Personalized mutation tracking in circulating-tumor DNA predicts recurrence in patients with high-risk early breast cancer

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Current Limitations

- Early breast cancer patients still face high recurrence risk, especially in high-risk subtypes.
- Current monitoring methods (e.g. imaging) lack sensitivity to detect minimal residual disease (MRD).
- Clinical use of ctDNA is promising but not yet standardized, and existing platforms are costly and complex.

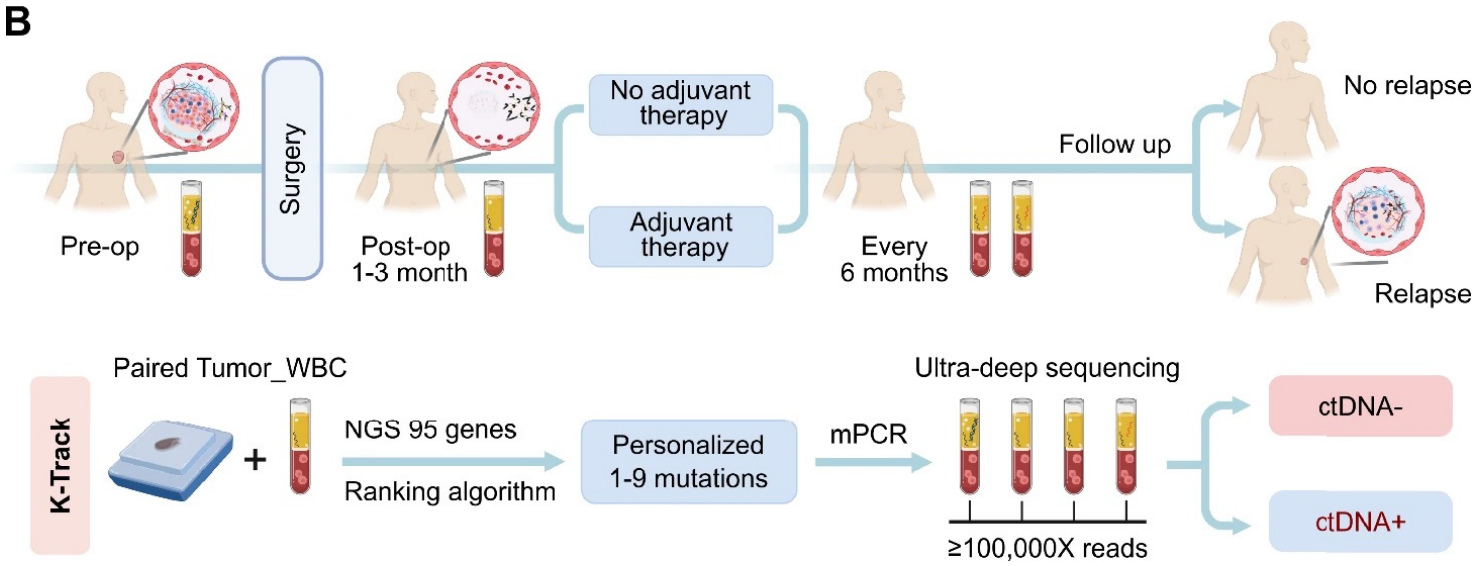
The Solutions

- Developed a cost-effective, tumor-informed ctDNA assay (K-TRACK) with targeted gene panel and 0.05% LOD.
- In a prospective study of 168 patients, ctDNA status before and after surgery strongly predicted recurrence.
- Showed potential for ctDNA to guide post-op surveillance and therapy decisions, especially in stage-III patients.

Key Findings

1. **Pre-operative ctDNA positivity (ctDNA+) had significantly shorter DFS and predicts high recurrence risk (HR=6.16),** notably in HER2+ and triple-negative subtypes (80–84% detection rate).
2. **Post-operative ctDNA clearance may guide adjuvant therapy decisions,** as ctDNA clearance was associated with superior outcomes (24-month DFS >90%), while persistent ctDNA indicated poor prognosis (24-month DFS = 0%) in 36.4% of stage III patients.
3. **Post-operative ctDNA monitoring during surveillance predicts recurrence with high accuracy (91% sensitivity, 99% specificity)** with a median lead time of 9.7 months.
4. **Case studies confirmed** ctDNA detection preceded clinical/radiologic relapse, supporting its role in **early intervention and personalized follow-up.**

ctDNA Analysis Workflow



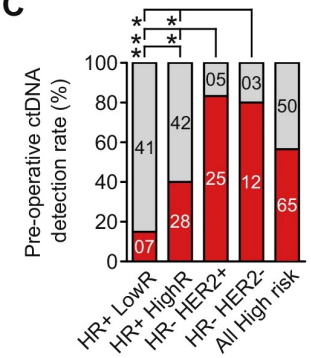
For each patient, paired tumor FFPE and WBC DNA samples were sequenced to identify tumor-specific mutations in 95 cancer-associated genes.

The top 1–9 mutations were used to detect ctDNA in serial plasma samples by bespoke multiplex PCR and ultra-deep sequencing.

ctDNA is a Powerful Prognostic Biomarker

1 Pre-operative ctDNA Associate with DFS and Recurrence Risk

C



ctDNA detection was significantly more common in aggressive breast cancer subtypes (~83.8% in HER2+ and 80.0% in TNBC)

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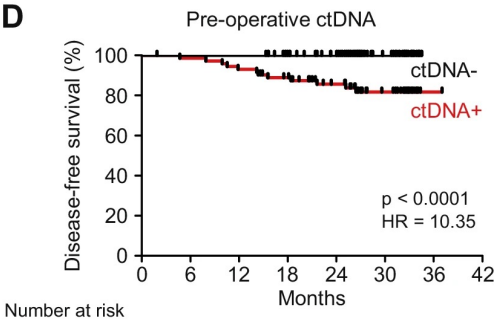

Number at risk

ctDNA+	72	72	68	61	49	29	3	0
ctDNA-	91	91	91	82	73	38	0	0

Patients with pre-operative ctDNA positivity (ctDNA+) had significantly shorter DFS, with a 5% lower 24-month DFS rate compared to ctDNA- patients

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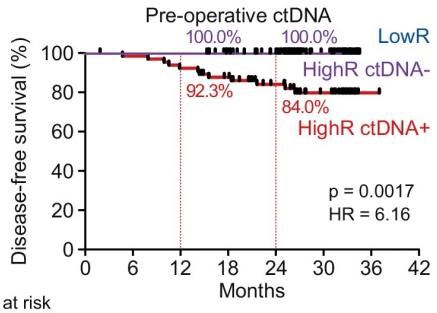

Number at risk

HighR ctDNA+	65	65	61	54	43	27	2	0
HighR ctDNA-	50	50	50	44	40	23	1	0
LowR	48	48	48	46	41	19	2	0

Pre-operative ctDNA+ identified patients with a 6-fold higher risk of recurrence, even within high-risk subgroups.

2 Post-operative ctDNA May Guide Adjuvant Therapy Decisions

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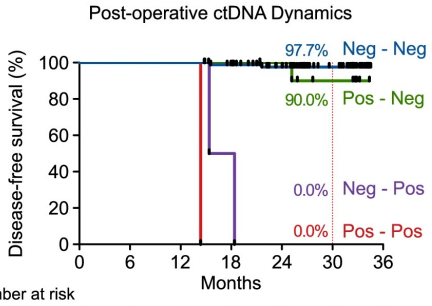


Number at risk

Pos - Pos	1	1	1	0	0	0	0
Neg - Pos	2	2	2	1	0	0	0
Pos - Neg	11	11	11	11	11	7	0
Neg - Neg	95	95	95	89	73	40	0

Persistence or emergence of ctDNA (Pos → Pos or Neg → Pos) indicates ineffective treatment and high risk of recurrence, with 0% DFS.

Clearance of post-operative ctDNA (Pos → Neg) was associated with superior outcome.

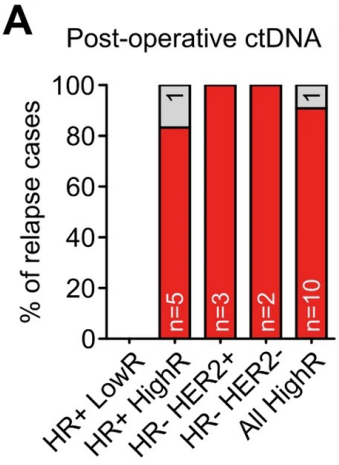
Post-operative ctDNA clearance by adjuvant therapy	Stage I-II	Stage III
Clearance (Pos → Neg)	100.0% (4/4)	63.6% (7/11)
No clearance (Pos → Pos or relapse)	0.0% (0/0)	36.4% (4/11)

36.4% (4/11) of stage-III patients had post-operative ctDNA persistence after adjuvant therapy.

This suggests that current adjuvant regimens may not be adequate and ctDNA-guided escalation for adjuvant therapy in stage-III BC might be worth exploring in the future.

3 Surveillance ctDNA Predicts Recurrence Early and Accurately

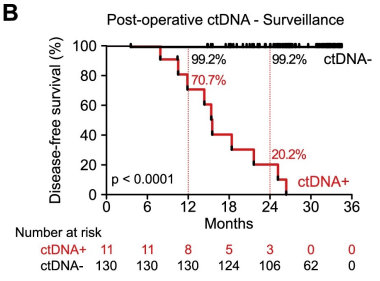
A



Surveillance ctDNA detection showed:

- **90.9%** Sensitivity
- **98.8%** Specificity
- **90.9%** PPV
- **98.8%** NPV
- **97.9%** Accuracy

B

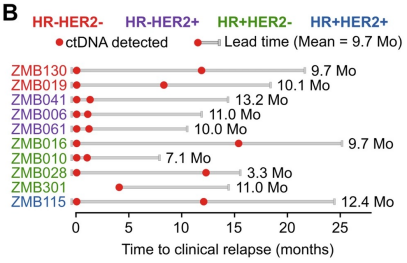


Number at risk

ctDNA+	11	11	8	5	3	0	0	
ctDNA-	130	130	130	124	106	62	0	

Patients with ctDNA+ during surveillance had dramatically worse DFS (20.2%) than ctDNA– patients (99.2%).

B



The ctDNA+ results all preceded clinical diagnosis by a median lead time:

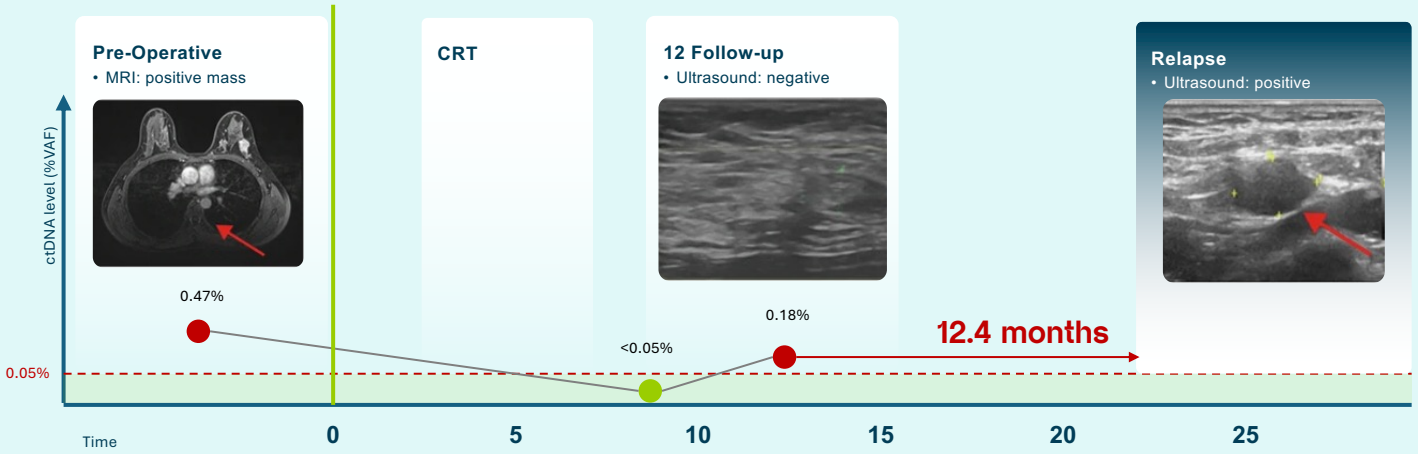
9.7 months
(3.3–13.2 months)

4 Case Studies

Case 1: Patient Information

Patient ZMB115
43-year-old – Female
HR+/HER2+, stage III (pT3N2M0) invasive ductal carcinoma

Pre-operative MRI showed a positive mass, and ctDNA level was 0.47%.
At 8 months post-surgery, ctDNA became undetectable.
However, at 12-month follow-up, ctDNA reappeared at 0.18%, **despite negative ultrasound and no symptoms.**
Only at month 24, supraclavicular lymph node metastasis was confirmed by ultrasound and biopsy.



Case 2: Patient Information

Case ZMB130
54-year-old – Female
HR–/HER2– (TNBC), stage III (pT3N2M0)

Pre-operative ultrasound confirmed tumor presence; ctDNA was 0.10%.
At 12 months, ctDNA was detected at 0.08%, **but ultrasound remained negative and patient was asymptomatic.**
9.7 months later, CT scan revealed liver and lung metastases, confirming disease progression.