Circulating Tumor DNA Dynamics and Anatomical Patterns of Relapse Following Curative Therapy in Early-Stage Breast Cancer

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Background

- Patterns of relapse in early-stage breast cancer (BC) following definitive treatment remain poorly defined, especially in the context of real-world ctDNA dynamics.[1]
- Understanding the anatomical distribution of the site of relapse and its association with ctDNA-based molecular residual disease (MRD) rates by tumor subtype and treatment history may inform surveillance strategies and enable earlier intervention with targeted therapies.

Methods

- This retrospective study explored the association between sites of relapse and ctDNA detection rates/dynamics in a real-world cohort (2019-2025) of patients with early-stage BC. Natera's proprietary real-world database, which is linked to the Forian claims database and results of tumor-informed ctDNA testing (Signatera™, Natera, Inc.), were utilized.
- Estrogen receptor (ER) and HER2 status were inferred from ICD-10 treatment codes. Relapse date was inferred from ICD-10 claims codes for secondary neoplasms. Patients with a diagnosis code of secondary neoplasms were categorized by the anatomic site(s) of recurrence. Based on the number of anatomical sites, patients were categorized into single-site relapse (recurrence in only one organ, e.g., bone, lung) or multi-site relapse (recurrence in two or more distinct anatomical locations). Other single-relapsed sites were observed but were excluded due to small numbers, heterogeneity, or inability to differentiate primary breast malignancies from secondary metastases.
- Inclusion/exclusion criteria: Patients with available subtype information who underwent Signatera ctDNA testing in the adjuvant setting (post-surgery and prior to relapse) were included. Patients with evidence of metastatic disease, non-standard systemic therapy, or only a single plasma sample collected on the same day as relapse were excluded.

Table 1. Patient and tumor characteristics (N=579)

Parameter	#Patients (%)
Median age in years (range)	57 (23-89)
Median RFS in years (range)	1.7 (0.1-9.2)
Median clinical follow-up in years (range)	2.8 (0.1-10.0)
Adjuvant treatment	
Yes	463 (80%)
No	116 (20%)
Treatment types	
Hormone therapy	257 (44.4%)
Chemotherapy	160 (27.6%)
Targeted therapy	140 (24.2%)
Immunotherapy	50 (8.6%)
Median number of timepoints for ctDNA testing in adjuvant setting (range)	2 (1-31)
LN status at surgery	
Positive	175 (30.2%)
Negative	404 (69.8%)

Parameter	#Patients (%)
Primary site of relapse	
Multi-site	268 (49.4%)
Bone	148 (25.6%)
Lung	43 (7.4%)
Liver	36 (6.2%)
LN	32 (5.5%)
Brain	26 (4.5%)
GI	8 (1.4%)
Subtypes	
TNBC	124 (21.4%)
HER2+	82 (14.2%)
ER+/HER2-	373 (64.4%)
Stages	
I	72 (12.4%)
II	148 (25.6%)
III	185 (32.0%)
unconfirmed	174 (30.0%)

Abbreviations: ER, estrogen receptor; GI, gastrointestinal; HER2, human epidermal growth factor receptor 2; LN, lymph node; RFS, recurrence-free survival; TNBC, triple-negative breast cancer.

Figure 1. ctDNA status/dynamics and anatomical patterns of relapse across breast cancer subtypes

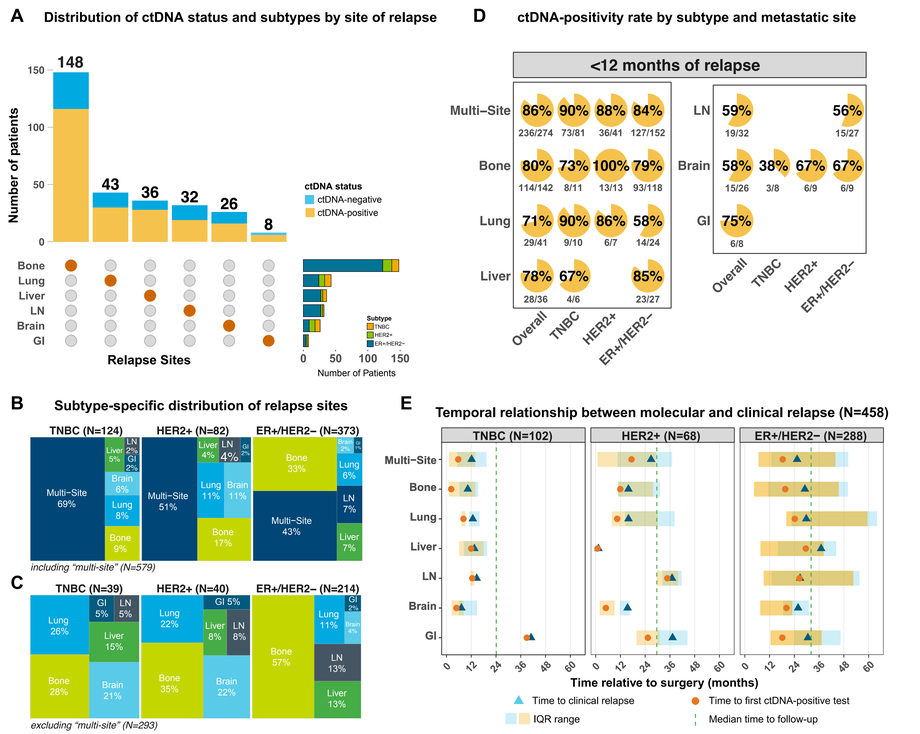


Figure 1: A. Stacked bar and upset plots show the number of patients with relapse at each anatomical site, stratified by anytime ctDNA status (in the adjuvant settings) and BC subtype. Bone and lung were the most frequent relapse sites, followed by liver and LN. **B-C.** Treemap plots display the proportional distribution of relapse sites across BC subtypes. Multi-site relapse dominated TNBC (69%) and HER2+ (51%), whereas ER+/HER2− cases most frequently relapsed to bone (33%), highlighting distinct metastatic tropism by subtype. When limited to single-site recurrences, bone remained the most common site in ER+/HER2− (57%), while TNBC (28%) and HER2+ (35%) in addition to bone showed a greater incidence of visceral (lung, brain, liver) relapses, underscoring biologic heterogeneity in relapse sites. **D.** A 2-dimensional matrix illustrating ctDNA detection rates among patients relapsing within 12 months of recurrence, stratified by subtype and site of recurrence. Each pie represents the detection rate (orange-fill) for a given site and a given subtype; detection rate for pie with more than 5 total number of patients are shown. ctDNA positivity exceeded 80% across most subtypes and sites, with the highest detection in multi-site and bone relapses, reflecting aggressive disease. **E.** Line plots show the median (including 25th to 75th quartiles) time to first positive ctDNA test relative to surgery vs. time to clinical relapse relative to surgery, by subtype and relapse site. The median time to follow-up for each subtype cohort (considering relapse and not-relapsed patients) is shown with vertical green-line. Across all subtypes, ctDNA detection consistently preceded clinical relapse, supporting ctDNA as an early marker of molecular recurrence. **Abbreviations:** BC, breast cancer; ctDNA, circulating tumor DNA; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LN, lymph node; TNBC, triple-negative breast cancer; GI, gastrointestinal; IQR, interquartile range.

Figure 2. ctDNA dynamics by relapse sites and subtypes

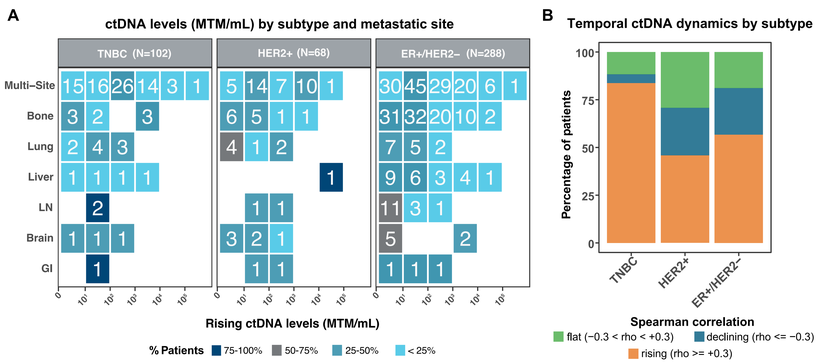


Figure 2: A. 2-dimensional grid matrix showing ctDNA levels (MTM/mL) across anatomical sites and BC subtypes using all ctDNA-positive time points in the adjuvant setting. For each patient, the median MTM/mL from surgery to relapse was calculated and categorized into six distinct ctDNA magnitude groups based on the intensity of ctDNA levels. The number and percentage of patients (row-wise within subtype) are shown per site and magnitude. Higher ctDNA levels were observed in multi-site, bone, and liver relapses, compared with lung, LN, brain, and GI single-sites, which showed lower ctDNA levels. **B.** Stacked bars display ctDNA trend over time as patients progress toward relapse, including only those with serial ctDNA time-points (≥3, N=194) and at least one ctDNA-positive result. Spearman correlation (ρ) was used to assess ctDNA trend. Values ≥ +0.3 or ≤ −0.3 defined rising or declining trajectories, respectively; |ρ| < 0.3 indicated a flat (non-significant) trend. Patients with multi-site and single-site recurrence show a clear rising trend in ctDNA MTM/mL preceding relapse, most prominent in TNBC (84%); however declining ctDNA MTM/mL levels are observed mainly HER2+ and ER+/HER2- (25%) with a smaller % achieving clearance. **Abbreviations:** BC, breast cancer; ctDNA, circulating tumor DNA; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LN, lymph node; TNBC, triple-negative breast cancer; GI, gastrointestinal; MTM/mL, mean tumor molecules per milliliter; rho (ρ), Spearman correlation coefficient.

Conclusions

- Distinct relapse patterns were observed across subtypes, with multi-site and bone relapses most common overall, and visceral (lung, liver, brain) recurrences more frequent in TNBC and HER2+ disease, reflecting biologic heterogeneity in relapse sites.
- ctDNA detection and MTM/mL levels varied by site of recurrence, indicating that ctDNA remains measurable across diverse metastatic locations despite differences in tumor DNA shedding potential.
- Serial ctDNA dynamics revealed rising trajectories in aggressive subtypes (TNBC), while declining profiles were more typical of HER2+ and ER+/HER2−, consistent with slower disease kinetics and treatment response variation.
- ctDNA detection consistently preceded clinical relapse across subtypes, highlighting the potential of real-world ctDNA monitoring to identify patients at high risk of recurrence and guide personalized surveillance and early intervention strategies.

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References

1. Magbanua MJM, et al. Cancer Cell. 2023 Jun 12;41(6):1091-1102.e4

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