

Kidney Cancer

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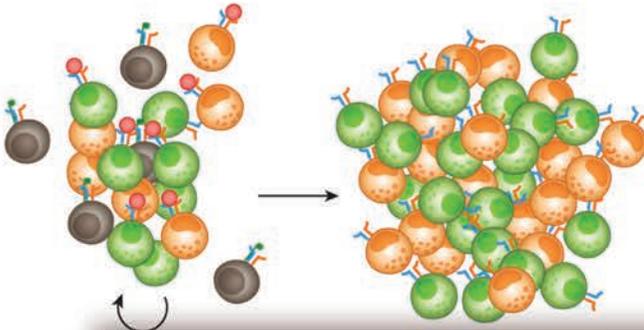
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Reinventing the Paradigm of
IL-2 Therapy: A Roundtable
Discussion Analyzes Potential
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Targeting the VHL-HIF Pathway:
Strategies and Challenges

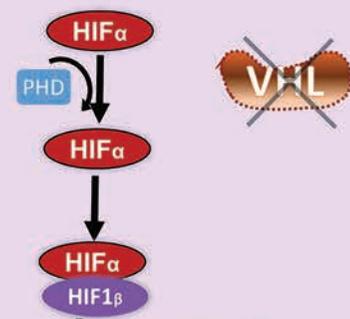
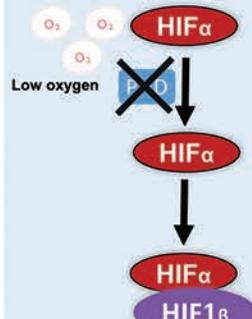
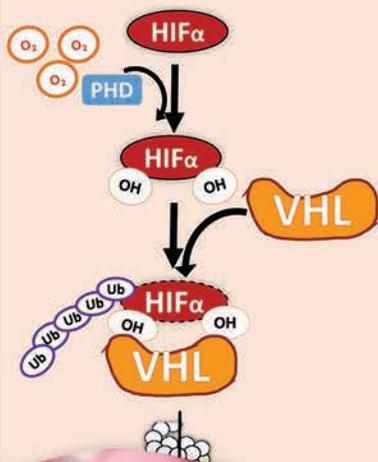
Is Circulating Tumor DNA Ready
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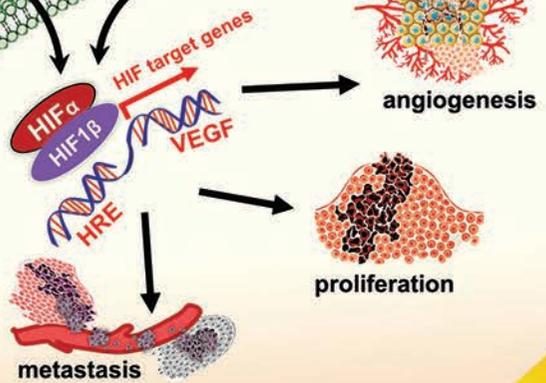
NORMOXIA

HYPOXIA

DYSREGULATED
VHL



cytoplasm
nucleus



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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-oncologists, and urologists.

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About the Cover

Illustrations and images from this issue offer an exciting glimpse at innovative strategies with potential impact on clinical practice. At top are the mechanisms of a combination using reformulated IL-2 therapy and a checkpoint inhibitor under investigation in a pivotal trial. The role of hypoxia inducible factor and VHL dysregulation is depicted in a second image, highlighting the need to target upstream pathways. Liquid biopsy is suggested in the image below, related to an article analyzing how this biomarker could play a key role in the precision medicine era.

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KCJ EDITOR'S MEMO

Consensus Statement Sets New Benchmark for Immunotherapy Guidelines in RCC



Robert A. Figlin, MD

“As a new standard in the field, every patient should receive an anti-PD-1-based therapy as initial treatment unless there is a specific contraindication to this approach. However, there remains a need for biomarkers to better predict patient response and to help decide the best treatment approach for each patient. Additionally, it remains to be determined whether new IO combinations including VEGFR TKIs will elicit properties of IO therapy, enabling the patient the ability to stop treatment with persistent benefit.”

These are among the conclusions of a comprehensive and thoroughly researched consensus statement with special relevance to those of us engaged in kidney cancer care. The document is The Society for Immunotherapy of Cancer Consensus Statement on Immunotherapy for the Treatment of Advanced Renal Cell Carcinoma (RCC).

Let's start with the credentials of the group spearheading this initiative. It was my privilege to serve among the 19 members of the Society for Immunotherapy of Cancer (SITC) panel, a group with literally hundreds of years of clinical practice and clinical trial experience. This subcommittee included expert physicians, nurses, scientists, and a patient advocate who regularly communicated via email, teleconference, and in-person between September 2018 and June 2019 to review existing new data and determine how to incorporate these results into updated RCC-specific consensus management guidelines. These resulting recommendations are meant to provide guidance to clinicians with the most up-to-date data and recommendations on how to best integrate immunotherapy into the treatment paradigm **[See treatment algorithm on page 30]** for patients with advanced RCC.

The need for such a document has never been more imperative, especially in view of the advances in IO therapy over the past decade. This overarching need inspired us to apply our knowledge to improve the management of patients with advanced RCC, including the emergence of IO in combination with TKIs, appropriate patient selection considerations, therapy

sequencing, response monitoring, adverse event management, and biomarker application. We are proud to present our efforts in the Consensus Statement and I urge you to review the recommendations referenced below. The accompanying clinical algorithm reflects part of the meticulous approach the panel followed in formulating the guidelines. But don't stop

(continued on page 30)

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- 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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Authors are required to submit their manuscripts in an electronic format, preferably by email to the Editor-in-Chief, Robert A. Figlin, MD, at robert.figlin@cshs.org. Please provide in a word processing program. Images should be submitted electronically as well.

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Spacing: One space after periods. Manuscripts should be double spaced.

References

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Example:

Lewczuk J, Piszko P, Jagas J, et al. Prognostic factors in medically treated patients with chronic pulmonary embolism. *Chest*. 2001;119:818-823.

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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.

Imaging of tumour response to immunotherapy.

Dromain C, Beigelman C, Pozzessere C, et al. *Eur Radiol Exp.* 2020 Jan 3;4(1):2. doi: 10.1186/s41747-019-0134-1.

Summary: The novel mechanism of action of immune checkpoint inhibitors (CPIs), with immune and T cell activation, leads to unusual patterns of response on imaging, with the advent of so-called pseudoprogression being more pronounced and frequently observed when compared to other anticancer therapies. Pseudoprogression, described in about 2-10% of patients treated with ICI, corresponds to an increase of tumour burden and/or the appearance of new lesions due to infiltration by activated T cells before the disease responds to therapy. To overcome the limitation of response evaluation criteria in solid tumors (RECIST) to assess these specific changes, new imaging criteria-so-called immune-related response criteria and then immune-related RECIST (ir-RECIST)-were proposed. The major modification involved the inclusion of the measurements of new target lesions into disease assessments and the need for a 4-week re-assessment to confirm or not confirm progression. The RECIST working group introduced the new concept of “unconfirmed progression”, into the ir-RECIST.

Conclusion: This paper reviews current immunotherapeutic approaches and summarizes radiologic criteria to evaluate new patterns of response to immunotherapy. Furthermore, imaging features of immunotherapy-related adverse events and available predictive biomarkers of response are presented.

NCI 6896: a phase I trial of vorinostat (SAHA) and isotretinoin (13-cis retinoic acid) in the treatment of patients with advanced renal cell carcinoma.

Molina AM, van der Mijl C, Christos P, et al. *Invest New Drugs.* 2020 Jan 3. doi: 10.1007/s10637-019-00880-7. [Epub ahead of print]

Summary: Preclinical studies suggest that histone deacetylase (HDAC) inhibitors may restore tumor sensitivity to retinoids and have synergistic anti-tumor activity when combined. This Phase I clinical trial evaluated the safety and preliminary efficacy of combining the oral HDAC inhibitor vorinostat and isotretinoin in patients with advanced renal cell carcinoma (RCC). Vorinostat was administered at 300 mg orally twice daily in combination with escalating doses. Vorinostat was administered at 300 mg orally twice daily in combination with escalating doses of isotretinoin in patients with advanced RCC. of isotretinoin for 3 consecutive days per week. A standard 3+3 dose escalation design was used. Dose limiting toxicities (DLT) were assessed during the first cycle to determine the maximum tolerated dose (MTD). Fourteen patients enrolled on the trial of which

12 were evaluable for toxicity (6 cohort 1; 3 cohort 2; 3 cohort 3) and 11 for tumor response. One patient in cohort 1 experienced a DLT (grade 3 depression). Common grade 1-2 toxicities included fatigue and GI effects (nausea, diarrhea, anorexia). MTD was established as vorinostat 300 mg with isotretinoin 0.5 mg/kg twice daily 3 days per week. Best responses in evaluable patients included 1 partial response and 9 stable disease, lasting a median of 3.7 months (range 1.8-10.4 months).

Conclusion: The combination of vorinostat and isotretinoin is safe, tolerable and associated with responses in patients with refractory metastatic RCC.

Outcomes based on age in the phase III METEOR trial of cabozantinib versus everolimus in patients with advanced renal cell carcinoma.

Donskov F, Motzer RJ, Voog E, et al. *Eur Cancer.* 2019 Dec 27;126:1-10. doi: 10.1016/j.ejca.2019.10.032. [Epub ahead of print]

Summary: Cabozantinib improved progression-free survival (PFS), overall survival (OS) and objective response rate (ORR) compared with everolimus in patients with advanced RCC after prior antiangiogenic therapy in the phase III METEOR trial (NCT01865747). Limited data are available on the use of targeted therapies in older patients with advanced RCC. Efficacy and safety in METEOR were retrospectively analyzed for three age subgroups: <65 (n = 394), 65-74 (n = 201) and ≥75 years (n = 63). PFS, OS and ORR were improved with cabozantinib compared with everolimus in all age subgroups. The PFS hazard ratios (HRs) were 0.53 (95% confidence interval [CI]: 0.41-0.68), 0.53 (95% CI: 0.37-0.77) and 0.38 (95% CI: 0.18-0.79) for <65, 65-74 and ≥75 years, respectively, and the OS HRs were 0.72 (95% CI: 0.54-0.95), 0.66 (95% CI: 0.44-0.99) and 0.57 (95% CI: 0.28-1.14). The ORR for cabozantinib versus everolimus was 15% vs 5%, 21% vs 2% and 19% vs 0%, respectively. No significant differences were observed in PFS or OS with age as a categorical or continuous variable. Grade III/IV adverse events (AEs) were generally consistent across subgroups, although fatigue, hypertension and hyponatremia occurred more frequently in older patients treated with cabozantinib. Dose reductions to manage AEs were more frequent in patients receiving cabozantinib than in those receiving everolimus. Dose reductions and treatment discontinuation due to AEs were more frequent in older patients in both treatment groups. **Conclusion:** Cabozantinib improved PFS, OS and ORR compared with everolimus in previously treated patients with advanced RCC, irrespective of age group, supporting use in all age categories. Proactive dose modification and supportive care may help to mitigate AEs in older patients while maintaining efficacy.

(continued on page 31)



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Implications of VHL-HIF Pathway Dysregulation in Renal Cell Carcinoma: Current Therapeutic Strategies and Challenges



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Introduction

Kidney cancer is among the 10 most common cancers in both men and women, leading to approximately 74,000 new cases and to more than 14,000 deaths annually in United States alone.¹ Early stage, localized renal cell carcinoma (RCC) has a significant cure fraction and a survival rate of 92%, whereas the treatment of late stage recurrent metastatic RCC remains highly challenging, with a minority of patients with metastatic RCC surviving past 5 years.² Given that RCC is chemo-resistant and radiation-resistant, novel targeted therapies were required for the prevention and management of advanced and/or metastatic RCC.

Studies found that the majority of localized and advanced clear cell RCCs (ccRCCs) are characterized by mutational inactivation and allelic loss of the von Hippel-Lindau (*VHL*) tumor-suppressor gene.³ The groundbreaking discoveries made by William G. Kaelin Jr., Sir Peter J. Ratcliffe and Gregg L. Semenza on the involvement of the *VHL* gene in various fundamental processes, including but not limited to sensing and adapting to the changing oxygen environment eventually led to the Nobel prize in physiology and medicine in 2019. These key insights not only paved the way for our understanding of a key factor in ccRCC tumorigenesis, but also provided the basis for the development of VHL-hypoxia pathway-targeted therapies that includes tyrosine kinase inhibitors (TKIs) for treatment of RCC and other diseases.

In this review, we outline key aspects of VHL-hypoxia inducible factor (HIF) pathway and their impact on tumorigenesis in VHL disease and sporadic ccRCC. We then explore the current status and future challenges for the RCC treatment landscape in the context of *VHL* loss and other biological factors.

Keywords: renal cell carcinoma, VHL-HIF pathway, loss of VHL, HIFs dysregulation, Mutational landscape of RCC, William Kaelin's discovery, therapeutic targets

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Implications of VHL Loss in VHL Disease and Sporadic ccRCC

von Hippel-Lindau disease is a rare autosomal dominant hereditary neoplastic disorder triggered by germline mutations in the *VHL* tumor-suppressor gene with an incidence of roughly 1 in 36,000 births.⁴ Individuals with VHL disease are at increased risk of recurrent and bilateral kidney cysts and ccRCC, as well as retinal, cerebellar and spinal hemangioblastomas, pheochromocytomas, pancreatic cysts, serous cystadenomas and neuroendocrine tumors, endolymphatic sac tumors and epididymal and round ligament cysts.⁵ The discovery of the *VHL* gene in 1993⁶ was driven by a desire to understand and treat VHL disease. The impact of this seminal discovery on our understanding of disease manifestations in patients with VHL disease and on individuals with sporadic ccRCC cannot be overstated. We now know that the majority of sporadic ccRCC cases also exhibit somatic loss-of-function mutations in the *VHL* gene,³ loss of 3p chromosome, or hypermethylation of the *VHL* locus.^{7,8}

The mechanistic understanding of VHL protein (pVHL) function, driven by Kaelin's group and others formed the cornerstone of our current understanding of ccRCC biology. Through additional work performed by a number of investigators and organizations including The Cancer Genome Atlas (TCGA), we now know *VHL* loss serves as the initiating truncal event for ccRCC tumorigenesis, eventually followed by additional mutational and chromosomal copy number altering changes that foster tumor growth and lethality.⁸⁻¹¹

The VHL- HIF Pathway

Bill Kaelin and colleagues were instrumental in characterizing the *VHL* gene and its function. In 1995, Iliopoulos, Kibel, Gray and Kaelin showed that the reintroduction of a wild-type but not a mutant *VHL* cDNA into the 786-0 *VHL*^(-/-) RCC cell line abrogated its ability to form tumors in nude mouse xenograft assays, reinforcing the concept that *VHL* is a *bona fide* tumor suppressor gene.¹² In the same year, the Kaelin group showed that pVHL interacts with with elongins C and B to form the

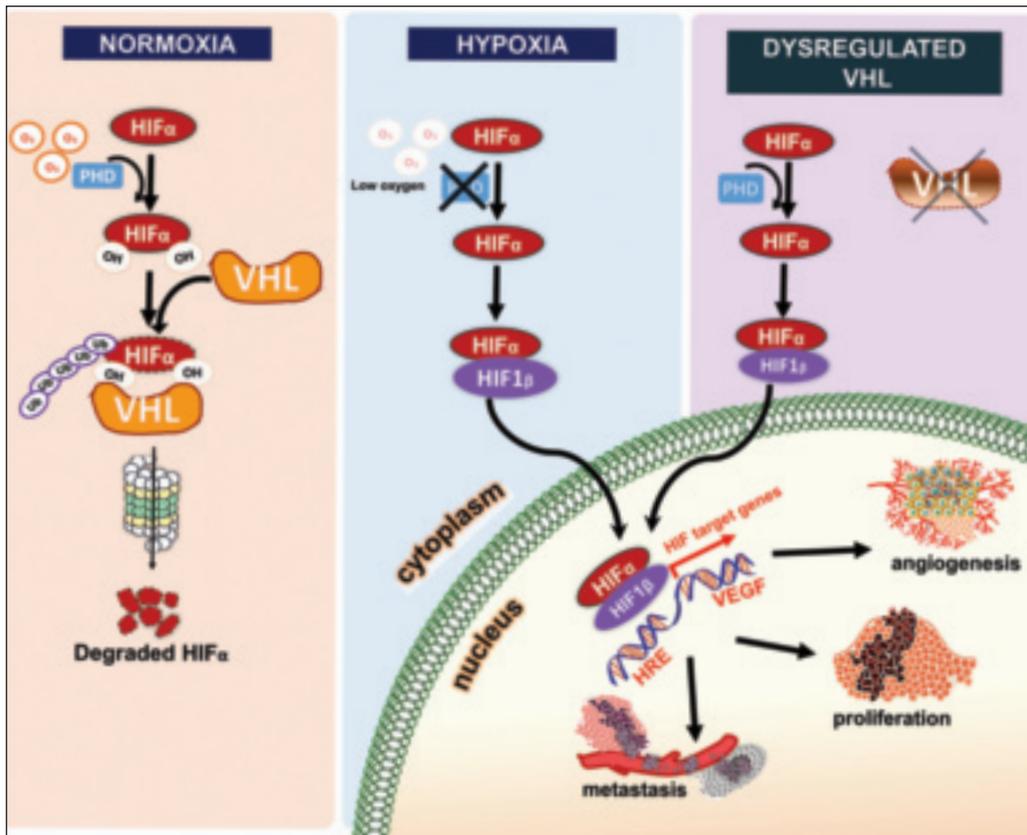


Figure 1. Mechanisms of VHL-mediated regulation of HIF α under normoxic, hypoxic and dysregulated VHL settings. Under normoxia, HIF α is hydroxylated by prolyl-4-hydroxylases enzymes (PHDs) via an oxygen-dependent enzymatic mechanism. The von Hippel Lindau (VHL) protein-E3 ligase complex recognizes HIF α and eventually HIF α are polyubiquitylated and degraded by the proteasome. Under hypoxia, prolyl hydroxylation of HIF α is impaired. As a consequence, HIF α accumulates in the cytoplasm, also forming complex with constitutively expressed HIF β . Similarly, in the presence of a mutated pVHL or in the absence of functional pVHL, HIF1 α and HIF2 α escape from such degradation, leading to stabilization of HIF α . Accumulated HIF α then dimerize with HIF β to generate transcriptionally active HIF complex in cytoplasm. This complex eventually translocates into the nucleus where it binds to HIF response element (HRE) to initiate the repertoire of hypoxia-induced genes eg: of VEGF-A and PDGF like growth factors related to adaptation to a low oxygen environment. The resulting transcriptional stimulation of the HRE and downstream overexpression of numerous genes involved in angiogenesis (eg. VEGF), proliferation (eg. EGFR), cell migration and invasion (eg. CXCR4), erythropoiesis (eg. EPO), which ultimately facilitate ccRCC tumorigenesis. **Abbreviations:** PI3K, phosphoinositide 3-kinase; HIF α , Hypoxia inducible factor alpha; Ub, Ubiquitin; VEGF, vascular endothelial growth factors; VEGFR, vascular endothelial growth factor receptor; PDGF, platelet derived growth factor; PDGFR, platelet derived growth factor receptor; CXCR4, C-X-C Motif Chemokine Receptor 4

VBC complex.¹³ In 1996, Iliopoulos *et al* demonstrated that pVHL was involved in negatively regulating hypoxia-inducible genes.¹⁴ Over the next few years, further refinement of the VBC complex,¹⁵ and the solution of the crystal structure of the VBC complex, led to a broader understanding of pVHL function.

The next major step was the identification of HIF as the substrate for the VBC complex. In 1991, Greg Semenza reported that HIF bound to enhancers near the human erythropoietin gene.¹⁶ Over the following decade Dr. Semenza and his colleagues further characterized HIF function, demonstrating its dimerization, DNA binding, and transactivation properties.¹⁷ In 1996 Jiang *et al* showed that vascular endothelial growth factor was HIF regulated.¹⁸

The third piece in the overall puzzle was the mecha-

nism of oxygen sensing, elegantly discovered by Peter Ratcliffe and colleagues. Dr. Ratcliffe's lab had been working on elucidating the key factors in erythropoietin gene activation since the early 1990s.¹⁹ In 1999, Maxwell *et al* reported that pVHL was required to degrade HIF in an oxygen and iron-dependent manner,²⁰ and in 2001 Jaakola *et al* reported this interaction was prolyl hydroxylation dependent.²¹

Further modeling showed that overexpression of a VHL-binding defective HIF2 α variant was sufficient for tumorigenesis in a mouse model, suggesting that HIF overexpression is one of the major drivers of the malignant phenotype. A review of the myriad functions of HIF1 α and HIF2 α show that each HIF isoform has both unique and overlapping target genes, including angiogenesis, metabolism and glycolysis²² (Figure 1).

Putting together all of these elements together, today we know that pVHL recognizes prolyl hydroxylated HIF α subunits in an oxygen dependent manner. Prolylhydroxylated HIF1 α and HIF2 α associate with the VBC complex, consisting of pVHL, elongin B, elongin C, cullin 2, and Rbx1.²³ HIF α subunits are polyubiquitylated and degraded by the proteasome, thereby tightly regulating cytoplasmic HIF α protein levels.

Conversely, hypoxic conditions impair the hydroxylation of HIF α and its subsequent degradation, leading to accumulation of HIF α , heterodimerization with ARNT (HIF1 β) and translocation to the nucleus to enable transcription of HIF α dependent genes. Similarly, in the presence of a mutated pVHL or in the absence of any pVHL expression, HIF1 α and HIF2 α are not degraded. Interestingly, HIF1 α and HIF2 α were found to exhibit contrasting roles in ccRCC xenograft mice models. HIF2 α reduction diminished tumor formation, whereas restoration of HIF2 α level resulted in a more pronounced tumor burden.^{24,25} Conversely, HIF1 α expression was associated with decreased xenograft tumor growth in mice models,²⁶ and knock-down of HIF1 α enhanced cell proliferation and tumor burden in animal model.^{24,27} These studies demonstrate that HIF1 α behaves as a tumor suppressor in RCC and

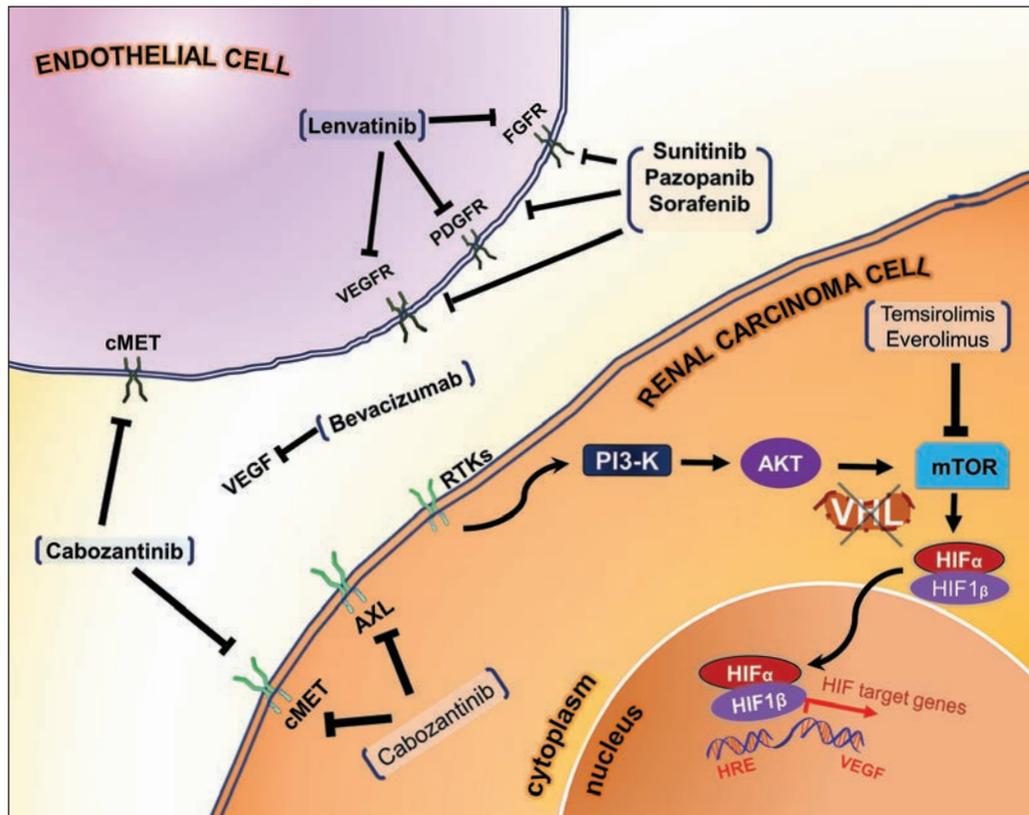


Figure 2. Mechanism of action of VHL-HIF axis based targeted therapies interfering critical molecular pathways in ccRCC. Pharmacological agents inhibit a variety of therapeutic targets of signaling cascades in both ccRCC and endothelial/stromal cells. The signal pathways in ccRCC can be interfered/blocked in different ways, including but not limited to (i) inhibition of VEGF (by bevacizumab); (ii) inhibition of tyrosine kinase activity of RTKs (by sunitinib, pazopanib, and sorafenib); and (iii) inhibition of mTOR (by temsirolimus and everolimus); (iv) blocking MET/AXL/VEGFR2 by a TKI – (by cabozantinib).

Abbreviations: vascular endothelial growth factor (VEGFR); mTOR, mammalian Target of Rapamycin, MAPK, mitogen-activated protein kinase; TKI, tyrosine kinase inhibitor. PI3K, phosphatidylinositol 3-kinase, AKT, protein kinase B; RTKs, receptor tyrosine kinases.

HIF2 α acts as an oncogenic driver. Taken together, HIF2 α is predominantly implicated in the pathogenesis of VHL-associated vascular tumors and pharmacologic blockade of HIF2 α may be an attractive therapeutic strategy for RCC treatment.

Mutational Landscape of RCC

Intriguingly, biallelic loss of *VHL* is not sufficient to generate tumors in model systems and additional genetic events are required to predispose *VHL* deficient cells to develop into ccRCC.²⁸ Studies using mouse embryo fibroblast cells or nonmalignant human tubular cells have shown that loss of *VHL* induces senescence. This finding suggests that additional events are needed for the malignant transformation of *VHL*-mutant proximal tubular cells.²⁹ This concept is supported by the observation that in addition to deletions in *VHL*, ccRCCs harbor mutations in a number of chromatin remodeling genes found on chromosome 3p, including Polybromo-1 (*PBRM1*)³⁰, *SETD2*³¹, and *BAP1*.³² Additionally, loss of 9p and 14q chromosomal regions is associated with increased probability of tumor lethality.⁹ How these mutations and copy number changes impact ccRCC biology and subsequent

response to therapy is an area of active research.

VHL-HIF Axis Based Targeted Therapies for ccRCC

The development of agents targeting the consequences of VHL loss shifted the treatment landscape from cytokine based immunotherapeutics, such as IFN and IL-2 towards targeted therapeutics fifteen years ago.^{33,34} Given that ccRCC are highly vascular tumors with overexpression of angiogenic vascular endothelial growth factor (VEGF) which is a downstream target of HIF, currently approved therapies include inhibitors of VEGF^{35,36} and VEGFR tyrosine kinases (TKIs).^{33,34,37-41} Patients with VHL disease also demonstrated some benefit from these agents, with a 33% objective response rate (ORR) in ccRCC after sunitinib treatment⁴² and a 51% ORR in ccRCC after pazopanib treatment.⁴³ The key challenge with all of these agents is that there is significant on and off target toxicity, and a near inevitable failure to cure or ultimately control tumor growth. There is no clear explanation for these findings, but there is

undoubtedly room for a further refinement of VHL-HIF axis blocking agents.

There is a cogent mechanistic rationale for targeting the VHL-HIF pathway proximally to inhibit as many downstream branchpoints as possible. In preclinical models, inhibition of HIF2 α appeared to be both necessary and sufficient to suppress ectopic blood vessel formation and decrease tumor growth.⁴⁴ Targeting HIF2 α is a very attractive but potentially daunting goal. Transcription factors are notoriously hard to develop small molecule inhibitors against due to their tight conformation, and the HIF isoforms were initially regarded as undruggable. Nonetheless, a series of small molecule inhibitors were recently developed against HIF-2 α . PT2399, a preclinical research compound, induced tumor regression in a *VHL*-defective ccRCC preclinical model.⁴⁵ PT-2399 displayed on-target antitumor activity against a significant percentage of *VHL*-mutated or deficient ccRCC lines and patient-derived xenografts.⁴⁵ PT2399 had greater activity than sunitinib, was active in sunitinib-progressing tumors, and was better tolerated⁴⁵ (Figure 2).

The first-in-class clinical HIF-2 α inhibitor PT-2385 caused dramatic tumor regressions in patient-derived

xenografts.⁴⁶ Clinical data from PT-2385 in pretreated patients with metastatic clear cell renal carcinoma (mRCC) were encouraging in a Phase I, dose-escalation trial, and demonstrated a favorable safety profile.⁴⁷ PT2977/MK-6482 is the second generation of the HIF2 inhibitor and was tested in a 55 patient phase Ib-II study.⁴⁸ This study, which was presented at the European Society of Medical Oncology meeting in the fall of 2019, described 55 patients with advanced cc RCC who had received at least one prior therapy and who were treated with 120 mg orally once daily dose of PT2977/MK6482. We found that PT2977/MK6482 was well tolerated and had a favorable safety profile. The most common Grade 3 adverse events and on-target effects of HIF2 inhibition were found to be anemia in 26% of patients and hypoxia in 15%, and only 2 patients experienced grade 4 toxicities. Despite having a study population treated with a median of three prior therapies, the ORR was 24%, the median progression-free survival (PFS) was an impressive 11 months (95% CI 6-17), and the 12-month PFS rate was 49%. PT2977/MK6482 is currently being tested in a randomized phase III study in patients with treatment refractory metastatic ccRCC (NCT04195750).

P2977/MK6482 is also being tested in patients with VHL disease (NCT03401788). This study has completed accrual, and the data are maturing. As there is currently no Food and Drug Administration approved therapy for VHL disease, we are anxiously awaiting the outcome of this trial to see if there is a potential registrational path for this agent in the treatment of VHL disease.

Recently, the approval of combination TKI- checkpoint blocking antibody therapy has resulted in a new treatment paradigm for many patients with ccRCC.^{49,50} Tissue based studies suggest antiangiogenic agents are capable of increasing T-cell recruitment to the tumor microenvironment,⁵¹ providing a mechanistic rationale for this type of combination therapy. Further investigations into the way VHL-HIF targeting agents can synergize with checkpoint blocking antibodies will undoubtedly further improve the treatment of patients with RCC.

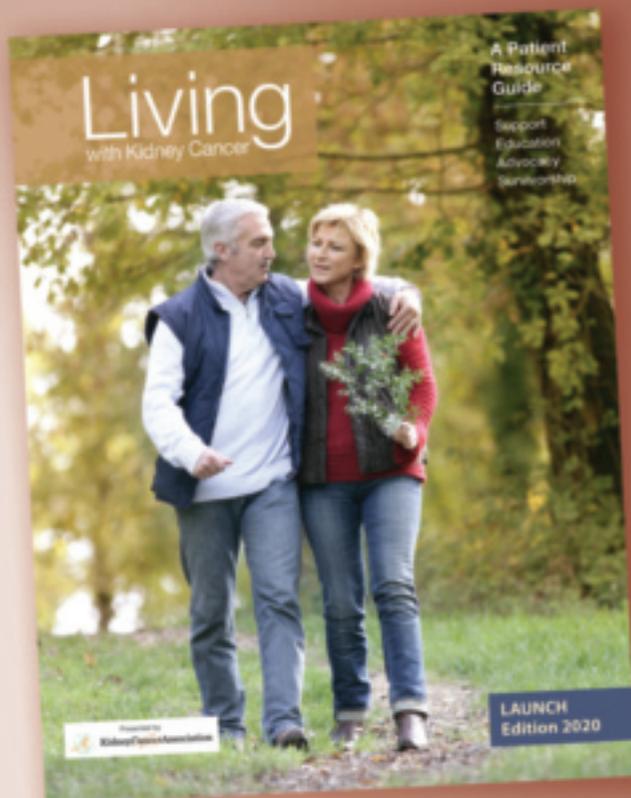
Concluding Remarks

The seminal work by Drs. Semenza, Ratcliffe and Kaelin have fundamentally changed our understanding of ccRCC biology and ushered in a completely new treatment paradigm for this disease and others. Elucidating the functional consequences of VHL loss has not only shed light on how cells sense and adapt to a hypoxic environment but has also paved the way for the development of a new class of target based therapeutic strategies to treat ccRCC. Although therapeutic agents targeting VEGF and VEGF receptors have demonstrated robust efficacy in clinical trials, few people have been cured. We await further development of HIF2 targeting agents to see whether they can move the treatment of ccRCC to the next level, either as monotherapy or in combination with other novel therapeutics. It is imperative we continue to strive for a better understanding of how these agents impact tumor biology and the surrounding microenvironment to allow us to develop even better treatments for patients with RCC.

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Reinventing the Paradigm of IL-2 Therapy: Pivotal Trial Could Change the Landscape of Combination Strategies in Advanced RCC



Robert A. Figlin, MD



Nizar Tannir, MD



Arif Hussain, MD

Objectives of the Roundtable Discussion

This roundtable discussion held on January 15, 2020 explores the potential impact and innovative clinical strategy of the PIVOT-09 trial involving bempegaldesleukin (BEMPEG; NKTR-214) combined with nivolumab as a novel combination therapy for renal cell carcinoma (RCC). In this discussion, three RCC cancer experts analyze the landscape of interleukin-2 (IL-2) therapy, and they also outline how a novel re-designed IL-2 molecule, comprising a PEGylated version of IL-2 (bempegaldesleukin; BEMPEG; NKTR-214), may deliver promising immunomodulatory capabilities. One of the goals of the PIVOT-09 trial is to evaluate the synergistic effect of BEMPEG with the checkpoint inhibitor (CPI) nivolumab (NIVO) in IMDC intermediate- or poor-risk patients and IMDC all-risk patients with previously untreated advanced renal cell carcinoma (aRCC). The discussion is led by Robert A. Figlin, MD, Editor-in-Chief of *Kidney Cancer Journal*. The panel members are Nizar Tannir, MD, Professor, Department of Genitourinary Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, and Arif Hussain, MD, Professor of Medicine, University of Maryland Greenebaum Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland.

■ The Development of NKTR-214 (BEMPEG)

Therapy: A Historical Perspective

Dr Figlin: Please describe the development of NKTR-214 and the properties that make it different from the historical IL-2 therapies that were developed in the 1990s.

Dr Hussain: Before we consider the development of NKTR-214, it is important to first review the background of IL-2 therapy and how it relates to recent treatment advances. The clinical treatment landscape of advanced RCC with a component of clear cell histology has been evolving dramatically over the last 14 years, since the approval of the first targeted therapies which target angiogenesis factors or further downstream factors at the level of the mammalian target of rapamycin (mTOR) complex.

The approval of these newer agents was based on the findings from randomized phase 3 trials that included various primary endpoints such as progression-free survival (PFS; e.g. sorafenib, sunitinib) or overall survival (OS; e.g. temsirolimus). The subsequent progress has been steady, both for initial treatment of patients presenting de novo with advanced disease, and those progressing after initial systemic therapies. These additional therapies have continued to build upon further targeting of receptor kinases (primarily vascular endothelial growth factor receptor [VEGFR], but potentially other targets as well such as MET, AXL, among others), demonstrating that progression on one tyrosine kinase inhibitor (TKI) does not preclude responses to other tyrosine kinase-targeting agents. In the last few years, an additional approach has also been incorporated into the treatment landscape of RCC with the demonstration of a positive impact of immune checkpoint inhibitor (CPI)-based therapy: a) in patients failing TKI-based treatments (nivolumab), or b) in patients as first-line therapy (nivolumab + ipilimumab). Furthermore, recent trials have also established a role for combination therapies in the first-line RCC setting that include TKI plus CPI (e.g. axitinib + pembrolizumab, axitinib + avelumab).

The role of IL-2 in RCC needs to be put in context with the current evolving treatments for metastatic renal cell carcinoma (mRCC) or aRCC, keeping in mind that HD intravenous IL-2 was in fact the first FDA-approved therapy for RCC (approved in 1992). The basis for use of HD IL-2 was a phase 2 pooled study in which approximately 12% of mRCC patients achieved a partial response, with 9% achieving complete response (CR).¹ Further, some of the responding patients could be converted into long-term cancer-free survivors upon resection of residual disease. Although the high incidence of significant side effects, including those related to capillary leak syndrome (CLS), necessitates close inpatient monitoring of high-dose interleukin-2 (HD IL-2) treatment, treatment by experienced providers and appropriate medical support can allow for successful administration of HD IL-2. The potential advantage of this approach is that if one is destined to respond, they do so

after 1–3 courses of therapy; such responding patients likely may not require other long-term systemic treatments, thus limiting prolonged treatment-related adverse events (TRAEs). By contrast, with targeted therapies and perhaps also immune CPIs, longer-term treatments are generally required, which can be associated with their own set of potentially prolonged AEs.

With HD IL-2 therapy there appears to be a plateau in terms of the proportion of patients with RCC achieving objective response and CR. Although to date there are no head-to-head comparisons, ORRs are often higher with single-agent TKI therapies, but CR rates lower than with HD IL-2. However, this paradigm may be shifting. For instance, TKI plus immune checkpoint blockade as upfront therapy (axitinib + pembrolizumab, axitinib + avelumab) demonstrates ORR of 50–59% and CR rates of 3–6%.² Interestingly, double immune checkpoint blockade with nivolumab plus ipilimumab in the first-line setting has resulted in ORR of 42% and CR rate of 9%. Thus, it appears that immune modulation in advanced aRCC is relevant to achieving clinical CR, and perhaps durable CR, albeit the proportion of patients achieving this remains low.³

An important question now is whether IL-2 can be integrated with some of the newer approaches to further improve treatment outcomes of RCC patients, given its purported immunomodulatory mechanisms of action, including enhanced expansion of antigen-specific clonal T cells and cytotoxic CD8 cells, stimulation of large granular lymphocytes (natural killer [NK] cells) and stimulation of B cells to secrete antibodies. The intense treatment schedule of HD IL-2, and the significant associated toxicities, have made it difficult to readily incorporate and test HD IL-2 with other treatments in combination therapies. Although lower-dose IV IL-2 and subcutaneous IL-2 have also been used to treat RCC, their anti-tumor activities are even more modest than HD IL-2. The challenge therefore has been to develop other formulations of IL-2 that: a) can recapitulate the clinical efficacy associated with HD IL-2, b) have lower side effects, c) be given in an outpatient setting, and d) be safely combined with other treatments to potentially improve anti-tumor activity.

BEMPEG incorporates recombinant human IL-2 into a polyethylene glycol moiety that favorably alters some of the pharmacokinetic and pharmacodynamic properties of HD IL-2. The gradual release of bound IL-2 from pegylated chains after IV administration allows for IL-2 to reach peak serum concentrations more gradually over 24–48 hours after administration compared with the rapid kinetics and short half-life of HD IL-2.² BEMPEG consequently has a 500-fold increase in AUC vs HD IL-2.⁴ These properties allow for less frequent dosing, and mitigate the rapid release of cytokines and hence cytokine release-associated AEs that often occur with HD IL-2 administration. Further, BEMPEG preferentially activates the intermediate affinity IL-2 receptors (IL-2R beta/gamma) over the high-affinity IL-2 receptors (IL-2R alpha/beta/gamma), leading to preferential activation and expansion of the desirable CD8⁺ T cells and NK cells rather than the immunosuppressive CD4⁺ T-regulatory (T_{reg}) cells. It is the location of PEG chains at the

IL2/IL2Ra interface that interferes with binding to high-affinity IL2Ra (CD25), while leaving binding to low-affinity IL2Rb (CD122). Its receptor-binding properties also lead to decreased activation of the high-affinity IL-2R on endothelial cells, and thus less likelihood of capillary leak syndrome. Altogether, these properties suggest that BEMPEG can be more readily incorporated into combination therapies.

Dr Tannir: HD IL-2 was approved by the FDA in 1992 based on seven single-arm phase 2 trials showing consistent ORRs of approximately 20% and CR rates of 7-10%, with approximately 85% of the CRs being durable.¹ Two major limitations of HD IL-2 therapy have limited its wide application: 1) significant toxicity, which in the early years was associated with a 4% fatality rate and 2) need for high-level training of healthcare providers to administer this therapy in an inpatient setting with close monitoring to manage challenging complications such as capillary leak syndrome, refractory hypotension requiring IV fluids, vasopressors, and intensive care unit monitoring, liver and/or renal dysfunction, neurotoxicity, sepsis, and gastrointestinal toxicity.⁵ The approvals of targeted therapies (VEGF-directed agents, mTOR inhibitors) have supplanted cytokine therapy, including HD IL-2, although few centers continue to administer HD IL-2 to selected patients who are candidates for this therapeutic approach. The recent approval of immune CPI-based therapies (e.g. nivolumab + ipilimumab, pembrolizumab + axitinib) as first-line therapies further eroded the use of HD IL-2.

The limitations of HD IL-2 have spurred the development of a more tolerable IL-2 therapy, which can harness the benefits of the immune system while minimizing the toxicity. BEMPEG was developed with these aims in mind. The inactive 6-PEG prodrug compound yields two active moieties after irreversible release in the circulation and shifts signaling preferentially through the IL-2R beta and gamma components, essentially acting as a CD122 agonist, away from the IL-2R alpha signaling that is responsible for much of the toxicity of HD IL-2. Additionally, the serum half-life of BEMPEG is 20 hours compared with 20 minutes for HD IL-2. The more favorable toxicity profile and longer half-life of BEMPEG allow for outpatient administration; the recommended dose of BEMPEG is on a three-week schedule.

■ Delineating the Pharmacologic Properties of BEMPEG

Dr Figlin: What are the results from the phase 1 monotherapy trials that delineate the pharmacologic properties, the AE profile, and how BEMPEG allows for intermittent dosing in cancer?

Dr Tannir: BEMPEG has been evaluated in solid tumors as a single agent in the phase 1 and phase 2 settings to establish dosing and safety, and to assess initial activity. It has been tested on an every-3-week schedule given IV; five dose levels were evaluated ranging from 0.003–0.012 mg/kg, with the maximal tolerated dose identified as 0.009 mg/kg and the phase 2 dose defined as 0.006 mg/kg every 3 weeks. The dose-escalation phase included 28 patients.⁶ Among these, only one patient experienced

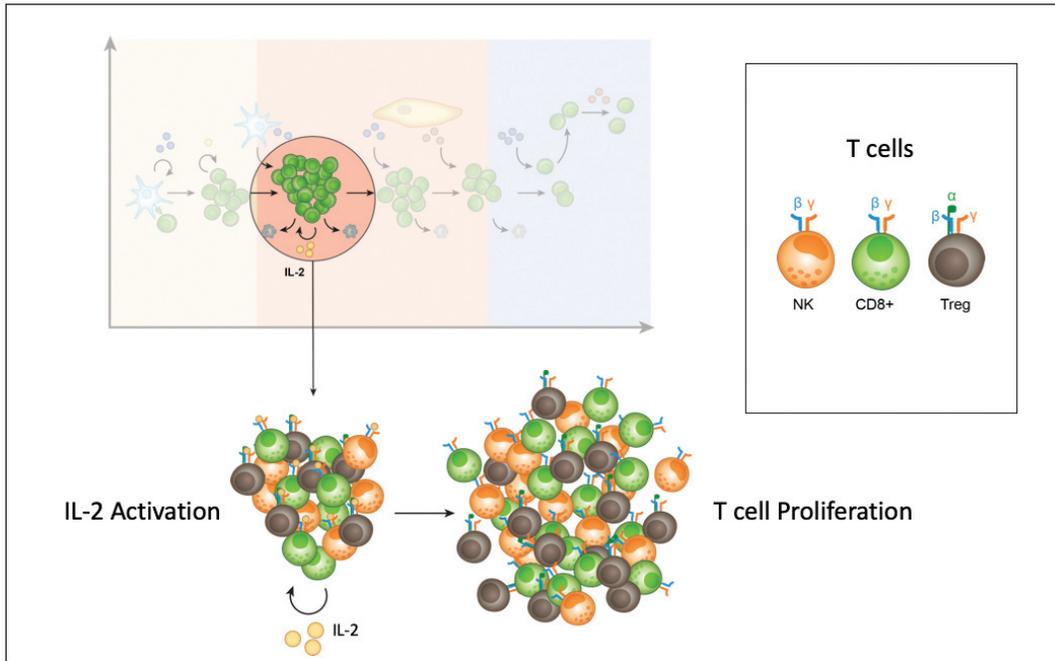


Figure 1. Tuning Receptor Selectivity

The IL-2 pathway regulates T-cell response and stimulates immune cell proliferation and activation of both tumor-killing CD8+ effector T cells and Natural Killer (NK) cells, and immuno-suppressive regulatory T cells (Treg).

two dose-limiting toxicities at 0.012 mg/kg: Grade 3 hypotension and Grade 3 syncope. Although the majority of patients had adverse events, Grade 3 TRAEs were observed in 6/28 (21.4%) patients, while none had Grade 4 AEs or capillary leak syndrome.⁶ The most common TRAEs in order of decreasing frequency included fatigue, flu-like symptoms, pruritus, hypotension, rash, decreased appetite, and arthralgia, with the AEs generally occurring 3–4 days post dosing. Hypotension was the only Grade 3 TRAE that occurred in more than one patient, being Grade 3 in four patients. The hypotension can be managed in the outpatient setting with judicious use of IV fluids during the day of BEMPEG infusion and additional increased oral fluid intake by patients.⁶

Dr Figlin: HD IL-2 monotherapy produced durable responses in about 10% of patients with RCC. Is it your hope that BEMPEG can accomplish similar results as monotherapy?

Dr Tannir: Novel combination therapies comprising the immune CPIs that block different inhibitory receptors on the T-cell (cytotoxic T-cell-associated protein-4, programmed death protein 1 [PD-1]) and VEGFR-TKIs such as axitinib, cabozantinib, and lenvatinib have revolutionized the field of RCC therapeutics. It was clear for investigators involved in the development of BEMPEG that the focus of studies with this novel immunotherapy agent had to be on strategies combining BEMPEG with nivolumab, or with nivolumab plus ipilimumab, rather than developing it as a single agent.

Dr Hussain: Based on some of the pharmacokinetic and pharmacodynamic properties, BEMPEG provides strong

rationale for evaluation in the clinical setting, particularly for RCC where there is an established role for IL-2-based therapy. In appropriately identified patients with good performance status, clear-cell RCC tumor histology, and no visceral/CNS/bone metastasis, HD IL-2 remains a viable treatment option despite the rapidly evolving RCC treatment landscape. It is certainly our hope that BEMPEG has at least a similar degree of activity to that observed with HD IL-2 in RCC, which would be an important step given its more favorable safety/ tolerability profile. This would contrast with low-dose IL-2 or subcutaneous IL-2, which generally have better tolerability profiles but are less active than HD IL-2. It should be noted

that BEMPEG is only being evaluated in combination.

■ Rationale for Combining BEMPEG With a CPI

Dr Figlin: Please describe the preclinical rationale for combining BEMPEG with immunotherapy in RCC.

Dr Hussain: The role of immunotherapy in RCC is well established, and in fact the initial therapies for RCC were based on immunomodulation via cytokines such as interferons and IL-2. The therapeutic benefit of immunomodulation in RCC has been further reinforced with the demonstration of increased anti-tumor effects of immune CPIs in RCC as compared with targeted therapy. CPIs help reactivate the anti-tumor properties of exhausted CD4⁺ and CD8⁺ T cells within tumors. Enhanced expression of PD-1 by activated T cells leads to down modulation of T cell activity upon engagement of PD-1 by its ligands such as programmed death ligand 1 (PD-L1) present on tumor cells, creating a tumor-permissive environment. This forms the basis for targeting PD-1 or PD-L1 with specific antibodies that, as noted, reactivate the T cells against the tumor cells. Given these dynamics between the tumor and the immune system, this provides a strong rationale to develop treatment strategies for RCC that incorporate the T-cell-promoting activities of IL-2 and the immune CPIs. Furthermore, BEMPEG can increase PD-1 expression on T cells and PD-L1 expression on tumor cells, providing relevant targets for immune checkpoint blockade. (Figures 1,2,3)

Dr Tannir: BEMPEG has been shown to increase tumor-infiltrating lymphocytes (TILs), T-cell clonality (expansion of CD4⁺, CD8⁺, and NK cells with little effect on T_{regs}), and PD-1 expression as determined by immunohisto-

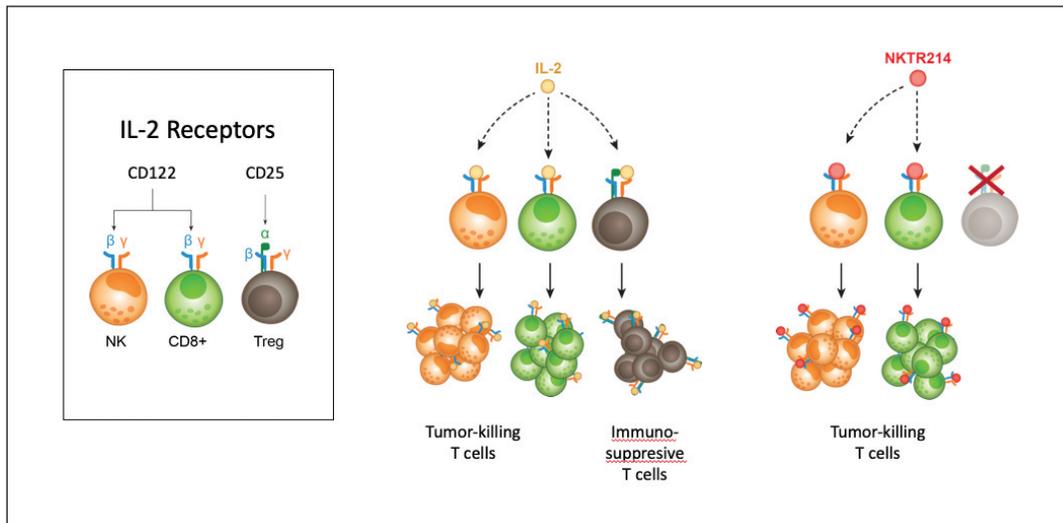


Figure 2. Tuning Receptor Selectivity
T cell proliferation is stimulated through the IL-2 receptor complexes CD122 (IL-2 $\beta\gamma$) on effector T cells and NK cells and CD25 (IL-2 $\alpha\beta\gamma$) on Tregs. IL-2 binds to both CD122 and CD25 while NKTR-214 has a biased action on the CD122 receptor.

chemistry. Low levels of baseline TILs and T-cell inflammation are predictive of a poor response to CPIs. BEMPEG combined with nivolumab has been shown by immunohistochemistry to convert baseline tumors from PD-L1 negative (<1%) to PD-L1 positive ($\geq 1\%$); hence, leveraging this conversion would increase the response to CPIs.

Dr Figlin: Please describe the phase 1 results of the combination of BEMPEG and nivolumab that served to inform the PIVOT-09 trial.

Dr Hussain: Based on large phase 3 trials, nivolumab as single-agent therapy has been approved in previously treated patients with clear-cell RCC, and more recently it is also approved in the first-line setting in combination with ipilimumab. Nivolumab plus IL-2-based therapy, such as with BEMPEG, would be a novel combination to evaluate for its relative clinical activity in RCC. In this regard, a phase 3 clinical trial (NCT03729245 A Study of NKTR-214 in Combination With Nivolumab Compared With the Investigator's Choice of a Tyrosine Kinase Inhibitor (TKI Therapy (Either Sunitinib or Cabozantinib Monotherapy) for Advanced Metastatic Renal Cell Carcinoma (RCC) is currently ongoing, and is evaluating BEMPEG plus nivolumab versus standard-of-care TKI in metastatic treatment-naïve clear-cell RCC across all IMDC patient risk groups (good, intermediate, poor), with co-primary endpoints being ORR and OS, and key secondary endpoint being PFS.⁷ This trial has been informed by an initial dose-escalation and dose-expansion study with the combination in patients with various advanced solid tumors, including RCC, melanoma, non-small-cell lung cancer (NSCLC) and urothelial cancer (PIVOT-02, NCT02983045). In the expansion phase, the recommended phase 2 dose for these agents were BEMPEG 0.006 mg/kg IV every 3 weeks plus nivolumab 360 mg IV every 3 weeks. To date, no unexpected AEs have resulted from the combination treatment, with the most

common TRAEs being flu-like symptoms, rash, pruritus, nausea, and decreased appetite. Importantly, BEMPEG does not appear to increase the side-effect profile of nivolumab. Amongst the almost 300 patients treated with the combination across several solid tumors, 14% experienced Grade 3 or higher TRAEs.⁸ Amongst the small number of patients with RCC treated with the combination to date, 12/26 (46%) have experienced a complete or partial response, which compares favorably with historical controls.

Dr Tannir: Yes, I agree with everything Arif has just said. In addition to what Arif notes, in the RCC expansion cohort of the PIVOT-02 trial, which combined BEMPEG at the dose of 0.006 mg/kg IV plus nivolumab 360 mg IV every 3 weeks, the ORR was in the range of 46% with low Grade 3/4 AE rates, and most AEs were Grade 1 and 2, with flu-like symptoms, fatigue, rash and pruritis starting 24 hours after the infusions and lasting 3–4 days. There was no increase in immune-related AEs compared with AEs observed with PD-1 antibodies alone.

■ Exploring the Phase 3 PIVOT-09 Trial and Its Endpoints

Dr Figlin: Let's consider some other aspects of the PIVOT-09 trial. Please describe the design of the pivotal trial, how you chose the comparator arm, the status of the trial, and your statistical endpoints to evaluate efficacy.

Dr Tannir: In most countries outside the US, Canada, and Western Europe, sunitinib or pazopanib remains the mainstay first-line therapy for patients with mRCC. Cabozantinib has been shown to produce a higher ORR and longer PFS compared with sunitinib in a randomized phase 2 trial of patients with advanced or metastatic clear-cell RCC with intermediate- or poor-risk disease. There are no data with cabozantinib as first-line therapy in patients with metastatic clear-cell RCC with favorable-risk disease, but it is anticipated that the clinical activity of cabozantinib would be at least comparable to sunitinib in patients with this risk group.

The phase 3 trial, PIVOT-09, is randomizing (1:1) treatment-naïve patients with advanced or metastatic clear-cell RCC to receive BEMPEG 0.006 mg/kg IV plus nivolumab 360 mg IV every 3 weeks (Arm A) or sunitinib 50 mg orally daily, 4 weeks on, 2 weeks off, or cabozantinib 60 mg orally daily (Arm B). Patients with any International mRCC Database Consortium IMDC prognostic risk group are eligible, and tumor tissue is required for PD-L1 testing. Stratification factors include the TKI choice (sunitinib vs cabozantinib) and IMDC prognostic

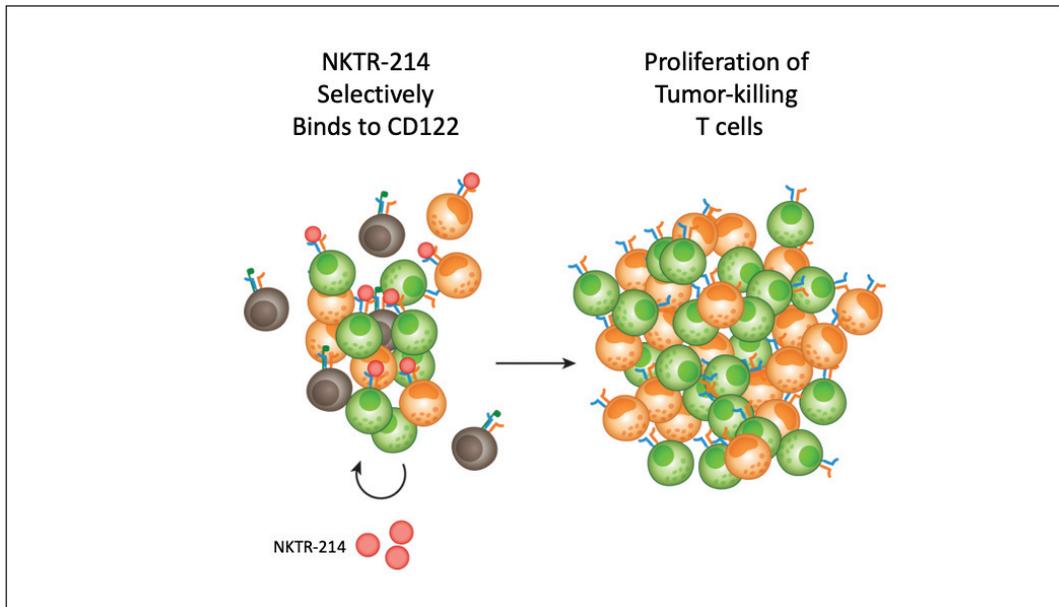


Figure 3. Tuning Receptor Selectivity
NKTR-214 preferentially stimulates proliferation of tumor-killing CD8+ effector T cells and Natural Killer cells without activating immunosuppressive regulatory T cells.

risk group. This trial aims to enroll a total of 600 patients at approximately 150 sites, although the vast majority of patients will be recruited from countries other than the US, Canada, and Western Europe. The co-primary endpoints are ORR by blinded independent central review (BICR) and OS. The key secondary endpoint is PFS by BICR. Other secondary endpoints include incidence of AEs, ORR using RECIST 1.1 by investigator and PD-L1 biomarker population, PFS by investigator and biomarker population, OS in biomarker population, and quality of life.

Dr Hussain: Recent phase 3 trials evaluating first-line therapies in RCC have used sunitinib as the standard comparator arm. The ongoing pivotal phase 3 trial with BEMPEG plus nivolumab is somewhat unique in this regard since the comparator arm is either sunitinib or cabozantinib, depending upon physician choice. This design takes into account the changing treatment patterns of RCC. Currently, there are three single-agent TKIs approved in the first-line setting, namely sunitinib, pazopanib, and cabozantinib. Among these, sunitinib and pazopanib are essentially similar in terms of treatment outcomes, with perhaps some differences in their respective side-effect profiles. Based on the CABOSUN trial, cabozantinib may have greater activity compared to sunitinib particularly among intermediate and poor risk RCC patients, and consequently is also being increasingly used as first-line monotherapy.⁹ In this respect, the comparator arm 'bar' against which BEMPEG plus nivolumab is being compared is perhaps higher than if the comparator arm included only sunitinib.

A major challenge to the successful development of BEMPEG plus nivolumab for RCC is that first-line therapies, particularly some of the newer combination therapies (nivolumab + ipilimumab, axitinib + pembrolizumab, axitinib + avelumab), show significant and favor-

able activity compared with single-agent sunitinib. The ongoing pivotal phase 3 trial of BEMPEG plus nivolumab must show similar activity, and perhaps even better activity, compared with the above combinations and against a standard-of-care arm that not only includes sunitinib but also cabozantinib.

Dr Figlin: Do you believe there are any tissue or laboratory-based biomarkers that could identify the potential beneficiaries of this approach?

Dr Hussain: To date, no clear biomarkers have been identified that reliably predict treatment outcomes to HD

IL-2 therapy. On the other hand, data across various malignancies support PD-L1 expression patterns as a potential predictor for response to immune checkpoint targeting, although there is increasing recognition that PD-L1 expression may not be adequately 'captured' during testing of tumor specimens as it is a dynamic marker. Further, although PD-L1 expression may identify subpopulations of responding RCC patients, those without significant PD-L1 expression can still respond to immune CPIs. Consequently, current RCC immune CPI treatment paradigms are essentially PD-L1 agnostic. It will be of interest to study and further define the role of PD-L1 testing in BEMPEG plus nivolumab RCC trials given that BEMPEG can in fact alter/enhance PD-1 and/or PD-L1 expression. Whether relative quantification of immune suppressor cells such as (myeloid-derived suppressor cells (MDSCs) and T_{regs} or/and TILs within tumors can serve as useful biomarkers to prognosticate or/and predict response to therapy remain important questions. Although exploratory, another open question would be whether there are certain cytokine signature patterns (for instance, within the circulation) at baseline and post therapy that can identify and potentially inform treatment outcomes for BEMPEG plus nivolumab or/and standard of care TKI therapy.

Dr Tannir: Immune profiling of blood and tissue and next-generation sequencing of tissue looking at prognostic and predictive markers for response to BEMPEG plus nivolumab and BEMPEG plus nivolumab and ipilimumab are ongoing.

■ Future development of BEMPEG

Dr Figlin: What other diseases or combinations will you be evaluating with respect to BEMPEG and its drug discovery platform?

Dr Hussain: The success of immune CPIs in many differ-

ent cancer types has established a paradigm for modulating the immune system for therapeutic benefit. Although IL-2-based treatments have, to date, focused primarily on melanoma and RCC, based on the immunomodulatory and potentially complementary effects of IL-2 with immune CPIs in reactivating and enhancing the immune system, this suggests that an approach integrating reformulated forms of IL-2 such as BEMPEG may have a broader role in treating malignancies beyond RCC. This may be a particularly viable approach if BEMPEG is integrated with immune CPIs or/and vaccine-based therapies in disease states where there is already a defined role for these latter treatments. It is of interest that pivotal phase 2/3 registrational trials are being carried out with BEMPEG and immune CPIs in other solid tumors, including melanoma, NSCLC, and urothelial cancers, which may provide a platform for further investigation into some of these other cancer types. It should be noted that metastatic prostate cancer is currently the only solid tumor for which a vaccine, sipuleucel-T, has been approved by the FDA. This autologous dendritic cell-based vaccine has been shown to improve OS, but not necessarily PFS, and significant room for improving upon this vaccine treatment remains. Whether BEMPEG or a CPI or both can be incorporated with sipuleucel-T to improve treatment outcomes in advanced prostate cancer is another potential area to consider.

As noted above, VEGF/VEGFR has been established as a pivotal axis in RCC angiogenesis and pathogenesis, targeting of which has led to significant improvements of RCC patients. Importantly, this axis not only enhances angiogenesis but also stimulates myeloid-derived suppressor cells (MDSCs) that contribute to an immunosuppressive and tumor-permissive environment. Thus, targeting VEGF/VEGFR can enhance anti-tumor immune responses by increasing T-cell trafficking into tumors, decreasing MDSC and T_{reg} activity, and producing immunosuppressive cytokines. The clinical relevance of targeting VEGF/VEGFR concurrently with immune modulation has now been well established in RCC based on several phase 3 trials that have shown positive outcomes with bevacizumab plus atezolizumab, axitinib plus pembrolizumab, and axitinib plus avelumab.^{10,11} Thus, the VEGF axis and the immune checkpoint axis provide a relevant framework in the context of BEMPEG, including exploratory co-targeting approaches that could evaluate BEMPEG plus VEGFR targeting, or even BEMPEG plus CPI plus VEGFR targeting in RCC and other solid tumors.

Recent work has identified a key role for PI3K-based signaling in immune cell function, particularly in immunosuppressive cells such as MDSCs. Targeting PI3K in combination with BEMPEG offers another potential opportunity to explore. In addition to the above, there have been significant efforts to modulate the metabolome, particularly glutamine metabolism, in RCC and other cancer types in combination strategies with TKIs and immune CPIs that could also inform further development of BEMPEG in the future.

Finally, although BEMPEG is being evaluated with nivolumab in the first-line setting in RCC via a pivotal ongoing phase 3 trial, another important and relevant aspect is to also explore and define a possible role for BE-

MPEG in the second-line or beyond settings in RCC patients progressing on initial immune CPI- or/and TKI-based therapies. For instance, is there any role or benefit to further immune modulation by BEMPEG among previously treated patients, either as single-agent therapy or more likely in combination with another targeting agent (e.g. PI3K inhibitor, glutaminase inhibitor, others)?

Dr Tannir: That is an accurate summary. I also would like to add that the two tumor types other than RCC where there is already promising preliminary data with the doublet of BEMPEG plus nivolumab are melanoma and urothelial carcinoma. In the melanoma expansion cohort of the PIVOT-02 trial, the ORR was 53% and CR 34%.

At the 2019 ASCO GU meeting, data were presented on the combination of BEMPEG and nivolumab from the PIVOT-02 cohort of first-line treatment of 41 patients with metastatic urothelial cancer who were cisplatin-ineligible or cisplatin-eligible and refused standard of care. The doublet of BEMPEG plus nivolumab was well tolerated. The most common TRAEs were Grade 1 and 2 flu-like symptoms, fatigue, rash, and pruritis. Six patients experienced a Grade 3 TRAE, which led to discontinuation of therapy in four patients (10%). There were no Grade 4 or 5 AEs. Among 27 evaluable patients for response, 13 patients had a complete or partial response for an ORR of 48%: 5 patients had CR and 8 patients had a partial response. Responses were noted in patients with PD-L1 <1% and in patients with PD-L1 ≥1%. In metastatic UC (mUC), responses were observed in patients with PD-L1-negative and CD8-TIL low tumors (4/8 or 50%) and CD3-TIL low tumors (3/7 or 43%).¹⁴ The combination of BEMPEG plus NIVO is being evaluated in several other tumor types, including RCC, melanoma, UC, and NSCLC.

Conclusion

Results from pivotal clinical trials have demonstrated the immunomodulatory and potentially complementary effects of IL-2 with CPIs in reactivating and enhancing the immune system. A novel therapeutic approach, integrating reformulated forms of IL-2 such as BEMPEG with the checkpoint inhibitor nivolumab may have translational impact as first-line therapy in treating RCC. Although BEMPEG is being evaluated with nivolumab in the first-line setting in RCC via a pivotal ongoing phase 3 trial, another important and relevant aspect is to also explore and define a possible role for BEMPEG in the second-line or beyond settings in RCC patients progressing on initial immune CPI- or/and TKI-based therapies.

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Back to Interleukin 2 After Four Decades: Review of the History, Biology, Novel Approaches and Clinical Trials



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Introduction

After the discovery of checkpoint inhibitors (IO), the treatment landscape of renal cell carcinoma (RCC) rapidly transformed from VEGF based treatments to IO and IO+TKI combinations. Within the last 5 years, the Food and Drug Administration (FDA) approved several IO and combination regimens for the treatment of renal cell carcinoma. In 2015, nivolumab was approved for second line treatment based on the Checkmate 025 study.¹ Then in 2018, nivolumab+ipilimumab, followed by pembrolizumab+axitinib and avelumab+axitinib in 2019, was approved for the first line treatment of RCC based on Checkmate 214, Keynote 426 and Javelin Renal 101 studies, respectively.²⁻⁴ These trials demonstrated improved efficacy of novel combinations compared to sunitinib in some patients; however, achieving durable response remained a challenge. Along with many other strategies addressing each mechanism, one of the approaches was to revisit IL-2 based treatments and evaluate the combinations with IO. This review will describe the historical discovery, development of Interleukin-2 (IL-2) immunotherapy, related biology and limitation in early studies. Additionally, we will review the novel approaches to modify IL-2 and summarize related clinical studies.

Discovery of IL-2 and Early Clinical Trials

The beginning of the IL-2 story started 45 years ago, in 1975, with the discovery of Dr Gallo and his team (Figure

Keywords: interleukin 2, IL-2, biology, renal cell carcinoma, CD25, T cells, immune response, toxicity, bempedaldesleukin, nivolumab, NKTR-214,

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1). For the first time, they showed that there is a selective growth of T lymphocytes when unfractionated normal bone marrow cells were cultured with the conditioned medium, which was obtained from phytohemagglutinin-stimulated normal human lymphocytes.⁵ Later, his group purified the so-called “human T-cell growth factor” (TCGF) from the phytohemagglutinin (PHA)-stimulated lymphocyte-conditioned media.⁶ Several other laboratories described many properties of this cytokine, using different names: Thymocyte Mitogenic Factor (TMF), Co-stimulator, Killer cell helper factor (KHF), Secondary cytotoxic T cell-inducing factor (SCIF). During the Second International Lymphokine Workshop, Switzerland in 1979, to decrease the redundancy and confusion, the interleukin nomenclature was revised, previously called TCGF, TMF, SCIF, KHF and then renamed as Interleukin-2.⁷ It was described as a cytokine that induces primary cytotoxic T cell responses and promotes and maintains the viability and proliferation of primary T cell lines in *in vitro* cultures. Following this, in 1983, the recombinant plasmid containing human interleukin 2 (IL-2) cDNA was identified in a cDNA library constructed from mRNA derived from phytohemagglutinin and phorbol ester induced splenocytes⁸ and from partially purified human leukaemic T-cell line IL-2 mRNA.⁹ Eventually, in 1992, the crystal structure of IL-2 was solved.¹⁰

In early preclinical studies, systemic administration of IL-2 to nude mice induced specific T-helper cells, cytotoxic cells and autoantibody production.^{11,12} It also demonstrated an antitumor effect with IL-2 dependent expansion of immune lymphocytes.^{13,14} Based on these early preclinical studies and an understanding of interleukin-2 biology, the phase I trial was performed in 1985. Ten patients with a variety of advanced stage malignancies unresponsive to conventional treatments were treated with at least 30 000 U/kg of IL-2 by bolus admin-

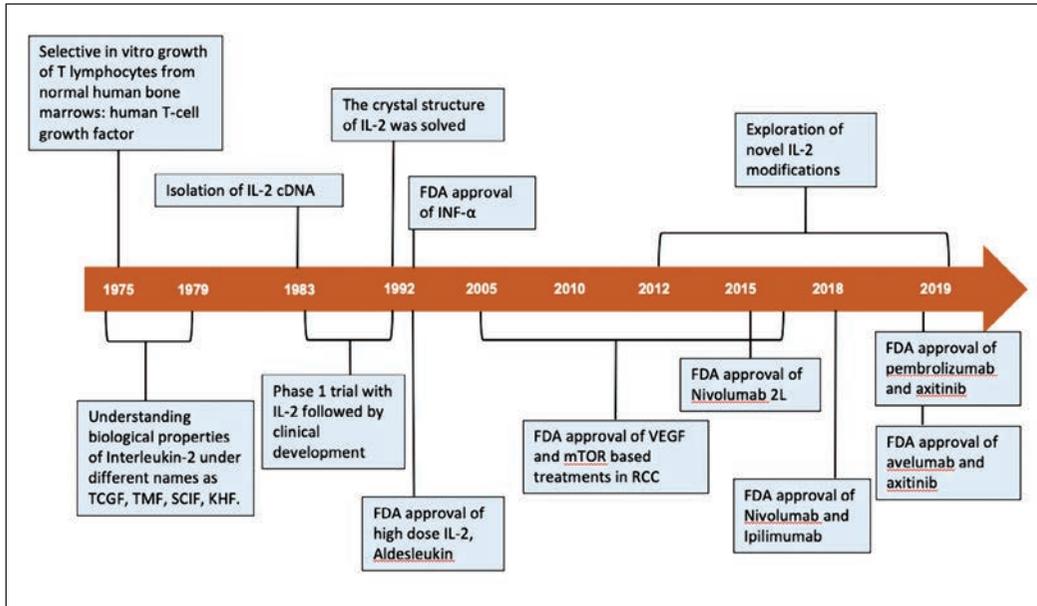


Figure 1. History of IL-2 and other immunotherapy agents in RCC.

istration three times a day, intravenously or intraperitoneally from 4 to 21 days in a single course. Three of six patients with metastatic melanoma experienced a partial response with >50% decrease in tumor volume. There was no response to treatment in the other four patients with colorectal or ovarian cancer. Marked lymphocytic infiltrate was noted in a patient with lesions accessible to repeated biopsies. This study was the first in human evidence that the administration of IL-2 could mediate the regression of cancer in some patients.¹⁵ Outside of this phase I trial a single patient with renal cell carcinoma with lung metastasis treated with IL-2 demonstrated a complete response.¹⁵

Following this promising result, in a phase 2 study, 283 patients with metastatic melanoma and RCC were treated with IL-2 at a dose of 720,000 IU/kg intravenously every 8 hours for a maximum of 15 doses per cycle; a 7% complete response and 10-13% partial response was observed in each subgroup. Three patients had treatment-related death. The major adverse event in these early studies was fever, chills, GI symptoms, weight gain, pulmonary edema, hypotension secondary to capillary leak syndrome.¹⁶ Based on responses in phase 1 and phase 2 trials, the FDA approved high-dose IL-2, Aldesleukin in RCC in 1992 and metastatic melanoma in 1998.¹⁷ However, due to the severe toxicity profile, only specialized centers were allowed to use high dose IL-2 immunotherapy.

Interleukin 2, IL-2 Receptor and Biology

Interleukin 2 gene, located on human chromosome 4, is heavily regulated with several transcription factors, including NF-κB, NF-AT and AP1 that are the downstream products of signaling pathways activated with TCR/CD3 trigger and CD28 costimulation on CD4+, CD8+ T-cells.^{18,19} It is also secreted by NK, NK-T cells, DCs and mast cells following activation.²⁰ The initial product of the gene is 153 amino acids protein, processed with the cleavage of 20 amino acid hydrophobic leader sequence

and O-linked glycosylation of threonine 3, important in cellular trafficking.²¹ It has Type I cytokine structure with 4 alpha helical bundles linked with a disulfide bond between cysteines 58 and 105.¹⁰ The IL-2 receptor has three non-covalently linked components called IL-2Rα (CD25, p55), IL-2Rβ (CD122, p75) and IL-2Rγ (CD132, p65). The IL-2 is able to bind monomeric IL-2Rα, dimeric IL-2Rβγ and trimeric IL-2Rαβγ forms of the receptor with low-, intermediate-, and high-affinity, respectively.^{22,23} Different immune cells express the receptor at different levels, either at resting or with stimulation. This provides diverse effects on immune cells.

In one end, it suppresses the immune response through Tregs, which express CD25 constitutively along with other subunits and have a high affinity to IL-2 (Figure 2). On the other end, it provides effector functions through Teff and NK cells. CD8+ and NK cells tend to express the intermediate affinity receptor, IL-2Rβγ. Activated T-cells transiently express CD25 to enhance their differentiation and proliferation as a response to IL-2. IL-2 activates mainly JAK-STAT, RAS-MAP and PI3K-AKT pathways to induce proliferation and effector functions of the immune cells.^{20,24} While PTEN, PD-1 and CTLA-4 inhibit PI3K-AKT pathway in Tregs, IL-2 also activates Mst1-Mst2 which amplifies STAT5 and maintains IL-2 induced Treg survival and stability.²⁵

Strategies to Modulate IL-2

During the early studies, the major limitations of IL-2 therapy were high toxicity with capillary leak syndrome, short half-life (15–30 min) and the requirement for high-dose to have adequate efficacy. Recent studies showed that limiting toxicity, including capillary leak syndrome and pulmonary edema, was mediated by CD25 stimulation of pulmonary endothelial cells.²⁶ Along with that, the strategies described below investigated new formulations of IL-2 that will prefer binding to CD122 and limit CD25 based stimulation on Tregs to improve both toxicity and efficacy profile (Figure 3). RG7461 (RO6874281) is a recombinant fusion protein comprised of an engineered form of IL-2 (IL-2v), carrying the mutations F42A, Y45A and L72G. It is located in the CD25-binding epitope of IL-2 and a human monoclonal antibody directed against fibroblast activation protein-alpha (FAP) which is strongly expressed on tumor-associated fibroblasts.^{27,28}

Upon administration of RG7461, the monoclonal antibody recognizes FAP and mediates retention and accumulation of IL-2v in malignant lesions. Due to the mutations on the CD25-binding epitope, IL-2v cannot bind to CD25 and does not activate Tregs. IL-2v maintains the ability to stimulate local immune response and acti-

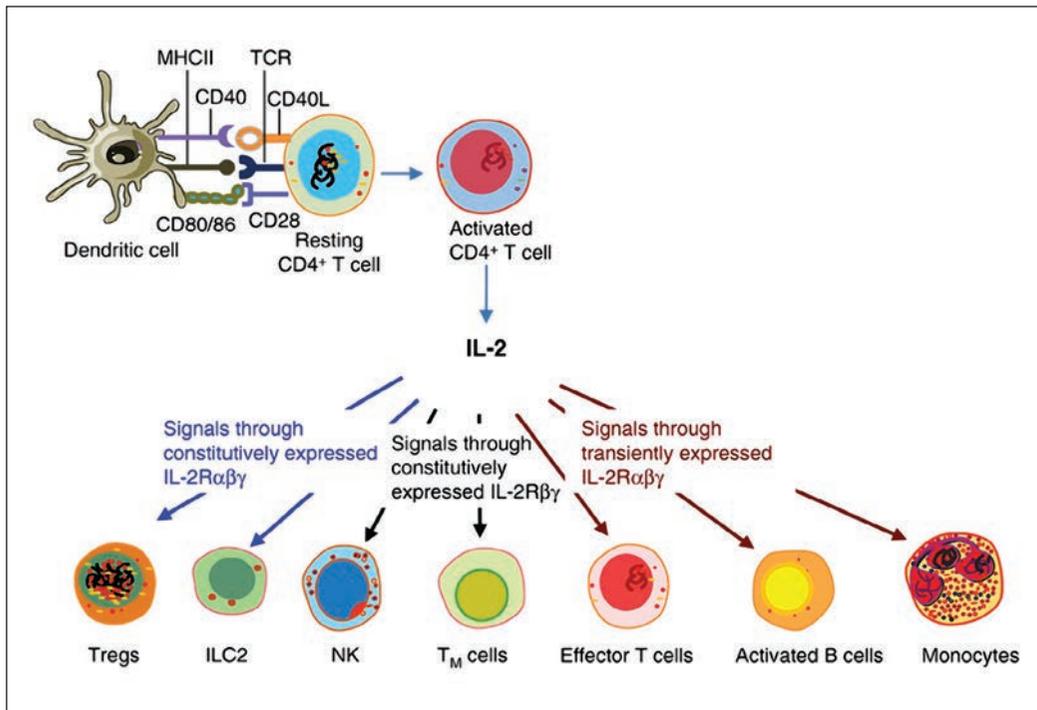


Figure 2. IL-2 plays multiple roles in shaping the immune response (Adapted from Mitra et al.).

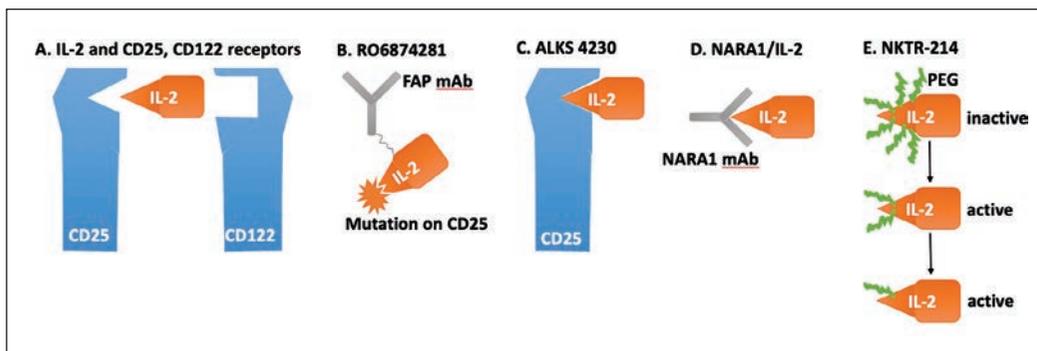


Figure 3. Basic structure of human recombinant IL-2 and novel modified IL-2 formulations. A. Recombinant human IL-2 has different epitopes binding to CD25 and CD122 receptors. B. RO6874281 is a fusion protein of FAP mAb and engineered IL-2 with mutations on CD25 binding region. C. ALKS 4230 is a fusion protein of IL-2 and CD25 receptor. D. NARA1 mAb is a monoclonal antibody against the IL-2 CD25 binding region. E. NKTR-214 is PEGylated IL-2 prodrug, becomes active as it dissociates from PEGs.

vate NK and effector T-cells. The Phase 1 study of RO6874281 (NCT02627274) investigated PK/PD and antitumor activity on 35 patients with metastatic solid tumors. Most frequent adverse events (>30%) were pyrexia, infusion related reactions, fatigue/asthenia, nausea, diarrhea, decreased appetite and elevated aspartate and/or alanine transaminase. The majority of events were mild or moderate (Grade 1/2). At the recommended dose of 20mg, RO6874281 rapidly expands CD8 and NK cells but not Tregs, both in peripheral blood and sequential tumor biopsies. Objective long-lasting (> 6 months) responses were observed in one patient with head and neck cancer, penile squamous cell carcinoma and checkpoint inhibitor-resistant malignant melanoma. Phase 1b/Phase 2 studies in combination with immune checkpoint inhibitors and other agents are currently underway. Immune triplets with atezolizumab plus bevacizumab and

the cell expansion and tolerability profile, 3 µg/kg/day dose was selected for an initial evaluation in combination with pembrolizumab and a 6 µg/kg/day dose was identified as the monotherapy recommended phase 2 dose for intravenous administration. At doses of 3 µg/kg/day and 6 µg/kg/day of ALKS 4230, 8 of 14 patients with evaluable initial scans had stable disease. Two of these patients had RCC: one with 4 lines of prior treatment and the other with 1 line of treatment. The most frequently reported adverse events were fever, chills and low-grade hypotension. The majority of the events were Grade 1-2 and no vascular leak syndrome observed. In addition, 25 patients with ovarian (7/25), colorectal (7/25), sarcoma (7/25), triple negative breast cancer (3/25), and PD-L1 negative NSCLC (1/25) were treated in the combination cohort received ALKS 4230 3 µg/kg/day dose and pembrolizumab. 10 of the 18 patients with evaluable scans achieved stable

RO6874281 (NCT03063762) is going to be tested in renal cell carcinoma (Table).

ALKS 4230 is a fusion protein comprised of modified IL-2 and CD25, the high affinity IL-2 receptor. This enables the compound to selectively bind the intermediate-affinity IL-2 receptor, CD122/CD132, thereby selectively expanding CD8+ and NK cells.^{29,30} IL2-CD25 fusion design of ALKS 4230 hinders its ability to bind to high-affinity IL2 receptor, which minimizes the activation of immunosuppressive Tregs. ARTISTRY-1 is the phase 1/2 study evaluating the safety and efficacy of ALKS 4230 as a monotherapy or in combination with pembrolizumab in advanced solid tumors.^{31,32} The initial analysis was reported in the 34th SITC meeting; 36 patients with a variety of solid tumor types, including melanoma (8/36), prostate (5/36) and renal cell carcinoma (5/36) were enrolled to the monotherapy dose escalation part of the study. Data from the five completed dose-escalation cohorts, spanning doses of 0.1 to 6 micrograms/kg of ALKS 4230, demonstrated dose-dependent pharmacodynamic effects on the numbers of circulating NK cells and CD8+ T cells and minimal non-dose dependent effects on immunosuppressive regulatory T cells. Based on

Table. Current trials with novel modified IL-2 formulations

Description and Interventions	Phase	Conditions	Status	NCT Number
RO6874281 + Pembrolizumab	1	Metastatic Melanoma	Recruiting	NCT03875079
RO6874281 + Atezolizumab + Bevacizumab	1	RCC	Active, not recruiting	NCT03063762
RO6874281 as a Single Agent or in Combination With Trastuzumab or Cetuximab	1	Solid Tumor, Breast Cancer, Cancer of Head and Neck	Recruiting	NCT02627274
RO6874281 + Atezolizumab or Gemcitabine or Vinorelbine	2	Advanced/Metastatic Head and Neck, Esophageal and Cervical Cancers	Recruiting	NCT03386721
ARTISTRY-1: ALKS 4230 intravenous as a single agent or in combination with pembrolizumab	1, 2	Advanced Solid Tumors	Recruiting	NCT02799095
ARTISTRY-2: ALKS 4230 subcutaneous as a single agent or in combination with pembrolizumab	1, 2	Advanced Solid Tumors	Recruiting	NCT03861793
ALKS 4230 + pembrolizumab	2	SCC of Head and Neck	Recruiting	NCT04144517
NKTR-214	1, 2	Advanced Solid Tumors	Completed	NCT02869295
DIRECT-01: NKTR-214 + VB10.NEO		Advanced Solid Tumors	Recruiting	NCT03548467
PROPEL: NKTR-214 + pembrolizumab	1, 2	NSCLC, Melanoma, Urothelial, SSC of Head and Neck, HCC	Recruiting	NCT03138889
NKTR-214 + Nivolumab	2	Sarcoma	Recruiting	NCT03282344
PIVOT-02: NKTR-214 + Nivolumab	1, 2	Melanoma, RCC, NSCLC, Urothelial, Metastatic Breast and Colorectal carcinoma	Recruiting	NCT02983045
Nivolumab + NKTR-214 vs Nivolumab Alone vs Standard of Care	3	Cisplatin ineligible muscle invasive bladder cancer	Not yet recruiting	NCT04209114.
Nivolumab + NKTR-214 vs Nivolumab	3	Melanoma	Recruiting	NCT03635983
PIVOT-10: NKTR-214 + Nivolumab	2	Cisplatin ineligible muscle invasive bladder cancer	Recruiting	NCT03785925
NKTR-214 + Nivolumab	1	Advanced Solid Tumors (Japanese)	Recruiting	NCT03745807
PIVOT-09: NKTR-214 + Nivolumab vs TKI (Cabozantinib or Sunitinib)	3	RCC	Recruiting	NCT03729245
REVEAL: NKTR-262 + NKTR-214 and NKTR-214 + Nivolumab	1, 2	Melanoma, Merkel Cell Carcinoma, Triple Negative Breast Cancer, SCC of Head and Neck, RCC, Colorectal Cancer, Sarcoma	Recruiting	NCT03435640
Avelumab + NKTR-214 with or without Talazoparib or Enzalutamide	2	SCC of Head and Neck, mCRPC	Not yet recruiting	NCT04052204
PORTER: NKTR-214 (Cohort A), Nivolumab (Cohort A, B and C), SBRT (Cohort B0, CDX-301 (Cohort B and C), Poly-ICLC (Cohort B), INO-5151 (Cohort C), Cellectra 2000	1	mCRPC	Recruiting	NCT03835533

disease and 2 had a partial response. One of those patients has triple negative breast cancer and another ovarian cancer. ARTISTRY-2 is an ongoing Phase 1/2 study designed to explore the safety, tolerability and efficacy of ALKS 4230 administered subcutaneously once weekly and once every 3 weeks.³³

NARA1 is a monoclonal antibody with a high affinity to the CD25 binding epitope of human IL-2³⁴ Compared to previous IL-2 and monoclonal antibody complexes, NARA1 and IL-2 binding do not affect the CD122 binding region on IL-2. IL-2/NARA1 complexes limit CD25 binding on cells Tregs and preferentially stimulate CD8+ T. In

vivo studies with a metastatic melanoma model showed that IL-2/NARA1 complex resulted in an efficient expansion of tumor-specific and polyclonal CD8+ T cells. These CD8+ T cells showed robust interferon- γ production and expressed low levels of exhaustion markers PD-1, LAG-3, and TIM-3. These effects resulted in potent anticancer immune responses and prolonged survival in the melanoma tumor models. The clinical development of NARA1 is in progress.

Bempegaldesleukin (BEMPEG, NKTR-214) is comprised of IL-2 bound to multiple releasable polyethylene glycol (PEG) chains through on average 6 lysine residues. The highly PEG-ylated form is an inactive prodrug when administered PEG chains slowly release and form active IL-2 with conjugated fewer PEG chains. The 1-PEG-IL2 and 2-PEG-IL2 are the most active form. Due to the short half-life, the unpegylated form of IL-2 is undetectable in vivo as it is eliminated faster than formed. The PEG chains on BEMPEG are located at the region of IL-2 that contacts the CD25, reducing its ability to bind and activate the heterotrimer,³⁵ thus preferentially activating and expanding effector CD8+ T and NK cells over Tregs³⁶. In the Phase 1 EXCEL study, 28 patients with advanced solid tumor malignancies were treated with BEMPEG at different doses.³⁷ The majority of patients had a diagnosis of metastatic RCC (15/28) or melanoma (7/28). The most common TRAEs included fatigue, flulike symptoms, pruritus, hypotension, rash, decreased appetite, and arthralgia and cough. The recommended phase II dose (RP2D) was determined to be 0.006 mg/kg q3w; 9 of 26 patients experienced maximum tumor reductions ranging from 2% to 30%. The best overall response included SD in 14 patients. In the peripheral blood analysis, CD4+ T cells, CD8+ T cells, and NK cells population significantly increased with the treatment. Tumor biopsies demonstrated increased CD8+ and NK cells in the tumor. Notably, there was an increased amount of CD8+ and PD-1+ T cells both in peripheral blood and in the tumor. There was a transient increase in Tregs in the peripheral blood after treatment, however this was not observed in tumor biopsies. Gene expression analysis from tumor biopsies revealed an increased expression of genes associated with T-cell infiltration and signaling (CD3G, CD3D, CD3E, CD247, and ZAP70; $P \leq 0.05$), T-cell activation and coinhibitory molecules (ICOS, TNFRSF9, PDCD1, CTLA4, TIGIT, and LAG3; $P \leq 0.05$), and of cytotoxic effector genes (PRF1, GZMB, GZMA, and GZMK; $P \leq 0.05$). Genes encoding for PD-L1 and PD-L2 (CD274, PDCD1LG2; $P \leq 0.05$), SOCS1, and IDO1 were also significantly increased. Several other studies evaluated the combination of checkpoint inhibitors and NKTR-214 listed in Table 1. The Phase 1/2 PIVOT-02 trial has evaluated the safety and efficacy of BEMPEG in combination with nivolumab in advanced solid tumors. Based on the dose-escalation phase patients received 0.006mg/kg and 360mg nivolumab IV every 3 weeks. The expansion cohort includes 5 different tumor types, including RCC, melanoma, NSCLC, urothelial and triple-negative breast cancer. Preliminary analysis of 34 metastatic urothelial cancer patients treated in this trial showed ORR was 48% (11/23; 95% CI 27–69%) with a 17% CR rate (4/23) and 70% (16/23) DCR. 6/10 (60%) PD-L1 negative tumor at baseline converted to PD-L1+ at

week 3.³⁸ With these promising results, the 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.

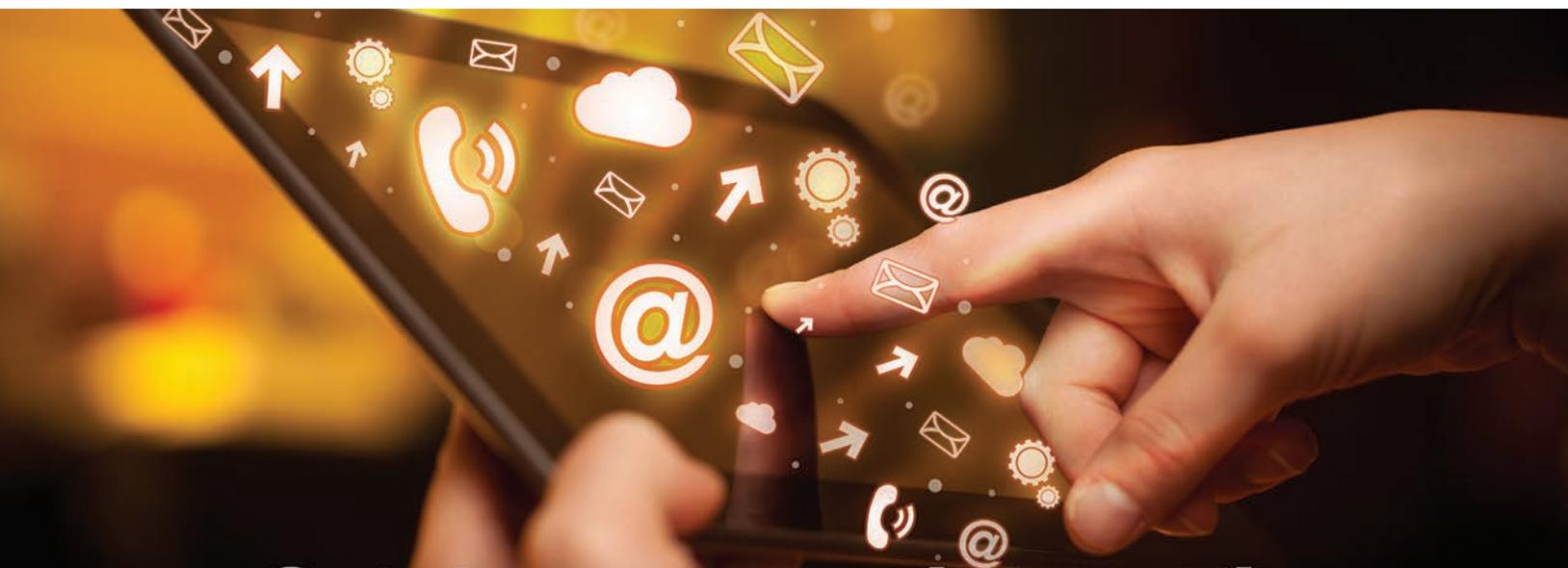
Conclusion

IL-2 is an excellent example of how a deep understanding of biology can help us to re-utilize “old” compounds on the shelf with enhanced capacities. Notably, the novel modified IL-2 and IO combination treatments showed the promise to overcome immune checkpoint monotherapy resistance. Additionally, they have a significantly better toxicity profile than the high dose IL-2 with availability to use in the outpatient setting. However, to date, we do not know which of the above mentioned approaches of combination treatments will be the best in RCC patients with the least toxicity and the best efficacy. Future biomarker based clinical and preclinical studies will help to elucidate these questions.

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Is Circulating Tumor DNA Ready for Prime Time? Evaluation of this Biomarker in the Era of Precision Medicine



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Incremental but exciting progress toward the development of a biomarker in metastatic renal cell carcinoma has put precision medicine on the verge of making dramatic changes in detection and surveillance. The utility of circulating tumor DNA is gaining converts to a technology with potential translational impact on clinical practice. But many issues remain to be elucidated before this tool can move beyond the hypothesis-generating stage to becoming integrated into clinical practice.

As new non-invasive tools emerge in the era of precision oncology, the landscape for diagnostic and prognostic markers in renal cell carcinoma (RCC) is dramatically changing. Next-generation sequencing (NGS) platforms, including techniques that analyze tumor tissue for somatic and germline cancer-associated gene alterations, are beginning to have an impact on kidney cancer diagnoses. Potentially - and therein lies the cautionary tale - innovative blood-based tests, also known as *liquid biopsies*, could begin to change the paradigm of RCC disease management, thereby overcoming issues posed by traditional radiological and histopathological examinations. The need for and the presentation of new research on reliable biomarkers in RCC remains a major focus, yet reliable and confirmatory evidence for such methods continues to be elusive for kidney cancer while substantial progress has been made in other solid tumors which readily employ treatment strategies based on actionable genetic data.

Aside from its non-invasive advantages, other benefits of liquid biopsies include multiple time point testing and its ability to facilitate the diagnosis and monitoring of evolving disease, offering clinicians a potentially contemporary and prognostic marker to effectively track a patient's clinical course. Liquid biopsies such as circulating tumor DNA (ctDNA) or circulating cell-free DNA (cfDNA) constitute two promising avenues of exploration in the era of precision oncology for RCC.

Keywords: circulating free DNA, circulating tumor DNA, liquid biopsy, biomarkers, diagnosis, tumor burden, hypermutation, metastatic renal cell carcinoma, methylation.

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Circulating cell-free DNA. With a simple blood test, the total quantity of DNA that is released into the peripheral blood circulation and captured comprises circulating cell-free DNA (cfDNA). Since this DNA is released from both normal and tumor cells, isolated DNA fragments in cfDNA are not only from the tumor but include normal cellular DNA that is released from other molecular processes like apoptosis, necrosis, and secretion of genomic DNA fragments.¹ The abundance and relative fragmentation of cfDNA has been suggested to be a biomarker for several solid tumors including RCC in numerous studies.²⁻⁸ However, additional metrics of cfDNA remain to be clarified and its clinical utility in RCC disease management has yet to be fully elucidated. With each study, differences in patient characteristics, RCC disease characteristics, and most importantly the platform used for cfDNA capture make it challenging to unify conclusions. Although controversy surrounds efforts to validate cfDNA as a clinical biomarker for RCC, recent studies reviewed in this report suggest its utility and promise for it being a non-invasive tool associated with potential high sensitivity and specificity for RCC management.

Circulating tumor DNA. Next generation sequencing (NGS) of circulating tumor DNA (ctDNA) is an attractive alternative to traditional tissue sequencing because it circumvents the need for repeated, invasive tissue biopsies to gain a contemporary mutational profile. In addition, ctDNA analyses may also provide a more comprehensive assessment of the total tumor as ctDNA is shed from separate heterogeneous tumor sites.⁹ While the role of ctDNA in other diseases like lung cancer and colorectal cancer is well established, studies of ctDNA in metastatic RCC (mRCC) are only hypothesis-generating to date. In contrast to cfDNA, ctDNA is derived from the tumor itself and usually represents a smaller fraction of cfDNA. ctDNA is thought to be shed into circulation by apoptotic and necrotic tumor cells in patients with cancer,^{10,11} highly prevalent in most advanced solid tumors except for brain tumors,¹² and has a half-life ranging from sixteen minutes to a few hours.¹³⁻¹⁵ Because advanced tumors, either pre-treated or at tumor progression, have a higher mitotic index and undergo more rapid cell cycling

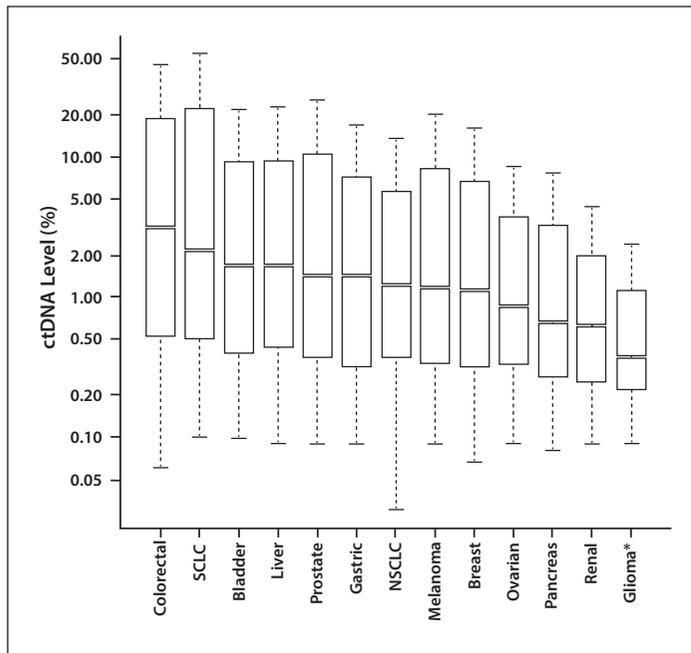


Fig. 1. In an analysis of 21,807 patients with treated, late-stage cancers across more than 50 cancer types, the recovered ctDNA for renal cancer is much less robust. (Adapted from reference 19.)

compared with normal tissue or earlier stage tumors, ctDNA constitutes a larger proportion of cfDNA in metastatic disease.^{16,17} Patients with high tumor burdens and aggressive disease have higher proportions of ctDNA, which may rise above 90% of cfDNA.¹⁸ The presence of multiple alterations in ctDNA may also represent selective treatment pressures and/or tumor heterogeneity, though, which complicate interpretation of identified variants. Ultimately, the goal of ctDNA is to derive actionable genomic information from a peripheral source to make real-time, personalized cancer treatment decisions.¹⁹

Investigational Uses of cfDNA and ctDNA: Potential Implications

Although the clinical utility of these assays is not ready for “prime time”, especially since integral biomarkers are not currently used to guide targeted therapy or immunotherapy in mRCC, a review of recent literature offers a glimpse of how these techniques can be applied as they move forward from the bench to the bedside. In a study by Wan et al.,²⁰ for example, results demonstrate how cfDNA may play a potential role in monitoring patients with RCC after nephrectomy. The objective of this and other such studies is to extend to RCC the significance of plasma/serum cfDNA identified post-surgically as studied in other solid tumors. Wan and colleagues focused on whether a quantitative analysis—before and after nephrectomy—could play an important role in monitoring patients during follow-up for detection of a recurrence in clear cell RCC (ccRCC). The pretreatment level of plasma cfDNA in patients with metastatic ccRCC (6.04 ± 0.72) was significantly higher than those with localized ccRCC (5.29 ± 0.53 , $p=0.017$) or controls (0.65 ± 0.29 , $p < 0.001$). Of patients with localized ccRCC, those with disease recurrence had a significantly higher plasma

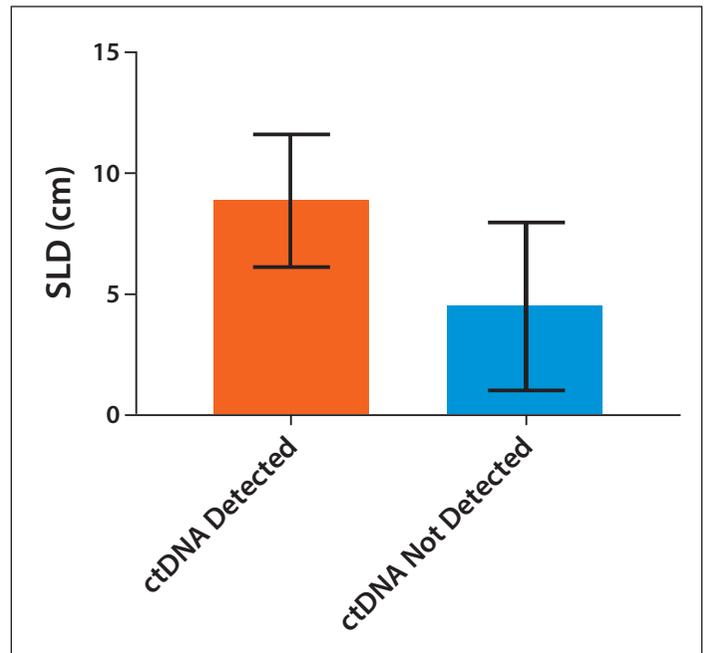


Fig. 2. Difference in sum of the longest dimension (SLD) of tumor in patients with detectable and non-detectable ctDNA. Mean SLD in was 8.81 cm in patients with detectable ctDNA, as compared to 4.49 in patients with non-detectable ctDNA ($p = 0.04$ by unpaired t-test). (Adapted from reference 24.)

cfDNA level than those without ($p=0.024$). Further, patients with a high plasma cfDNA level had a significantly higher recurrence rate than those with a low plasma cfDNA level before and after nephrectomy ($p= 0.018$).

Although the follow-up was relatively short (36 months), the results from Wan et al. highlight potentially a “minimal residual disease” state which would be helpful in monitoring ccRCC patients after surgery. Traditional nomograms which currently help predict recurrence include variables like disease stage, high Fuhrman grade or large tumor size, and adding serum testing after surgery to characterize this biological disease state may add predictive power. Several observations suggest the potential value of cfDNA in this setting: there was a significant difference of plasma cfDNA levels between low and high Fuhrman grade; patients with high disease stage (T3) and large tumor size (>3 cm) had significantly higher plasma cfDNA levels than those with lower stage and smaller tumors. Also, the average cfDNA level was significantly higher in metastatic tumors (N+ and/or M+) than in localized tumors before nephrectomy.

One of the challenges with ctDNA studies in RCC has been uncovered in large pan-cancer studies, which show a relatively low recovered ctDNA quantity in patients with kidney cancers when compared to other solid tumors. In an analysis of 21,807 patients with treated, late-stage cancers across more than 50 cancer types, the recovered ctDNA for renal cancer is much less robust (Figure 1).¹⁰ Understanding why ctDNA loads remain low for this cancer may help fuel new RCC-specific methods to improve ctDNA detection. Nevertheless, several recent reports are capturing the narrative of how ctDNA can be used for clinical care.

One report by Hahn and colleagues²¹ assessed whe-

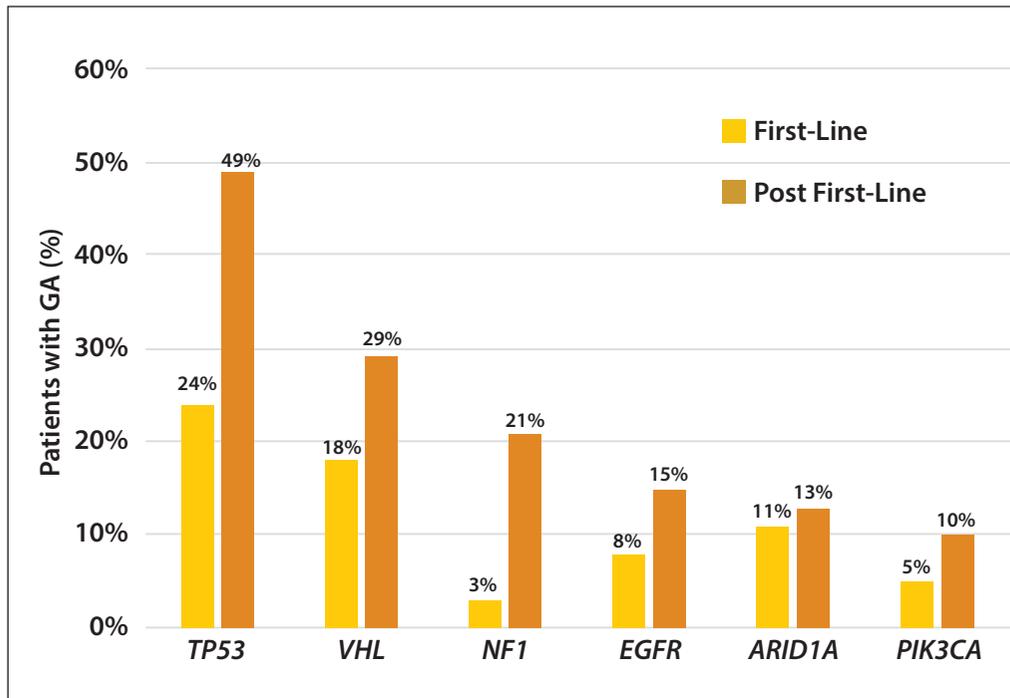


Fig. 3 – Notable differences in genomic alteration (GA) frequency in patients documented as receiving first-line therapy versus post first-line therapy (p values were as follows: TP53: $p = 0.02$; NF1: $p = 0.01$; VHL: $p = 0.26$; EGFR: $p = 0.6$; PIK3CA: $p = 0.3$). (Adapted from reference 25.)

ther genomic alterations detected by ctDNA NGS are truly representative of those alterations detected in tumor tissues. Are these NGS platforms interchangeable or complementary? Understanding this key distinction impacts how and when to integrate ctDNA testing during clinical care. In this first report to correlate ctDNA with matched tumor tissue NGS, there is mixed news. When the study controlled for genes tested by both platforms, the median mutation rate for ctDNA was similar to tissue (median 3.0 vs 1.0) but the concordance rate between the two platforms was only 8.6%. This result is comparable to findings in other solid tumors on concordance. The “take-home” message from this study is that ctDNA NGS offers the advantage of a decreased risk for sample collection and an improved ease of repetitive testing over tumor tissue NGS, and that these two platforms may be used in concert with each other rather than as a substitute. Since this avenue of investigation is still in the preliminary stage, appropriate use of ctDNA in this context remains an area of active research.

CtDNA and Checkpoint Inhibitor Therapy

A key question in this era of precision medicine is to what extent ctDNA might be applied to correlate with response to immune checkpoint inhibitors. Recently, Khagi et al.²² studied whether hypermutated ctDNA correlated with immune checkpoint inhibitor response in solid tumors. In this study of 69 patients with various malignancies including melanoma, lung cancer, and head and neck cancer, 63 patients (91% of the cohort) had at least one ctDNA alteration detected. Characterizing these alterations further, the authors found many patients with “variants of unknown significance (VUS)”, which refers to a variant identified through genetic test-

ing whose significance on disease remains unknown. The authors found an association between ctDNA VUS on progression-free survival (PFS) and overall survival (OS) with immune checkpoint blockade therapy. For example, at two months, landmark survival analyses of responder’s versus non-responders to checkpoint inhibitor therapy with VUS >3 showed a median PFS of 23 versus 2.3 months ($p = 0.004$). The preliminary conclusions from this study – still investigational – is that tissue tumor mutational burden as determined by liquid biopsy could also have a role in predicting response to immunotherapy.

A closely related case report by Dizman et al.²³ of ctDNA changes in a patient with metastatic RCC who achieved an exceptional response to nivolumab therapy adds personalized context to the clinical

utility of ctDNA in metastatic RCC. In this case, the patient’s disease had progressed after treatment with bevacizumab and subsequently cabozantinib. In addition to several genomic alterations from a tissue-based assessment, unique alterations were noted in ctDNA at baseline. After 4 weeks of therapy with nivolumab, the patient had a significant clinical response to immune checkpoint blockade therapy. Interval ctDNA analysis during nivolumab therapy showed no alterations, highlighting paralleled changes in ctDNA with therapy response.

An additional metric highlighted from this case report is whether the rate of ctDNA change, termed ctDNA velocity, may be used as a surrogate for therapy response. In this case report, 6 distinct genomic alterations were identified. Although this is not a clear surrogate for mutational burden, Dizman et al.²³ refer to other reports that link the presence of increased mutational load with response to checkpoint inhibition. Additionally, timing of ctDNA changes seen during therapy may represent markers for cell turnover and therefore surrogates of treatment response. As prior research has demonstrated differences in radiographic tumor burden with ctDNA (Figure 2),²⁴ dynamic measurements which incorporate serial changes in ctDNA like velocity could have significant implications particularly in challenging scenarios like pseudoprogression.

Single-Time Point and Evolutionary Changes in ctDNA

A new generation of studies extending the above efforts into large cohorts of treated RCC patients provides additional insights into the utility of ctDNA as a tool which may capture evolving disease with therapy. In a large cohort of 220 patients with metastatic RCC, Pal et al.²⁵ assessed ctDNA profiles of patients treated with first-line

and later lines of therapy. In their cohort, the most frequent identified alterations included *TP53* (35%), *VHL* (23%), *EGFR* (17%), *NF1* (16%), and *ARID1A* (12%). This cohort of patients remains the largest assessment of ctDNA sequencing in metastatic RCC to date. Variations seen across first-line and refractory settings suggests underlying mechanisms for therapeutic resistance (Figure 3, e.g. *TP53* mutations), as well as identification of alterations which may prompt non-conventional therapy selection for certain patients.

As noted above, the excitement for ctDNA to guide targeted therapy in RCC has started to gain traction. For instance, inhibition of the *MET* pathway remains an active area of investigation, and evidence for *MET* alteration identification across solid tumors is increasing. To investigate this further, Ikeda et al.²⁶ performed ctDNA digital sequencing (using a 54-70 gene panel) in a pancreatic cohort of 438 patients, 263 of whom had tissue sequencing for comparison. *MET* alterations were seen in 7.1% of patients which correlated with presence of bone metastases; *TP53* and *PTEN* abnormalities were also found to be correlated as well. Importantly, *MET* alterations were detected at a lower frequency in tissue (1.14%) compared to ctDNA (7.1%), again highlighting that ctDNA analyses complement standard tissue sequencing.

To further characterize the complexities of applying ctDNA as a biomarker for metastatic RCC, we performed a large cohort analysis incorporating a comparative genomics approach with matched primary tissues at Memorial Sloan Kettering Cancer Center.²⁷ In our cohort, 110 metastatic ccRCC patients underwent a single-time point collection for ctDNA, and the median time between ctDNA collection and previously collected tissue used for comparison was 24 months. Although the mutational profiles were similar between these two tissue platforms – with *VHL* and *PBRM1* alterations recovered with the highest frequency in both blood and tissue, there remained discordance between the total number of alterations recovered. For instance, the majority of *VHL* and *PBRM1* alterations were only identified in primary tissue and not in ctDNA. Alterations of these genes found in ctDNA, though, were always found in the matched primary tissue. In sum, investigating other methodologies which use an enriched RCC specific gene-set panel or higher sequencing depth may improve and enhance ctDNA detection and concordance in this patient population.

With the focus on ctDNA undergoing closer scrutiny, application of this tool in varied disease stages has been explored and presented at scientific symposia. A report by Correa et al.²⁸ of a cohort of 42 patients with stage I-IV RCC who underwent complete surgical resection demonstrated the impact of ctDNA on prognosis. At baseline, for example, ctDNA was detected in 41% of patients and was significantly associated with increased tumor size, advanced tumor stage, and poorly differentiated tumors. Postoperatively, 8 of 8 ctDNA-positive patients relapsed while only 16 of 33 ctDNA-negative patients relapsed. This report concludes that ctDNA values have the potential to be used as a prognostic marker across multiple disease settings.

Future Directions

Looking ahead, future studies need to address a wide range of issues to determine the translational impact of ctDNA in RCC. A few notable areas of exploration include:

1. Robust ctDNA testing with matched tissues NGS data to provide reliable sensitivity, specificity and positive/negative predictive metrics.
2. Studies to “benchmark” each assay, delineating how each of these platforms work and how they can be used in clinical practice.
3. An improved understanding of which relevant alterations need to be identified and their relationship to a disease stage (e.g. prognostic or predictive power, understanding genomic changes and their relationship to therapeutic resistance).
4. Correlation of clinical variables like disease sites or treatment effects with ctDNA variables like ctDNA velocity or load to improve upon clinical significance during assay development.
5. Discovery of disease states like “minimal residual disease” after curative intent surgeries, or responding/progressive disease states for systemic therapy monitoring.

Conclusion

Cell free and circulating tumor DNA assessments are non-invasive tools which can provide pertinent and serial genomic tumor assessments. Although the experience of ctDNA has not advanced to the stage where it can be considered an actionable routine part of clinical practice for RCC disease management, all signs point toward it becoming integrated as a complementary tool to current tissue sequencing efforts. As new technology emerges on the forefront – including integration of epigenomics or analyses of other circulating substances like exosome-derived DNA, ensuring that these assays are benchmarked and robustly tested in the RCC population remains crucial. Studies such as these can propel the use of these innovative tools and usher in a new era of precision testing for patients with kidney cancers.

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In the Next Issue of *Kidney Cancer Journal*

- Highlights and Analyses from the 2020 Scientific Sessions of the American Society of Clinical Oncology
- Targeted Therapy Following Immuno-oncology Approaches in RCCa: Challenges and Guidelines

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EDITOR'S MEMO

(continued from inside front cover)

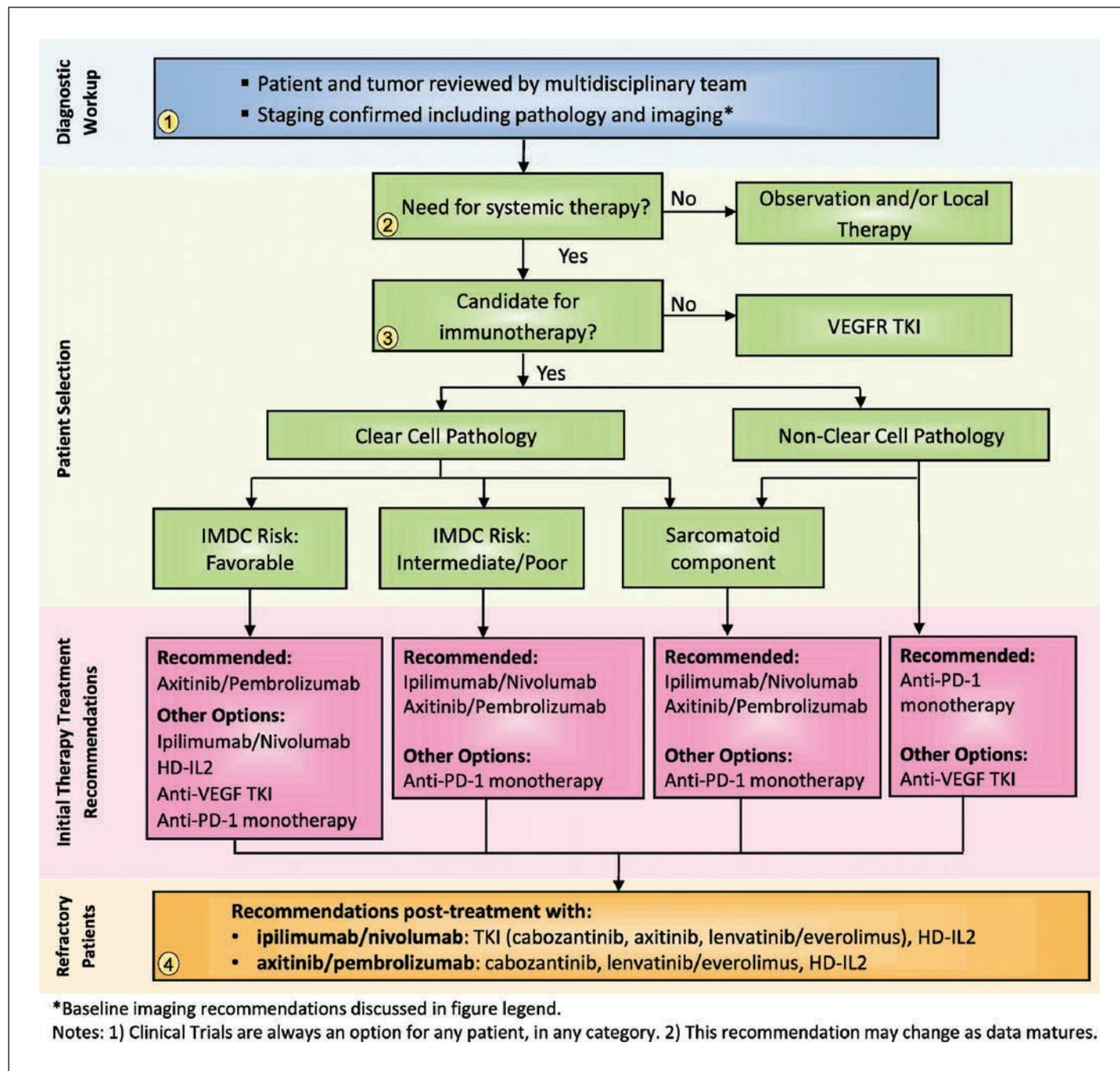
there. The full set of recommendations set a new benchmark for guidelines in this setting. As is the case with all Consensus Statements, an update will follow eventually, but for now, this is the gold standard.

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Robert A. Figlin, MD
Editor-in-Chief

Immunotherapy treatment algorithm for patients with advanced renal cell carcinoma



Combination with Stereotactic Body Radiotherapy Offers a Promising Strategy to Overcome Resistance to Immunotherapy in Advanced Renal Cell Cancer.

Sun X, Na A, et al. *J Oncol.* 2019 Nov 28;2019:1483406. doi: 10.1155/2019/1483406. eCollection 2019.

Summary: Among various attempts at overcoming resistance to immunotherapy, stereotactic body radiotherapy (SBRT) has been found to potentiate the activity of immunotherapy agents through several potential mechanisms, including normalization of microvessels to alleviate tumor hypoxia, improvement in efficient delivery of drugs, abundant neoantigen exposure, and recruitment of antitumor immune cells to alter the immunosuppressive tumor microenvironment. Preclinical studies and clinical case reports have predicted that the combination of SBRT, an immunotherapy, may lead to remarkable results.

Conclusion: This review aims to provide the biological basis for the feasibility of combining SBRT to overcome immunotherapy resistance and to review the currently available clinical evidence of this combination therapy in patients with advanced RCC.

Transcriptomic signatures related to the obesity paradox in patients with clear cell renal cell carcinoma: a cohort study.

Sanchez A, Furberg H, Kuo F, et al. *Lancet Oncol.* 2019 Dec 20. pii: S1470-2045(19)30797-1. doi: 10.1016/S1470-2045(19)30797-1. [Epub ahead of print]

Summary: Obesity is associated with an increased risk of developing clear cell renal cell carcinoma (RCC) but, paradoxically, obesity is also associated with improved oncological outcomes in this cancer. Because the biological mechanisms underlying this paradoxical association are poorly understood, this study identified transcriptomic differences in primary tumour and peritumoral adipose tissue between obese patients and those at a normal weight. This cohort study assessed data from five independent clinical cohorts of patients with clear cell RCC aged 18 years and older. Overweight patients were excluded from each cohort for our analysis. The study assessed patients from the COMPARZ phase 3 clinical trial, a cohort from the Cancer Genome Atlas (TCGA), and a Memorial Sloan Kettering (MSK) observational immunotherapy cohort. We assessed overall survival in obese patients (those with a body-mass index [BMI] ≥ 30 kg/m²) and in patients with a normal weight (BMI 18.5-24.9 kg/m², as per WHO's BMI categories), defined as the time from treatment initiation (in the COMPARZ and MSK immunotherapy cohorts) or surgery (in the TCGA cohort) to the date of any-cause death or of censoring on the day of the last follow-up. We also evaluated and validated transcriptomic differences in the primary tumors of obese patients compared with those of a normal weight. The final cohort for overall survival analysis comprised 129 (64%) participants. Overall survival was longer in obese patients than in those with normal weight in the TCGA cohort, after adjustment for stage or

grade (adjusted HR 0.41, 95% CI 0.22-0.75), and in the COMPARZ clinical trial after adjustment for International Metastatic RCC Database (IMDC) risk score (0.68, 0.48-0.96). In the MSK immunotherapy cohort, the inverse association of BMI with mortality (HR 0.54, 95% CI 0.31-0.95) was not significant after adjustment for IMDC risk score (adjusted HR 0.72, 95% CI 0.40-1.30). Tumors of obese patients showed higher angiogenic scores on gene-set enrichment analysis-derived hallmark gene set angiogenesis signatures than did those of patients at a normal weight, but the degree of immune cell infiltration did not differ by BMI. The study found increased peritumoral adipose tissue inflammation in obese patients relative to those at a normal weight, especially in peritumoral fat near the tumour.

Conclusion: The study found that aspects of the tumor microenvironment vary by BMI in the tumor and peritumoral adipose tissue, which might contribute to the apparent survival advantage in obese patients with clear cell RCC compared with patients at a normal weight. The complex interplay between the clear cell RCC tumor and peritumoral adipose tissue microenvironment might have clinical relevance and warrants further investigation.

The effect of antibiotics on clinical outcomes in immune-checkpoint blockade: a systematic review and meta-analysis of observational studies.

Wilson BE, Routy B, Nagrial A, et al. *Cancer Immunol Immunother.* 2019 Dec 21. doi: 10.1007/s00262-019-02453-2. [Epub ahead of print]

Summary: Pre-clinical and early clinical data suggests the microbiome plays an important role in oncogenesis and influences response to immune checkpoint blockade (ICB). The objective of this systematic review and meta-analysis was to determine whether antibiotics affect overall survival (OS) and progression free survival (PFS) in patients with solid malignancies treated with ICB. A systematic search of EMBASE, MEDLINE and conference proceedings was conducted for observational studies examining the effect of antibiotics on ICB. A random effects study-level meta-analysis was performed with pooling of the hazards ratio (HR) for OS and PFS. Meta-regression was used to determine the impact of the timing of antibiotic exposure on OS. 766 studies were identified, and 18 studies met the inclusion criteria. Of the 2889 patients included, 826 (28.6%) were exposed to antibiotics. The most common malignancies were lung (59%), renal cell carcinoma (RCC) or urothelial carcinoma (16.3%) and melanoma (18.7%). OS was prolonged in those without antibiotic exposure (pooled HR 1.92, 95% CI 1.37-2.68, $p < 0.001$). The effect of antibiotics on OS was greater in studies defining antibiotic exposure as 42 days prior to initiation of ICB (HR 3.43, 95% CI 2.29-5.14, $p < 0.0001$). PFS was also longer in patients who did not receive antibiotics (pooled HR 1.65, 95% CI 1.3-2.1, $p < 0.0001$).

CONCLUSION: In patients receiving ICB, OS and PFS are longer in patients who are not exposed to antibiotics. Antibiotic use in the 42 days before starting ICB appears to be most detrimental to outcome. **KCJ**



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