Personalized Mutation Tracking in Circulating tumor DNA Detects Recurrence in High-risk Early Breast Cancer

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12th Asia-Pacific Breast Cancer Summit™ Reaching New Heights in Breast Cancer Care

Abstract #89

BACKGROUND

- **Minimal residual disease (MRD)** refers to a small number of cancer cells that remain in the body after curative-intent treatment, causing relapse and metastasis. Current tumor biomarkers and imaging methods have limited sensitivity and specificity to monitor MRD for solid tumors.
- **Circulating tumor DNA (ctDNA)** is tumor-derived DNA fragments circulating in the bloodstream → a promising biomarker to monitor MRD.
- **Current ctDNA-based MRD assays remain mostly inaccessible and unaffordable.**

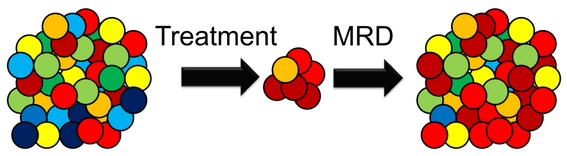


Figure 1. MRD in solid tumors. MRD, or the remaining cancer cells after curative-intent treatment, is the leading cause for relapse and metastasis.

Yoshida et al. J Exp Clin Cancer Res (2015)

METHOD

- **Tumor-informed personalized ctDNA tracking assay**
- **Streamlined and cost-effective design**

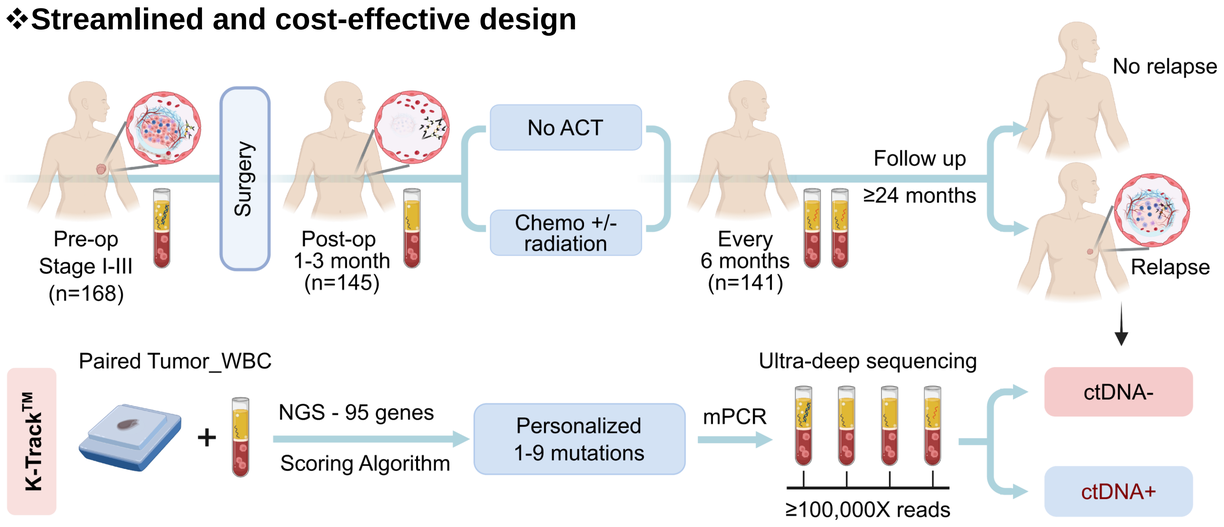


Figure 2. Study design and workflow of K-Track™ assay. **(A)** 168 breast cancer patients indicated for curative-intent surgery were enrolled. Serial plasma samples were collected before surgery, after surgery and at scheduled visits. FFPE samples were also collected. **(B)** gDNA of paired FFPE and WBC samples were sequenced to identify all tumor-specific somatic mutations in 95 cancer-associated genes. Top mutations were ranked and selected to detect ctDNA presence in plasma samples by a multiplex PCR assay and ultra-deep sequencing at an average depth of 100,000X.

Table 1. Patients Demographics

Characteristics	N = 168
Median age (range), year	52 (25 – 85)
Invasive carcinoma	
Ductal	164 (97.6)
Lobular	3 (1.8)
Not available	1 (0.6)
TNM stage	
I	41 (24.8)
II	81 (47.9)
III	33 (20.0)
NA (I-III)	13 (7.3)

Characteristics	N = 168
Receptor status[1]	
HR+ HER2-	93 (54.8)
Ki67 Low (<10%)	30 (17.9)
Ki67 High	63 (37.5)
HR+ HER2+	27 (16.1)
HR- HER2+	30 (17.9)
HR- HER2-	16 (9.5)
NA	02 (1.2)
Risk of recurrence[2]	
HR+ Low risk	48 (28.5)
HR+ High risk	72 (42.9)
HR- HER2+	30 (17.9)
HR- HER2-	16 (9.5)
NA	02 (1.2)
Germline *BRCA1/2*	5 (2.4)

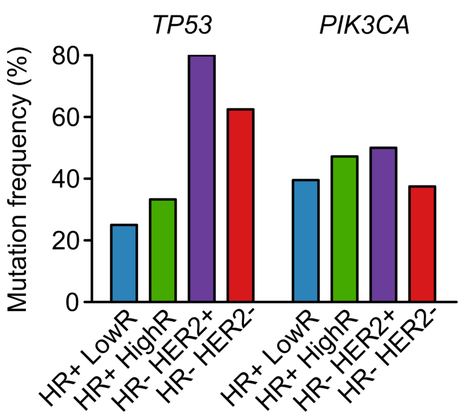
[1] Receptor status was determined by IHC

[2] **High risk criteria for HR+ patients**

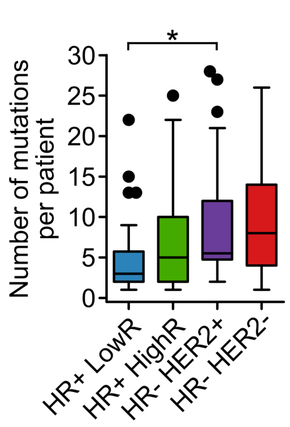
- ≥15% predicted risk of death within 10 years using ePREDICT V2.1, or
- Tumor size T>5 cm, or N≥4 nodes, or
- N=1-3 nodes and at least one of the following: T>3 cm, grade 3, high genomic risk defined as Oncotype Dx Recurrence Score >26.

PRE-OPERATIVE DETECTION OF ctDNA

A – Top mutated genes



B – Mutational burden



C – Pre-operative plasma

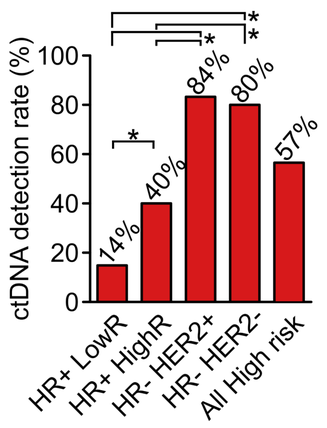
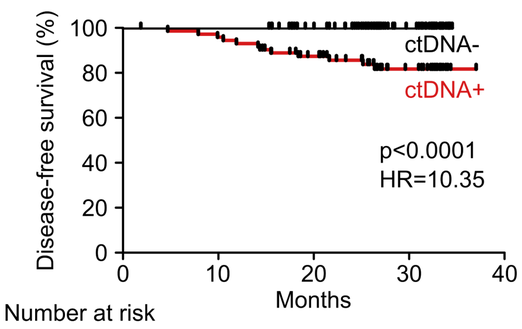


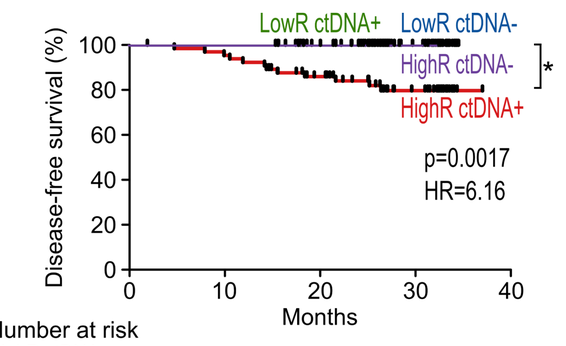
Figure 3. Analysis of FFPE and pre-operative plasma samples. **(A)** Mutation frequency of the top 2 mutated genes in FFPE samples. **(B)** The number of somatic mutations identified per patient was significantly higher in the HR-HER2+ compared to the HR+ groups. **(C)** Pre-operative ctDNA detection rate was significantly higher in the HR- compared to the HR+ groups.

PROGNOSTIC VALUE OF ctDNA

A – PRE-operative ctDNA

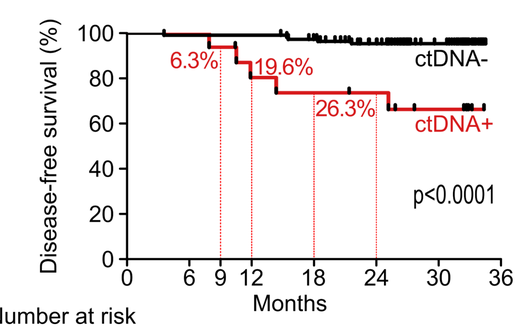


Number at risk					
ctDNA+	72	70	56	29	0
ctDNA-	91	91	79	38	0

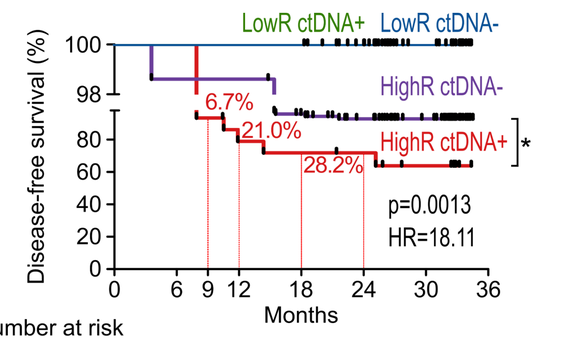


Number at risk					
HighR ctDNA+	65	63	50	27	0
HighR ctDNA-	50	50	42	23	0
LowR ctDNA+	7	7	7	3	0
LowR ctDNA-	41	41	37	16	0

B – POST-operative ctDNA (Landmark – after surgery only)

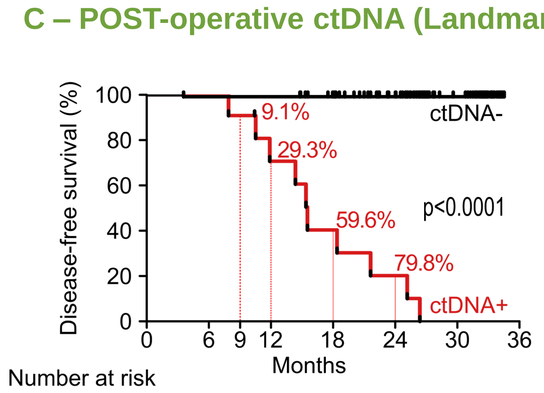


Number at risk							
ctDNA+	16	16	13	12	11	7	0
ctDNA-	112	112	112	107	91	54	0

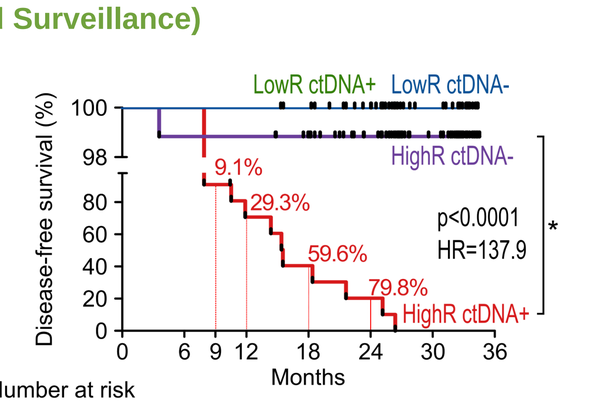


Number at risk							
HighR ctDNA+	15	15	12	11	10	7	0
HighR ctDNA-	72	72	72	67	58	37	0
LowR ctDNA+	1	1	1	1	1	1	0
LowR ctDNA-	40	40	40	40	34	18	0

C – POST-operative ctDNA (Landmark and Surveillance)



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



Number at risk							
HighR ctDNA+	11	11	8	5	3	1	0
HighR ctDNA-	86	86	86	82	71	45	0
LowR ctDNA+	0	0	0	0	0	0	0
LowR ctDNA-	44	44	44	43	35	18	0

Figure 4. Prognostic value of pre- and post-operative ctDNA. **(A-C)** Kaplan-Meier plots showed that patients with ctDNA detection before surgery, and particularly after surgery were more likely to relapse than those with ctDNA(-)

EARLY RELAPSE DETECTION

A – Relapse detection rate

B – Lead time ahead of clinical diagnosis

Figure 5. Molecular relapse detection by ctDNA. **(A)** The sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of post-operative ctDNA status in detecting clinical relapse. **(B)** Diagram showing the lead time from post-operative ctDNA detection to clinical diagnosis in all patients that relapsed.

CONCLUSION

- Patients with ctDNA+ at any time after surgery had a significantly higher risk of relapse (HR=137.9) compared to those with ctDNA-. Median lead time ahead of clinical diagnosis was 9.7 months (up to 13.2 months).
- **K-Track™ assay** is streamlined, reliable and affordable MRD assay.

Ethical approval Approved by the institutional ethics committees of the University of Medicine and Pharmacy (#300/HDDD) and Thu Duc city Hospital (#17/HDDD).

Acknowledgement We especially thank the doctors, nurses and our patients for participating in the study.

Funding This study was funded by Gene Solutions JSC, Vietnam.