoding the SWI/SNF family proteins PBRM1, SMARCA4 and ARID1A (47.1%), the histone methyltransferases SETD2 and MLL (23.8%), and the histone deubiquitinase BAP1 (12.1%). Alterations in PTEN, TSC1, and other components of the PI3K/AKT/mTOR pathway were observed in 28% of ccRCCs. Other findings of significance included loss of one CDKN2A allele in 16.2% of ccRCCs (mostly through deletion of 9p21.3), and mutation of TP53 in 2.6% of cases.

In a similar analysis of non-ccRCC, we found distinct mutated genes and SCNA alterations highlighting the diverse molecular landscape of RCC. In pRCC, we identified 10 significantly mutated genes including MET, SETD2, and NF2. Activating MET mutations were identified in 15% (10/65) of pRCC analyzed, including 4 previously unreported mutations. Furthermore, 70% of pRCC samples had amplification of chromosome 7, containing MET, and these samples had higher levels of MET expression, consistent with the role of MET in type I pRCC. These findings were confirmed in a subsequent analysis of the pRCC TCGA cohort, where activating MET mutations were identified in 17% (14/75) of type I pRCC samples, which exhibited near universal gain of chromosomes 7 and 17. In contrast, copy number analysis of type II pRCC revealed two distinct subtypes, one with relatively few SCNA and another with a high degree of aneuploidy and frequent chromosome 9p loss. DNA methylation studies revealed a subset of pRCC (9/160) referred to as the CpG Island Methylator Phenotype (CIMP), eight of which were categorized as type II pRCC histologically. CIMP tumors were characterized by universal hypermethylation of CDKN2A, frequent fumarate hydratase (FH) mutations (including somatic mutations, 57%), and worse survival relative to non-CIMP pRCC. Loss of FH and the subsequent accumulation of fumarate has been demonstrated to result in deregulation of the nuclear factor erythroid 2-related factor (NRF2)/antioxidant response element (ARE) pathway through direct effects of fumarate on KEAP1, an inhibitor of NRF2. This was first demonstrated in hereditary leiomyomatosis and renal cell cancer (HLRCC), an inherited form of type II pRCC arising from germline inactivation of FH. Consistent with the notion that FH loss leads to deregulation of the NRF2/ARE pathway, expression of NQO1, a canonical transcriptional target of NRF2, was highest in CIMP tumors. Targeted sequencing in sporadic type II pRCC revealed activating mutations in the NRF2/ARE pathway in four of five cases. Genomic analysis of the TCGA cohort supported this finding with activating mutations in the NRF2/ARE pathway in 16.7% (10/60) of cases. An NRF2/ARE gene transcription program is a distinguishing feature of type II pRCC.

Analysis of chRCC revealed distinct molecular alterations defining this subgroup. chRCC can be classified histologically into classical and eosinophilic subtypes, the latter characterized by abundant eosinophilic cytoplasm and densely packed mitochondria. Analysis of the TCGA cohort of 66 chRCC identified molecular distinctions between these two subgroups. All 47 cases of classical chRCC in the TCGA cohort demonstrated characteristic chromosomal losses of chromosomes 1, 2, 6, 10, 13, and 17 whereas only half (10/19) of the eosinophil variants did. Studies from TCGA and our own group found that TPS3 mutations were significantly enriched (P = 2.3E-5) in the classic chRCC subtype. The two most frequently mutated genes across chRCC variants are TPS3 (31.1%) and PTEN (9%). Mutually exclusive mutations in genes of the PI3K/AKT/mTOR pathway were observed in 23% of cases. Interestingly, analysis of mitochondrial DNA in chRCC revealed recurring mutations in genes encoding components of complex 1 of the electron transport chain (ETC), although these mutations were not associated with changes in the expression of genes implicated in oxidative phosphorylation. chRCC are particularly enriched in mutations involving metabolic genes, including deleterious mutations in PDHB (which encodes a critical component of the pyruvate dehydrogenase complex) and PRKG2 (encoding one of three subunits of AMP-Kinase (AMPK)). These findings reinforce the long-standing implication that metabolic derangements in RCC can contribute to oncogenesis.

Integrated transcriptome and protein expression analysis (using reverse protein phase arrays) in TCGA revealed distinct metabolic patterns both across and within histological subtypes of RCC. ccRCC was characterized by overexpression of glycolytic and fatty acid synthesis enzymes as well as suppression of Krebs and ETC programs. This contrasts with pRCC and chRCC, which generally expressed intermediate and high levels of Krebs and ETC proteins, respectively. Decreased expression of AMPK (which inhibits fatty acid synthesis and mTOR), and increased ribose sugar metabolism, correlated with higher stage and worse prognosis in ccRCC. The CIMP subtype of pRCC demonstrated the highest level of ribose sugar metabolism across all RCC subtypes. A metabolically divergent subset of chRCC was identified, which demonstrated decreased levels
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of AMPK and ETC genes, increased ribose sugar metabolism, and demonstrated a profoundly worse outcome than metabolically traditional chRCC.24 This metabolically divergent subtype of chRCC lacked the typical chromosomal aberrations of chRCC, and four of the six displayed sarcomatoid de-differentiation. Taken as a whole, these landmark studies contributed tremendously to our understanding of the molecular biology of RCC, and the findings are driving the development of molecularly-based prognostic models across histological subtypes in RCC.

Identification of Prognostic and Predictive Biomarkers
Successful prognostication of patients is critical to both the clinical practice of oncology and research, as it influences treatment approaches and may be used to stratify patients in clinical trials. Classically, the most robust prognostic variables were related to histology (sub-type, grade, tumor size), clinical features (performance status and pace of disease), or laboratory parameters. For localized disease treated with curative nephrectomy, the two main prognostic models are the UCLA Integrated Scoring System (UISS) and the Mayo Clinic Stage, Size, Grade, and Necrosis (SSIGN) score.54-55 The UISS score integrates pathological (tumor size and nuclear grade) with clinical (ECOG performance status) variables, whereas the SSIGN score utilizes strictly pathological variables. Despite the frequent use of the aforementioned models in patient prognostication, a recent analysis has called into question the predictive power of these nomograms.56

Utilizing prospectively collected data from the ASSURE trial,57 a randomized phase III placebo-controlled trial of sunitinib and sorafenib in the adjuvant setting, Correa and colleagues analyzed 8 recurrence prediction models including the SSIGN and UISS tools. They reported that the predictive power for each model’s predefined outcome, as measured by the co-occurrence index (C-index), fell considerably for all models in the dataset. However, among these models, the SSIGN score performed best with a C-index of 0.688 (95% CI: 0.686 – 0.689). Most other nomograms provided only marginal improvement relative to the TNM staging system.56 Integrating underlying genomic information with clinical and histological variables is one strategy to improve these models. We previously demonstrated that BAP1 and PBRM1 expression by immunohistochemistry (IHC) are highly correlated with mutation status (P = 3E-58 and 4E-23 respectively)58 and that BAP1/PBRM1 expression by IHC are independent predictors of both disease-free survival and overall survival in the localized disease setting.59-61 PBRM1 and BAP1 expression status did not, however, add independent prognostic information to the SSIGN score in a multivariate analysis of nearly 1,500 cases of localized RCC.62 One possible explanation is that BAP1-mutant tumors are significantly more likely to demonstrate higher nuclear grade, stage, and necrosis than PBRM1-mutant tumors.34-37 Thus, despite progress, additional strategies to stratify patients in the localized disease setting remain needed.

For advanced disease, the most commonly used prognostic models are the Memorial Sloan Kettering Cancer Center (MSKCC) risk model68 and the International Metastatic RCC Database Consortium (IMDC) risk model.69 Both models integrate clinical variables (time from diagnosis to systemic treatment and performance status) and laboratory variables including calcium and hemoglobin (as well as lactate dehydrogenase in the MSKCC model vs platelet and neutrophil counts in the IMDC model) but are agnostic to the molecular biology of the underlying disease. Many of the key driver mutations in RCC have been shown to have negative (BAP1, SETD2, PTEN, and TP53) and positive (PBRM1) prognostic significance.24,34,53,60 although it is important to interpret the prognostic implications of these mutations in the context of histology. For example, PBRM1 mutations which tend to associate with favorable prognosis in ccRCC were strongly associated with reduced survival in type I pRCC (p<0.0001).24 Regardless, retrospective analysis of two phase III trials in metastatic RCC, COMPARZ (first-line sunitinib vs pazopanib) and RECORD-3 (first-line sunitinib vs everolimus) identified PBRM1, BAP1, and TP53 as having independent prognostic value.40 Their prognostic significance persisted after multivariate analysis with the traditional variables in the original MSKCC model. This resulted in the development of a genomically-annotated MSKCC risk model which stratifies patients into four risk groups (favorable, good, intermediate, and poor) versus three in the original. The genomically-annotated model had a more balanced distribution across these groups and an improved ability to predict overall survival. The C-index of the original model was 0.567 (95% CI: 0.529 – 0.604) vs 0.637 (95% CI: 0.529 – 0.604) with the new model.40 While promising, this model will need to be validated in the prospective setting.

Prognostic models have also been developed using gene expression data. One of the first such attempts in ccRCC was by Brannon and colleagues who collected transcriptomic data in 47 ccRCC primary tumor samples. Unsupervised clustering analyses revealed two subgroups, referred to as clear cell types A and B (ccA/B), which had distinctly different clinical outcomes with a median survival of 59 vs 36 months respectively in a validation cohort of 177 patients (P=0.004).61 A 34 gene panel (Clear-code34), which could delineate ccA and ccB subtypes, was found to better predict relapse-free survival and cancer-specific survival than both the UISS and SSIGN models in a cohort of nearly 500 patients with localized RCC.62 Similar findings were seen in a smaller metastatic cohort (54 patients), however, only ccB classification added prognostic value when incorporating the IMDC and MSKCC risk models.63

More recently, cluster of cluster analysis including DNA copy number, mRNA, microRNA, DNA methylation, and protein expression data from the TCGA cohort revealed 9 unique clusters across all histological subtypes.53 three of which were enriched in histologically defined ccRCC (e.1, e.2, and e.3). These demonstrated significant differences in survival, with e.3 demonstrating the poorest prognosis and e.2 demonstrating the most favorable. Tumors in the e.3 cluster tended to have frequent loss of the CDKN2A gene, frequent BAP1 mutations, and overexpression of cell cycle and hypoxia-related genes.53 Additionally, e.3 was enriched in inflammatory gene expression relative to e.2, and expression of PDCD1 (encoding for PD1) and CTLA4 were independent predictors of poor prognosis across the ccRCC.
subtypes. The e2 subgroup, on the other hand, was found to demonstrate significant overlap with the previously described cCA subtype which is distinguished by overexpression of angiogenesis-related genes such as FLT4, FLT1, and VEGFR. Thus, given that the most widely utilized treatments in metastatic RCC are immune checkpoint inhibitors, angiogenesis inhibitors, or combinations of the two, transcriptomic information regarding inflammation or angiogenesis may also have predictive potential.

Unlike prognostic biomarkers which inform on the spontaneous trajectory of the disease, predictive biomarkers have the potential to inform on treatment selection, which may be tailored to the particular patient. Beyond the obvious benefit of selecting the most efficacious treatment, predictive biomarkers have the potential to reduce adverse events as well as cost from exposures to non-beneficial drugs. The use of gene expression profiles to predict treatment response was considered in an exploratory analysis of the IMmotion150 trial, a phase 2 study comparing the combination of atezolizumab (a PD-L1 inhibitor) and bevacizumab (a neutralizing VEGF antibody) to atezolizumab (atezo) or sunitinib monotherapy. McDermott and colleagues showed that predefined gene signatures of angiogenesis (Angio), immune T cell infiltration (T eff), and myeloid inflammation (Myeloid) may have predictive potential. They found that patients with the Angio high gene signature had an improved progression-free survival (PFS) compared to those with Angio low with the angiogenesis inhibitor, sunitinib. Interestingly, PBRM1 mutations were found to be enriched in the Angio high subgroup. In line with these findings, a similar analysis of the COMPARZ trial evaluating two anti-angiogenic agents, sunitinib and pazopanib, found that high expression of angiogenesis genes was associated with improved response rates. Furthermore, tumors with PBRM1 mutations demonstrated significantly higher angiogenesis gene expression scores than those with BAP1 mutations. In the IMmotion150 trial, patients with the T eff high gene signature had improved PFS with atezo+bev compared to the sunitinib and the atezo arms. Intriguingly, when examining the effect of myeloid signature on outcomes within the T eff high population, a PFS advantage of atezo+bev over atezo was observed in the T eff high/Myeloid-high subgroup (n=66) but not in the T eff high/Myeloid-low subgroup (n=66). This finding may identify a particular population of patients which would benefit from combination ICI and TKI therapy. However, this is a relatively small study and has not yet been reproduced in larger datasets.

These studies highlight the potential role of the tumor microenvironment (TME) on clinical outcomes. Bioinformatics techniques can be leveraged to gather information about the TME from RNA extracted from tumors. One such approach is to utilize single gene set enrichment analysis (ssGSEA) in which signature gene panels attributed to particular cell types are utilized to disentangle a heterogeneous tumor. Our group expanded upon this method by exploiting patient-derived tumorgrafts (patient tumors implanted in mice), where the human TME is ultimately replaced by the host. By focusing on human RNA and studying the transcriptome of the tumorgraft, one is left with the human TME transcriptome. Utilizing this dissection algorithm and a cutoff of 20-fold to distinguish TME vs tumor genes, an empirically derived TME (eTME) signature was obtained. Clustering analyses of the ccRCC TCGA (KIRC) tumors according to the eTME revealed two subgroups, an inflamed subtype (IS) and a non-inflamed subtype (NIS). Interestingly, the IS was enriched for BAP1 mutations (P = 7.7E-5) and demonstrated a worse prognosis compared to NIS. Furthermore, the eTME-IS subtype correlated with systemic inflammatory markers such as elevated platelet counts and decreased hemoglobin levels, as well as worse prognosis in three distinct cohorts totaling approximately 1,000 patients. This correlation draws a link between inflammatory subtypes of RCC and key prognostic variables in IMDC or MSKCC models. Notably, the presence of such variables is associated with intermediate/poor risk disease, which suggests that inflammatory tumors are particularly aggressive in patients. Interestingly, in the Checkmate 214 trial comparing the combination of nivolumab and ipilimumab to sunitinib, the intermediate/poor risk groups appeared to derive the most benefit from ICI, which is consistent with the predictive potential of the IS subtype. Thus, the combined use of NGS and bioinformatics has potential to predict responses to therapy and is an active area of investigation.

Intratumoral Heterogeneity

One important disadvantage of the aforementioned studies is the use of a limited number of tumor samples per patient (typically just one), as this may fail to capture intratumoral heterogeneity (ITH). In one of the earliest attempts to measure ITH in ccRCC, Gerlinger and colleagues performed multiregional WES of two primary tumors and demonstrated that only 31% of the somatic mutations were ubiquitous amongst all sampled regions. Furthermore, previously described “cc-A” and “cc-B” gene expression patterns could be identified in spatially distinct areas of an individual patient’s tumor, highlighting how ITH can confound efforts to establish effective prognostic models based on analyses of a single sample. This laid the groundwork for the TRAcking Cancer Evolution through therapy (Rx) (TRACERx) consortium, which prospectively collects tumor samples and performs multiregional sequencing, when possible, over time. In a recently published report, Turalichtig and colleagues sequenced 1,206 primary tumor samples from 101 patients with the use of a 110 gene panel, allowing for an unprecedented view of the molecular diversity within a single tumor. Multiregional sampling allowed for the detection of clonal and subclonal somatic mutations. Thus, the prevalence of PBRM1 (55%), SETD2 (25%), BAP1 (19%) and other driver mutations could be more accurately calculated. Interestingly, while BAP1 and PBRM1 mutations could be identified in the same tumor, they were typically located in spatially distinct regions, consistent with previous reports that these mutations anticorrelate with one another and are found in different areas of the same tumors.

Genomic data obtained from spatially distinct regions provided the ability to assess the timing of mutations, and thus patterns of tumor evolution could be inferred. In the TRACERx studies, seven distinct patterns could be identified utilizing rule-based clustering. However, 36.6% (37/101) of cases could not be assigned an evolutionary subtype. Subtypes were assessed for nuclear grade, stage, microvascular invasion, genomic instability, and degree of ITH. The most aggressive subtype, based on the aforementioned parameters, was the “multiple clonal drivers” subtype, which contained truncal aberrations in two or more of the following: BAP1, PBRM1, SETD2, or PTEN. It is important to note that in the “multiple driver mutations” subtype, the temporal relationships of driver mutations were indistinguishable, which separates this group from subsets defined
by subclonal (sequential) aberrations in driver genes. The “multiple clonal drivers” subtype includes BAP1/PBRM1-deficient tumors previously described which we and others noted to portend a poor prognosis. The “BAP1 driven” evolutionary subtype, characterized by truncal VHL and BAP1 mutations was also found to have aggressive histological characteristics, with decreased DFS and OS. Three PBRM1-driven subtypes were noted to have sequential loss of PBRM1 followed by either loss of SETD2, activation of PI3K signaling, or distinct SCNAs. Consistent with this, in 11/101 cases, PBRM1 loss was noted to precede SETD2 loss, but the converse was not seen. The least aggressive subtype was the VHL monodriver subtype, which likely represents sampling early in the disease course. When compared with “multiple clonal driver” and “BAP1 driven” subtypes, the three PBRM1 subtypes tended to have increased ITH, less aggressive biology, and demonstrated a more attenuated disease course. Conversely, the “multiple clonal driver,” “BAP-1 driven” and, perhaps unexpectedly, “VHL wild-type” evolutionary subtypes tended to demonstrate rapid progression to metastases. Decreasing ITH tended to correlate with a more aggressive disease course, consistent with the notion that an aggressive clone would “out-compete” other clones. On the other hand, evolutionary subtypes with greater degrees of ITH demonstrated a more attenuated disease course. Tumors with a high degree of ITH may, however, also harbor a diverse reservoir of cancer cells which are resistant to therapy, providing a potential explanation for the mixed responses sometimes exhibited by patients. Capturing the full degree of ITH may be challenging, as on average at least 7 biopsies are required to detect over 75% of variants. Though challenging to implement clinically, techniques are evolving that allow the dissection of the whole tumor followed by single-cell sequencing to provide molecular information at the cellular level. Other approaches include lysing large quantities of tumor material with deep sequencing in an attempt to comprehensively catalogue all mutations. Future prognostic and predictive models will likely need to incorporate methods to capture ITH to faithfully predict patient outcomes.

**Leveraging Next Generation Sequencing for Therapeutic Gain**

A deeper understanding of the biological underpinnings of RCC has led to novel therapeutic opportunities. This has resulted in a dramatic shift in the treatment landscape over the past decade. Immuno-therapy and ICI+TKI combinations are now frontline therapies and objective response rates as high as 60% are seen. Future directions will focus on identifying the correct agent for the correct patient, as well as developing novel therapies. NGS has allowed the identification of activating mutations in oncogenes, and these have been very effective targets in cancers such as melanoma and non-small cell lung carcinoma. Oncogenes are not, however, commonly mutated in RCC and thus have not been an area of therapeutic gain. One exception is the MET oncogene in which activating mutations have been observed in nearly 20% of type I pRCC. Cabozantinib is a TKI with activity not only against the VEGFR, but also MET. However, how much MET targeting contributes to its activity against ccRCC is unclear. In a phase III trial comparing second line cabozantinib to everolimus in ccRCC, cabozantinib was found to result in an improved OS rate (HR 0.66 [95% CI: 0.53 – 0.83]; p = 2.6E-4), as well as improved PFS (HR 0.51 [95% CI: 0.41 – 0.62]; p < 1E-4). MET overexpression by IHC did not predict, however, PFS...
A deeper understanding of the biological underpinnings of RCC has led to novel therapeutic opportunities. This has resulted in a dramatic shift in the treatment landscape over the past decade. Immunotherapy and ICI+TKI combinations are now frontline therapies and objective response rates as high as 60% are seen.

One initial report utilizing a shRNA library directed against 88 kinases in VHL-deficient RCC cell lines identified CDK6, MET, and MAP2K1 as potential targets. More recently, an expanded shRNA library targeting ~1000 genes identified EZH1 depletion to be synthetically lethal with VHL loss. EZH1/2 are histone methyltransferases which can functionally act to trimethylate lysine residue 27 on histone 3 (H3K27). Interestingly, constitutive HIF signaling mediates relative H3K27 hypermethylation, potentially explaining EZH1 and VHL synthetic lethality. Pharmacological inhibition of EZH1/2 in VHL-deficient RCC cell lines recapitulated these findings, however, the compounds were toxic in mice models. Whereas EZH2 was not identified as exhibiting synthetic lethality in the aforesaid screen, there is preclinical evidence that EZH2 inhibitors may be effective in the setting of BAP1 deficiency. Mice with isolated BAP1 deficiency in hematopoietic precursors develop myelodysplastic syndrome. In these models, BAP1 deficiency results in increased EZH2 expression and methylation of H3K27. Interestingly, EZH2 depletion by both genetic and pharmacologic methods abrogated the tumorigenic effect of BAP1 loss. In ccRCC, increased levels of EZH2 expression by IHC are associated with higher grade and worse outcomes. In addition, RCC-derived cell lines deficient in BAP1 overexpress EZH2, and are sensitive to EZH2 inhibitors in vitro. Furthermore, in a sunitinib-resistant xenograft model of RCC, the EZH2 inhibitor EPZ011929 demonstrated rescue of sunitinib sensitivity through epigenetic reprogramming (BAP1 status was not reported in this study). At the time of preparation of this manuscript there are no RCC specific clinical trials involving EZH2 inhibitors.

An alternative downstream target of HIF-2, CCND1 (encoding Cyclin D1) is also overexpressed in VHL-deficient (HR 0.41 [95% CI: 0.53 – 0.83] vs HR 0.58 [95% CI: 0.43 – 0.79]). Retrospective analysis of 112 patients with non-cRC treated with cabozantinib demonstrated efficacy with 27% (30/112) of patients achieving an objective response. For the small subset of patients with genomic data, 40% (4/10) of RCC patients with MET mutations demonstrated partial responses. While these data did not reach statistical significance, prospective trials investigating cabozantinib (as well as other MET inhibitors) in MET-driven RCC are ongoing (i.e. NCT03091192).

While targeting driver mutations may provide benefit to a subset of RCC patients, techniques leveraging tumor suppressor genes therapeutically are needed to benefit the larger population. One strategy to tackle loss-of-function mutations in tumor suppressor proteins has been to inhibit downstream effector pathways. In the setting of VHL loss, HIF-2α accumulates and binds HIF-1β, and the heterodimer upregulates the expression of hundreds of genes important to tumor growth including VEGF. Given the key role HIF-2 mediated transcription in ccRCC development, direct inhibition of HIF-2 has substantial potential. Transcription factors such as HIF-2 have classically been regarded as “undruggable,” as they lack catalytic pockets suitable for targeting by small molecules. However, characterization of the atomic structure of HIF-2 identified a highly structured pocket that could be bound by small molecule inhibitors. Compounds with improved pharmacological properties were subsequently developed through iterative structure-based design. This led to the discovery of PT2385 and PT2399, which were shown to be potent and highly selective inhibitors leading to the dissociation of HIF-2 complexes. Preclinical testing of PT2399 in our laboratory demonstrated decreased tumor growth across ~50% of ccRCC tumourgrafts analyzed (P<0.0001), including in sunitinib resistant tumors. However, prolonged therapy with PT2399 led to the development of acquired resistance in tumourgraft models. Sequencing of tumourgrafts with acquired resistance to PT2399 led to the identification of point mutations which restored dimerization in the presence of inhibitors, one of which was subsequently identified in patient tumors that developed resistance to HIF-2 inhibition. In a phase I trial, PT2385 demonstrated a favorable safety profile and disease control lasting greater than 4 months in 40% (21/52) of patients, despite heavy pretreatment with a median of 4 prior therapies. PT2977, a second generation inhibitor with more consistent drug circulating levels, demonstrated a similar safety profile to PT2385 in recently reported results of a phase I trial in ccRCC. Anemia, which is thought to be an on-target effect through suppression of erythropoietin, was the most common adverse event, and only 4% (2/55) of patients stopped therapy due to adverse events. The patients in this trial were heavily pretreated, 62% (34/55) had greater than three lines of therapy, including TKI and ICI therapy. In spite of this, a promising efficacy signal was seen; the median PFS was 11 months (95% CI: 6 – 17), 24% (13/55) of patients experienced a partial response (56% (31/55) demonstrated stable disease (NCT02974738). A phase II trial of (now MK-6482) in combination with cabozantinib is ongoing (NCT03634340), as well as phase II trials of both agents in VHL syndrome related ccRCC (NCT03108066, NCT03401788).

An alternative approach to targeting tumor suppressor genes leverages “synthetic lethality,” where loss of two genes results in cell death whereas loss of either gene does not. Since VHL is lost in nearly all ccRCC, identifying molecular targets that exhibit synthetic lethality with VHL loss is an attractive strategy. Several groups, including our own, have developed high-throughput screening platforms of chemical libraries that are capable of identifying compounds which exhibit selective killing of VHL deficient cells. In one of the first such studies, Turcotte and colleagues screened a panel of ~64,000 small molecules in parallel on VHL deficient RCC4 cells and RCC4 cells with re-introduced VHL. They found that STF-62247 was able to selectively induce apoptosis in VHL deficient cells, likely through inhibition of protein trafficking. Utilizing the same screen, STF-31 which acts through inhibition of GLUT1, was also found to have preferential toxicity among VHL deficient cells. Employing a strategy where differentially labeled VHL deficient and reconstituted RCC cell lines were co-cultured, we identified homoharringtonine (HHT) as a hit compound. Furthermore, HHT demonstrated efficacy in ~30% of tested tumourgrafts. While these screens have the potential to identify promising compounds, other strategies utilize short hairpin RNA (shRNA) libraries to identify gene combinations which exhibit synthetic lethality.
RCC. Cyclin D1 binds the CDK4/6 kinases resulting in phosphorylation and inactivation of the retinoblastoma (RB) protein, with subsequent progression through the cell cycle. Of note, RB loss is an uncommon event in ccRCC, and thus cell cycle progression likely remains CDK4/6 dependent. The CDK4/6 inhibitor palbociclib demonstrated a G0/G1 cell-cycle arrest, induction of late apoptosis, and blockade of RB phosphorylation in multiple RCC cell lines. Abemacibib, another inhibitor of the CDK4/6 enzyme, was shown to diminish tumor growth in combination with sunitinib in mouse tumourgraft models, and a phase I trial of the combination is now actively recruiting (NCT03 905889). Recently, Nicholson and colleagues demonstrated synthetic lethality between CDK4/6 and VHL in ccRCC cell lines as well as in a Drosophila model, suggesting a fundamental dependency between these two gene products. Furthermore, the anti-proliferative effects of CDK4/6 inhibition were synergistic with HIF-2 inhibition in mouse xenograft models of disease, suggesting the combination of CDK4/6 inhibitors with the HIF-2 antagonists described above may also have therapeutic potential.

Another emerging strategy is to target the NRF2 pathway. As previously alluded to, NRF2 is negatively regulated by KEAP1, and under conditions of oxidative stress KEAP1 is bound by p62 releasing NRF2 to localize in the nucleus and bind ARE. The NRF2/ARE pathway regulates a number of genes involved in oxidative stress regulation, drug metabolism, and cell proliferation. Among other functions, NRF2 plays a vital role in overcoming oxidative stress and treatment resistance. Accordingly, overexpression of NRF2 has been implicated as a negative prognostic marker in several tumor types. Overexpression of NRF2/ARE-controlled genes are a distinguishing feature of type II pRCC, and elevated expression of NQO1 is associated with worsened outcomes. Activating mutations in ccRCC are less frequent than type II pRCC, however, emerging evidence suggests epigenetic silencing of KEAP1 may contribute to NRF2/ARE deregelation in ccRCC. Consistent with a potential role of NRF2/ARE in ccRCC, NRF2 depletion via shRNA was recently shown to decrease proliferation and increase sensitivity to sunitinib in the 786-O ccRCC cell line. Several flavonoids have been demonstrated to have non-specific NRF2 inhibition, possibly through stimulating ARE depletion via KEAP1, and under conditions of oxidative stress KEAP1 is negatively regulated by NRF2 via the KEAP1 binding domains are also in development.

**Conclusion**

Next generation sequencing has provided unprecedented insight into the biology of RCC. The field has moved dramatically from the discovery of the VHL gene by genetic linkage analysis to the simultaneous and comprehensive analysis of genomic, transcriptomic, metabolomics, and proteomic data across multiple regions of a single tumor. Novel bioinformatic strategies and insightful experimental designs are revealing new molecular profiles of tumors and the tumor microenvironment. The culmination of these technologies has already resulted in refined prognostic opportunities based on molecular, pathological, and clinical variables. We are now rapidly shifting towards developing and validating predictive models with the ultimate goal to deliver on precision medicine.

**References**

Revisiting IL-2 Therapy in Renal Cell Carcinoma: A Case Report of a Patient Treated With Pegylated IL-2, Bempegaldesleukin (NKTR-214)

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This cohort in PIVOT-02 is ongoing and full data analyses for the cohort are not yet available. Therefore, this patient case should be interpreted with caution.

Abstract
IL-2 is a well-known stimulatory cytokine involved in differentiation and activation of T-cells and natural killer (NK) cells. FDA approved high-dose IL-2, aldesleukin for the treatment of metastatic renal cell carcinoma (mRCC) in 1992 based on the phase 2 trial that showed 7% complete response and 15% overall response rate. However, due to its severe toxicities, such as vascular leak syndrome, pulmonary edema, and cardiac toxicity, that required inpatient administration, it has limited use. After the discoveries of immune checkpoint inhibitors (CPI), the treatment landscape of mRCC has rapidly evolved. Different combinations of CPI or with tyrosine kinase inhibitors (TKI) have been approved for first-line treatment. Despite that, achieving a durable response or overcoming resistance to CPI therapy is an unmet medical need. Revisiting IL-2 therapy and combining with CPI may overcome this and achieve better and more durable responses.

Bempegaldesleukin (BEMPEG; NKTR-214) is a CD122-preferential IL-2 pathway agonist that has been shown to increase tumor-infiltrating lymphocytes (TIL), T-cell clonality and PD-1 expression.(Figure 1). With its pro-drug design, BEMPEG achieves rapid and sustained activation of the IL-2 pathway, and minimizes toxicity vs native IL-2 allowing for administration in an outpatient setting. Additionally, BEMPEG combined with the CPI nivolumab (NIVO) has been shown to convert baseline tumors from PD-L1 negative (<1%) to PD-L1 positive (≥1%). PIVOT-02 (NCT02983045) is a phase 1/2 study of BEMPEG in combination with NIVO and other anti-cancer therapies in patients with advanced solid tumors. Here we present a clinical vignette of a patient who was successfully treated with BEMPEG plus NIVO as part of the PIVOT-02 trial. Some details have been modified to protect the privacy of the individual.

Case Report
Mr. F is a 50 to 55 year-old man who initially presented with sudden onset gross hematuria and was found to have a 7cm right renal mass. He underwent laparoscopic right radical nephrectomy and pathology revealed clear cell RCC, pT3a N0 M0, Fuhrman grade 2. He was initially treated on a phase 2 randomized single-blind study of Vitespen (HSPPC-96, Oncophage) for immune response assessment following treatment of patients with resectable RCC at intermediate risk for recurrence. He was followed up with CT chest, abdomen, and pelvis every 3, then 6 and 12 months.

He did not have any evidence of recurrence until 6 years after the nephrectomy, when restaging CT showed a 2 x 3cm hypervascular lesion at the pancreatic tail and multiple subcentimeter bilateral pulmonary nodules concerning for metastasis. Endoscopic ultrasound-guided fine-needle aspiration of the pancreas lesion showed metastatic clear cell RCC. Based on the International Metastatic RCC Database Consortium (IMDC) risk stratification, he was categorized as having favorable-risk disease. Mr. F enrolled on the PIVOT-02 trial. His PD-L1 status at baseline was PD-L1 negative, and his initial baseline SLD was 55mm. The patient was enrolled during the dose escalation phase, where he received BEMPEG 0.003mg/kg and NIVO 240mg every 2 weeks. After 8 months, his dose of BEMPEG was increased to the recommended Phase 2 dose, 0.006mg/kg, and the NIVO increased to 360 mg every 3 weeks. At week 16, the patient...
achieved a 35% reduction in SLD from baseline (55 to 35). He achieved a complete response after 18 months and completed two years, a total of 40 cycles, of treatment in Q1-2019 (Figure 2).

The patient did not experience any grade 2 or higher adverse events (AEs) during his therapy, and he remains physically active and asymptomatic from his cancer.

Discussion
In the above clinical vignette, we described a patient who had a complete response with BEMPEG plus NIVO without any serious side effects. There are preclinical data and phase I BEMPEG monotherapy data supporting these findings and explaining the rationale of the PIVOT-02 trial. Earlier studies have shown that high concentrations of IL-2 cause CD8+ effector T-cell growth and activation, however low concentrations of Tregs. This pleiotropic effect is due to IL-2 receptor components and binding properties. IL-2Rbγ, the low-affinity IL-2 receptor mostly expressed on CD8+ T cells and NK cells; the high-affinity IL-2Rbγ, a heterotrimeric receptor expressed on Tregs.

The IL-2 component of BEMPEG was specifically engineered to have preferential binding to IL-2Rbγ. This provides preferential activation and expansion of CD8+ T and NK cells over Tregs in the tumor microenvironment, potentially explaining improved efficacy. There are six lysine residues on IL-2 conjugated to PEG chains as part of the BEMPEG compound. Because of PEGylation the compound is initially inactive and slowly releases PEG chains after infusion. 2-PEG-IL-2 and 1-PEG-IL-2 are the most active versions (Figure 2). In contrast to the traditional IL-2 infusion, immediate high concentrations, and related toxicity, the PEGylation allows time for tissue distribution, potentially decreasing toxicity and allowing for outpatient administration.

The first-in-human study of BEMPEG monotherapy (the EXCEL study), assessing safety and tolerability enrolled 28 patients including 15 RCC, 7 melanoma and 6 other tumors. Patients were treated with BEMPEG every 2 or 3 weeks at different doses, 0.003mg/kg, 0.006mg/kg, 0.009mg/kg and 0.012mg/kg. The most common side effects were fatigue (71%), flu-like symptoms (68%), pruritus (64%), hypotension (57%), rash (50%), decreased appetite (46%), and arthralgia and cough (each 32%). 5 of 28 (18%) patients developed grade 3 hypotension, which was managed with IV fluids, and patients continued the treatment. After hydration guidelines were implemented, no patients experienced Grade 3 or greater AEs for hypotension. One patient discontinued the treatment due to infusion-related reaction at 0.009mg/kg. Overall, BEMPEG had a favorable safety profile, and 0.006mg/kg dose recommended for further studies. Biomarker analysis demonstrated the proliferation of CD4+, CD8+ T cells and NK cells in the peripheral blood and increased the expansion of CD8+ T cell and NK cells in the tumor. There was a transient increase in the Treg population in peripheral blood, but not in the tumor. Notably, there was an increased amount of CD8+ and PD-1+ T cells both in peripheral blood and in the tumor.3

These preclinical and clinical studies demonstrating BEMPEG activity of increasing effector immune cell infiltration and PD-1+ T cells in the tumor microenvironment led to the Phase 1/2 PIVOT-02 trial evaluating the safety and efficacy of BEMPEG in combination with NIVO in advanced solid tumors. Based on the dose-es-
clation phase data, the recommended phase 2 dose is 0.006mg/kg and 360mg nivolumab IV every 3 weeks. This q3w administration of BEMPEG allows for a new generation of antigen specific T cells without leading to exhaustion. The expansion cohort includes 5 different tumor types, including RCC, melanoma, NSCLC, urothelial and triple-negative breast cancer. Preliminary results from 38 untreated metastatic melanoma patients showed 53% ORR and CR rate of 34%, which was durable and deepened over time. Our patient was also observed to have a deepening of response over time and was able to achieve a complete response with minimal side effects (Figure 2). It is also interesting that our patient had a similar response to that which was seen in the patients with RCC who were anti–PD-1 treatment-naive, who ended the dose escalation (BEMPEG) with stable disease and within, 1 month started NIVO and experienced rapid tumor reductions, resulting in partial responses. Our patient also supports this prior hypothesis that BEMPEG may have conditioned the TME by expanding activated TILs, thereby potentially providing synergy with therapies that block inhibitory signals, such as PD-1/PD-L1.

The biomarker analysis confirmed the prior observation of effector T cell clonal expansion in tumor microenvironment and conversion of PD-L1 negative tumor to PD-L1 positive tumor. Similar findings were observed in the preliminary analysis of 34 metastatic urothelial cancer patients treated in this trial. In the efficacy evaluable population, overall ORR was 48% (11/23, 95% CI 27–69%) with a 17% CR rate (4/23) and 70% (16/23) DCR. The most common treatment-related AEs (TRAE, >30%) were fatigue (59%), pyrexia (38%), chills (32%), and flu-like symptoms (32%). Grade ≥ 3 TRAEs occurred in 18% of patients, and 8.8% discontinued due to TRAEs. 6/10 (60%) PD-L1 negative tumor at baseline converted to PD-L1+ at week. Phase 2 part of the PIVOT-02 trial continues recruiting patients and final results are pending. Based on the above mentioned promising preliminary data, PIVOT-09, the Phase 3 study of BEMPEG in combination with NIVO compared with the investigator’s choice of a TKI therapy (either sunitinib or cabozantinib monotherapy) for advanced mRCC started recruiting in December 2018.

Conclusion
In conclusion, we presented a patient with mRCC who achieved a deepening and complete response with BEMPEG plus NIVO and summarized the preclinical and clinical data related to BEMPEG. PIVOT-02 and PIVOT-09 are ongoing studies; the results of these studies potentially will provide a novel treatment combination for patients and further improve outcomes of patients with mRCC.

References
Survival Outcome of Pazopanib and Sunitinib as First-line Targeted Therapy in Metastatic Renal Cell Carcinoma

A ‘real world’ retrospective review of practice in a tertiary cancer center in the North-West of England

Abstract

Background: The use of multi-receptor tyrosine kinase inhibitor (TKI) targeting the tumor angiogenesis pathway has shifted the treatment paradigm as well as improved outcomes among patients with mRCC. Sunitinib (S) and pazopanib (P) are two widely used TKIs options among treatment naïve patients. Here, we present our experience and survival outcomes of these two drugs used in a UK tertiary cancer center.

Methods: mRCC patients who had received either of these agents as first line therapy were identified through the Renal Cancer Database (2005-2015). Outcomes of interest include response rate, progression free survival (PFS) and overall survival (OS). Subgroup analysis was performed based on prognostic variables to assess survival impact.

Results: A total of 665 patients were identified (S=397, P=268). The majority of patients were categorized as having worse (intermediate to poor) prognostic risk groups (93% IMDC; 71% MSKCC). Cytoreductive nephrectomy rate in this population was 62%. Objective response rate (ORR) was 22%; ORR 25% (S) v 20% (P). Median PFS and OS of the entire cohort was 10.5 and 16.1 months respectively. Median OS in good-risk IMDC group reached 47 months but 21 and 7 months in the intermediate and poor-risk groups respectively. Survival outcomes for both drug groups were comparable when stratified by prognostic risks.

Conclusion: In this large population-based retrospective review, the survival performance of either TKI was impressive and comparable to published evidence among favorable-risk patients. However, the overall survival is lower than expected for the entire cohort and is likely attributable to the high proportion of patients with less than favorable prognostic risk in this real world cohort and the correspondingly lower benefits of this subgroups to these drugs.

Introduction

Renal cell carcinoma is the most common cancer of the kidney and its incidence is increasing rapidly worldwide. In England, kidney cancer is the third most common urological cancer after prostate and bladder cancer with an incidence in excess of 4000 and 2500 per 100,000 cases per year for male and female respectively. Unfortunately, around 30% of patients present with advanced or metastatic disease and relapse rate despite curative surgery is as high as 40%. The understanding of tumor angiogenesis pathway in RCC has led to the successful utilization of anti-angiogenic agents in the last decade. This signalling process is mediated by binding of stimulatory protein such as vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) to the external domain of transmembrane receptor which in turn activates the subsequent intracellular tyrosine kinase activity with cascading effect on cancer cell proliferation and metastasis. Sunitinib is a first generation small molecule that inhibits multiple receptor tyrosine kinases. Targets of sunitinib include vascular endothelial growth factor receptors (VEGFR1, VEGFR2, and VEGFR3), platelet-derived growth factor receptors (PDGFR and PDGFR), and several other kinase receptors. It received FDA approval for use in mRCC in 2006. Pazopanib is a second-generation small-molecule TKI with higher selective activity against

Keywords: renal cell carcinoma, pazopanib, sunitinib, first-line therapy, survival outcomes.

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similar receptors range and was FDA-approved in 2009. For a long while, sunitinib and pazopanib were the only two approved oral TKIs for patients with treatment-naïve mRCC in the UK. Consistent PFS benefits have been demonstrated in separate randomized controlled clinical studies of sunitinib (versus Interferon alpha) and pazopanib (versus placebo) as the first-line treatment of mRCC. Pazopanib was later compared directly to sunitinib in the COMPARZ trial showing essentially non-inferior efficacy among 1110 patients. Investigator-led median PFS and OS were similar for both agents at around 10 and 29 months respectively.

There are several reports on the real world outcomes of the use of targeted therapies in mRCC. Ruiz-morales et al documented the use of pazopanib versus sunitinib in the first-line setting using data from the International mRCC Database Consortium (IMDC), but the UK is not part of this consortium. In this article, we report the outcomes on the use of these two drugs in a large tertiary referral center between 2005 - 2015 with the aim to evaluate and compare their population-based efficacy and extent of survival benefits and this will serve as an important benchmark for future outcome comparisons in this region.

**Patient selection, treatment and methods**

Patients who received pazopanib or sunitinib as first-line therapy for mRCC (from April 2005 to August 2015) were identified from our Renal Cancer database. Patients who had previous cytokines therapy or were treated with either of these agents as monotherapy within a clinical trial of first-line setting were included in this analysis. Patient demographics and relevant clinical data were retrieved from electronic patient record. Treatment response was evaluated using CT scan assessments based on RECIST criteria approximately every 3 months. Progression-free survival (PFS) was calculated from start of treatment to date of progression or death or last date patient known to be progression-free. Overall survival (OS) was calculated from start of treatment to date of death of any cause or last date patient known to be alive. Last observation date was September 2, 2016.

**Statistical analysis**

Survivals were analysed using Kaplan-Meier method and Log-rank tests were used to compare difference between covariates (SPSS software version 20).

**Limitation**

The assessment of treatment response reflects that of standard clinical practice and therefore will not be as robust as that in a clinical trial setting and may have an impact on interpretation of PFS result. Also, due to the retrospective nature of a clinical audit, the complete and accurate assessment of adverse events was not feasible and therefore not presented here.

**Ethical consideration**

This is a retrospective clinical review with no intervention beyond standard of care; therefore ethics committee approval is not required. This project was approved and registered with The Christie NHS Trust Foundation trust audit department (Registration reference: SE17/1957).

**Results**

**Patients**

A total of 665 patients were identified from The Christie Renal Cancer Database. Of these, 577 (86.8%) patients were treated within standard clinical practice while 88 (13.2%) received pazopanib or sunitinib within a clinical trial. Higher proportions of patients within the pazopanib group were of worse prognosis and performance status. Sixty-two percent of this patient series had cytoreductive nephrectomies. Other patient characteristics and demographics are as outlined in Table 1. Clear cell was the predominant histology subtype seen in both treatment

| Table 1. Patient demographic and clinical characteristics including prognostic risks |
|---------------------------------------------|------------------|------------------|
| **Number (%)** | **Sunitinib N = 397** | **Pazopanib N = 268** | **Combined N = 665** |
| Age (Median, Range) | 65, 20-92 | 68, 39-90 | 66, 20-92 |
| Gender | Male | Female | Male | Female | Combined |
| | 249 (63%) | 148 (37%) | 163 (61%) | 105 (39%) | 412 (62%) | 253 (38%) |
| MSKCC | Good | Intermediate | Poor | Good | Intermediate | Poor | Good | Intermediate | Poor | Good | Intermediate | Poor | Good | Intermediate | Poor | Good | Intermediate | Poor | Good | Intermediate | Poor | Good | Intermediate | Poor |
| | 138 (35%) | 198 (50%) | 61 (15%) | 56 (21%) | 155 (58%) | 57 (21%) | 194 (29%) | 353 (53%) | 118 (18%) |
| IMDC | Favourable | Intermediate | Poor | Favourable | Intermediate | Poor | Favourable | Intermediate | Poor | Favourable | Intermediate | Poor | Favourable | Intermediate | Poor | Favourable | Intermediate | Poor | Favourable | Intermediate | Poor |
| | 23 (6%) | 286 (72%) | 88 (22%) | 26 (10%) | 165 (62%) | 77 (28%) | 49 (7%) | 451 (68%) | 165 (25%) |
| Nephrectomy | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No |
| | 254 (64%) | 143 (36%) | 161 (60%) | 107 (40%) | 415 (62%) | 250 (38%) |
| Performance Status | 0 | 1 | 2 or more | 0 | 1 | 2 or more | 0 | 1 | 2 or more | 0 | 1 | 2 or more | 0 | 1 | 2 or more | 0 | 1 | 2 or more | 0 | 1 | 2 or more |
| | 134 (34%) | 186 (47%) | 77 (22%) | 65 (24%) | 119 (45%) | 84 (31%) | 199 (30%) | 305 (46%) | 161(24%) |
populations (85% pazopanib, 77% sunitinib). A higher percentage of patients with non-clear cell (papillary, chromophobe, or translocation) and unclassified RCC received treatment with sunitinib. Histology detail was not available in less than 10% of both cohorts (Table 2).

**Treatment and subsequent therapy**
Only 52% and 59% of patients in the sunitinib and pazopanib groups started treatment at full dose. Median duration on treatment was 6.5 and 6.2 months for sunitinib and pazopanib group, respectively. Forty-two percent (42%) of patients who discontinued first line sunitinib or pazopanib received second line treatment. Subsequent treatments were predominantly axitinib (P 51%, S 40%) and everolimus (P 35%, S 38%) while very small number (less than 5%) of patients received other treatment such as nivolumab, HD IL-2 or treatment within clinical trial during this period. Proportionately more patients in the pazopanib group were able to receive any subsequent treatment (58%) compared to sunitinib group (42%).

**Response rate**
Overall Response Rate (ORR) was 22% of which 2% were complete; 43% of patients had SD as best response while over a quarter had PD. ORR was 25% and 21% in Sunitinib and pazopanib arm respectively.

**Survival**
Median follow up was to 40.2 months. One year survival estimated to be above 60%. Median PFS and OS of the entire cohort are 10.5 and 15.8 months respectively (see Tables 3, 4).

Median PFS of pazopanib cohort (8.3 months) was lower than Sunitinib (10.4 months P = 0.02) but median OS was not significantly different (S 17.7 v P 14.8 months P = 0.67).

**Discussion**
The median OS in our study population is notably lower compared to contemporary trial or real-world reports of similar treatment setting (16 versus 23-28 months). Of significance, only about 7% of patients in our study population had favorable IMDC risk category which is in stark contrast to at least 25-27% in COMPARZ or IMDC.

### Table 2. RCC histology subtypes within the patient cohort and distribution within treatment groups.

<table>
<thead>
<tr>
<th></th>
<th>Clear cell</th>
<th>Non-clear cell</th>
<th>Unclassified/NOS</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pazopanib</td>
<td>231 (86)</td>
<td>7 (3)</td>
<td>9 (3)</td>
<td>21 (8)</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>310 (79)</td>
<td>34 (9)</td>
<td>36 (9)</td>
<td>• (4)</td>
</tr>
</tbody>
</table>
The proportion of patients with non-clear cell pathology, which is usually associated with poorer outcome, was also comparatively higher in this study cohort. In a review done by Kidney Cancer UK in 2016, survival outcomes in the UK as a whole is poorer compared to other western advanced countries or European counterparts. The possible contributing factors highlighted was the low proportion of patients receiving more than one line of treatment and the generally restricted overall number of approved and effective treatment available during the study period. Another relevant factor to the poor survival outcome in this region is likely the very high proportion (41%) of highly deprived neighborhoods served by this referral center compared to only 6-7% in other parts of England (ONS 2015). The findings here indirectly highlighted the absolute importance of public awareness and early diagnosis to further improve survival outcome of kidney cancer. The approval of several newer agents by NICE in recent times including cabozantinib and ipilimumab and nivolumab combination immunotherapy among patients with I/P prognostic risk, for example, is extremely timely and will likely bring about improvement in survival outcome in general and for this region.

The outcome of mRCC patients with favorable prognostic risk (IMDC or MSKCC) in this series is excellent.

### Table 3. Table showing median PFS in Favorable, Intermediate, Poor risk and overall population.

<table>
<thead>
<tr>
<th>IMDC</th>
<th>Median PFS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>29.2</td>
</tr>
<tr>
<td>Intermediate</td>
<td>13.0</td>
</tr>
<tr>
<td>Poor</td>
<td>4.1</td>
</tr>
<tr>
<td>Overall</td>
<td>10.5</td>
</tr>
</tbody>
</table>

### Table 4. Table showing median OS in Favorable, Intermediate, Poor and overall population.

<table>
<thead>
<tr>
<th>IMDC</th>
<th>Median OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>47.3</td>
</tr>
<tr>
<td>Intermediate</td>
<td>20.8</td>
</tr>
<tr>
<td>Poor</td>
<td>7.4</td>
</tr>
<tr>
<td>Overall</td>
<td>15.8</td>
</tr>
</tbody>
</table>

![Figure 3a.](image1.png)

![Figure 3b.](image2.png)

![Figure 3c.](image3.png)

![Figure 3. Kaplan-Meier curves comparing PFS between Pazopanib versus Sunitinib according to MSKCC a) good b) intermediate and c) poor prognostic subgroups. No statistical difference shown.](image4.png)
Kidney Cancer Journal

(median survival of more than 3 years similar to that of COMPARZ trial) irrespective of TKI type. The median PFS is also comparable to COMPARZ (investigator review) at around 10 months. Taking the unequal distribution of patient with poorer risks between the two drug types into consideration, efficacy and survival are comparable between sunitinib and pazopanib and remaining true when compared among matched prognostic subgroups. This real world evidence reaffirms the important role of VEGF pathway inhibition as one the main therapeutic strategy in mRCC, particularly among patients with favorable prognostic risk. The lower benefits among patients belonging to intermediate and poor prognostic risk categories in this real world data set is in keeping with published research data and represents an on-going challenge in mRCC.

Results from Checkmate-214 study represent a breakthrough in this area. In this phase 3 randomized controlled trial comparing ipilimumab and nivolumab with sunitinib among patient populations of predominantly I/P prognostic risk category, combination checkpoint inhibitors showed superior PFS in patients with less than favorable risk group while patients with favorable risk achieved better outcome with sunitinib. At 30 months update review, median OS was not reached in the combination group (versus 37.9 months) and a complete response rate of 11%. Translational work done in the IMMOTION 150 and 151 study by Rini et al found that patients with favorable prognostic risk is characterized by a predominantly angiogenesis gene expression signature which correlated with improved PFS when exposed to VEGF-directed therapy. Patients with a predominantly immune underlying gene expression signature fared better when exposed to immunotherapy in these trials. These early findings provided some insight into the differential susceptibility of tumors to VEGF or immune checkpoint pathway blockade or even both among the different clinical prognostic risk categories although the full understanding is still evolving.

There is other progress in the management of patients with I/P risk categories. Following positive results of the METEOR trial in second line setting, the CABOSUN study was set up to evaluate the efficacy of Cabozantinib compared to sunitinib among mRCC patients with Intermediate or Poor IMDC risk group (~20% Poor IMDC risk). Cabozantinib is a third generation VEGF pathway directed tyrosine kinase inhibitor poised with additional target activity against AXL and MET receptor. In CABOSUN, ORR was significantly higher in Cabozantinib group at 46% versus 18% and an impressive 34% reduction in

Figure 4. Kaplan-Meier curves comparing OS between Pazopanib versus Sunitinib according to MSKCC a) good b) intermediate and c) poor prognostic subgroups. No statistical difference shown.
• Round Table Discussion: How a Pivotal Trial Could Usher in New Era of IL-2 Therapy

• HIF-2alpha: What Is the Impact of the Nobel Prize-Winning Research?

• Targeted Therapy Following Immuno-oncology Approaches in RCCa

• Circulating Tumor DNA in Advanced Renal Cell Carcinoma – Is It Ready for Prime Time?
risk of progression or death (hazard ratio 0.66; 95% CI 0.46-0.95 P =0.012) was seen with Cabozantinib. This represents another significant stride given the unmet need of this adverse risk group.

**Conclusion**

There has been definite stride in the survival outcome among patients mRCC through therapeutic exploitation of the VEGF-pathway in recent decade. However the benefit of the commonly utilised agents such as sunitinib and pazopanib so far has been largely limited to patients with Favourable prognostic risk. The strikingly discrepant outcome defined by prognostic risks emphasises the importance of risk-stratifying mRCC patients in clinical practice and personalising appropriate treatment based on this.

The arrival of newer and more effective treatments and combination offers optimism to improving survival outcomes of mRCC patients particular those of I/P risk groups. Better understanding of differential underlying immune versus vascular-driven disease process through relentless translational research and biomarker-based treatment personalisation will be the next crucial step in advancing the care of our patients.

**References**


**List of Abbreviations**

TKI = Tyrosine Kinase Inhibitor; NHS = National Health Service; NICE= National Institute for Health and Care Excellence; ECOG PS = Eastern Cooperative Oncology Group Performance status; mRCC= Metastatic Renal Cell Carcinoma; VEGF= Vascular Endothelial Growth Factor; MSKCC= Memorial Sloan-Kettering Cancer Centre; ORR= Overall Response Rate; ONS = Office for National Statistic; CR = Complete Response; CCR= Complete Response Rate; SD= Stable Disease; PR = Partial Response; PD = Progressive Disease; PD1 = Programmed cell death 1; EORTC= European Organisation for Research and Treatment of Cancer; HD-IL2 = High Dose Interleukin 2; UK = United Kingdom. **KCJ**

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**EDITOR’S MEMO**

*(continued from inside front cover)*

Except for the sarcomatoid variant of RCC, for which immune-based therapy is effective, the practical utility of biomarkers to select therapy remains what some observers have called “the unattainable holy grail” in kidney cancer.

As we look toward, still other unresolved issues remain to be addressed in 2020. There is the need to consider subsets of patient populations for whom the treatment algorithm requires more clarification. For example, patients who present with both synchronous metastatic disease and primary tumor together constitute a group who deserve systemic therapy as part of a front-line approach.

This population tends to be underrepresented in clinical trials and a majority of them who are enrolled in such studies receive nephrectomy instead of upfront immuno-therapy-based approaches that would yield an equally favorable benefit. The editors look forward to exploring new results on this and other critical issues.

On behalf of the *Kidney Cancer Journal* and its Board of Editors, I wish you a new year filled with an improved outlook for your patients as we integrate knowledge gained from symposia such as the IKCS and look forward to clinical applications.

**Ulka Vaishampayan, MD**

**Guest Editor**

Chair, Division of Solid Tumor Oncology
Director of Phase 1 Therapeutics
Karmanos Cancer Institute
Charles Martin Endowed Chair, Professor of Oncology
Wayne State University, Detroit, Michigan
novel way of describing disease control and toxicity during an off-treatment period, was longer in patients who received nivolumab/ipilimumab in combination versus those who received sunitinib, according to an innovative analysis of the CheckMate 214 dataset. The study was presented by Meredith M. Regan, MD of the Dana-Farber Cancer Institute.

Dr Regan and colleagues previously defined TFS as the interval between the time point of immune checkpoint inhibitor (ICI) discontinuation and initiation of subsequent systemic treatment (*J Clin Oncol.* 2019 Sep 9;JCO1900345. doi:10.1200/JCO.19.00345). Development of this novel outcome measure was based on the fact that ICI-treated patients are observed to have continued benefit from treatment or develop treatment-related adverse events (TRAEs) after treatment discontinuation.

**Incidence of Brain Metastases Is 4.5% in Asymptomatic Metastatic RCC**

MIAMI—Asymptomatic brain metastases are encountered in 4.5% of asymptomatic metastatic renal cell carcinoma (mRCC) patients according to a large multi-institutional retrospective analysis of 1597 patients’ data. The study was presented by Dr. Ritesh Kotecha of Memorial Sloan Kettering Cancer Center.

Through a collaborative effort of scientists from the Institut Gustav Roussy and Memorial Sloan Kettering Cancer Center, investigators analyzed the data of 1597 asymptomatic mRCC patients who were screened for brain metastases for inclusion in 68 clinical trials. A total of 71 patients, representing the 4.5% of the cohort, were found to have brain metastases. Per IMDC criteria, 26%, 61%, and 13% of the cohort were in favorable-, intermediate-, and poor-risk groups, respectively. A majority of the patients were treatment-naïve (32%) or had received one prior line of treatment (43%). A total of 86% of the patients had more than one extracranial metastatic site, and the most common accompanying extracranial metastatic site was lung (92%). Patients with metastatic disease at initial presentation comprised 60% of the patient population. The investigators reported a median overall survival (OS) of 10.3 months (95% CI, 7–17.9 months).

**Living With Kidney Cancer, New Patient Magazine**

The Kidney Cancer Association is launching a patient and caretaker magazine called “Living with Kidney Cancer”. This complimentary magazine is available for you to have in your offices, waiting rooms, or to give specifically to patients. Sign-up to receive your complimentary magazines here: [https://tinyurl.com/LivingWithKidneyCancer](https://tinyurl.com/LivingWithKidneyCancer)

**William Kaelin’s 2019 Nobel Prize in Physiology Linked to Kidney Cancer Molecular Mechanisms**

William G. Kaelin Jr., MD, has won the 2019 Nobel Prize in Physiology or Medicine with two other physician-scientists for unraveling a molecular mechanism that not only is crucial to survival, but is entwined with cancer and other diseases, especially kidney cancer. Dr Kaelin is the Sidney Farber Professor of Medicine at Harvard Medical School, and an investigator at the Howard Hughes Medical Institute.

Dr Kaelin and his collaborators deciphered the core molecular events that explain how almost all multi-cellular animals tune their physiology to cope with varying quantities of life-sustaining oxygen in a unique signaling scheme. Their findings could lead to new therapeutics for a wide range of disorders — including cancer, cardiovascular disease, anemia, and macular degeneration. This oxygen-sensing mechanism involves the tumor-suppressor protein VHL, which is mutated in many kidney cancers, and proteins known as hypoxia inducible factors, HIF-1α and HIF-2α. Kaelin showed that HIF-2α drives certain kidney cancers and recently discovered how HIF-1α is hijacked by triple-negative breast cancers. He is developing therapeutic strategies for targeting these molecules and others implicated in cancer, such as mutated enzymes IDH1 and IDH2, with designer drugs.

**2019 Top Stories on Oncology? Pembrolizumab Plus Axitinib for RCC Heads the List**

The pembrolizumab plus axitinib study is the renal cell carcinoma top story for 2019, according to Eric Jonasch, MD, a leading investigator on RCC trials and Professor, Department of Genitourinary Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston.

Writing in the journal Oncology, Dr Jonasch reported: KEYNOTE-426 was a randomized phase III study designed to compare the combination of axitinib and pembrolizumab against sunitinib in patients with untreated clear cell renal cell carcinoma.\(^1\) The study demonstrated superior overall survival, response rate, and progression-free survival in patients treated with the pembrolizumab plus axitinib combination, while showing relatively low levels of toxicity. This combination also appeared to show benefit across all risk strata—good-, intermediate-, and poor-risk patients all had superior outcomes in the combination arm. Although the complete response rate of 6% was a bit lower than the rate seen in the CheckMate 214 study published a year earlier,\(^2\) the combination of efficacy, favorable toxicity, and benefit across all risk groups makes the combination of pembrolizumab plus axitinib a compelling choice for patients with metastatic RCC choosing a front-line therapy.
