Circulating Tumor DNA as a Biomarker in Patients With Stage III and IV Wilms Tumor: Analysis From a Children's Oncology Group Trial, AREN0533

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abstract

PURPOSE The utility of circulating tumor DNA (ctDNA) analyses has not been established in the risk stratification of Wilms tumor (WT). We evaluated the detection of ctDNA and selected risk markers in the serum and urine of patients with WT and compared findings with those of matched diagnostic tumor samples.

PATIENTS AND METHODS Fifty of 395 children with stage III or IV WT enrolled on Children's Oncology Group trial AREN0533 had banked pretreatment serum, urine, and tumor available. Next-generation sequencing was used to detect ctDNA. Copy-number changes in 1q, 16q, and 1p, and single-nucleotide variants in serum and urine were compared with tumor biopsy data. Event-free survival (EFS) was compared between patients with and without ctDNA detection.

RESULTS ctDNA was detected in the serum of 41/50 (82%) and in the urine in 13/50 (26%) patients. Agreement between serum ctDNA detection and tumor sequencing results was as follows: 77% for 1q gain, 88% for 16q deletions, and 70% for 1p deletions, with κ-coefficients of 0.56, 0.74, and 0.29, respectively. Sequencing also demonstrated that single-nucleotide variants detected in tumors could be identified in the ctDNA. There was a trend toward worse EFS in patients with ctDNA detected in the serum (4-year EFS 80% *v* 100%, *P* = .14).

CONCLUSION ctDNA demonstrates promise as an easily accessible prognostic biomarker with potential to detect tumor heterogeneity. The observed trend toward more favorable outcome in patients with undetectable ctDNA requires validation. ctDNA profiling should be further explored as a noninvasive diagnostic and prognostic tool in the risk-adapted treatment of patients with WT.

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INTRODUCTION

Wilms tumor (WT) is the most common malignant kidney tumor in the pediatric population,[1] with 500 new cases being diagnosed annually in the United States. Although outcomes of children diagnosed with WT have significantly improved over the past few decades, there remain significant challenges to optimizing therapy such that all patients are cured while side effects of therapy are minimized. A subset of patients experience relapse after therapy, and survival after relapse is unacceptably low.[2-4] Recent clinical research efforts have focused on identifying patients who are at increased risk of relapse, demonstrating the prognostic relevance of specific tumor genomic features at diagnosis, including loss of heterozygosity (LOH) of 16q and 1p and, more recently, gain of 1q.[5,6] On the basis of these observations, the Children's

Oncology Group (COG) recently implemented treatment intensification for patients with LOH of 1p and 16q tumor genetics, resulting in marked improvements in outcome.[7]

Liquid biopsy is a novel technology that is recently being applied to adult and pediatric malignancies to detect, quantify, and profile ultrarare strands of circulating tumor DNA (ctDNA) as a screening method.[8-10] These technologies are already used clinically to select patients for targeted therapies in the setting of colorectal cancer, NSCLC, and breast cancer.[11-17] Previous studies indicate that ctDNA is detectable in a range of pediatric solid tumors; however, those studies exploring ctDNA in patients with WT have been limited by sample size and cohorts of patients with a mix of risk-defining clinical features.[9,18-26] Whether recently identified stratifying genomic features, namely 16q and 1p LOH and 1q

CONTEXT

Key Objective

Is circulating tumor DNA (ctDNA) detectable in the blood and/or urine of patients with stage III and IV Wilms tumor with next-generation sequencing? If ctDNA is detectable, can prognostically relevant somatic variants be identified? Is detection associated with outcome?

Knowledge Generated

In this retrospective cohort, ctDNA was detected in the majority of serum but less than half of urine samples from the same patients. Identification of deletions of chromosomal arms 1p and 16q and gains of 1q were detectable in ctDNA. In most but not all patients, ctDNA findings correlated with genomic alterations in corresponding tumors. All recurrences and deaths occurred in patients who had detectable levels of ctDNA at the time of diagnosis.

Relevance

This study demonstrates that ctDNA analysis may improve prognostication and risk-stratified treatment selection for patients with Wilms tumor with advantages of using minimally invasive technology and potential for overcoming challenges of tumor heterogeneity.

copy gains, can be identified in the blood of patients with WT using liquid biopsy has yet to be explored.

In this study, we used a next-generation sequencing approach to profile cell-free DNA extracted from banked serum and urine samples collected from patients with stage III or IV WT. We leveraged genome-wide assessment of copy-number alterations (CNAs) to determine which patients had detectable levels of ctDNA, explore whether the presence of ctDNA in the blood or urine of these patients correlates with outcome, and evaluate whether ctDNA can serve as an alternative source of tumor material for detecting the presence of 1q copy gains and 16q and 1p LOH.

PATIENTS AND METHODS

Patient Selection

Our cohort consisted of patients who had previously been enrolled and treated on the COG prospective AREN0533 trial with a diagnosis of stage III or IV WT for which matched serum, urine, and tumor samples were banked and available at the COG biopathology center.[7,27] All patients provided written informed consent. Eligible patients were those younger than 15 years diagnosed on the basis of imaging with a stage III or IV WT. Patients with stage III WT were eligible for AREN0533 only if tumors were found to have LOH in 1p and 16q. All participating institutions from which data and samples are derived obtained institutional review board approval before study conduct.

Sample Preparation, DNA Extraction, and Sequencing

Specimen collection and processing procedures as well as the details of standard DNA extraction techniques and sequencing library preparation are detailed in the Data Supplement (online only). All samples were profiled for the detection of CNAs with ultra-low-passage whole-genome sequencing (ULP-WGS) and analyzed with the ichorCNA software as previously described.[22,28]

A targeted next-generation sequencing assay, OncoPanel, was performed to detect mutations in formalin-fixed paraffin-embedded tumor tissue, serum, and urine ctDNA (additional details on sequencing and analysis are presented in the Data Supplement).[29,30]

Annotation of Recurrent CNAs

CNAs of chromosomal arms 1p, 1q, and 16q were identified from ULP-WGS data by extracting the log2 fold-change for data from the segmental files generated from the ichorCNA algorithm. The low coverage of this technique required that we rely on a large segment of the chromosome to confidently identify the copy-number variants. In this study, copy-number losses and gains were called if they involved at least half of the chromosome arms of interest as indicated by the start and stop genomic coordinates in the segmental files.

Statistical Methods

Fifty patients were eligible for inclusion in this study. Descriptive statistics were computed as relative frequencies for categorical variables and means, standard deviations, and ranges for continuous variables. Previously analyzed individual patient data for LOH of 1p, LOH 16q, combined LOH 1p AND 16q, and 1q gain from AREN03B2 were included for comparison.

To evaluate ctDNA detection rates overall, the distribution of % ctDNA detected in urine and serum were separately plotted as box/violin plots. Rates of marker positivity were calculated separately for each marker, specimen type, and source (current study v AREN03B2). Raw rates of agreement between specimen sources and associated 95% CIs were computed. As these rates can be skewed by underlying prevalence of a marker as well as chance sampling

variability, kappa coefficients for inter-rater reliability in detecting each marker were also computed.[31]

Event-free survival (EFS; defined as the time from enrollment on AREN0533 to the earliest of disease relapse, progression, secondary malignancy, or death due to any cause) and overall survival (OS; defined as the time from enrollment to death due to any cause) were compared by ctDNA detection (yes v no) using Kaplan-Meier estimates and log-rank tests, where patients not experiencing an event of interest were right-censored at their last known disease assessment (EFS) or last known vital status (OS).

At the request of reviewers, potential associations between ctDNA detection in serum or urine and disease burden variables (metastatic disease, tumor diameter, lymph node status, local stage, and renal sinus involvement) were tested with Fisher exact tests. All analyses were performed using the R program for statistical computing.

RESULTS

ctDNA Is Detectable in the Serum and Urine of Patients With WT

Descriptive statistics for the included 50 patients are given in Table 1. Eighty-six percent of patients presented with stage III disease at the primary tumor site, defined by an upfront biopsy, positive lymph nodes, positive margins, tumor rupture, or peritoneal implants. Ninety-two percent of included patients presented with stage IV disease. All patients were initially considered to have favorable-histology WT, but five patients (10%) were found to have focal or diffuse anaplasia at delayed nephrectomy.

ctDNA was detected in the serum of 82% of patients, whereas ctDNA was only detected in the urine of 27% of patients. The lower limit of detection for ctDNA by ULP-WGS with ichorCNA analysis is 3% of the total cell-free DNA extracted from a sample.[28] The maximum amount of ctDNA detection in the serum and urine was 66% and 90%, respectively (Fig 1A). All five patients found to have anaplasia at delayed resection had detectable levels of ctDNA in the serum, and 2/5 anaplastic patients additionally had detectable ctDNA in the urine. In reviewer-requested ad hoc analyses, ctDNA detection in serum was more likely with increased local stage ($P = .051$ among all patients; $P = .036$ in the subset with upfront nephrectomies wherein stage was not due to biopsy). Other disease burden variables explored were not associated with ctDNA detection in serum. No disease burden variables were associated with ctDNA detection in urine (Data Supplement).

Somatic Variants Identified From Tumor Biopsies Can Be Detected in ctDNA

Chromosomal CNAs of 1p, 16q, and 1q are associated with outcome in patients with WT.[5,6] We next studied whether these variants could be detected in the tumors or liquid biopsy samples by ULP-WGS. Rates of detection of LOH 1p,

LOH 16q, LOH 1p AND 16q were fairly stable across specimen sources (Fig 1B and Data Supplement), whereas rates of detection of 1q gain were substantially higher in serum ctDNA (63.3%) and urine ctDNA (66.7%) than in matched diagnostic tumor analyzed contemporaneously as part of this project (44.4%) or as reported previously on AREN03B2 (32.4%; Table 2).[7,32] This enrichment could be attributed, at least in part, to the significantly higher rate of ctDNA detection in the serum among patients whose tumors demonstrated gain in 1q, which was noted in ad hoc analyses ($P = .01$; Fig 1B and Data Supplement). In fact, all patients that were found to have a 1q gain in the tumor by ULP-WGS had detectable levels of ctDNA in the serum. Rates of agreement in biomarker classification among pairs of specimen types ranged from 67% to 90% for LOH 1p, 75% to 88% for LOH 16q, 80% to 90% for combined LOH 1p AND 16q, and 77% to 89% for 1q gain (Table 3 and Figs 1C-1E). In general, across the markers evaluated, agreement was lowest between urine and tumor and highest between serum and tumor, where tumor was presently analyzed or reported from AREN03B2.

Kappa coefficients for agreement between analytic sources ranged from 0.11 to 0.78 (slight to substantial) for LOH 1p; 0.53-0.74 (moderate to substantial) for LOH 16q; –0.04 to 0.61 (none to moderate) for combined LOH 1p AND 16q; and 0.56-0.77 (moderate to substantial) for 1q gain. For all but 1q gain, the strongest association was between serum ctDNA and tumor; for 1q gain, the strongest association (kappa = 0.77) was between urine ctDNA and tumor as analyzed on AREN03B2 (Table 3).

Panel sequencing of tumor, serum, and urine samples with detectable levels of tumor DNA content also revealed that single-nucleotide variants (SNVs) detected in one tissue type were often detected in other tissue types. We focused our analysis of somatic SNVs on genes known to be recurrently mutated in WT samples and targeted in our existing OncoPanel hybrid capture assay (WT1, CTNNB1, DICER1, MYCN, TP53, BCOR, BCORL1, ASXL1, MAP3K4, and ARID1A).[33] In patients with available tumor sequencing and sufficient ctDNA levels in serum for sequencing, we found that the most commonly mutated genes were CTNNB1 and WT1, consistent with previous reports (Fig 2).[33] We also found that the majority of SNVs detected in tumors were also detected at lower allelic fraction in ctDNA but detection of some SNVs also suggested subclonal heterogeneity within the patient's disease (Fig 2).

Rare Events Occur in Patients With Detectable ctDNA

Within our cohort of 50 patients, those with ctDNA detected in their serum trended toward worse long-term EFS (4-year EFS = 80.41%) than those without (4-year EFS = 100%; log-rank $P = .14$; Fig 3A). Patients with ctDNA detected in serum had a 4-year OS of 82.79%, whereas those without ctDNA detected in serum had a 4-year OS of 100% (log-

TABLE 1. Baseline Characteristics of Patients

Characteristic	Overall (N = 50)
Sex, No. (%)	
Female	26 (52)
Male	24 (48)
Age, months	
Mean (SD)	53 (26)
Range	7-169
Race, No. (%)	
Black or African American	11 (22)
Unknown	3 (6)
White	36 (72)
Ethnicity, No. (%)	
Hispanic or Latino	8 (16)
Not Hispanic or Latino	40 (80)
Unknown	2 (4)
Disease stage, No. (%)	
III	4 (8)
IV	46 (92)
Local tumor stage, No. (%)	
I	1 (2)
II	6 (12)
III	43 (86)
Lymph node status, No. (%)	
Negative	13 (45)
Positive	16 (55)
Not sampled[a]	21
Initial diagnostic procedure, No. (%)	
Complete nephrectomy	29 (58)
Lung biopsy	1 (2)
Renal biopsy	20 (40)
Tumor diameter, cm	
Mean (SD)	13 (3)
Range	6-21
Missing/unknown	20
Histology, No. (%)	
Diffuse anaplastic WT	4 (8)
Favorable histology WT	45 (90)
Focal anaplastic WT	1 (2)

Abbreviations: SD, standard deviation; WT, Wilms tumor.

[a]The diagnostic procedure was biopsy.

rank P = .20; Fig 3B). Although the overall sample size and few EFS and OS events in this 50-patient cohort precluded statistical significance, the nearly 20% difference in long-term EFS and 17% difference in long-term OS may be clinically significant and warrants further validation in a prospective cohort. Removing patients from this analysis whose diagnoses were updated to anaplastic WT at post-chemo delayed resection had minimal impact on these comparisons (Data Supplement).

The trends toward worse EFS and OS observed among patients with ctDNA detected in serum were also observed for patients with ctDNA detected in urine, but with a lower level of discrimination. Patients with ctDNA detected in urine had a 4-year EFS of 76.92% and 4-year OS of 76.92%, whereas those without had a 4-year EFS of 88.57% and 4-year OS of 91.43% (EFS log-rank P = .39 and hazard ratio = 1.85; OS log-rank P = .13 and hazard ratio = 3.20; Data Supplement). Excluding patients with anaplasia at delayed resection had a more substantial impact on relative EFS and OS (Data Supplement).

DISCUSSION

The past several decades of clinical trials for patients with WTs has resulted in a dramatic improvement in outcomes, with the majority of patients now cured. However, patients who experience relapses have a markedly worse outcome even when treated with intensified regimens. Furthermore, emerging evidence demonstrates that the use of clinical measures of disease burden alone does not risk stratify as precisely as integration of histopathologic and molecular features of a patient's disease. It also remains unclear how to include molecular prognostic features present in only subclonal populations of tumor cells. Ongoing efforts to implement these additional prognostic biomarkers of outcome suggest that improved precision of risk stratification could further reduce the rate of disease progression or relapse after initial therapy and mitigate risks of long-term toxicities by facilitating further intensity reduction for some patients without jeopardizing outcomes.

Liquid biopsies are emerging as a new tool for prognostication and precision medicine in cancer.[11-18,24] Previous studies have demonstrated that the detection of ctDNA in the blood of patients with WT was feasible, but these studies were too small to demonstrate associations with other prognostic features or outcomes.[19,21-23,25] In this study, we profiled samples collected from a cohort of 50 patients with matched tumor, serum, and urine who were treated on the COG study AREN0533 for patients with newly diagnosed stage III and IV disease. We found that the majority of these patients shed sufficient levels of ctDNA into their serum to be reliably detectable by a low-sensitivity next-generation sequencing approach that identifies segmental and chromosomal copy-number variants. By contrast, only a minority of patients had detectable levels of ctDNA in their urine, suggesting that more sensitive assays or larger sample volumes may be needed to make urine-based liquid biopsies useful in this disease.

ULP-WGS can be used to detect ctDNA when it comprises at least 3% of the cell-free DNA content isolated from a
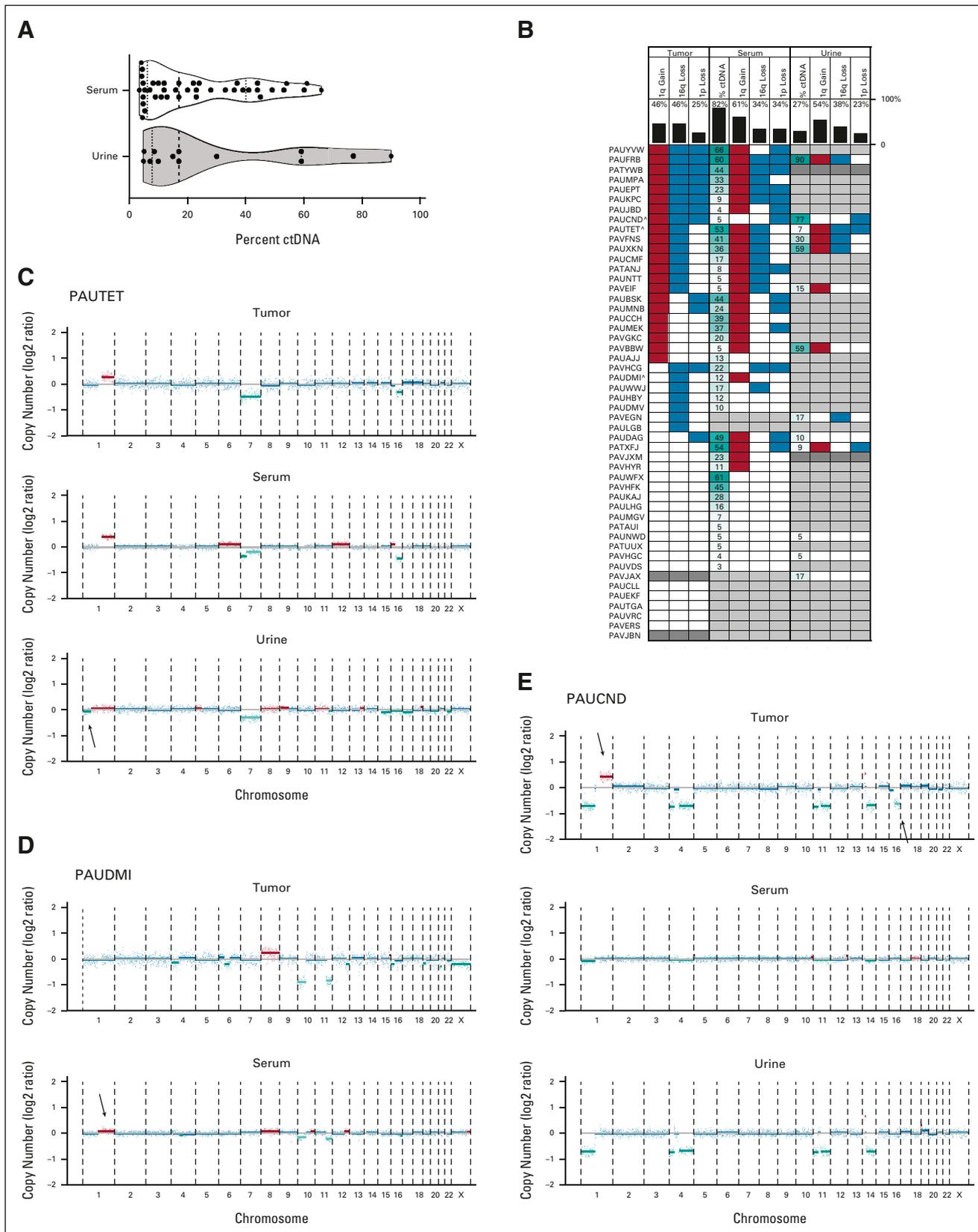
FIG 1. Detection of ctDNA by identification of copy-number variants in serum and urine of patients with WT. (A) Violin plot of ctDNA levels in serum and urine samples restricted to cases with ctDNA above the limit of detection by ULP-WGS. Dashed vertical line within each violin plot indicates the median and the dotted lines indicate the boundaries between the first and second quartiles and the third and fourth quartiles. (continued on following page)

FIG 1. (Continued). Probability densities are reflected by the upper and lower borders of the violin plot. Individual ctDNA data are indicated by black dots. (B) Summary of CNAs in chromosomes 1p, 1q, and 16q by case across sample types. Tissue is indicated at the top of the plot, data type indicated in the column label, and vertical black bars indicate the percent of total evaluable samples containing the variant (or with detectable ctDNA). Red blocks indicate copy gains, blue indicates copy losses, white indicates that a variant was not detected, dark gray indicates an unevaluable sample (no tissue or insufficient DNA for sequencing), and light gray indicates that no ctDNA was detectable in the sample. Percent ctDNA content indicated by number with higher ctDNA content shaded in dark teal and lower ctDNA by light teal. Cases plotted in (C-E) include "a" in the patient identifier. (C-E) Genome-wide plots represent the log2 ratio for each data point with blue data equivalent to two copies of the genomic location, teal equal to copy-number loss, and red equal to copy-number gains. Chromosomal segmental medians are also plotted as horizontal lines with light teal segments representing likely subclonal events. Arrows indicate CNAs that are not present in all matched samples from the patient. Case identifiers are present at the top of the panels. CNA, copy-number alteration; ctDNA, circulating tumor DNA; WT, Wilms tumor.

liquid biopsy sample.[28] In previous studies of other pediatric solid tumors, we have found that this limit of detection is a convenient and clinically relevant cutoff for distinguishing patients with high ctDNA ($\geq$ 3%) from patients with low ctDNA (< 3%).[24] Consistent with this previous work, we found that none of the patients enrolled on this study with low (undetectable) ctDNA in their serum experienced a relapse and all survived. Although this difference in PFS and OS was not significant, these findings justify further validation in prospective cohorts and suggest that ctDNA levels could further refine risk stratification for patients with stage III-IV WT. We also found that all patients later found to have localized or diffuse anaplasia in their tumors at the time of surgery, shed ctDNA into the serum and urine, further supporting the concept that detection of ctDNA by ULP-WGS may be associated with more

aggressive disease in patients with WT, an important finding to also validate in an expanded cohort.

In this study, we demonstrated that somatic variants present in the tumor could be detected in the serum and urine of patients with detectable levels of ctDNA. These findings demonstrate the practice changing potential of liquid biopsies to detect the presence of validated prognostic molecular events in the context of up-front chemotherapy without biopsy. Although the level of correlation between tumor profiling and ctDNA samples was high, we also observed discrepancies between samples that suggest the presence of subclonal heterogeneity in these patients, most of whom had metastatic disease. In other cancers, ctDNA has been shown to better capture the presence of subclonal variants than
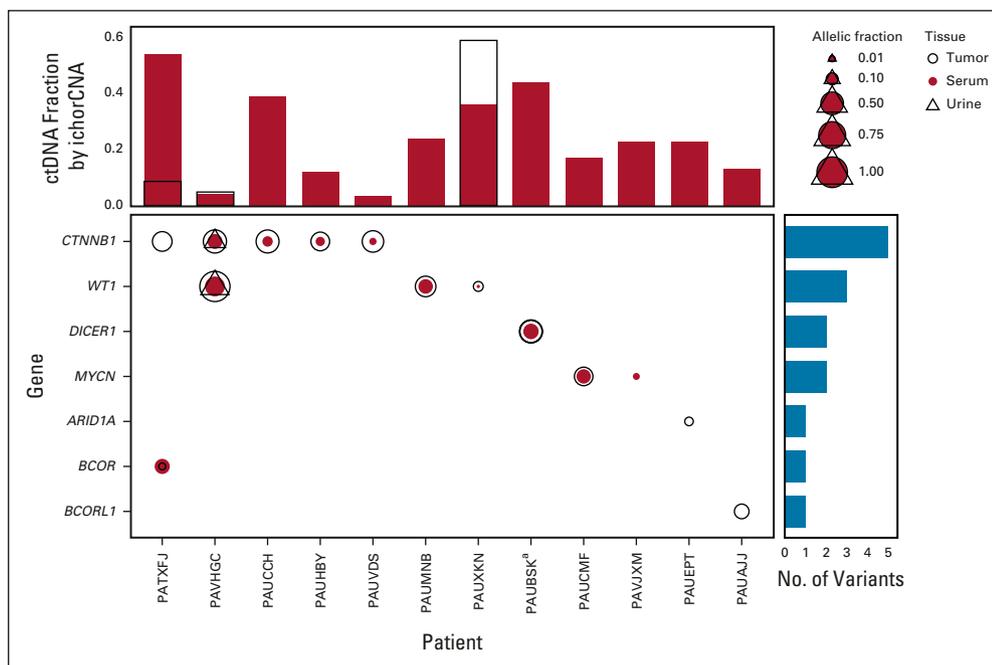
FIG 2. SNVs identified in tumors and matched ctDNA samples. Plotted are somatic variants identified in genes frequently mutated in WTs. Open circles indicate variants identified in tumors, red circles indicate variants identified in serum, and open triangles identified from urine. The size of the symbols reflects the relative allelic fraction of the identified events. Vertical bar plots reflect the fraction of ctDNA identified in the serum (red bars) and urine (open bars with black outline). Horizontal blue bars summarize the number of variants identified in the indicated gene. [a]A case with two variants in the DICER1 gene identified in both the tumor and the serum. ctDNA, circulating tumor DNA; SNV, single-nucleotide variant; WT, Wilms tumor.

TABLE 2. Detection Rates for Somatic Variants by Specimen Type

	LOH 1p			LOH 16q			LOH 1p and 16q			1q Gain		
Sample	Negative, No. (%)	Positive, No. (%)	NA	Negative, No. (%)	Positive, No. (%)	NA	Negative, No. (%)	Positive, No. (%)	NA	Negative, No. (%)	Positive, No. (%)	NA
Tumor AREN03B2	38 (77.6)	11 (22.4)	1	29 (60.4)	19 (39.6)	2	41 (83.7)	8 (16.3)	1	25 (67.6)	12 (32.4)	13
Tumor	36 (76.6)	11 (23.4)	3	26 (56.5)	20 (43.5)	4	39 (83)	8 (17)	3	20 (55.6)	16 (44.4)	14
Serum	27 (67.5)	13 (32.5)	10	26 (65)	14 (35)	10	34 (85)	6 (15)	10	11 (36.7)	19 (63.3)	20
Urine	10 (76.9)	3 (23.1)	37	8 (66.7)	4 (33.3)	38	12 (92.3)	1 (7.7)	37	3 (33.3)	6 (66.7)	41

NOTE. Each marker restricted to patients with available data from AREN03B2.

Abbreviations: ctDNA, circulating tumor DNA; LOH, loss of heterozygosity; NA, not available either because of missing data, insufficient tumor tissue, or undetectable ctDNA levels.

solitary biopsies, especially in patients with metastatic disease.[9,16,20,34-37] In five cases from our study, we detected a 1q gain in the serum but not in the matched tumor, and in two cases, 1q gain was detected in the tumor but not the serum, even when samples were profiled by using the same technology (ULP-WGS), suggesting the possible utility of a more comprehensive profiling strategy for risk stratification at diagnosis. However, it is still unclear how to interpret the detection of these somatic events when present in subclonal fractions. Interestingly, we did not identify *TP53* mutations in either the tumors or ctDNA from the five patients found to have anaplasia at delayed resection. It is possible these cases were representative of *TP53* wild-type anaplasia[31] or that our sequencing techniques were not sensitive enough to detect rare subclones containing these variants. We anticipate that more comprehensive detection of clonal and subclonal CNVs and SNVs will be possible by cell-free DNA profiling as more sensitive sequencing approaches are implemented.[38] Additional studies will be needed to determine whether ctDNA can better identify clinically relevant tumor genetic biomarkers than tumor biopsies in WT and to determine

how to incorporate subclonal variants into risk-stratification strategies.

One limitation of this study is that our cohort was enriched for patients with stage IV disease because only a subset of patients with stage III disease, those with LOH of 1p and 16q, were eligible for AREN0533. Levels of detectable ctDNA could be lower in patients with biologically less aggressive stage III disease or patients with localized WT, which will need to be further studied. This patient cohort was only a subset of the patients enrolled on AREN0533, which could have introduced unexpected selection bias. Another limitation of this study is that only serum was available from these patients, and samples demonstrated a high degree of contamination with cellular DNA.[39-41] For this reason, accurate ctDNA levels could not be confidently calculated (beyond detect or not detect), and this precluded studying whether the quantity of ctDNA was directly associated with an increased risk of relapse or death. Furthermore, only small volumes of urine were available for our study. Given the potential to collect much larger volumes of urine from patients, this analysis does not rule out the possibility that urine could be a promising source of material for ctDNA

TABLE 3. Kappa Coefficient for Inter-Rater Reliability and Raw Rate of Agreement (and 95% CI for the Rate) for Each Somatic Variant and Pair of Specimen Types

	LOH 1p		LOH 16q		LOH 1p and 16q		1q Gain	
Group	Kappa	Rate of Agreement (95% CI)	Kappa	Rate of Agreement (95% CI)	Kappa	Rate of Agreement (95% CI)	Kappa	Rate of Agreement (95% CI)
Tumor and tumor AREN03B2	0.41	0.79 (0.64 to 0.89)	0.59	0.8 (0.66 to 0.91)	0.40	0.84 (0.7 to 0.93)	0.65	0.83 (0.67 to 0.94)
Serum and tumor AREN03B2	0.29	0.7 (0.53 to 0.83)	0.74	0.88 (0.73 to 0.96)	0.34	0.84 (0.7 to 0.93)	0.56	0.77 (0.58 to 0.9)
Urine and tumor AREN03B2	0.42	0.77 (0.46 to 0.95)	0.53	0.75 (0.43 to 0.95)	−0.04	0.82 (0.68 to 0.93)	0.77	0.89 (0.52 to 1)
Serum and tumor	0.78	0.9 (0.77 to 0.97)	0.71	0.85 (0.71 to 0.94)	0.61	0.9 (0.78 to 0.97)	0.65	0.83 (0.68 to 0.93)
Urine and tumor	0.11	0.67 (0.35 to 0.9)	0.68	0.83 (0.52 to 0.98)	−0.04	0.8 (0.66 to 0.9)	0.66	0.83 (0.52 to 0.98)

NOTE. Each result was restricted to those with available marker data from both specimen sources, and for comparability across rows, all marker-specific results are further restricted to the subset of patients who had available data for the given marker from AREN03B2.

Abbreviation: LOH, loss of heterozygosity.
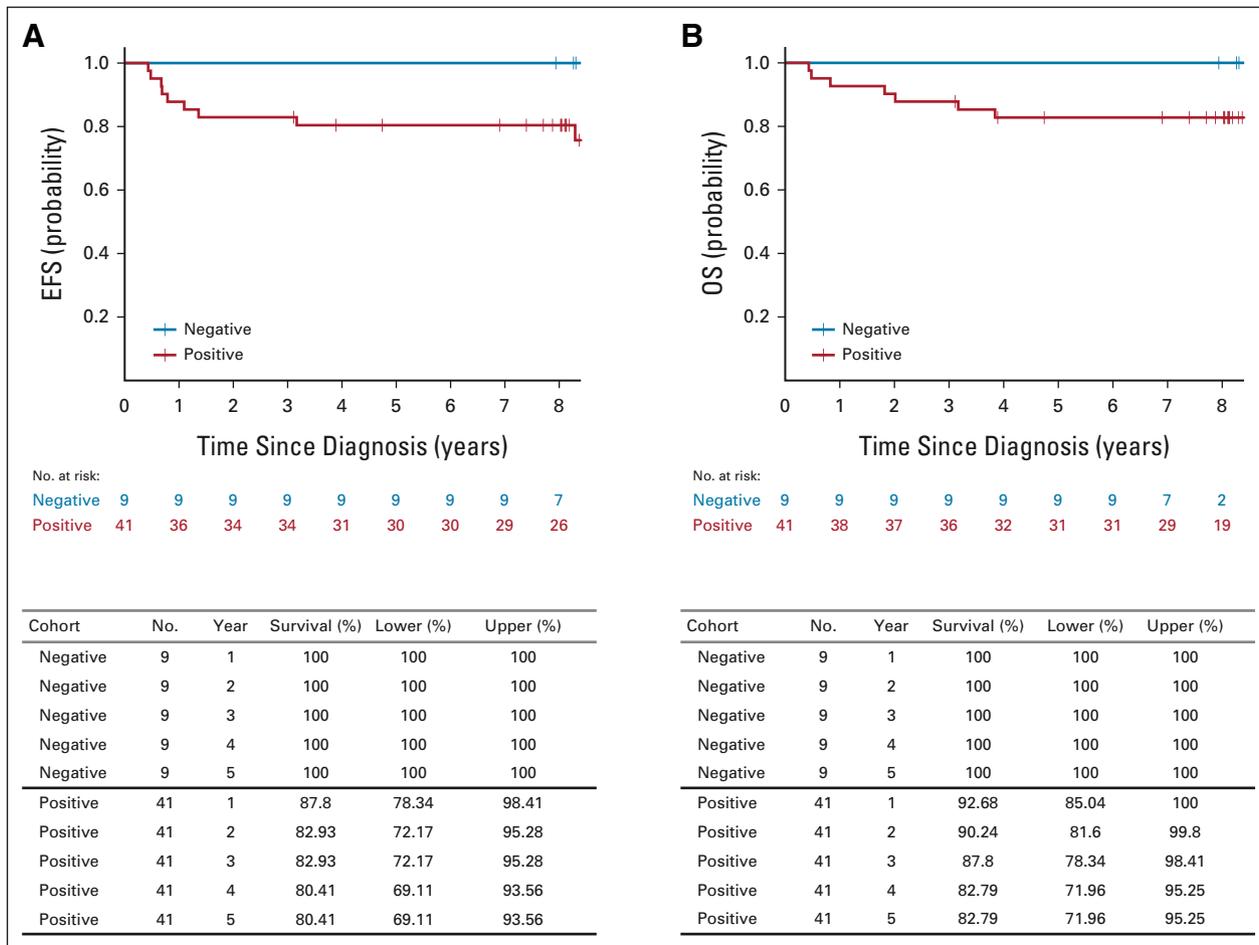
FIG 3. EFS and OS in patients with and without detectable ctDNA in the serum: (A) EFS and (B) OS by ctDNA detection in the serum of patients with stage III and IV WT. ctDNA, circulating tumor DNA; EFS, event-free survival; OS, overall survival; WT, Wilms tumor.

analysis. Moreover, as only pretreatment samples were available for this cohort, we were not able to evaluate whether changes in ctDNA detection on and after therapy were associated with prognosis or relapse, but we recognize this as an important area of future study.[17,20,42-44] Each of these limitations can be overcome through routine collection of serial blood and urine samples using validated ctDNA-optimized sample handling protocols, such as the use of cell-stabilizer tubes, in future clinical trials.[39-41] Finally, leveraging ULP-WGS for detection of segmental CNAs could have resulted in some false-negative results for focal CNVs picked up by array profiling for the AREN03B2 study, potentially accounting for some of the rare discrepancies seen in our analysis. Improvements in computational analysis would be expected to shorten the

minimum length of CNVs detectable by ULP-WGS to one megabase, equivalent to the length needed to call an alteration in the AREN03B2 protocol.

In summary, to our knowledge, our study is the first to define the feasibility of detecting and quantifying ctDNA from liquid biopsy samples collected from patients with WT enrolled on a multi-institutional prospective clinical trial, with available correlative treatment and outcome data. The findings highlight the potential advantage of these assays in refining risk-stratified treatment strategies that have already improved outcome for patients with this pediatric malignancy. We believe prospective validation of these results is warranted, and studies to determine whether ctDNA studies in patients with higher-risk and lower-risk disease would also be informative.

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DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

EQUAL CONTRIBUTION

E.A.M. and B.D.C. jointly and equally supervised this work.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at DOI https://doi.org/10.1200/JCO.22.00098.

DATA SHARING STATEMENT

Upon publication, sequencing data will be available through the dbGAP repository (https://www.ncbi.nlm.nih.gov/gap/) at accession number phs002847. Clinical data for the study cohort (study ID: AREN0533, NCTN Trial Number: NCT00379340) are available through the NCTN/NCORP Data Archive (https://nctn-data-archive.nci.nih.gov/). Sequencing and clinical data from both databases are linkable using universal patient-specific research identifiers.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Circulating Tumor DNA as a Biomarker in Patients With Stage III and IV Wilms Tumor: Analysis From a Children's Oncology Group Trial, AREN0533

Jeffrey S. Dome
Patents, Royalties, Other Intellectual Property: Rockland Immunochemicals

Lisa R. Diller
Stock and Other Ownership Interests: Novartis, Amgen, Roche, CRISPR Therapeutics, Baxter, LabCorp, Apellis Pharmaceuticals, AstraZeneca, Intellia Therapeutics, Novo Nordisk
Patents, Royalties, Other Intellectual Property: My husband holds a patent with Massachusetts General Hospital for a drug currently in clinical trials. The drug is an inhibitor of BMP-1 (Alk2) receptor and is being studied in anemia of myelodysplastic syndrome and high-hepcidin level–associated anemias

Brian D. Crompton
Employment: Acceleron Pharma
Stock and Other Ownership Interests: Acceleron Pharma
Research Funding: Gradalis

No other potential conflicts of interest were reported.