Variant allele frequency changes in TP53 predict pembrolizumab response in patients with metastatic urothelial carcinoma

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Abstract. Prognoses for patients with metastatic urothelial carcinoma (mUC) have improved with pembrolizumab treatment, an immune checkpoint inhibitor, but clinical benefits are limited to a subset of patients. Therefore, a non-invasive biomarker to predict pembrolizumab response is required. The present study retrospectively examined genomic alterations in 25 plasma circulating tumor DNA (ctDNA) samples using targeted sequencing of 77 genes from 16 patients with mUC during pembrolizumab treatment. A total of 11 (68.8%) patients demonstrated ≥2 genomic alterations, including *TP53* mutations (as defined by ctDNA-positive status). The proportion of responders to pembrolizumab in the ctDNA-positive group was higher compared with that in the ctDNA-negative group (72.7 vs. 20.0%). Furthermore, among all detected genomic alterations, variant allele frequency decreases in *TP53* during pembrolizumab treatment were mainly associated with therapeutic response. Collectively, these data suggest that profiling of ctDNA in plasma, particularly *TP53*, may be useful for predicting and monitoring therapeutic responses to pembrolizumab in patients with mUC.

Introduction

Patients with metastatic urothelial carcinoma (mUC) have a poor prognosis, with a 5-year survival rate of ~15% using conventional treatment regimens (1,2). The current standard treatment for patients with mUC, cisplatin-based chemotherapy, has a long history for mUC treatment but median overall survival (OS) times for patients with mUC remain at 14-15 months (3). Patients demonstrating mUC recurrence after first-line treatment, especially those with progression during first-line treatment, have a particularly poor prognosis. The introduction of immune checkpoint inhibitors (ICIs) as a second-line treatment has resulted in longer durations of treatment response and improved prognoses, but only for responders; the proportion of those patients is limited to 15-30% (4-6). Therefore, a non-invasive biomarker to evaluate response to ICIs is urgently required.

Regarding tissue-based biomarkers for ICI responses, several studies have reported the significance of tumor programmed death-1 (PD-L1) expression, tumor mutation burden (TMB) and microsatellite instability (MSI) (7-9). However, tissue biopsies present several problems in terms of invasiveness, accessibility and frequency of use in contrast to blood-based biomarkers obtained by liquid biopsy (10). Of note, plasma circulating tumor DNA (ctDNA) from liquid biopsies has shown particular promise in screening patients who may benefit from ICIs (11). In addition, ctDNA is useful for detecting minimal residual disease, monitoring disease status during treatment and predicting response to therapeutic drugs with a significant lead time to radiographic imaging (12-14). However, the significance of plasma ctDNA as a biomarker to evaluate pembrolizumab response in patients with mUC has not yet been fully investigated.

Therefore, the present study investigated the association between plasma ctDNA analysis and response to pembrolizumab in patients with mUC.

Materials and methods

Patients and sample collection. A total of 25 plasma samples from 16 patients with mUC were prospectively collected using PAX gene circulating free DNA (cfDNA) tubes (PreAnalytiX GmbH) at the University of Tsukuba Hospital (Tsukuba, Japan) between February 2018 and April 2021. Of these 25 samples, 16 were collected prior to administration of pembrolizumab (used as a second-line therapy) and nine were collected after administration of pembrolizumab in conjunction with computed tomography evaluation of clinical response. All

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patients provided informed, written consent to participate in the present study and the protocol was approved by the University of Tsukuba Hospital Institutional Review Board (Tsukuba, Japan; approval no, #H28-104). Clinical data were obtained from hospital charts. Response to pembrolizumab was evaluated according to the Response Evaluation Criteria in Solid Tumor (RECIST) guidelines (version 1.1) (15). In the present study, response to pembrolizumab was classified as complete response (CR), partial response (PR) and stable disease (SD).

Survival analysis. For analysis of OS, death from any cause was defined as an event and patients without any events were censored at the last follow-up visit. For progression-free survival (PFS) analysis, progressive disease (PD) evaluated by RECIST was defined as an event. The number of months from the date of administration of pembrolizumab to the event or censored date was calculated for OS and PFS.

Sample preparation and targeted sequencing. The extracted plasma cfDNA was subjected to a ctDNA analysis using the Avenio ctDNA Expanded Kit (Roche Diagnostics) designed to detect 77 genetic alterations, including single nucleotide variants (SNVs), indels and copy number variants (CNVs), according to the manufacturer's instructions. DNA concentration was assessed using a Qubit fluorometer (Thermo Fisher Scientific, Inc.). Prepared libraries were sequenced using a NextSeq 500 (Illumina, Inc) and analyzed using Roche AVENIO ctDNA Analysis Software (version 2.0.0; Roche Sequencing Solutions). Somatic variants were called and filtered with human reference genome hg38 using the same software and default logical and operation filter sets according to the manufacturer's instructions. Patients who carried ≥2 alterations (SNV, indels or CNVs) were defined as ctDNA-positive.

Statistical analysis. All statistical analyses were performed using R (version 4.0.2; R Foundation) and GraphPad Prism (version 8; GraphPad Software; Dotmatics). Kaplan-Meier survival curves were generated and the differences between the curves were compared with the log-rank test. For continuous variables, differences between groups were compared using the Mann-Whitney U-test. $P<0.05$ was considered to indicate a statistically significant difference.

Table I. Patient characteristics (n=16).

Characteristic	Value
Age, years	70.0 (51-81)
Sex	
Male	12 (75.0)
Female	4 (25.0)
Follow-up time, months	9.5 (1.0-24.7)
Histology	
UC	13 (81.3)
UC with small cell component	2 (12.5)
UC with sarcomatoid variant	1 (6.2)
Chemotherapy regimen	
GC	9 (56.3)
GCa	4 (25.0)
GC+GCa	2 (12.5)
EP	1 (6.2)
Performance status	
0	7 (43.8)
1	4 (25.0)
NA	5 (31.2)
Clinical response	
PR	4 (25.0)
SD	5 (31.3)
PD	7 (43.7)
Tumor location	
Bladder	10 (62.5)
Upper urinary tract	6 (37.5)
Visceral metastasis	
Present	8 (50.0)
Absent	8 (50.0)

Values are expressed as n (%) or the median (range). UC, urothelial carcinoma; GC, gemcitabine and cisplatin; GCa, gemcitabine and carboplatin; EP, etoposide and cisplatin; NA, not available; PR, partial response; PD, progressive disease; SD, stable disease.

Results

Patient characteristics. The characteristics of patients with mUC (n=16) treated with second-line pembrolizumab are presented in Table I. The median age of the patient pool was 70.0 years (range, 51-81 years) and the majority were male (75.0%). The most common histology was UC (n=13), followed by UC with small cell component (n=1) and UC with sarcomatoid variant (n=1). In terms of tumor location, 10 (62.5%) patients had bladder tumors and six (37.5%) had upper urinary tract tumors. Half of all patients presented with visceral metastasis.

As first-line chemotherapy before pembrolizumab administration, nine (56.3%) patients received a combination therapy of gemcitabine and cisplatin (GC), four (25.0%) patients received a combination therapy of gemcitabine and carboplatin (GCa), and one (6.2%) patient received a combination therapy of etoposide and cisplatin (EP). The remaining two patients (12.5%) received GC followed by GCa therapy as first- and second-line chemotherapies. Most patients (68.8%) demonstrated a good initial performance status of 0-1 at the time of pembrolizumab administration and second-line therapy resulted in nine (four PR and five SD; 56.3%) responders and seven non-responders (PD; 43.8%).

Genomic alteration profiles in plasma ctDNA and clinical outcomes. The genomic alteration profiles in plasma ctDNA collected from the 16 patients at baseline were analyzed. Unfiltered and filtered somatic variant data for each patient were collected. In total, 43 somatic variants (35 SNVs, one indel and seven CNVs) were identified with a median VAF of 2.0% (range, 0.08-55.8%) (Table SI). The observed genomic alteration profiles of analyzed plasma ctDNA were examined (Fig. 1). The median number of genomic alterations was

Table II. Patient characteristics stratified by ctDNA status.

Characteristic	ctDNA-positive (n=11)	ctDNA-negative (n=5)
Age, years	71.0 (59-81)	69.0 (51-74)
Sex		
Male	8 (72.7)	4 (80.0)
Female	3 (27.3)	1 (20.0)
Follow-up time, months	10.4 (1.0-24.7)	5.6 (2.4-11.9)
Chemotherapy regimen		
GC	6 (54.5)	3 (60.0)
GCa	2 (18.2)	0 (0.0)
GC+GCa	2 (18.2)	2 (40.0)
EP	1 (9.1)	0 (0.0)
Performance status		
0	6 (54.5)	1 (20.0)
1	2 (18.2)	2 (40.0)
NA	3 (27.3)	2 (40.0)
Tumor location		
Bladder	8 (72.7)	2 (40.0)
Upper urinary tract	3 (27.3)	3 (60.0)
Visceral metastasis		
Present	5 (45.5)	3 (60.0)
Absent	6 (54.5)	2 (40.0)
WBC count	6,000 (2,900-9,700)	8,900 (3,600-22,400)
Neutrophil count	3,830 (1,302-6,383)	6,602 (2,045-20,026)
Lymphocyte count	1,428 (378-2,408)	907 (417-1,958)
NLR	2.7 (1.3-12.1)	7.3 (1.7-40.0)[a]

[a]P<0.05 vs. ctDNA-positive group. Values are expressed as n (%) or the median (range). ctDNA, circulating tumor DNA; GC, gemcitabine and cisplatin; GCa, gemcitabine and carboplatin; EP, etoposide and cisplatin; NA, not available; WBC, white blood cell; NLR, neutrophil-lymphocyte ratio.

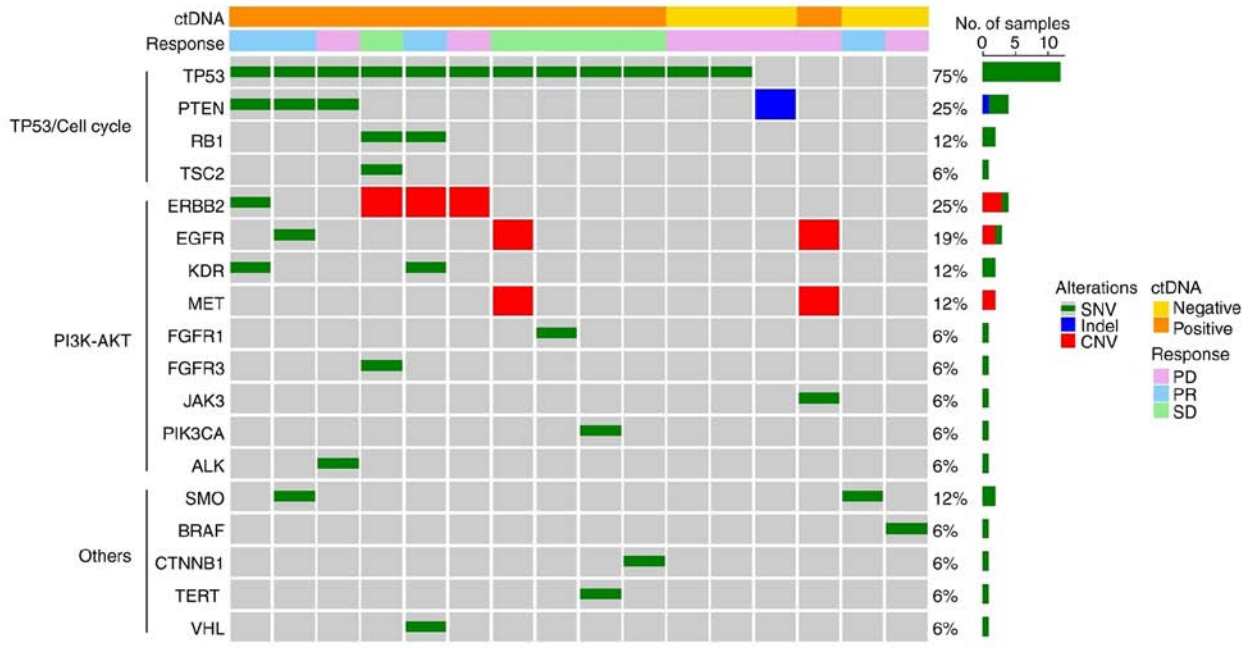

Figure 1. Genomic alteration profiles of plasma ctDNA analyzed by targeted sequencing. Patients who demonstrated ≥2 alterations were defined as ctDNA-positive. ctDNA, circulating tumor DNA; SNV, single nucleotide variant; CNV, copy number variant; PD, progressive disease; PR, partial response; SD, stable disease.

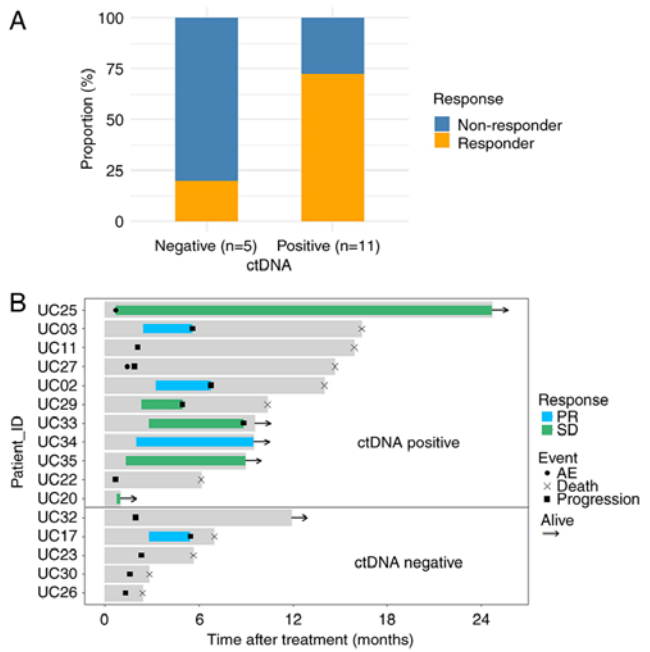

Figure 2. Proportions of (A) patient responses and (B) response statuses in individual patients over time stratified by ctDNA status. ctDNA, circulating tumor DNA; PR, partial response; SD, stable disease; AE, adverse event.

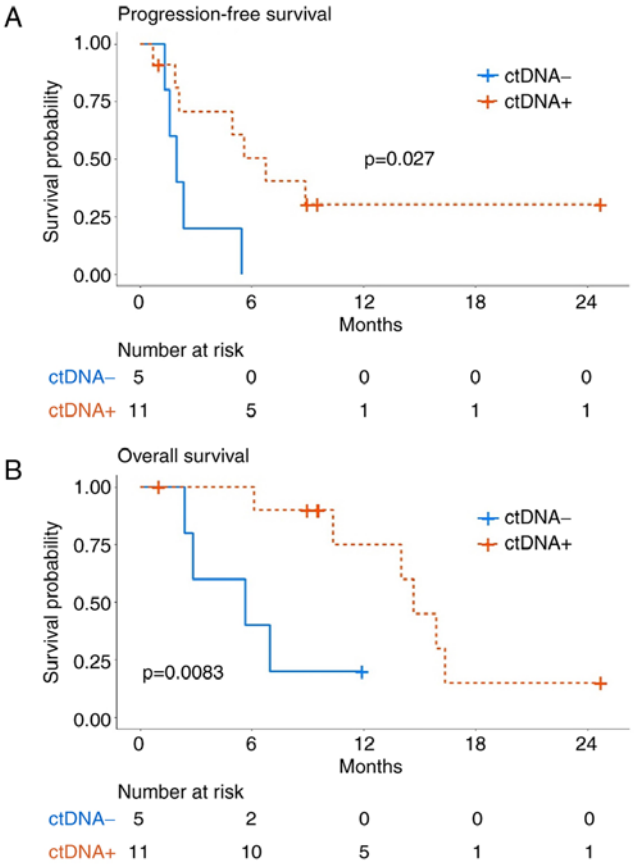

Figure 3. (A) Progression-free survival and (B) overall survival of patients treated with pembrolizumab stratified by ctDNA status. + in the Kaplan-Meier curves indicate censored data-points. ctDNA, circulating tumor DNA.

2.5 (range, 1-6) and all patients demonstrated ≥1 alterations. The most commonly altered gene was *TP53* (75%), followed by *ERBB2* (25%), *PTEN* (25%) and *EGFR* (19%). In terms of *TP53* mutations, the most frequent subtype was missense (67%), followed by nonsense (25%) and splicing mutations (8%). Among ctDNA-positive patients, defined as those who demonstrated ≥2 genomic alterations, 10/11 (91%) had *TP53* SNVs. There were no significant differences in cell-free DNA concentration after stratification by ctDNA positivity (Fig. S1). The characteristics of patients with mUC were also stratified by ctDNA status (Table II). The neutrophil-lymphocyte ratio of patients in the ctDNA-negative group was significantly higher than that in the ctDNA-positive group (7.28 vs. 2.65).

Association between mutation frequency data from target sequencing and response to pembrolizumab. The association between mutation profiles and pembrolizumab response was analyzed. The proportion of responsive patients in the ctDNA-positive group was higher than that in the ctDNA-negative group (72.7 vs. 20.0%) (Fig. 2A). After stratification by ctDNA positivity, median response times in the ctDNA-positive group were significantly longer compared with those in the ctDNA-negative group (3.2 vs. 0 months) (Fig. 2B). Furthermore, 4/11 (36.4%) patients in the ctDNA-positive group demonstrated a sustained response at data cutoff, while none in the ctDNA-negative group had such a response.

The association between ctDNA positivity and patient prognoses, as well as response to pembrolizumab, was analyzed. The median PFS of patients in the ctDNA-positive group was significantly longer compared with that in the ctDNA-negative group (3.9 vs. 2.0 months) (Fig. 3A) and the median OS demonstrated a similar trend (14.7 vs. 5.6 months) (Fig. 3B). These results suggested that the mutation frequency in plasma ctDNA was associated with clinical response to pembrolizumab and disease outcomes.

Association between changes in the VAF of each mutation in plasma ctDNA and pembrolizumab response. The association between mutation frequency and clinical response during pembrolizumab treatment using samples from eight (50.0%) of 16 patients collected during pembrolizumab treatment was examined. There was no association between changes in the mutation frequency and individual clinical responses (Fig. S2). Next, the association between changes in the VAF of each mutation and individual clinical responses was examined. The UC03 patient who demonstrated PR with shrinking lymph node metastasis had decreased VAF in *TP53*, *RB1* and *KDR*, whereas the VAF in *ERBB2* and *RAF1* was increased (Fig. 4A). Another patient (UC22), who demonstrated PD with enlargement of lymph node metastasis, had increased VAF in *TP53*, *AKL* and *GNA11* and decreased VAF in *PTEN* (Fig. 4B). Among these mutated genes, only the VAF change in *TP53* was associated with clinical response. In terms of the VAF changes for each mutated gene in the other six patients, only *TP53* was associated with clinical response during pembrolizumab treatment (Fig. 4C). These results suggested that the VAF changes in *TP53* during pembrolizumab treatment accurately reflected the therapeutic response.

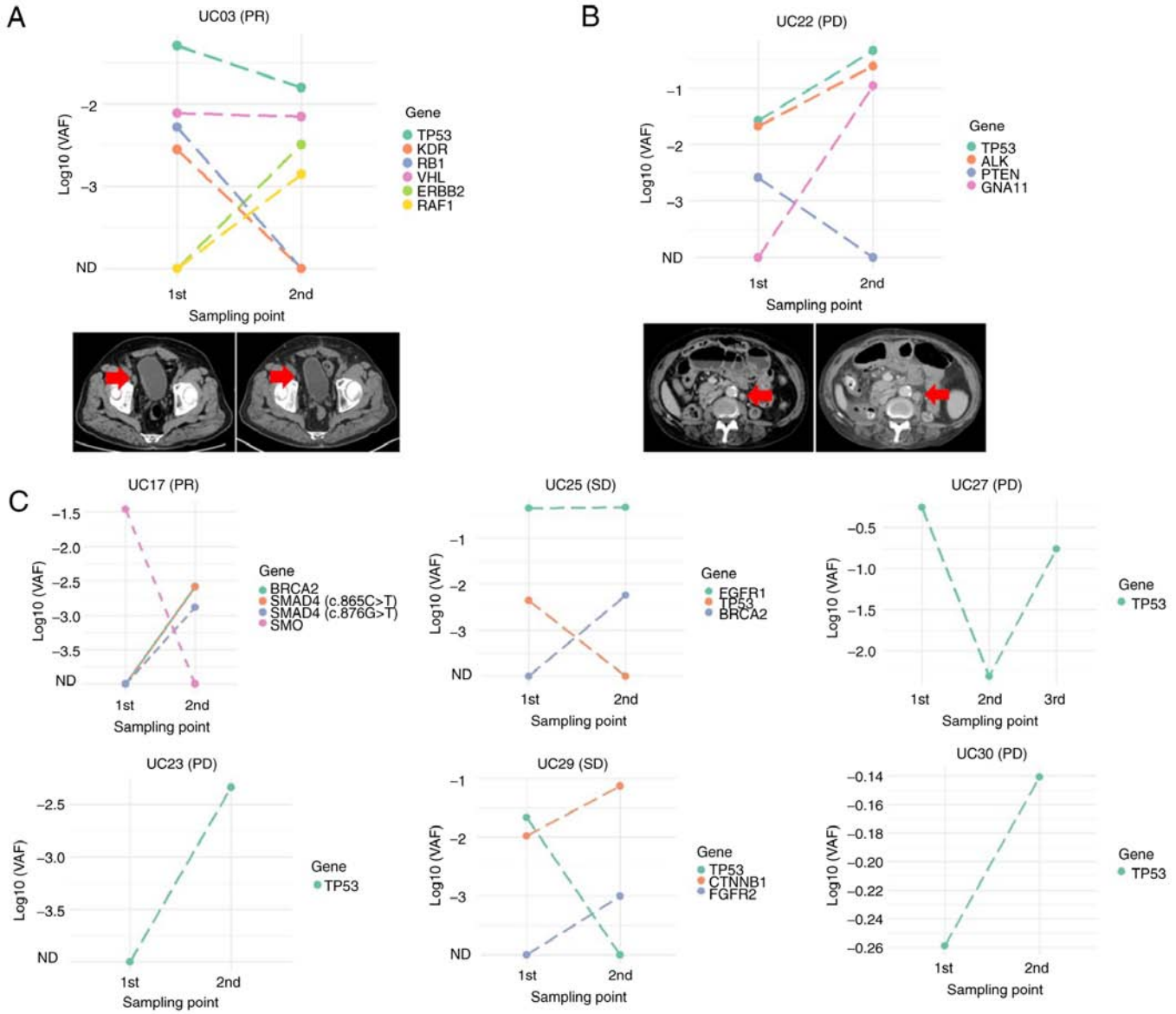

Figure 4. Changes in variant allele frequency for each mutation in eight patients with (A) PR, (B) PD and (C) other disease statuses. Computed tomography images indicate changes of lymph node metastasis (red arrows). Samples were collected up to three times during pembrolizumab treatment. PD, progressive disease; PR, partial response; VAF, variant allele frequency; ND, not detected.

Discussion

The present study, using genomic alteration profiling of plasma ctDNA collected from 16 patients with mUC at baseline, demonstrated that ctDNA-positive status was associated with patient response to pembrolizumab treatment. Furthermore, analysis of consecutive samples from eight patients during treatment demonstrated that decreases in *TP53* VAF accurately reflected therapeutic response. These results suggested that profiling of plasma ctDNA was useful in predicting and monitoring the therapeutic pembrolizumab response in patients with mUC.

Blood-based biomarkers, which may be detected by liquid biopsy in a non-invasive manner, are useful in screening responsive patients (11); however, there are no currently established and approved blood-based biomarkers for predicting responses to ICI. While TMB is recognized as a tissue-based biomarker for ICI response (8,16), previous studies suggest that elevated TMB in blood, a liquid biopsy-style marker as detected by ctDNA assays, is also associated with responses and prognoses in patients treated with ICI (17-19). Si *et al* (18) previously reported that blood TMB ^{3}20 mutations/megabase was useful in predicting clinical benefit from ICI treatment compared with chemotherapy. In addition, Kim *et al* (19) reported that ctDNA mutational load scores correlate with clinical pembrolizumab responses in metastatic gastric cancer. Although the present study did not examine the blood TMB, an association between ctDNA-positive status and ICI response was demonstrated. On the other hand, Laukhtina *et al* (20) reported an association between high baseline ctDNA levels and worse disease-free survival and OS. This may be due to different profiling of the mutated genes, including the percentage of *TP53* mutations, between the previous studies and the present study. A number of studies previously reported that *TP53* mutations are associated with superior clinical benefits to ICI treatment (21-23). In line with these studies, the present study demonstrated that

TP53 mutations were associated with an improved patient response to ICI. Sun *et al* (22) reported that *TP53* missense mutations were associated with better clinical benefit in their local clinical cohort, but nonsense mutations were not. Similar trends were also reported in the MSK study cohort, while the Checkmate-012 study cohort reported an opposite trend (22). In the present study, *TP53* missense mutations were the most common subtype of *TP53* mutations.

A recent study reported that ctDNA clearance during ICI treatment in advanced urothelial carcinoma is associated with better clinical outcomes (24), which led the present study to investigate VAF changes for each mutation during second-line pembrolizumab treatment. The tumor suppressor *TP53* is the most frequently mutated cancer-associated gene in urothelial bladder cancer (25), a result also demonstrated by the present study. Thorsson *et al* (26) suggested that driver mutations, such as *TP53*, by inducing genomic instability, may alter the immune environment through the production of neoantigens and paradoxically produce better response to ICIs. However, among the genomic alterations detected in the present study, decreases in *TP53* VAF were associated with improved clinical response during pembrolizumab treatment. Similarly, Parkinson *et al* (27) reported that, in high-grade serous ovarian carcinoma, *TP53* VAF changes during chemotherapy correlated with clinical response. The findings of the present study, which suggested that decreases in *TP53* VAF during pembrolizumab treatment were associated with therapeutic response, open up new possibilities for frequent liquid biopsies to screen first-line non-responders for second-line feasibility.

The present study has several limitations. First, the number of patients studied was small, so larger patient cohorts are necessary for future studies. Second, technological restrictions limited the assessable genes to 77; therefore, the blood TMB and MSI status of samples could not be identified. Third, as the study protocol did not include any tissue analysis, the TMB and PD-L1 expression could not be evaluated, both of which have been previously reported to correlate with ICI response (7-9). Studies have reported that sequential ctDNA analysis can identify responders to ICI therapy faster when compared with radiographic imaging (12,13). As the present study protocol did not determine the timings of liquid biopsies and radiographic evaluation, it was difficult to assess the time saved for ctDNA analysis over radiographic imaging. Finally, variants associated with clonal hematopoiesis of indeterminate potential could be mistaken for tumor-specific variants.

In conclusion, plasma ctDNA analysis in patients with mUC treated with pembrolizumab demonstrated an association of *TP53* mutation frequency with clinical response of patients. Furthermore, decreases in *TP53* VAF during pembrolizumab treatment was associated with clinical response. These results suggest that plasma ctDNA analysis of patients with mUC could potentially be used for predicting both pembrolizumab response and monitoring disease status.

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Availability of data and materials

Sequence data generated during the current study are available in the Japanese Genotype-phenotype Archive (https://ddbj.nig.ac.jp/resource/jga-study/JGAS000622). The generated somatic variant data for each patient is presented in Table SI.

Authors' contributions

YN, TK and BJM contributed to the study design and technical and material support. KH, YN, SK, KT, MS, AH, HNe and HNi performed sample preparation. KH, YN and TK analyzed the acquired data. KH and YN drafted the manuscript. KH, YN, SK, BJM, HNe and HNi revised the manuscript critically. KH and YN confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol and data processing were approved by the University of Tsukuba Hospital Institutional Review Board (approval no. H28-104). Written informed consent was obtained from all patients involved in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. von der Maase H, Sengelov L, Roberts JT, Ricci S, Dogliotti L, Oliver T, Moore MJ, Zimmermann A and Arning M: Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. J Clin Oncol 23: 4602-4608, 2005.
2. Babaian RJ, Johnson DE, Llamas L and Ayala AG: Metastases from transitional cell carcinoma of urinary bladder. Urology 16: 142-144, 1980.
3. Clark PE, Spiess PE, Agarwal N, Bangs R, Boorjian SA, Buyyounouski MK, Efstathiou JA, Flaig TW, Friedlander T, Greenberg RE, *et al*: NCCN guidelines® insights bladder cancer, version 2.2016 featured updates to the NCCN guidelines. J Natl Compr Canc Netw 14: 1213-1224, 2016.
4. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, Loriot Y, Necchi A, Hoffman-Censits J, Perez-Gracia JL, *et al*: Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: A single-arm, multicentre, phase 2 trial. Lancet 389: 67-76, 2017.
5. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, Vogelzang NJ, Climent MA, Petrylak DP, Choueiri TK, *et al*: Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med 376: 1015-1026, 2017.

6. Sharma P, Retz M, Siefker-Radtke A, Baron A, Necchi A, Bedke J, Plimack ER, Vaena D, Grimm MO, Bracarda S, *et al*: Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): A multicentre, single-arm, phase 2 trial. Lancet Oncol 18: 312-322, 2017.
7. Petrelli F, Ghidini M, Ghidini A and Tomasello G: Outcomes following immune checkpoint inhibitor treatment of patients with microsatellite instability-high cancers: A systematic review and meta-analysis. JAMA Oncol 6: 1068-1071, 2020.
8. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, Barron DA, Zehir A, Jordan EJ, Omuro A, *et al*: Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet 51: 202-206, 2019.
9. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, *et al*: Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 375: 1823-1833, 2016.
10. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, *et al*: Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 6: 224ra24, 2014.
11. Snyder A, Morrissey MP and Hellmann MD: Use of circulating tumor DNA for cancer immunotherapy. Clin Cancer Res 25: 6909-6915, 2019.
12. Bardelli A and Pantel K: Liquid biopsies, what we do not know (yet). Cancer Cell 31: 172-179, 2017.
13. Goldberg SB, Narayan A, Kole AJ, Decker RH, Teysir J, Carriero NJ, Lee A, Nemati R, Nath SK, Mane SM, *et al*: Early assessment of lung cancer immunotherapy response via circulating tumor DNA. Clin Cancer Res 24: 1872-1880, 2018.
14. Christensen E, Birkenkamp-Demtröder K, Sethi H, Shchegrova S, Salari R, Nordentoft I, Wu HT, Knudsen M, Lamy P, Lindskrog SV, *et al*: 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. J Clin Oncol 37: 1547-1557, 2019.
15. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, *et al*: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45: 228-247, 2009.
16. Jardim DL, Goodman A, de Melo Gagliato D and Kurzrock R: The challenges of tumor mutational burden as an immunotherapy biomarker. Cancer Cell 39: 154-173, 2021.
17. Rizvi NA, Cho BC, Reinmuth N, Lee KH, Luft A, Ahn MJ, van den Heuvel MM, Cobo M, Vicente D, Smolin A, *et al*: Durvalumab with or without tremelimumab vs standard chemotherapy in first-line treatment of metastatic non-small cell lung cancer: The MYSTIC phase 3 randomized clinical trial. JAMA Oncol 6: 661-674, 2020.
18. Si H, Kuziora M, Quinn KJ, Helman E, Ye J, Liu F, Scheuring U, Peters S, Rizvi NA, Brohawn PZ, *et al*: A blood-based assay for assessment of tumor mutational burden in first-line metastatic NSCLC treatment: Results from the MYSTIC study. Clin Cancer Res 27: 1631-1640, 2021.
19. Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, Liu XQ, Sher X, Jung H, Lee M, *et al*: Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. Nat Med 24: 1449-1458, 2018.
20. Laukhtina E, Hassler MR, Pradere B, Yanagisawa T, Quhal F, Rajwa P, Sari Motlagh R, König F, Pallauf M, Kawada T, *et al*: Circulating tumour DNA is a strong predictor of outcomes in patients treated with systemic therapy for urothelial carcinoma. Eur Urol Focus 8: 1683-1686, 2022.
21. Hellmann MD, Nathanson T, Rizvi H, Creelan BC, Sanchez-Vega F, Ahuja A, Ni A, Novik JB, Mangarin LMB, Abu-Akeel M, *et al*: Genomic features of response to combination immunotherapy in patients with advanced non-small-cell lung cancer. Cancer Cell 33: 843-852.e4, 2018.
22. Sun H, Liu SY, Zhou JY, Xu JT, Zhang HK, Yan HH, Huan JJ, Dai PP, Xu CR, Su J, *et al*: Specific TP53 subtype as biomarker for immune checkpoint inhibitors in lung adenocarcinoma. EBioMedicine 60: 102990, 2020.
23. Liu S, Geng S, Shi N, Zhang L, Xue W, Li Y and Jiang K: Survival prediction of patients treated with immune checkpoint inhibitors via KRAS/TP53/EGFR-single gene mutation. Front Pharmacol 13: 878540, 2022.
24. Ravi P, Ravi A, Riaz IB, Freeman D, Curran C, Mantia C, McGregor BA, Kilbridge KL, Pan CX, Pek M, *et al*: Longitudinal evaluation of circulating tumor DNA using sensitive amplicon-based next-generation sequencing to identify resistance mechanisms to immune checkpoint inhibitors for advanced urothelial carcinoma. Oncologist 27: e406-e409, 2022.
25. Lamy A, Gobet F, Laurent M, Blanchard F, Varin C, Moulin C, Andreou A, Frebourg T and Pfister C: Molecular profiling of bladder tumors based on the detection of FGFR3 and TP53 mutations. J Urol 176: 2686-2689, 2006.
26. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, Porta-Pardo E, Gao GF, Plaisier CL, Eddy JA, *et al*: The immune landscape of cancer. Immunity 48: 812-830.e14, 2018.
27. Parkinson CA, Gale D, Piskorz AM, Biggs H, Hodgkin C, Addley H, Freeman S, Moyle P, Sala E, Sayal K, *et al*: Exploratory analysis of TP53 mutations in circulating tumour DNA as biomarkers of treatment response for patients with relapsed high-grade serous ovarian carcinoma: A retrospective study. PLoS Med 13: e1002198, 2016.