



Prognostic Value of Residual Circulating Tumor DNA in Metastatic Pancreatic Ductal Adenocarcinoma

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Background: Circulating tumor DNA (ctDNA) is a potential biomarker in pancreatic ductal adenocarcinoma (PDAC). However, studies on residual ctDNA in patients post-chemotherapy are limited. We assessed the prognostic value of residual ctDNA in metastatic PDAC relative to that of carbohydrate antigen 19-9 (CA19-9).

Methods: ctDNA analysis using a targeted next-generation sequencing panel was performed at baseline and during chemotherapy response evaluation in 53 patients. Progression-free survival (PFS) and overall survival (OS) were first evaluated based on ctDNA positivity at baseline. For further comparison, patients testing ctDNA-positive at baseline were subdivided based on residual ctDNA into ctDNA responders (no residual ctDNA post-chemotherapy) and ctDNA non-responders (residual ctDNA post-chemotherapy). Additional survival analysis was performed based on CA19-9 levels.

Results: The baseline ctDNA detection rate was 56.6%. Although clinical outcomes tended to be poorer in patients with baseline ctDNA positivity than in those without, the differences were not significant. Residual ctDNA post-chemotherapy was associated with reduced PFS and OS. The prognosis of ctDNA responders was better than that of non-responders but did not significantly differ from that of ctDNA-negative individuals (no ctDNA both at baseline and during post-chemotherapy). Compared with ctDNA responses to chemotherapy, a $\geq 50\%$ decrease in the CA19-9 level had less effect on both PFS and OS based on hazard ratios and significance levels. ctDNA could be monitored in half of the patients whose baseline CA19-9 levels were within the reference range.

Conclusions: Residual ctDNA analysis post-chemotherapy is a promising approach for predicting the clinical outcomes of patients with metastatic PDAC.

Key Words: Biomarkers, CA19-9, Circulating tumor DNA, Neoplasm metastasis, Pancreatic ductal adenocarcinoma, Prognosis, Residual neoplasm

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INTRODUCTION

Pancreatic cancer poses a formidable challenge in oncology; it

is the seventh leading cause of cancer-related deaths worldwide, and its incidence is predicted to double in the coming decades in the United States and Europe, raising concerns [1–4].

Pancreatic ductal adenocarcinoma (PDAC) accounts for approximately 90% of pancreatic cancer cases, and more than 50% of the patients diagnosed as having PDAC exhibit systemic metastases [5]. Current systemic treatments, including the multi-drug chemotherapy regimen FOLFIRINOX consisting of folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin, modestly improve survival outcomes. However, the median overall survival (OS) of patients with metastatic PDAC remains < 1 yr [6–9].

The lack of reliable prognostic biomarkers poses a major challenge in establishing treatment strategies for patients with advanced PDAC [8]. A commonly used blood-based biomarker is the tumor marker carbohydrate antigen 19-9 (CA19-9). However, the clinical utility of CA19-9 is restricted by several limitations, including the potential of being falsely elevated because of conditions other than PDAC, rendering it less reliable for assessing treatment response [10–12]. Additionally, CA19-9 cannot be monitored in patients who are CA19-9 non-producers because of Lewis-antigen negativity [12, 13].

Circulating tumor DNA (ctDNA) has emerged as a useful tool in oncology [14] and serves as a prognostic biomarker in several advanced cancers, providing valuable insights for guiding clinical discussions regarding expected treatment outcomes [15]. The prognostic and predictive values of ctDNA in patients with advanced PDAC are being increasingly recognized. Although some studies have revealed that evaluating residual ctDNA post-chemotherapy can be useful for predicting treatment responses and clinical outcomes in metastatic PDAC, related research remains limited [8, 16–18], necessitating further study before clinical application.

We investigated the prognostic value of ctDNA in patients with metastatic PDAC using a targeted next-generation sequencing (NGS) panel. We performed ctDNA analysis on plasma samples at baseline and during chemotherapy response evaluation. Using the results, we performed survival analysis and compared the prognostic value of ctDNA with that of CA19-9.

MATERIALS AND METHODS

Patients and sample collection

The study protocol was approved by the Institutional Review Board/Ethics Committee of Yonsei University College of Medicine, Seoul, Korea (approval No. 4-2019-1004), and informed consent was obtained from all participants. All methods were performed in accordance with relevant guidelines and regulations.

We initially enrolled 64 patients diagnosed as having meta-

static PDAC at Severance Hospital in Seoul, Korea, between February 2020 and October 2022. Blood samples to obtain plasma for ctDNA analysis were collected at two time points: at baseline (before initiation of chemotherapy) and during chemotherapy response evaluation. Chemotherapy response was primarily evaluated at two weeks after four cycles of FOLFIRINOX or at four weeks after two cycles of gemcitabine with nab-paclitaxel. The second ctDNA assay was essential for determining the presence of residual ctDNA during chemotherapy response evaluation. Nine patients were lost to follow-up before the second assay, and two patients refused to undergo the second ctDNA assay. Hence, in this study, we primarily focused on the remaining 53 patients with metastatic PDAC.

Study design

Tier 1/2 mutations are those with clinical significance, whereas tier 3 mutations are considered variants of uncertain significance based on the Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer [19]. In this study, ctDNA was defined as cell-free DNA (cfDNA) containing at least one tier 1/2 mutation. Patients were categorized into three groups according to ctDNA positivity at baseline and residual ctDNA status post-chemotherapy: ctDNA non-responders (patients with ctDNA at baseline who also had residual ctDNA post-chemotherapy), ctDNA responders (patients with ctDNA at baseline but no residual ctDNA post-chemotherapy), and ctDNA negatives (patients without ctDNA both at baseline and post-chemotherapy).

Initially, we analyzed the cfDNA mutations at baseline and stratified progression-free survival (PFS) and OS in the function of the presence of ctDNA at baseline. Furthermore, we performed a survival analysis based on the number of tier 1/2 mutations detected at baseline, categorizing patients into four groups with zero, one, two, or more than two mutations. Subsequently, we stratified PFS and OS based on the three defined groups (ctDNA non-responders, ctDNA responders, and ctDNA negatives). To compare the prognostic performance of CA19-9 with that of ctDNA, we performed survival analyses based on the baseline CA19-9 level and its changes post-chemotherapy. We used the median baseline level and a 50% decrease as respective cut-offs for grouping. Fig. 1 provides a schematic overview of the study design.

ctDNA analysis

cfDNA was extracted from 3–4 mL of plasma using a Magnetic Serum/Plasma Circulating DNA Kit (Dxome, Seongnam, Korea).

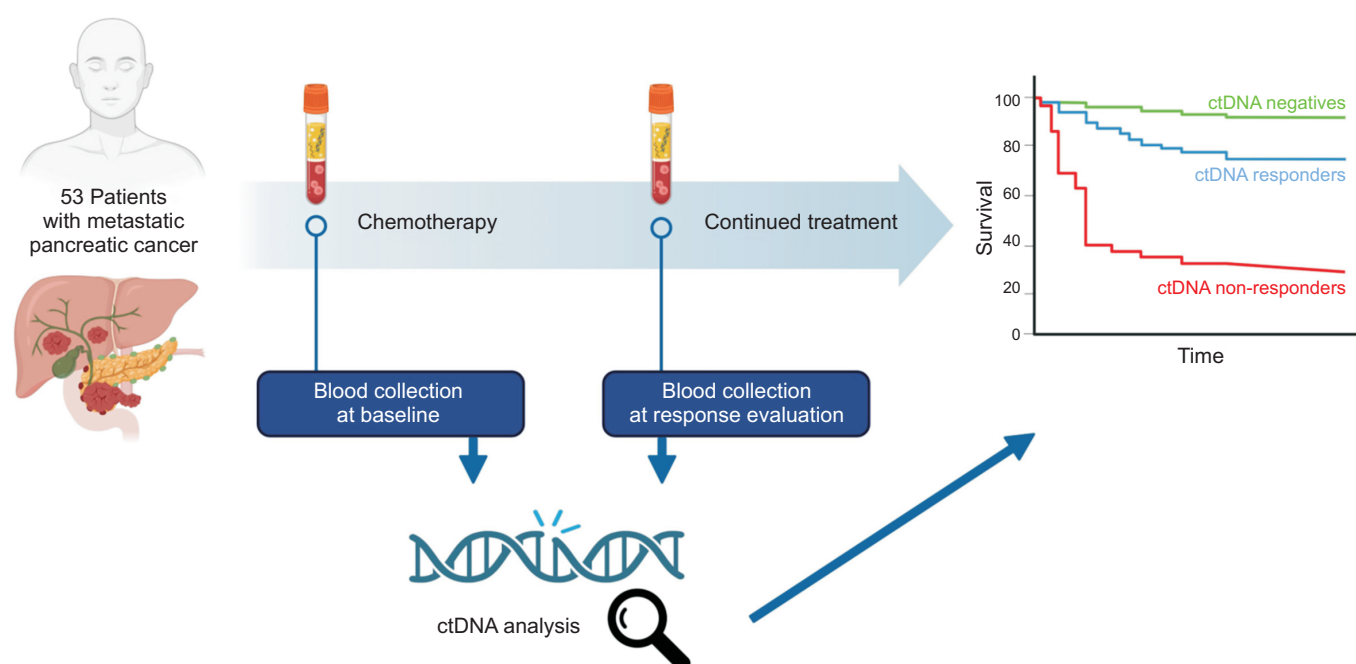


Fig. 1. Study overview. This study involved 53 patients with metastatic PDAC. ctDNA was analyzed in blood samples at baseline and post-chemotherapy. The primary objective was to explore the prognostic value of residual ctDNA on survival.
Abbreviation: ctDNA, circulating tumor DNA.

cfDNA size and concentration were measured using TapeStation 4200 (Agilent Technologies, Santa Clara, CA, USA) and a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), respectively. Libraries were prepared from 5–30 ng cfDNA using DxSeq Library Prep reagent (Dxome). A targeted NGS panel comprising 40 genes (pancreatobiliary panel) was used. However, for seven samples from five patients, the TMB500 panel, which comprises 531 genes, was employed (Supplemental Data Table S1). In the samples analyzed using the TMB500 panel, ctDNA analysis was performed exclusively on 33 genes included in the pancreatobiliary panel. Paired-end sequencing was performed using a NovaSeq 6000 System (Illumina) with 2×151 bp reads. A position-indexing sequencing algorithm (PiSeq; Dxome) was used to detect ctDNA mutations, with a limit of detection of 0.24% [20]. The variant allele fraction (VAF) was calculated by dividing the number of mutant allele reads by the total number of reads covering the locus. For patients with multiple mutations, the maximum VAF for each patient was used to determine the median VAF. Matched peripheral blood mononuclear cell sample sequencing was performed to eliminate clonal hematopoiesis of indeterminate potential when ctDNA was detected, except in one patient (PC_195), because of a low DNA concentration.

CA19-9 analysis

CA19-9 levels were measured using a Cobas 8000 modular analyzer (Cobas e801; Roche Diagnostics GmbH, Mannheim, Germany) employing the Roche Elecsys CA19-9 reagent (Roche). The upper limit of the reference range was 37 U/mL. The lower and upper limits of quantification were set to 2 and 20,000 U/mL, respectively. The sample values for these quantification limits were assumed to be 2 and 20,000 U/mL.

Statistical analysis

All statistical analyses were conducted using R (version 4.2.1; R Core Team, 2022). All tests were two-sided, and results with $P < 0.05$ were considered statistically significant. The Wilcoxon rank-sum test and Fisher's exact test were used for continuous and categorical data, respectively. Survival analyses were performed using Kaplan–Meier estimates, the log-rank test, and Cox regression. We performed univariable Cox regression analysis of the ctDNA assay results, followed by multivariable Cox regression analysis incorporating clinical variables (age, sex, baseline CA19-9 level, changes in the CA19-9 level post-chemotherapy, number of metastatic sites, and chemotherapy regimen) that demonstrated significance in the respective univariable analyses. The R packages “survival” and “survminer” were used for survival analysis.

RESULTS

Patient characteristics

The characteristics of the 53 patients finally included in the study are presented in Table 1. The majority (86.8%, N=46) of patients received FOLFIRINOX as first-line chemotherapy, nine of whom received a modified regimen. The typical number of chemotherapy cycles before response evaluation was four for FOLFIRINOX and two for gemcitabine plus nab-paclitaxel. One patient received a single dose of gemcitabine plus nab-paclitaxel after four cycles of modified FOLFIRINOX prior to response evaluation. The median number of days from the last chemotherapy session to response evaluation was 14 (interquartile range [IQR], 14–16) for FOLFIRINOX and 28 (27–31) for gemcitabine plus nab-paclitaxel. We observed no significant differences in patient characteristics among ctDNA non-responders, ctDNA responders, and ctDNA negatives, except for sex. The median PFS and OS, in days, were 227 (95% confidence interval [95% CI], 195–326) and 625 (598–not reached [NR]), respectively. The median duration from baseline to censoring for the nine patients who were lost to follow-up before their chemotherapy response evaluation was 25 (20–37) days. Because of this short observation period, only one survival event was confirmed among these patients. Detailed clinical information, including that of the nine patients who were lost to follow-up, is provided in Supplemental Data Table S2.

ctDNA analysis at baseline and during chemotherapy response evaluation

Median cfDNA concentrations were 6,066.7 pg/mL (4,293.3–11,933.3) at baseline and 17,750.0 pg/mL (10,221.8–28,036.4) during chemotherapy response evaluation, respectively. The samples were sequenced to a median average depth of 17,829 (15,188–20,084) and 19,193 (16,512–22,220) reads at baseline and during chemotherapy response evaluation, respectively (Supplemental Data Table S3). Fig. 2 illustrates the mutations detected in the ctDNA analysis. Median VAFs at baseline were 2.4% (1.4–9.1) for all mutations and 2.2% (1.3–8.6) specifically for tier 1/2 mutations. Mutations across all tier 1/2/3 mutations were the most frequently detected in *KRAS* (49.1%), followed by *TP53* (34.0%), *SMAD4* (13.2%), and *CDKN2A* (7.5%). For tier 1/2 mutations specifically, the detection rates were similar, with the same frequencies observed for *KRAS*, *TP53*, and *CDKN2A*, whereas *SMAD4* had a slightly lower detection rate (11.3%). Tier 1/2 mutations were found in 30 of the 53 patients, yielding a ctDNA detection rate of 56.6%. Among these patients, 26 (86.7%) harbored tier 1/2 *KRAS* mutations. Ten patients had one tier 1/2 mutation, nine had two, eight had three, and three had four tier 1/2 mutations. Among the 20 patients with two or more tier 1/2 mutations, 16 (80.0%) had both *KRAS* and *TP53* mutations. In total, 64 tier 1/2 mutations were detected, with 41 (64.1%) being missense. All 26 *KRAS* mutations identified were missense. Baseline ctDNA was detected in eight out of nine patients who were lost to follow-up before chemotherapy response evaluation (Supplemental Data Table S4). When the

Table 1. Characteristics of 53 patients with metastatic PDAC

Characteristic	Total (N=53)	ctDNA non-responders (N=13)	ctDNA responders (N=17)	ctDNA negatives (N=23)	P
Age, yrs, median (IQR)	63 (60–68)	68 (63–70)	62 (55–72)	63 (59.5–68)	0.073
Sex, N (%)					
Male	32 (60.4)	12 (22.6)	9 (17.0)	11 (20.8)	0.022
Female	21 (39.6)	1 (1.9)	8 (15.1)	12 (22.6)	
Number of metastatic sites, N (%)					
Single	25 (47.2)	4 (7.5)	8 (15.1)	13 (24.5)	0.339
Multiple	28 (52.8)	9 (17.0)	9 (17.0)	10 (18.9)	
First-line chemotherapy, N (%)					
FOLFIRINOX*	46 (86.8)	10 (18.9)	16 (30.2)	20 (37.7)	0.448
Gemcitabine + nab-paclitaxel	7 (13.2)	3 (5.65)	1 (1.9)	3 (5.65)	
CA19-9, U/mL, median (IQR)	402 (69.5–1,764)	2,125 (126–5,990)	292 (120–988)	238 (44.25–598)	0.122

*Of the 46 patients treated with FOLFIRINOX, nine received a modified regimen.

Abbreviation: IQR, interquartile range; FOLFIRINOX, combination of folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin; PDAC, pancreatic ductal adenocarcinoma.

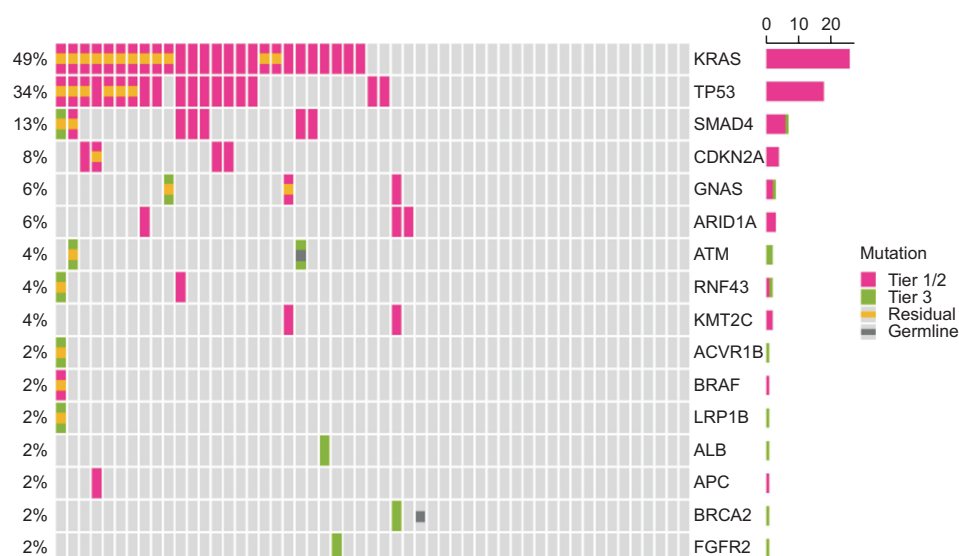


Fig. 2. Oncoprint of mutations detected in cfDNA at baseline. Columns depict patients; rows depict genes with mutation frequencies. A gene with multiple mutations was ranked as tier 1/2 when any of the mutations met the criteria for these tiers. Residual ctDNA post-chemotherapy is highlighted in yellow, and patients with pathogenic germline variants are indicated in dark gray.

results from these nine patients were included, the detection rate was 61.3%. Of the 30 patients with detectable ctDNA at baseline, 13 (43.3%) had residual ctDNA during chemotherapy response evaluation (ctDNA non-responders). In contrast, 17 patients (56.7%) had no residual ctDNA (ctDNA responders). Two patients had germline pathogenic variants (patient IDs: PC_141 and PC_203). Detailed mutation data are provided in Supplemental Data Table S4.

Prognostic value of baseline ctDNA

Fig. 3 presents the survival analysis results based on the presence of baseline ctDNA. Patients with baseline ctDNA tended to have shorter PFS and OS than those without, although the differences were not significant (median PFS of 177 vs. 368 days, $P=0.062$; median OS of NR vs. 966 days, $P=0.16$) (Fig. 3A, B). Survival analysis based on the number of tier 1/2 mutations at baseline, categorized as zero, one, two, or more than two, did not yield statistically significant differences in PFS ($P=0.26$) and OS ($P=0.52$) (Supplemental Data Table S5). To compare the prognostic value of ctDNA with that of CA19-9, survival analysis was performed based on the baseline CA19-9 level. PFS and OS did not significantly differ between the groups with baseline CA19-9 levels above and below the median of 402 U/mL (Fig. 3C, D; $P=0.60$ for PFS and $P=0.32$ for OS). Univariable Cox regression analysis of the baseline clinical variables did not yield significant results. Detailed information on the survival analysis is provided in Table 2 and Supplemental Data Table S5.

Prognostic value of residual ctDNA post-chemotherapy

The survival analysis results according to the presence of residual ctDNA after a series of chemotherapy cycles are presented in Fig. 4A, B. In univariable Cox regression, the hazard ratio (HR) for PFS was 4.40 (1.94–10.00) and 3.66 (1.54–8.67) for ctDNA non-responders compared with ctDNA negatives and ctDNA responders, respectively (Fig. 4A). Additionally, the HR for OS was 4.35 (1.39–13.62) and 4.72 (1.22–8.20) for ctDNA non-responders compared with ctDNA negatives and ctDNA responders, respectively (Fig. 4B). To compare the prognostic value of ctDNA with that of CA19-9, a survival analysis was performed based on the changes in the CA19-9 level post-chemotherapy (Fig. 4C, D). Ten patients with baseline CA19-9 levels <37 U/mL were grouped separately, among whom five had detectable ctDNA at baseline. The HRs for PFS and OS for the group with a <50% decrease in the CA19-9 level compared with those of the group with a ≥50% decrease were 2.61 (1.21–5.64) and 2.63 (0.68–10.13), respectively. The group with a ≥50% decrease in the CA19-9 level had better clinical outcomes than the group with baseline CA19-9 levels <37 U/mL, with HRs for PFS and OS of 0.34 (0.13–0.85) and 0.20 (0.05–0.78), respectively. The HRs for PFS and OS for ctDNA non-responders, even after adjusting for the three CA19-9 groups, were significantly high at 3.32 (1.37–8.03) and 4.28 (1.09–16.84), respectively, compared with those for ctDNA responders. Detailed information on the survival analysis is provided in Table 2 and Supplemental Data Table S5.

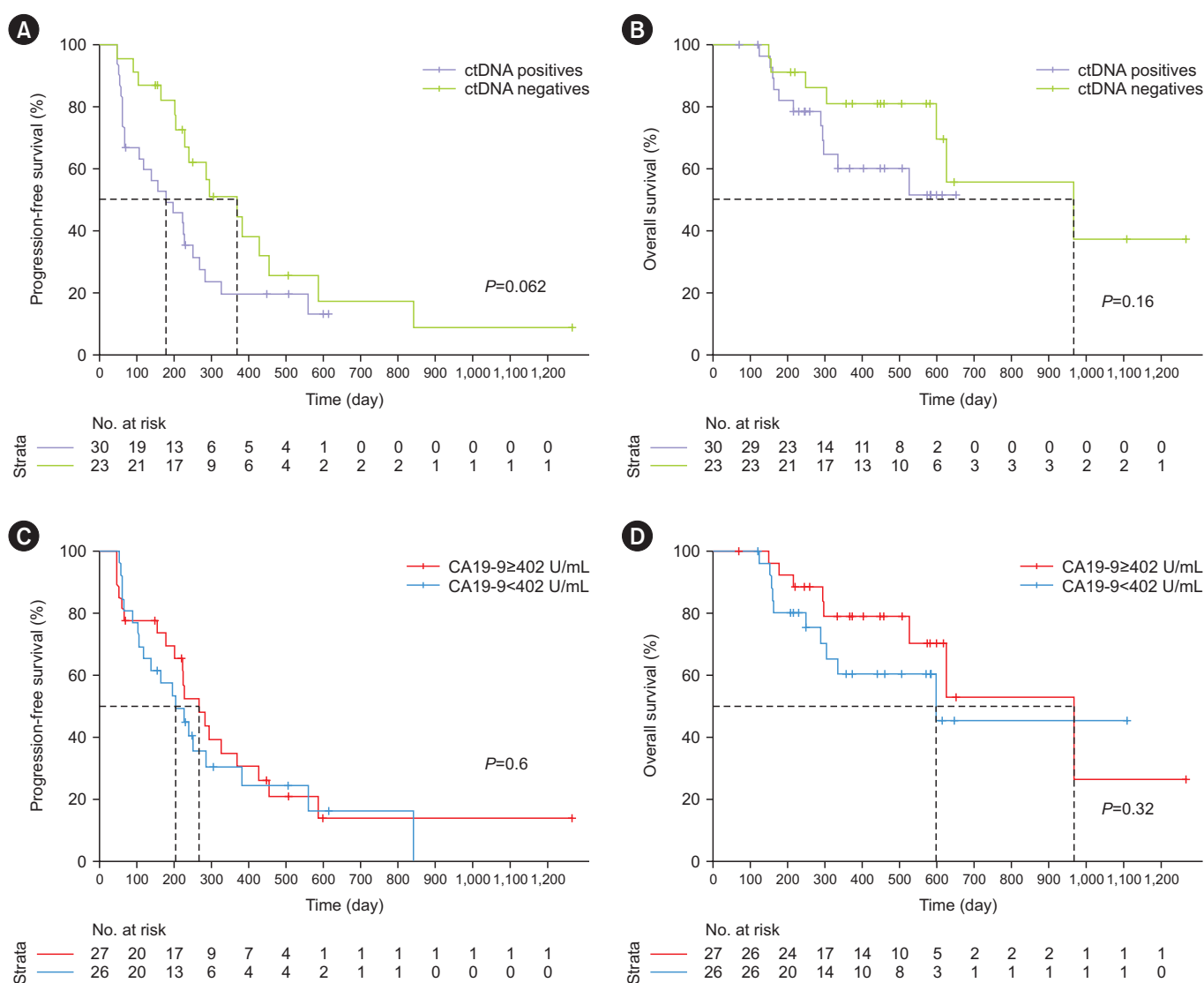


Fig. 3. Kaplan-Meier survival curves for ctDNA and CA19-9 at baseline. (A) Progression-free survival (PFS) according to baseline ctDNA. (B) Overall survival (OS) according to baseline ctDNA. (C) PFS according to baseline CA19-9. (D) OS according to baseline CA19-9.

DISCUSSION

The ctDNA detection rate varies depending on assay sensitivity [21, 22]. Studies have reported varying detection rates at baseline in advanced PDAC, ranging from 25% to 100%, with recent studies using targeted NGS reporting a detection rate of approximately 65% [16, 17, 23]. We found a baseline ctDNA detection rate of 61.3% when including the nine patients who were lost to follow-up before chemotherapy response evaluation, suggesting that the sensitivity of our ctDNA assay is acceptable.

Baseline ctDNA levels in advanced PDAC have been linked to poorer prognosis [8, 16, 17]. Although our observations also

suggested a trend toward worse clinical outcomes with baseline ctDNA positivity, this trend was not significant. This discrepancy can be attributed to differences in patient cohorts. Notably, the nine patients excluded from the survival analysis had high baseline ctDNA detection rates but were lost to follow-up, likely because of poor life expectancy that led to early treatment discontinuation or discharge. Had these patients been included, the prognostic gap between ctDNA-positive and -negative groups might have been statistically significant.

A few studies have demonstrated that residual ctDNA post-chemotherapy is associated with poor clinical outcomes in advanced PDAC [8, 16, 17]. Furthermore, a reduction in the *KRAS*

Table 2. Summary of Cox regression results

Parameter	Univariable				Multivariable			
	HR for PFS (95% CI)	<i>P</i>	HR for OS (95% CI)	<i>P</i>	HR for PFS (95% CI)	<i>P</i>	HR for OS (95% CI)	<i>P</i>
ctDNA at baseline								
Positives vs. negatives	1.84 (0.96–3.52)	0.07	2.06 (0.74–5.73)	0.16				
ctDNA response								
Non-responders vs. negatives	4.40 (1.94–10.00)	<0.001	4.35 (1.39–13.62)	0.01	4.02 (1.59–10.16)	0.003	4.72 (1.28–17.41)	0.02
Non-responders vs. responders	3.66 (1.54–8.67)	0.003	4.72 (1.22–18.20)	0.02	3.32 (1.37–8.03)	0.008	4.28 (1.09–16.84)	0.04
Responders vs. negatives	1.20 (0.56–2.57)	0.63	0.92 (0.23–3.75)	0.91	1.21 (0.55–2.69)	0.64	1.10 (0.25–4.80)	0.90
CA19-9 at baseline								
CA19-9, U/mL, continuous value	1.00 (1.00–1.00)	0.34	1.00 (1.00–1.00)	0.91				
≥402 U/mL vs. <402 U/mL	0.85 (0.45–1.58)	0.60	0.62 (0.25–1.59)	0.32				
CA19-9 change								
Decrease <50% vs. baseline <37 U/mL	0.88 (0.39–1.97)	0.76	0.51 (0.17–1.51)	0.23	0.59 (0.24–1.45)	0.25	0.34 (0.10–1.11)	0.07
Decrease <50% vs. decrease ≥50%	2.61 (1.21–5.64)	0.01	2.63 (0.68–10.13)	0.16	1.80 (0.77–4.20)	0.17	1.44 (0.32–6.54)	0.64
Decrease ≥50% vs. baseline <37 U/mL	0.34 (0.13–0.85)	0.02	0.20 (0.05–0.78)	0.02	0.33 (0.13–0.84)	0.02	0.23 (0.06–0.96)	0.04
Age	1.02 (0.98–1.05)	0.38	1.03 (0.98–1.10)	0.26				
Sex								
Male vs. female	1.80 (0.93–3.49)	0.08	2.75 (0.90–8.40)	0.08				
No. of metastasis sites								
Single vs. multiple	1.32 (0.69–2.51)	0.41	1.68 (0.62–4.50)	0.31				
Chemotherapy regimen								
FOLFIRINOX vs. gemcitabine plus nab-paclitaxel	1.77 (0.67–4.70)	0.25	2.34 (0.76–7.21)	0.14				

Abbreviations: CI, confidence interval; FOLFIRINOX, combination of folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

ctDNA level may serve as an early marker of treatment response, whereas CA19-9 changes are less reliable [8]. Our findings are in line with these previous findings. Notably, even among patients with baseline ctDNA positivity, PFS and OS in ctDNA responders showed no significant differences compared with those of ctDNA-negative patients, whereas ctDNA non-responders demonstrated poorer outcomes. This finding highlights the significance of residual ctDNA and the importance of follow-up testing.

Although CA19-9 is commonly used for monitoring advanced PDAC, its reliability remains controversial [8, 10, 12, 13, 24]. In our study, ctDNA responses showed a stronger association with both PFS and OS than decreases in the CA19-9 level and remained a major predictor of survival even when adjusted for the

CA19-9 level. In addition, ctDNA monitoring was applicable for half of the patients for whom CA19-9 monitoring was difficult because their baseline CA19-9 levels were within the reference range. These findings suggest that the prognostic value of ctDNA is not diminished by the routine evaluation of CA19-9, and instead, ctDNA may replace or complement CA19-9 in the monitoring of patients with advanced PDAC.

Our study has some limitations. First, as mentioned above, differences in patient cohorts may influence the prognostic results. Second, we did not establish a cut-off for clinically significant ctDNA levels. Instead, we analyzed survival outcomes based on the presence or absence of detectable ctDNA. The sensitivity of ctDNA detection can vary depending on assay types and conditions [21–23], potentially influencing the signifi-

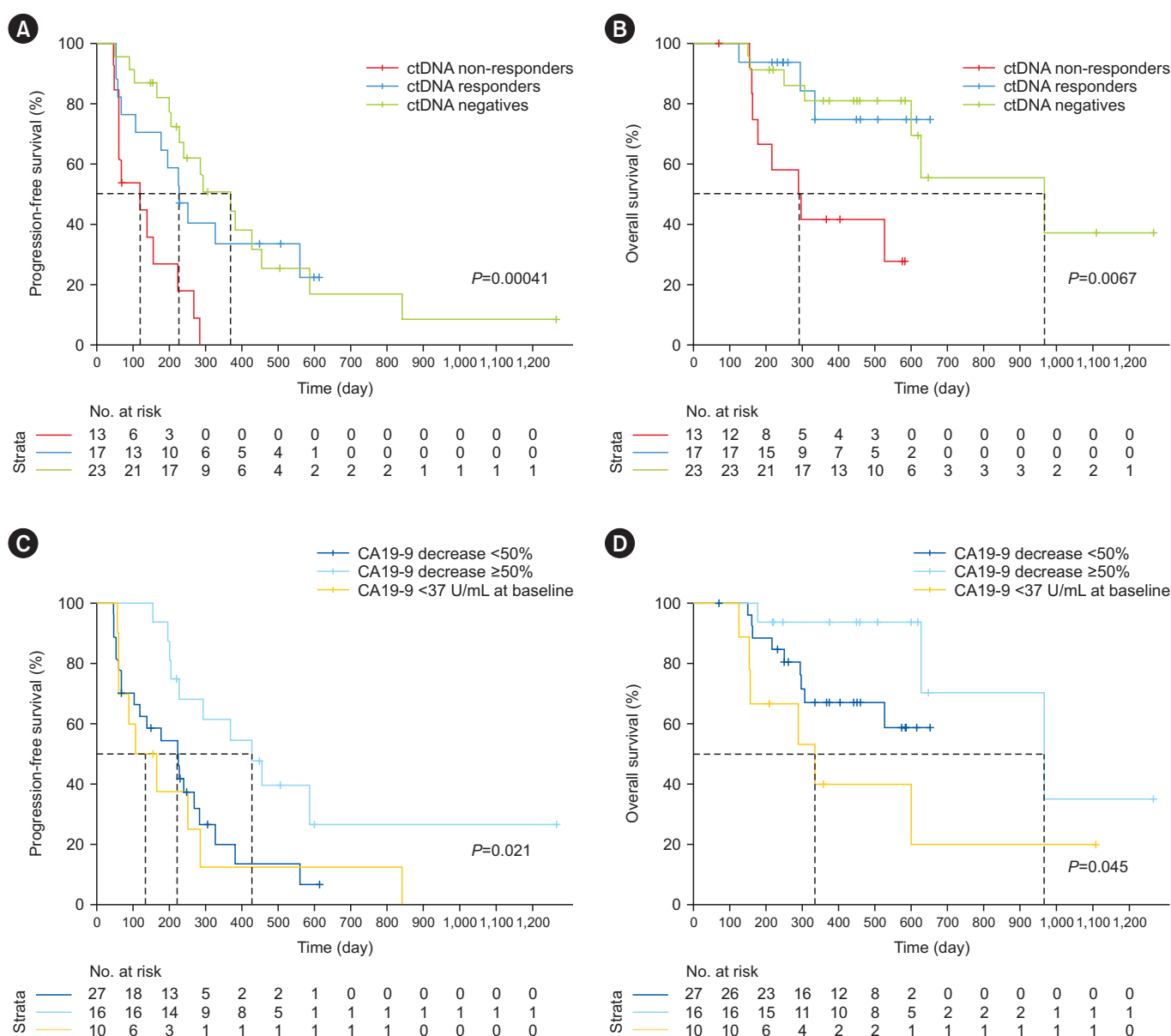


Fig. 4. Kaplan-Meier survival curves for residual ctDNA and CA19-9 level changes post-chemotherapy. (A) Progression-free survival (PFS) for ctDNA non-responders, responders, and negatives. (B) Overall survival (OS) in the aforementioned groups. (C) PFS based on CA19-9 level reduction: <50%, ≥50%, and baseline <37 U/mL. (D) OS for the respective CA19-9 groups.

cance of detectable ctDNA. Therefore, determining the cut-off will enable more accurate and in-depth prognostic stratification, and can be a focus of future research [15]. Third, we did not investigate the optimal timing of blood sampling for residual ctDNA analysis. However, a previous study revealed that an increase in ctDNA 14 days post-treatment is associated with later disease progression [8]. In our study, most patients underwent blood sampling for residual ctDNA analysis 14 days after their last chemotherapy. Therefore, our timing is unlikely to be inap-

propriate; however, further research may help identify more optimal timings.

In conclusion, our study suggests that testing for residual ctDNA post-chemotherapy in patients with metastatic PDAC may be a promising prognostic approach, offering a more specific stratification of clinical outcomes. Further studies are required to validate our findings and to establish the appropriate timing and cut-off levels, which can influence prognosis, for ctDNA analysis.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via <https://doi.org/10.3343/alm.2024.0345>

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AUTHOR CONTRIBUTIONS

Kim H: data curation, investigation, visualization, writing–original draft preparation. Lee J: data curation, investigation, writing–review and editing. Park MR: investigation, writing–review and editing. Choi Z: investigation, writing–review and editing. Han SJ: investigation, writing–review and editing. Kim D: investigation, writing–review and editing. Shin S: funding acquisition, investigation, resources, writing–review and editing. Lee ST: conceptualization, methodology, resources, writing–review and editing. Choi JR: conceptualization, methodology, resources, supervision, writing–review and editing. Park SW: conceptualization, funding acquisition, methodology, resources, supervision, writing–review and editing. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

ST Lee and JR Choi are members of the board of directors at Dxome, SJ Han and D Kim are employees at Dxome Co., Ltd., and Z Choi was a former employee of Dxome Co., Ltd.

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