Complementary Role of Circulating Tumor DNA Assessment and Tissue Genomic Profiling in Metastatic Renal Cell Carcinoma

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ABSTRACT

Purpose: The role of circulating cell-free tumor DNA (ctDNA) as an adjunct to tissue genomic profiling is poorly defined in metastatic renal cell carcinoma (mRCC). In this study, we aim to validate previous findings related to genomic alteration (GA) frequency in ctDNA and determine the concordance between ctDNA and tissue-based profiling in patients with mRCC.

Experimental Design: Results of 839 patients with mRCC who had ctDNA assessment with a Clinical Laboratory Improvement Amendments (CLIA)-certified ctDNA assay between November 2016 and December 2019 were collected. Tissue-based genomic profiling was collected when available and concordance analysis between blood- and tissue-based testing was performed.

Results: ctDNA was assessed in 839 patients (comprising 920 samples) with mRCC. GAs were detected in 661 samples (71.8%). Tissue-based GAs were assessed in 112 patients. Limiting our analyses to a common 73-/74-gene set and excluding samples with no ctDNA detected, a total of 228 mutations were found in tissue and blood. Mutations identified in tissue (34.7%; 42/121) were also identified via ctDNA, whereas 28.2% (42/149) of the mutations identified in liquid were also identified via tissue. Concordance between ctDNA and tissue-based profiling was inversely related to the time elapsed between these assays.

Conclusions: This study confirms the feasibility of ctDNA profiling in the largest mRCC cohort to date, with ctDNA identifying multiple actionable alterations. It also demonstrates that ctDNA and tissue-based genomic profiling are complementary, with both platforms identifying unique alterations, and confirms that the frequency of unique alterations increases with greater temporal separation between tests.

Introduction

Current management of metastatic renal cell carcinoma (mRCC) entails targeted therapy and immune therapy approaches (1). Targeted therapy for mRCC is directed at angiogenic mediators such as VEGF and its cognate receptor, or downstream moieties such as the mTOR. Immune therapy has evolved from cytokine-based treatments such as IL-2 and IFN-α to checkpoint inhibitors, which are themselves targeted entities directed at various proteins expressed at the T-cell–cancer cell interface (1, 2).

Despite the fact that mRCC is managed with an array of targeted agents, there is paradoxically no recommended testing of molecular targets for selection of patients before implementation of therapy. Although there have been multiple efforts to characterize single-gene biomarkers or multigene signatures as predictors of immune or targeted therapy response, none have been fully validated at this time (3–5).

A key limitation of molecular profiling performed via tissue biopsy is the inability to account for temporal and spatial heterogeneity without performing multiple biopsies throughout the course of a patient's treatment, which is not always viable (6). Spatial heterogeneity is the uneven distribution of genetically diverse tumor cell populations within the primary tumor and between other disease sites. Temporal heterogeneity is the evolution of tumor genomics over time due to treatment pressure and the natural disease course (6, 7). Many existing attempts at biomarker validation use baseline tissue, which may not reflect temporal evolution in the tumor, and in fact several clinical trials have demonstrated that failure to account for changes in the genetic make-up of a tumor over time may result in patients having worse outcomes or being treated with ineffective therapies (8, 9). In mRCC, the recent TRACERx series has elegantly defined the potential for branched evolution of different genomic clones with significant implications for disease phenotype (10).

One way to address temporal evolution is to consider circulating tumor biomarkers. Several investigators have characterized circulating tumor cells in mRCC (11, 12). Our group and others have also

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Translational Relevance

In metastatic renal cell carcinoma (mRCC), current treatment options consist of targeted therapies and immune therapies. Despite the common use of targeted agents in mRCC, there is no standard testing before the implementation of therapy. Tissue-based genomic testing is limited due to temporal and spatial heterogeneity, whereas genomic testing with circulating cell-free tumor DNA (ctDNA) can overcome these difficulties with minimal invasive risk. In this study of the largest cohort of patients with mRCC to date, we showed that ctDNA can be detected in the majority of patients with mRCC, and potentially actionable and predictive biomarkers can be identified through the assessment of blood. We also demonstrated that ctDNA and tissue-based testing are complementary, and concordance is increased when sampling is closer in time, whereas the frequency of the unique alterations is increased when there is a greater temporal separation.

previously reported the feasibility of circulating cell-free tumor DNA (ctDNA) in smaller series (13, 14). Herein, we address the paucity of literature comparing blood- and tissue-based genomic profiling in mRCC, demonstrating that these are complementary approaches by expanding on our initial experience 5-fold.

Materials and Methods

Patient selection

We retrospectively analyzed a de-identified database containing results from consecutive patients with mRCC who underwent clinical testing with a validated, next-generation sequencing (NGS) cell-free DNA (cfDNA) assay (Guardant360, Guardant Health), with testing ordered between November 2016 and December 2019. Patient age, sex, and cancer type were extracted from test request forms. ctDNA was assessed using Guardant360 (Guardant Health), a Clinical Laboratory Improvement Amendments (CLIA)-certified, College of American Pathologists (CAP)-accredited, New York State Department of Health–approved, clinical ctDNA assay with analytic and clinical validation previously reported (15, 16). Blood draw, ctDNA extraction, and sequencing procedures have been described in detail previously (15, 17). Briefly, ctDNA is extracted from the plasma aliquot derived from two 10-mL blood draws collected in Streck Cell-Free DNA BCT (Streck, Inc.; ref. 15). The assay is a targeted NGS panel with complete or critical exonic coverage of 73/74 genes and includes analysis of sequence alterations [single-nucleotide variants (SNV), small insertions/deletions, splice site alterations], amplifications, and fusions in a subset of genes. The reportable range for SNVs, indels, fusions, and amplifications is ≥0.04%, ≥0.02%, ≥0.04%, and ≥2.12 copies, respectively, with a >99.9999% per-position analytic specificity (15, 17). The assay evolved from a 73-gene to a 74-gene panel during the course of this study.

Tissue assessment

Patients with ctDNA assessment at the City of Hope Comprehensive Cancer Center (Duarte, CA) and Huntsman Cancer Institute (Salt Lake City, UT) with an available tissue-based genomic profiling were selected for further study. Three CLIA-certified commercial platforms were used, including: (i) a targeted NGS platform encompassing 324 cancer genes and a subset of genes assessed for rearrangement (FoundationOne CDx; Foundation Medicine), (ii) a targeted NGS platform with 595/648 genes and rearrangements in a subset of genes (Tempus xT; Tempus), or (iii) a whole-exome sequencing (WES) platform (GEM ExTra; Ashion Analytics). Techniques for each platform are described previously elsewhere in detail (18–20).

This study was approved by institutional review boards at City of Hope Comprehensive Cancer Center, Huntsman Cancer Institute, and Guardant Health. Study was conducted in accordance with Declaration of Helsinki, and informed written consent was obtained from all the participating subjects or their legal guardians.

Results

Patient characteristics

In total, ctDNA results were available from 839 patients (597 male and 242 female) with a median age of 64 (range, 22–96). Among 198 patients where pathologic information was available, the most frequent histology was clear cell (68.7%), followed by papillary (13.1%) and clear cell carcinoma with sarcomatoid features (5.6%; **Table 1**). Serial ctDNA tests were available for 39 patients, resulting in a total of 920 samples for analysis.

A total of 112 patients also had tissue genomic profiling data available. Eighty-seven patients (77.7%) were tested using a targeted NGS panel, whereas 25 patients (22.3%) were assessed with WES. In this series, 81 (72.3%) patients were male and the median age was 67 (range, 37–89 years; Supplementary Table S2). The most frequent histology was clear cell (85.7%), followed by papillary (8.0%). In total, 64 patients (57.1%) had received systemic treatment before ctDNA collection, and the median (range, 1–6) line of treatment at time of collection was one. The most frequent first-line treatments rendered within this group were sunitinib (34.4%), pazopanib (12.5%), and cabozantinib (9.4%). The median time between tissue- and blood-based genomic profiling was 9.8 months (IQR, 1.15–23.7 months).

ctDNA assessment

Of 920 samples assessed, ≥1 ctDNA genomic alteration (GA) was detected in 661 (71.8%). Among the 839 patients tested, this reflects a finding of ≥1 GA in 612 patients (72.9%). A median of two GAs was detected per sample (IQR, 1–4 GAs). When limited to only assumed functional alterations (i.e., excluding synonymous variants and variants of uncertain significance), the most frequently mutated genes were *TP53* (36.9%), *VHL* (22.2%), and *TERT* (7.2%). Of the 1,061 GAs noted in total (excluding variants of uncertain significance and synonymous variants), there was a higher proportion of insertions/

Table 1. Patient characteristics.

Number of patients	839
Median age, years (range)	64 (22–96)
Gender, *n* (%)	
Female	242 (28.8%)
Male	697 (71.2%)
ctDNA analysis	
Number of tests	920
ctDNA detected, *n* (%)	661 (71.8%)
Median number of alterations (range)	2 (1–39)
Median maxVAF (range)	0.72 (0.0–72.8)
Histopathology, *n* (%)	
Clear cell	136 (68.7%)
Papillary	26 (13.1%)
Clear cell with sarcomatoid features	11 (5.6%)

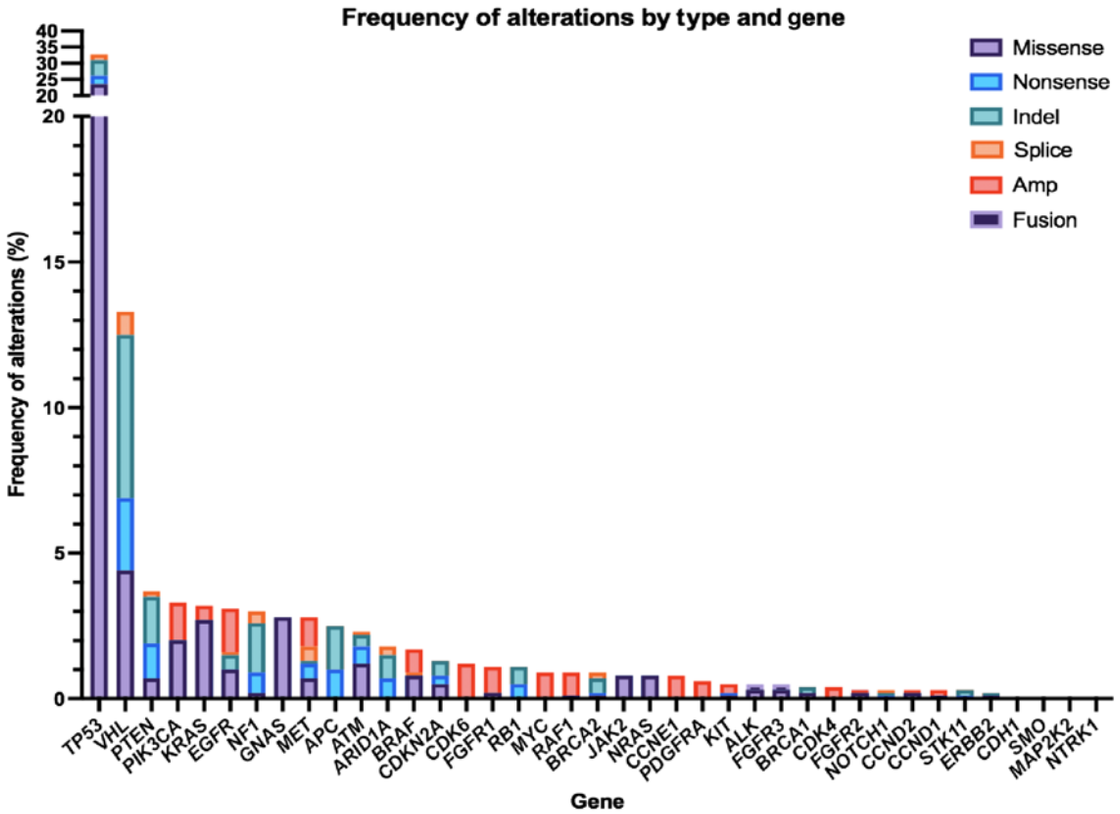

Figure 1.
Landscape of ctDNA alterations in all patients with mRCC.

deletions and SNVs compared with gene amplifications (87.7%; **Fig. 1**). Among 39 patients with ctDNA assessment at more than 1 timepoint, we examined changes in the detection of alterations in certain genes over time. As illustrated in Supplementary Fig. S1, we did see changes in the frequency of certain genes over time, though there were no discernable or consistent trends. This is likely because this is a clinical cohort, so there was no specified timing for when ctDNA needed to be drawn. Given this, it seems likely that treatment effects (e.g., ctDNA falling because a patient is responding to therapy) played a role in these changes.

Comparison of ctDNA and tissue

All 112 patients with available tissue-based genomic profiling results had detectable GAs in their tissue samples. The most commonly mutated genes with functional alterations were *VHL* (67.0%), *PBRM1* (40.2%), and *SETD2* (18.8%). Notably, several of the most commonly mutated genes (e.g., *PBRM1, SETD2*, and *KDM5C*) were assessed in tissue but were not included on the ctDNA panel. Of note, at least one ctDNA alteration was detected in 63.6% (42/66) of samples from Huntsman Cancer Center, and 54.3% (25/46) of samples from City of Hope. For concordance assessment, patients without any genomic alterations or who had only synonymous alterations in their ctDNA were excluded from the analysis. When examining 67 patients with mutations in genes assessed by both ctDNA and tissue platforms (excluding synonymous variants), a total of 228 GAs was detected (**Fig. 2**; Supplementary Table S1). Of these, 34.2% of the GAs identified on tissue were also identified by ctDNA, whereas 28.2% (42/149) of alterations detected via blood were also detected by tissue. One hundred and seven (46.9%) and 79 (34.6%) GAs were exclusively detected in blood and tissue, respectively (**Fig. 3**). When samples that were collected within 6 months of each other were analyzed, 50.8% (29/57 GAs) identified on tissue were also detected via blood, and this increased further to 60.8% (28/46 GAs) in samples collected within 3 months of each other.

Several unique findings in ctDNA warrant mention. First, targetable GAs in *EGFR* were detected in 4 patients exclusively in ctDNA (**Fig. 2**). Mutations in A647T (2 patients) and V843I represent previously reported resistance mechanisms to EGFR-TKIs; the fourth patient in this series had *EGFR* amplification (21, 22). Second, *TERT* promoter mutations (which our group has previously identified as a predictor of immunotherapy resistance) occurred in 7 patients (23). Third, consistent with phylogenetic subgroups identified in the TRACERx study, we identified patients with *PBRM1* mutations detected in tissue, with subsequent evolution of mutations in mTOR-related genes in ctDNA.

Discussion

We provide herein ctDNA results from the largest cohort of patients with mRCC to date. Our data address the paucity of information on the utility of ctDNA in mRCC and confirm that most patients have detectable GAs in ctDNA. Furthermore, a detailed comparison of

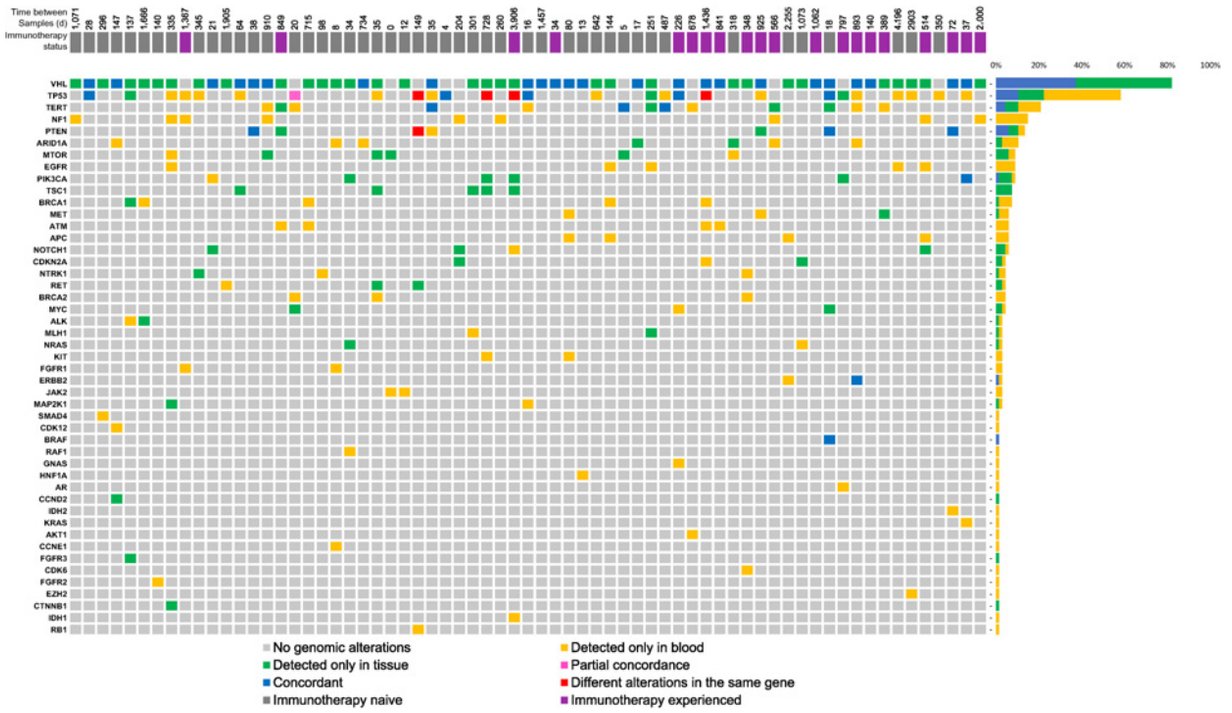

Figure 2.
Comparison of GAs detected in blood- and tissue-based platforms. Analysis limited to 74 genes interrogated by ctDNA platform.

tissue- and blood-based genomic profiling highlights the complementarity of these techniques; although a proportion of alterations are shared, multiple exclusive GAs were captured on ctDNA, including actionable mutations (e.g., *EGFR, ALK, MET*). Tumors are highly dynamic and the timeframe between both genomic tests is critical. Consistent with previous studies, there were higher levels of agreement between tissue and liquid assays when samples were contemporaneous (24, 25). In a subset of patients, we observed a pattern consistent with the branched evolution observed in the TRACERx Renal study. As in TRACERx, we observed an increase in alterations in the mTOR pathway in ctDNA specimens collected "distal" to tissue specimens.

Using ctDNA assessment for molecular testing to identify patients who may qualify for targeted therapy is standard of care in other malignancies, such as lung cancer. For instance, in patients with lung cancer being treated with first-generation *EGFR* inhibitors, such as erlotinib, ctDNA assessment for *EGFR* T790M resistance mutations may suggest potential benefit from osimertinib (26). It has been well established that the yield of ctDNA varies across different malignancies. Diseases that often present with widespread and bulky metastatic burden (e.g., non–small cell lung cancer, NSCLC) have a high ctDNA yield. In contrast, locally aggressive diseases such as glioblastoma (which has the added caveat of blood–brain barrier shielding) have lower rates of ctDNA detectability. RCC has low levels of ctDNA and falls near the end of this spectrum (16). A single institution study conducted by the authors of this article did show a strong association between measured tumor burden and ctDNA detection within mRCC (27). In addition, the timing of the ctDNA blood draw can also impact the level of ctDNA shed; studies have consistently shown that in the advanced-disease setting, treatment-naïve tumors and tumors progressing on therapy are more likely to shed ctDNA, whereas slow-growing tumors or tumors that are actively responding to therapy are less likely to shed ctDNA (28, 29). Notably, the ctDNA detection rates for the samples provided from both City of Hope and Huntsman Cancer Center fell below the rates seen across the broader ctDNA cohort. This may in part be due to the unique use of ctDNA in these centers. At both institutions, ctDNA profiling is often pursued when tissue-based profiling is not feasible, as in the case of low tumor burden or inaccessible tumors.

On the basis of these results, we propose that the utility of ctDNA is 2-fold. First, actionable mutations defined by ctDNA may yield unique therapeutic insights. For example, if we observed mutations in the mTOR pathway (genes including *TSC1, TSC2*, and *MTOR*) in a patient who is progressing on standard front-line regimens (e.g., nivolumab/ipilimumab or axitinib/pembrolizumab), a regimen, including an mTOR inhibitor (such as lenvatinib/everolimus), might be chosen as second-line therapy (30). Of course, actionable mutations may point to targets not normally considered in RCC and provide clinical trial opportunities. For instance, several patients in our series displayed mutations in *EGFR*. If refractory to existing targeted therapies and immunotherapy, we propose that these patients could attempt to secure compassionate use of next-generation EGFR-TKIs. We have previously reported a rare series of papillary patients with RCC with *ALK* fusions, seen in 4% of patients with NSCLC but rarely in RCC. All 3 patients in this series responded to the small-molecule ALK-inhibitor alectinib (31).

The second potential benefit of ctDNA assessment lends itself to the predictive benefit of selected mutations. Our group has recently reported a large experience comparing patients receiving VEGF-targeted therapy or immunotherapy for mRCC (23). In this series, *TERT* alterations were found to be associated with immunotherapy resistance. Interestingly, in three cases within this study, *TERT*

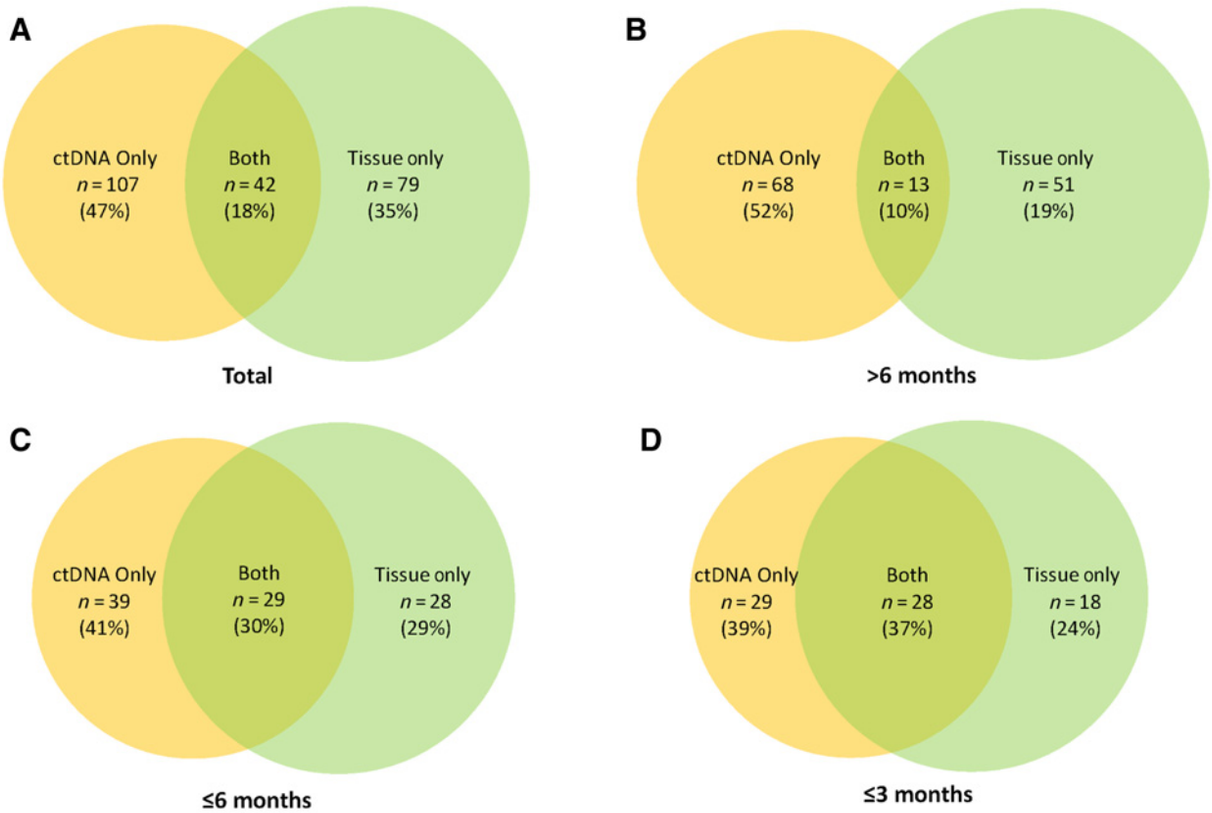

Figure 3.

The frequency of overlapping and exclusive genomic alterations of ctDNA and tissue-based platforms in (**A**) overall cohort and in subgroups separated by (**B**) more than 6 months, (**C**) equal or less than 6 months, and (**D**) equal or less than 3 months.

mutations were found to be exclusive to ctDNA and these evolved after patients had progressed on prior immunotherapy. Multiple studies have shown the *PBRM1* mutation to be associated with more benefit with single-agent immune checkpoint blocker in patients with RCC progressing on VEGF-targeted agents (3, 32). Although the ctDNA platform used herein did not capture *PBRM1* mutations, mutations in this gene were seen in a considerable number of patients in our cohort receiving tissue-based profiling.

Connecting tissue-based *PBRM1* mutations to exclusive mutations seen subsequently in ctDNA allows us to reconstruct the evolutionary patterns proposed by Turajlic and colleagues (10) in the recently published TRACERx Renal studies. The study defines populations with distinct disease phenotypes, including those with rapid progression or attenuated progression. Attenuated progression appears to be more consistent with a branched evolution of GAs, with many originating from *VHL* or *PBRM1* mutations, and subsequent alterations in *SETD2* or genes along the mTOR pathway. Our observation of patients with a branched evolution from *VHL/PBRM1→MTOR/TSC1* mutation (e.g., those with a tissue *VHL/PBRM1* mutation and exclusive mutation of *MTOR/TSC1* in ctDNA) supports these observed patterns. True to the TRACERx Renal study, these patients did have an attenuated pattern of progression with estimated survivals ranging from 19 to 113 months.

Limitations of this study include the lack of clinical data for the overall cohort; only in a select subset of patients with available tissue were robust clinicopathologic and treatment data available. The submission of clinical data was at the discretion of the treating physician; in many cases, pathology reports or treatment-related data were not supplied. In particular, the limited histologic information in the overall dataset makes it challenging to interpret the lower-than-normal frequency of certain canonical alterations in clear cell RCC (i.e., *VHL*). In the overarching cohort, we identified functional *VHL* alterations in 22% of patients, not far from the estimate of 20% of patients with *VHL* alterations in blood of our "concordance" cohort, with both blood and tissue available. In this concordance cohort, 67% of patients had functional *VHL* alterations using tissue-based assessment. A deeper look at this cohort suggests a median maximum variant allele frequency (maxVAF) of 1.10% in patients with *VHL* detected in blood, as compared with 0.30% in patients with no *VHL* detected (which falls below the assay's median level of detection of 0.4%; ref. 16). Although several factors have been shown to impact the %cfDNA (e.g., disease burden, rate of tumor growth, response to treatment), overall the maxVAF is an indicator of tumor shed, and it has been well established that decreased tumor shed is a limiting factor in the sensitivity of liquid biopsy assays (28, 33). In this case, it seems likely that decreased tumor shed, potentially due to smaller disease burden, slow tumor growth, ongoing response to therapy, or a combination of all three, led to some of these *VHL* alterations occurring below the liquid biopsy assay's limit of detection, therefore decreasing the observed frequency of *VHL* alterations in the cohort.

Our comparison of ctDNA profiling against tissue genomic profiling is also challenged by the use of three distinct tissue-based

platforms. Presumably, WES would identify a broader spectrum of GAs as compared with hotspot sequencing. In addition, we acknowledge that the utilization of ctDNA testing by these academic centers may not be reflective of how ctDNA testing is being used by the broader community (as illustrated by the atypical DNA detection rates within our cohort). Therefore, these results may not fully reflect findings from a broader study with more typical ctDNA use. We also chose to limit our assessment of concordance to those patients who had detectable ctDNA. This was to avoid confounding by patients who may have had undetectable ctDNA on account of clinicopathologic features (e.g., lower cumulative disease burden) and because patients who had attempted tissue molecular testing that could not be completed (e.g., because tissue quality/quantity was not sufficient for molecular analysis) were not included in this analysis. We also did not have uniform data on specific systemic agents and our population of patients was not treatment naïve.

Conclusions

In summary, we present the largest series of patients with mRCC evaluated for ctDNA alterations to date. Our findings add substantially to the paucity of data currently available on the utility of ctDNA in mRCC. In addition, our concordance study between tissue and ctDNA suggests a truly complementary role of ctDNA and tissue-based genomic profiling. In addition to the clinical implications of ctDNA assessment (e.g., identifying novel targets and offering potentially predictive biomarkers for therapeutic response), these results add significantly to our understanding of tumor evolution.

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Authors' Contributions

Z.B. Zengin: Conceptualization, resources, data curation, software, formal analysis, supervision, validation, investigation, visualization, methodology, writing–original draft, writing–review and editing. **C. Weipert:** Conceptualization, data curation, formal analysis, supervision, validation, investigation, visualization, methodology, writing–original draft, writing–review and editing. **N.J. Salgia:** Data curation, writing–original draft. **N. Dizman:** Data curation, writing–original draft. **J. Hsu:** Data curation, writing–original draft. **L. Meza:** Data curation, writing–original draft. **A. Chehrazi-Raffle:** Data curation, writing–original draft. **R. Muddasani:** Data curation, writing–original draft. **S. Salgia:** Data curation, writing–original draft. **J. Malhotra:** Data curation, writing–original draft. **N. Chawla:** Data curation, writing–original draft. **E.J. Philip:** Writing–original draft. **L. Kiedrowski:** Data curation. **B.L. Maughan:** Data curation. **N. Rathi:** Data curation. **D. Goel:** Data curation. **T.K. Choueiri:** Supervision, writing–original draft, writing–review and editing. **N. Agarwal:** Conceptualization, resources, data curation, supervision, writing–original draft, writing–review and editing. **S.K. Pal:** Conceptualization, resources, data curation, formal analysis, supervision, validation, investigation, visualization, methodology, writing–original draft, writing–review and editing.

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