original report

Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients With Urothelial Bladder Carcinoma

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abstract

PURPOSE Novel sensitive methods for early detection of relapse and for monitoring therapeutic efficacy may have a huge impact on risk stratification, treatment, and ultimately outcome for patients with bladder cancer. We addressed the prognostic and predictive impact of ultra-deep sequencing of cell-free DNA in patients before and after cystectomy and during chemotherapy.

PATIENTS AND METHODS We included 68 patients with localized advanced bladder cancer. Patient-specific somatic mutations, identified by whole-exome sequencing, were used to assess circulating tumor DNA (ctDNA) by ultra-deep sequencing (median, 105,000×) of plasma DNA. Plasma samples (n = 656) were procured at diagnosis, during chemotherapy, before cystectomy, and during surveillance. Expression profiling was performed for tumor subtype and immune signature analyses.

RESULTS Presence of ctDNA was highly prognostic at diagnosis before chemotherapy (hazard ratio, 29.1; P = .001). After cystectomy, ctDNA analysis correctly identified all patients with metastatic relapse during disease monitoring (100% sensitivity, 98% specificity). A median lead time over radiographic imaging of 96 days was observed. In addition, for high-risk patients (ctDNA positive before or during treatment), the dynamics of ctDNA during chemotherapy was associated with disease recurrence (P = .023), whereas pathologic downstaging was not. Analysis of tumor-centric biomarkers showed that mutational processes (signature 5) were associated with pathologic downstaging (P = .024); however, no significant correlation for tumor subtypes, DNA damage response mutations, and other biomarkers was observed. Our results suggest that ctDNA analysis is better associated with treatment efficacy compared with other available methods.

CONCLUSION ctDNA assessment for early risk stratification, therapy monitoring, and early relapse detection in bladder cancer is feasible and provides a basis for clinical studies that evaluate early therapeutic interventions.

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ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Urothelial carcinoma is a common malignant disease, with 430,000 new cases diagnosed and 165,000 deaths recorded globally in 2012.[1] Localized, muscle-invasive bladder cancer (MIBC) is treated with radical cystectomy, but 20% of patients with node-negative and 80% with node-positive disease at surgery will experience metastatic relapse.[2] Neoadjuvant chemotherapy improves survival,[3] and treatment with gemcitabine and cisplatin is a commonly used regimen that results in significant downstaging in 40% to 50% of patients.[4,5] Currently, detection of relapse and monitoring of response to treatment in the metastatic setting is performed by standard computed tomography scan. Although imaging techniques offer an assessment of the tumor burden, the monitoring potential is restricted by a suboptimal detection limit and inherent variability in measurements.[6,7] Early detection of metastatic relapse and/or progression and evaluation of treatment efficacy, therefore, are major clinical challenges in this disease setting. Identification of metastatic relapse after cystectomy at an early time point, where relapse is not detectable by radiographic

imaging, could aid in the selection of patients who may benefit from early/adjuvant treatment.

The use of circulating tumor DNA (ctDNA) as a biomarker for disease staging at diagnosis, tumor burden, early detection of metastatic relapse, disease surveillance, and therapeutic treatment response is an emerging field in multiple cancer types.[8-15] In bladder cancer, we and others have previously reported proof-of-concept data documenting that ctDNA is detectable in plasma and urine and that high levels of ctDNA are associated with later clinical disease progression and metastatic disease.[16-19] We therefore hypothesized that longitudinal analysis of ctDNA in patients with MIBC would demonstrate prognostic and predictive power at key time points and provide early evidence of metastatic disease.

To our knowledge, this report is the largest and most comprehensive study of ctDNA in patients with bladder cancer to date. We document that ctDNA is a powerful biomarker for prognosis and early detection of metastatic disease. Furthermore, we show that ctDNA dynamics during treatment is a predictor of chemotherapy response and patient outcome.

PATIENTS AND METHODS

Additional information can be found in the Data Supplement.

Patients and Clinical Samples

Ninety-nine patients diagnosed with MIBC and who were receiving neoadjuvant chemotherapy before cystectomy were prospectively enrolled between 2013 and 2017 at Aarhus University Hospital in Denmark. Treatment and surveillance were done according to Danish national guidelines, which adhere to the European Guidelines for patients with bladder cancer.[20] Blood samples were collected at uniformly scheduled clinical visits and before each chemotherapy cycle. Pathologic downstaging after chemotherapy was defined as Ta,CIS,N0 or less after treatment. Detailed follow-up data were available for all patients; clinical end points were obtained from computed tomography scan results (recurrence-free survival) and from the nationwide civil registry (overall survival). For details, see the Data Supplement. Sixty-eight patients were selected for exome sequencing and ctDNA analysis on the basis of the following criteria: neoadjuvant/first-line chemotherapy for localized MIBC; plasma samples obtained before and during chemotherapy, before and after cystectomy; and available DNA from a tumor biopsy. All patients provided written informed consent, and the study was approved by The National Committee on Health Research Ethics (#1302183).

Exome Sequencing and Bioinformatics Analysis

Libraries of tumor and matching germline DNA were prepared using 100 to 500 ng DNA and captured by SeqCap EZ MedExomeV1_hg19 or MedExomePlusV1_hg19 panel (Roche, Basel, Switzerland). Sequencing data were processed according to Genome Analysis Toolkit Best Practices, and single nucleotide variants and insertions and deletions were called using MuTect2 (Broad Institute, Cambridge, MA). Exome sequencing metrics are listed in the Data Supplement.

Plasma Multiplex Polymerase Chain Reaction Next-Generation Sequencing

For each patient, 16 patient-specific somatic variants were selected as previously described.[21] Cell-free DNA (cfDNA) was extracted from a median of 7.5 mL of plasma using QIAamp Circulating Nucleic Acid Kit (QIAGEN, Hilden, Germany). Libraries were created and sequenced as previously described.[14] Quality control was performed throughout the workflow (Data Supplement). In total, 651 (99%) of 656 plasma samples passed the sample quality control process. A plasma sample with at least two variants with a confidence score above a predefined algorithm threshold (0.97) was defined as ctDNA positive. Mutation calls from plasma samples are listed in the Data Supplement.

Statistical Analyses

Assessment of statistical significance was performed using Wilcoxon rank sum or Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables. Survival analyses were carried out in R using packages survminer, survival, and coxphf (https://cran.r-project.org). Recurrence assessment was not available for patients 4519 and 3889, and these patients were excluded from analyses where recurrence status is considered. Furthermore, cystectomy was not completed for patients 4175 and 4250. Recurrence status after cystectomy was therefore not possible to evaluate, and these patients were similarly excluded. Recurrence rates 12 months after cystectomy were based on imaging data up to 14 months after cystectomy to allow for variability in scheduling of imaging.

RESULTS

Patient Characteristics and Primary Tumor Analysis

In total, 68 patients with localized MIBC fulfilled the inclusion criteria, with a median follow-up of 21 months after cystectomy. We observed a recurrence rate of 20% (n = 13) among the 64 patients with available recurrence evaluation. Whole-exome sequencing (WES) of tumor and matched germline DNA was performed at a mean target coverage of 104× (range, 31× to 251×) for tumor samples and 66× (range, 35× to 120×) for germline samples, which identified an average of 488 mutations (range, 11 to 3,536 mutations) per patient (Data Supplement). A summary of mutation frequency, mutational signatures, frequently mutated genes, and clinical data is shown in Figure 1.

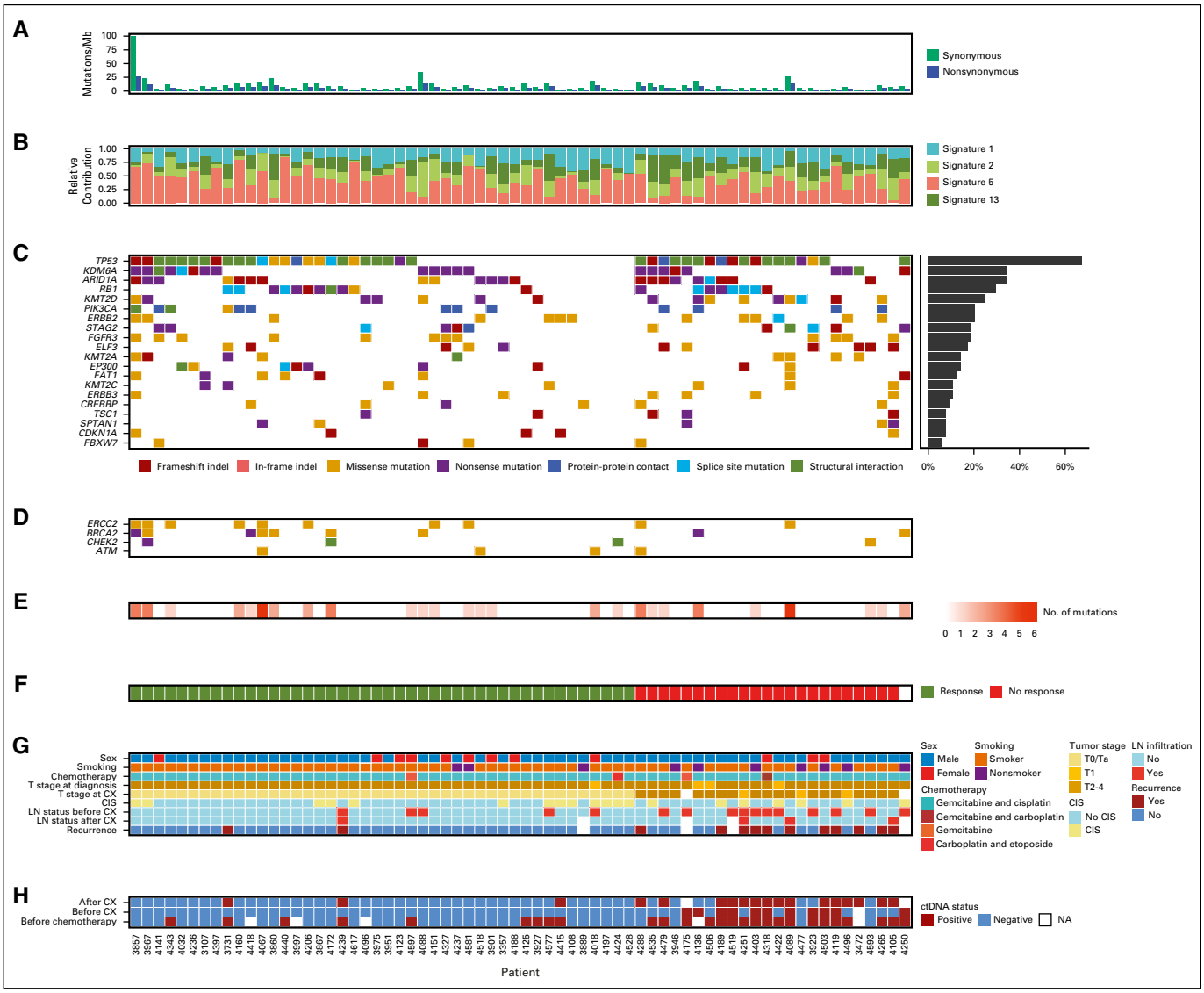

FIG 1. Summary of clinical, histopathologic, and molecular parameters for all patients. (A) Rate of synonymous and nonsynonymous mutations called from whole-exome sequencing. Of note, the tumor of patient 3857 was hypermutated with a mutational burden of 126 mutations/megabase (Mb) and displayed a *POLD1* mutation that previously has been associated with hypermutators.[22] (B) The relative contribution of the four most prevalent bladder cancer–associated mutational signatures. (C) Mutations in frequently mutated genes in muscle-invasive bladder cancer (The Cancer Genome Atlas).[23] (D) Deleterious mutations in DNA damage response (DDR)–associated genes mutated in more than 5% of the 68 samples. (E) Absolute number of deleterious DDR mutations detected from a total of 34 DDR-related genes.[24] (F) Chemotherapy response evaluation. (G) Clinical and histopathologic characteristics. (H) Summarized circulating tumor DNA (ctDNA) status. CIS, carcinoma in situ; CX, radical cystectomy; indel, insertion and deletion; LN, lymph node; NA, not available.

ctDNA Monitoring by Ultra-Deep Multiplex Polymerase Chain Reaction–Based Next-Generation Sequencing

A predefined and previously validated ctDNA analysis pipeline was applied to 656 plasma samples procured from the 68 patients.[14] In brief, unique patient-specific assays were designed for 16 highly ranked somatic mutations, and multiplex polymerase chain reaction next-generation sequencing was performed on plasma cfDNA. A sample was called ctDNA positive if two or more target variants were detected, as previously described.[14] Sample-level analytic sensitivity was previously determined to be greater than 95% at a 0.01% ctDNA concentration level.[25] Plasma samples were sequenced to a median target coverage of 105,000× (error rate: transitions, 0.0063%; transversions, 0.0033%; Data Supplement).

ctDNA Detection for Prognosis and Relapse Detection

Throughout the disease courses, presence or absence of ctDNA was strongly correlated with patient outcomes (Fig 2; Figs 3A to 3C). Of note, ctDNA-positive samples obtained during follow-up and outside of treatment were generally followed by additional ctDNA-positive samples (Fig 2). However, at very-low ctDNA levels, we observed two

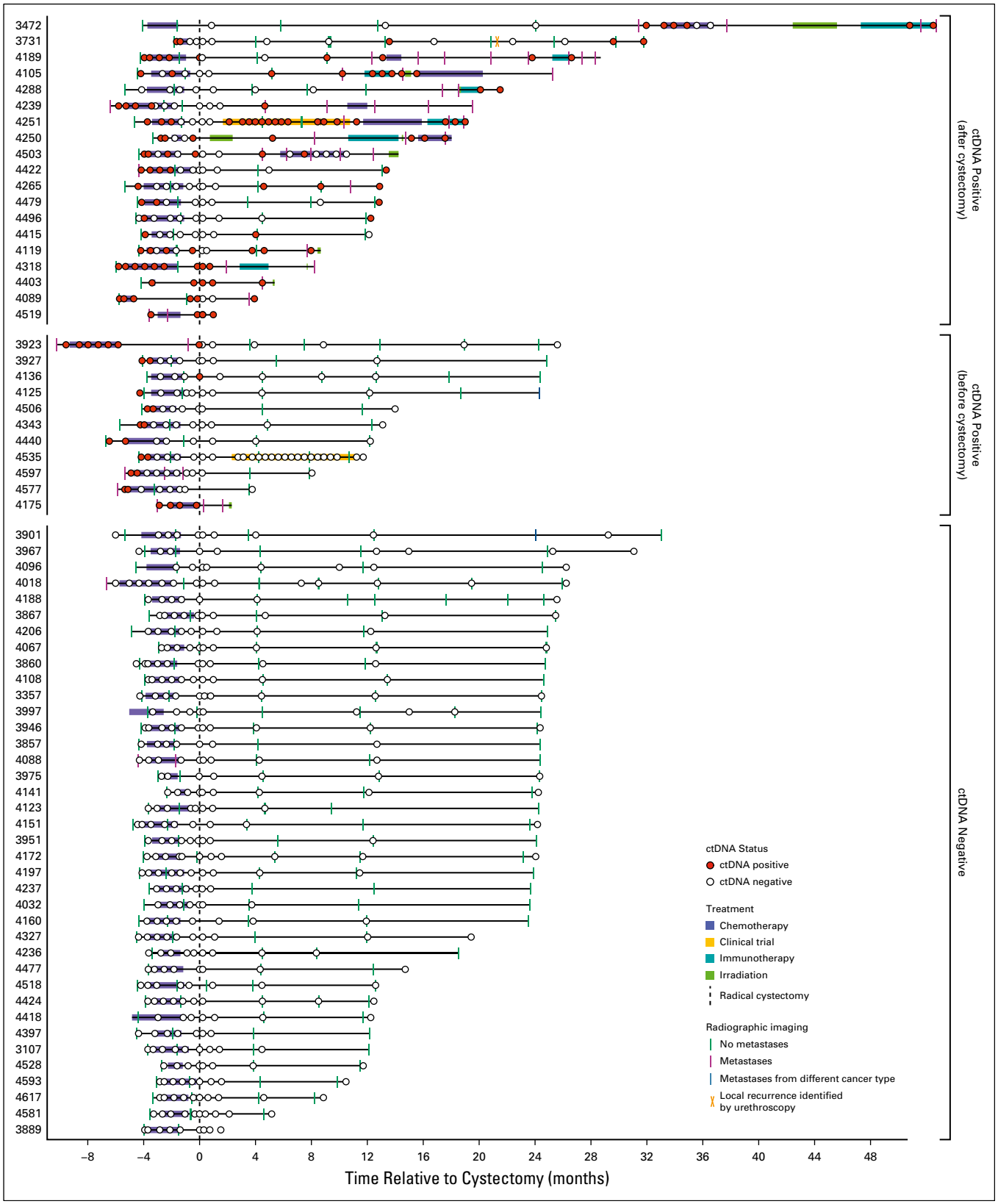

FIG 2. Longitudinal representation of circulating tumor DNA (ctDNA) results for all analyzed samples from 68 patients. Horizontal lines represent the disease courses of the patients. Circles represent ctDNA status. Treatment and imaging information is indicated for each patient. Patients are separated into three groups on the basis of ctDNA status. The top panel shows patients ctDNA-positive after cystectomy, the middle panel shows patients ctDNA-positive before cystectomy only, and the bottom panel shows ctDNA-negative patients. Patient 3731 was diagnosed with a Ta tumor by urethroscopy.

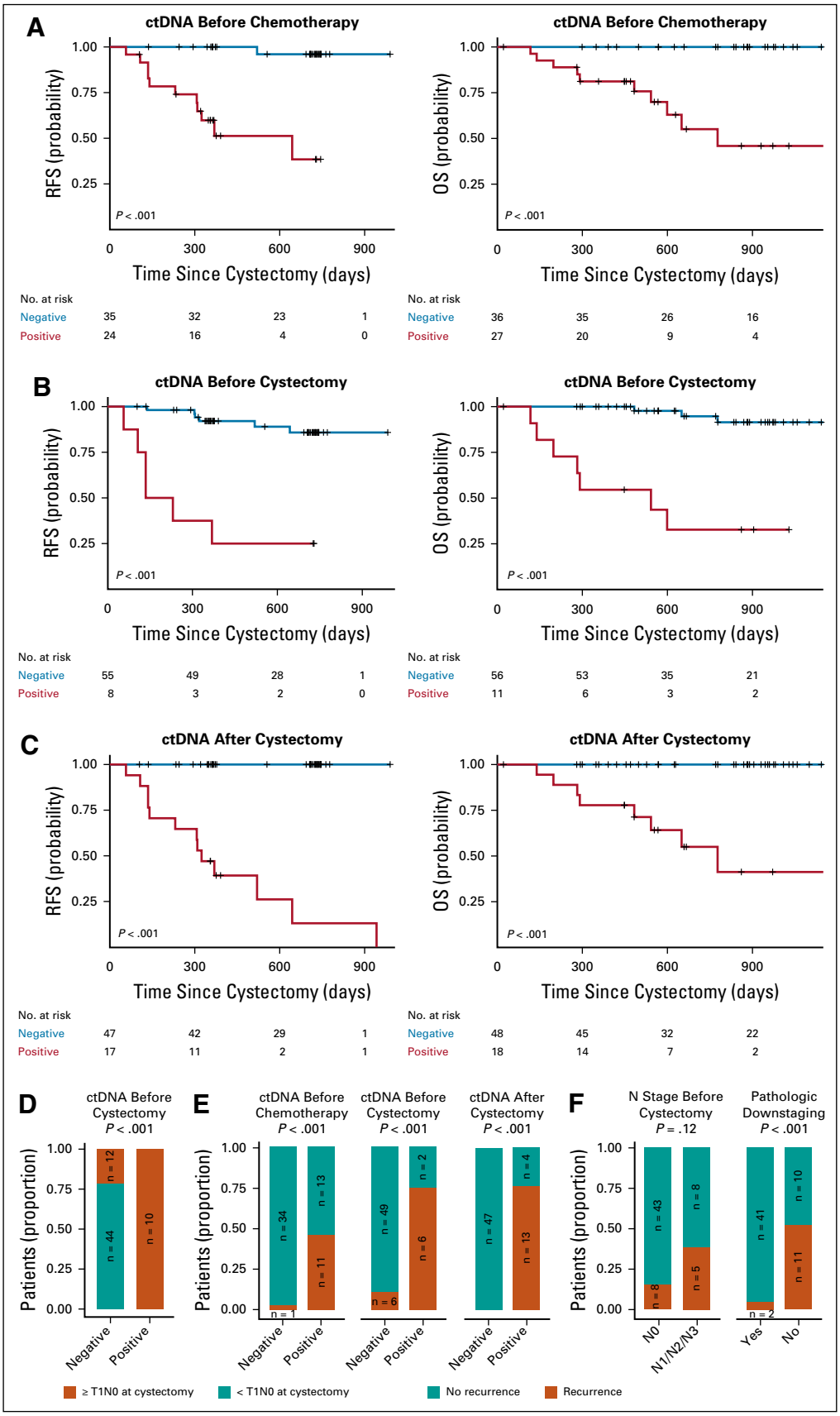

FIG 3. Prognostic value of circulating tumor DNA (ctDNA) detection. Kaplan-Meier survival analysis shows probability of recurrence-free survival (RFS) and overall survival (OS) stratified by ctDNA status (A) before chemotherapy, (B) before cystectomy, and (C) after cystectomy. (D) Association between ctDNA status before cystectomy and pathology status at cystectomy. (E) Dichotomized association between disease recurrence and ctDNA status before chemotherapy, before cystectomy, and after cystectomy. (F) Association between disease recurrence and lymph node status before cystectomy and response to chemotherapy. Of note, one ctDNA-positive patient died before clinical evaluation (4519) and one ctDNA-negative patient dropped out of the study before clinical evaluation (3889).

exceptions (patients 3731 and 4415; approximately 0.6 copies/mL plasma) and here, subsampling may influence ctDNA detection and repeatability.[26]

Three time points are of significant interest. First, ctDNA status at diagnosis before chemotherapy was strongly prognostic (Fig 3A). In bladder cancer, the first intervention is transurethral resection of bladder tumor; plasma ctDNA status after transurethral resection of bladder tumor may serve as a proxy to measure minimal residual disease. For patients who were ctDNA positive at this time point, we observed overall and 12-month recurrence rates of 46% (11 of 24 patients) and 42% (10 of 24 patients), respectively. Of note, only 3% of patients (one of 35) who were ctDNA negative at this first time point experienced a recurrence during the study ($P < .001$; 12 months, 0% [zero of 35 patients; $P < .001$]). The detection of ctDNA at this early time point is therefore a strong prognostic factor for the long-term clinical outcome after chemotherapy and cystectomy (HR, 29.1; $P = .001$; Data Supplement).

The second time point (after chemotherapy and before cystectomy) was also prognostic of patient outcome (Fig 3B). In ctDNA-positive patients, we observed an overall and 12-month recurrence rate of 75% (six of eight patients). In ctDNA-negative patients, the overall and 12-month recurrence rates were 11% (six of 55 patients; $P < .001$) and 7% (four of 55 patients; $P < .001$), respectively. Presence of ctDNA before cystectomy was associated with pathology at cystectomy as 100% of ctDNA-positive patients at this time point had residual tumor (stage ≥ T1) and/or lymph node metastases identified at cystectomy (Fig 3D). Furthermore, 100% of patients (36 of 36) with pT0 at cystectomy were ctDNA negative. For this second time point, we observed an HR of 12.0 ($P < .001$; Data Supplement).

Third, and most significantly, plasma ctDNA status during disease surveillance after cystectomy was highly prognostic (Fig 3C). We observed an overall recurrence rate of 76% (13 of 17 patients) and a 12-month recurrence rate of 59% (10 of 17 patients) in ctDNA-positive patients. In ctDNA-negative patients, the recurrence rate was 0% at both time points (zero of 47 patients; $P < .001$). The status of ctDNA at any time point after cystectomy was stronger than any other predictive factor, such as lymph node status before cystectomy and pathologic downstaging (Figs 3E and 3F). In addition, in multivariable Cox proportional hazards regression analysis, ctDNA status was the strongest predictor of recurrence-free survival after cystectomy (HR, 129.6; $P < .001$; Data Supplement).

Serial ctDNA Measurements for Disease Surveillance

ctDNA dynamics (ie, changes in ctDNA levels measured in consecutive samples) and detection of relapse during disease courses are shown for selected patients in Fig 4A. For example, for patient 4251, ctDNA was detected 64 days after cystectomy, and clinical relapse was detected 309 days after cystectomy (lead time, 245 days). Similarly, for patient 4189, ctDNA was detected 273 days after cystectomy, and clinical relapse was detected 369 days after cystectomy (lead time, 96 days). Overall, for patients with metastatic relapse and detectable ctDNA, we found ctDNA analysis to have a median lead time of 96 days (−83 to 245 days; $P = .023$) over conventional imaging (Fig 4B). Restriction of analyses to patients with simultaneous plasma and radiographic imaging identified eight patients, five of whom showed a lead time in recurrence detection for ctDNA analyses. The remaining three patients showed simultaneous recurrence detection, and the resulting median lead time for all eight patients was 107 days (0 to 186 days; $P = .059$). Disease courses and ctDNA detection and dynamics are shown in the Data Supplement for all 68 patients.

For evaluation of potential clinical performance of the ctDNA test, we calculated sensitivity and specificity measures by restricting our analysis to only include plasma samples where 180 days or more (approximately two times the median lead time) of follow-up was available for patients with nonmetastatic disease. Using these criteria, serial analysis of ctDNA during surveillance after cystectomy identified metastatic relapse with 100% (13 of 13 patients) sensitivity and 98% (48 of 49 patients) specificity.

To assess the impact of heterogeneity between primary tumors and metastases on serial ctDNA measurements, we performed WES of cfDNA in four plasma samples from three patients. Samples were sequenced to a mean target coverage of 307× (272× to 340×), and between 508 and 1,294 mutations were identified. We compared all mutations identified in the plasma WES data to the associated WES data from the primary tumor to assess mutational changes acquired during metastatic evolution (Fig 4C). We found a high degree of similarity between the mutational landscapes of the primary tumors and the cfDNA, which indicated a limited clonal evolution during the disease course of the selected patients. On average, we identified 62 mutations in ctDNA present at the time of metastases, which had not been detected in the primary tumors (0.5% to 61.2% increase in number of mutations compared with the primary tumors).

Serial ctDNA Measurements for Therapy Response Monitoring

Clinically useful predictive biomarkers of response to treatment are not currently available. Multiple tumor-centric markers are being investigated; however, the best tool to evaluate treatment response remains pathologic downstaging after treatment. Pathologic downstaging (≤ pTa,CIS,N0) was observed for 66% of patients (44 of 67; pT0N0, 54%) and was significantly associated with a lower frequency of disease recurrence, as expected (HR, 0.1; $P < .001$; Data Supplement). However, only 52% of nonresponding patients (11 of 21) with available recurrence evaluation had disease recurrence, suggesting

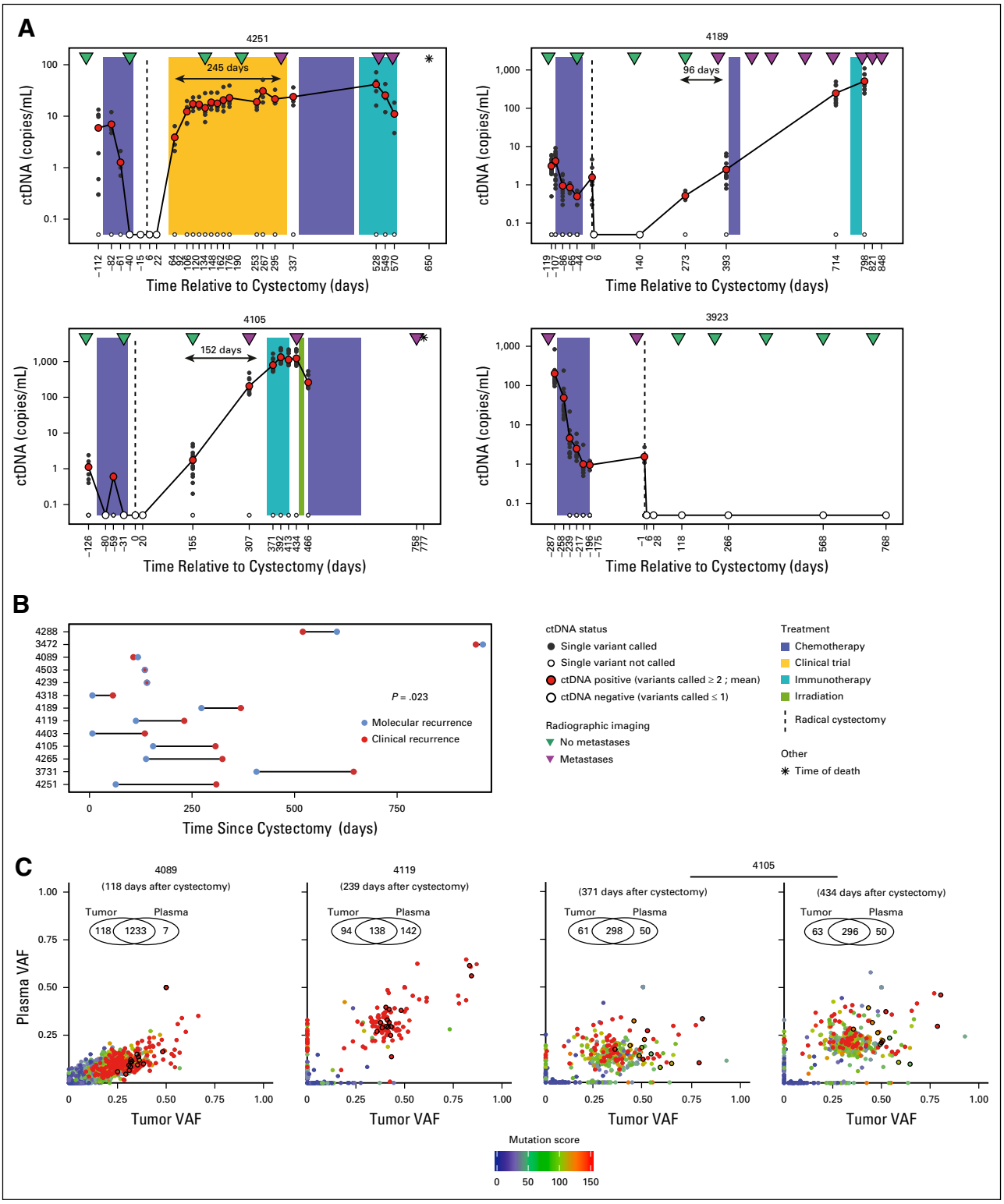

FIG 4. Tracking circulating tumor DNA (ctDNA) changes in individual disease courses and measures of heterogeneity. (A) Representation of detailed disease courses, applied treatments, and longitudinal ctDNA analyses from selected patients. (B) Lead time in days shown as differences between molecular recurrence (ctDNA positive) and clinical recurrence (radiographic imaging positive). *P* value was calculated using a paired Wilcoxon rank sum test. (C) Comparison of whole-exome sequencing data from primary tumors and cell-free DNA at metastatic relapse. Plasma samples were selected on the basis of ctDNA targets at 10% allele frequency or above in the multiplex polymerase chain reaction next-generation sequencing assays. Individual mutations are color-coded according to statistical probability (strength) of the mutation call. Venn diagrams represent the number of mutations identified exclusively in the tumor, plasma, or both. VAF, variant allele frequency.

that pathologic downstaging is suboptimal for evaluating treatment efficacy. Our ctDNA results demonstrated that the presence and dynamics of ctDNA during chemotherapy were correlated to pathologic downstaging. In total, 85% of ctDNA-negative patients (35 of 41) showed pathologic downstaging. Patients who were initially ctDNA positive but with subsequent clearance of ctDNA (ie, ctDNA no longer measurable) showed a response rate of 53% (nine of 17 patients), whereas patients without clearance of ctDNA showed a response rate of 0% (zero of eight patients; Figs 5A and 5B). Of note, for patients who were ctDNA positive before or during treatment, the dynamics of ctDNA during chemotherapy was significantly associated with disease recurrence, whereas pathologic downstaging was not significantly associated with disease recurrence (Fig 5C), which indicates that ctDNA measurements may be a better tool for evaluating treatment efficacy. Overall, our results suggest that the presence of ctDNA identifies patients with a high risk of developing metastatic spread and that the dynamics of ctDNA during treatment further inform both chemotherapy response and outcome. Of note, although pathologic downstaging is a strong predictor of outcome, ctDNA informs chemotherapy response and outcome during treatment and before cystectomy.

We also assessed possible predictive biomarkers of treatment response in the primary tumors. Analysis of mutational processes[27] showed a significantly higher contribution of the trinucleotide mutational signature 5 (P = .024) in patients who responded to chemotherapy (Figs 1 and 5D). A high contribution of mutational signature 5 was significantly associated with *ERCC2* mutation status (Fig 5E), which indicated a correlation to DNA damage response mechanisms as previously described.[28] Patients with *ERCC2* mutations were associated with a higher rate of response to chemotherapy, although not significantly (Fig 5F). Finally, transcriptional analysis of the tumors (n = 46) showed that molecular subtypes and immune signatures were not significantly associated with response to chemotherapy and ctDNA status (Data Supplement). In conclusion, clinical parameters and molecular features of the primary tumor were associated with treatment response and outcome, but ctDNA monitoring remained the strongest predictor of outcome and therapy response in high-risk patients (ctDNA positive; Data Supplement).

DISCUSSION

This study documents several important findings for ctDNA analysis for patients with bladder cancer: (i) ctDNA serves as a prognostic biomarker already before chemotherapy, (ii) ctDNA dynamics during chemotherapy reflect response to treatment and patient outcome, and (iii) ctDNA identifies disease recurrence in the postsurgery setting with high sensitivity and specificity and a positive lead time compared with radiographic imaging. On the basis of these findings, new paradigms for ctDNA-guided patient management should be investigated in future clinical trials. Suggestions for ctDNA-guided management concepts are presented in the Data Supplement. Patients who are ctDNA negative before chemotherapy seem to have a low risk of recurrence after cystectomy when treated with the current standard of care (recurrence rate only 3% in this study). Because of the low risk of micrometastatic spread, these patients may be eligible for cystectomy without neoadjuvant chemotherapy (Data Supplement). Patients who are ctDNA positive before chemotherapy seem to be at high risk of recurrence (recurrence rate in this study, 46%). ctDNA might be an indicator of early disease dissemination with micrometastases, and assessment of response to treatment in this patient group is therefore crucial. In our study, we observed ctDNA before or during chemotherapy in 43% of patients (27 of 63). For patients with clearance of ctDNA during treatment, we observed pathologic downstaging in 53%, whereas for patients without clearance of ctDNA, none were found to be downstaged. Of note, for patients who were ctDNA positive before or during chemotherapy, ctDNA dynamics during chemotherapy showed a superior association with patient outcome compared with pathologic downstaging. We therefore propose that ctDNA-positive patients be monitored using ctDNA analysis during chemotherapy to assess treatment efficacy. Patients with ctDNA clearance, which suggests responsiveness to chemotherapy, may be offered additional cycles of chemotherapy before cystectomy. For patients without clearance of ctDNA, the potential benefits of other therapeutic strategies can be explored (Data Supplement).[29]

Detection of ctDNA after cystectomy serves as direct evidence of occult carcinoma cells and thus remnant disease. ctDNA was detected in 17 patients after cystectomy, and 13 of these were diagnosed with a recurrence. ctDNA-based recurrence detection displayed a lead time of up to 245 days (median, 96 days) compared with radiographic imaging. Of note, for three of four ctDNA-positive patients without a recurrence diagnosis, no follow-up was available after the positive blood test. The observed lead time in recurrence detection provides a window of opportunity for earlier initiation of therapy, which could improve treatment efficacy and thereby survival (Data Supplement).[30] Similar findings have been observed in other cancer types but with great variability in the observed lead times.[31] This might reflect a bias in the frequency of plasma sampling compared with imaging. In our study, however, we observed a similar lead time in recurrence detection when restricting our analyses to time points with simultaneous plasma sampling and imaging.

Earlier work has demonstrated *ERCC2* mutations[32] and gene expression–based subtypes[33,34] to be predictors of chemotherapy response. Here, we observed limited predictive power associated with the tumor-centric biomarkers, and the *ERCC2*-related mutational signature was the strongest predictor of response in this work.

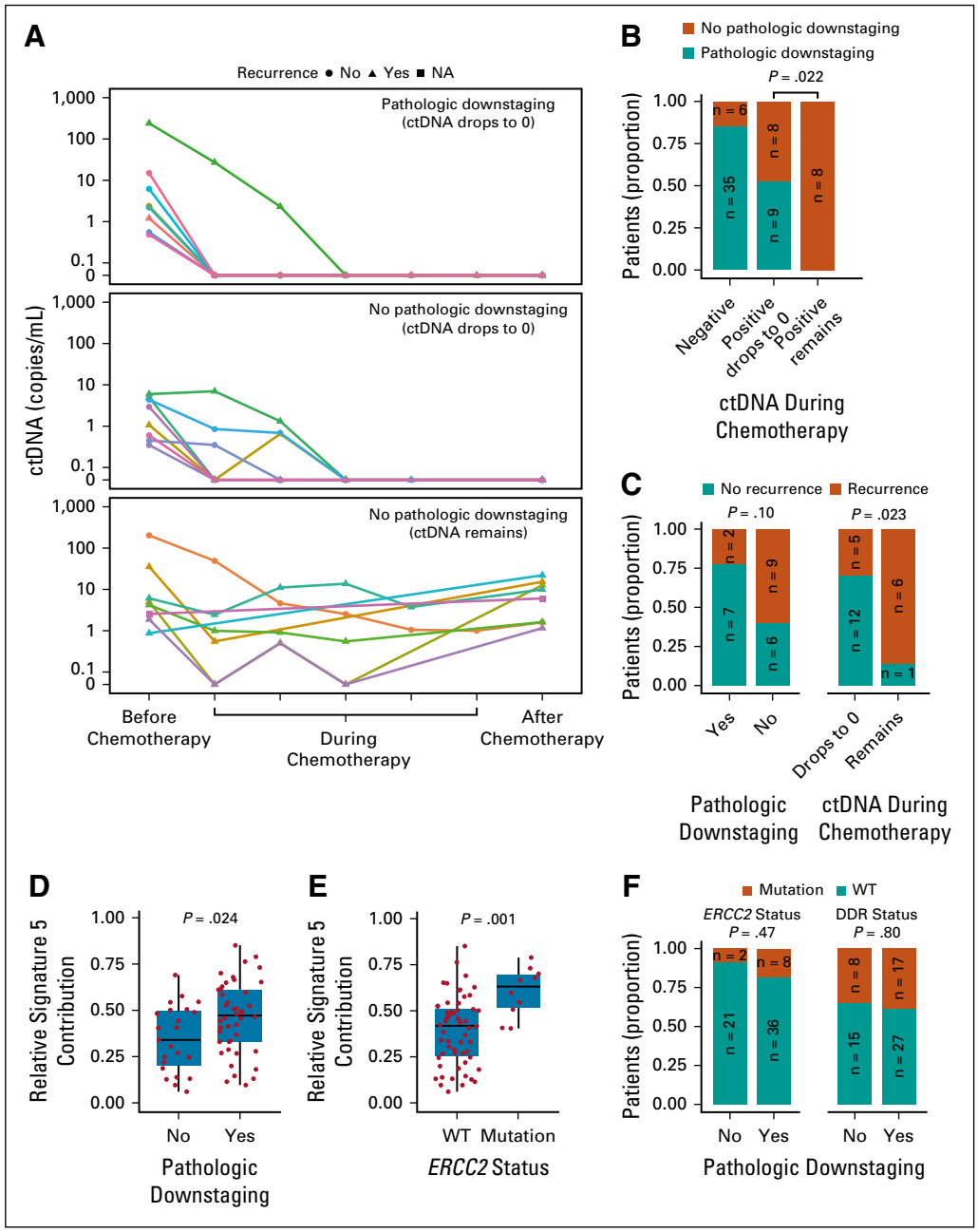

FIG 5. Predictive markers of chemotherapy response. (A) The level of circulating tumor DNA (ctDNA) is shown for all patients with detectable ctDNA before, during, and after chemotherapy. Patients are grouped by response to chemotherapy (≤ pTa,CIS,N0 at cystectomy) and ctDNA dynamics. (B) Association between ctDNA and response to chemotherapy for ctDNA-negative patients (at all time points), patients in whom ctDNA level dropped to zero, and patients in whom ctDNA remained. (C) Association between recurrence and pathologic downstaging (left) and ctDNA dynamics during chemotherapy (right) for patients ctDNA-positive before chemotherapy. The association was evaluated using Fisher's exact test instead of Cox proportional hazards regression modeling because of the low number of patients (n = 24) included in this subanalysis. (D) Relative mutational signature 5 contribution for all patients stratified by response to chemotherapy. (E) Relative mutational signature 5 contribution in relation to *ERCC2* mutation status. (F) Proportion of patients who responded to chemotherapy in relation to *ERCC2* and DNA damage response (DDR) mutation status. WT, wild type.

A low number of ctDNA molecules were detected in many samples (down to two molecules), which makes the case for highly sensitive and specific NGS-based methods. The selection of clonal mutations on the basis of WES of the primary tumor makes it possible to perform ultra-deep sequencing of the patient-specific mutations in plasma ctDNA; the disadvantage of not being able to detect novel mutations that arise during tumor evolution and disease

dissemination exists. We performed deep WES of cfDNA from plasma for a subset of patients and observed heterogeneity between primary tumors and plasma at the time of metastatic relapse, but of note, all clonal mutations selected from the primary tumors were also detected in the plasma sample at relapse. Earlier work has shown high levels of genetic heterogeneity between primary tumors and metastases[35-37]; however, our data document that genetic heterogeneity is not affecting assay performance when clonal mutations are selected.

In conclusion, we have found ctDNA testing in patients with bladder cancer who undergo chemotherapy and cystectomy to be highly sensitive and specific for early risk stratification of patients, prediction of treatment response, and early detection of metastatic relapse. ctDNA biomarkers are superior to tumor-centric biomarkers (mutations and subtypes) for predicting treatment efficacy, and novel randomized clinical trials should be initiated to determine the clinical impact of ctDNA-stratified therapeutic approaches.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

Disclosures provided by the authors and data availability statement (if applicable) are available with this article at DOI https://doi.org/10.1200/JCO.18.02052.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients With Urothelial Bladder Carcinoma

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