Serial Postoperative Circulating Tumor DNA Assessment Has Strong Prognostic Value During Long-Term Follow-Up in Patients With Breast Cancer

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ABSTRACT

PURPOSE Here, we report the sensitivity of a personalized, tumor-informed circulating tumor DNA (ctDNA) assay (Signatera) for detection of molecular relapse during long-term follow-up of patients with breast cancer.

METHODS A total of 156 patients with primary breast cancer were monitored clinically for up to 12 years after surgery and adjuvant chemotherapy. Semiannual blood samples were prospectively collected, and analyzed retrospectively to detect residual disease by ultradeep sequencing using ctDNA assays, developed from primary tumor whole-exome sequencing data.

RESULTS Personalized Signatera assays detected ctDNA ahead of clinical or radiologic relapse in 30 of the 34 patients who relapsed (patient-level sensitivity of 88.2%). Relapse was predicted with a lead interval of up to 38 months (median, 10.5 months; range, 0-38 months), and ctDNA positivity was associated with shorter relapse-free survival ($P < .0001$) and overall survival ($P < .0001$). All relapsing triple-negative patients (n = 7/23) had a ctDNA-positive test within a median of 8 months (range, 0-19 months), while the 16 nonrelapsed patients with triple-negative breast cancer remained ctDNA-negative during a median follow-up of 58 months (range, 8-99 months). The four patients who had negative tests before relapse all had hormone receptor–positive (HR+) disease and conversely, five of the 122 nonrelapsed patients (all HR+) had an occasional positive test.

CONCLUSION Serial postoperative ctDNA assessment has strong prognostic value, provides a potential window for earlier therapeutic intervention, and may enable more effective monitoring than current clinical tests such as cancer antigen 15-3. Our study provides evidence that those with serially negative ctDNA tests have superior clinical outcomes, providing reassurance to patients with breast cancer. For select cases with HR+ disease, decisions about treatment management might require serial monitoring despite the ctDNA-positive result.

ACCOMPANYING CONTENT

- Data Sharing Statement
- Data Supplement

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INTRODUCTION

Breast cancer is the most commonly diagnosed cancer in men and women combined and is the leading cause of cancer-related deaths in women.[1] The current standard of care for women with early-stage breast cancer for most patients consists of surgery and (neo)adjuvant chemotherapy and/or endocrine therapy with the aim of eliminating microscopic minimal residual disease (MRD).[2,3] However, up to 30% of patients with breast cancer ultimately relapse with life-threatening metastases after their primary treatment.[4] Hence, there is an urgent need to develop more sensitive technologies capable of detecting MRD and following patients with breast cancer after primary treatment with the aim of identifying whether interventions in patients with MRD might be helpful in improving outcomes. Personalized circulating tumor DNA (ctDNA) measurements have been shown to predict relapse in lung and

CONTEXT

Key Objective
To determine the value of a personalized, tumor-informed circulating tumor DNA assay (Signatera) for early detection of relapse in patients with breast cancer.

Knowledge Generated
The assay predicted relapse in 30 of 34 patients with variable lead intervals. In patients with triple-negative breast cancer, the test was consistently predictive, but a minority of patients with hormone receptor–expressing breast cancer were not detected and others in this subtype had a positive test with no evidence of relapse as yet.

Relevance
The test is highly predictive of recurrence in patients with breast cancer, especially the triple-negative subtype. For patients with hormone receptor–positive breast cancer, the test needs to be used with care since a small proportion of patients relapse with a negative test and others whose test is positive have not yet relapsed.

colon cancers,[5,6] and there is some evidence that treatment given to patients showing a positive test in the absence of overt recurrence might be of benefit.[7]

For patients with breast cancer, several groups—including our own—have shown that ctDNA detection can antedate metastatic recurrence.[8-10] We presented the preliminary results of the Exploratory Breast Lead Interval Study (EBLIS) in 2019.[11] After 2 years of follow-up, approximately 50% of the predicted events (18 relapses) had occurred. After an interim analysis, the trial management group recommended laboratory assessment of serial plasma in the first 50 patients. Of these, 49 patients had successful tumor whole-exome sequencing (WES) enabling ctDNA (Signatera bespoke, multiplex PCR next generation sequencing) assay design, wherein we reported a lead interval of up to 2 years (median of 8.9 months; range, 0.5-24 months) between detection of ctDNA and clinical detection of overt metastatic disease. The results indicated that the tumor-informed, ctDNA assay was prognostic of recurrence in the majority of patients (16/18).[11]

Concerning the late adjuvant setting, in a recent report, 83 patients with hormone receptor–positive (HR+) breast cancer were followed up for a median of 10.4 years from diagnosis. Eight patients had a positive ctDNA test; six were MRD-positive before overt clinical recurrence, with a median lead interval of 12.4 months, while two ctDNA-positive patients had not relapsed at the time of last follow-up.[12] However, this study only had a median of two samples per patient, and as patients with HR+ breast cancer remain at risk for many years, more information is needed on ctDNA dynamics in HR+ breast cancer.

Here, we report results for the entire EBLIS cohort, to our knowledge, the largest breast cancer cohort with the longest ctDNA based follow-up to date, where a total of 156 patients with primary breast cancer were followed for up to 12 years with semiannual blood sampling. A total of 1,136 plasma samples from the 156 patients were profiled for ctDNA detection with personalized Signatera assays following our previously validated approach.[11]

METHODS

Patients and Samples

EBLIS is a multicenter, prospective cohort study, funded by Cancer Research UK and the National Institute for Health Research that opened to recruitment in 2012. Patients must meet all the inclusion criteria to be considered eligible for this study. All patients provided written informed consent before entry into the study. None of the assay results were shared with either clinicians or patients. Patients were age 18 years or older, have had histologically confirmed breast cancer, and must have completed all surgery and chemotherapy within 3 years of entry into the study. They had to have an Adjuvant! Online risk of relapse at >65% relapse or mortality of >50% at 10 years. The trial protocol was approved by the Riverside Research Ethics Committee (REC:13/LO/115; IRAS:126462). The primary objective was to determine the lead interval between detection of ctDNA in plasma and clinical detection of overt metastatic disease. A cohort of 188 patients were monitored with semiannual blood sampling for ctDNA analysis, along with concomitant clinical examination as described previously (Fig 1A; Table 1; Data Supplement, Tables S1-S2d).[11] The study census date (last date of follow-up) was December 31, 2021. All patients had provided consent for the publication of the study.

Signatera Assay Design and Analysis

Personalized, tumor-informed Signatera ctDNA assays were developed, from primary tumor WES data, targeting 16 high-ranked, clonal, somatic single-nucleotide variants (SNVs) that were used to detect ctDNA in plasma. Details of the methodology and workflow have been reported

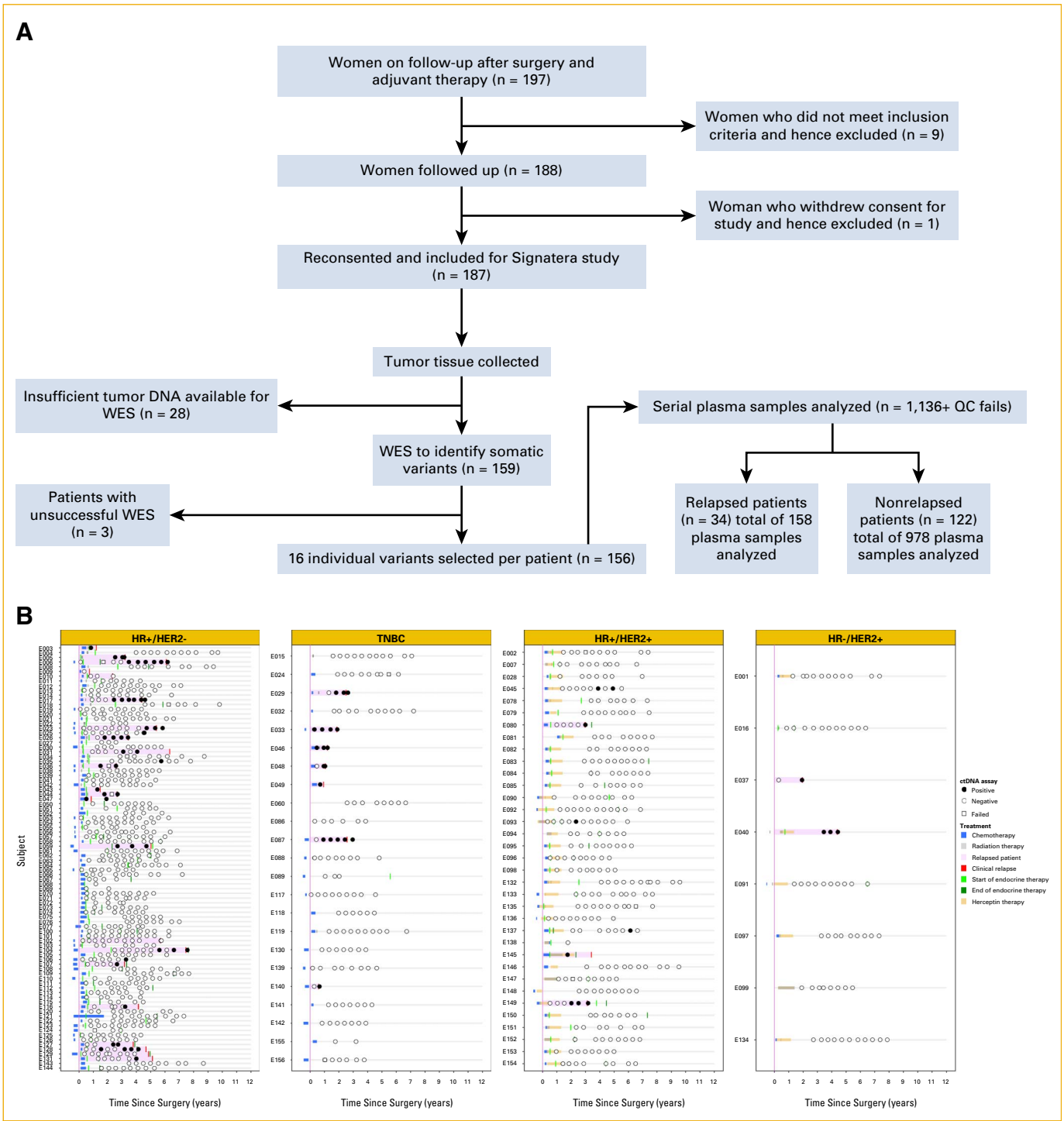

FIG 1. EBLIS study flow diagram and patient timeline summaries showing detection of ctDNA ahead of clinical relapse. (A) Patient recruitment and collection of clinical samples. For the 156 women with breast cancer monitored in this study, exonic alterations were determined through paired-end sequencing of FFPE tumor-tissue specimens and matched normal DNA. Patient-specific Signatera assays were designed to include 16 somatic mutations identified from whole-exome sequencing data. Serial plasma samples were analyzed with the corresponding custom assay panels using the Signatera workflow in a blinded manner in a CLIA-certified laboratory. A total of 1,141 plasma samples were analyzed for ctDNA detection. (B) Each patient's time since surgery showing longitudinal ctDNA assay samples, treatment and relapse status and results summary of each patient's (n = 156) treatment regimen by subgroup along with results of serial plasma samples (n = 1,136) analyzed. CLIA, Clinical Laboratory Improvement Amendments; ctDNA, circulating tumor DNA; EBLIS, Exploratory Breast Lead Interval Study; FFPE, formalin-fixed paraffin-embedded; QC, quality control; TNBC, triple-negative breast cancer; WES, whole-exome sequencing.

TABLE 1. Patient and Tumor Baseline Characteristics

	Molecular Subtype (No.)				
Variable	HR+/HER2− (90)	HR+/HER2+ (35)	TNBC (23)	HER2+ (8)	All Subjects (156)
Age, years					
≤40	8	8	2	0	18
41-60	57	18	14	5	94
61-80	25	9	5	3	42
>80	0	0	2	0	2
Age at diagnosis, years					
Mean (SD)	53.9	51.8	57.1	61.1	54.3
Median	54	50	57	57	54
Min-max	26-80	29-79	34-87	48-80	26-87
Size of tumor, mm					
Mean (SD)	41.1	35.9	24.8	34.5	37.1
Median	32	30	25	31	30
Min-max	5-150	1-100	5-50	27-60	1-150
Tumor type					
IDC	73	34	22	6	135
ILC	16	1	0	1	18
Other	1	0	1	1	3
Type of surgery					
Mastectomy	56	21	10	5	92
Breast conservation	30	14	13	2	59
Bilateral mastectomy	2	0	0	0	2
Other	2	0	0	1	3
Tumor grade					
1	1	0	0	0	1
2	57	3	2	0	62
3	32	32	21	8	93
Histology					
Left	46	22	13	5	86
Right	44	13	10	3	70
HER2 status					
Positive	0	35	0	8	43
Negative	90	0	23	0	113
ER status					
Positive	89	33	0	0	122
Negative	1	2	23	8	34
PgR status					
Positive	64	12	0	0	76
Negative	24	20	23	8	75
Not documented	2	3	0	0	5
Staging					
IA	1	0	6	0	7
IIA	2	3	2	0	7
IIB	26	12	9	5	52
IIIA	36	16	4	2	58
IIIB	0	0	0	0	0
IIIC	24	4	2	1	31
Unknown	1	0	0	0	1

NOTE. After screening and recruitment, patients were followed up with six monthly blood samples for up to 10 years. HER2 status was determined by using IHC and FISH. A patient was considered to have HER2-positive cancer if IHC had a HER2 3+ score and/or a positive FISH test.
Abbreviations: ER, estrogen receptor; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; HR+, hormone receptor–positive; IDC, infiltrating ductal carcinoma; IHC, immunohistochemical; ILC, infiltrating lobular carcinoma; PgR, progesterone receptor; SD, standard deviation; TNBC, triple-negative breast cancer.

previously.[5,6,11] All tests were carried out in a Clinical Laboratory Improvement Amendments–certified laboratory.

Statistical Analyses

The study sample size was described previously.[11] Clinical characteristics of patients were summarized using descriptive statistics, including means, medians, or range for continuous variables, and frequency and percentage for categorical variables. The Wilcoxon matched-pairs signed rank test was used to compare mean variant allele frequency (VAF) at the first ctDNA-positive time point and the last time point before relapse. Sensitivity was defined as the number of patients with preclinical metastasis detected by ctDNA over the total number of patients with clinical relapse. Specificity was defined as the number of patients who were ctDNA-negative during the clinical follow-up period over the total number of patients who have not relapsed. The primary and secondary outcome measures were relapse-free survival (RFS) and overall survival (OS), respectively. RFS was assessed by standard radiologic criteria measured from date of surgery to verified first radiologic recurrence (local or distant). OS was defined as the time from date of surgery to the date of death or last follow-up date (December 31, 2021, or death). Primary associative analysis used a univariable approach with categorical ctDNA status (negative and positive). RFS and OS were compared between patients with positive and negative ctDNA status at the first blood sample time point (baseline) and any time point by using Kaplan-Meier and log-rank tests. Hazard ratios (HRs) for RFS and OS were estimated using a univariable Cox proportional hazard model. Multivariable Cox regression models were used to determine the impact of ctDNA on RFS and OS while controlling for clinicopathologic factors. An exploratory analysis was performed in a subgroup of the cohort with complete data on cancer antigen (CA)15-3 and ctDNA. Fisher's exact test was used to evaluate the correlation between the measurements of ctDNA and CA15-3. All statistical tests were two-sided. A P value of <.05 was regarded as statistically significant for results. All statistical analyses were performed using R (R Foundation for Statistical Foundation, R version 4.0.1, *survival* [version 3.2-7] and *survminer* [version 0.4.8], R Core Team, Vienna, Austria).

RESULTS

Here, we report full results from EBLIS, to our knowledge, the largest breast cancer cohort with the longest ctDNA follow-up to date. A total of 188 patients with primary breast cancer, recruited after surgery and adjuvant chemotherapy, were followed up with semiannual blood sampling for ctDNA analysis. After review of all available formalin-fixed paraffin-embedded surgical tissue blocks, 29 patients did not have sufficient residual tumor for WES. In the remaining 159 patients, paired tumor and genomic DNA samples were subjected to WES; samples from two patients failed WES quality control requirements, and tumor WES for a third patient identified too few somatic variants, leaving 156 patients for ctDNA testing (see the flow diagram: Fig 1A). The landscape of somatic mutations detected in the 156 primary tumor DNA samples was similar to other breast cancer series, with *TP53* and *PIK3CA* being the most commonly mutated genes (Data Supplement, Fig S1).

The clinicopathologic characteristics of the patient cohort are presented in Table 1. The median follow-up was 77 months (range, 8-140 months; Table 2). Patient clinical characteristics, blood sample time points, CA15-3 levels, tests to confirm metastasis, and treatment schedules are provided in the Data Supplement (Tables S1-S2d).

Long-Term Postoperative Follow-Up of Patients With Breast Cancer With the Signatera Residual Disease Test

Multiple plasma samples (n = 1,136) for ctDNA evaluation were available from all 156 patients, with a median of 8

TABLE 2. Median Follow-Up and Lead Interval by Molecular Subtype

	Molecular Subtype (No.)				
Variable	HR+/HER2− (90)	HR+/HER2+ (35)	TNBC (23)	HER2+ (8)	All Subjects (156)
Follow-up months, median (range)	94 (27-131)	73 (8-140)	58 (8-99)	86 (25-113)	77 (8-140)
Treatment, No. (%)					
NACT	29 (32)	8 (23)	10 (43.5)	1 (12.5)	48
ACT	57 (63)	27 (77)	10 (43.5)	5 (62.5)	99
None	4 (6)	0 (0)	3 (13)	2 (25)	9
Patients who relapsed, No. (%)	22 (24.4)	3 (8.6)	7 (30.4)	2 (25)	34 (21.8)
Relapses detected, No. (%)	18 (81.8)	3 (100)	7 (100)	2 (100)	30 (88.2)
Lead interval months, median (range)	13 (2-38)	6 (4-13)	8 (0-19)	15.7 (11.6-19.8)	10.5 (0-38)
No. of blood samples per patient, median (range)	8 (1-11)	8 (1-11)	6 (1-11)	9.5 (3-11)	8 (1-11)

NOTE. Median follow-up was from the date of surgery (months). Lead interval was from detection of ctDNA in plasma to clinical detection of overt metastatic disease.

Abbreviations: ACT, adjuvant chemotherapy; ctDNA, circulating tumor DNA; HER2+, human epidermal growth factor receptor 2–positive; HR+, hormone receptor–positive; NACT, neoadjuvant chemotherapy; TNBC, triple-negative breast cancer.

(range, 1-11) samples per patient. These included 121 plasma samples from continued follow-up of 31 patients who had not relapsed at the first reporting census date (interim analysis, June 30, 2018).[9] In the full cohort, time from surgery to first blood sample ranged from 3 to 57 months (median, 16 months; Data Supplement, Table S2a). Personalized Signatera assays detected ctDNA in a total of 46 of the 1,136 plasma samples (Fig 1B; Data Supplement, Tables S3a and S3b).

Thirty-four patients (21.7%) had been diagnosed with clinical recurrence at the last date of follow-up (Table 3). Plasma ctDNA was detected ahead of clinical or radiologic relapse in 30 of the 34 relapsed patients, with a patient-level sensitivity of 88.2% (Table 2; Fig 1B). Considering molecular subtypes, the patient-level sensitivity was 81.8% for the HR+/human epidermal growth factor receptor 2 (HER2)− group; however, 100% of relapses were detected through ctDNA in the HR+/HER2+, triple-negative breast cancer (TNBC), and HER+ groups (Table 2). Metastatic relapse was predicted with a lead interval between ctDNA detection and relapse of up to 38 months (median, 10.5 months; range, 0-38 months), updating the lead interval of 2 years (median, 8.9 months; range, 0.5-24 months) reported in the first 49 patients.[9] The longest lead time to molecular relapse was observed in HR+/HER2− patients (median, 13 months; range, 2-38 months) and HR−/HER2+ patients (median, 15.7 months; range, 11.6-19.8 months). Patients with HR+/HER2+ (median, 6 months; range, 4-13 months) and TNBC (median, 8 months; range, 0-19 months) had the shortest time to molecular relapse (Fig 1B; Table 2). Of note, there were no new positive ctDNA results in the TNBC cohort after 19 months and during a median follow-up of 58 months (range, 8-99 months).

The four relapsed patients not detected in the study (E09, E010, E0102, and E0129) were all HR+/HER-; two had bone recurrence (one with axillary lymph node involvement), one had a malignant pleural effusion and no other sites of metastasis, and one had an isolated local recurrence to bone (Fig 1B; Table 3; Data Supplement, Table S2c). Of the remaining 122 nonrelapsed patients, 116 patients were consistently ctDNA-negative across 941 plasma tests over up to 12 years after their primary surgery. Four patients (E035, E093, E106, and E137) had a single ctDNA-positive sample detected with low VAF, followed by a negative test, three with two variants and one with five variants detected (Fig 1B; Data Supplement, Tables S3a-S3c). Another patient (E045) had two of nine plasma samples that were termed ctDNA-positive, each of which was followed by a negative test. All five of these patients had HR+ breast cancer, and none had relapsed by the study census date (December 31, 2021). The disease status for these five patients was subsequently reviewed; at April 30, 2023, none had yet relapsed.

One other patient with a ctDNA-positive result (E025) was diagnosed with primary lung cancer. Her last blood sample on study had two variants detected, raising the possibility of recurrent breast cancer as opposed to primary lung cancer but the patient withdrew participation on the study, precluding access to the lung cancer tissue for molecular comparison.

Rising ctDNA VAF and Mean Tumor Molecules/mL Antedates Relapse

The mean tumor molecules per mL (MTM/mL) was calculated on the basis of the mean of ctDNA molecules detected per mL of the patient's plasma. There was a positive correlation (rho, 0.75; $P < .001$) between the MTM/mL and VAF (Data Supplement, Table S3c). The number of variants, mean VAF, and MTM/mL varied between patients, with significantly higher VAF ($P = .0028$) and MTM/mL values ($P < .001$) observed at the time closest to relapse compared with the first ctDNA-positive sample. Moreover, patients who relapsed showed significantly higher median MTM/mL values compared with the five patients who did not relapse (0.60 [0.15-5.70] *v* 0.12 [0.06-128.2], $P = .011$). Although statistically significant, this trend is based on a small number of patients who did not relapse.

Association Between Circulating Tumor DNA and Clinical Outcomes

The impact of ctDNA status on clinical outcomes was assessed. Patients with a positive ctDNA test had poorer RFS (HR, 52.98 [95% CI, 18.32 to 153.20]; $P < .0001$) and a significantly reduced OS (HR, 53.69 [95% CI, 7.01 to 411.49]; $P < .0001$; Figs 2A and 2B). This includes those patients with ctDNA detected in the first postsurgical plasma sample (HR, 30.15 [95% CI, 13.76 to 66.05]; $P < .0001$ for RFS) and (HR, 19.32 [95% CI, 6.66 to 56.01]; $P < .0001$ for OS; Figs 2C and 2D). Moreover, in multivariable models incorporating clinicopathologic variables, ctDNA status remained the most significant factor associated with RFS and OS ($P < .0001$; Data Supplement, Table S4).

Circulating Tumor DNA and Other Monitoring Tests

Concurrent ctDNA analyses and CA15-3 measurements were available for 100 patients. CA15-3 status was defined as positive and negative at a cutoff value of 30 U/mL. The Fisher's exact test showed a borderline significant correlation between ctDNA status and CA15-3 status ($P = .053$; Data Supplement, Table S5a). Multivariate analysis indicated that ctDNA was independent of CA15-3 in predicting RFS and OS. Here, positive ctDNA status was significantly associated with shorter RFS [HR, 30.89 [94% CI, 10.05 to 94.99]; $P < .001$) and OS (HR, 35.52 [95% CI, 4.41 to 285.96]; $P < .001$), whereas CA15-3 was not (Data Supplement, Table S5b).

DISCUSSION

The Signatera assay detected ctDNA up to 3 years before overt breast cancer relapse in the EBLIS patient population. The prognostic association is particularly striking for

TABLE 3. Clinical and ctDNA Characteristics in Patients With Clinical Relapse

Publication ID	Type of Recurrence	Site of Metastasis	Time From Surgery to Relapse, Days	Lead Time, Days	ctDNA-Positive at First Plasma Time Point	ctDNA-Positive at Any Plasma Time Point
E003	Metastatic	Pleura, lymph nodes, liver, and bone[a]	435	133	Yes	Yes
E005	Metastatic	Nodal disease right hilum and mediastinum[a]	1,183	263	Yes	Yes
E006	Metastatic	Right mediastinum and bilateral cervical nodes	2,242	973	No	Yes
E009	Metastatic	Sternum, pelvis, and vertebrae[a]	256	Not available	No	No
E010	Local	Sternum	857	Not available	No	No
E017	Metastatic	Sternoclavicular joint, skin, and lung	1,611	721	No	Yes
E023	Metastatic	Liver	[b]	Not available	No	Yes
E026	Metastatic	Spine	1,263	611	No	Yes
E029	Metastatic	Lung[a]	918	258	No	Yes
E031	Metastatic	Skin on right lower back[a]	1,428	301	No	Yes
E033	Metastatic	Intraclavicular fossa and sentinel lymph nodes	680	570	Yes	Yes
E036	Metastatic	Bone and bladder[a]	951	405	Yes	Yes
E037	Metastatic	Lung[a]	717	610	Yes	Yes
E040	Metastatic	Bone[a]	1,617	259	Yes	Yes
E043	Metastatic	Liver, lung, bone, and bile duct[a]	535	68	Yes	Yes
E044	Local	Local nodes and bone mets[a]	968	323	Yes	Yes
E046	Metastatic	Bone, liver, and pleura	439	263	Yes	Yes
E047	Metastatic	Local nodes and intrapulmonal nodes	302	114	Yes	Yes
E048	Metastatic	Bone and pleura	372	199	Yes	Yes
E049	Metastatic	Not known[a]	336	79	Yes	Yes
E059	Metastatic	Bone	1,849	856	Yes	Yes
E080	Metastatic	Skin and bone mets	1,232	137	No	Yes
E087	Local	Unresectable nodal recurrence[a]	940	596	No	Yes
E102	Metastatic	Axillary LNs and bone mets	2,092	Not available	No	No
E104	Metastatic	LNs, liver, and spinal mets	2,753	695	No	Yes
E107	Metastatic	Bone mets at L2 and L5 of spine	1,164	194	No	Yes
E116	Metastatic	Bone mets	1,524	116	No	Yes
E127	Metastatic	Bone, pleural, and nodes outside axilla[a]	1,389	512	No	Yes
E128	Metastatic	CNS, bone, and liver mets	1,722	1,147	No	Yes
E129	Metastatic	Pleural	1,786	Not available	No	No
E131	Metastatic	Bone and liver mets	1,887	421	No	Yes
E140	Metastatic	CNS	206	0	No	Yes
E145	Metastatic	Skin, bone, pleural, and liver[a]	827	188	Yes	Yes
E149	Metastatic	Skin and bone	1,148	408	No	Yes

NOTE. Lead time refers to the time (in days) from the first positive plasma sample to clinical occurrence.
Abbreviations: ctDNA, circulating tumor DNA; LN, lymph node.
[a]Patients are deceased.
[b]Excluded as dates affected by COVID-19.

patients with TNBC, as all seven patients with TNBC who relapsed had a positive ctDNA result before overt relapse. Additionally, none of the other patients with TNBC became ctDNA-positive after 19 months of monitoring, and during a median follow-up of 58 months (range, 8-99 months), which corresponds to the expected time frame of breast cancer recurrences in this subtype.[13]

The correlation between ctDNA detection and recurrence from HR+ breast cancer is also strong, but discordances were observed that may be attributable to underlying tumor biology—of note, four HR+ patients developed recurrent disease despite persistent ctDNA negativity. Additionally, five HR+ patients had one or two positive ctDNA samples with no diagnosis of recurrence despite prolonged follow-up, as shown using a similar personalized ctDNA technology in a smaller series for two HR+ patients.[10] Although these cases are technically considered false-positive results, one could postulate that these are situations in which indolent micrometastatic disease is present and transiently sheds

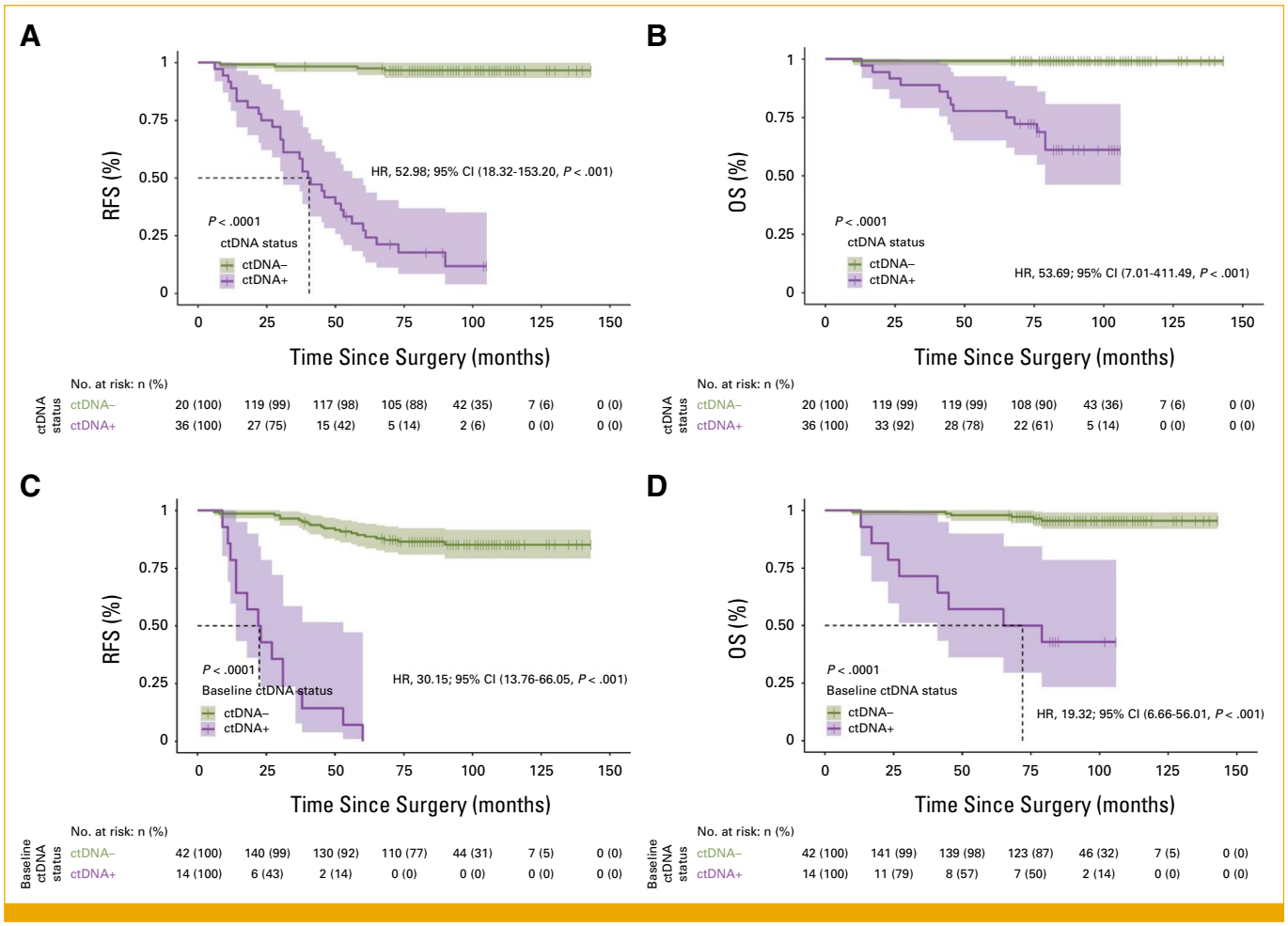

FIG 2. Personalized ctDNA detection in serial plasma samples predicts relapse-free survival and overall survival. (A) Relapse-free survival according to the detection of ctDNA in any follow-up plasma sample after surgery (HR, 52.98 [95% CI, 18.32 to 153.20]; $P < .0001$). (B) Overall survival according to the detection of ctDNA in any follow-up plasma sample after surgery (HR, 53.69 [95% CI, 7.01 to 411.49]; $P < .0001$). (C) Relapse-free survival according to the detection of ctDNA in the first postsurgical plasma sample (HR, 30.15 [95% CI, 13.76 to 66.05]; $P < .0001$). (D) Overall survival according to the detection of ctDNA in the first postsurgical plasma sample (HR, 19.32 [95% CI, 6.66 to 56.01]; $P < .0001$). n = 156 patients. ctDNA, circulating tumor DNA; HR, hazard ratio; OS, overall survival; RFS, relapse-free survival.

ctDNA as a result of biologic changes (eg, holding endocrine therapy). The presence or absence of ctDNA detection should be interpreted differently in the context of HR+/HER2– disease and might require serial monitoring; ctDNA positivity can serve as a measure of tumor activity, which among other things can be affected by ongoing treatment (eg, endocrine therapy). With milder phenotype, prolonged treatment duration, underestimated adherence to therapy, relapse destinations such as bone brain, or local progression (associated with lower ctDNA availability rate), HR+/HER2– disease is more challenging than other breast cancer subtypes for ctDNA detection. Although more evidence is needed to better understand the significance of occasional ctDNA positivity followed by serially negative results during the course of treatment, it is possible that more information on therapy adherence could help resolve this question. The results presented here support the use of ctDNA in clinical trials to determine if this technology can improve outcomes. Similar to colorectal cancer, the velocity of ctDNA concentration increase in subsequent tests,[14] known to be associated with time to clinical progression, might provide useful insights into the biology of HR+ breast cancer subtype.

Several studies have been published that studied ctDNA in the setting of breast cancer surveillance monitoring,[15–17] but these focus on patients with TNBC, which is known to be associated with higher levels of ctDNA.[9] These and other studies have evaluated smaller numbers of patients, and/or used other assays, so, it is difficult to compare our results with other groups. In general, however, the findings reported herein support the use of ctDNA defined by clonal somatic SNVs (Signatera), hotspot mutations,[9,10] breakpoint junctions,[10] or amplifications[18] to detect MRD and predict relapse. Importantly, serial longitudinal assessments are helpful to confirm the trajectory of ctDNA changes—particularly in patients with indolent HR+ breast cancer. Persistently negative ctDNA results strongly correlated with

the lack of disease recurrence and may therefore provide reassurance to patients.

In conclusion, the EBLIS study demonstrates that serial postoperative ctDNA analysis has strong prognostic value and allows for earlier detection of recurrence than by scans in many patients, while repeated negative tests can provide reassurance to patients. This provides a potential window that could enable the design of trials to assess the impact of earlier therapeutic interventions, which may lead to improved clinical outcomes, particularly in the setting of more aggressive subtypes (ie, TNBC). For patients with HR+ breast cancer, who remain at risk of relapse for many years, a negative test does not rule out the possibility of relapse, and for those where ctDNA is detected, a repeated Signatera test may be needed to confirm a positive test. In particular, confirming ctDNA concentration increase in subsequent tests might be more informative. Earlier intervention opportunities may allow better and more timely treatment with switch of endocrine therapy, but properly controlled randomized studies will be needed to determine if this is the case. Our study has some limitations: all blood tests were assayed retrospectively. Thus, it is not possible to state conclusively that patients did not have evidence of metastatic disease on conventional scanning as was shown in a recent study.[16]

All told, however, our results suggest that ctDNA testing may add to existing recommendations for symptom assessments, physical examination, and routine breast imaging as a means of monitoring patients with breast cancer after completion of definitive local therapy with or without adjuvant chemotherapy.

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DISCLAIMER

E.K., H.S., D.R., and B.Z. are employees of Natera, Inc, and own stock, or options to stock, in the company. E.C.d.B., R.M., and D.S. are employees of AstraZeneca and hold AstraZeneca shares. B.A. is now an employee of Inivata Ltd. R.H. is now an employee of Nonacus Ltd. D.F.-G. is now an employee of GEICAM.

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