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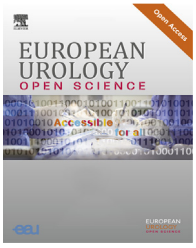

European Association of Urology

Urothelial Cancer

Circulating Tumor DNA and Response to Cisplatin-based Chemotherapy in Patients with Metastatic Urothelial Carcinoma Enrolled in CALGB 90601 (Alliance)

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Abstract

Background and objective: Cisplatin-based chemotherapy has been a cornerstone of therapy for advanced/metastatic urothelial cancer (mUC). However, no genomic characteristics have been validated as prognostic biomarkers for this therapy. We sought to identify prognostic biomarkers using plasma cell-free (cf)DNA collected in a phase 3 cooperative group trial.

Methods: We analyzed pretreatment cfDNA from a cohort nested in CALGB 90601 (Alliance), a first-line trial of gemcitabine/cisplatin with bevacizumab or placebo in mUC. We examined associations between cfDNA features and overall survival (OS), progression-free survival (PFS), and treatment response.

Key findings and limitations: Baseline cfDNA was sequenced from 201 patients with mUC. There was no statistically significant association between alterations in DNA damage response (DDR) genes and response to cisplatin-based chemotherapy (12/24; 50% response rate in DDR+ vs 60/145; 41% response rate in DDR−; p = 0.4), OS (hazard ratio [HR] 0.78, 95% confidence interval [CI] 0.50–1.22; p = 0.3) or PFS (HR 0.77, 95% CI 0.48–1.22; p = 0.3), although the DDR analysis was underpowered owing to the low frequency of DDR gene alterations. Higher variant allele frequency (VAF) in circulating tumor (ct)DNA was associated with shorter OS (HR 2.51, 95% CI 1.26–5.00; p = 0.009) and PFS (HR 2.18, 95% CI

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1.02–4.67; p = 0.045). Shorter OS was associated with cfDNA alterations in *TERT* (HR 1.59, 95% CI 1.15–2.19; p = 0.005), *PIK3CA* (HR 1.91, 95% CI 1.20–3.04; p = 0.006), and *ERBB2* (HR 1.64, 95% CI 1.08–2.49; p = 0.019).

Conclusions and clinical implications: Among patients with mUC treated with cisplatin-based chemotherapy, high pretreatment VAF in ctDNA and alterations in the *TERT* promoter, *PIK3CA*, and *ERBB2* were associated with poor prognosis.

Patient summary: We looked at the link between tumor DNA present in blood and outcomes after chemotherapy for patients with advanced bladder cancer. Higher amounts of tumor DNA in blood and mutations in specific cancer genes were linked to worse survival. The results may help in the design of new studies to improve survival for patients with advanced bladder cancer.

This trial is registered on ClinicalTrials.gov as NCT00942331.

1. Introduction

Cisplatin-based chemotherapy remains part of the treatment paradigm for locally advanced and metastatic urothelial carcinoma (mUC). Although specific genomic features such as DNA damage response (DDR) genes such as *ERCC2* have emerged as promising predictors of cisplatin sensitivity in nonmetastatic UC [1–5], no prognostic genomic features have been validated for the metastatic setting.

Bladder cancer is characterized by intratumoral, intertumoral, and temporal genomic heterogeneity [6–9], such that matched mUC tumors before and after chemotherapy may share only 28.4% of mutations [7]. Such heterogeneity highlights the disadvantages of relying on archival tumor sequencing in mUC. Evaluation of circulating tumor (ct) DNA—the portion of plasma cell-free (cf)DNA originating from tumor [10]—can overcome these challenges via noninvasive sampling of multiple tumor sites in real time [11].

In light of the critical knowledge gap pertaining to prognostic genomic features in mUC, we analyzed baseline cfDNA in a subset of patients from CALGB 90601 (Alliance), a phase 3 cooperative group trial of first-line cisplatin-based chemotherapy for mUC. Among cases with sufficient archival tumor for DNA sequencing, genomic profiles from matched cfDNA and tumor were compared. We sought to clarify the significance of alterations in DDR genes such as *ERCC2* as biomarkers of sensitivity to cisplatin-based chemotherapy in the metastatic setting, as well as the variant allele frequency (VAF) in ctDNA and other genomic features.

2. Patients and methods

A detailed description of the methodology is provided in the Supplementary material.

2.1. Study population

Patients in the study population participated in the National Cancer Institute–sponsored trial Cancer and Leukemia Group B (CALGB, now part of the Alliance for Clinical Trials) 90601, a randomized, double-blind, phase 3 trial comparing gemcitabine/cisplatin plus either bevacizumab or placebo for mUC [12]. Patients with ≥5 ml of baseline plasma available were included in the current study. Participants signed an institutional review board–approved, protocol-specific informed consent document.

2.2. Genomic sequencing

Baseline plasma samples and matched germline DNA were analyzed using MSK-ACCESS, a tumor-uninformed platform that examines mutations and copy-number alterations in 129 genes as well as select gene fusions and rearrangements [13,14]. MSK-ACCESS uses unique molecular indexes and has a depth of coverage of >15 000× that allows a detection threshold of 0.1% for allele frequency [14]. Matched archival tumor was sequenced using the MSK-IMPACT platform [15].

2.3. Statistical analysis

The primary endpoint of CALGB 90601 was overall survival (OS). Progression-free survival (PFS) and response were secondary endpoints. In CALGB 90601, there was no statistically significant difference in OS or response between treatment arms, and no clinically meaningful difference in PFS [12]. Therefore, data from both arms were pooled for the current analysis. OS and PFS were estimated using the Kaplan-Meier method and compared using the log-rank test. Associations of survival with clinical and genomic characteristics were analyzed via Cox proportional-hazards models.

To account for the nonlinear association of VAF with clinical endpoints and the impact of the upper tail of each patient's VAF distribution, we examined PFS and OS as a function of the cube root–transformed 75th percentile ($\sqrt[3]{}$VAF 75pc) [16]. The aim of this transformation was to stabilize VAF variance and reduce the effect of extreme values and provide a more symmetric distribution to meet statistical model assumptions [17].

Multivariable models were adjusted for Eastern Cooperative Oncology Group performance status (0 vs 1; patients with performance status ≥2 were excluded from CALGB 90601) and the presence versus absence of visceral metastases, as these are established prognostic factors for advanced UC [18]. Multivariable models examining *PIK3CA*

and *ERBB2* were also adjusted for ctDNA VAF. To adjust for multiple comparisons, p values were adjusted using the Benjamini-Hochberg method, and a false discovery rate of 5% ($q < 0.05$) was used to identify significant genes.

3. Results

Of 506 patients enrolled between July 2009 and December 2014 in CALGB 90601, 212 (42%) provided consent for blood collection and had sufficient baseline plasma for inclusion in the current study, with cfDNA sequencing successful for 201 (95%) (Supplementary Fig. 1). The median cfDNA concentration was 0.74 ng/μl (interquartile range 0.45–1.48, range 0.09–27.2). The median cfDNA mass was 44.6 ng (interquartile range 27.1–88.8, range 5.3–1633.3).

The 201 patients included in the current study had similar baseline characteristics to the overall CALGB 90601 cohort (Table 1). Median follow-up for patients alive at last follow-up was 70.6 mo in the cfDNA cohort and 76.3 mo original trial cohort. In the cfDNA cohort, median OS was 14 mo (95% confidence interval [CI] 13–18) for patients treated with chemotherapy plus bevacizumab compared to 16 mo (95% CI 15–18) for patients treated with chemotherapy plus placebo. Median PFS was 7.8 mo (95% CI 6.7–9.5) with chemotherapy plus bevacizumab versus 7.6 mo (95% CI 6.7–8.5) with chemotherapy plus placebo. These results are comparable to findings for the overall CALGB 90601 cohort, in which median OS was 15 mo versus 14 mo, and median PFS was 8.0 mo versus 6.7 mo with bevacizumab versus placebo, respectively.

Table 1 – Baseline characteristics for the cell-free DNA cohort and the overall CALGB 90601 cohort

Parameter	Cell-free DNA cohort (n = 201)	CALGB 90601 cohort (n = 506)
Treatment arm, *n* (%)		
Bevacizumab	101 (50)	252 (50)
Placebo	100 (50)	254 (50)
Age group, *n* (%)		
<70 yr	143 (71)	375 (74)
≥70 yr	58 (29)	131 (26)
Race, *n* (%)		
Asian	2 (1)	7 (1)
Black or African American	8 (4)	18 (4)
Other or unknown	2 (1)	17 (3)
White	189 (94)	464 (92)
Female, *n* (%)	39 (19)	109 (22)
Ethnicity, *n* (%)		
Hispanic or Latino	4 (2)	21 (4)
Not Hispanic or Latino	194 (97)	472 (93)
Other or not reported	3 (1.5)	13 (3)
Primary tumor site, *n* (%)		
Bladder	153 (76)	375 (74)
Upper tract	41 (20)	107 (21)
Other	7 (3.5)	24 (5)
Baseline ECOG performance status, *n* (%)		
0	113 (56)	275 (54)
1	87 (44)	228 (45)
Unknown	1 (<1)	3 (<1)
Preoperative chemotherapy, *n* (%)	29 (14)	66 (13)
Presence of visceral metastases, *n* (%)	140 (70)	348 (69)
Creatinine clearance <60 ml/min, *n* (%)	35 (17)	90 (18)
Measurable disease at baseline, *n* (%)	181 (90)	459 (91)

ECOG = Eastern Cooperative Oncology Group.

Among the 201 patients in the current analysis, visceral metastases, poor performance status, and renal insufficiency were associated with shorter PFS and OS, as expected [18]. Treatment arm (bevacizumab vs placebo) was not associated with outcomes (Supplementary Table 1).

3.1. Landscape of genomic alterations observed in cfDNA

Genomic alterations in significantly mutated genes as defined by The Cancer Genome Atlas (TCGA) bladder cancer cohort [20], as well as other genes selected for biological relevance (*TERT*, *BRAF*, *BRCA1*, and *MDM2*), observed at a frequency of >2% in cfDNA for our cohort are shown in Fig. 1A. Alterations in *TERT* (primarily the *TERT* promoter) were most frequent; *FGFR3* alterations were detected in 19% of patients, consistent with prior studies [20,21].

A subset of 107 patients (53%) in this cfDNA cohort had sequencing of matched archival tumors using the MSK-IMPACT platform [15]. Assessment of genomic regions covered by both the MSK-IMPACT and MSK-ACCESS cfDNA sequencing panels revealed that the percentage of patients with gene alterations pertinent to mUC were similar between cfDNA and matched tumor (Fig. 1B and Supplementary Table 2) and the TCGA cohort (Supplementary Fig. 2) [20]. However, in comparison to matched archival tumor, a higher rate of alterations was detected in cfDNA for several oncogenes, including *PIK3CA* (17% vs 13%), *RB1* (14% vs 12%), *ERCC2* (6.5% vs 4.7%), *BRAF* (5.6% vs 4.7%), and *KRAS* (5.6% vs 3.7%). While 57.1% (n = 24) of DDR gene alterations were detected in both cfDNA and matched tumor tissue, 21.4% (n = 9) were detected in tumor alone, and 21.4% (n = 9) in cfDNA alone.

3.2. DDR genes

Oncogenic DDR gene alterations were identified in pretreatment cfDNA in 27 (13%) cases, including 11 *ATM* (5.5%); nine *ERCC2* (4.5%); six *BRCA1* (3%) and two *BRCA2* (1%) alterations. One patient had oncogenic *ATM* and *BRCA1* mutations.

Neither oncogenic DDR gene alterations nor alterations in other notable biological pathways were associated with response to chemotherapy (Supplementary Table 3). Similarly, there was no statistically significant association between oncogenic DDR gene mutations and OS or PFS (Fig. 2 and Supplementary Fig. 3). Although oncogenic alterations in the *BRCA2* DDR gene in cfDNA were associated with shorter OS (univariable hazard ratio [HR] 6.06, 95% CI 1.45–25.3; p = 0.014), there were only two oncogenic *BRCA2* alterations and the result would not be statistically significant after adjustment for multiple comparisons. Stratification of DDR gene and pathway analyses by treatment arm (bevacizumab vs placebo) did not significantly alter the results (Supplementary Table 4).

Assessment of the association of clinical outcomes with any cfDNA *ERCC2* alteration (n = 11, including *ERCC2* variant of unknown significance) revealed no statistically significant association with OS (HR 0.93, 95% CI 0.50–1.72;

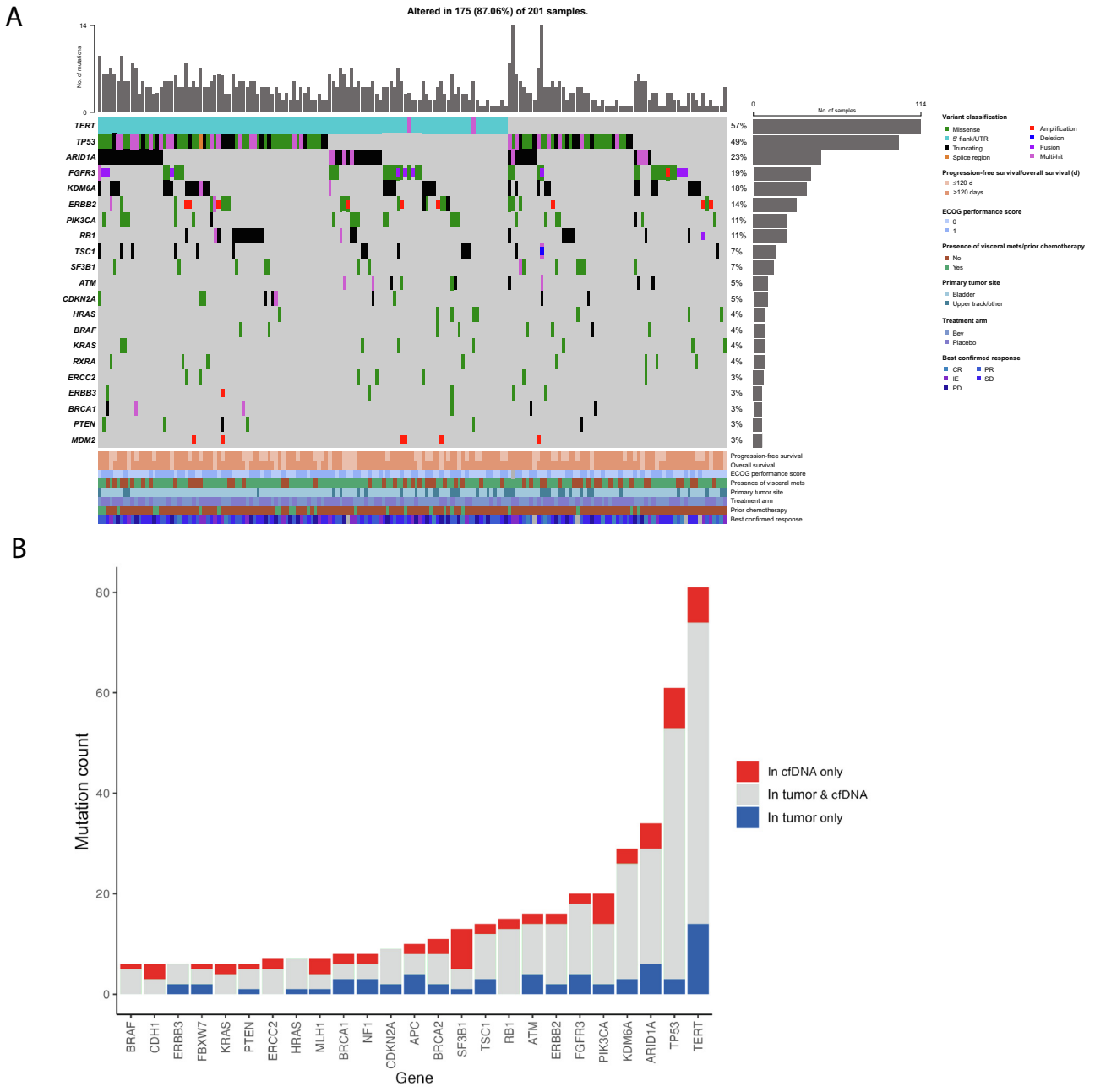

Fig. 1 – (A) Oncoprint of the landscape of genomic alterations among 201 patients with advanced urothelial cancer enrolled in CALGB 90601 (Alliance) detected via sequencing of cfDNA collected before treatment. (B) Mutation counts among 107 patients with sequencing of both cfDNA and matched tumor. The mutation count indicates the number of patients with a mutation in that gene detected in either cfDNA alone, tumor alone, or in both cfDNA and matched tumor. For any given gene, a single patient could only be counted once (ie, even a patient with 2 distinct mutations in a single gene would only be counted once for that gene). Only mutations covered by both the MSK-IMPACT tumor sequencing panel and the MSK-ACCESS cfDNA sequencing panel are included in the counts. Genes with more than five mutations (5%) are shown. Bev = bevacizumab; cfDNA = cell-free DNA; CR = complete response; IE = inevaluable; mets = metastases; PD = progressive disease; PR = partial response; SD = stable disease; UTR = untranslated region.

$p = 0.8$), PFS (HR 0.85, 95% CI 0.46–1.58; $p = 0.6$), or response (a response occurred in 5/11 patients [45%] with any cfDNA *ERCC2* alteration; $p > 0.9$). Notably, the statistical power of the DDR analysis was limited by a low frequency of oncogenic DDR gene alterations detected in cfDNA (13%). Oncogenic *ERCC2* alterations in cfDNA were observed in only 4.5% of cases, compared to 11% in the TCGA cohort [20].

3.3. *ctDNA VAF*

Multiple measures of the ctDNA VAF were associated with shorter OS and PFS (Table 2). To account for the nonlinear association of VAF with clinical endpoints and the impact of the upper tail of each patient's VAF distribution, we examined $\sqrt[3]{}$VAF 75pc and identified an inverse relation-

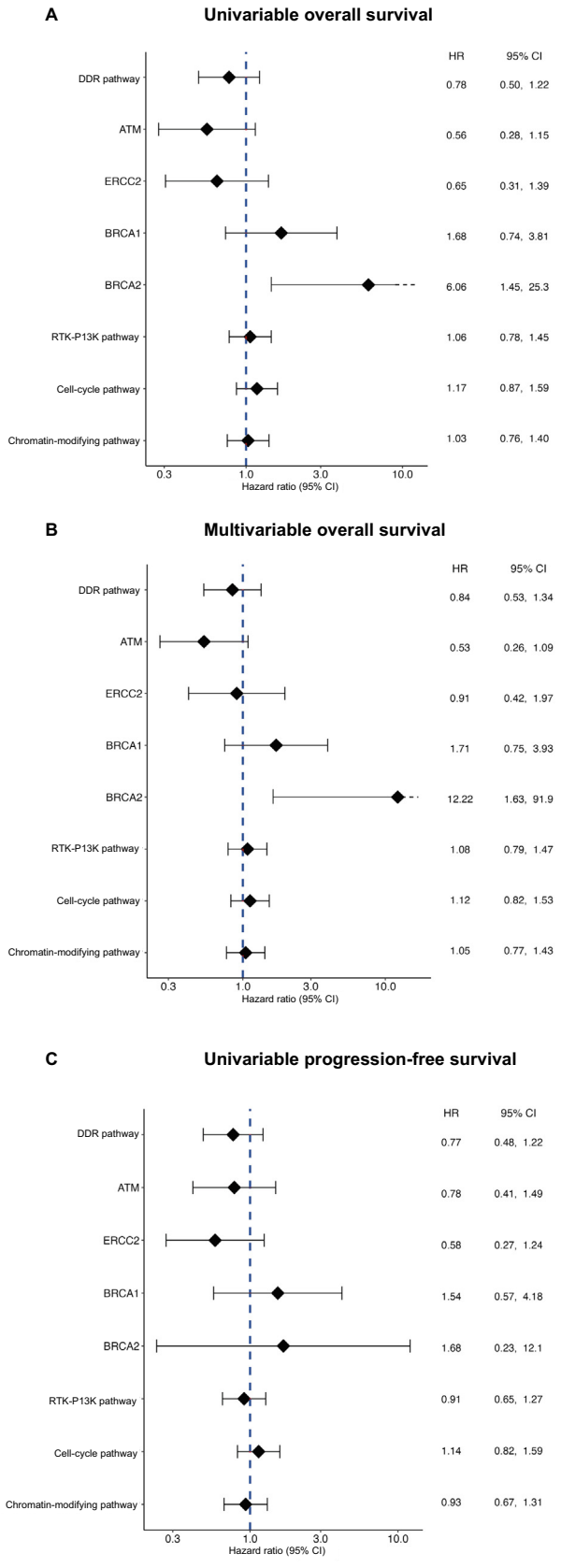

Fig. 2 – Associations of DDR genes and select functional pathways with (A,B) overall survival and (C) progression-free survival. For multivariable models (panel B), each gene or pathway was assessed in a separate multivariable model adjusted for ECOG performance status (0 vs 1) and the presence versus absence of visceral metastases. CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; DDR = DNA damage response and repair; HR = hazard ratio; RTK = receptor tyrosine kinase.

ship with OS and PFS (Fig. 3). These associations persisted after adjusting for visceral metastases and performance status (OS: HR 2.51, 95% CI 1.26–5.00; $p = 0.009$; PFS: HR 2.18, 95% CI 1.02–4.67; $p = 0.045$) and did not significantly change after stratification by treatment arm (Supplementary Tables 5 and 6).

3.4. *Non-DDR genes*

Alterations in *PIK3CA*, *ERBB2*, and *TERT* in cfDNA were associated with shorter OS, with HRs of 1.74 (95% CI 1.12–2.71; $p = 0.014$), 1.62 (95% CI 1.08–2.42; $p = 0.019$), and 1.74 (95% CI 1.12–2.71; $p = 0.014$), respectively, before adjusting for multiple comparisons (q values of 0.14, 0.14, and 0.032; Fig. 3 and Supplementary Table 7).

After multivariable adjustment for visceral metastases, performance status, and $\sqrt[3]{}$VAF 75pc, the association with OS remained for *PIK3CA* alterations (HR 1.91, 95% CI 1.20–3.04; $p = 0.006$) and *ERBB2* alterations (HR 1.64, 95% CI 1.08–2.49; $p = 0.019$). Concordance probability estimates (CPE) for models including *PIK3CA* and *ERBB2* were 0.63 (95% CI 0.59–0.67) and 0.63 (95% CI 0.59–0.67), respectively.

TERT alterations remained associated with shorter OS after adjustment for visceral metastases and performance status (HR 1.59, 95% CI 1.15–2.19; $p = 0.005$). *TERT* alterations were highly correlated with ctDNA VAF ($p < 0.001$) and were therefore not included in a multivariable model with VAF because of collinearity. In models of OS adjusted for visceral metastases and performance status, inclusion of *TERT* alteration status versus $\sqrt[3]{}$VAF 75pc resulted in CPEs of 0.62 (95% CI 0.58–0.66) and 0.62 (95% CI 0.58–0.66), respectively.

There was an insufficient number of events in most biomarker groups to formally test for interactions with bevacizumab exposure. We conducted an exploratory assessment of bevacizumab exposure and *TERT*, since *TERT* promoter alterations were prevalent and associated with poor survival. However, there was no meaningful differential effect of bevacizumab on the association of *TERT* with clinical outcomes (Supplementary Table 8 and Supplementary Fig. 4).

4. Discussion

In a cohort of patients with mUC enrolled in a phase 3 trial, genomic alterations detected in pretreatment cfDNA were generally concordant with those in matched archival tumor. However, a numerically higher rate of alterations in several prominent oncogenes was detected in cfDNA. Although we did not detect a statistically significant association between clinical outcomes and DDR gene alteration, the statistical power was insufficient because of a low frequency of DDR gene alterations in this cohort. High VAF in pretreatment ctDNA was associated with short OS and PFS, and ctDNA alterations in the *TERT* promoter, *PIK3CA*, and *ERBB2* were associated with shorter OS, independent of clinical prognostic factors. If further validated, these markers may serve to identify the patients most likely to benefit from clinical trials and novel treatment approaches.

Table 2 – Univariable associations of the ctDNA VAF with overall survival and progression-free survival (*n* = 201)

Parameter	Overall survival			Progression-free survival		
	HR (95% CI)	*p* value	*q* value[a]	HR (95% CI)	*p* value	*q* value[a]
Log VAF 75pc	1.13 (1.05–1.22)	**0.001**	0.003	1.11 (1.03–1.21)	**0.006**	0.015
Log median VAF	1.13 (1.05–1.22)	**0.002**	0.003	1.12 (1.03–1.21)	**0.005**	0.015
Log sum VAF	1.1 (1.04–1.18)	**0.002**	0.003	1.09 (1.02–1.17)	**0.01**	0.015
Log maximum VAF	1.12 (1.04–1.21)	**0.002**	0.003	1.11 (1.02–1.20)	**0.011**	0.015
$\sqrt[3]{}$VAF 75pc	3.48 (1.74–6.96)	**<0.001**	0.003	3.04 (1.44–6.42)	**0.004**	0.015
VAF 75pc tertiles						
VAF ≤0.018	Reference			Reference		
VAF >0.018–0.082	1.12 (0.77–1.63)	0.5	0.5	1.11 (0.74–1.66)	0.6	0.6
VAF >0.082	1.81 (1.25–2.63)	**0.002**	0.003	1.51 (1.01–2.25)	**0.044**	0.052

75pc = 75th percentile; CI = confidence interval; ctDNA = circulating tumor DNA; HR = hazard ratio; VAF = variant allele frequency.
[a] False discovery rate correction for multiple testing.

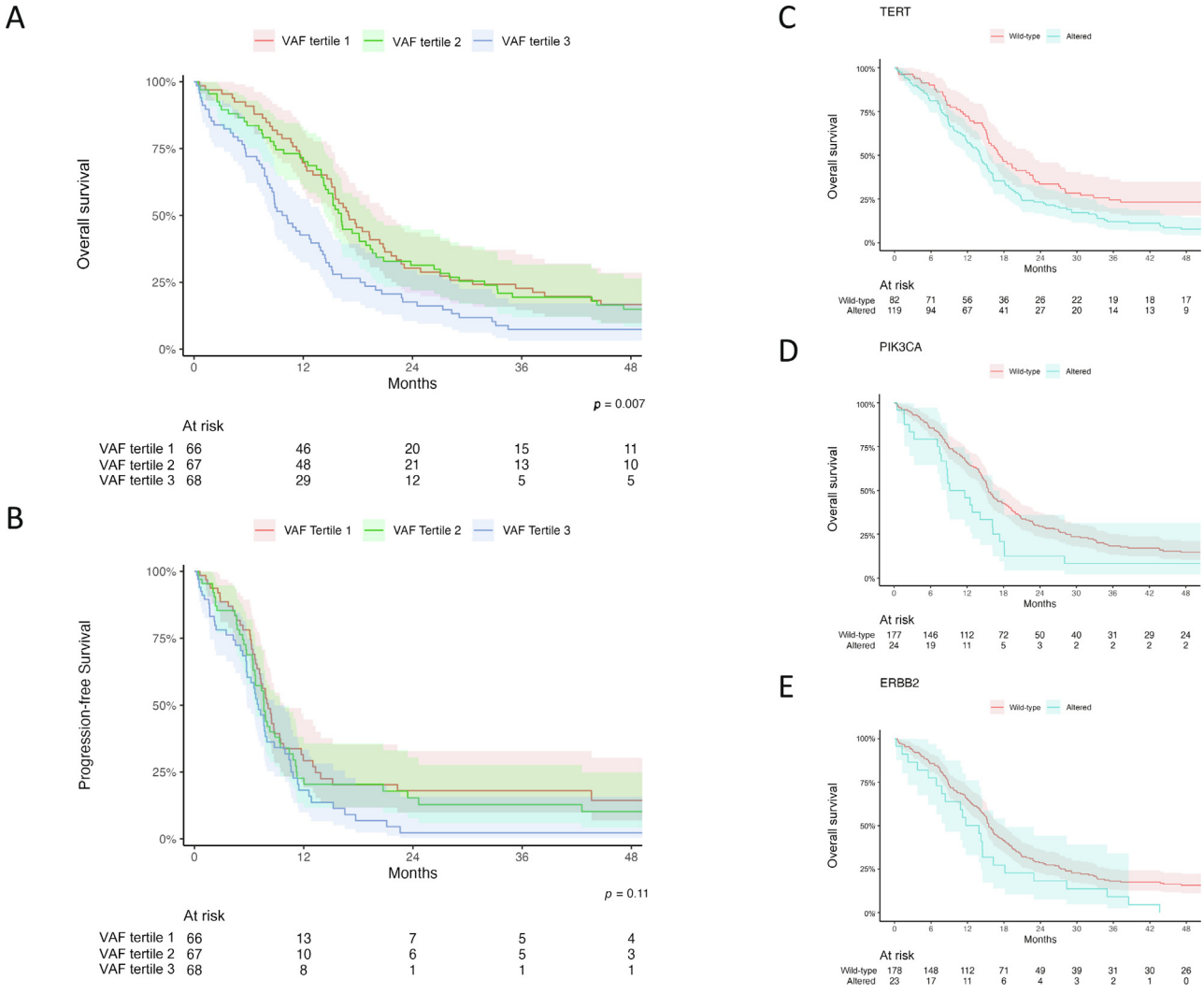

Fig. 3 – (A) Overall survival and (B) progression-free survival by tertiles for the 75th VAF percentile, and overall survival by cell-free DNA status for (C) *TERT*, (D) *PIK3CA*, and (E) *ERBB2*. Shaded areas indicate 95% confidence intervals. VAF = variant allele frequency.

Multiple studies have established DDR gene alterations as promising markers of cisplatin sensitivity in localized muscle-invasive bladder cancer [2–5]. The most extensively validated DDR gene, *ERCC2*, is a nucleotide excision repair protein in which helicase domain alterations confer cisplatin sensitivity in orthotopic xenograft models [1–3]. Notably, statistical power in the current study was limited by a low frequency of DDR gene alterations detected in cfDNA (*n* = 27, or 13%). Oncogenic *ERCC2* alterations in cfDNA were observed in only nine cases (4.5%), compared to 11% in the TCGA [20], and associations between *ERCC2* and longer OS (HR 0.65; *p* = 0.3) and PFS (HR 0.58; *p* = 0.2) did not reach statistical significance. Ultimately, the completion of ongoing randomized trials to definitively clarify the value of DDR

genes in the context of neoadjuvant chemotherapy remains crucial to the field (eg, NCT03609216).

To the best of our knowledge, this is the largest study in mUC to demonstrate an association between high baseline VAF in ctDNA and shorter OS and PFS, even after adjusting for clinical prognostic variables. Notably, a recent cfDNA analysis from KEYNOTE-361 showed no statistically significant association between baseline maximum VAF in cfDNA and mUC outcomes after platinum-based chemotherapy [22]. However, there were some evidence of an association, and the findings in that study were limited by statistical imprecision and uncertainty, as the cfDNA analysis included only 125 patients. Furthermore, baseline VAF in ctDNA was prognostic for OS in other, more heterogeneous mUC cohorts (n = 53 and 103) [23,24]. If validated, our findings highlight the potential utility of cfDNA for prognostication and clinical trial stratification.

TERT, *PIK3CA*, and *ERBB2* are all implicated in bladder cancer biology and were associated with poor prognosis in our cohort. *TERT* promoter alterations occur in 73% of bladder tumors [25] and were associated with shorter survival when present in UC tumors in a cohort of patients with variable disease stages [25]. Our findings regarding *PIK3CA* corroborate a smaller analysis of mUC cfDNA that also found that *PIK3CA* alterations were associated with shorter OS [23], although data on the prognostic significance of *PIK3CA* alterations in localized UC are mixed [26]. In muscle-invasive bladder cancer, *ERBB2* missense mutations have been associated with excellent response to neoadjuvant chemotherapy; however, despite a pathologic complete response to chemotherapy, 3/11 patients with *ERBB2*-mutant muscle-invasive bladder cancer in that study developed distant recurrence [27]. Ultimately, the prognostic significance of these biomarkers for UC requires further study and validation in both the localized and metastatic settings. The association of *ERBB2* with poor OS observed in our analysis is especially pertinent given mUC response rates of 33–83% to regimens containing HER2-directed antibody-drug conjugates such as trastuzumab deruxtecan and disitamab vedotin [28,29].

Enfortumab vedotin plus pembrolizumab recently supplanted cisplatin-based chemotherapy as the preferred first-line therapy for mUC [30,31]. However, gemcitabine/-cisplatin remains a crucial regimen for mUC, for example in the second line following enfortumab vedotin plus pembrolizumab [30,31]. Therefore, the current findings remain highly relevant. In fact, the rapidly evolving therapy landscape for mUC compels the next generation of clinical trials to redefine the role of platinum chemotherapy in competition with other recently approved and investigational agents. Such trials would greatly benefit from stratification by prognostic cfDNA biomarkers, such as those explored in the current study if properly validated.

Our study has several limitations. First, only a subset of patients in CALGB 90601 had sufficient plasma for cfDNA analysis, raising potential for sampling bias. However, there were no observable clinical differences between our cfDNA cohort and the overall trial cohort. Second, while half of the patients in this cohort received gemcitabine/cisplatin (plus placebo), the other half received gemcitabine/cisplatin plus bevacizumab. However, there was no clinically meaningful impact of bevacizumab on outcomes in comparison to placebo [12]. Moreover, we stratified analyses by bevacizumab exposure and observed no significant difference in our findings. Finally, our cfDNA platform was limited to a panel of selected genes, and therefore genomic features outside the regions covered, as well as epigenomic and transcriptional features, could not be assessed [14]. While the panel covered multiple DDR genes, including *ERCC2* [1–3], it did not include all DDR genes [14,32]. However, the 129 genes covered by our New York State Department of Health–approved platform were selected on the basis of genomic data from >25 000 solid tumors to encompass the most recurrent oncogenic mutations and all clinically actionable genes in solid tumor oncology. The limited panel also allowed ultra-deep sequencing (depth of coverage >15 000×) to ensure de novo detection of low-frequency mutations down to a VAF of 0.5% [14]. Nonetheless, further study is warranted to investigate genomic and transcriptional features beyond those interrogated by our assay.

5. Conclusions

In an analysis of pretreatment cfDNA from a cohort of patients with mUC nested within a phase 3 trial of cisplatin-based chemotherapy, we found that detection of alterations in several notable oncogenes was more frequent in cfDNA than in matched archival tumor. We did not observe a statistically significant association between DDR gene alterations in cfDNA and clinical outcomes, but the analysis was underpowered because of a low frequency of DDR gene alterations. Higher VAF in ctDNA was prognostic for shorter OS and PFS, and alterations in the *TERT* promoter, *PIK3CA*, and *ERBB2* in ctDNA were poor prognostic markers, even after adjusting for clinical factors. If validated, these biomarkers may guide clinical trial stratification and identify patients likely to benefit from investigational therapies.

Author contributions: Jonathan E. Rosenberg had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Rosenberg, Guercio, Whiting, Ballman, Halabi, Bajorin, Beltran, Morris, Berger, Seshan, Iyer.
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2.4. Data availability

The study data are available via cBioPortal [19].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euros.2025.03.009.

References

[1] Li Q, Damish AW, Frazier Z, et al. ERCC2 helicase domain mutations confer nucleotide excision repair deficiency and drive cisplatin sensitivity in muscle-invasive bladder cancer. Clin Cancer Res 2019;25:977–88.
[2] Liu D, Plimack ER, Hoffman-Censits J, et al. Clinical validation of chemotherapy response biomarker ERCC2 in muscle-invasive urothelial bladder carcinoma. JAMA Oncol 2016;2:1094–6.
[3] Van Allen EM, Mouw KW, Kim P, et al. Somatic ERCC2 mutations correlate with cisplatin sensitivity in muscle-invasive urothelial carcinoma. Cancer Discov 2014;4:1140–53.
[4] Iyer G, Balar AV, Milowsky MI, et al. Multicenter prospective phase II trial of neoadjuvant dose-dense gemcitabine plus cisplatin in patients with muscle-invasive bladder cancer. J Clin Oncol 2018;36:1949–56.
[5] Miron B, Hoffman-Censits JH, Anari F, et al. Defects in DNA repair genes confer improved long-term survival after cisplatin-based neoadjuvant chemotherapy for muscle-invasive bladder cancer. Eur Urol Oncol 2020;3:544–7.
[6] Meeks JJ, Al-Ahmadie H, Faltas BM, et al. Genomic heterogeneity in bladder cancer: challenges and possible solutions to improve outcomes. Nat Rev Urol 2020;17:259–70.
[7] Faltas BM, Prandi D, Tagawa ST, et al. Clonal evolution of chemotherapy-resistant urothelial carcinoma. Nat Genet 2016;48:1490–9.
[8] Liu D, Abbosh P, Keliher D, et al. Mutational patterns in chemotherapy resistant muscle-invasive bladder cancer. Nat Commun 2017;8:2193.
[9] Thomsen MBH, Nordentoft I, Lamy P, et al. Comprehensive multiregional analysis of molecular heterogeneity in bladder cancer. Sci Rep 2017;7:11702.
[10] Corcoran RB, Chabner BA. Application of cell-free DNA analysis to cancer treatment. N Engl J Med 2018;379:1754–65.
[11] Diehl F, Schmidt K, Choti MA, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med 2008;14:985–90.
[12] Rosenberg JE, Ballman KA, Halabi S, et al. Randomized phase III trial of gemcitabine and cisplatin with bevacizumab or placebo in patients with advanced urothelial carcinoma: results of CALGB 90601 (Alliance). J Clin Oncol 2021;39:2486–96.

[13] Tsui DWY, Cheng ML, Shady M, et al. Tumor fraction-guided cell-free DNA profiling in metastatic solid tumor patients. Genome Med 2021;13:96.
[14] Rose Brannon A, Jayakumaran G, Diosdado M, et al. Enhanced specificity of clinical high-sensitivity tumor mutation profiling in cell-free DNA via paired normal sequencing using MSK-ACCESS. Nat Commun 2021;12:3770.
[15] Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn 2015;17:251–64.
[16] Chapin WJ, Till JE, Hwang WT, et al. Multianalyte prognostic signature including circulating tumor DNA and circulating tumor cells in patients with advanced pancreatic adenocarcinoma. JCO Precis Oncol 2022;6:e2200060.
[17] Manikandan S. Data transformation. J Pharmacol Pharmacother 2010;1:126–7.
[18] Bajorin DF, Dodd PM, Mazumdar M, et al. Long-term survival in metastatic transitional-cell carcinoma and prognostic factors predicting outcome of therapy. J Clin Oncol 1999;17:3173–81.
[19] Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401–4.
[20] Robertson AG, Kim J, Al-Ahmadie H, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell 2017;171:540–556.e25.
[21] Guercio BJ, Sarfaty M, Teo MY, et al. Clinical and genomic landscape of FGFR3-altered urothelial carcinoma and treatment outcomes with erdafitinib: a real-world experience. Clin Cancer Res 2023;29:4586–95.
[22] Powles T, Chang YH, Yamamoto Y, et al. Pembrolizumab for advanced urothelial carcinoma: exploratory ctDNA biomarker analyses of the KEYNOTE-361 phase 3 trial. Nat Med 2024;30:2508–16.
[23] Shohdy KS, Villamar DM, Cao Y, et al. Serial ctDNA analysis predicts clinical progression in patients with advanced urothelial carcinoma. Br J Cancer 2022;126:430–9.
[24] Vandekerkhove G, Lavoie JM, Annala M, et al. Plasma ctDNA is a tumor tissue surrogate and enables clinical-genomic stratification of metastatic bladder cancer. Nat Commun 2021;12:184.
[25] Isharwal S, Audenet F, Drill E, et al. Prognostic value of TERT alterations, mutational and copy number alterations burden in urothelial carcinoma. Eur Urol Focus 2019;5:201–4.
[26] Patel VG, McBride RB, Lorduy AC, et al. Prognostic significance of PIK3CA mutation in patients with muscle-invasive urothelial carcinoma (UC). J Clin Oncol 2016;34(15 Suppl):e16002.
[27] Groenendijk FH, de Jong J, Fransen van de Putte EE, et al. ERBB2 mutations characterize a subgroup of muscle-invasive bladder cancers with excellent response to neoadjuvant chemotherapy. Eur Urol 2016;69:384–8.
[28] Galsky MD, Conte GD, Foti S, et al. Primary analysis from DS8201-A-U105: a phase 1b, two-part, open-label study of trastuzumab deruxtecan (T-DXd) with nivolumab (nivo) in patients (pts) with HER2-expressing urothelial carcinoma (UC). J Clin Oncol 2022;40(6 Suppl):438.
[29] Sheng X, Zhou L, Yang K, et al. Disitamab vedotin, a novel humanized anti-HER2 antibody-drug conjugate (ADC), combined with toripalimab in patients with locally advanced or metastatic urothelial carcinoma: an open-label phase 1b/2 study. J Clin Oncol 2023;41(16 Suppl):4566.
[30] Flaig TW, Spiess PE, Abern M, et al. NCCN Guidelines® insights: bladder cancer, version 3.2024. J Natl Compr Canc Netw 2024;22:216–25.
[31] Powles T, Bellmunt J, Comperat E, et al. ESMO Clinical Practice Guideline interim update on first-line therapy in advanced urothelial carcinoma. Ann Oncol 2024;35:485–90.
[32] Teo MY, Bambury RM, Zabor EC, et al. DNA damage response and repair gene alterations are associated with improved survival in patients with platinum-treated advanced urothelial carcinoma. Clin Cancer Res 2017;23:3610–8.