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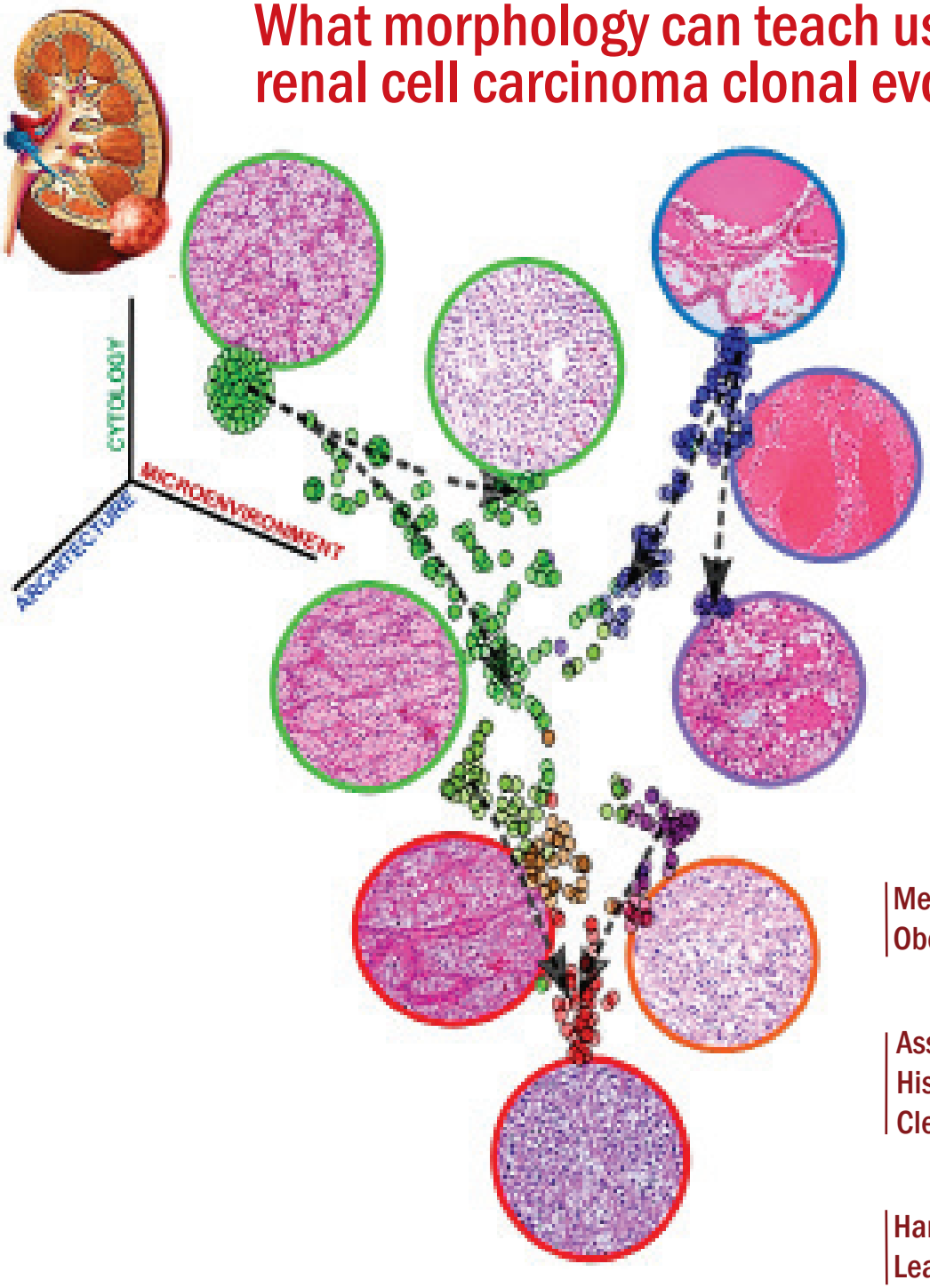
What morphology can teach us about renal cell carcinoma clonal evolution



Mechanistic Insights into the Obesity Paradox and Implications

Assessment of Intratumoral Histologic Heterogeneity in Clear Cell Renal Cell Carcinoma

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ABOUT THE COVER
Integrated ontological evaluation according to three fundamental axes (tumor cytology, architecture and the microenvironment) identifies distinct trajectories of morphological evolution in clear cell renal cell carcinoma and sheds light on the therapeutic vulnerabilities of different morphologic subtypes.

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KCJ Editor's Memo

ASCO20 Takeaways And Exploring New Horizons In Precision Oncology


Robert A Figlin, MD

For the first time in its 56-year history, the ASCO Annual Scientific Meeting was held entirely virtually due to the global outbreak of the COVID-19 that swept the world. Despite the odds, the 3-day scientific program was broadcasted to a record-breaking audience of more than 42,700 attendees from 130 countries. Moreover, the content of the conference has been viewed more than 2.5 million times as of June 4, 2020. During the conference sessions, attendees were able to view 5300 abstracts and more than a hundred on-demand and broadcast sessions and over 2300 poster and oral presentations throughout the weekend. "Although the pandemic prevented us from gathering in Chicago, it didn't stop us from fulfilling our mission of sharing knowledge to accelerate progress for millions of people worldwide living with cancer" said Howard A. Burris III, MD, FACP, FASCO, the president of ASCO. This is clearly evident in the way attendees networked with their peers via chat and one-on-one video calls and social media. "#ASCO20", the official meeting hashtag, was used in more than 45,000 tweets from more than 8,800 Twitter users and this meeting generated more than three-quarters of a billion engagements including likes, shares, and comments on social media. This year's scientific sessions highlighted a remarkable progress and the tremendous efforts underway to capitalize the full potential of immunotherapy and targeted therapy, find the best treatment settings and combinations and match them to the right patients. In a way, this ASCO20 meeting was groundbreaking as it overcame the pandemic barrier and brought together a record-breaking number of clinicians from across the globe to fulfill the mission of sharing knowledge to accelerate progress against cancer.

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KCJ Editorial Advisory

KCJ Author Guidelines

Scope of Manuscripts

Kidney Cancer Journal considers the following types of manuscripts for publication:

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

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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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Manuscripts will be peer reviewed. Accepted manuscripts will be edited for clarity, spelling, punctuation, grammar, and consistency with American Medical Association (AMA) style. Authors whose manuscripts are not initially accepted may have the opportunity to revise the manuscript based on recommendations from peer reviewers and at the discretion of the Editor-in-Chief.

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Length: Full-length manuscripts should not exceed 4000 words, including references. Please limit the reference list to 50 citations. Manuscripts should be accompanied by figures and/or tables. Generally 4-5 figures and 2-3 tables are preferred for each manuscript. Please include a brief description to accompany these items, as well as a legend for all abbreviations. Manuscripts should not contain an abstract but an introduction is recommended.

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

References

All submissions should have references that are referred to in the text by superscripted numbers and that conform to AMA style. 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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What morphology can teach us about renal cell carcinoma clonal evolution

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ABSTRACT

While cancer is a clonal process, cumulative evidence suggest that tumors are rather heterogenous and are composed of multiple genetically-distinct subclones that arise at different times and either persist and co-exist, expand and evolve, or are eliminated. A paradigm of tumor heterogeneity is renal cell carcinoma (RCC). By exploiting morphological traits and building upon a framework around three axes (architecture, cytology and the microenvironment), we review recent advances in our understanding of RCC evolution leading to an integrated molecular genetic and morphologic evolutionary model with both prognostic and therapeutic implications. The ability to predict cancer evolution may have profound implications for clinical care and is central to oncology.

KEYWORDS: Kidney cancer • ccRCC • genomics • intratumoral heterogeneity • ontology • tumor evolution • morphology

INTRODUCTION

Metastasis is a complex process wherein tumor cells evade immune surveillance, dislodge from primary tumors, invade a vascular/lymphatic conduit, and disseminate to secondary sites to form new masses. Each step involves a selection process between the tumor cells and the host. Fundamental to this process (and not much dissimilar to evolution across species), is the plasticity of tumor cells and their diversity, which increases their fitness. Multiregion genomic sampling analyses have shown that tumors evolve from an ancient clone that over time evolves into multiple genetically-distinct subclones and acquire metastatic competency. Dissemination to metastatic sites may be an early or late event. This intra-tumoral heterogeneity poses a significant problem for cancer management. Focusing on renal cell carcinoma (RCC), the prototypical model of tumor heterogeneity, we discuss its implications.

RCC of clear cell type: not a single disease

Clear cell RCC (ccRCC) accounts for over 70% of all RCC. Like many cancers, ccRCC has significant variability. Small ccRCCs (<4 cm in size) exhibit a relatively indolent behavior, however, a small subset of these tumors are aggressive[1]. Surgery is the treatment of choice for localized or locoregional disease (stage I-III). Up to 25% of patients with apparently localized ccRCC relapse after surgery[2]. Fifteen percent of patients present with metastatic disease, and up until recently, metastatic RCC was largely incurable. The 5-year survival rate for patients with metastatic RCC is ~10%, though more durable responses have been recently observed in patients receiving combination immunotherapy[3-5].

ccRCC can spread through both lymphatics and hematogenously, and is remarkably predisposed to intravascular growth. It can colonize at a wide range of secondary distant sites. Intriguingly, the site of metastasis may confer variable prognosis; liver metastasis is associated with a worse prognosis, while pancreatic metastasis has good prognosis[6-9]. Additionally, the metastatic course may be variable, both in terms of spatial and temporal patterns. Metastases may be solitary or oligometastatic with long latency periods or associated with rapid multiorgan dissemination. Isolated metastases can be managed with focal therapies (surgery, ablation or stereotactic radiation) or by active surveillance[10,11].

Disseminated metastatic ccRCC requires systemic therapy including inhibitors of the vascular endothelial growth factor (VEGF)/ VEGF receptor (VEGF/R-I) and mammalian target of rapamycin (mTOR) inhibitors. These have variable responses and are largely palliative[2]. More recently, immune checkpoint inhibitors (ICIs) of the PD-1 and CTLA-4 pathways have been approved by the FDA[4,5]. In 2020, frontline systemic treatment is defined by immune checkpoint inhibitor (ICI) combination therapies such as ICI doublets (ipilimumab and nivolumab) or ICI with targeted therapies (pembrolizumab or avelumab with axitinib)[4,12,13]. Even with these combinations, over 50% of patients experience progressive disease. Multiple other therapeutic options are

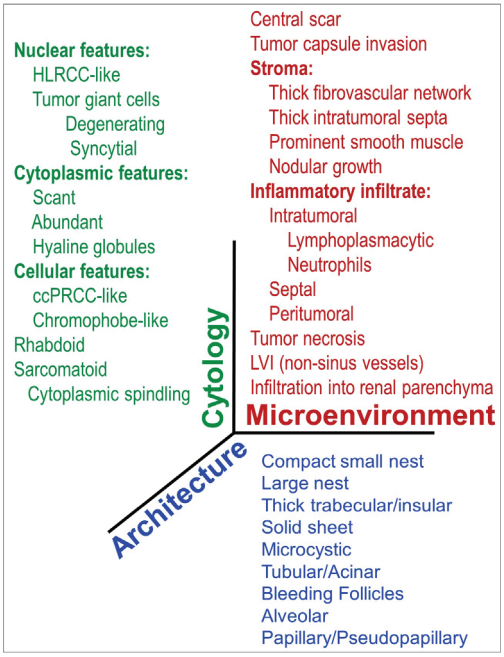

Fig. 1 | A multiscale framework to dissect ccRCC heterogeneity according to three fundamental axes: tumor cytology, architecture and the microenvironment. 33 different parameters were evaluated in 549 ccRCCs (Cai *et al.*, EBioMedicine 2020).

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Conflicts of interest disclosures: None.

	Pattern present (%)		
	≤4 cm (n = 250)	>4 cm (n = 299)	p
Microcystic	110 (44.0%)	77 (25.8%)	<0.0001
Tubular/Acinar	82 (32.8%)	78 (26.1%)	0.085
Bleeding Follicles	87 (34.8%)	59 (19.7%)	<0.0001
Compact Small Nests	191 (76.4%)	217 (72.6%)	0.31
Large Nests	122 (48.8%)	195 (65.2%)	0.0001
Alveolar	48 (19.2%)	98 (32.8%)	0.0003
Papillary/ Pseudopapillary	16 (6.4%)	38 (12.7%)	0.013
Thick Trabecular/ Insular	37 (14.8%)	131 (43.8%)	<0.0001
Solid Sheet	27 (10.8%)	137 (45.8%)	<0.0001
Median present (interquartile range)	3 (2-4)	4 (2-5)	<0.0001

Table 1 | Clear cell renal cell carcinoma tumor size and frequency of architectural features (as patterns co-occur, the composite frequencies exceed 100%).

being investigated ranging from targeted therapies such as HIF-2 inhibitors, inhibitors of metabolism (e.g. glutaminase inhibitors), and a plethora of immunotherapies.

Despite substantial progress and a broad spectrum of systemic therapies, precision medicine and biomarker development have lagged behind. The success and optimal utilization of treatment options will depend on our understanding of tumor biology as well as intra-tumoral heterogeneity (ITH). In summary, the wide clinical course of ccRCC patients may reflect the underlying intra-tumor and inter-patient heterogeneity. Precision medicine will benefit from better understanding of the tumor evolution and ITH.

Clear cell RCC, a prototype of genomic intra-tumoral heterogeneity

The wide spectrum of clinical behavior and variable response to therapy is mirrored at the molecular level by inter- and intra-tumoral heterogeneity[14-16]. Molecularly, ccRCC is quite intriguing, as genes that are frequently mutated in other common tumor types are rarely mutated in ccRCC[17]. Converesely, frequently mutated genes in ccRCC are rarely mutated in other tumors. Sporadic ccRCC is characterized by *VHL* mutation (or methylation) in >80% of tumors, and loss of heterozygosity of 3p (where *VHL* resides) in ~90% of cases[18,19].

ccRCC has long been considered the archetype for genomic ITH starting with the seminal publication by Gerlinger and colleagues demonstrating the value of multi-region sequencing in understanding cancer evolution[20]. More recently, multiregional sequencing experiments have been performed on a much larger scale, including 1,206 spatially-distinct primary tumor areas from 101 RCC patients profiled by the TRAcking Cancer Evolution through therapy (Rx) (TRACERx) consortium. These studies have provided unprecedented detail on ITH and showed that as tumor cells proliferate, spatial and temporal subclones with different mutations and somatic copy number alterations (SCNA) evolve and co-exist in different areas. Other than *VHL* and *PBRM1,* driver mutations *in SETD2, BAP1, KDM5C, MTOR, PIK3CA, PTEN, p53,* and *KDM6A* are frequently found at a subclonal level[16,18]. Based on patterns of tumor evolution and inferred mutation timing, ~60% of ccRCC (64/101) can be grouped into seven evolutionary subtypes[16]. "VHL monodriver" tumors are characterized by low grade, low stage, indolent behavior and minimum ITH. In contrast, tumors that are characterized by high grade and rapid progression to metastases include 3 subtypes: "BAP1 driven," "VHL wild-type," and "multiple clonal drivers." The "BAP1 driven" evolutionary subtype is characterized by truncal *VHL* and *BAP1* mutations; and no *VHL* alteration was detected in the "VHL wild-type" evolutionary subtype. The "multiple clonal drivers" exhibited truncal aberrations in two or more of the following genes: *BAP1, PBRM1, SETD2,* or *PTEN*. Tumors with intermediate aggressiveness were characterized by low-intermediate grade, increased ITH, parallel evolution, and attenuated disease progression. Three PBRM1-driven subtypes were noted in this group, which had sequential loss of *PBRM1* followed by loss of *SETD2*, activation of PI3K/ AKT/ mTOR signaling pathway, or specific SCNAs.

These results support findings from a prior single sample sequencing study that led us to propose an integrated molecular genetics and pathological classification of sporadic ccRCC[15]. We discovered that mutations in *BAP1* tend to anticorrelate with mutations in *PBRM1*, while *PBRM1* and *SETD2* often co-occur[15,21,22]. *BAP1*-mutant tumors tend to be of high grade and are associated with worse survival (HR, 2.7; p=0.044), compared to *PBRM1*-mutant tumors that are typically of low grade and associated with better outcomes although when *SETD2* is also mutated, *PBRM1*-mutant tumors are more aggressive[15,21-25].

In the TRACERx study, decreasing ITH correlated with an aggressive disease course, suggesting that a competent, aggressive clone may outgrow co-existing clones. Reduced ITH of these tumors may make them potentially

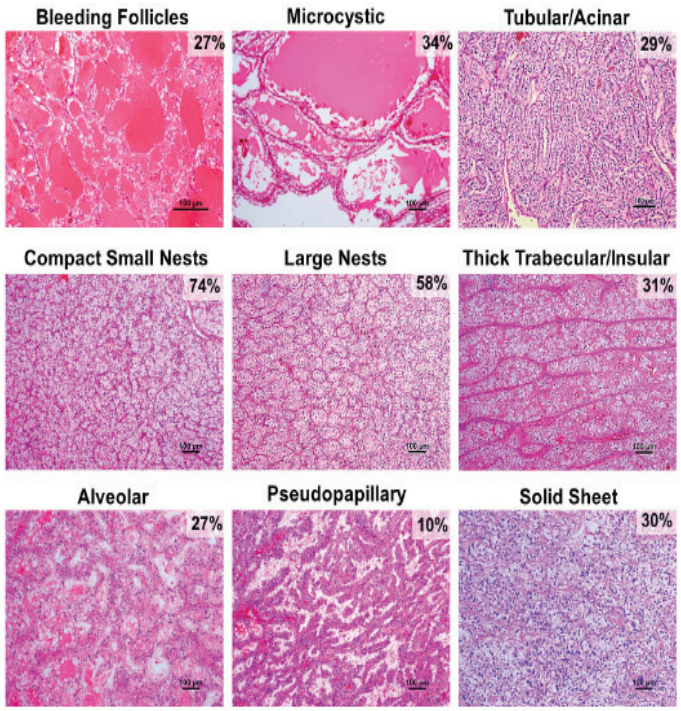


Fig.2 | Architectural patterns of ccRCC. Nine architectural patterns with representative H&E-stained images and their frequency in ccRCC. As patterns co-occur in tumors, the composite frequencies exceed 100% (For more details, Cai *et al*., EBioMedicine 2020).

more vulnerable to therapy. On the other hand, tumors with high degree of ITH comprising diverse subpopulations of cancer cells, may be more likely to have a mixed response to therapy, unless a truncal event is targeted.

Capturing the full degree of ITH is challenging. It has been proposed that at least 7 biopsies are required to detect over 75% of variants[16]. An alternative might be representative sequencing (Rep-Seq). By homogenizing larger quantities of tumor material, Rep-Seq may accurately deconvolute clonal structures[26]. However, these tools are subject to sampling bias and are expensive. Nevertheless, what is clear is that accurate prognostic and predictive models will likely need to address ITH to faithfully predict tumor behavior.

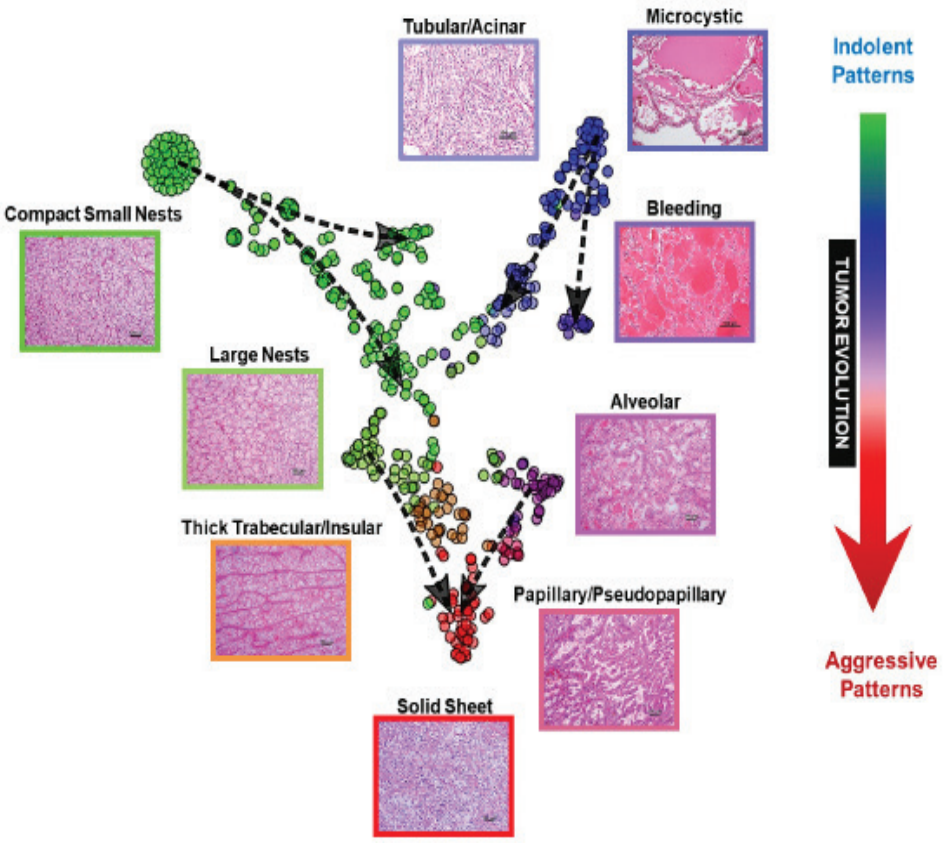


Fig. 3 | Map of ccRCC architectures and evolutionary model. t-distributed stochastic neighbor embedding (t-SNE) map of primary ccRCC tumors based on architectural composition with superimposed vectors using pure patterns as anchors and overlaying tumor size and grade for directionality (dashed arrows).

Morphologic intra-tumoral heterogeneity

The ITH so elegantly highlighted with molecular tools has been appreciated for decades by pathologists[27-29]. Indeed, categorization of new entities often starts with recognition of different morphologic patterns and these entities are subsequently determined to have divergent genomics, biology and behavior (e.g. clear cell papillary RCC). In addition, histopathologic analyses are the current standard of care for routine diagnosis and prognosis of RCC (and cancer in general). Currently ccRCC grading is largely based on morphologic assessment of nucleolar size. More recently, differentiation patterns like sarcomatoid and rhabdoid have been shown to impact prognosis and may even be predictive. This implies that morphology can capture tumor aggressiveness. However, multiregional molecular genetic studies have largely been performed agnostic to morphology. A contributing factor may be that molecular genetic analyses are optimally performed from frozen tissues while morphology is routinely assessed on formalin fixed tissue.

Morphology, a phenotypic reflection of the underlying genome and transcriptome, not only allows for interrogating molecular data at a phenotypic level but also provides clues about tumor–environment interactions. The tumor interaction with its microenvironment is important, and it is noteworthy that most FDA-approved therapies today do not target cancer cell themselves, but rather target host cells either in the vasculature or of the immune system. In addition, morphology may be useful proxy to evaluate the effects of genomic alterations and may shed light on genomic evolutionary subtypes and their therapeutic vulnerabilities. However, up until recently, the significance of these different morphological phenotypes has remained unexplored.

Recently, our multidisciplinary team sought to systematically dissect and comprehensively explore the implications of ccRCC phenotypes. Our goals were: (1) to devise a framework to deconvolute the phenotypic complexity of ccRCC; (2) to establish an ontological classification capturing the breadth of ccRCC phenotypes; (3) to explore the biological and clinical implications of subtypes identified; (4) to develop an evolutionary model of tissue phenotypes; and (5) to expand the approach by objectivizing it using digital pathology[30]. Unlike prior morphologically-based cataloguing efforts[29], we did not focus on high-grade areas alone, but sought instead to evaluate representative areas of the entire tumor so as to infer the phenotypic evolutionary process.

We developed a multiscale atlas based on three fundamental axes: tumor cytology, architecture and the microenvironment (Figure 1). We defined 9 distinct architectural and 24 unique cytologic and tumor microenvironment (TME) features (Figure 2). We systematically applied this framework assessing the frequency of these individual features across 549 ccRCCs. The analysis was carried out by two pathologists independently. While they both trained at the same institution, the concordance rate was 95%[30]. This study sets a paradigm for de-convoluting phenotypic complexity and established a comprehensive morphologic ontology of ccRCC.

Widespread intra-tumoral architectural heterogeneity

Similar to genomic studies, a large number of spatially distinct morphologic architectural phenotypes/ patterns are observed in ccRCCs. More than 85% (476/549) of the tumors in our study had multiple patterns with an average of 3 architectures per tumor (*std. dev.*, 1.4; *range*, 1-7 patterns/tumor)[30]. In addition, widespread variability in the tumor microenvironment and cytologic heterogeneity was also observed. At times, different cytologic features were observed within the same architectural pattern. Conversely, similar cytologic features were observed across different architectural patterns.

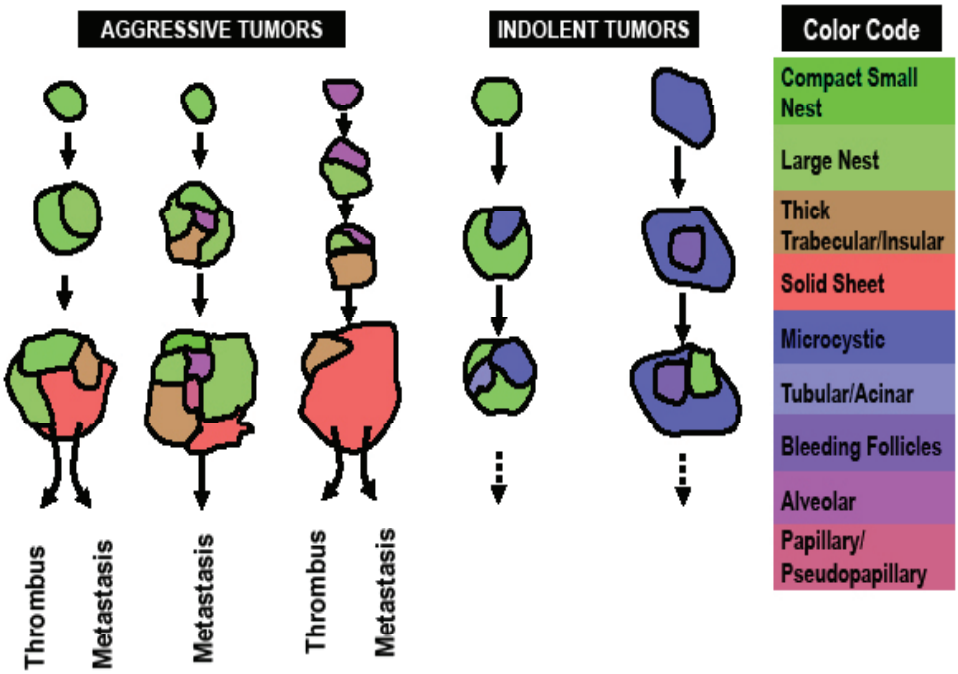


Fig. 4 | Architectural evolutionary model. Color-coded cartoon to show the broad directionality of the architectural evolution likely influencing future tumor behavior.

Smaller tumors were generally characterized by reduced architectural diversity than larger ones ($p<0.0001$), suggesting that morphological heterogeneity reflected tumor plasticity over time. The most common architecture in small tumors was compact small nests. In contrast, large tumors where characterized by thick trabecular/insular and solid sheet patterns (Table 1). Tumors with high morphological ITH were associated with features of aggressive biology including high nucleolar grade, high TNM stage, and metastasis (all $p<0.0001$). Overall, these patterns of morphologic ITH show similar trends as those of genetic ITH reported with multiregional sequencing[16].

Phenotypic patterns illustrate tumor aggressiveness and predict patient outcomes

To determine the clinical significance of each unique phenotype, we sought to quantify their relationship with known clinical parameters. In doing so, we wanted to go beyond the traditional paradigm of ccRCC behavior being predicted solely by its most aggressive area/subclone and explore how individual components contributed to the outcome. This required a comprehensive pattern assessment within a tumor. Chi-square analyses of individual patterns with clinical parameters revealed a subset of architectural, cytologic, and TME features (irrespective of their extent or the presence of other features) that were informative of tumor aggressiveness and were significantly associated with high nucleolar grade, TNM stage, and metastases[30]. Architectures could be broadly categorized into indolent (microcystic, tubular/acinar, bleeding follicle, and compact small nests) and aggressive (alveolar, papillary/ pseudopapillary, thick trabecular/insular, and solid sheet). Certain features such as infiltration into the renal parenchyma, a feature not currently considered for RCC staging, was prognostic even if found in isolation[30]. Morphologic patterns correlated with tumor grade, and subgroup analyses showed that while aggressive patterns were observed more frequently in tumors of higher grade/ stage, grade alone underestimated the aggressiveness of some low-grade ccRCCs. After controlling for conventional variables such as nucleolar grade and sarcomatoid/ rhabdoid patterns in multivariate analysis, the presence of a subset of features (tubular/acinar pattern, chromophobe RCC (ChRCC)-like pattern, infiltration into the renal parenchyma, and necrosis) was predictive of disease-free survival (DFS)[30]. These data suggest that architecture, cytologic and TME features may help refine current prognostic algorithms.

Leveraging spatial relationships of architectural patterns to identify recurrent evolutionary trajectories

One advantage of morphological analyses over random sampling genomic studies is visualization of the spatial relationship of the different architectures within a tumor. Clonal evolution is thought to result from the interplay of random mutations (and possibly epigenetic changes) and non-random selection. In spite of the presumed random nature, lineage constraints and the microenvironment may influence evolutionary trajectories. Accordingly, conditioned by the presence of a specific mutation or characteristic microenvironment, additional mutations may vary greatly in their fitness impact and thus their likelihood of fixation. This may explain, for example, the low frequency of tumors with simultaneous mutations in *BAP1* and *PBRM1*.

Like the TRACERx studies, our results represent a snapshot of a single time point in the evolutionary history of a tumor. To infer tumor evolution, we made the following assumptions. First, larger tumors arise from smaller tumors and their patterns likely emerge from those present in smaller tumors. Second, higher grade tumors are unlikely to evolve into lower grade tumors, but lower grade tumors may evolve to higher grade. Third, co-existing architectures likely evolve from one another or a common precursor. Fourth, while analyses of particular tumors provide snapshots, trends in the evolutionary process may be inferred from the collective analysis of tumors in (presumably) different stages of evolution.

The most common architectural pattern was compact small nests, prevalent in ~75% of the tumors in varying amounts. The compact small nest pattern was consistently associated with low nuclear grade, and was the only pattern seen in some small ccRCCs (of the 73 tumors with a single pattern, 78% were comprised solely of compact small nests), suggesting that it may be a truncal pattern. In a subset of ccRCCs, spatially separate subclones with more aggressive architectures were present sometimes giving the appearance of an overgrowth pushing the compact small nest pattern to the periphery. The more aggressive architectural patterns were often spatially adjacent to the more indolent patterns, suggesting that they arose from the more indolent pattern. The transition to distinct subclones was either gradual with progressive changes in nuclear features, or abrupt with strikingly higher-grade nuclei and distinctly different architectural phenotypes. In a subset of single-pattern tumors (8%; 6/73) a more aggressive pattern made

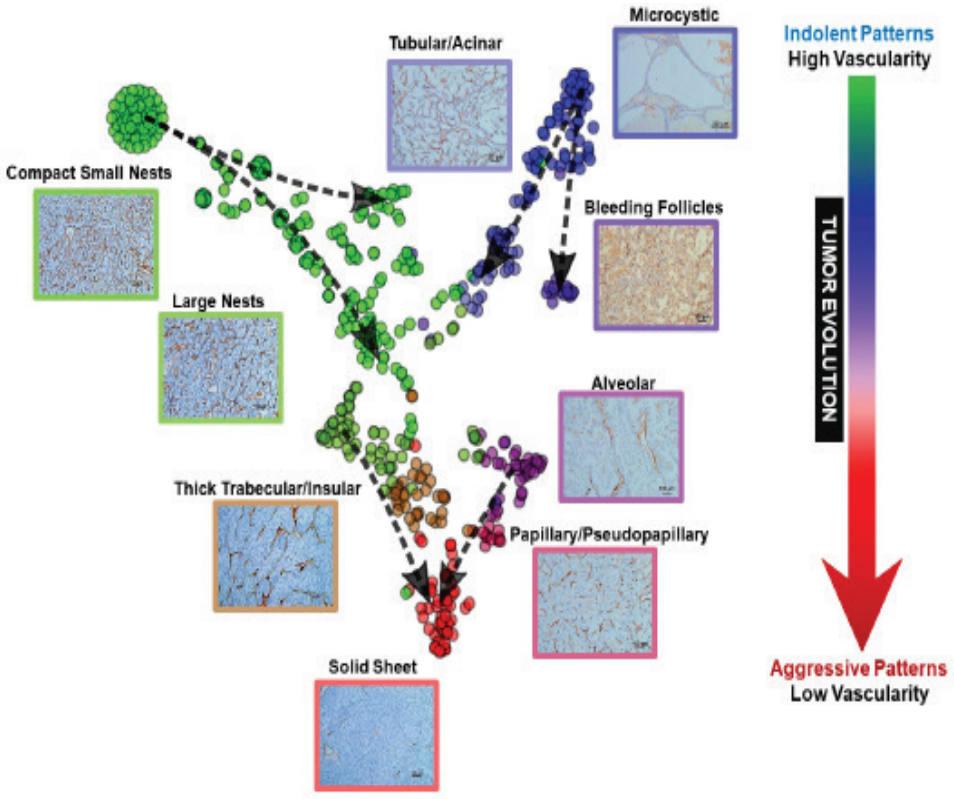


Fig. 5 | Vascular framework as highlighted by CD31 immunohistochemical labeling in ccRCC architectures and evolutionary model. Representative CD31 IHCs from primary ccRCC tumors based on architectural pattern. The vascular network becomes more spaced out as the tumor evolves from indolent to more advanced patterns.

up the entire tumor and these tumors were uniformly large (mean size, 9.5 cm) and of high grade.

The compact small nest pattern is the prototype of ccRCC. In routine clinical practice, the presence of compact small nests, even when restricted to a small area, is used to characterize RCC as ccRCC. This pattern closely resembles the low-grade tumors observed in genetically engineered mouse models with loss of *Vhl* and *Pbrm1*[31-33]. Consistent with this notion, human ccRCCs with compact small nests show diffuse membranous carbonic anhydrase (CAIX) expression, a downstream effect of VHL loss, and a large subset show PBRM1 loss by immunohistochemistry (IHC).

To dissect the interplay between patterns, we undertook a mathematical approach. We reasoned that if two architectures co-occurred in tumors more frequently than expected based on their individual frequencies, the process was non-stochastic. We quantitated co-occurrence based on conditional probabilities and developed a co-occurrence matrix. We applied this approach not just to architectures, but also to cytological and stromal features. This analysis revealed significant preferential associations. For example, tumors with solid sheet patterns also contained thick trabecular/insular, large nests and compact small nests patterns. Interestingly, intra-tumoral lymphocytic infiltrates were most frequently associated with sarcomatoid and rhabdoid features, which may contribute to the better response of sarcomatoid tumors to ICI therapies[34].

The co-occurrence (or exclusion) of particular patterns likely reflects evolutionary constraints. In order to further dissect the process, we generated t-distributed stochastic neighbor embedding (t-SNE) maps[35]. t-SNE is a machine learning algorithm that reduces high-dimensional data to two dimensions where the distance between objects is proportional to their similarity (e.g. similar objects cluster together). We applied t-SNE to ccRCC architectures. As expected, t-SNE identified several clusters as well as transitions between the clusters. To infer evolution, we superimposed tumor size as well as nuclear grade onto the t-SNE map. We used tumors with pure architectures as anchors and added vectors from small to large and from low to high grade (Figure 3). This revealed a "V" shaped evolutionary process consistent with a model where a majority of tumors start as either compact small nests or microcystic/cystic and progress to more advanced patterns converging into a solid sheet.

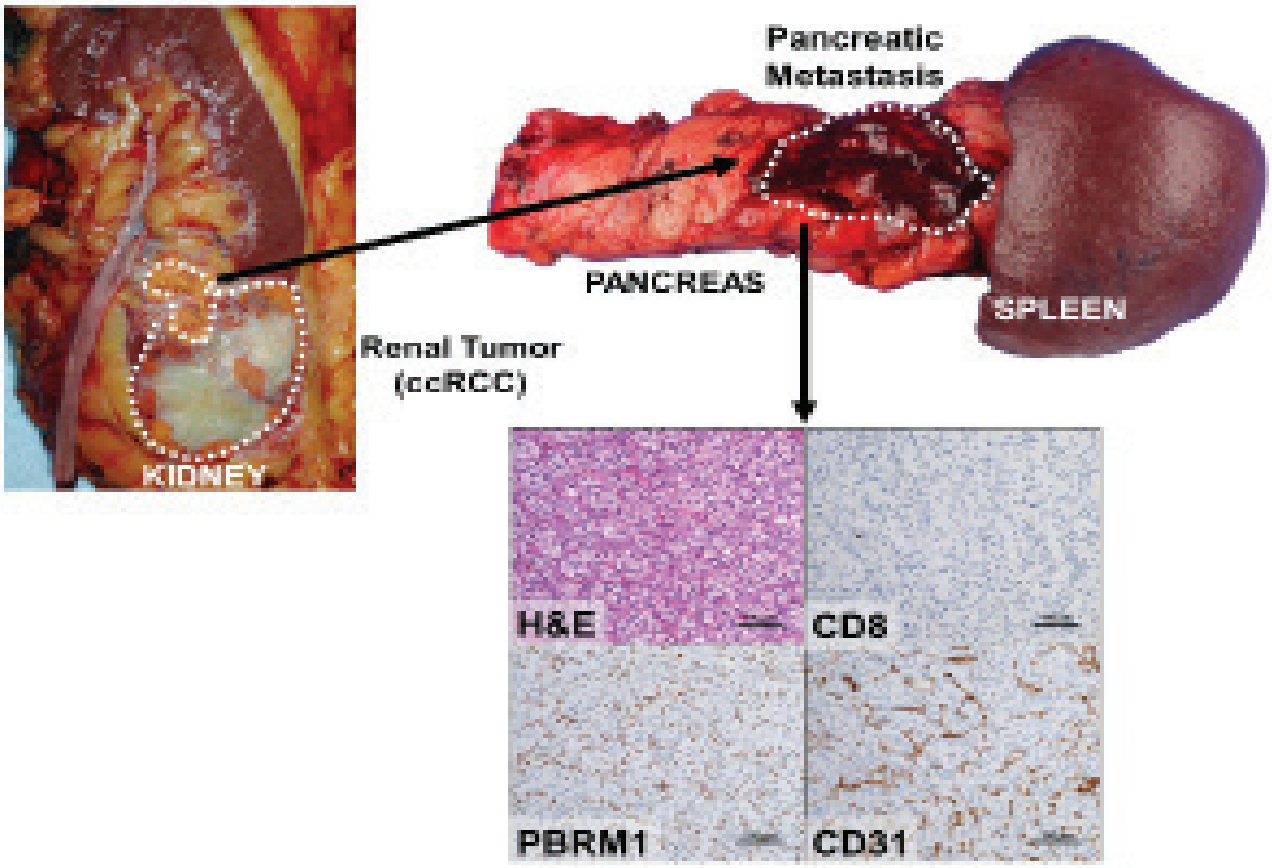
We sought opportunities to 'test' the model. We reasoned that we may use tumor outgrowth into the vasculature, where one can infer directionality, as a model. Thrombus formation occurs later in the evolutionary trajectory of ccRCC, after a primary tumor has developed, and offers an opportunity to infer the direction of growth. We compared the architectural compositions of primary tumors and matched thrombi. Consistent with the t-SNE plot trajectories, we found that in every case, the architectural patterns observed in the thrombi, which were often more aggressive, were already present in the matched primary sample[30]. Interestingly, in some cases, the advanced architectural pattern in the thrombus was only a minority pattern in the kidney tumor. Indolent patterns such as microcystic and bleeding follicles were infrequently found in the thrombus, even when they comprised a significant proportion of the kidney tumor. Overall, we found that the more advanced architectural patterns in the kidney tumor often seeded the tumor thrombus providing additional support to our evolutionary model (Figure 4).

Another context where we thought we may test our evolutionary model was provided by our tumorgraft (or patient-derived xenograft) program. Over the course of a decade, the UT Southwestern Kidney Cancer Program/SPORE has transplanted tumors from over 900 patients orthotopically in mice[36,37]. The process of engraftment in mice recapitulates to some extent the process of metastases[38], as engraftment requires small tissue samples to be able to survive and grow at a site distant from the primary tumor (in fact, in a different host), and only ~15% of tumors stably engraft. We have previously shown that samples from metastatic sites engraft at higher frequency than those from primary tumors[36]. In addition, engraftment predicts for reduced patient survival[37]. Thus, efficient engraftment in mice may serve as a surrogate for tumor aggressiveness. We asked therefore whether there were differences in engraftment rates of the different patterns. We correlated sample architectures with stable engraftment in mice and found that the odds of engraftment of aggressive patterns compared to indolent patterns were 3 (95% CI 1.31, 6.92; p = 0.0104)[30]. Thus, engraftment efficiency, a measure of tumor aggressiveness, was higher in our more advanced patterns (Figure 4).

Overall, these data in tumor thrombi and tumorgrafts are consistent with a model where more aggressive morphologically appearing subclones have greater fitness and arise from lower fitness patterns.

Vascularity is predictive of response to anti-angiogenic therapy

Beyond analyses of tumor evolution, we explored whether there may be an association between morphological patterns and drug sensitivity. We observed extensive variability in vascularity across phenotypes and hypothesized that tumors devoid of a vascular network may be less likely to respond to anti-angiogenic therapies, which largely target VEGFR-2 on endothelial cells. Interestingly, vascularity was maximal in indolent architectures and



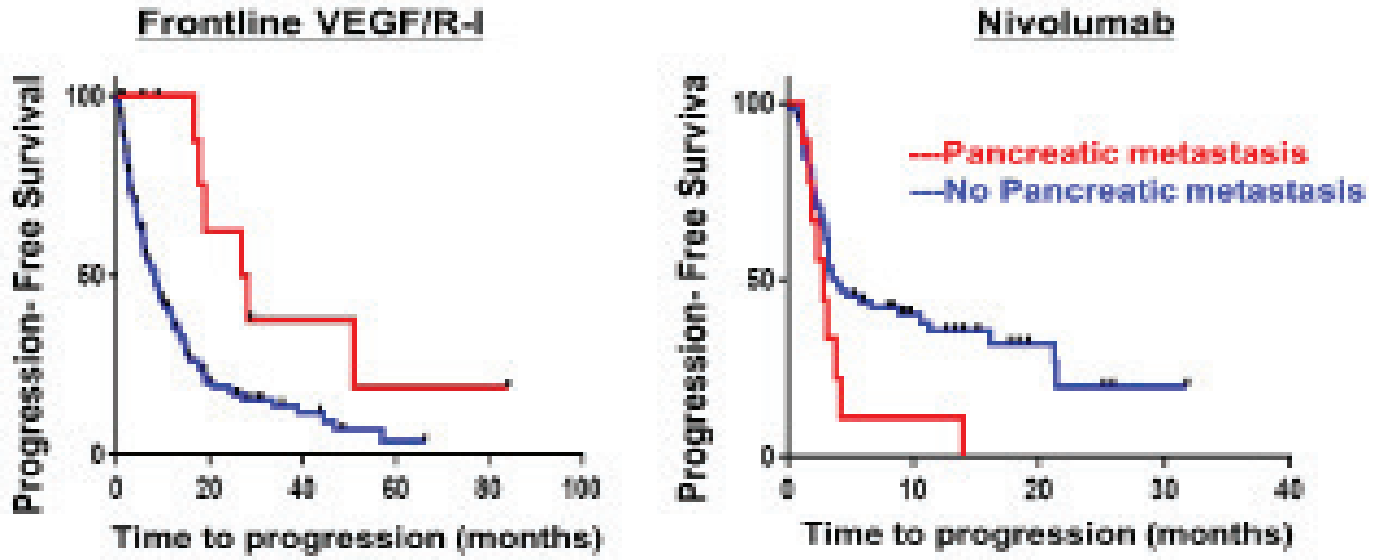


Fig. 6 | ccRCC metastatic to the pancreas is characterized by PBRM1 loss, compact small nests, a prominent vascular network, and response to VEGF inhibitors. Representative H&E and corresponding CD31, CD8 and PBRM1 IHC images from a pancreatic metastasis illustrating the typical compact small nest morphologic phenotype, increased vasculature (CD31), lack of CD8 T lymphocytes and PBRM1 loss. Kaplan Meier survival analyses for PFS in patients treated with first-line VEGF/R-I (HR 0.34 [95% CI 0.15-0.77]; p=0.007) vs. nivolumab (HR 2.15 [95% CI 1.04-4.46]; p=0.034) stratified by presence (red line) or absence (blue line) of pancreatic metastasis) (modified from Singla *et al.*, JCI Insight 2020).

most spaced out in aggressive architectures (Figure 5). Notably, the opposite was true of inflammation, which was over-represented in more aggressive architectures. These results are in line with previous empirical analyses of the tumor microenvironment, which revealed largely non-overlapping angiogenic and inflammatory signatures[39]. Importantly, these data suggest a coordinated evolutionary process of the tumor and its microenvironment.

Retrospective analyses of IMmotion150 and COMPARZ trials suggested that tumors responsive to VEGF/R-I exhibit an angiogenic gene expression signature[40-45] and we asked whether there was an association between a vascular stroma and response to VEGF/R-I in our cohort. We focused on patients with metastatic ccRCC that received VEGF/R-I in the frontline, and found that tumors composed predominantly of patterns characterized by reduced vascularity had shorter time to progression[30].

In contrast, compact small nests architectures are characterized by an extensive vascular network and might be expected to respond to VEGF/R-I. These tumors, however, are less aggressive and less frequently metastasize. Interestingly, however, we found a correlation between tumors with compact small nests and tumors with pancreatic metastases. In addition, when multiple architectures coexisted in the primary, there was an enrichment for compact small nests among pancreatic metastases[8]. We asked if there was an association of ccRCC metastatic to the pancreas and response to VEGF/R-I. We observed that patients with pancreatic metastasis benefited from VEGF/R-I to a greater extent than those without (progression free survival (PFS) HR 0.34 [95% CI 0.15-0.77]; p=0.007). Conversely, these patients were refractory to ICI therapy (HR 2.15 [95% CI 1.04-4.46]; p=0.034) (Figure 6).

Finally, given that the compact small nest architecture closely resembles the architecture of *Vhl/Pbrm1* deficient ccRCC in genetically engineered mouse models, we asked whether there was an association between the phenotype and underlying mutations. The rate of PBRM1 loss among pancreatic tumors was 84%, significantly higher than what might be expected among metastatic ccRCC[8]. Overall, these data suggest that loss of *VHL* and *PBRM1* results in a compact small nest architecture that induces an extensive vascular network from the host, which is possibly related to the activation of hypoxia-inducible factors following *VHL* inactivation, and amplified by the loss of *PBRM1*[46].

In summary, these studies provide a systematic and comprehensive ontology that captures the breath of ccRCC morphologies and their clinical significance. They utilize morphology to deconvolute tumor complexity and improve our current understanding of ccRCC pleiotropy. By analyzing patterns of spatial co-occurrence, our studies identify distinct trajectories of morphological evolution. Advanced architectures, which form terminal nodes, appear more likely to expand from the primal and more indolent architectures, and demonstrate differential response to therapy. The ability to predict cancer evolution may have profound implications for clinical care and is central to oncology. Our histopathological phenotypes and molecular studies indicate that, at least in part, tumor evolution evolves along particular paths. Despite its stochastic nature, lineage and micro-environmental constraints may allow for a limited set of predictable subsequent evolutionary trajectories. Characterizing repeated evolution in cancer would have significant implications both for understanding the biology of tumor progression, and for the ability to stratify patients in the clinic.

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Mechanistic Insights into the Obesity Paradox and Implications for Therapy

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ABSTRACT
Exciting progress has been made towards uncovering mechanisms which underlie the paradoxical impact of obesity in renal cell carcinoma, suggesting new treatment strategies in this patient population. Enhancing our understanding of the complex interactions within the tumor microenvironment may allow us to identify novel targets for therapeutic action.

KEYWORDS: • **Renal Cell Carcinoma** • **Obesity Paradox** • **Immunotherapy** •

INTRODUCTION

The obesity epidemic has unfortunately only continued to worsen in the United States and across the globe. Obesity is determined by measuring one's body mass index (BMI), weight divided by height squared. For adults, several weight classes have been defined, including normal weight (18.5 to 25 kg/m^2), overweight (>25 to <30 kg/m^2), and class I through III obesity (30 to <35 kg/m^2, 35 to <40 kg/m^2, and >40 kg/m^2, respectively). Trends suggest that approximately 1 in 2 adults will be obese in the United States in the year 2030, and severe obesity will become the most common weight class amongst women and low-income earning adults.[1] With thirteen cancers linked with obesity[2] and expenditures for obesity-related cancers accounting for an adjusted two-fold higher cost compared with non-obese cancers,[3] understanding the biological impact of obesity and leveraging these insights to improve outcomes is paramount.

Clinical Insights into the Obesity Paradox

As in other malignancies, obesity remains a significant independent risk factor for the development of renal cell carcinoma (RCC).[4-6] This risk remains weight dependent, as an incremental increase in BMI by 1 (kg/m^2) is associated with a rise in risk of RCC by 4%.[5] Uniquely, though, several independent reports have demonstrated an inverse association between BMI and mortality, termed the "*obesity paradox*" – obese patients have more favorable outcomes when compared to their non-obese counterparts. While many have argued that this trend may be due to confounding factors and analytic biases, other reports which have attempted to overcome these biases demonstrate this protective trend.[7] In the localized setting, a recent meta-analysis of >1,500 patients showed a superior overall survival (OS) for patients with clear cell RCC who were overweight and obese (HR 0.57, 95% CI 0.43-0.76).[8] Reported disease-specific patterns support this trend, as obesity has been linked to a lower risk of lymph node metastasis,[9] and other factors associated with poorer prognosis, such as renal vein invasion, have been associated in male patients with lower BMI.[10] Several independent reports confirm this protective trend in metastatic clear cell RCC,[11-15] and preliminary integration of BMI into validated risk models like the International Metastatic RCC Database Consortium (IMDC) model has yielded several insights on potentially improving predictive performance.[16] While prospective validation of tools like this is needed, the ease of computing a patient's BMI during a clinical examination presents an attractive modifier that can likely be rapidly adopted in clinical practice.

Several reports have investigated the predictive and prognostic impact of obesity in patients with advanced clear cell RCC treated with systemic agents. In the seminal work by Albiges *et al.* investigators illustrated these trends in two large cohorts of patients treated with VEGFR-targeted tyrosine kinase inhibitors (TKIs) and mTOR inhibitors in the first-line and second-line settings.[17] Patients from the curated IMDC database and a pooled validation cohort consisting of cases from prospective clinical trials were used for data analysis, totaling an impressive 4,657 patients for study. Patients were stratified based upon BMI ≥25 kg/m^2 (which includes both overweight and obese patients), and patients with high BMI were found to have a superior OS (adjusted HR = 0.84, 95% CI = 0.74-0.93).[17] Notably, this association was present in patients with IMDC intermediate- and poor-risk disease but did not reach significance in the favorable-risk population.

As obesity is characterized as a chronic, pro-inflammatory state, there has been a shift in focus to understanding the impact of BMI within the immune checkpoint inhibitor (ICI) era. McQuade *et al.* demonstrated a clinical association between BMI and ICI therapy outcomes in patients with advanced melanoma, with a superior response and survival in obese men compared with normal weight men.[18] Interestingly, this protective association was not seen in female melanoma patients. For patients with advanced RCC, a single institutional cohort of metastatic RCC patients treated with immunotherapy at Memorial Sloan Kettering Cancer Center demonstrated that obesity was associated with superior clinical outcomes, but this did not appear to be independent of IMDC risk.[19] In a multi-institutional

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cohort, RCC patients with high BMI were found to have improved progression-free survival (PFS) and superior overall survival (OS).[20] This trend remained significant only in the overweight (25-30 kg/m²) but not the obese group. Pan-cancer studies that include metastatic RCC patients highlight this across tumors treated with ICI.[21,22] As a corollary to these findings, as patients who benefit from ICI therapies are more likely to experience immune related adverse events (irAEs),[23] a meta-analysis demonstrated that obesity was associated with an increased risk for irAE development across multiple tumor types.[24]

With the introduction of VEGFR TKI and ICI combinatorial strategies, it remains to be seen whether these trends persist with the use of both VEGFR TKI and ICI therapies together. In the subgroup analysis of the randomized phase III study of axitinib plus avelumab, superiority over sunitinib was found regardless of BMI status.[25] Efforts to characterize the impact of novel targeted agents with ICI therapies in this context to identify if trends remain consistent underway.

Obesity and Renal Cell Carcinoma – Mechanistic Insights

With multifactorial metabolic and immune-regulated changes associated with obesity, it has been challenging to decipher the specific mechanisms which may underlie this paradoxical trend in patients with advanced RCC. Several questions remain — does obesity lead to change in natural immunity which impairs tumor surveillance or does obesity contribute to the evolution of a distinct tumoral phenotype? Prior research has demonstrated that obesity may activate mTOR and hypoxia-inducible factor-1 (HIF-1) signaling in immune cells,[26] highlighting overlapping pathways pertinent in clear cell RCC tumorigenesis. With more longitudinal studies focused on uncovering cellular mechanisms related to obesity, we can better link disease pathophysiology with these clinical trends.

In the search for differences in RCC tumor phenotypes in the obese setting, several investigators have looked to specific mutational or gene-expression profiles from obese and normal weight patients. RCC-specific alterations like *VHL* and *PBRM1* have been shown to occur in similar frequencies in these two groups.[27] Molecular subtyping by ClearCode34 a gene-expression profiling tool, highlights that obese and diabetic patients are more likely to harbor clear cell type A tumors characterized by angiogenesis dominant profiles.[28] Translational work using the Cancer Genome Atlas (TCGA) dataset also found that tumoral expression of fatty acid synthase (FASN), a key enzyme involved in lipogenesis and the production of long-chain fatty acids, correlates with BMI in clear cell RCC patients.[17] In that study, investigators found that patients with low FASN tumors had superior OS, and high FASN expression was found more often in patients with IMDC poor-risk versus favorable-risk disease.[17]

Obesity's effect on immune dysregulation has been well established and exploring these downstream consequences of obesity, particularly in RCC, has led to several interesting observations. In preclinical models of diet-induced obese (DIO) mice, widespread changes in cytokines and chemokines were seen in tumor-bearing mice compared to controls,[29] and dendritic-cell (DC)-based immunotherapy was associated with faster tumor growth in obese mice. Therapeutic failure in this context was proposed to stem from an increase in immunosuppressive DCs within the tumor microenvironment (TME), and a lower influx of CD8+ effector T cells. Other reports have also detailed that DIO mice have higher levels of myeloid-derived suppressor cells (MDSCs),

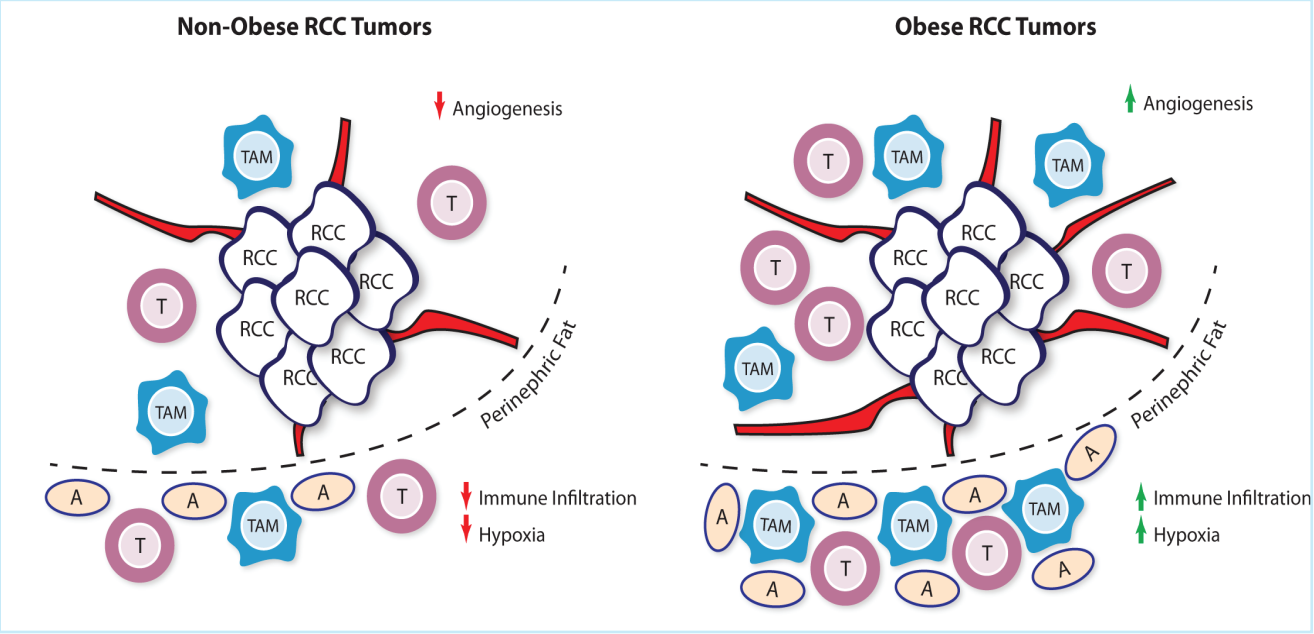
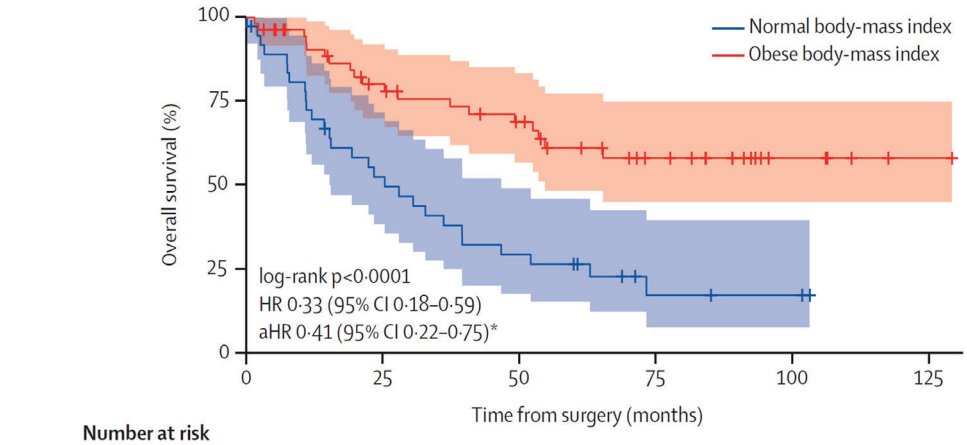


Fig. 1 | Obese and Non-Obese Renal Cell Carcinoma Tumor Microenvironment. RCC tumors from obese patients were found to have increased angiogenesis signatures which may promote response to VEGFR tyrosine kinase inhibitor therapy. The peritumoral fat tissue in obese RCC patients, compared to normal weight patients, also harbors increased hypoxia and immune cell infiltration signatures. These immune cells may act as a reservoir for infiltration upon checkpoint inhibitor therapy. RCC: renal cell carcinoma ; TAM: tumor-associated macrophage; T: T cell; A: Adipocyte.

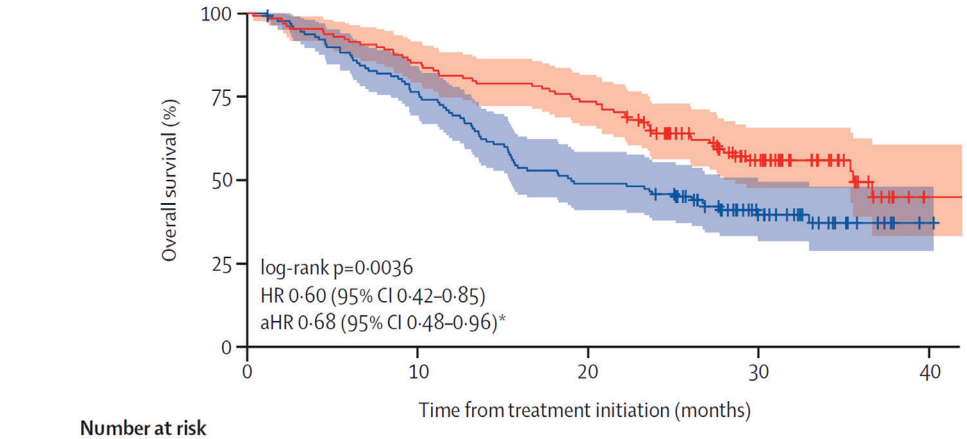
with elevated DCs and tumor-associated macrophages which traffic to the TME via CCL2.[30] While obese patients with clear cell RCC have been shown to have fewer circulating PD-1+/CD8+ T cells, other circulating immune populations remain similar when compared to normal-weight patients.[31]

In follow up to the pivotal work by McQuade *et al.*,[18] Wang and colleagues investigated potential mechanisms that may underlie the obesity paradox in relation to ICI therapy.[32] Using melanoma and animal models and patient samples, investigators consistently found upregulation of PD-1 expression and low T cell proliferation in obese patients. Within the TME, isolated T cells had upregulation of immune checkpoint proteins including PD-1, Tim3, and Lag3 expression, and significant upregulation of specific T cell profiles associated with anergy and senescence. Interestingly, researchers found that PD-1 CD8+ expression correlated with elevated levels of leptin, a hormone commonly implicated in obesity. Utilizing a leptin-deficient tumor model, mice treated with leptin were found to have faster tumor growth and higher T cell exhausted phenotypes, suggesting the critical role leptin signaling may have in immunity and the role ICI therapy may have in restoring immune sensitivity with leptin-mediated dysregulation.[32] Others have shown high levels of leptin in RCC tissues associated with a shorter PFS,[33] but a direct link between leptin and T cell function in this context for patients with RCC remains unclear.

Given the differences in immune regulation that are seen in obese patients, Sanchez and colleagues investigated whether immune infiltration within the RCC tumor and peritumoral microenvironment might shed light into other components which may affect clinical outcomes (Figure 1).[27] The investigators compiled three different data sets comprising of patients from COMPARZ, the randomized phase III study of sunitinib and pazopanib, TCGA, and an observational cohort of patients with metastatic clear cell RCC treated at Memorial Sloan Kettering Cancer Center. Using gene set enrichment profiling and immune deconvolution analysis, the degree and type of immune populations within each tissue sample was estimated for each of these tumors. Firstly, they confirmed the protective effect of obesity in this population compared to normal-weight patients in both the COMPARZ (all advanced metastatic) and TCGA (mostly early stage) cohorts (Figure 2). Interestingly, results with ICI therapy did not remain significant in regards to obesity when adjusted for IMDC risk. In the COMPARZ cohort, they performed transcriptional analysis and found that tumors from patients who were obese had significant upregulation of several pathways involved in hypoxia, angiogenesis, TGF-β signaling, and epithelial -mesenchymal transition, and upregulation of metabolic pathways like adipogenesis, fatty acid metabolism, and glycolysis. With these changes in angiogenesis within the microenvironment, the investigators propose that these changes may explain the increased sensitivity to VEGFR TKI therapy. They then focused their work on the tumor microenvironment and did not find a significant difference in the degree of total immune infiltration within the TME and found that tumors from patients who were obese harbored lower expression of immune checkpoint molecules. Extending their search towards



Number at risk (number censored)						
Normal body-mass index	37 (0)	18 (2)	10 (2)	3 (6)	2 (7)	0 (9)
Obese body-mass index	55 (0)	37 (8)	28 (12)	16 (20)	5 (31)	1 (35)



Number at risk (number censored)					
Normal body-mass index	128 (0)	97 (1)	62 (1)	28 (26)	1 (51)
Obese body-mass index	128 (0)	109 (0)	94 (0)	45 (29)	2 (69)

Fig. 2 | Overall Survival of Obese and Normal Weight clear cell RCC patients. Kaplan-Meier curves comparing obese and non-obese clear cell RCC patients from the TCGA (mostly early stage) (A) and COMPARZ (all advanced metastatic) (B) cohorts, showing obese patients have longer overall survival, and longer overall survival when treated with first-line VEGFR tyrosine kinase inhibitor therapy. Shaded areas are 95% confidence interval. [Figure adapted and modified from Sanchez A et al., Lancet Oncology, 21(2), 283-293, 2020].

the peritumoral fat, they did identify a significant difference in inflammatory signatures in these regions when compared to the peritumoral environment of tumors from normal-weight patients, with higher levels of classically activated M1 TAM phenotypes. With the degree of immune infiltration and immune sensitivity of RCC tumors, this study prominently underscores the importance of peritumoral signaling interactions and extended our view of the TME to surrounding tissue compartments.

Harnessing Obesity-Related Mechanisms for Future Discovery

While the reports discussed here lay the groundwork for understanding mechanistic differences related to obesity and immunity, translating these findings into clinical practice remain to be done. A common limitation often faced in many of these studies is that a single BMI data point may not reflect the longitudinal biological changes which occur during therapy. Hence, understanding whether other measurements related to obesity may also serve as surrogates for underlying physiologic changes in muscle, fat and metabolic fitness is vital. For instance, cross-sectional imaging can be used to calculate the skeletal muscle index (SMI) or skeletal muscle radiodensity (SMD), surrogates for body muscle mass, and a lower SMI at baseline has been associated with a shortened OS.[34] SMI also appears to be prognostic in RCC patients treated with targeted agents.[35] Other measures like subcutaneous fat have been shown to be highly correlated with BMI but not significantly associated with survival outcomes for RCC patients.[36] With novel software acquisition tools which can automate capture of these different muscle and fat compartments, prospective studies which can track long-term physiologic changes related to metabolic syndrome may uncover novel insights with these clinical trends.

Expanding biomarker efforts which may capture the inflammatory changes associated with obesity also is an area of active investigation. Changes in the neutrophil-to-lymphocyte ratio (NLR) has shown to be a marker for early response to immunotherapy,[37] and likely needs to be interpreted in the context of NLR changes which may occur with obesity. Other circulating markers which relate to underlying physiologic changes may also be more apt for this job. Low expression of miR-204-5p, a microRNA associated with obesity, was significantly associated with higher disease recurrence and may enhance risk-based prognostic models.[38] Serum adiponectin has been shown to inversely correlate with BMI in RCC patients, yet tissue expression was not associated with disease aggressiveness or survival.[39] In addition to leptin, it has been postulated that sex hormones may play a role in modulating ICI therapy, as trends were seen in the melanoma series only in male patients.[18] Sun and colleagues raise further questions as they preliminarily showed RCC disease risk differences between pre-menopausal and post-menopausal women.[40] Lastly, with the growing understanding of the relationship between the gut microbiome and obesity gut dysbiosis-related changes with ICI therapy[41], linking these findings may help inform other biomarker discovery efforts in the future.

Looking forward, augmenting cancer immunotherapy with obesity-related strategies to improve immune cell fitness or by inhibiting obesity-related immune dysregulation remains a high priority. Several drugs that are commonly used in this patient population have previously been shown to have beneficial effects when combined with ICI therapy. Fenofibrate, a common lipid-lowering drug, was found to synergize with ICI blockade in melanoma models via activation of the PPARα pathway.[42] Metformin, a diabetes drug commonly implicated in cancer risk reduction, was also shown to synergize with ICI therapy via increased tumor oxidative phosphorylation and reduced tumor hypoxia.[43] Given the upregulation of pseudohypoxia-related pathways in *VHL*-driven clear cell RCC, understanding this combinatorial strategy in a RCC tumor model may uncover unique insights. As leptin partly exerts activity through activation of Jak/STAT pathways, many have proposed downstream modulation of these specific pathways with currently approved in combination with ICI therapy. As immune related changes have been shown after even lifestyle adjustments like dietary modifications, exercise and psychoeducation,[44] understanding the contribution of these interventions within this population may ultimately improve ICI outcomes and the relative overall health of our patients.

Extrapolating these paradigms outside of clear cell RCC histology may uncover new insights and applications for rare, non-clear cell RCC tumors where there remains a high unmet need. Data from the Kaiser Permanente network highlighted that the obesity paradox was associated with clear cell and chromophobe tumors but not papillary RCC, and this was confirmed in their meta-analysis showing a relative risk of 1.8 and 2.2 for clear cell and chromophobe histologies, respectively, and 1.2 for papillary tumors.[11] A large case-control series in the United States and Europe also confirmed this same subtype-specific trend.[13] As chromophobe RCC tumors are notoriously resistant to ICI therapy[45], evaluating whether immune microenvironment shifts which may be related to the obesity paradox are paralleled to the changes seen in clear cell RCC may help refine immune-based strategies in this specific tumor subtype.

FINAL REMARKS

The field has rapidly evolved from the first sighting of obesity-related trends to a more mechanistic grasp on the links between obesity, immunity, and cancer biology. As new surrogates and biomarkers are developed that interrogate individual tumors within their surrounding tissue borders and microenvironment, combinatorial strategies which leverage this metabolic data to enhance immune cell fitness and efficacy remains on the horizon. Further, with advances in our understanding of the physiologic impact of obesity itself, we will soon be better positioned to improve the health and lives of our patients. With accelerating rates of obesity worldwide and the rising health and economic tolls, now, more than ever, it is critical to undertake further exploration of mechanism-based strategies to improve precision-based care in this patient population.

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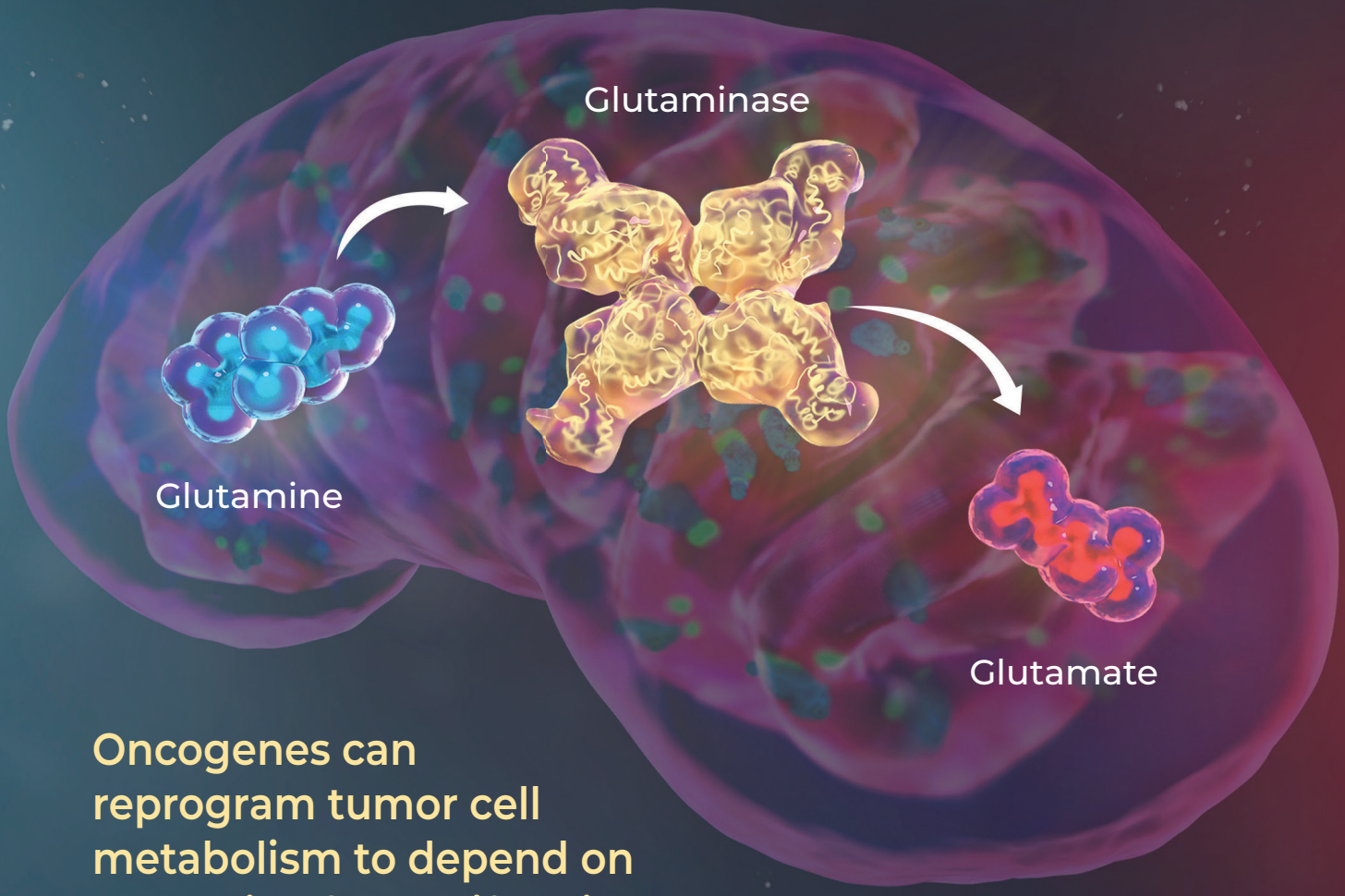

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Harnessing Big Data with Machine Learning in Precision Oncology

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ABSTRACT

While multi-level molecular "omic" analyses have undoubtedly increased the sophistication and depth with which we can understand cancer biology, the challenge is to make this overwhelming wealth of data relevant to the clinician and the individual patient. Bridging this gap serves as the cornerstone of precision medicine, yet the expense and difficulty of executing and interpreting these molecular studies make it impractical to routinely implement them in the clinical setting. Herein, we propose that machine learning may hold the key to guiding the future of precision oncology accurately and efficiently. Training deep learning models to interpret the histopathologic or radiographic appearance of tumors and their microenvironment—a phenotypic microcosm of their inherent molecular biology—has the potential to output relevant diagnostic, prognostic, and therapeutic patient-level data. This type of artificial intelligence framework may effectively shape the future of precision oncology by fostering multidisciplinary collaboration.

INTRODUCTION

Undoubtedly, we have become deeply immersed in an oncologic era defined by omics. In our quest to achieve precision medicine, we have attempted to unpeel several omic layers in cancer, including the genome, epigenome, transcriptome, proteome, lipidome, glycome, metabolome, and microbiome. These approaches have yielded an unparalleled wealth of data and breakthrough discoveries that have enabled us to better deconvolute the biology and aggressiveness of tumors, decipher the process of metastatic dissemination and tropism, characterize patterns of inheritance, identify candidate biomarkers, and understand mechanisms of therapeutic response and resistance. For the clinician and the individual patient, however, this information becomes relevant only if it can be translated into informing prognosis, guiding therapeutic decisions, and improving outcomes overall. As more sophisticated omic levels are introduced, the integration of these data becomes increasingly complex, and the clinical interpretation is made even more challenging. Furthermore, the necessary technical and bioinformatics expertise coupled with the financial expense of executing omic studies makes it impractical to routinely implement such studies in the clinical setting.

As we delve into deeper layers of tumor omics, it is worth taking a step back and revisiting these tumors on a more phenotypic level—both pathologically and radiographically—which is easily overlooked with this newfound molecular knowledge. In particular, a closer assessment of the histopathology and morphologic architecture of tumors and their microenvironment reveals that, indeed, the appearance of tumor cells and their surroundings under the microscope can serve as a microcosm of the molecular milieu that defines these tumors. That is, precise histologic features conceivably appear the way they do as a consequence of omics. Likewise, with improvements in anatomical and functional imaging techniques, correlating the radiographic appearance of tumors with omics—an emerging field termed radiomics—may similarly reveal heretofore uncaptured information about a tumor's biology from radiography alone. Novel approaches that integrate the pathologic and radiographic phenotypes of cancers with their inherent omics may thus serve as a powerful means by which to glean information about their biological behavior and potentially inform clinical outcomes and therapeutic responsiveness in a more cost-sensitive and practical manner.

This concept forms the fundamental basis of machine learning in precision oncology. Iterative artificial intelligence strategies in medicine are designed to distill big data into practically useful means by streamlining the analysis, decreasing cost, increasing accuracy via automation, and yielding clinically relevant information at the patient level. Conceptually, a deep learning model could be trained to harness and integrate clinical data, radiographic data, histopathologic data, and molecular (omic) data to yield information that could be used by the clinician in counseling patients and in guiding treatment strategies (Figure 1). Following training and validation of the initial model, the machine learning algorithm would then be able to output similar information with a high degree of accuracy, but with the need for less inputs.

As a case study, clear cell renal cell carcinoma (ccRCC) nicely exemplifies these concepts. By virtue of its considerable histologic and molecular intratumoral heterogeneity, broad spectrum of biological and clinical behavior, and recent groundbreaking discoveries, ccRCC serves as a robust platform to illustrate the utility of machine learning in guiding precision oncology. Arguably the most comprehensive molecular characterization of ccRCC to date, Clark et al.

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Disclosures: None

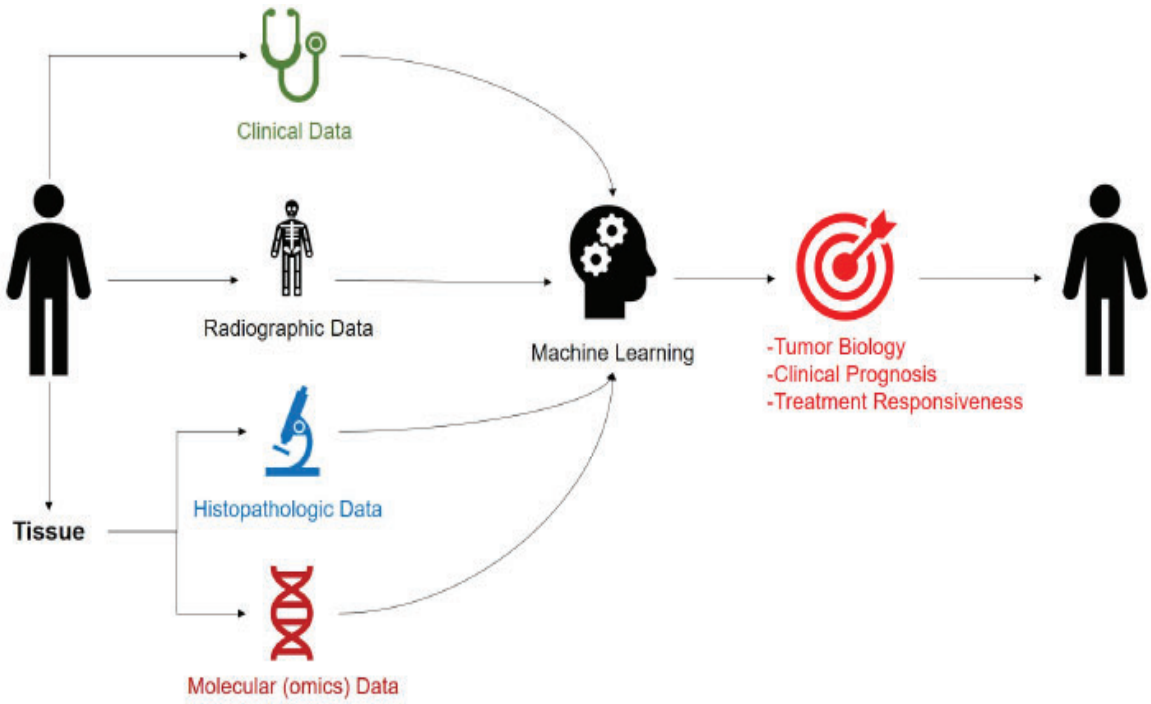

Fig. 1 | Simplified schematic conceptually depicting large-scale integration of big data into a machine learning algorithm for precision oncology. Inputs from a single patient, including clinical, radiographic, histopathologic, and/or molecular data, can be used to train a machine learning model to accurately and efficiently predict personalized, clinically relevant data including information about the biological behavior of the patient's tumor(s), clinical prognosis, and treatment responsiveness.

recently conducted a multi-level omics analysis of ccRCC tumors and matched normal tissue by combining genomics, epigenomics, transcriptomics, proteomics, and phosphoproteomics.[1] Through proteogenomic integration, they discerned the functional impact of genomic alterations in ccRCC and further characterized novel immune signatures in the tumor microenvironment. Their findings provide evidence for rational selection of personalized therapies for patients based on ccRCC pathobiology, which is urgently needed in an era in which multiple frontline therapeutic regimens are available for metastatic ccRCC without a clear algorithmic approach.[2] On a biological and prognostic level, the study by Clark et al. extends the recent findings of the TRACERx Renal Consortium, who, in a series of three elegant studies, used multiregional targeted genomic analyses to describe clonal evolutionary patterns and explain metastatic competence of ccRCC tumors.[3-5]

Given the cost and difficulty of conducting analyses of such depth, Cai et al. employed an alternative approach based entirely on histopathology to develop a systematic ontology of ccRCC phenotypic variability across tumor architecture, cytology, and the microenvironment.[6] Remarkably, the authors reveal that a meticulous analysis of the histopathology alone may recapitulate clonal evolutionary trajectories, portend patient outcomes, and even inform differential response to therapies, in much the same way that Clark *et al.* and the TRACERx Renal Consortium implicated using molecular data.[1,3-5] They suggest that the morphologic appearance of tumors captures their molecular environment on a phenotypic level. Logically, this rather traditional approach of analyzing tumors pathologically, when integrated with multi-level omics, may serve as the basis to train future deep learning models that may then require less data input to yield the same prognostic and therapeutic patient-level information relevant to personalized medicine.

Indeed, a similar conceptual framework for artificial intelligence can be applied across other cancer types as well. As we traverse an age of big data, the challenge we will increasingly face is not so much how to generate more information, but rather how to use the information we gather. Through multidisciplinary integration of radiology, pathology, bioinformatics, and bench science, machine learning will likely hold the key to harnessing this information efficiently and help translate precision oncology into a clinical reality.

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Assessment of Intratumoral Histologic Heterogeneity in Clear Cell Renal Cell Carcinoma: Opportunities to Inform Molecular Studies and Therapeutic Approach?

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The past four and a half decades have witnessed amazing progress in the management of renal cell carcinoma (RCC), in large part due to developments in histologic classification. As recently as 1975[1], the histologic diversity of RCC encompassed only two types – clear cell and granular cell RCC. Careful histologic observations over the next two decades, validated by cytogenetic correlations[2] led to greater understanding of RCC as being composed of numerous subtypes each with unique clinical, morphologic, and prognostic implications[3]. Continuing interest in histopathology and growing use of molecular analyses have taken classification of RCC to a greater level of diagnostic complexity, in turn facilitating improved clinical efficacy. Clear cell papillary RCC[4,5], MiT family translocation RCC[6], Fumarate Hydratase and Succinate Dehydrogenase-deficient RCCs[7,8], *TCEB1* mutated RCC[9], and ALK-rearranged RCC[10] to name a select few, are now diagnosed based on an integrated morphologic and molecular approach. Contemporary practice thus now separates many entities from clear cell RCC, rendering a more pristine clinicopathologic entity, with more consistent morphologic and molecular features.

Treatment paradigms in RCC have rapidly evolved in parallel, influenced by discovery of the molecular underpinnings of different RCCs subtypes. Principal among these is clear cell RCC, with its defining relationship to loss of VHL function, versus numerous non-clear cell RCC subtypes. Indeed, much of the value of contemporary diagnostic practice for kidney tumors is recognition and classification of a tumor as clear cell RCC, with its attendant prognostic (more aggressive than many RCCs) and targeted treatment (tyrosine kinase inhibitors, immunotherapy) implications[11]. Clear cell RCC remains the most common type, exhibiting variable and often enigmatic clinical behavior, due to incomplete prognostication by clinical and histopathologic variables. While numerous different mutations may be detected from a single tumor, and many tumors be shown to be heterogeneous across subclones[12], current reporting in histopathology has been limited to stage, grade, lymphovascular invasion, necrosis, and cytologic changes such as rhabdoid and sarcomatoid cytomorphology.

Into this milieu, Kapur and Brugarolas have provided for the readership of *Kidney Cancer Journal* a thought-provoking review of their recent scholarship in assessment, analysis, and experimental modeling of the intratumoral heterogeneity of clear cell RCC (see Page 68 of this issue), touching on both molecular and histologic aspects[13]. Throughout, they draw an analogy between the well understood intratumoral molecular heterogeneity of clear cell RCC and the pathologist's intuition that the tumor's frequent intratumoral histomorphologic heterogeneity reflects this phenomenon. Recognizing that although clear cell RCC can be pathologically defined as a unique subtype, it is neither a single disease among different tumors, nor among clones within a single tumor. Drawing from their experience modeling the molecular heterogeneity of this disease and its progression[14–16], the authors detail their recently reported comprehensive and quantitative histologic assessment of unique histologic patterns within (treatment naïve) clear cell RCCs[13]. These 33 patterns were sourced from three conceptual "axes", specifically, architectural patterns, cytologic features, and histopathologic features of the tumor microenvironment. Data from detailed histologic feature assessment of over 500 clear cell RCCs were then correlated to key conventional pathologic parameters (grade, TNM stage, etc.), prognosis, and response to therapy. Remarkably, analyses based in their histomorphologic assessments recapitulated similar trends to those seen in prior studies based in genomic assessment.

Their project documents in unprecedented and quantitative detail the range of pattern heterogeneity between clear cell RCC tumors, with lower heterogeneity seen in smaller tumors than larger tumors, as well as a significant association between pattern heterogeneity and aggression. From the pathologist's standpoint, intriguing, too, were observations of certain patterns predictive of

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survival, even after multivariable adjustment for the aforementioned, conventionally reported parameters. Striking to us, particularly, were the findings of analyses, based on adapting fundamental assumptions about tumor biology and evolution to the spatial relationships between histologic patterns. These analyses allowed them to infer even phylogenetic relationships between patterns. Thus they observed that the small nested or cystic/microcystic patterns, seen so routinely diagnostically, represent founder patterns, from which subclones outgrow with more aggressive patterns, converging on aggressive solid patterns. Preferential co-occurrence of certain patterns allowed inference that they were related, including observations with therapeutic relevance such as association of intratumoral lymphocytic infiltrates with sarcomatoid and rhabdoid features. Adroitly, they employed the paradigm of this disease's natural history to assess inferences from their models, for example, validating patterns inferred to reflect aggression in primary tumors as present in later stages, such as thrombus formation.

Going forward, there are multiple implications of the authors' ontological analyses of histologic patterns, and we suspect these go beyond their potential to nominate additional "univariate" prognostic parameters for pathologists to assess and report diagnostically. Awareness of the ontological relationships between different clones/patterns and establishing which reflect adverse prognosis or treatment resistance could be used to more capably manage RCC, not least by assisting in selecting the most appropriate tumor sample for precision medicine approaches. The power of (and accomplishments of) reductionist approaches like molecular studies to inform our understanding of the genesis of cancer and its progression should not be understated. Yet, we cannot help wonder as artificial intelligence/machine learning approaches begin to assist in refinement, quantitation, and objectification of "next gen histologic" features like those assessed by Kapur et al., whether we are about to discover the prognostic and predictive power of higher order features of the cancer system, too.

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Newsworthy, late-breaking information from Web-based sources, professional societies, and government agencies

Latest Outcomes from the Phase 3 JAVELIN Renal 101 Trial (N = 886; NCT02684006) were Published.

Biomarker analyses of baseline tumor samples from the phase 3 JAVELIN Renal 101 trial (NCT02684006) demonstrated that first-line avelumab + axitinib versus sunitinib significantly prolonged progression-free survival (PFS) in advanced renal cell carcinoma (aRCC). In this retrospective analysis of the large (n = 886), controlled, exploratory clinical biomarker dataset, authors have provided evidence to confirm the immunomodulatory role of anti-angiogenic therapy, defined molecular features that differentiate therapy-specific outcomes in first-line aRCC and highlighted previously unappreciated biologically and clinically significant determinants of PFS benefit with an ICI + VEGFR TKI combination versus VEGFR TKI alone according to the results published in *Nature Medicine*.

The investigators in this study identified important biological features associated with differential PFS between the treatment arms, including new immunomodulatory and angiogenesis gene expression signatures (GESs), previously undescribed mutational profiles and their corresponding GESs, and several HLA types. Similar to findings in KEYNOTE-426 (pembrolizumab + axitinib versus sunitinib), these observations suggest that PD-L1 expression (on TCs or ICs) may have limited positive-predictive value in RCC and are in contrast with findings from the CheckMate 214 (ipilimumab + nivolumab versus sunitinib) and IMmotion 150 (atezolizumab + bevacizumab vs sunitinib) trials. These findings provide insight into the determinants of response to combined PD-1/PD-L1 and angiogenic pathway inhibition and may aid in the development of strategies for improved patient care in aRCC. This research outcome may inform personalized therapeutic strategies for patients with aRCC and other tumor types.

Reference: Motzer, R.J., Robbins, P.B., Powles, T. et al. Avelumab plus axitinib versus sunitinib in advanced renal cell carcinoma: biomarker analysis of the phase 3 JAVELIN Renal 101 trial. Nature Medicine (2020). Published online. https://doi.org/10.1038/s41591-020-1044-8

AVEO Oncology Announces FDA Acceptance for Filing of a New Drug Application for Tivozanib as a Treatment of Relapsed or Refractory Renal Cell Carcinoma

BOSTON - AVEO Oncology recently announced that the US FDA accepted for filing its New Drug Application (NDA) seeking approval for tivozanib, the Company's next-generation VEGFR-TKI, as a treatment for relapsed or refractory renal cell carcinoma (RCC).

"The acceptance of our NDA filing marks yet another important milestone for AVEO, as we pursue our goal of providing RCC patients whose disease has relapsed or become refractory to multiple lines of therapy with a meaningful new treatment option," said Michael Bailey, president and chief executive officer. "We look forward to working closely with the FDA over the coming months during their review of our application. In parallel, we continue to focus on commercial-readiness to ensure we are well positioned to support the potential launch of tivozanib, subject to approval."

The NDA submission is based on AVEO's pivotal Phase 3 study, TIVO-3, comparing tivozanib to sorafenib in 3rd and 4th line RCC, including results recently presented at the American Society of Clinical Oncology 2020 Virtual Scientific Program. As previously announced, the TIVO-3 trial met the primary endpoint of progression free survival (PFS) (HR=0.73; p=0.02) and the secondary endpoint of overall response rate (ORR) (18% vs. 8%; p=0.02). The final OS hazard ratio (HR), which assesses the overall relative risk of death, was 0.97 (95% CI: 0.75-1.25; p=0.82), favoring tivozanib and improving from the previously reported interim HR of 0.99. Updated median OS, representing a single point in time in the OS curve, was 16.4 months for tivozanib (95% CI: 13.4-22.2) and 19.2 months for sorafenib (95% CI: 15.0-24.2). These OS HR results are similar to those of prior VEGFR TKI vs. VEGFR TKI studies in RCC. The application is also supported by three additional trials, including an active comparator-controlled Phase 3 study, TIVO-1, comparing tivozanib to sorafenib in first line RCC, and two Phase 2 studies, Study 902, the open-label, crossover clinical study of tivozanib for patients who progressed on sorafenib in TIVO-1, as well as placebo-controlled Study 201 in first line RCC. TIVO-3 provides the first positive superiority study to help guide this important treatment decision and, furthermore, offers this highly refractory patient population a favorable tolerability profile as indicated by fewer dose reductions, interruptions and discontinuations over a less selective VEGFR TKI in sorafenib." Said Dr. Sumanta Pal, MD, co-director, Kidney Cancer Program, at City of Hope Comprehensive Cancer Center.

19th Annual Meeting of the IKCS 2020

The 19th annual meeting of the International Kidney Cancer Symposium (IKCS 2020) organized by Kidney Cancer Association (KCA) is scheduled on November 6th and 7th virtually. This annual event is an opportunity for physicians, researchers, academics, and industry professionals from across the globe to join together and exchange ideas which will direct the future of kidney cancer research and treatment in the ultimate pursuit of a cure. The hot topics include (i) immunotherapy and emerging therapies for rcc discussion and round table, (ii) metabolism as a target for rcc discussion, (iii) multimodality therapy for metastatic rcc and an international considerations panel, (iv) non-clear cell trial design round table, (v) health disparities and crisis management: lessons from covid-19 (vi) the woodfire tumor board. Registration and full details on the agenda is available online through the Association's website, kcameetings.org/ikcs. Submission deadline is September 18, 2020 at 11:59 p.m. CST. All accepted abstracts will be published in our Kidney Cancer Journal.

The 2020 ASTRO Annual Meeting

The 2020 ASTRO Annual Meeting has transitioned from a live meeting to an enhanced virtual educational experience.

The meeting will include all of the programming you are accustomed to, only this year it will be in an interactive online format including a robust program of educational and scientific sessions, live SA-CME opportunities, a poster hall with narration from poster presenters, and a virtual Exhibit Hall where you can visit booths to learn and connect with industry colleagues. The meeting opens on October 25 and will be available for 30 days to ensure you have access to all the presentations and materials. This year's Annual Meeting will be uniquely redesigned to ensure that attendees from around the globe continue to access timely scientific and education session. The PRO and ARRO programs will provide curated content addressing issues specific to community practitioners and residents.

Liquid Biopsy Shows High Accuracy in Detecting Early-Stage Renal Cell Carcinoma

A novel plasma DNA assay has shown remarkable accuracy in identifying patients with renal cell carcinoma (RCC) across all stages of disease, making it easier to detect at early-stage, according to the recent report published in Nature Medicine. If validated, this assay could potentially be used initially as a screening test for people who have a family history of kidney cancer or who previously had kidney cancer. This is especially very important as currently, no FDA-approved or recommended screening method is available for the early detection of RCC in the general population.

Of all extracranial tumors, RCC sheds the least amount of cell-free DNA (cfDNA) so cfDNA-based methods alone are insufficient for detecting RCC. Therefore, Cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq), could be potentially efficient in identifying RCC. The investigators in this study used a cfMeDIP-seq approach on plasma and urine cfDNA to detect RCC, which was the first such application of cfMeDIP-seq on urine cfDNA for cancer detection and demonstrated for the first time that this assay can accurately detect RCC by measuring urine cfDNA

Testing was performed on 148 samples, including 99 from cases of stage I to IV RCC, 21 samples of stage IV urothelial bladder cancer, and 28 samples from healthy, cancer-free controls. Across the training test sets, RCC samples had a higher median methylation score than control samples and had a mean area under the receiver operating characteristic (AUROC) curve of 0.990 (95% CI, 0.985-0.995). Among urine cfDNA samples, the mean AUROC for patients with RCC compared with healthy controls was 0.858 (95% CI, 0.831-0.885).

The authors noted that following further validation, this screening method, alone or in combination with imaging, could transform clinical management by enabling early detection of RCC and reducing unnecessary kidney biopsies and nephrectomies.

Reference: Nuzzo PV, Berchuck JE, Korthauer K, et al. Detection of renal cell carcinoma using plasma and urine cell-free DNA methylomes. *Nature Med.* Published online June 22, 2020. doi:10.1038/s41591-020-0933-1

EDITOR'S MEMO *(continued from Page 66)*

It is exciting to see the promising results from KEYNOTE-426, KEYNOTE-146, COSMIC-313 and PDIGREE that highlighted optimal strategies for combining and sequencing treatment modalities of targeted and immunotherapies. The initial results of the open-label phase 2 study of MK-6482 that targets hypoxia inducible factor signaling has opened up an avenue of a new class of therapy for treatment of VHL-associated ccRCC. Some other hot topics especially new approaches exploiting PARP inhibitors, glutaminase inhibitors, newer personalized medicine around immunotherapies, and new tyrosine kinase inhibitor strategies were also presented in ASCO20 plenary sessions.

Despite revolutionary approaches in the RCC treatment, it is apparent that intra-tumoral heterogeneity poses a significant problem for cancer management. Precision oncology approaches harnessing knowledge of heterogeneous tumor is crucial to tailor those therapies to ultimately target and improve prognosis and outcomes for patients. The article in this issue by Payal Kapur and James Brugarolas *et al* present an intriguing molecular genetic and morphologic evolutionary model especially focusing on prototypical model of tumor heterogeneity in renal cell carcinoma. This systematic and comprehensive ontology that captures the breath of ccRCC morphologies has profound implications both for understanding the biology of tumor progression, and for the ability to stratify patients in the clinic. Undoubtedly, this knowledge sets a paradigm for de-convoluting phenotypic complexity and establishes a comprehensive morphologic ontology of ccRCC. The other article by Ritesh Kotecha dicusses the mechanistic insights into the potential mechanisms underlying the counter-intuitive phenomenon known as obesity paradox in clear cell renal cell carcinoma. Emerging trends discussed in this article highlight that differences in the tumour microenvironment could hold the key to apparent survival advantage of obese patients with clear cell RCC versus patients at a normal weight and also emphasize such studies merit careful consideration for designing clinical trials in the future. In the *Letter to the Editor* column, Nirmish Singla and Shyamli Singla illustrate that the deep machine learning may be harnessed to inform clinical prognosis and therapeutic responsiveness using clear cell renal cell carcinoma as a prototype and also envision that such artificial intelligence approach may effectively shape the future of precision oncology.

Robert A. Figlin, MD
Editor-in-Chief

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.

The society for immunotherapy of cancer consensus statement on immunotherapy for the treatment of advanced renal cell carcinoma (RCC). Rini BI, Battle D, Figlin RA, et al. J Immunother Cancer. 2019;7(1):354. Published 2019 Dec 20. doi:10.1186/s40425-019-0813-8

Abstract: The approval of immunotherapeutic agents and immunotherapy-based combination strategies in recent years has revolutionized the treatment of patients with advanced renal cell carcinoma (aRCC). Nivolumab, a programmed death 1 (PD-1) immune checkpoint inhibitor monoclonal antibody, was approved as monotherapy in 2015 for aRCC after treatment with a VEGF-targeting agent. In April 2018, the combination of nivolumab and ipilimumab, a CTLA-4 inhibitor, was approved for intermediate- and poor-risk, previously untreated patients with aRCC. Then, in 2019, combinations therapies consisting of pembrolizumab (anti-PD-1) or avelumab (anti-PD-ligand (L) 1) with axitinib (a VEGF receptor tyrosine kinase inhibitor) were also approved to treat aRCC and are likely to produce dramatic shifts in the therapeutic landscape. To address the rapid advances in immunotherapy options for patients with aRCC, the Society for Immunotherapy of Cancer (SITC) reconvened its Cancer Immunotherapy Guidelines (CIG) Renal Cell Carcinoma Subcommittee and tasked it with generating updated consensus recommendations for the treatment of patients with this disease.

Results of the ADAPT Phase 3 Study of Rocapuldencel-T in Combination with Sunitinib as First-Line Therapy in Patients with Metastatic Renal Cell Carcinoma. Figlin RA, Tannir NM, Uzzo RG, et al. . Clin Cancer Res, 2020 (26) (10) 2327-2336; DOI: 10.1158/1078-0432.CCR-19-2427

Purpose: Rocapuldencel-T is an autologous immunotherapy prepared from mature monocyte-derived dendritic cells (DC), coelectroporated with amplified tumor RNA plus CD40L RNA. This pivotal phase III trial was initiated to investigate the safety and efficacy of a combination therapy dosing regimen of Rocapuldencel-T plus sunitinib in patients with metastatic renal cell carcinoma (mRCC).

Results: Between 2013 and 2016, 462 patients were randomized 2:1, 307 to the combination group and 155 to the SOC group. Median OS in the combination group was 27.7 months [95% confidence interval (CI) 23.0-35.9] and 32.4 months (95% CI, 22.5-) in the SOC group HR of 1.10 (95% CI, 0.83-1.40). PFS was 6.0 months and 7.83 months for the combination and SOC groups, respectively [HR = 1.15 (95% CI, 0.92-1.44)]. The ORR was 42.7% (95% CI, 37.1-48.4) for the combination group and 39.4% (95% CI, 31.6-47.5) for the SOC group. Median follow up was 29 months (0.4-47.7 months). On the basis of the lack of clinical efficacy, the ADAPT trial was terminated on February 17, 2017. Immune responses were detected in 70% of patients treated with Rocapuldencel-T, and the magnitude of the immune response positively correlated with OS. No serious adverse events attributed to the study medication have been reported to date.

Conclusions: Rocapuldencel-T did not improve OS in patients treated with combination therapy, although the induced immune response correlated with OS. Moreover, we identified two potential survival-predictive biomarkers for patients receiving DC based immunotherapy, IL-12 produced by the DC vaccine and higher numbers of T regulatory cells present in the peripheral blood of patients with advanced RCC.

Stool Microbiome Profiling of Patients with Metastatic Renal Cell Carcinoma Receiving Anti-PD-1 Immune Checkpoint Inhibitors. Salgia NJ, Bergerot PG, Maia MC, et al. Stool Microbiome Profiling of Patients with Metastatic Renal Cell Carcinoma Receiving Anti-PD-1 Immune Checkpoint Inhibitors. Eur Urol. 2020;S0302-2838(20)30543-1. doi:10.1016/j.eururo.2020.07.011

Abstract: Preclinical models and early clinical data suggest an interplay between the gut microbiome and response to immunotherapy in solid tumors including metastatic renal cell carcinoma (mRCC). We sought to characterize the stool microbiome of mRCC patients receiving a checkpoint inhibitor (CPI) and to assess treatment-related changes in microbiome composition over the course of CPI therapy. Stool was collected from 31 patients before initiation of nivolumab (77%) or nivolumab plus ipilimumab (23%) therapy, of whom 58% experienced clinical benefit. Greater microbial diversity was associated with clinical benefit from CPI therapy (p = 0.001), and multiple species were associated with clinical benefit or lack thereof. Temporal profiling of the microbiome indicated that the relative abundance of Akkermansia muciniphila increased in patients deriving clinical benefit from CPIs. This study substantiates results from previous CPI-related microbiome profiling studies in mRCC.

Summary: We compared the composition and diversity of the gut microbiome in patients receiving immunotherapy for renal cell carcinoma. We found that higher microbial diversity is associated with better treatment outcomes. Treatment response is characterized by changes in microbial species over the course of treatment.

Clinical Activity of Ipilimumab Plus Nivolumab in Patients With Metastatic Non-Clear Cell Renal Cell Carcinoma. Gupta R, Ornstein MC, Li H, et al. Clin Genitourin Cancer. 2019;S1558-7673(19)30363-5. doi:10.1016/j.clgc.2019.11.012

Introduction: Ipilimumab plus nivolumab has been approved for intermediate- and poor-risk metastatic renal cell carcinoma (RCC). However, the activity in non-clear cell RCC (nccRCC) is unknown.

Results: Eighteen patients were identified. The median age was 59 years (range, 32-81 years), 77.8% were men, and the Eastern Cooperative Oncology Group performance status was 0 (38%) or 1 (50%). The median treatment duration was 2.4 months (range, 0.7-12.3 months). The non-clear cell histologic types included 6 papillary, 5 chromophobe, 3 unclassified, 2 adenocarcinoma of renal origin, 1 translocation, and 1 medullary. Most had an intermediate (66%) or poor (22%) International Metastatic Database Consortium risk. The best objective response included 6 partial responses (PRs; 33.3%) and 3 with stable disease (16.7%). Of the patients with a PR, the median time to the best response was 3.0

months, and median duration of the PR was 4.3 months. The median progression-free survival was 7.1 months. All-grade TRAEs were noted in 11 patients (61.1%) and included colitis (22%), hepatotoxicity (16%), rash (11%), and fatigue (11%). Eleven patients (61%) had TRAEs requiring high-dose glucocorticoids (> 40 mg of prednisone equivalent daily).
Conclusions: Ipilimumab plus nivolumab demonstrated objective responses and notable toxicity in patients with nccRCC.

HIF-1a and HIF-2a differently regulate tumour development and inflammation of clear cell renal cell carcinoma in mice. Hoefflin R, Harlander S, Schäfer S, et al. Nat Commun. 2020;11(1):4111. 2020. doi:10.1038/s41467-020-17873-3
Abstract: Mutational inactivation of VHL is the earliest genetic event in the majority of clear cell renal cell carcinomas (ccRCC), leading to accumulation of the HIF-1a and HIF-2a transcription factors. While correlative studies of human ccRCC and functional studies using human ccRCC cell lines have implicated HIF-1a as an inhibitor and HIF-2a as a promoter of aggressive tumour behaviours, their roles in tumour onset have not been functionally addressed. Herein we show using an autochthonous ccRCC model that Hif1a is essential for tumour formation whereas Hif2a deletion has only minor effects on tumour initiation and growth. Both HIF-1a and HIF-2a are required for the clear cell phenotype. Transcriptomic and proteomic analyses reveal that HIF-1a regulates glycolysis while HIF-2a regulates genes associated with lipoprotein metabolism, ribosome biogenesis and E2F and MYC transcriptional activities. HIF-2a-deficient tumours are characterised by increased antigen presentation, interferon signalling and CD8+ T cell infiltration and activation. Single copy loss of HIF1a or high levels of HIF2A mRNA expression correlate with altered immune microenvironments in human ccRCC. These studies reveal an oncogenic role of HIF-1a in ccRCC initiation and suggest that alterations in the balance of HIF-1a and HIF-2a activities can affect different aspects of ccRCC biology and disease aggressiveness.

Angiogenic and immune-related biomarkers and outcomes following axitinib/pembrolizumab treatment in patients with advanced renal cell carcinoma. Martini JF, Plimack ER, Choueiri TK, et al. Clin Cancer Res. 2020; clincanres.1408.2020. doi:10.1158/1078-0432.CCR-20-1408
Purpose: Combined axitinib/pembrolizumab is approved for advanced renal cell carcinoma (aRCC). This exploratory analysis examined associations between angiogenic and immune-related biomarkers and outcomes following axitinib/pembrolizumab treatment.
Results: Higher baseline tumor levels of CD8 showed a trend toward longer PFS (hazard ratio [HR] 0.4; P = 0.091). Higher baseline serum levels of CXCL10 (P = 0.0197) and CEACAM1 (P = 0.085) showed a trend toward better ORR and longer PFS, respectively. Patients for whom IL-6 was not detected at baseline had longer PFS vs patients for whom it was detected (HR 0.4; P = 0.028). At C2D1 and/or EOT, mainly immune-related biomarkers showed any association with better outcomes. The genes CA9 (P = 0.084), HIF1A (P = 0.064), and IFNG (P = 0.073) showed trending associations with ORR, and AKT3 (P = 0.0145), DDX58 (P = 0.0726), GZMA (P = 0.0666), LCN2 (NGAL; P = 0.0267), and PTPN11 (P = 0.0287) with PFS.
Conclusions: With combined axitinib/pembrolizumab treatment in patients with aRCC, mostly immune-related biomarkers are associated with better treatment outcomes. This exploratory analysis has identified some candidate biomarkers to consider in future prospective testing. ClinicalTrials.gov identifier: NCT02133742.

A pan-cancer analysis of PBAF complex mutations and their association with immunotherapy response. Hakimi AA, Attalla K, DiNatale RG, et al. Nat Commun. 2020;11(1):4168. Published 2020 Aug 20. doi:10.1038/s41467-020-17965-0
Abstract: There is conflicting data regarding the role of PBAF complex mutations and response to immune checkpoint blockade (ICB) therapy in clear cell renal cell carcinoma (ccRCC) and other solid tumors. We assess the prevalence of PBAF complex mutations from two large cohorts including the pan-cancer TCGA project (n = 10,359) and the MSK-IMPACT pan-cancer immunotherapy cohort (n = 3700). Across both cohorts, PBAF complex mutations, predominantly PBRM1 mutations, are most common in ccRCC. In multivariate models of ccRCC patients treated with ICB (n = 189), loss-of-function (LOF) mutations in PBRM1 are not associated with overall survival (OS) (HR = 1.24, p = 0.47) or time to treatment failure (HR = 0.85, p = 0.44). In a series of 11 solid tumors (n = 2936), LOF mutations are not associated with improved OS in a stratified multivariate model (HR = 0.9, p = 0.7). In a current series of solid tumors treated with ICB, we are unable to demonstrate favorable response to ICB in patients with PBAF complex mutations.

PBRM1 loss defines a nonimmunogenic tumor phenotype associated with checkpoint inhibitor resistance in renal carcinoma. Liu XD, Kong W, Peterson CB, et al. Nat Commun. 2020;11(1):2135. Published 2020 May 1. doi:10.1038/s41467-020-15959-6
Abstract: A non-immunogenic tumor microenvironment (TME) is a significant barrier to immune checkpoint blockade (ICB) response. The impact of Polybromo-1 (PBRM1) on TME and response to ICB in renal cell carcinoma (RCC) remains to be resolved. Here we show that PBRM1/Pbrm1 deficiency reduces the binding of brahma-related gene 1 (BRG1) to the IFNa receptor 2 (Ifngr2) promoter, decreasing STAT1 phosphorylation and the subsequent expression of IFNg target genes. An analysis of 3 independent patient cohorts and of murine pre-clinical models reveals that PBRM1 loss is associated with a less immunogenic TME and upregulated angiogenesis. Pbrm1 deficient Renca subcutaneous tumors in mice are more resistance to ICB, and a retrospective analysis of the IMmotion150 RCC study also suggests that PBRM1 mutation reduces benefit from ICB. Our study sheds light on the influence of PBRM1 mutations on IFN-STAT1 signaling and TME, and can inform additional preclinical and clinical studies in RCC.

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