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Current utilization of circulating tumor DNA (ctDNA) monitoring in patients with stage I-III breast cancer

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Research Article

Keywords: breast cancer, circulating tumor DNA, ctDNA, molecular residual disease, MRD, surveillance, real world evidence

Posted Date: November 1st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11529663/v1

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Additional Declarations: The authors declare potential competing interests as follows: Marla Lipsyc-Sharf (MLS): reports receiving non-monetary publication support from Natera and research funding via an American Society of Clinical Oncology Career Development Award (paid to institution); honoraria for lectures or educational events from Eli Lilly, Natera, and Stemline Therapeutics; participation on advisory boards for AstraZeneca, Eli Lilly, Exact Sciences, Guardant Health, Stemline Therapeutics, and TerSera Therapeutics. Bhakti Dwivedi (BD): is an employee of Natera, Inc., receives a salary and may own stock and/or stock options. Samuel Rivero-Hinojosa (SRH): is an employee of Natera, Inc., receives a salary and may own stock and/or stock options. Meenakshi Malhotra (MM): is an employee of Natera, Inc., receives a salary and may own stock and/or stock options. Philip Miller (PM): is an employee of Natera, Inc., receives a salary and may own stock and/or stock options. Ekaterina Kalashnikova (EK): is an employee of Natera, Inc., receives a salary and may own stock and/or stock options. Rena D. Callahan

(RDC): reports research grants from Roche, Celcuity, and Lilly; consulting fees from Gilead, Stemline, AstraZeneca, Daiichi Sankyo (DSI), Novartis, Pfizer, Arvinas, Roche, Natera, SAGA, Hologic, and Tempus; honoraria for lectures or educational events from Gilead, Stemline, AstraZeneca, DSI, Novartis, Lilly, Pfizer, Natera, Hologic, and Tempus. Nimmi S. Kapoor (NSK): reports research grants from the Jonsson Comprehensive Cancer Center, Association of Women Surgeons Foundation, and Hologic Inc.; consulting fees from IceCure Medical Inc., Aura Health Technologies, Inc., and Elucent Medical, Inc.; support for travel from Viewpoint Medical, Inc.; receipt of equipment, materials, drugs, medical writing, gifts, or other services from Hologic, Inc. Aashini Master (AM): reports consulting fees from Gilead Sciences and a leadership or fiduciary role with the Keep A Breast Foundation. Nicholas P. McAndrew (NPM): reports receiving non-monetary publication support from Novartis; research grants (paid to institution) from Novartis, Pfizer, Daiichi Sankyo, Arvinas, Loxo, SeaGen, Mersana, Eisai, and Merck; consulting fees from AstraZeneca; honoraria for lectures or educational events from Gilead; support for travel to scientific meetings from Novartis; service on guideline panels for NCCN and ASCO. Kelly E. McCann (KEM): reports research grants (paid to institution) from H3 Biomedicine, Immunomedics, Gilead, Pfizer, Roche/Genentech, Merck, Eli Lilly, Olema Pharmaceuticals, AstraZeneca, Macrogenics, G1 Therapeutics, Ambrx, Sanofi, Zymeworks, Duality Biologics, Novartis, Puma Biotech, BriaCell, and Marengo Therapeutics; consulting fees from Daiichi Sankyo, Eli Lilly, AstraZeneca, Novartis, Pfizer, TerSera Therapeutics, Puma Biotech, Halda Therapeutic OpCo, Inc., Gilead, and BioNTech; honoraria for lectures or educational events from Eli Lilly; participation on advisory boards for Daiichi Sankyo, Eli Lilly, Biotheranostics, Astr

Abstract

While testing for circulating tumor DNA (ctDNA) in the surveillance setting of early-stage breast cancer is available and is currently being used nationwide, it is unknown how breast oncologists are utilizing ctDNA testing and whether testing patterns and/or results are currently impacting clinical care. To further understand this utilization, we identified 7,044 patients with stage I-III breast cancer who underwent personalized, tumor-informed ctDNA testing for molecular residual disease (MRD) in the postsurgical setting between 2019–2025. ctDNA results and clinicopathologic data were abstracted from available clinical records and insurance claims. For 5692/7044 patients with ≥ 2 ctDNA tests after definitive surgery, the median (range) time of initial testing was 7.87 (0.0-47.3) months after surgery; median interval between ctDNA tests was 3.4 (0.2–31.4) months. The negative predictive value (NPV) of a single negative ctDNA test was high for all tumor subtypes, with the highest NPVs (> 97%) observed within 12 months of the test. MRD detection within 3 months post-surgery was strongly associated with worse distant recurrence-free survival (DRFS; HR: 6.5, 95%CI: 4.1–10.3, p = 1.99e-13). ctDNA levels increased closer to the time of clinical recurrence and varied by tumor subtype; for patients with a positive ctDNA test within 3 months before clinical recurrence, the median (range) ctDNA concentration was 19.0 (0.03-14,248) and 4.41 (0.01-2,493) MTM/mL for triple-negative and estrogen receptor-positive HER2-negative breast cancers, respectively. In a subset analysis, positive ctDNA testing impacted clinical care for 36/44 (81.8%) patients with detectable ctDNA, leading to treatment changes in 7 patients, 4 of whom achieved ctDNA clearance following treatment change. These findings offer key insights into how personalized, tumor-informed ctDNA testing is being used in clinical practice as part of the management of care for patients with early breast cancer.

Introduction

Despite improvements in screening, breast surgery, radiation, and systemic therapies, breast cancer remains the most frequently diagnosed cancer and the second-leading cause of cancer-related death among women in the United States[1]. Of over 40,000 patients that die from metastatic breast cancer each year, ~ 75% had early-stage breast cancer that ultimately relapsed[2]. Current local therapy for early-stage breast cancer includes surgery and, when indicated, radiation. Adjuvant systemic therapies, like chemotherapy and targeted therapies, are recommended to reduce the risk of recurrence[3].

Currently, breast cancer recurrence is detected by evaluating symptoms, history, and physical examinations. Prior meta-analyses, incorporating studies from over twenty years ago, suggest that surveillance using traditional laboratory testing (e.g., alkaline phosphatase) or imaging (e.g., chest radiographs, abdominal ultrasounds, and/or bone scans) does not substantially improve long-term survival[4–7]. However, novel technologies can identify evidence of micrometastatic recurrence, often far in advance of imaging and testing that was available at that time. Specifically, plasma testing for circulating tumor DNA (ctDNA) can potentially detect molecular residual disease (MRD) in breast cancer and other malignancies prior to detection of symptomatic and/or radiographically-evident metastatic

disease[8–16]. Detection of MRD via tumor-informed, personalized ctDNA testing specifically is strongly prognostic for disease recurrence in patients with early-stage breast cancer[12]. Indeed, post-surgery ctDNA monitoring with Signatera™ (Natera, Inc) is approved for insurance coverage reimbursement by the Centers for Medicare & Medicaid Services for patients with early breast cancer, and private insurers are increasingly covering ctDNA testing for all breast cancer stages[17]. While testing is available and being used throughout the United States, it is unknown how providers are utilizing ctDNA testing and whether testing patterns and/or results are currently impacting clinical care[18].

To address this clinical question, this study evaluated the clinicopathologic characteristics, ctDNA testing cadence, ctDNA dynamics, ctDNA concentration, and patient outcomes in a large dataset of patients with stage I-III breast cancer (N = 7044) to understand how ctDNA testing is being used in current practice as part of care for patients with early breast cancer in the adjuvant setting.

Methods

ctDNA utilization analysis in a real-world cohort

Using Natera's proprietary real-world database, which is linked to the Forian claims database, we identified patients with early-stage breast cancer (stage I-III) who underwent commercial tumor-informed ctDNA testing in the United States from 2019 to 2025. All tumor subtypes were included: estrogen receptor-positive, HER2-negative (ER+/HER2-); HER2-positive (HER2+); and triple negative breast cancer (TNBC). To the best of our knowledge, this cohort did not include any patients on clinical trial lines and no participants of clinical trials were included. A total of 7,044 patients with early-stage breast cancer who had postsurgical tumor-informed plasma ctDNA testing performed in the adjuvant treatment and surveillance periods and had available recurrence-free survival data were included in this retrospective analysis (Fig. 1A). Treatment and follow-up, including the cadence and application of ctDNA testing, were at the discretion of the treating physician. Clinicopathologic data were de-identified and abstracted from available clinical documentation, pathology reports, and insurance claims data (Table 1). A waiver of the consent process and of the requirement for documentation of informed consent was granted according to 45 CFR 46.116(d) and 45 CFR 46.117(c)(2), respectively, under Natera's IRB-approved protocol 21–058, and was conducted in accordance with the Declaration of Helsinki.

Table 1
Early breast cancer ctDNA real-world evidence cohort

Characteristic	Overall	TNBC	HER2+	ER+/HER2-
Total number of patients (N)	7044	2068	1071	3905
Follow-up (years), median [IQR]	2.46 (1.43–4.11)	2.15 (1.22–3.46)	2.33 (1.44–4.01)	2.71 (1.54–4.49)
Cancer Stage				
I	2806 (39.8)	604 (29.2)	432 (40.3)	1770 (45.3)
II	2551 (36.2)	863 (41.7)	395 (36.9)	1293 (33.1)
III	1687 (23.9)	601 (29.1)	244 (22.8)	842 (21.6)
Histology				
Ductal	4930 (70.0)	1546 (74.8)	841 (78.5)	2543 (65.1)
Lobular	772 (11.0)	116 (5.6)	46 (4.3)	610 (15.6)
Mixed	181 (2.6)	25 (1.2)	15 (1.4)	141 (3.6)
Missing	1161 (16.5)	381 (18.4)	169 (15.8)	611 (15.6)
Lymph nodes				
Positive	2952 (41.9)	860 (41.6)	455 (42.5)	1637 (41.9)
Recurrence				
Distant Relapse	434 (6.2)	157 (7.6)	61 (5.7)	216 (5.5)
Metastatic Sites				
Brain only	24 (0.3)	10 (0.5)	8 (0.7)	6 (0.2)
Bone only	92 (1.3)	19 (0.9)	13 (1.2)	60 (1.5)
Lung only	47 (0.7)	26 (1.3)	9 (0.8)	12 (0.3)
Other/multiple sites	271 (3.8)	102 (4.9)	31 (2.9)	138 (3.5)
No relapse	6610 (93.8)	1911 (92.4)	1010 (94.3)	3689 (94.5)
Neoadjuvant Therapy				
Yes	2671 (37.9)	1107 (53.5)	602 (56.2)	962 (24.6)
No	4373 (62.1)	961 (46.5)	469 (43.8)	2943 (75.4)
Neoadjuvant Chemotherapy				
Yes	2102 (29.84)	1092 (52.8)	563 (52.57)	447 (11.45)

Characteristic	Overall	TNBC	HER2+	ER+/HER2-
No	569 (8.08)	15 (0.73)	39 (3.64)	515 (13.19)
NA	4373 (62.08)	961 (46.47)	469 (43.79)	2943 (75.36)
Adjuvant Therapy				
Chemotherapy	2168 (30.8)	1078 (52.1)	394 (36.8)	696 (17.8)
Hormone therapy	3781 (53.7)	0 (0.0)	370 (34.5)	3411 (87.3)
Immunotherapy	211 (3.0)	188 (9.1)	6 (0.6)	17 (0.4)
CDK4/6 inhibitor	502 (7.1)	0 (0.0)	6 (0.6)	496 (12.7)
HER2-targeted therapy	824 (11.7)	8 (0.4)	815 (76.1)	1 (0.0)
Number of patients with single or multiple ctDNA tests in adjuvant setting				
Single	1352 (19.2)	387 (18.7)	199 (18.6)	766 (19.6)
≥ 2	5692 (80.8)	1681 (81.3)	872 (81.4)	3139 (80.4)
ctDNA status at first post-surgery adjuvant testing time point				
Negative (-)	6494 (92.2)	1882 (91.0)	996 (93.0)	3616 (92.6)
Positive (+)	550 (7.8)	186 (9.0)	75 (7.0)	289 (7.4)
ctDNA dynamics anytime post-surgery prior to date of relapse or end of follow-up				
All (-)	6183 (87.8)	1771 (85.6)	951 (88.8)	3461 (88.6)
All (+)	352 (5.0)	123 (5.9)	46 (4.3)	183 (4.7)
(-) to (+)	179 (2.5)	70 (3.4)	16 (1.5)	93 (2.4)
(+) to (-)	146 (2.1)	43 (2.1)	26 (2.4)	77 (2.0)
Mixed (Fluctuating)	184 (2.6)	61 (2.9)	32 (3.0)	91 (2.3)
ctDNA dynamics within 0–6 mos post-surgery (independent of recurrence or follow-up)				
All (-)	895 (12.7)	330 (16.0)	135 (12.6)	430 (11.0)
Single (-)	1024 (14.5)	309 (14.9)	131 (12.2)	584 (15.0)
All (+)	81 (1.1)	42 (2.0)	8 (0.7)	31 (0.8)
Single (+)	48 (0.7)	22 (1.1)	5 (0.5)	21 (0.5)
(-) to (+)	21 (0.3)	9 (0.4)	3 (0.3)	9 (0.2)
(+) to (-)	67 (1.0)	21 (1.0)	9 (0.8)	37 (0.9)
Mixed (Fluctuating)	12 (0.2)	5 (0.2)	3 (0.3)	4 (0.1)

Characteristic	Overall	TNBC	HER2+	ER+/HER2-
No Timepoint	4896 (69.5)	1330 (64.3)	777 (72.5)	2789 (71.4)

ctDNA utilization analysis in a single institution cohort

ctDNA utilization analysis in a single institution cohort

To better understand ctDNA utilization patterns for breast cancer surveillance, a subset analysis was performed in a cohort of patients from a single institution (University of California Los Angeles, UCLA) between 2019–2024. Among 383 patients identified with a history of early-stage breast cancer and available ctDNA testing after definitive surgery, a comprehensive retrospective chart review was performed with a data cutoff of 12/31/2024. Clinicopathologic data abstracted for this sub-cohort are detailed in Table 2. Oncology office visit documentation was used by institutional clinical investigators to best approximate the reason for ctDNA testing (provider-initiated, patient-initiated, or unknown), management change due to positive ctDNA results (change in imaging frequency, ctDNA cadence, or medication), dates of distant recurrences (date of biopsy if available; if unavailable, date of imaging was used), and dates of additional clinical events (including date of breast surgery). “Restaging scans” were defined as cross-sectional imaging (either CT chest/abdomen/pelvis with bone scan or PET/CT imaging) performed in the absence of documented clinical symptoms or signs of recurrence, as abstracted from oncology notes. “Change in ctDNA cadence” was defined based on documentation in oncology notes indicating a plan to increase the frequency of ctDNA testing in response to a positive ctDNA result, relative to prior testing intervals. This research was conducted with appropriate institutional review board approvals (University of California Los Angeles IRB #24–000177 and IRB #24-5588) and performed in accordance with the Declaration of Helsinki.

Table 2
Single institution ctDNA actionability cohort

Characteristic	Number of patients, n (%)
Total number of patients (n)	383
Age at surgery, years, median (range)	53.9 (32–93)
Tumor subtype at diagnosis	
ER+/HER2-	242 (63)
HER2+	66 (17.2)
TNBC	73 (19)
Stage	
I	90 (23.5)
II	173 (45.2)
III	112 (29.2)
Unavailable	8 (2.1)
ctDNA status*	
Positive	44 (11.5)
Negative	339 (88.5)
Recurrence status	
Event Free	360 (94)
Recurrence	23 (6)
Recurrence by ctDNA status	
ctDNA-	7 (2.1)
ctDNA+	16 (36.4)
*ctDNA status at any time post-surgery	
Supplemental Tables	

Personalized mPCR-NGS assay and data analysis

A personalized, tumor-informed, multiplex PCR assay (Signatera™, Natera, Inc.) was used to detect and quantify ctDNA (as previously[19]). Briefly, whole exome sequencing (WES) was performed on formalin-fixed paraffin-embedded (FFPE) tumor blocks and matched-normal DNA blood samples. Based on the

WES results, multiplex PCR primers were designed for 16 tumor-specific, somatic single-nucleotide variants for each patient. Plasma samples with ≥ 2 variants detected were defined as ctDNA positive. ctDNA concentration was reported as mean tumor molecules/mL (MTM/mL) of plasma.

Statistical Methods and Analysis

Missing data, inherent to real-world datasets derived from clinical documentation and claims, were handled using available-case analyses without imputation, with incomplete variables excluded from multivariable models. Cadence for ctDNA testing was determined as a time interval between subsequent ctDNA tests for patients who had at least two ctDNA tests performed. For survival analysis, the post-operative window was defined as a post-surgical time point if collected from 0 to 3 months post-surgery and prior to adjuvant therapy as per clinical documentation and claims data. The analysis was landmarked to encompass a reasonably broad post-operative window that would include a sufficient number of patients with post-surgery and pre-adjuvant therapy ctDNA testing timepoints. Adjuvant/Surveillance window was defined as the period after the post-operative window, during which a patient received adjuvant therapy and/or surveillance ctDNA testing up to clinical confirmation of disease relapse or end of follow-up. Association of ctDNA with survival outcomes was determined by evaluating distant recurrence-free survival (DRFS) —determined by claims codes for secondary neoplasms in the overall cohort and by standard clinical metrics (radiological findings and/or tissue confirmation) in the single-institution subcohort—and was measured from the date of surgery to the first documented indication of distant recurrence, and was censored at the last follow-up (last clinical contact or last blood draw/collection date) or death.

Trends in ctDNA testing, including time to first postsurgical test and ctDNA testing cadence, were evaluated for all patients undergoing testing after 2021, following the increased utilization of tumor-informed ctDNA testing outside of the research setting. The cumulative incidence of clinical recurrence over time following an initial post-surgical negative ctDNA test was used to calculate the negative predictive value (NPV) for recurrence. Molecular lead time was defined as the time between a positive ctDNA test and clinical disease recurrence. Retrospective lead time assessment in this study remained exploratory, given that testing and scans were not performed at regular intervals. Qualitatively, ctDNA patterns or dynamics indicating persistently negative ctDNA results or initial positive ctDNA result(s) followed by one or a series of negative ctDNA results were considered “longitudinally MRD-negative/cleared”, and patterns indicating persistently positive ctDNA results, initially negative ctDNA result(s) followed by one or a series of positive ctDNA result(s), or fluctuating dynamics (such as positive ctDNA results testing followed by a negative ctDNA test and subsequent positive ctDNA test(s) or negative ctDNA results testing followed by a positive ctDNA test and subsequent negative ctDNA test) were considered "longitudinally MRD-positive”. To determine the prognostic impact of early longitudinally MRD-positive ctDNA dynamics, a 6-month landmark timepoint from surgery was set; ctDNA dynamics were determined for patients with ≥ 2 ctDNA tests between 0 and 6 months from surgery, and DRFS was compared at fixed time points from the 6-month landmark.

Categorical variables were compared using the chi-squared and Fisher's exact test. The medians of numerical factors were compared between categorical variable groups using the pairwise Wilcoxon test. Survival analyses were performed using R software version 4.5.0 using the survival (version 3.7.0), survminer (version 0.4.9), and coxphf (version 1.13.4) packages. Exact P values from Cox regression hazard models were calculated using the Rmpfr (version 0.9.5) package[20]. Survival distributions were estimated using the Kaplan–Meier method, and differences in survival were evaluated using the log-rank test. Cox proportional hazard models were used to assess prognostic factors associated with DRFS.

Results

Patient cohort and cadence of ctDNA testing

Among 7,044 patients with early-stage breast cancer (ER+/HER2-: n = 3905, HER2+: n = 1071, TNBC: n = 2068, Table 1), a total of 28,328 plasma samples ordered by 1554 providers were obtained in the post-surgery and surveillance settings, with a median of 3 ctDNA tests per patient, and the median time for first ctDNA testing post-surgery was 7.87 months after surgery (range: 0.0–47.3). For patients with ER+/HER2- tumors, median time to first ctDNA testing was 8.02 months (range: 0.06–47.3) vs. 9.15 months (range: 0.16–43.8) in HER2 + and 7.3 months (range: 0.0-46.8) in TNBC **(**Fig. 1B**)**. Among all patients with ≥ 2 post-surgical ctDNA tests, the median interval between tests was 3.2 months (range: 0.2–27.0). The median (range) ctDNA testing intervals among patients according to breast cancer receptor subtype were: ER+/HER2-: 3.3 (0.2–23.8) months; HER2 + breast cancer: 3.0 (0.4–19.5) months; and TNBC: 3.1 (0.3–27) months (Fig. 1C). Median (interquartile range; IQR) follow-up for all patients was 2.46 years (1.43–4.11). The results of a ctDNA test impacts subsequent testing cadence. Among patients with ≥ 2 postoperative ctDNA tests, time-to-next test (median [range]) tended to be shorter following a positive ctDNA test result (1.84 [0.1–16.3] months) than after negative ctDNA test results (3.21 [0.13–37.25] months) (**Supplemental Table 1**). The testing cadence and relapse rate among patients grouped by sequential ctDNA testing outcomes are detailed in **Supplemental Table 2**.

Association of post-operative ctDNA detection with patient outcomes

Among patients with eligible ctDNA time points within the post-operative window (n = 1135), 11% (overall: 125/1135; by subtype: TNBC: 12.6%, 54/430; HER2+: 9.28%, 13/140; ER+/HER2-: 10.2%, 58/565) had at least one ctDNA-positive test (MRD-positive), and 89.0% (1010/1135) had only ctDNA-negative tests (MRD-negative). Of the 125 patients who tested MRD-positive in the post-operative window 70% (88/125) underwent adjuvant systemic therapy and the event rate observed is reflective of the length of follow-up. Of MRD-positive patients, 28% (35/125) experienced clinical relapse with a median follow-up of 12.7 mos (range: 5.51–35.1 mos), compared to 4.65% (47/1010) of MRD-negative patients with median follow-up of 22.5 mos (range: 4.75–54.9 mos). ctDNA levels were significantly higher in MRD-positive patients who relapsed (n = 35) compared to patients who did not relapse during the follow-up

period (n = 90) (median 0.680 vs. 0.418 MTM/mL, respectively, p = 0.047) (**Supplemental Fig. 1**). MRD-positivity within the 3-month post-operative window was significantly associated with shorter DRFS (log-rank p < 0.0001, Fig. 2A; for subtype-specific DRFS see **Supplemental Fig. 2**) with a 2-yr marginal DRFS rate of 58% (95% CI: 46–72) vs. 92% (95% CI: 89–95) in MRD-negative patients. Multivariate Cox proportional hazard analysis indicated MRD positivity in postoperative window to be the most significant prognostic factor for DRFS when compared with other clinicopathological factors (HR: 6.5, 95% CI: 4.1-10.32, p = 1.99e-13, Fig. 2B**)**, including breast cancer subtype. MRD positivity anytime postoperative was associated with significantly inferior DRFS (Time-dependent CoxPH HR: 31.01, 95% CI: 25.18–38.19), p < 0.0001, Fig. 2C).

Predictive value of negative ctDNA testing and early ctDNA dynamics

To estimate the potential implications of a negative initial postsurgical ctDNA result (n = 3,942), we assessed the cumulative negative predictive value (NPV) of ctDNA testing for recurrence and analyzed its temporal variation. Among all cases, the NPV (95% CI) remained high throughout long-term follow-up, starting at 99.16% NPV at 0–3 months and 98.93% (95% CI: 98.56–99.23) at 0–6 months, 97.94% (97.45–98.36) at 12 months, 96.82% (96.23–97.35) at 24 months, and maintaining high NPV of 96.32% (95.68–96.88) at 36 months **(**Fig. 3A). The high NPV of a negative initial ctDNA test was seen across all subtypes; starting at 99.13% (98.67–99.47) for ER+/HER2- tumors, 99.02% (98.21–99.53) for TNBCs, and 99.49% (98.52–99.89) for HER2 + cancers at 3 months. NPV for all subtypes remained high 48-months post testing: 96.64% (95.82–97.33) for ER+/HER2- tumors, 95.02% (93.50-96.27) for TNBCs, and 97.13% (95.44–98.32) for HER2 + cancers. Additionally, among those patients with multiple ctDNA testing timepoints between 0–6 months after surgery, those with longitudinally negative ctDNA dynamics had substantially better prognosis vs. those with longitudinally positive ctDNA dynamics, up to at least two years later (Fig. 3B).

Evaluating patterns of ctDNA concentration and molecular recurrence

We explored molecular patterns of recurrence in patients who had confirmed clinical recurrence by assessing longitudinal changes in ctDNA levels for patients who had a positive ctDNA test post-surgery and prior to recurrence (n = 305, Fig. 3C). In general, we observed longer molecular lead times for ER+/HER2- breast cancers (median 3.93 months, range 0-39.2 months) and shorter for TNBC (median 3.74 months, range 0-28.9 months). For patients with clinical recurrence ≤ 3 months after ctDNA detection, ctDNA levels also varied by subtype, with median (range) ctDNA concentrations of 19.0 (0.03-14,248), 4.41 (0.01-2,493), and 4.12 (0.03-2,879) MTM/mL in TNBC, ER+/HER2-, and HER2 + subtypes, respectively (Fig. 3C). In contrast, patients who had > 3 month molecular lead time between a positive ctDNA test and confirmed disease recurrence tended to have lower initial levels of ctDNA (TNBC: 0.97 (0.03–716); ER+/HER2-: 1.67 (0.05-7,665); HER2+: 1.37 (0.04-4,136) MTM/mL (Fig. 3C, bottom panel). In

many cases, a positive ctDNA test was found prior to confirmation of metastatic disease in the lung, brain, bone, and other/multiple sites (Fig. 3C).

Practice patterns of postsurgical ctDNA detection in early breast cancer

To evaluate real-world practice patterns of ctDNA testing in clinical decision-making in the adjuvant/surveillance setting, a subset analysis in 383 patients with early-stage breast cancer (ER+/HER2-: 63.2% (242/383); HER2+: 17.2% (66/383), and TNBC: 19.1% (73/383) was performed (Table 2). Among these patients, 11.5% (44/383) were MRD-positive at any post-surgery time point, and 88.5% (339/383) were MRD-negative. Among patients with MRD-positivity, 36.4% (16/44) experienced distant recurrence up until the date of data cut-off, compared to only 2.1% (7/339) of MRD-negative patients. Tumor stage distribution differed significantly among the MRD-positive and MRD-negative groups. Of patients who had a positive MRD test after surgery, 5 (11%), 16 (36%), and 23 (52%) had stage I, II, and III disease, respectively, vs. 86 (25.4%), 155 (45.7%), and 90 (26.5%), respectively, with 8 stage unspecified, among patients who remained MRD negative (Chi-sq. p = 0.0029, **Supplemental Fig. 3**).

Of the 44 patients with ctDNA-positive results at any time post-surgery, a total of 81.8% (36/44) experienced change(s) in clinical management, including altered imaging frequency, increased ctDNA monitoring cadence, or cancer medication adjustments (Fig. 4A). Specifically, 72.7% (32/44) of patients underwent restaging scans, and of these, 11/32 (34.4%) of patients had radiographic evidence of distant metastases, with seven identified without symptoms (Fig. 4A). While 65.6% (21/32) initially showed no distant recurrence on imaging, 4 later developed distant metastasis, and 6 later developed local recurrence (local to the breast, lymph node, or chest wall) (Fig. 4A). Additionally, 15.9% (7/44) had adjustments made to their cancer medication; four of these seven patients achieved clearance of ctDNA (Fig. 4B). The remaining 3 had rising ctDNA levels (**Supplemental Fig. 4**); none of the 7 patients who had their treatment altered following a ctDNA-positive result experienced distant recurrence up until the date of censorship, with a median follow-up interval after the ctDNA-positive result of 7.93 (range 4.16–24.72) months.

Discussion

To our knowledge, this is the largest analysis of postsurgical ctDNA testing in the adjuvant/surveillance setting of early breast cancer. We found that most patients in this cohort underwent initial ctDNA testing within the first year after surgery and that, on average, testing was performed every 3 months irrespective of tumor subtype. ctDNA dynamics within 6 months of surgery was highly prognostic of DRFS. Additionally, we investigated the NPV of a single negative ctDNA test and observed excellent NPVs within 12 months of a negative test, with an NPV of of 98.93% (95% CI: 98.56–99.23) at 6 months post-ctDNA testing. In general, ctDNA levels were highest in patients with TNBC with overall shorter retrospective lead times; in patients with ER+/HER2- BC ctDNA levels were generally lower with overall longer retrospective lead times. Furthermore, in a subset of patients, we identified how ctDNA results

influenced clinical care, including changes in ctDNA testing, imaging, and/or treatment, consequently influencing the lead time in this real-world setting.

Our study, which includes over 7,000 patients tested with the same personalized, tumor-informed ctDNA assay, contributes to the growing body of real-world evidence on ctDNA testing in the post-surgical and adjuvant surveillance settings for EBC. A recent study reported that post-surgical ctDNA detection was significantly associated with a shorter recurrence-free interval in early-stage breast cancer (n = 114 patients)[21]. Another real-world study reported that ctDNA detection in TNBC during long-term follow-up preceded clinical diagnosis of recurrence by 8 (SD 2.98) months (n = 42 patients)[22]. Large, real-world quantitative data in the adjuvant setting of multiple breast cancer subtypes and stages have been sparse; however, this data may offer helpful insight into contemporary testing patterns and outcomes.

In breast cancer, ongoing clinical trials and studies have reported a strong association of ctDNA positivity with poor prognosis, though interventional trials have not yet shown whether ctDNA surveillance improves survival or quality-of-life for patients. The c-TRAK DNA (NCT03145961) study assessed ctDNA via digital PCR in patients with high- and moderate-risk early-stage TNBC [23] and found that nearly three-quarters of patients already had radiographically-detectable metastatic disease at the start of ctDNA testing. It is possible that this was due to the enrollment of high-risk patients with TNBC in the study. In contrast, a recent report from the LEADER trial indicated that among patients with ER+/HER2– disease who tested ctDNA + in the post-operative setting, only 27% (4/15) had radiographic recurrence detected at the time of ctDNA detection[24]. Additionally, the DARE trial reported that 73% of patients with ER+/HER2– disease who tested ctDNA+ post-operatively were imaging-negative for metastatic disease at the time of positive ctDNA test[25]. Whole exome or genome sequencing for tumor-informed testing might be necessary for more sensitive MRD detection before clinical recurrence, allowing a longer interval from ctDNA detection to clinical recurrence. In the subset analysis of this real-world cohort, detection of ctDNA in post-operative surveillance was shown to trigger imaging 72.7% of the time, leading to change in clinical management. However, it is still unknown whether therapeutic intervention before radiographic recurrence improves overall outcomes. For radiographically evident metastatic disease, lower tumor burden at time of diagnosis has been associated with improved prognosis[26,27], though ongoing investigational trials will test whether identification of metastasis at even lower tumor burden (MRD that is not radiographically detectable) will confer improved morbidity or survival[25,28–30].

Recent studies in early breast cancer have suggested that ctDNA dynamics during treatment may differ in treatment responders vs. non-responders. In the DARE study (NCT04567420), ctDNA surveillance with a personalized, tumor-informed assay was performed in 507 patients with stage II-III ER+/HER2- BC who were on adjuvant endocrine therapy[25]. In DARE, ctDNA clearance during treatment was associated with improved RFS, wherein patients with a persistently ctDNA-negative result showed excellent survival with RFS of 99.4% at 36 months[25]. Additionally, ctDNA testing has been performed retrospectively in pivotal early-stage breast cancer trials to better understand ctDNA dynamics in different clinical situations and

treatment paradigms. The monarchE study in patients with ER+/HER2-, node positive, high-risk early breast cancer (n = 910 with ctDNA testing) demonstrated that persistently negative ctDNA testing or ctDNA clearance while on adjuvant treatment was associated with improved invasive disease-free survival (IDFS) [31].

Limitations of this study include its retrospective nature, the heterogeneity in the patient population, and the interpretation of outcomes and clinical features inferred from real-world claims codes and medical records. Missing data, inherent to real-world datasets derived from clinical documentation and claims, were handled using available-case analyses without imputation, with incomplete variables excluded from multivariable models. ctDNA results were derived from samples analyzed by commercial ctDNA testing and were not collected according to a prescribed timing schedule; these variables limit the scope of our analyses. Additionally, there is a relatively short follow-up time in this study, particularly for ER+ breast cancer in which most recurrences occur more than five years after initial diagnosis. Our findings are largely hypothesis-generating and reflect real-world ctDNA testing practices being used in the care and management for patients with early breast cancer.

Our study generates several questions that remain unaddressed, warranting evaluation in future observational and interventional trials. Specifically, it's unclear whether negative ctDNA results influenced clinical care (e.g., imaging, treatments, or other blood tests) in the observed cohorts. Currently, there has been no change in clinical guidance to omit evidence-based treatment based on negative ctDNA testing, though clinical trials (including SIGNAL-ER 101, NCT07214532) are underway to assess appropriate treatment de-escalation opportunities. Another key question is whether ctDNA testing results, or surveillance blood tests, impact patient anxiety, fear of recurrence, or overall quality-of-life. While some studies on prognostic testing indicate that patients desire such testing, even without actionable results, our group and others are actively investigating this area.

We present here the largest to date real-world cohort characterizing practice patterns of ctDNA testing in the post-surgical setting across all breast cancer subtypes. Our findings demonstrate that early initiation of tumor-informed ctDNA testing (0–3 months post-surgery) for breast cancer can identify true MRD recurrence at time points prior to clinical manifestation or imaging positivity and may enable timely intervention. Evaluation of ctDNA practice patterns in a single-institution cohort found that > 80% of positive ctDNA cases led to changes in clinical management (re-staging scans, adjusted testing, adjustment of systemic therapy). These findings offer key insights into how personalized, tumor-informed ctDNA testing is being used in clinical practice as part of the management of care for patients with early breast cancer.

Abbreviations

BC
Breast cancer
ctDNA

circulating tumor DNA
DRFS
distant recurrence-free survival
EBC
early stage breast cancer
ER
estrogen receptor
HER2
human epidermal growth factor receptor 2
IDFS
invasive disease-free survival
MRD
molecular residual disease
MTM/mL
mean tumor molecules/mL
NAT
neoadjuvant therapy
NED
no evidence of disease
NGS
next generation sequencing
NPV
negative predictive value
OR
odds ratio
pCR
pathologic complete response
PCR
polymerase chain reaction
RFS
recurrence-free survival
TNBC
triple-negative breast cancer
WES
whole exome sequencing

Declarations

PRIOR PRESENTATION: The data reported here were presented in part in poster presentations at the 2024 ASCO annual meeting (Lipsyc-Sharf, J Clin Oncol 42, 2024 [suppl 16; abstr 549]) and at the 2025

ASCO annual meeting (Lipsyc-Sharf,10.1200/JCO.2025.43.16_suppl.560, abstr 560)

CLINICAL TRIAL NUMBER: Not applicable.

ACKNOWLEDGEMENTS: This research was supported by an ASCO Conquer Cancer Foundation: Career Development Award to PI Marla Lipsyc-Sharf (Grant ID: 2025CDA-7252610831)

AUTHOR CONTRIBUTIONS: A.B., M.D.L.-S., and A.A.R. contributed to conceptualization. C.C., B.D., E.K., M.D.L.-S., and S.R.-H. contributed to data curation. Formal analysis, methodology, and validation: B.D., E.K., and S.R.-H. M.D.L.-S. contributed to funding acquisition and writing – original draft. S.A., A.B., M.J.B., R.D.C., C.C., B.D.C., K.F., N.S.K., A.L., M.D.L.-S., A.K.M., N.P.M., K.E.M., J.M.P., and M.S.S. contributed to investigation and resources. M.D.L.-S., M.C.L., and A.A.R. contributed to project administration and supervision. B.D., E.K., M.D.L.-S., M.M., P.M., and S.R.-H. contributed to visualization. M.M. and P.M. contributed to writing – original draft. Software: No contributions claimed. All authors contributed to writing – review & editing.

DECLARATION OF COMPETING INTERESTS:

- Marla Lipsyc-Sharf (**MLS**): reports receiving non-monetary publication support from Natera and research funding via an American Society of Clinical Oncology Career Development Award (paid to institution); honoraria for lectures or educational events from Eli Lilly, Natera, and Stemline Therapeutics; participation on advisory boards for AstraZeneca, Eli Lilly, Exact Sciences, Guardant Health, Stemline Therapeutics, and TerSera Therapeutics.
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- Nimmi S. Kapoor (**NSK**): reports research grants from the Jonsson Comprehensive Cancer Center, Association of Women Surgeons Foundation, and Hologic Inc.; consulting fees from IceCure

Medical Inc., Aura Health Technologies, Inc., and Elucent Medical, Inc.; support for travel from Viewpoint Medical, Inc.; receipt of equipment, materials, drugs, medical writing, gifts, or other services from Hologic, Inc.

- Aashini Master (**AM**): reports consulting fees from Gilead Sciences and a leadership or fiduciary role with the Keep A Breast Foundation.
- Nicholas P. McAndrew (**NPM**): reports receiving non-monetary publication support from Novartis; research grants (paid to institution) from Novartis, Pfizer, Daiichi Sankyo, Arvinas, Loxo, SeaGen, Mersana, Eisai, and Merck; consulting fees from AstraZeneca; honoraria for lectures or educational events from Gilead; support for travel to scientific meetings from Novartis; service on guideline panels for NCCN and ASCO.
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- The other authors declare no competing financial or non-financial interests.

DATA AVAILABILITY: All data necessary to interpret, replicate, and build upon the findings of this study are presented in the article and its supplementary files. The de-identified clinical datasets generated and/or analyzed during the current study are not publicly available due to patient privacy and confidentiality protections, but are available from the corresponding author on reasonable request. Access can be obtained by emailing the corresponding author, Dr. Marla Lipsyc-Sharf

(MLipsycSharf@mednet.ucla.edu), with a brief research proposal, institutional affiliation, and IRB/ethics documentation. Requests will be acknowledged and reviewed within 2–3 weeks; upon approval, a data transfer/use agreement will be executed and de-identified data shared via secure transfer for scientific verification and replication purposes only, with no redistribution, secondary use, or commercial use, for the duration specified in the agreement.

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Figures

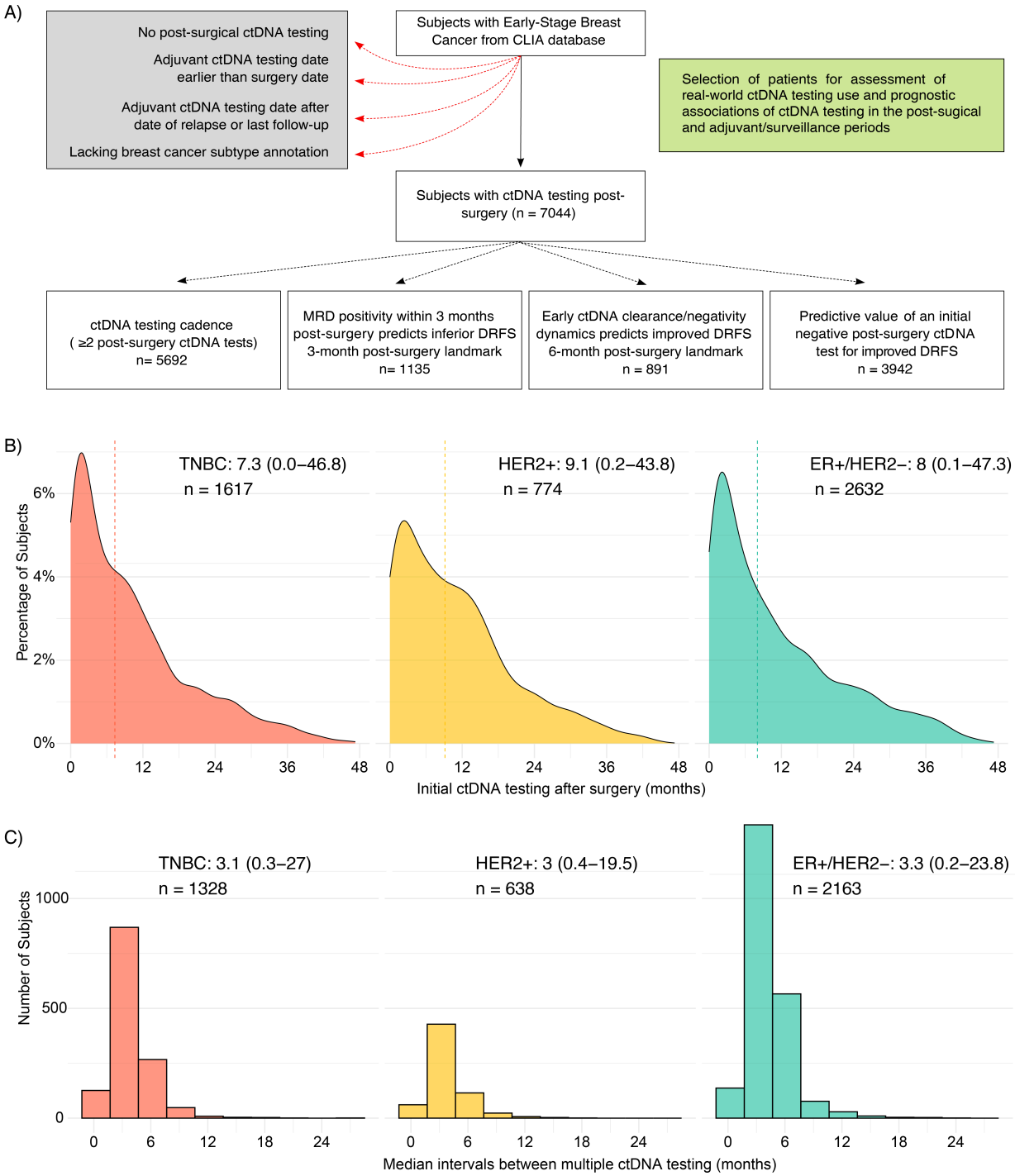
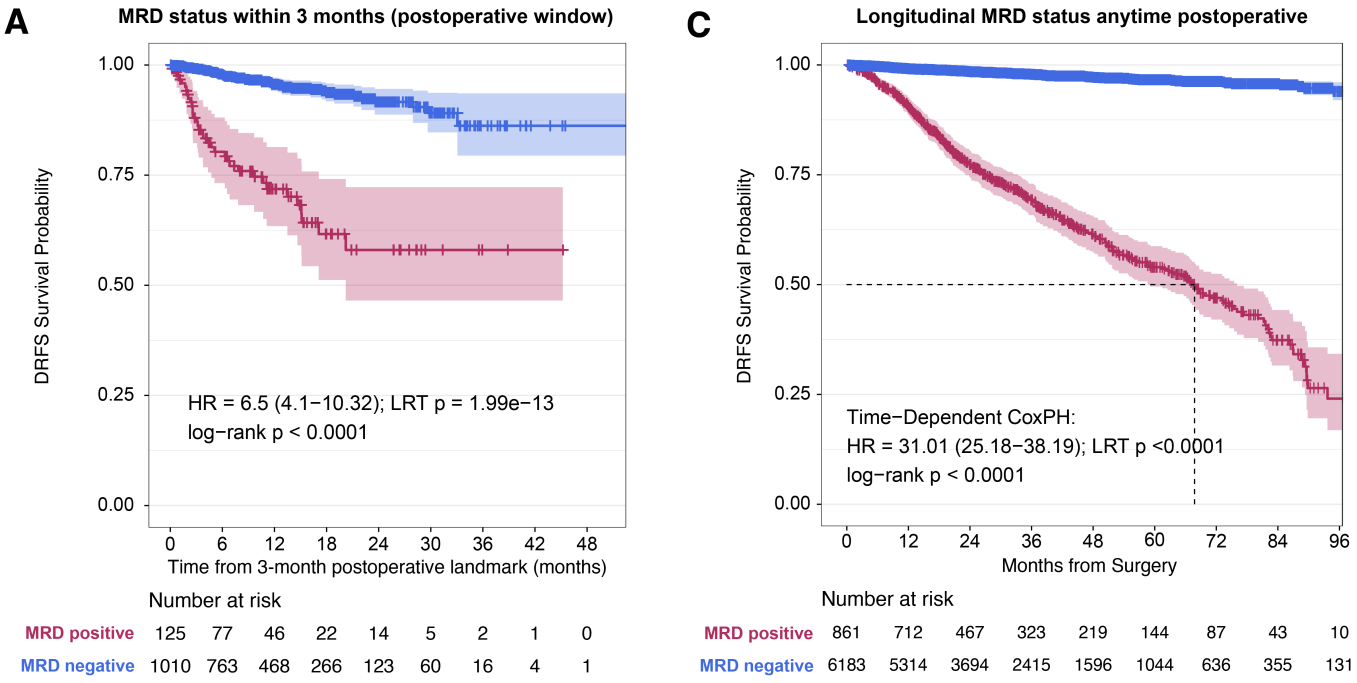


Figure 1

Consort diagram of a real-world cohort dataset, along with timing of first ctDNA test and cadence of subsequent testing. A. A total of 7,044 patients who underwent tumor-informed ctDNA testing in the post-surgical setting for early breast cancer were included. **B.** Area chart depicting time from surgery to the first ctDNA test performed in the adjuvant setting by receptor subtype; for all patients: median 7.87 (range 0.0–47.3) month (n=5,023), data from patients who had surgery from 2021 onward. Vertical

dashed lines represent the median. **C.**Bar plots depicting the median interval between successive ctDNA tests among patients with ≥2 tests, by receptor subtype; for all patients: median 3.2 months (range: 0.2-27.0) months (n=4,129).



B

Multivariate CoxPH Model – DRFS

Variable	HR (95% CI)	p–value	Hazard Ratio
MRD status ≤3 mo. postoperative : (+) vs. (–)	6.50 (4.10–10.32)	1.99e–13	
TNBC vs. HER2+	2.87 (1.22–6.74)	0.0156	
ER+/HER2– vs. HER2+	1.29 (0.53–3.12)	0.574	
LN: (+) vs (–)	1.48 (0.93–2.36)	0.0946	
NAT: Received vs. Not	1.27 (0.80–2.02)	0.305	

Figure 2

Prognostic significance of molecular residual disease (MRD) at 3-months post-surgery. A. Kaplan-Meier survival analysis indicated that patients who were ctDNA-positive in the post-operative period (MRD+) (n=125) experienced significantly inferior DRFS compared to those who were ctDNA-negative (n=1010, MRD–). **B**. Multivariate Cox proportional hazards modeling indicated that MRD positivity was an independent prognostic risk factor for increased hazard of poorer DRFS, when considering breast cancer subtype, lymph node status, and whether a patient received neoadjuvant therapy. C) A time-dependent Cox analysis treating MRD status as a time-varying covariate for all patients with longitudinal follow-up showed a significant association between anytime MRD positivity and recurrence. LN = lymph node; MRD= molecular residual disease; NAT= neoadjuvant therapy.

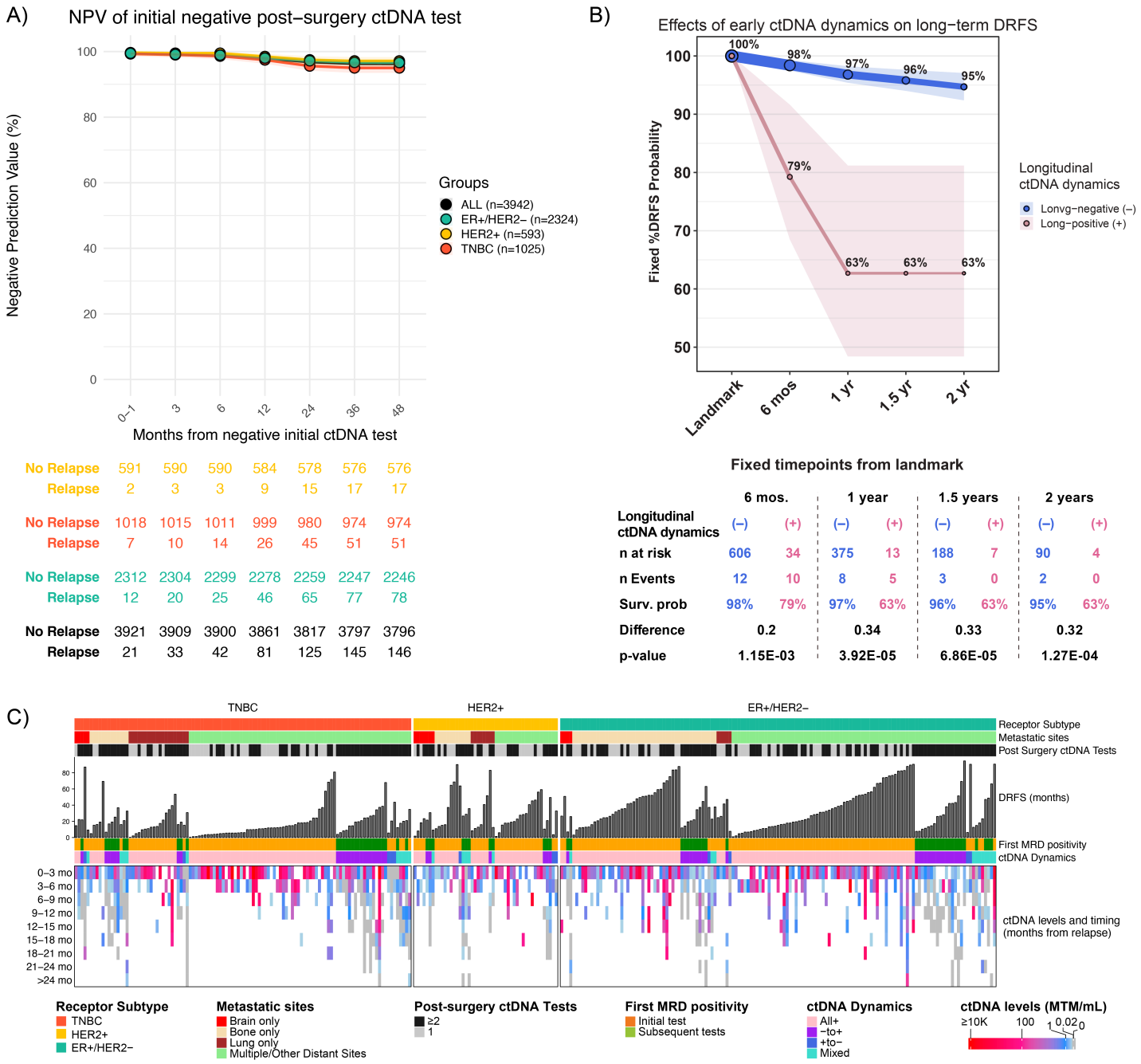


Figure 3

Predictive value and prognostic insights of tumor-informed ctDNA testing in early breast cancer.A) Among patients who were ctDNA-negative at their first test post-surgery and had at least 24 months of follow-up (n=3,942), the plot shows the negative predictive value (NPV) for subsequently predicting a recurrence event over different timeframes after an initial negative ctDNA test. Shaded areas indicate 95% confidence intervals. The NPV of a single negative test in the adjuvant setting remains very high over time for all subtypes, with the highest NPV being within the first year after the negative result (97-99.5%). Cumulative relapse events over time are tabulated below for HER2+ (yellow), TNBC (red), ER+/HER2– (green), and all patients (black). **B)** Fixed DRFS probability at set time intervals (6, 12, 18, 24 months) between patients with longitudinally MRD-negative/cleared (All-, +to-) and longitudinally MRD-

positive (All+, -to+) ctDNA dynamics was estimated using the KM method. Survival probabilities and corresponding 95% CI were calculated using Greenwood's equation at each landmark year. ctDNA dynamics were derived for n = 891 patients with ≥2 ctDNA tests between post-surgery and 6 months landmark. **C)** Molecular patterns of relapse in patients with anytime ctDNA positive tests who went on to have clinically confirmed relapse (n=305). The panel at the bottom represents ctDNA levels (MTM/mL) detected at tests performed within the indicated time intervals prior to the detection of recurrence. ctDNA levels (MTM/mL) for positive tests are represented by blue (low levels) to red (high levels) color gradient. For patients with >1 test performed within a single time interval, the heatmap shows the maximum MTM/mL value detected during that interval.

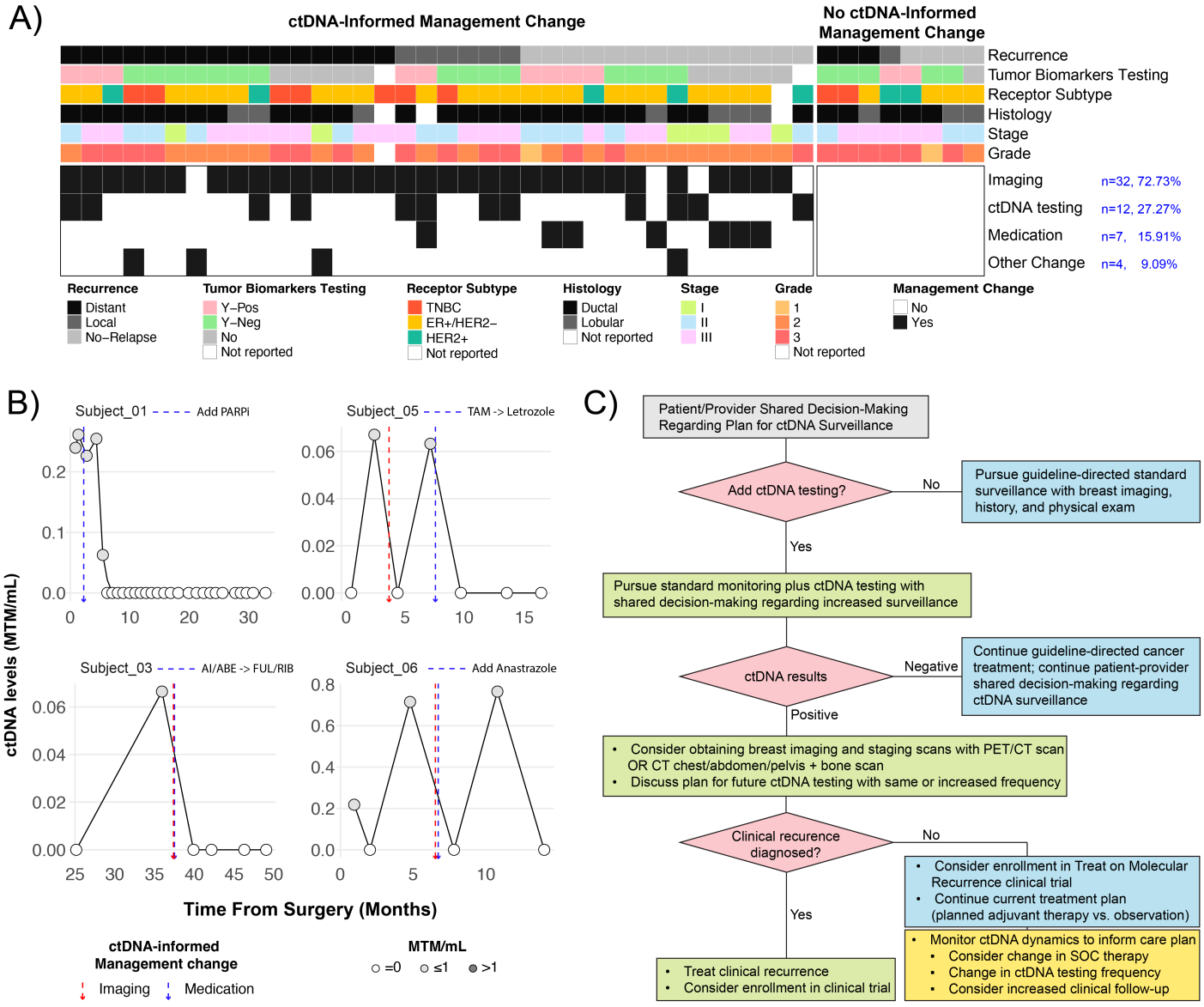


Figure 4

ctDNA-informed changes in the clinical management of patients with early-stage breast cancer

A)Characteristics of patients in ctDNA actionability cohort who were MRD positive at any time post-

surgery (n=44). Columns indicate an individual patient, and rows indicate tumor characteristics (subtype, histology, stage, grade), clinical characteristics (recurrence outcomes, therapy, biomarker testing status), and changes in management of clinical care (ctDNA-informed restaging scan, ctDNA testing cadence; medication, other change in care management). **B)**Treatment diagrams for individual patients from ctDNA actionability cohort who underwent ctDNA-informed change in medication and went on to achieve ctDNA clearance. **C)** Decision tree describing the most common decision-making patterns observed for tumor-informed ctDNA monitoring of MRD into shared decision-making for patients who have undergone surgery for early-stage breast cancer. PARPi, PARP inhibitor; AI, aromatase inhibitor; ABE, abemaciclib; FUL, fulvestrant; RIB, ribociclib.

Supplementary Files

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