

Circulating Tumor DNA Predicts the Response and Prognosis in Patients With Early Breast Cancer Receiving Neoadjuvant Chemotherapy

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PURPOSE Many patients with breast cancer still relapse after curative treatment. How to identify the ones with high relapse risk remains a critical problem. Circulating tumor DNA (ctDNA) has recently become a promising marker to monitor tumor burden. Whether ctDNA can be used to predict the response and prognosis in patients with breast cancer receiving neoadjuvant chemotherapy (NAC) is unknown. Our study aimed to evaluate the clinical value of the presence and dynamic change of ctDNA to predict the tumor response and prognosis in patients with breast cancer treated with NAC.

MATERIALS AND METHODS Fifty-two patients with early breast cancer who underwent NAC were prospectively enrolled. Serial plasma samples before, during, and after NAC and paired tumor biopsies were harvested and subjected to deep targeted sequencing using a large next-generation sequencing panel that covers 1,021 cancer-related genes.

RESULTS Positive baseline ctDNA was detected in 21 of 44 patients before NAC. Most patients with positive ctDNA had one or more mutations confirmed in paired primary tumor. The ctDNA level after 2 cycles of NAC was predictive of local tumor response after all cycles of NAC (area under the curve, 0.81; 95% CI, 0.61 to 1.00). ctDNA tracking during NAC outperformed imaging in predicting the overall response to NAC. More importantly, positive baseline ctDNA is significantly associated with worse disease-free survival ($P = .011$) and overall survival ($P = .004$) in patients with early breast cancer, especially in estrogen receptor–negative patients.

CONCLUSION Our study demonstrated that ctDNA can be used to predict tumor response to NAC and prognosis in early breast cancer, providing information to tailor an individual's therapeutic regimen.

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INTRODUCTION

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death in women worldwide.¹ Although early breast cancer is a curable disease, up to 40% of such patients still relapse after surgery.² It is believed that breast cancer is a systemic disease and that clinically undetectable micrometastases often happened before the diagnosis.^{3,4} How to distinguish patients with early breast cancer with high relapse risk from those with low risk remains a critical and challenging clinical question.

Neoadjuvant chemotherapy (NAC) is often used in patients with locally advanced or triple-negative/Her2-positive early breast cancer before surgery to decrease tumor size and enable breast-conserving surgery. The tumor response to NAC can provide the information regarding in vivo treatment efficacy and long-term prognosis of patients. Monitoring the early response

to NAC is key to the clinical decision to continue, change, or stop NAC. The current consensus to monitor response to NAC is clinical examination backed up by radiologic and sonographic measures.⁵ However, these measures are quite subjective and have poor inter- and intraobserver reproducibility.⁶ Moreover, given the heterogeneity of cancer, a reliable method to examine the overall response from local, regional, and micrometastatic lesions in patients with early breast cancer is still lacking.

Circulating tumor DNAs (ctDNAs) are mutated gene fragments that are exclusively shed by cancer cells into blood,⁷ which can be detected by digital polymerase chain reaction (dPCR)⁸ or sequencing. In patients with metastatic breast cancer, ctDNA by dPCR was reported to outperform classic tumor biomarker (CA15-3/CEA) and circulating tumor cells in monitoring disease progression⁹ as well as identifying resistant clonal evolution during therapy.¹⁰ In

ASSOCIATED CONTENT

Appendix

Data Supplement

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

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CONTEXT

Key Objective

Can the presence and dynamic change of circulating tumor DNA (ctDNA) predict the tumor response and prognosis in patients with breast cancer receiving neoadjuvant chemotherapy (NAC)?

Knowledge Generated

Using a next-generation sequencing panel of 1,021 genes, positive baseline ctDNA was identified in 21 of 44 patients with early breast cancer before NAC. The ctDNA change after 2 cycles of NAC was predictive of local tumor response. Dynamic change of ctDNA during NAC outperformed imaging in predicting the overall response to NAC. Positive ctDNA before NAC was associated with significantly worse disease-free survival and overall survival, especially in estrogen receptor (ER)-negative patients.

Relevance

Our study demonstrates that ctDNA has significant value to complement imaging to monitor tumor response and predict prognosis in patients with early breast cancer, providing information to tailor an individual's therapeutic regimen. The high relapse rate in ER-negative patients with positive ctDNA indicates that it may be necessary to escalate treatment in these high-risk patients.

patients with early breast cancer after surgery, ctDNA by dPCR predicted metastasis at 7.9-11 months earlier than clinical relapse.^{11,12} A few studies also explored the role of ctDNA in patients with breast cancer who received NAC. It was reported that ctDNA change during NAC was correlated with tumor response¹³⁻¹⁵ or relapse,^{16,17} although two of them only used dPCR to detect a single gene (*met-RASSF1A*¹⁴ or *TP53*¹⁶), and the other study only correlated the ctDNA disappearance after the first cycle of NAC with two patients who achieved pathologic complete response.¹³

Advances in next-generation sequencing (NGS)¹⁸ technology have enabled the rapid identification and broad coverage of tumor-specific genomic alterations in cell-free DNA (cfDNA) of individual patients.¹⁹⁻²¹ The NGS assay provides an opportunity to screen more genes at a single time point and avoid missing mutations absent in biopsy because of intratumor heterogeneity. Here, using a large NGS panel that covers 1,021 cancer-related genes, we compared the change of ctDNA and imaging during NAC to determine whether ctDNA is superior to imaging in predicting tumor response during NAC and prognosis in patients with early breast cancer.

MATERIALS AND METHODS

Study Design

This was an observational, prospective, single-center study to investigate whether ctDNA can predict tumor response and survival in early breast cancer. Serial plasma samples were collected from patients scheduled to receive NAC. All patients were prospectively recruited from Sun Yat-sen Memorial Hospital between 2013 and 2015. The study was approved by the ethics committee of Sun Yat-sen Memorial Hospital and registered on ClinicalTrials.gov (NCT03260192). Written informed consent was obtained from all participants. For patient cohort, sample collection

and processing, and NGS sequencing and data analysis, please see the Data Supplement.

Statistical Analysis

The primary end point of this study was to evaluate the clinical value of the presence and dynamic change of ctDNA to predict the tumor response and prognosis in patients with breast cancer treated with neoadjuvant chemotherapy. Survival analysis was performed using the Kaplan-Meier method and the log-rank test. The correlations of baseline ctDNA presence with clinicopathological markers were assessed using Pearson χ^2 test. All *P* values are two sided. Receiver operating characteristic (ROC) models were constructed and compared by pROC package.²² Cutoff points of the ROC models were calculated by pROC packages. All statistical analyses were performed with GraphPad Prism version 6.0 or R version 3.4.1.

RESULTS

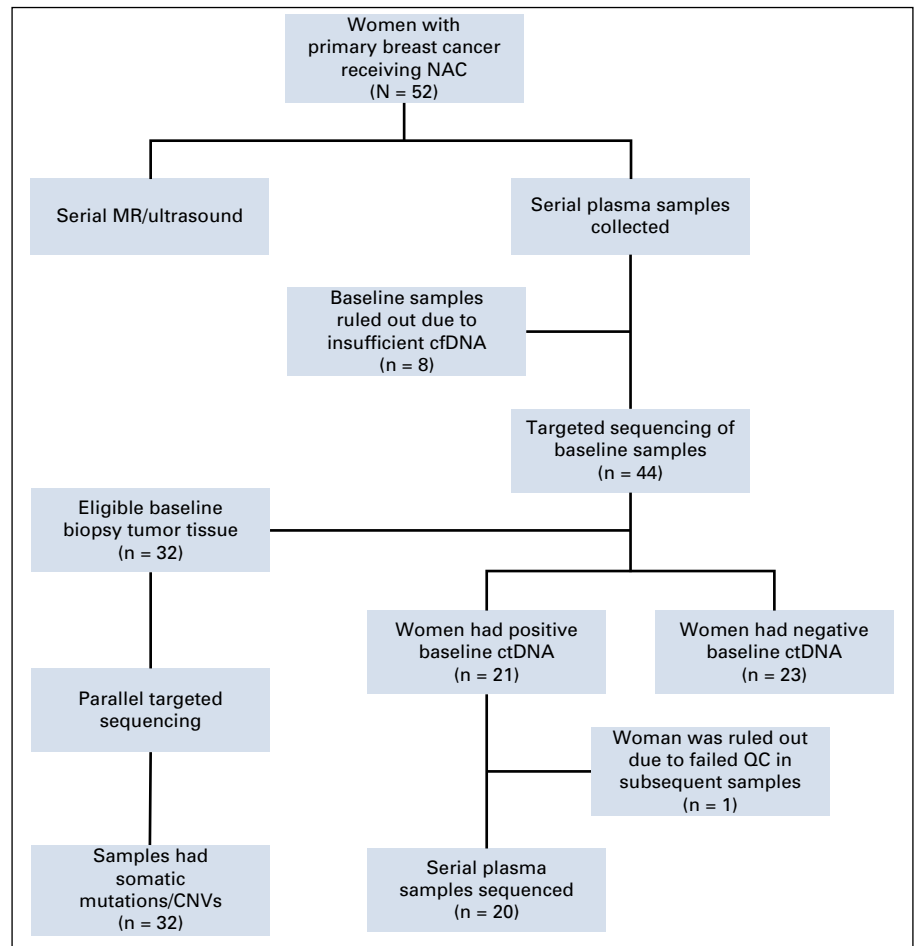
Clinicopathological Features of Patients

A total of 52 patients with early breast cancer who received NAC were prospectively enrolled in the study since 2013. Eight patients were excluded from further study because of insufficient cfDNA in plasma before NAC (Fig 1).

The clinicopathological features of the 44 patients in the study are listed in Table 1 and the Data Supplement. All patients received 3-8 cycles of anthracycline/taxane-based NAC before surgery. Most of them were T2 or above (approximately 95%) or with lymph node metastasis (approximately 80%). The plasma was collected from patients before NAC (baseline), during NAC, and before surgery, with the tumor tissue biopsy specimens and matched blood cells collected before NAC.

According to RECIST1.1²³ criteria, the majority of patients were responsive to NAC (complete response [CR], 3/44;

FIG 1. Study flowchart. cfDNA, cell-free DNA; CNV, copy number variation; ctDNA, circulating tumor DNA; MR, magnetic resonance; NAC, neoadjuvant chemotherapy; QC, quality control.



partial response [PR], 24/44; CR/PR, 27/44; 61%) and the rest were irresponsive (stable disease [SD], 16/44; progressive disease [PD], 1/44; SD/PD, 17/44; 39%). After surgery, the patients were followed up every 6 months. During the follow-up (median, 46 months; range, 11-68 months), 11 patients (25%) had distant metastasis, and 9 of them died of metastatic breast cancer.

Baseline Mutated DNAs in Plasma and Tumor

All baseline plasma samples, matched blood cells, and 32 paired tumor biopsy samples underwent parallel targeted NGS to screen for point mutations and structural variants, using the NGS panel of 1,021 cancer-related genes that covers a region of 1.1 MB (Data Supplement).²⁴ After deduplication, the median depths of unique coverage were 984x for primary tumor and 1,225x for plasma DNA. The analysts were blinded to clinical and follow-up information during the analysis of sequencing data.

Among the 44 patients, 21 patients were found to have positive baseline ctDNA. A total of 72 mutations in 46 genes were found in these 21 samples, with 1-8 (median, 3) mutations per sample (Data Supplement). *TP53* (15/21), *P13KCA* (5/21), *GAB2* (3/21), and *IRS2* (3/21) were the most frequently mutated genes in plasma (Fig 2A). There

was no significant association between baseline ctDNA positivity and tumor size, lymph node status, ERBB2 status, and Ki67 index (Table 1). However, breast cancers with positive ctDNA were more likely to be negative for estrogen receptor (ER) or progesterone receptor ($P < .05$), which is consistent with a previous report.¹² Also, higher baseline ctDNA levels were significantly associated with larger tumor size, negative ER, and triple-negative breast cancer subtype (Appendix Fig A1).

The objective response rate (ORR) after NAC in ctDNA-positive patients was lower than that in ctDNA-negative patients (47.6% v 73.9%), although the difference was not statistically significant ($P = .13$), which might be due to the limited sample size ($n = 44$).

The mutated DNA is often found to be different between tumor and plasma, with the former having more mutations and the latter representing the heterogeneity of tumor. Thus, we also sequenced the 32 available baseline tumor biopsy samples and compared them with the paired baseline plasma samples. Somatic mutations were found in all 32 tumor biopsy samples. In total, 114 mutations were identified in 63 genes, with 1-19 (median, 3) mutations per tumor sample (Data Supplement). Among the 114 mutations, 33 were detected in both tumor and plasma, and 81

TABLE 1. Clinical Characteristic of the Study Cohort (N = 44)

Clinical Characteristic	All Patients (N = 44)	PBC (n = 21)	NBC (n = 23)	P(PBC vNBC)
Clinical tumor stage				
1	2	0	2	.66
2	23	11	12	
3	14	8	6	
4	5	2	3	
Clinical nodal status				
Negative	9	2	7	.18
Positive	35	19	16	
Molecular subtype				
HR+/HER2-	21	8	13	.09
HR+/HER2+	8	2	6	
HR-/HER2+	9	7	2	
TNBC	6	4	2	
ER status, %				
< 10	15	11	4	.03
≥ 10	29	10	19	
Progesterone receptor status, %				
< 10	22	15	7	.02
≥ 10	22	6	16	
ERBB2				
Negative	27	12	15	.81
Positive	17	9	8	
Ki67, %				
< 14	5	2	3	1.00
≥ 14	39	19	20	
Objective response				
PR/CR	27	10	17	.13
SD/PD	17	11	6	

Abbreviations: CR, complete response; ctDNA, circulating tumor DNA; NBC, negative baseline ctDNA; PBC, positive baseline ctDNA; PD, progressive disease; PR, partial response; SD, stable disease; TNBC, triple-negative breast cancer.

mutations were only detected in tumor. In addition, 29 mutations were only detected in the plasma, but not in tumor, of these 32 patients (Fig 2B). *TP53* (20/32), *PI3KCA* (13/32), *NF1* (5/32), and *GATA3* (4/32) were the most frequently mutated genes in tumor (Fig 2C).

In the 32 patients with both tumor and plasma sequencing data, 14 had concordant/partially concordant mutations in plasma and tumor, 16 patients had mutations only in tumor with negative ctDNA, and the other two patients (P034 and P036) had completely different mutations in tumor and plasma (Fig 2B). In the 16 patients with positive ctDNA, 14 (87.5%) of them had one or more mutations confirmed independently in tumor sequencing data, indicating that most positive ctDNA represents tumor-specific mutations in

the primary tumors. Among the 33 concordant mutations found in both tumor and plasma of the 14 patients, *TP53* (12/14) and *PI3KCA* (4/14) mutations were the most frequent (Fig 2D).

Changes of ctDNA and Tumor Response During NAC

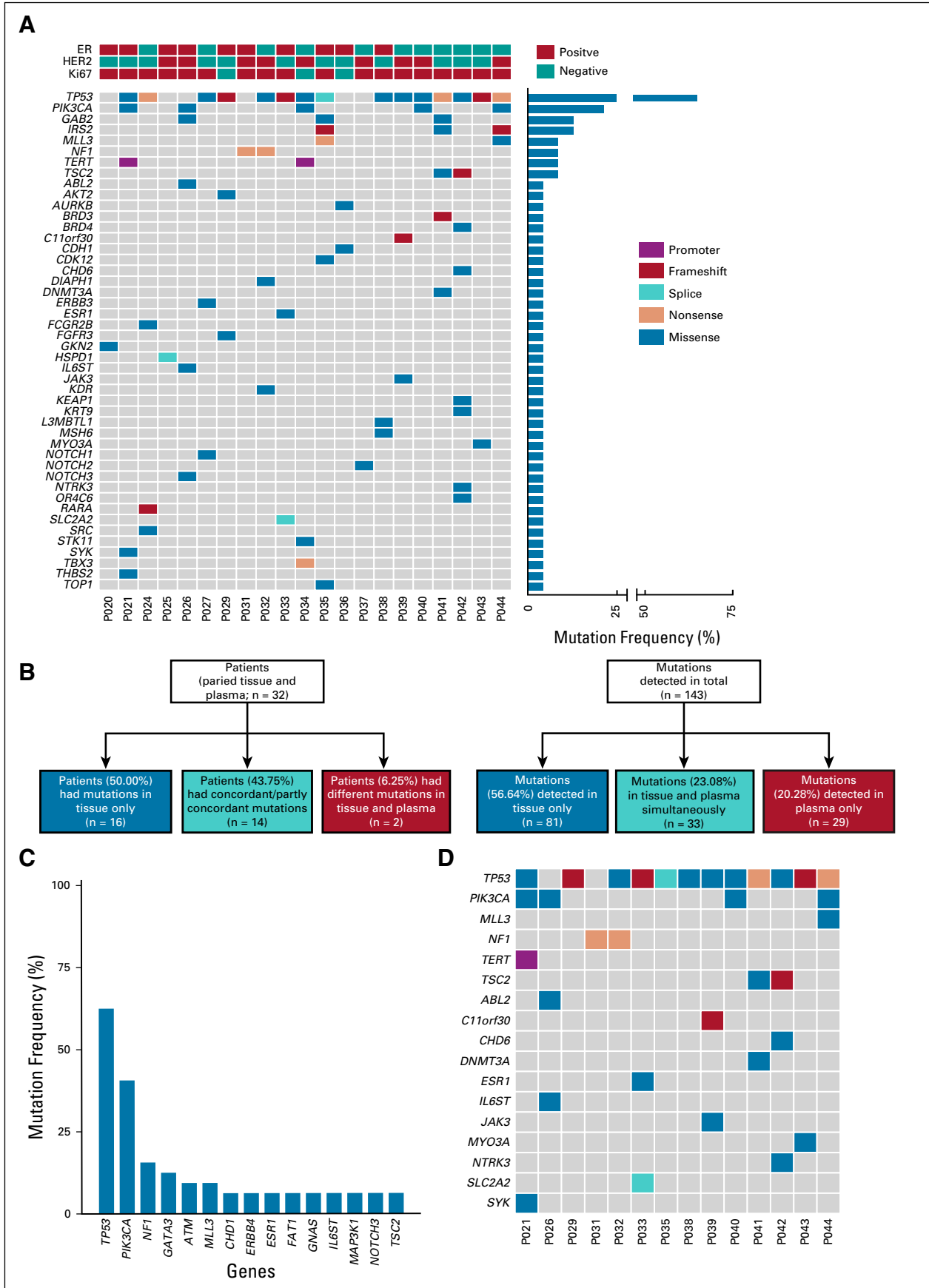
The current gold standard to monitor tumor response during NAC is ultrasound or magnetic resonance imaging, although it is subjective and sometimes falls behind the histologic changes. To explore whether ctDNA surpasses imaging in monitoring or predicting overall tumor response, all plasma samples collected during NAC in the 20 patients (1 patient was excluded because of insufficient cfDNA in subsequent plasma samples) with positive baseline ctDNA were sequenced and compared with baseline data to track the dynamic changes of ctDNA during NAC (Data Supplement).

In this study, tumor response was defined as CR/PR/SD/PD using RECIST1.1 criteria when the imaging data of local/regional tumor before surgery was compared with that before NAC. SD/PD was classified as no response, and CR/PR was classified as response. Of the 20 assessable patients, 11 did not respond to NAC (including a patient with PD), and 9 responded to NAC (response rate, 45%).

However, patient 038 (P038), who was defined as PR according to imaging, had an increasing ctDNA amount (clonal variant allele frequency [VAF]) during all cycles of NAC,²⁵ and a persisting high ctDNA even after surgery, although she did not have any clinical metastasis before surgery. Follow-up showed that she had multiple distant metastases (liver, lung, and bone) at 21 months and died at 46 months after surgery (Appendix Fig A2). The increase of ctDNA in this case indicated the early micrometastases undetected by imaging did not respond to NAC well, although the primary tumor did, suggesting that ctDNA is better than imaging of local tumor to monitor the overall response to NAC.

Next we did a longitudinal analysis of ctDNA changes during NAC and correlated it with local tumor response. P038 was excluded from this analysis because of discrepant tumor and ctDNA data. The ctDNA amount (clonal VAF) in baseline plasma was set as 100%, and those in subsequent plasma from the same patient were normalized to the baseline value. It was found that patients who responded to NAC had more decreasing ctDNA than patients who did not respond to NAC (Fig 3A). The difference was significant after the second cycle ($P = .03$) and all cycles of NAC ($P = .02$) but was not statistically significant after the first cycle of NAC ($P = .290$; Figs 3B-3D).

Next, we constructed ROC²² curves to compare the efficacy of ctDNA amount (clonal VAF) and ultrasound to predict the response to NAC (Fig 4). It was found that clonal VAF after 2 cycles, clonal VAF before surgery, and ultrasound after 2 cycles were all predictive of the response to NAC before surgery. The clonal VAF after 2 cycles (area under the curve



[AUC], 0.81; 95% CI, 0.61 to 1.00) and before surgery (AUC, 0.82; 95% CI, 0.64 to 1.00) had higher predictive value than the ultrasound after 2 cycles (AUC, 0.76; 95% CI, 0.58 to 0.94), although the difference was not statically significant. The optimal criteria of clonal VAF in predicting the response to NAC, as determined by the ROC plots, are 40% drop in clonal VAF after 2 cycles and 60% drop in clonal VAF before surgery, respectively. The optimal criterion of ultrasound after 2 cycles was 20% decrease in the longest diameter of tumor in our study.

Concordant mutations between tumor and plasma were also identified in two patients with smaller tumor and negative lymph nodes (T2N0M0). Notably, the concordant mutations were still found in the plasma before surgery, and both patients remained SD until surgery (Appendix Fig A3), suggesting that ctDNA can be used to track the response of early tumors (stage IIA) during NAC.

ctDNA and the Prognosis of Patients

To determine whether ctDNA can predict the survival of patients with breast cancer, we analyzed the association between ctDNA and patient prognosis.

Baseline ctDNA positivity had a significant impact on the prognosis of patients with breast cancer, because the patients with positive ctDNA before NAC had significantly shorter disease-free survival (DFS) and overall survival (OS) than those with negative ctDNA ($P = .011$; hazard ratio [HR], 5.72; 95% CI, 1.74 to 18.81 for DFS; $P = .004$; HR, 11.27; 95% CI, 2.99 to 42.45 for OS; Figs 5A and 5B). Subgroup analysis demonstrated that this impact was mainly derived from ER-negative patients. There was no significant difference in the DFS and OS of ER-positive patients with positive or negative baseline ctDNA ($P = .57$; HR, 1.75; 95% CI, 0.23 to 13.39 for DFS; $P = .72$; HR, 1.66; 95% CI, 0.095 to 28.94 for OS; Figs 5C and 5D). Nevertheless, the difference of DFS and OS in ER-negative patients was surprisingly large between those with positive and negative ctDNA ($P = .04$; HR, 5.11; 95% CI, 1.08 to 24.18 for DFS; $P = .033$; HR, 5.46; 95% CI, 1.14 to 26.10 for OS; Figs 5E and 5F). The ER-negative patients with negative baseline ctDNA had 100% DFS and OS, whereas those with positive baseline ctDNA had a DFS/OS of only approximately 36%.

Among the 20 patients with positive baseline ctDNA (one patient ruled out due to lack of subsequent samples), 6 of them turned negative and 14 remained positive for the ctDNA before surgery. The relapse rate in the patients with

persistent ctDNA was 50%, and that in the patients with negative ctDNA before surgery was 33%. The difference was more contrasting in ER-negative patients. Six out of 8 (75%) ER-negative patients with persistent positive ctDNA relapsed, whereas only 1 out of 3 (33%) such patients with negative ctDNA before surgery relapsed, although the difference was not statistically significant because of small sample size (Appendix Figure A4).

DISCUSSION

In this study, we used targeted deep sequencing of cfDNA with a panel of 1,021 cancer-related genes to screen for mutated ctDNA in patients with early breast cancer and found that 21 out of 44 patients had positive ctDNA. ctDNA tracking during NAC outperformed imaging in predicting the response to NAC, both in local response and general tumor burden (patient 038). More importantly, positive ctDNA before NAC is significantly associated with worse prognosis in patients with early breast cancer, with the significance mainly derived from ER-negative patients.

Many patients with early breast cancer still relapse after surgery because of clinically undetectable micrometastases. Current imaging methods are not sensitive enough to detect micrometastatic lesions; even the most sensitive positron emission tomography-computed tomography has the resolution limit at 4 mm.²⁶ Recently, ctDNA has been suggested to be an ideal tumor biomarker because of its specificity, stability, and sensitivity. It is shown to be better than traditional biomarkers and imaging in predicting the relapse or progression in both early and metastatic cancer.^{9,11,12,27-29} However, the best method to measure ctDNA is uncertain. dPCR is cheaper and easier, but it only measures one or few known mutations. Personalized dPCR is highly sensitive but requires sequencing of tumor tissue and individualized bespoke probes. NGS measures many mutations simultaneously and can identify resistant clones newly emerged during treatment, but it is also expensive. To our knowledge, this study used the largest NGS panel so far to look for ctDNA in patients with early breast cancer.

Using the large 1,021-gene panel, somatic mutations were found in all the tumor biopsies. More importantly, positive ctDNA was found in 47% of patients with early breast cancer and 73% of ER-negative patients, which was consistent with previous reports.^{12,16,17,30,31} The majority of the ctDNA was confirmed in tumor mutation data, suggesting that ctDNA can be used to detect tumor-specific

FIG 2. The baseline somatic mutations found in the plasma and tissue of patients with early breast cancer. (A) The baseline plasma mutations in the 21 patients with positive circulating tumor DNA. Left panel shows mutations in individual patients with different ER and Her2 positivity; right panel shows mutation frequency of individual genes. (B) Mutation profiles in the 32 paired tumor biopsy and plasma samples. Left panel shows the concordance of mutations in the 32 patients; right panel shows the distribution of 143 mutations found in tissue and/or plasma. (C) The mutation frequency of the top 15 mutated genes found in baseline tumor biopsy. (D) The concordant mutated genes in both tumor and plasma of the 14 patients.

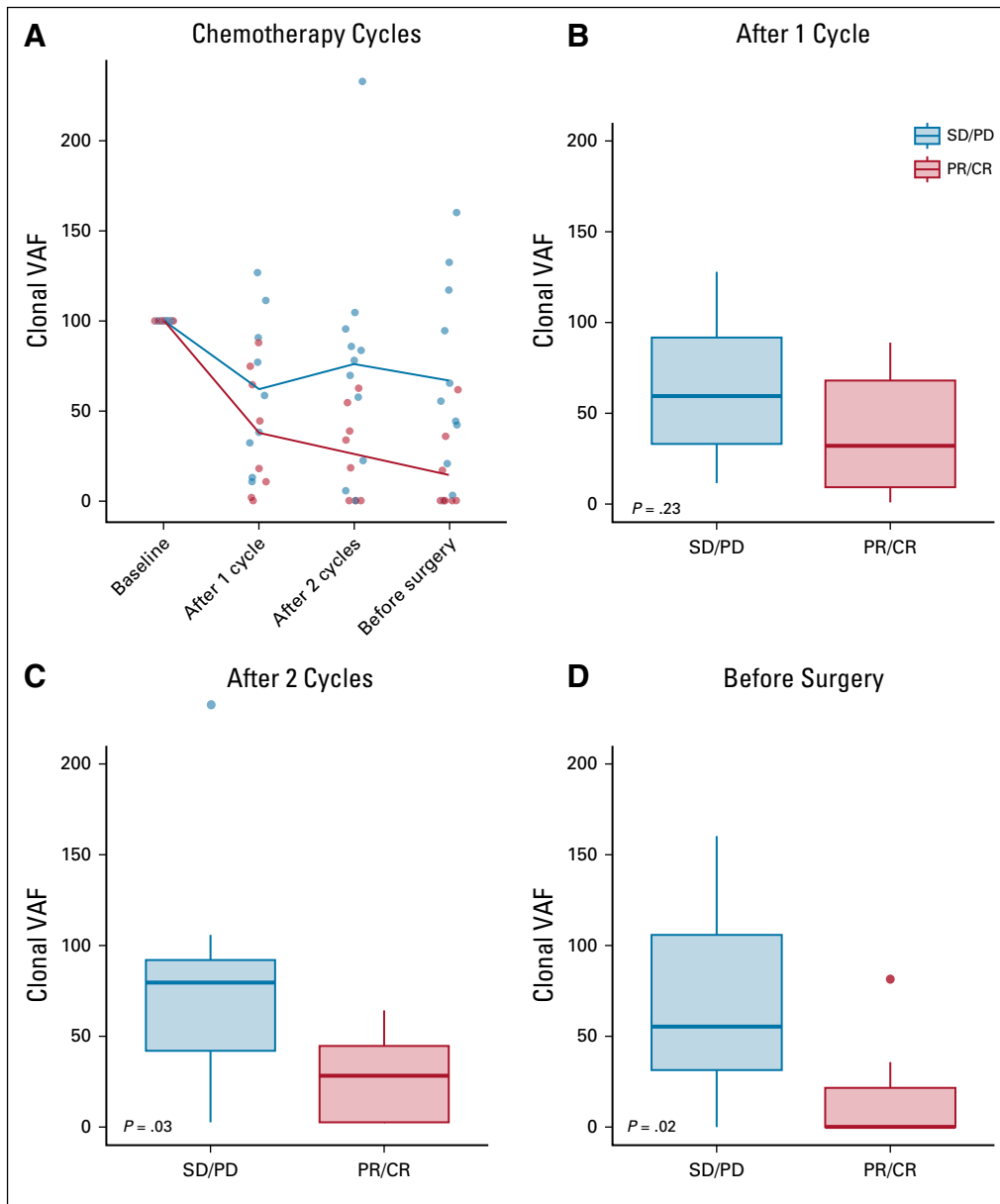


FIG 3. Dynamic changes of circulating tumor DNA amount (clonal variant allele frequency [VAF]) and tumor response to neoadjuvant chemotherapy (NAC). (A) The trend of clonal VAF during NAC in response and no-response patients. (B) After 1 cycle of NAC, the difference of clonal VAF between response and no-response patients was not significant. (C, D) Clonal VAF in no-response patients was significantly higher than that in response patient after (C) 2, and (D) all cycles of NAC. CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

mutations in patients with early breast cancer. In the 21 patients with positive baseline ctDNA, 16 patients had paired plasma and tumor biopsy samples for NGS analysis, and 14/16 had at least one mutation confirmed in primary tumors. With the high specificity, at 98.72%, the discrepancy between the mutations in plasma and the mutations in tumor may be explained by the small tumor biopsy specimen not representing the whole primary tumor or micrometastasis. Similar to previous studies, we found that *TP53* and *PI3KCA* were the most commonly mutated

genes both in tumor and plasma. But this study also identified 24% (5/21) of patients with positive ctDNA who had mutations other than *TP53* and *PI3KCA*.

Interestingly, our data showed that dynamic change of ctDNA can be used to monitor and predict the response to NAC in early breast cancer. After one cycle of NAC, patients who eventually responded well to NAC began to have lower clonal VAF than that of patients with no response. After 2 cycles of NAC, patients who responded well showed significantly lower clonal VAF than patients with no response.

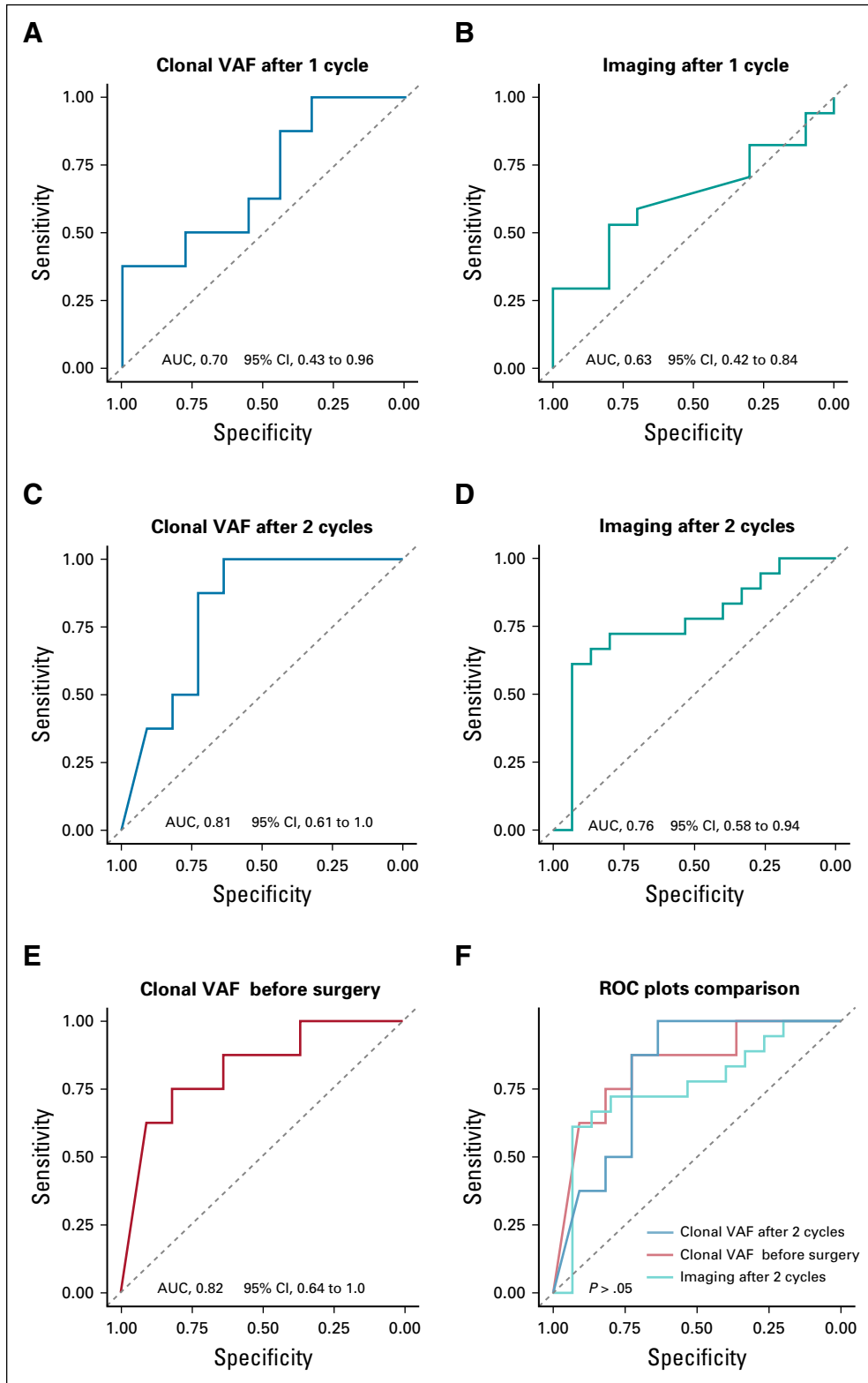


FIG 4. Receiver operating characteristic (ROC) analysis shows the accuracy of clonal variant allele frequency (VAF) to predict tumor response to neoadjuvant chemotherapy (NAC). (A) Tumor response according to clonal VAF after 1 cycle of NAC. (B) Imaging after 1 cycle of NAC. (C) Clonal VAF after 2 cycles of NAC. Imaging after 2 cycles of NAC. (E) Clonal VAF after all cycles of NAC (before surgery). (F) ROC plot comparison. AUC, area under the curve.

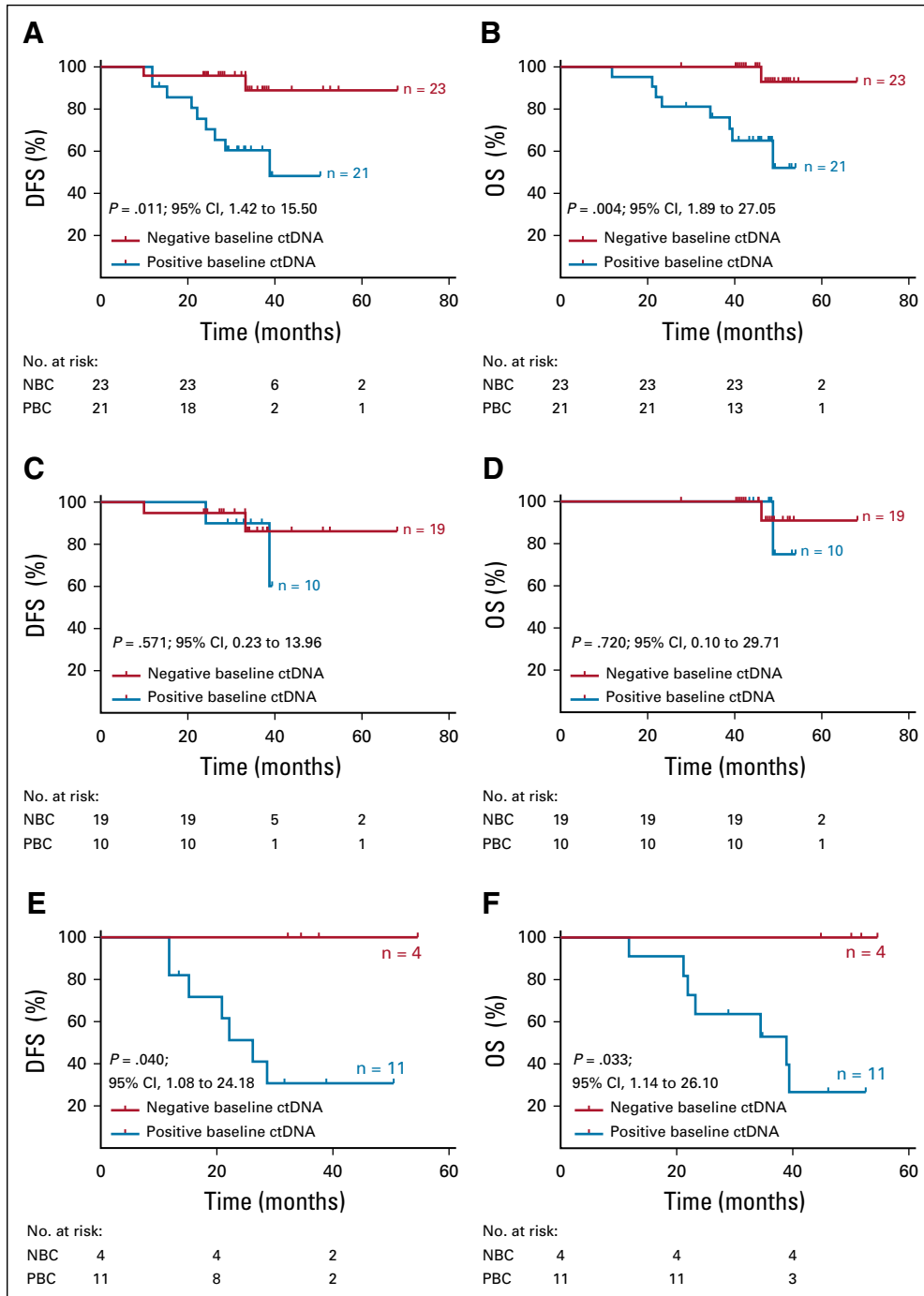


FIG 5. Kaplan-Meier curve compares the disease-free survival (DFS) and overall survival (OS) in patients with breast cancer with positive or negative baseline circulating tumor DNA (ctDNA). (A) DFS, and (B) OS in the 44 patients with early breast cancer. (C) DFS, and (D) OS in the 29 ER-positive patients. (E) DFS, and (F) OS in the 15 ER-negative patients. NBC, negative baseline ctDNA; PBC, positive baseline ctDNA.

The change of ctDNA level after 2 cycles of NAC predicted local tumor response to NAC better than ultrasound, although this was not statistically significant. More importantly, patient O38 had increasing high levels of ctDNA even when partial remission of primary tumor was confirmed by

imaging. Early recurrence of this case suggested the development of resistance in undetectable micrometastases during NAC. This indicates that ctDNA is superior to imaging in monitoring the overall response to NAC in early breast cancer.

Our results showed that baseline ctDNA has strong prognostic value, especially in ER-negative patients. Interestingly, none of the four ER-negative patients with negative baseline ctDNA relapsed during the follow-up, which was strikingly different from the high relapse rate in ER-negative patients with positive baseline ctDNA. This finding is inconsistent with a previous report that showed postsurgery ctDNA, but not baseline ctDNA, predicted early relapse in breast cancer.¹² However, the follow-up study of the aforementioned report, which used dPCR assay, showed that positive baseline ctDNA was a marker of poor prognosis, with an HR similar to our study (5.8 v 5.7).¹⁷ These data together indicate that the presence of ctDNA before NAC is a marker of poor prognosis that helps physicians to distinguish the patients with high risk of metastasis from those with low risk.

Unfortunately, a significant portion of patients in our study did not have plasma samples drawn after surgery, preventing the comparison between baseline and postsurgery ctDNA. Other limitations of this study include limited sample size and suboptimal NAC cycles, which hinder further subgroup analysis.

In conclusion, our study demonstrates the feasibility and validity of ctDNA in patients with early breast cancer receiving NAC. Serial tracking of ctDNA has significant potential to complement imaging-based tumor assessment during NAC in early breast cancer. Moreover, the ctDNA presence before NAC correlates with worse prognosis, especially in ER-negative patients. ctDNA analysis may help to identify high-risk patients for escalating the treatment.

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EQUAL CONTRIBUTION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

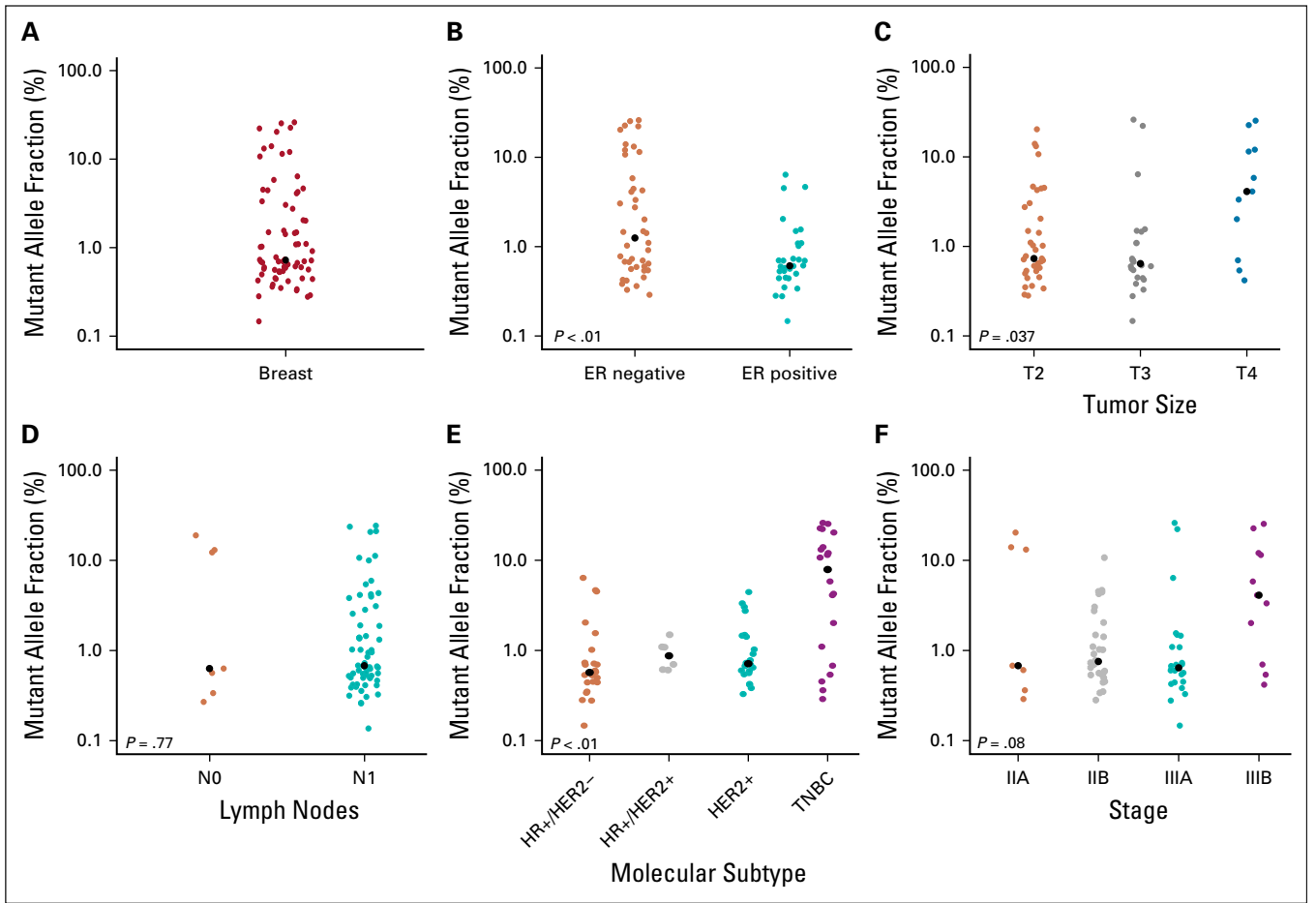


FIG A1. Circulating tumor DNA distribution in patients with breast cancer. TNBC, triple-negative breast cancer.

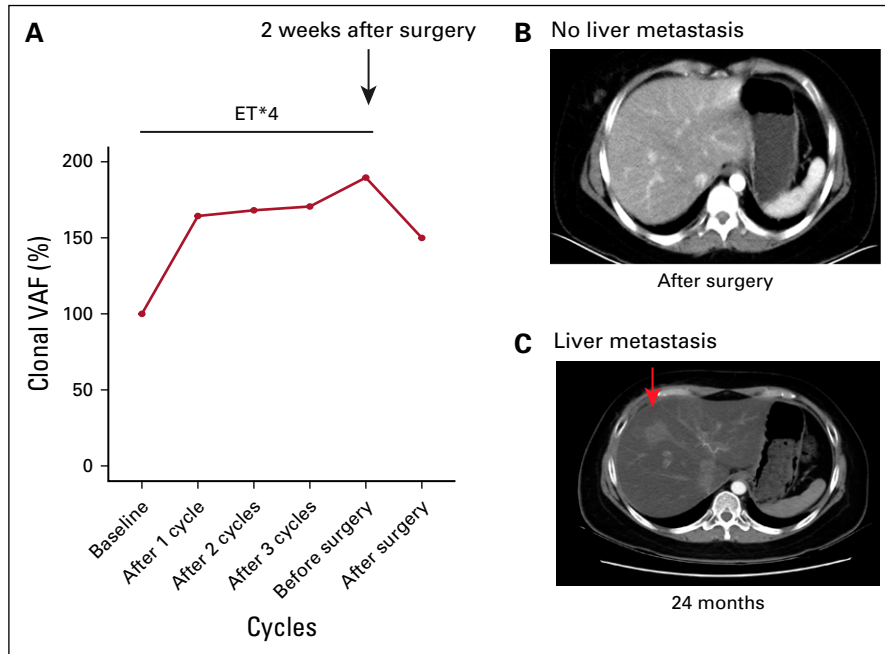


FIG A2. The increasing circulating tumor DNA during neoadjuvant chemotherapy and metastasis after surgery in patient 038. VAF, variant allele frequency. ET*4, 4 cycles of epirubicin and docetaxel.

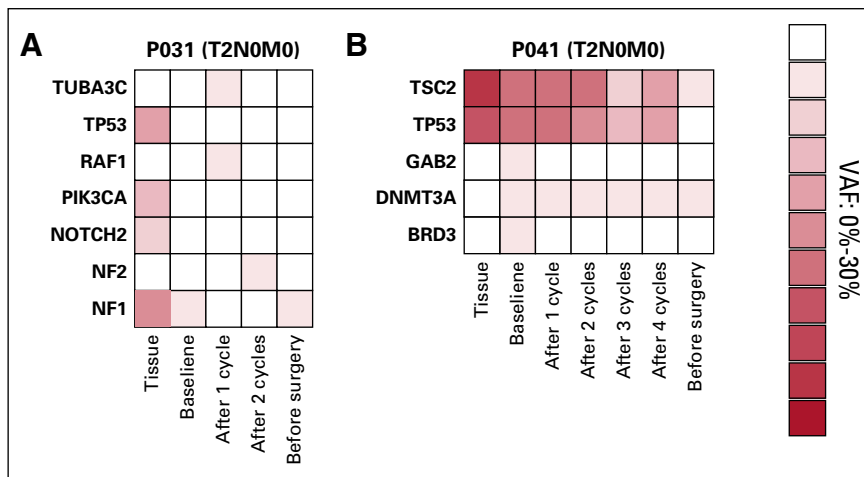


FIG A3. Mutations detected in the paired tumor biopsy and serial plasma samples from two T2N0M0 patients. (A) Patient 031 (P031). (B) Patient 041 (P041).

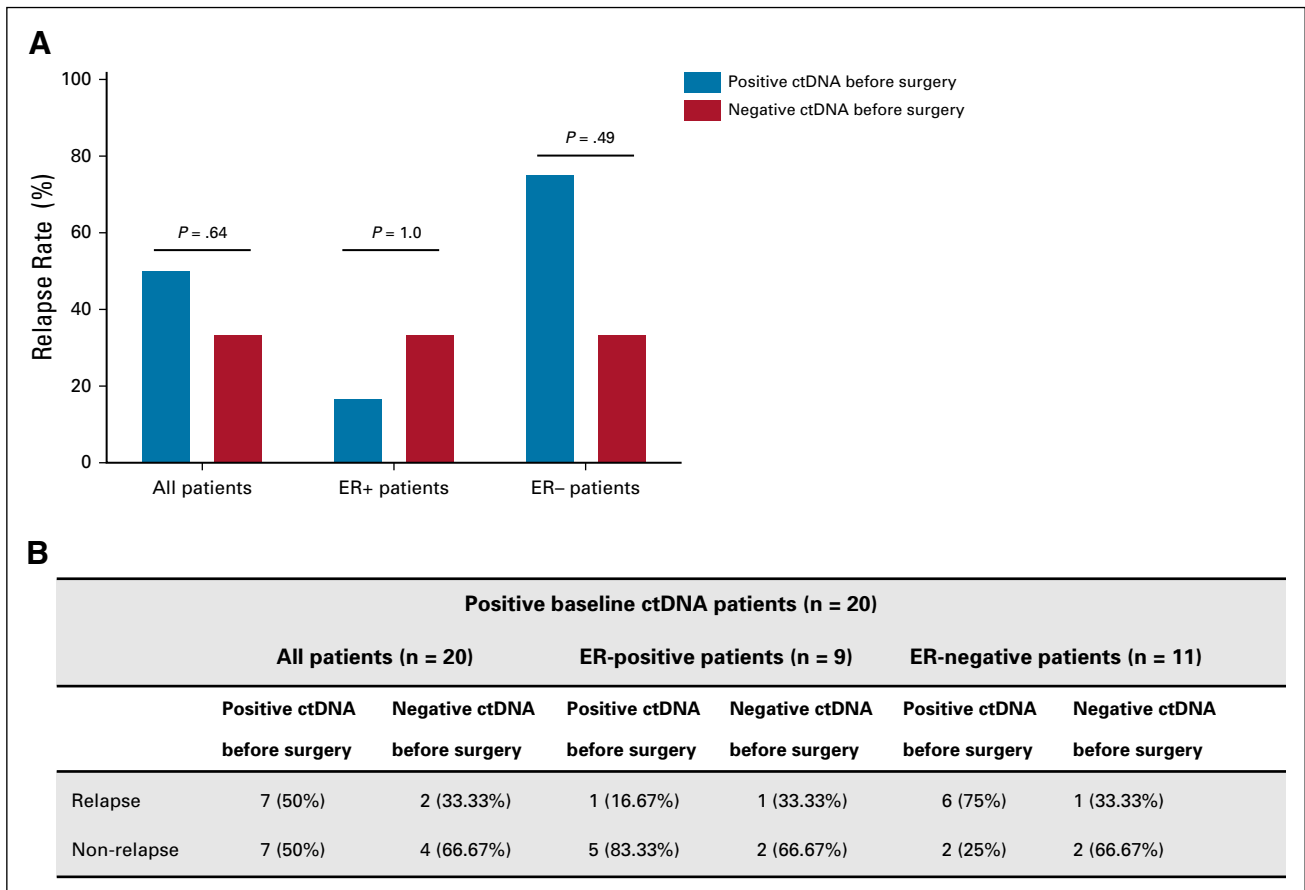


FIG A4. (A) Bar chart and (B) table show different relapse rates in the patients with positive baseline circulating tumor DNA (ctDNA; n = 20) who remained positive (n = 14) or turned negative (n = 6) before surgery.