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Characterization of Metastatic Urothelial Carcinoma via Comprehensive Genomic Profiling of Circulating Tumor DNA

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AUTHOR CONTRIBUTIONS

Neeraj Agarwal: Conceptualization, data curation, writing-original draft, and writing-review and editing. **Sumanta K. Pal**: Conceptualization, data curation, and writing-review and editing. **Andrew W. Hahn**: Writing-original draft and writing-review and editing. **Roberto H. Nussenzveig**: Data curation, writing-original draft, and writing-review and editing. **Gregory R. Pond**, **Sumati V. Gupta**, **Jue Wang**, **Mehmet A. Bilen**, **Gurudatta Naik**, **Pooja Ghatalia**, **Christopher J. Hoimes**, **Dharmesh Gopalakrishnan**, **Pedro C. Barata**, **Alexandra Drakaki**, **Bishoy M. Faltas**, **Lesli A. Kiedrowski**, **Richard B. Lanman**, **Rebecca J. Nagy**, **Nicholas J Vogelzang**, and **Ulka N. Vaishampayan**: Data curation and writing-review and editing. **Kenneth M. Boucher**: Statistical analysis, writing-review and editing. **Guru Sonpavde** and **Petros Grivas**: Conceptualization, data curation, writing-original draft, and writing-review and editing.

CONFLICT OF INTEREST DISCLOSURES

Neeraj Agarwal has acted as a paid consultant for Pfizer, Novartis, Merck, Genentech, Eisai, Exelixis, Clovis, and EMD Serano for work performed outside of the current study. Sumanta K. Pal has acted as a paid consultant for Genentech, Aveo, Eisai, Roche, Pfizer, Novartis, Exelixis, Ipsen, Bristol-Myers Squibb, and Astellas as well as received honoraria from Genentech for work performed outside of the current study. Lesli A. Kiedrowski, Richard B. Lanman, and Rebecca J. Nagy all are full-time employees of and shareholders in Guardant Health Inc. Ulka N. Vaishampayan has acted as a paid consultant for Pfizer, Exelixis, Bristol-Myers Squibb, and Bayer and has received honoraria from Genentech, Bayer, Bristol-Myers Squibb, and Exelixis for work performed outside of the current study. Guru Sonpavde has received grants and personal fees from Bayer, Pfizer, and Merck; personal fees from Sanofi, Novartis, Eisai, Janssen, Amgen, AstraZeneca, Genentech, Argos, Agensys, EMD Serano, UptoDate, Clinical Care Options, Physicians Education Resource, Research to Practice, and OncLive; and grants from Amgen-Onyx, Celgene, and Boehringer-Ingelheim. Petros Grivas has received personal fees from Genentech and Bristol-Myers Squibb for consulting, an educational unbranded speaker program, and fees to the Cleveland Clinic Foundation; Dendreon, Bayer, Merck, and Mirati for consulting and fees to the Cleveland Clinic Foundation; Oncogenex and Pfizer (fees to the Cleveland Clinic Foundation); Exelixis for consulting; Astra Zeneca for consulting and fees to the Cleveland Clinic Foundation; and Biocept, ClovisOncology, and EMD Serono for consulting for work performed outside of the current study.

Additional supporting information may be found in the online version of this article.

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Abstract

BACKGROUND—Biomarker-guided clinical trials are increasingly common in metastatic urothelial carcinoma (mUC), yet patients for whom contemporary tumor tissue is not available are not eligible. Technological advancements in sequencing have made cell-free circulating DNA (cfDNA) next-generation sequencing (NGS) readily available in the clinic. The objective of the current study was to determine whether the genomic profile of mUC detected by NGS of cfDNA is similar to historical tumor tissue NGS studies. A secondary objective was to determine whether the frequency of genomic alterations (GAs) differed between lower tract mUC (mLTUC) and upper tract mUC (mUTUC).

METHODS—Patients from 13 academic medical centers in the United States who had a diagnosis of mUC between 2014 and 2017 and for whom cfDNA NGS results were available were included. cfDNA profiling was performed using a commercially available platform (Guardant360) targeting 73 genes.

RESULTS—Of 369 patients with mUC, 294 were diagnosed with mLTUC and 75 with mUTUC. A total of 2130 GAs were identified in the overall mUC cohort: 1610 and 520, respectively, in the mLTUC and mUTUC cohorts. In the mLTUC cohort, frequently observed GAs were similar between cfDNA NGS and historical tumor tissue studies, including tumor protein p53 *(TP53) (P =* 1.000 and .115, respectively), AT-rich interaction domain 1A *(ARID1A) (P =* .058 and .058, respectively), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha *(PIK3CA) (P*

= .058 and .067, respectively), erb-b2 receptor tyrosine kinase 2 *(ERBB2) (P =* .565 and .074, respectively), and fibroblast growth factor receptor 3 *(FGFR3) (P =* .164 and .014, respectively). No significant difference was observed with regard to the frequency of GAs between patients with mLTUC and mUTUC.

CONCLUSIONS—Among patients with mUC for whom no tumor tissue was available, cfDNA NGS was able to identify a similar profile of GAs for biomarker-driven clinical trials compared with tumor tissue. Despite the more aggressive clinical course, cases of mUTUC demonstrated a circulating tumor DNA genomic landscape that was similar to that of mLTUC.

Keywords

bladder cancer; circulating tumor DNA; metastatic urothelial carcinoma; next-generation sequencing; upper tract urothelial carcinoma

INTRODUCTION

In 2016, a total of 76,690 new cases of urothelial carcinoma (UC) were diagnosed in the United States.[1] Of patients with muscle-invasive UC, approximately 50% develop metastatic disease.[2] Metastatic urothelial carcinoma (mUC) is incurable, with high cancer-related mortality. The backbone of first-line treatment for mUC remains cisplatin-based chemotherapy regimens, yet approximately 50% of patients are ineligible for these regimens due to preexisting medical comorbidities.[3,4] Five novel immune checkpoint inhibitors were recently approved for the treatment of patients with mUC.[5–9] However, the prognosis for these patients remains grim, with only 10% to 25% of patients reported to respond to immune checkpoint inhibitors.

UC has one of the highest tumor mutation burdens across all malignancies, and many genes are thought to contribute to tumor progression, including tumor protein p53 (*TP53*), retinoblastoma transcriptional corepressor 1 (*RB1*), and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*).[10] Despite the multiplicity of genomic alterations, to the best of our knowledge, no targeted therapies are currently approved for the treatment of mUC, most likely due to a lack of optimal patient selection in clinical trials. Biomarker-guided clinical trials are increasingly common, due in part to the growing availability of next-generation sequencing (NGS), ushering in an era of precision oncology. Recent clinical trials of targeted therapies in patients with mUC harboring specific genomic alterations (GAs) have shown promise.[11,12]

To date, all biomarker-guided clinical trials in patients with mUC have relied on NGS of tumor tissue to capture the mutational profile of a tumor.[13,14] Biopsies to obtain tumor tissue are expensive, labor-intensive, and invasive; do not address intrapatient tumor heterogeneity; and often are technically and logistically difficult. These limitations often delay treatment with biomarker-guided therapy either on or off clinical trials. Cell-free circulating DNA (cfDNA) NGS, also referred to as “liquid biopsy,” is an attractive alternative that can provide a real-time profile of a tumor’s mutational landscape in a dynamic (serial) fashion, attempting at the same time to recapitulate tumor heterogeneity and treatment resistance mechanisms. Herein, we compared the mutational landscape detected in circulating tumor

DNA (ctDNA) with tumor tissue in what to the best of our knowledge is the largest cohort of patients with UC presented to date (369 patients; 294 with metastatic lower tract UC [mLTUC]) and 75 with metastatic upper tract UC [mUTUC]). Because UTUC is a distinct clinical entity with a more aggressive clinical course compared with LTUC, we believe the current study is the first to provide a comparison of GAs in ctDNA from patients with mLTUC versus those with mUTUC.[15,16]

MATERIALS AND METHODS

In this study, two 10-cc Streck tubes of blood were collected from patients with a diagnosis of mUC (either mLTUC or mUTUC) at 13 academic medical centers across the United States and sent to Guardant Health Inc for cfDNA NGS. cfDNA NGS was performed by Guardant Health (Guardant360; Redwood City, California), a Clinical Laboratory Improvement Amendments (CLIA)-licensed, College of American Pathologists-accredited, New York State Department of Health-approved clinical laboratory, using their standard collection protocol. This comprehensive genomic test performs complete exon sequencing of all critical exons (those with known hotspots) and reports all 4 major classes of GAs (single-nucleotide variants [SNVs] in 73 genes, indels in 23 genes, fusions in 6 genes, and copy number amplifications [CNAs] in 18 genes). As per Guardant360's standard protocol, blood is collected in two 10-mL Streck tubes to obtain 5.0 ng to 30.0 ng of cfDNA from plasma and analyzed as described previously.[17] Patient demographics were obtained by retrospective review of specimen submissions for both cohorts (Table 1). Wholly deidentified data were used for the current analysis. Approval for the study, including a waiver of informed consent and a Health Insurance Portability and Accountability Act (HIPAA) waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817).

Two-sided Fisher's exact tests were used to compare the frequency of GAs between ctDNA NGS and historical tumor tissue studies (see Supporting Table 1). The false discovery rate method of Benjamini and Hochberg was used to adjust the *P* value for multiple comparisons.[18] The specific GAs and mutational landscape identified in both patient cohorts, mLTUC and mUTUC, were compared (see Supporting Table 2). Hotspot mutations were identified using MutationMapper (v 1.0.1), as described in Cerami et al.[19] The different types of GAs detected were grouped into mutational pathways for further analysis. The pathways used were DNA damage, cell cycle regulation, phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR), WNT/B-catenin, SWItch/sucrose nonfermentable (SWI/SNF), signal transduction, receptor tyrosine kinase (RTK), and RAS/RAF/mitogen-activated protein kinase (MAPK). The specific genes used for each pathway can be found in the legend for Figure 1.

RESULTS

Between October 2014 and April 2017, comprehensive somatic genomic profiling testing (CGP) of cfDNA from 369 patients with a diagnosis of mUC was performed (male:female ratio of 273:96 and a median age of 69 years [range, 39–91 years]). Of these 369 patients, 294 were diagnosed with mLTUC and 75 were diagnosed with mUTUC. GAs were

identified in 336 patients (91.1%); 265 patients had mLTUC (90%) and 71 patients had mUTUC (95%). A total of 2130 GAs were identified in the overall mUC cohort: 1610 and 520, respectively, in the mLTUC and mUTUC cohorts. Patient demographic data for both cohorts are listed in Table 1.

An average of 5.4 GAs (range, 0–35 GAs) and 6.8 GAs (range, 0–101 GAs), respectively, per patient were detected in the mLTUC and mUTUC cohorts. The 10 most frequent alterations in the mLTUC cohort were in *TP53* (48%), AT-rich interaction domain 1A (*ARID1A*) (17%), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) (14%), neurofibromin 1 (*NF1*) (12%), Erb-B2 receptor tyrosine kinase 2 (*ERBB2*) (10%), telomerase reverse transcriptase (*TERT*) (10%), fibroblast growth factor receptor 2 (*FGFR2*) (10%), *FGFR3* (10%), *MET* (9%), and *BRCA1* (9%) (Fig. 2A). For patients with mLTUC, frequently observed GAs were similar between ctDNA NGS and historical tumor tissue studies, including *TP53* (*P* = 1.000 and .115, respectively), *ARID1A* (*P* = .058 and .058, respectively), *PIK3CA* (*P* = .058 and .067, respectively), *ERBB2* (*P* = .565 and .074, respectively), and *FGFR3* (*P* = .164 and .014, respectively) (Fig. 3A)[20,21] (see Supporting Table 1). The 10 most frequent alterations in the mUTUC cohort were *TP53* (51%), *PIK3CA* (23%), *ARID1A* (20%), *TERT* (17%), epidermal growth factor receptor (*EGFR*) (14%), *BRCA1* (11%), *ERBB2* (11%), *FGFR3* (11%), *NF1* (11%), and *MET* (10%) (Fig. 2B). In patients with mUTUC, the frequency of GAs significantly differed between ctDNA NGS and historical tumor tissue studies for *TP53* (*P* = .000 and .000) and *FGFR3* (*P* = .000 and .000) (Fig. 3B) [22,23] (see Supporting Table 1). The 3 most common CNAs in the mLTUC cohort were in Raf-1 proto-oncogene, serine/threonine kinase (*RAF1*) (13.8%); cell cycle (cyclin E1) (*CCNE1*) (13.3%); and *ERBB2* (11.4%). In contrast, the 3 most common CNAs in the mUTUC cohort were in *PIK3CA* (15.6%), *CCNE1* (15.6%), and *RAF1* (10.9%). A total of 9 *FGFR3-TACC* gene fusions were detected in the overall cohort (6 in the mLTUC cohort and 3 in the mUTUC cohort).

Somatic alterations in *TP53* were prevalent in both cohorts; however, position R248Q/R/W, within the DNA-binding domain, a hotspot gain-of-function mutation, was present at a greater frequency (19 cases; 7.3%) in the mLTUC cohort compared with the mUTUC cohort (2 cases; 3%) (Fig. 4).[26] Hotspot mutations in *PIK3CA* (E545K and E542K), *FGFR3* (S249C), and *ERBB2* (S310F/Y) were detected as well, albeit at similar frequencies between cohorts (Fig. 4).[27–30]

GA-based pathway analysis was segregated by cohort (Figs. 1A and 1B). Overall, frequent alterations were found in genes coding for components of signal transduction pathways, DNA damage, and RAS/RAF/MAPK. Alterations in the PI3K/AKT/mTOR pathway, cell cycle pathway, and SWI/SNF pathway also were observed, although alterations in the individual genes comprising these pathways were less frequent. Overall frequencies of pathway-specific GAs were similar for both cohorts, although minor distinctions could be noted in the DNA damage and signal transduction pathways (Fig. 1C). Pathway analysis indicated targeted therapies against genes within the DNA damage, RTK, or RAS/RAF/MAPK pathways may have a greater chance of success. A trend toward an increased number of GAs in several genes within the mUTUC versus mLTUC cohort was observed (eg,

PIK3CA [23% vs 14%], *TERT* [17% vs 10%], and *EGFR* [14% vs 8%]) (see Supporting Table 2).

DISCUSSION

Biomarker-driven oncology (precision oncology) has the goal of driving clinical decisions and therapeutics based on the identification of actionable targets within a tumor's mutational landscape. cfDNA NGS is an ideal platform for upfront as well as recurrent testing at the time of disease progression in patients with advanced cancer due to its ease of collection, patient safety, ability to capture tumor heterogeneity, and reasonable cost compared with repeat tissue biopsy plus tissue NGS.[31,32] The primary objective of the current study was to characterize the mutational landscape of patients with mUC in cfDNA and compare these with historical tumor tissue NGS controls. A secondary objective was to compare the GAs in those with LTUC and UTUC. To the best of our knowledge, the current study is the second largest cohort (365 cases) to characterize the mutational landscape in UC to date and the largest plasma-based NGS evaluation of mUC.

When compared with previous reports of CGP in tumor tissue of LTUC by Ross et al (295 cases)[21] and The Cancer Genome Atlas (TCGA; 412 cases), the mutational landscape and frequency of GAs detected in ctDNA from the current study cohort (294 mLTUC cases) are very similar (Fig. 3A)[20,21] (see Supporting Table 1). In all 3 studies, *TP53* was the most common GA in LTUC (median, 48%; range, 48%−56%). The prevalence of potentially targetable GAs in LTUC, such as *PIK3CA* (median, 20%; range, 14%−22%), *FGFR3* (median, 14%; range, 10%−21%), and *ERBB2* (median, 12%; range, 10%−17%) also was similar between all 3 cohorts (see Supporting Table 1). Similar to studies using NGS of tumor tissue, mutations detected by NGS of cfDNA in *FGFR3, PIK3CA,* and *ERBB2* were more frequent at known hotspots (Fig. 4).[20] *ERBB2* amplifications (7%) and SNVs (5% in LTUC) serve as an example of a much needed candidate gene for targeted therapy in patients with mUC. The majority of the characterized sequence alterations in *ERBB2* observed were S310F/Y (15 cases), although there were 2 other cases in the extracellular domain as well as 9 in the protein kinase domain.

In the current study, cfDNA NGS was performed using a commercially available, CLIA-certified, and College of American Pathologists-accredited platform (Guardant360) that tests for GAs in 73 genes. In contrast, the tissue-based studies by Ross et al and TCGA were based on either large gene capture panels (>230 genes) or whole-exome sequencing, respectively. Therefore, due to the absence of select genes in our panel, several common GAs identified in the tumor tissue cohorts were not tested in the current study, including *MLL2, KDM6A,* and cyclin-dependent kinase inhibitor 2B (*CDKN2B*). A possible approach to remedy this issue would be to increase the number of genes examined in the cfDNA panel, perhaps up to ≥400 genes as currently used in several other commercially available panels for tumor tissue NGS. Significantly, Guardant Health plans to release a 500-gene, plasma-based NGS panel before the end of 2018, which will enable such future investigations with liquid biopsy. There is evidence with this assay that targetable alterations with variant allele fractions <0.1% may respond to "matched therapy," and therefore exquisite ctDNA sensitivity is critical.[33] Nevertheless, because to our knowledge none of the

excluded genes currently have targeted therapies with proven clinical benefit available, their absence does not limit the clinical usefulness of this cfDNA NGS panel for precision treatment in patients with mUC. Furthermore, only somatic alterations are reported in Guardant360 reports, not germline alterations. Germline alterations are called and excluded as per a proprietary bioinformatics process performed at Guardant Health Inc.

UTUC comprises only approximately 5% of all cases of UC, and therefore there have been far fewer genomic profiling studies of UTUC than LTUC.[34] Although to our knowledge the current study is the first analysis of GAs in mUTUC performed by cfDNA NGS, it also is to our knowledge the second largest CGP of UTUC after a study of 83 patients by Sfakianos et al.[22] In comparison with tumor tissue NGS studies by Sfakianos et al[22] and Moss et al (31 cases), [23] the results of the current study using cfDNA NGS detected a similar landscape of GAs, albeit at different frequencies than previously reported (Fig. 3B)[22,23] (see Supporting Table 1). In the current study cohort, we identified significantly fewer *FGFR3* alterations and a far greater prevalence of *TP53* alterations in ctDNA compared with tumor tissue NGS studies (Fig. 3B)[22,23] (see Supporting Table 1). However, a similar prevalence was noted for *PIK3CA* and *ARID1A* genes in all 3 studies (Fig. 3B)[22,23] (see Supporting Table 1). There are several possible explanations for the discrepancy in the frequency of GAs detected between studies of UTUC. All 3 studies have relatively small cohorts (<100 patients) and reflect different tumor stages and tumor DNA sources (primary tumor tissue DNA vs ctDNA). Approximately 53% of patients in the tissue-based NGS study by Sfakianos et al[22] had noninvasive bladder cancer (pTa or pT1), whereas all patients in the current study cohort had metastatic disease treated with possible interim therapies that may have induced clonal selection pressure at several time points. A prior study reported that early-stage UC is characterized by a greater prevalence of *FGFR3* mutations; in contrast, an increased frequency of *TP53* alterations is found in patients with advanced stage disease.[35] Despite the discordance in the frequency of GAs detected among the 3 cohorts, a consistent mutational landscape was noted when comparing tumor tissue and cfDNA NGS, thereby supporting the use of liquid biopsies to expedite the enrollment of patients in biomarker-guided clinical trials in mUC.

Despite a similar histologic appearance, UTUC is characterized by a more aggressive clinical course with a poorer prognosis compared with LTUC.[15,16] In the current study, we also reported what to our knowledge is the first comparison of the ctDNA mutational landscape in mLTUC and mUTUC. We did not observe a significant difference in the frequency of GAs between patients with mLTUC (294 patients) and mUTUC (75 patients). These results suggest that GAs alone do not fully explain the different clinical courses observed between mLTUC and mUTUC. This is consistent with previous studies that have demonstrated that epigenetic changes are more common in UTUC, and epigenetic alterations are not detected by NGS of ctDNA.[36] To our knowledge to date, 2 additional studies with much smaller cohorts have used tumor tissue NGS to compare the frequency of GAs between UTUC and LTUC.[22,23] In a cohort of 31 patients with UTUC, Moss et al reported a similar landscape of GAs in tumor tissue compared with the TCGA's LTUC cohort.[23] In contrast, significant differences in the frequency of GAs in tumor tissue were noted in a study of 83 patients with UTUC and 102 patients with LTUC. The results

indicated that *FGFR3* and *HRAS* alterations were more common in UTUC, whereas *TP53* and *RB1* alterations were more common in LTUC.[22]

Liquid biopsies have garnered increasing attention because they may capture a contemporary profile of a tumor's genomic landscape while avoiding many of the technical and logistical complications associated with tumor tissue biopsy. However, to the best of our knowledge, only limited data are available regarding cfDNA NGS in UC, with only 2 studies performed in much smaller cohorts currently reported for LTUC.[24,25] Including the current study, all 3 studies reported that >50% of patients with LTUC had detectable ctDNA alterations. Furthermore, the landscape of GAs detected in cfDNA NGS was similar in all 3 studies, albeit at different frequencies, most likely due to distinct cohort sizes and patients (Fig. 3C)[24,25] (see Supporting Table 1). Barata et al reported significant discordance in the mutational landscape in an intrapatient comparison of paired tumor tissue and ctDNA with advanced LTUC.[25] Vandekerkhove et al analyzed levels of ctDNA in patients with both localized LTUC and mLTUC, and found that patients with localized LTUC had significantly less to no ctDNA compared with patients with metastatic disease.[24] They also noted a direct relationship between the levels of ctDNA, the frequency of mutant alleles, and the presence of distant metastasis.

The cost of the cfDNA test depends on the platform/assay used and may be less expensive compared with tumor tissue NGS, especially if the gene panel is smaller. Considering the recent approval by the US Food and Drug Administration of a tumor tissue-based NGS platform, it will be interesting to assess the implications for the cost of cfDNA assays.[37] There is a growing body of literature regarding cfDNA NGS, including for example the report that if cfDNA NGS is repeated on the same patient with UC over time (especially with intervening therapies) tumor heterogeneity and evolution may be noted, which also is observed in other genitourinary malignancies.[25,38] There also is variability depending on the assay used; different companies may use different gene panels, platforms, bioinformatics methods, and cutoff levels of calling variants. The dynamic nature and ease of the cfDNA NGS can render it attractive for the possible earlier detection of treatment resistance or disease recurrence/progression, as well as for the assessment of resistance mechanisms. There are emerging data that cfDNA may correlate with tumor burden and treatment response.[39, 40] Ongoing research is aiming to answer these questions regarding the appropriate use of ctDNA NGS.

Although to the best of our knowledge no targeted therapies currently are approved for the treatment of mUC, ctDNA NGS tests can be used for multiple GAs that have matched targeted therapies currently under investigation. In the current study, *PIK3CA* was found to be altered in 14% of patients with LTUC and 23% of patients with UTUC. There is significant interest in *PIK3CA* in mUC, with 10 novel agents currently being studied in phase 1 or 2 clinical trials.[25] *ERBB2* was another commonly altered gene in all cohorts of LTUC (Fig. 1A). There are many *ERBB2* inhibitors currently approved for the treatment of other malignancies, yet to our knowledge none is approved for UC. Despite poor response rates to monotherapy with lapatinib or trastuzumab, especially in unselected patients, a recent report suggested that the combination of trastuzumab plus pertuzumab is promising, and studies of combinations of trastuzumab with small-molecule antihuman epidermal

growth factor receptor 2 tyrosine kinase inhibitors, such as lapatinib, neratinib, or tucatinib, are ongoing.[41–45]

One limitation of the current study was that no clinical data were available with which to correlate clinical outcomes or prior lines of treatment for metastatic disease with GAs detected. In addition, we did not have primary or metastatic tumor tissue available with which to compare GAs detected between tissue and cfDNA NGS platforms and thus we believe studies with paired tumor tissue and cfDNA analysis can validate the current study results further. Guardant360 reports variant allele frequencies with a higher sensitivity/lower limit of detection than tissue-based assays and comprehensive plasma-based assays and thus reports frequencies that are <1%, while maintaining near-perfect specificity. Due to tumor heterogeneity, allele frequencies in ctDNA are representative of multiple subclones, some of which are major whereas some are minor. It is not feasible to determine with certainty whether GAs that occur at similar frequencies originate from the same clone. Finally, normal DNA controls were not performed in the current study.

Conclusions

To the best of our knowledge, no targeted therapies currently are approved for the treatment of mUC, but biomarker-based patient selection remains critical. Currently, to enroll in any biomarker-guided clinical trials in mUC, patients must have tumor tissue NGS available. The results of the current study demonstrate that cfDNA-based liquid biopsy detects a similar profile of GAs when compared with tumor tissue NGS in patients with mUC. Therefore, cfDNA-based liquid biopsy could guide and expedite clinical trial enrollment, especially in those patients who do not have archived tumor tissue readily available or in cases in which fresh tumor tissue biopsies are not feasible. Based on the totality of available data, it appears logical that tumor tissue and/or cfDNA may be used for clinical trial eligibility and stratification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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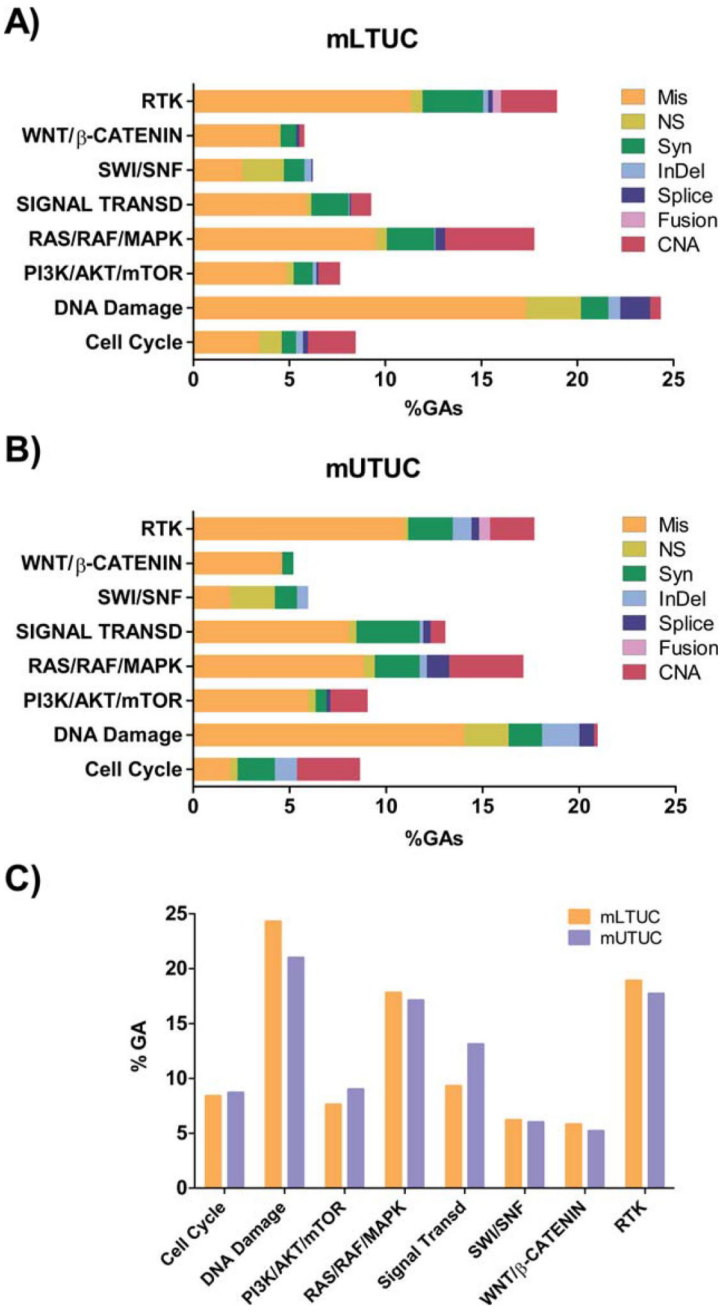

Figure 1.

Mutational landscape pathway analysis for the type of genomic alteration (GA) in (A) metastatic lower tract urothelial carcinoma (mLTUC) and (B) metastatic upper tract urothelial carcinoma (mUTUC) and (C) the overall frequency of GAs in both cohorts. Pathway definitions are: receptor tyrosine kinase (RTK) (epidermal growth factor receptor [*EGFR*]; fibroblast growth factor receptor 1 [*FGFR1*]; *FGFR2; FGFR3; KIT*; *MET;* MPL proto-oncogene, thrombopoietin receptor [*MPL*]; and platelet-derived growth factor receptor alpha [*PDGFRA*]), cell cycle (cyclin E1 [*CCNE1*], cadherin-1 [*CDH1*], cyclin-dependent kinase 4 [*CDK4*], *CDK6,* cyclin-dependent kinase inhibitor 2A [*CDKN2A], CDKN2B,* F-

box and WD repeat domain containing 7 [*FBXW7*], and *RB1*), DNA damage *(ATM, BRCA1, BRCA2,* cyclin D1 [*CCND1*], MutL homolog 1 [*MLH1*], and *TP53*), PI3K/AKT/mTOR (AKT serine/threonine kinase 1 [*AKT1*], mechanistic target of rapamycin kinase [*MTOR*], *PIK3CA,* phosphatase and tensin homolog [*PTEN*], serine/threonine kinase 11 [*STK11*], and TSC complex subunit 1 [*TSC1*]), RAS/RAF/MAPK (*BRAF; ERBB2;* guanine nucleotide-binding protein subunit alpha-11 [*GNA11*]; *HRAS; KRAS;* mitogen-activated protein kinase 1 [*MAP2K1]; MAP2K2;* mitogen-activated protein kinase 1 [*MAPK1]; MAPK3,* neurofibromin 1 [*NF1*]; *NRAS;* Raf-1 proto-oncogene, serine/threonine kinase [*RAF1*]; Ras-like without CAAX 1 [*RIT1*]; and A-Raf proto-oncogene, serine/threonine kinase [*ARAF*]), signal transduction (anaplastic lymphoma kinase [*ALK*]; androgen receptor [*AR*]; discoidin domain receptor tyrosine kinase 2 [*DDR2*]; estrogen receptor 1 [*ESR1*]; GATA-binding protein 3 [*GATA3*]; G protein subunit alpha Q [*GNAQ*]; *GNAS; MYC; NOTCH1;* neurotrophic receptor tyrosine kinase 1 [*NTRK1*]; *NTRK3;* tyrosine-protein phosphatase nonreceptor type 11 [*PTPN11*]; *RET;* Ras homolog gene family, member A [*RHOA*]; ROS proto-oncogene 1, receptor tyrosine kinase [*ROS1*]; and SMAD family member 4 [*SMAD4*]), and SWI/SNF *(ARID1A).* Abbreviations: CNA, copy number amplificiation; InDel, insertion/deletion; Mis, missense; NS, nonsense; Syn, synonymous; splice, splice-site.

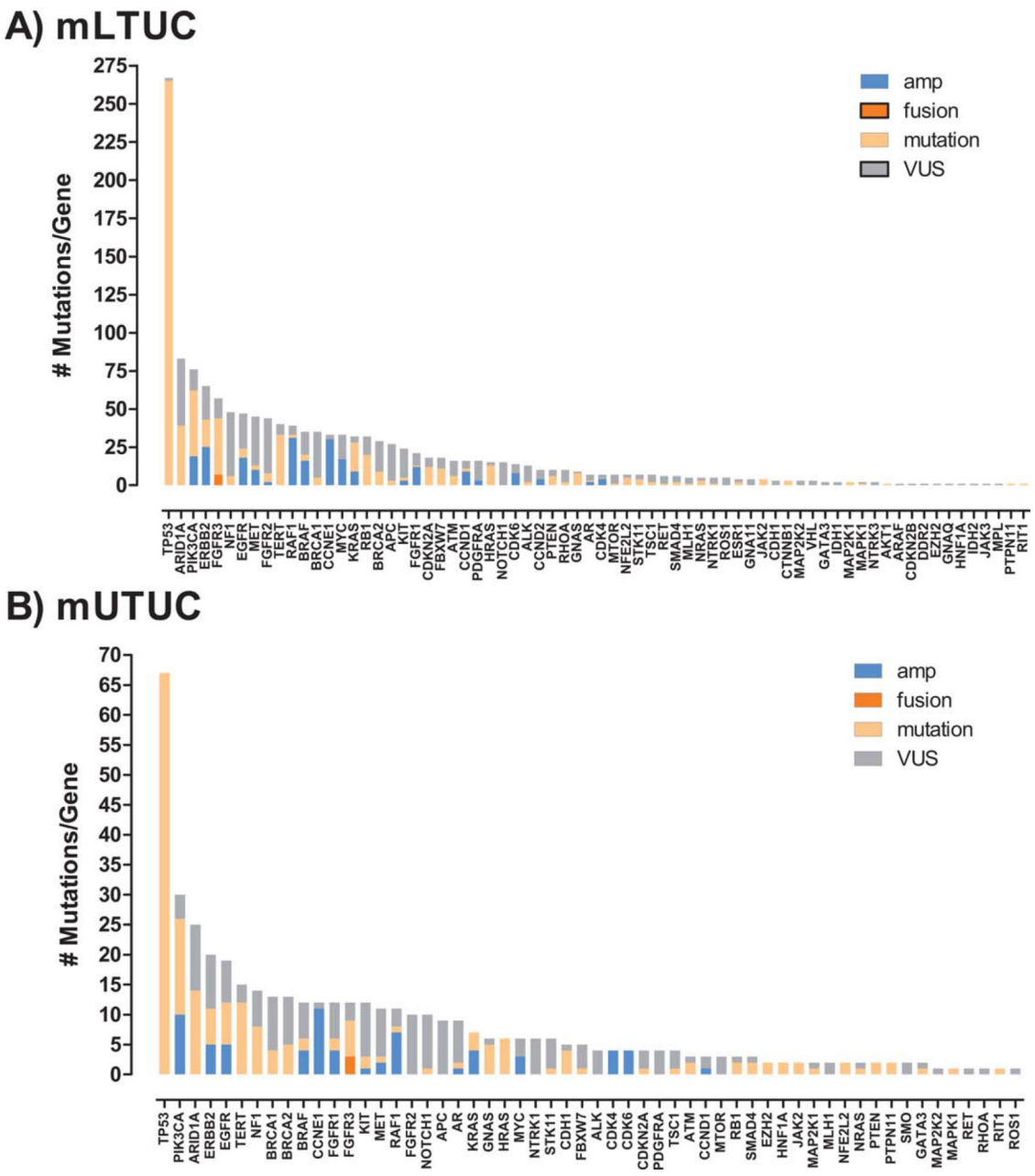

Figure 2.

Distribution of genomic alterations in (A) 265 patients with metastatic lower tract urothelial carcinoma (mLTUC) and (B) 71 patients with metastatic upper tract urothelial carcinoma (mUTUC). VUS indicates variant of unknown significance.

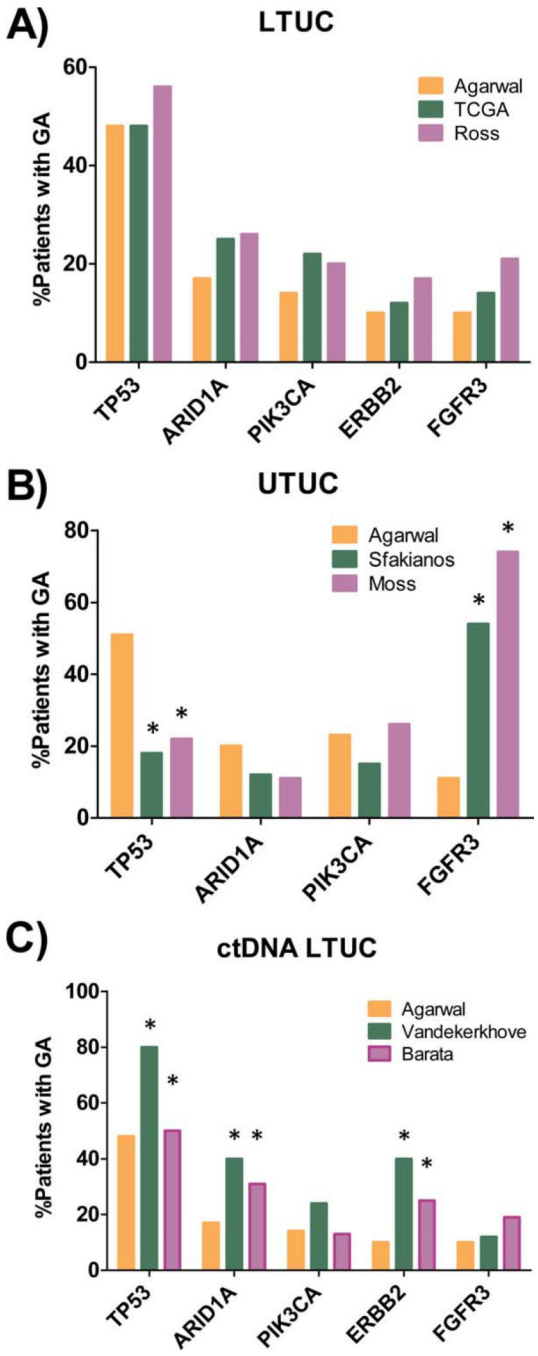

Figure 3.

Frequency of genomic alterations (GAs) in tumor protein p53 (*TP53*), AT-rich interaction domain 1A *(ARID1A),* phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha *(PIK3CA),* Erb-B2 receptor tyrosine kinase 2 *(ERBB2),* and fibroblast growth factor receptor 3 *(FGFR3)* in different reported data sets. (A) Comparisons of the frequency of tissue-based GAs identified in the 5 genes between the tissue-based studies from The Cancer Genome Atlas (TCGA)[20] and Ross et al[21] with the circulating tumor DNA (ctDNA) in lower tract urothelial carcinoma (LTUC) in the current study. (B) Comparisons of the frequency of

GAs identified in the 5 genes between the tissue-based studies from Sfakianos et al[22] and Moss et al[23] with the ctDNA in upper tract urothelial carcinoma (UTUC) in the current study. (C) Comparisons of the frequency of GAs between data sets in the current study, Vandekerkhove et al,[24] and Barata et al[25] in ctDNA from LTUC. Asterisk indicates a statistically significant difference ($P<.05$) between the ctDNA next-generation sequencing results in the current study and both tumor tissue studies.

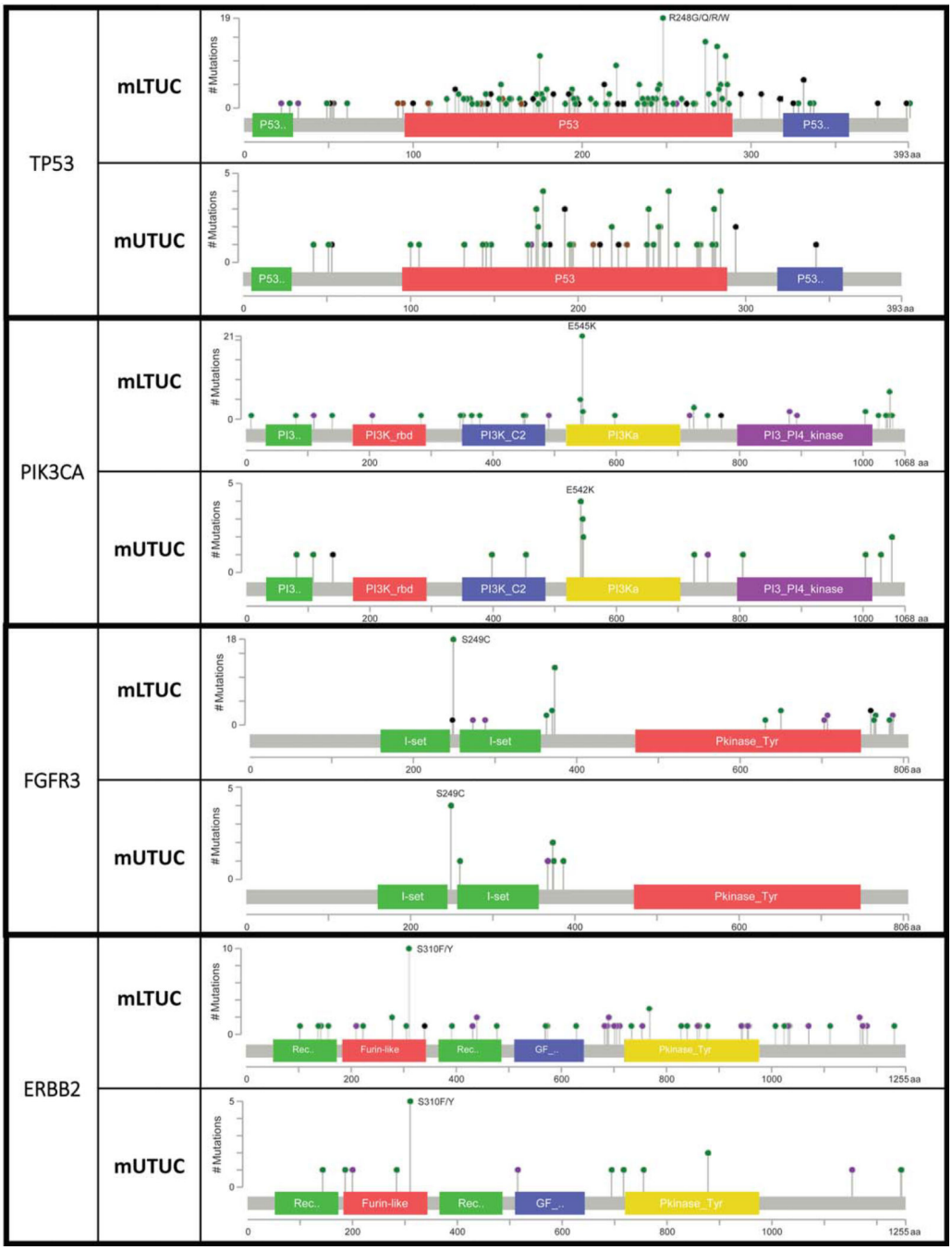

Figure 4.

Lollipop plot highlighting genomic alterations of select genes (tumor protein p53 [*TP53*], phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha [*PIK3CA*], fibroblast growth factor receptor 3 [*FGFR3*], and Erb-B2 receptor tyrosine kinase 2 [*ERBB2*]) in metastatic upper tract urothelial carcinoma (mUTUC) and metastatic lower tract urothelial carcinoma (mLTUC). Hotspot amino acid substitutions are indicated for each gene. Circles are colored with respect to the corresponding mutation types, and the height of the line is proportional to the number of mutations at the specified position. In the case of different

mutation types at a single position, the color of the circle is determined with respect to the most frequent mutation type. Mutation types and corresponding color codes are as follows. Green circle indicates missense mutations; brown circle, truncating mutations (nonsense, nonstop, frameshift deletion, frameshift insertion, and splice site); black circle, inframe mutations (inframe deletion and inframe insertion); purple circle, other mutations (all other types of mutations).

TABLE 1.

Patient Characteristics

Diagnosis	mLTUC	mUTUC
No. of patients	294	75
Patients with GA, no. (%)	265 (90)	71 (95)
Median age (range), y	69 (31–91)	69 (40–90)
Female, no. (%)	66 (22)	30 (40)
Male, no. (%)	228 (78)	45 (60)
Time between diagnosis and G360, mo		
Average	24	12
Median	11	6
Range	0–147	0–49
Samples for which dx date was unknown, no. (%)	223 (67)	58 (72)
Genomic alterations		
SNV, no. of positive tests (% of tests with GAs)	292 (98)	77 (100)
CNA, no. of positive tests (% of tests with GAs)	94 (31)	24 (31)
Indels, no. of positive tests (% of tests with GAs)	23 (8)	11 (14)
Fusions, no. of positive tests (% of tests with GAs)	6 (2)	3 (4)

Abbreviations: CNA, copy number amplifications; dx, diagnosis; G360, Guardant360; GA, genomic alteration; mLTUC, metastatic lower tract urothelial carcinoma; mUTUC, metastatic upper tract urothelial carcinoma; SNV, single-nucleotide variant.