Personalized Serial Circulating Tumor DNA (ctDNA) Analysis in High-Risk Early Stage Breast Cancer Patients to Monitor and Predict Response to Neoadjuvant Therapy and Outcome in the I-SPY 2 TRIAL

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Introduction

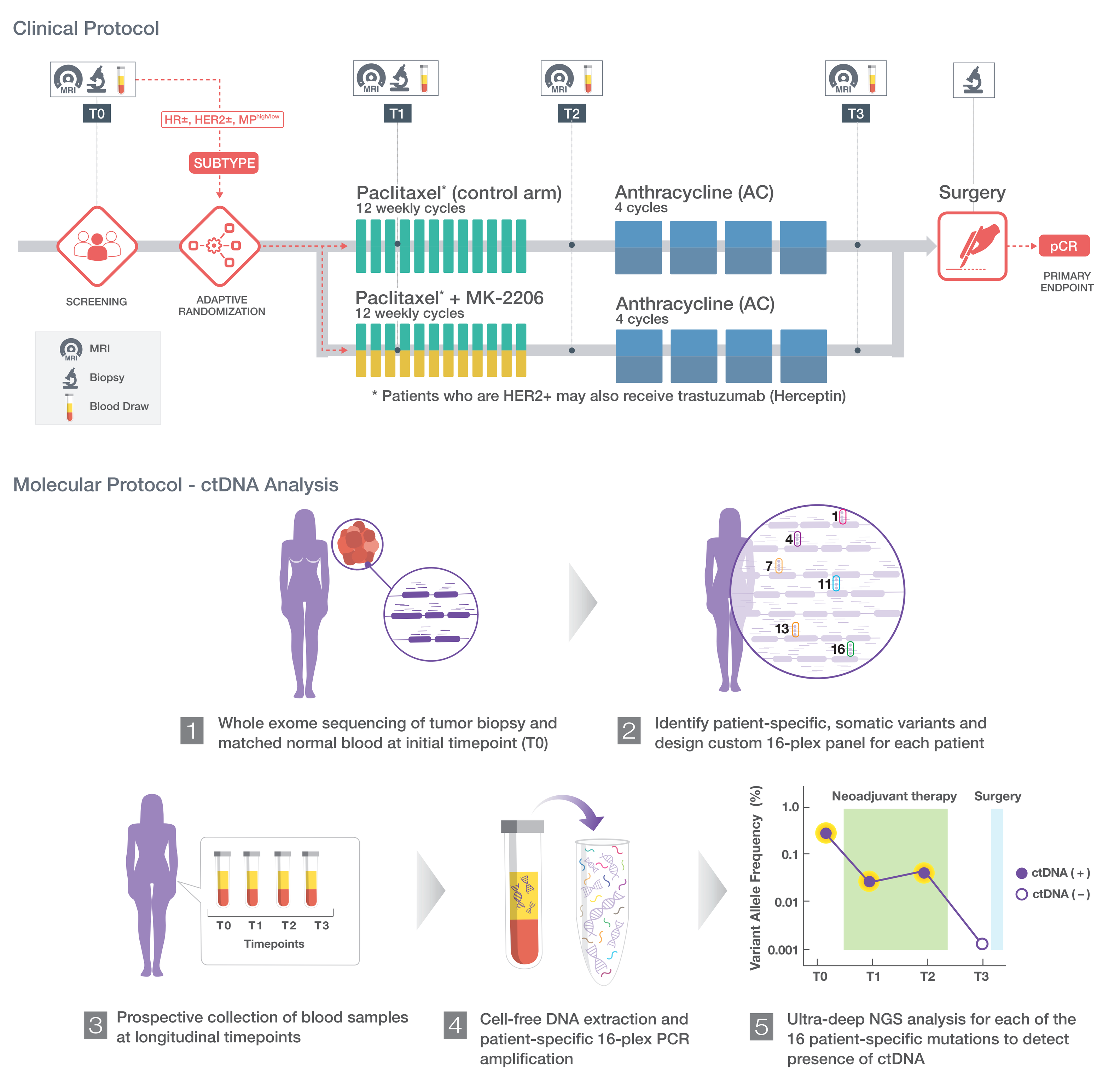
- Analysis of circulating tumor DNA (ctDNA) offers a minimally invasive approach for monitoring treatment response and resistance to treatment in patients with breast cancer.
- Serial ctDNA testing during neoadjuvant therapy has the potential to provide early indicators of emerging resistance and disease progression.
- We previously reported early detection of lung cancer recurrence using a patient-specific ctDNA detection approach;[1,2] an improved version of this assay was developed (Signatera™ RUO) and made available for clinical validation testing in the neoadjuvant setting.

Objectives

- The aim of the study was to analyze ctDNA from high-risk early breast cancer patients who received neoadjuvant therapy and curative surgery in MK-2206 and control arms of the adaptive randomized I-SPY 2 TRIAL (NCT01042379).
- We hypothesize that:
  1. Assessment of ctDNA levels early in treatment improves the performance of molecular and imaging-based predictors of pathologic complete response (pCR) to neoadjuvant therapy.
  2. Levels of ctDNA after neoadjuvant therapy are associated with residual cancer burden and recurrence [distant recurrence-free survival (DRFS)].

Methods

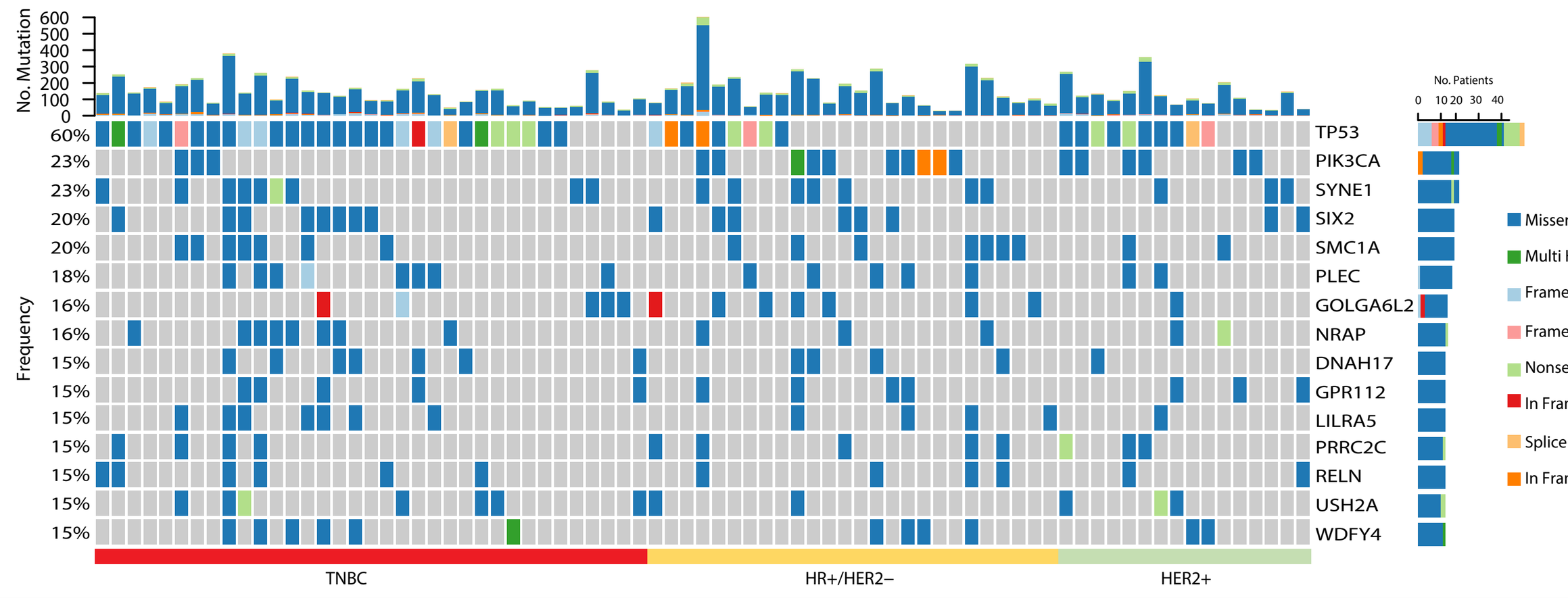
Figure 1. Schematic of Study Protocol



A total of 150 high-risk stage II and III breast cancer patients were treated in the I-SPY 2 trial's MK-2206 and control arms. Of those, 90 had sufficient tumor and buffy coat/plasma samples for NGS and Signatera™ analysis. Of those, 8 had samples that failed NGS QC testing and were excluded from the analysis. Serial ctDNA analysis (T0, T1, T2, and T3) was performed in a final cohort of 82 patients. Sixteen patient-specific somatic variants detected in pretreatment tumor biopsy were chosen as targets for ctDNA detection in serial plasma samples.

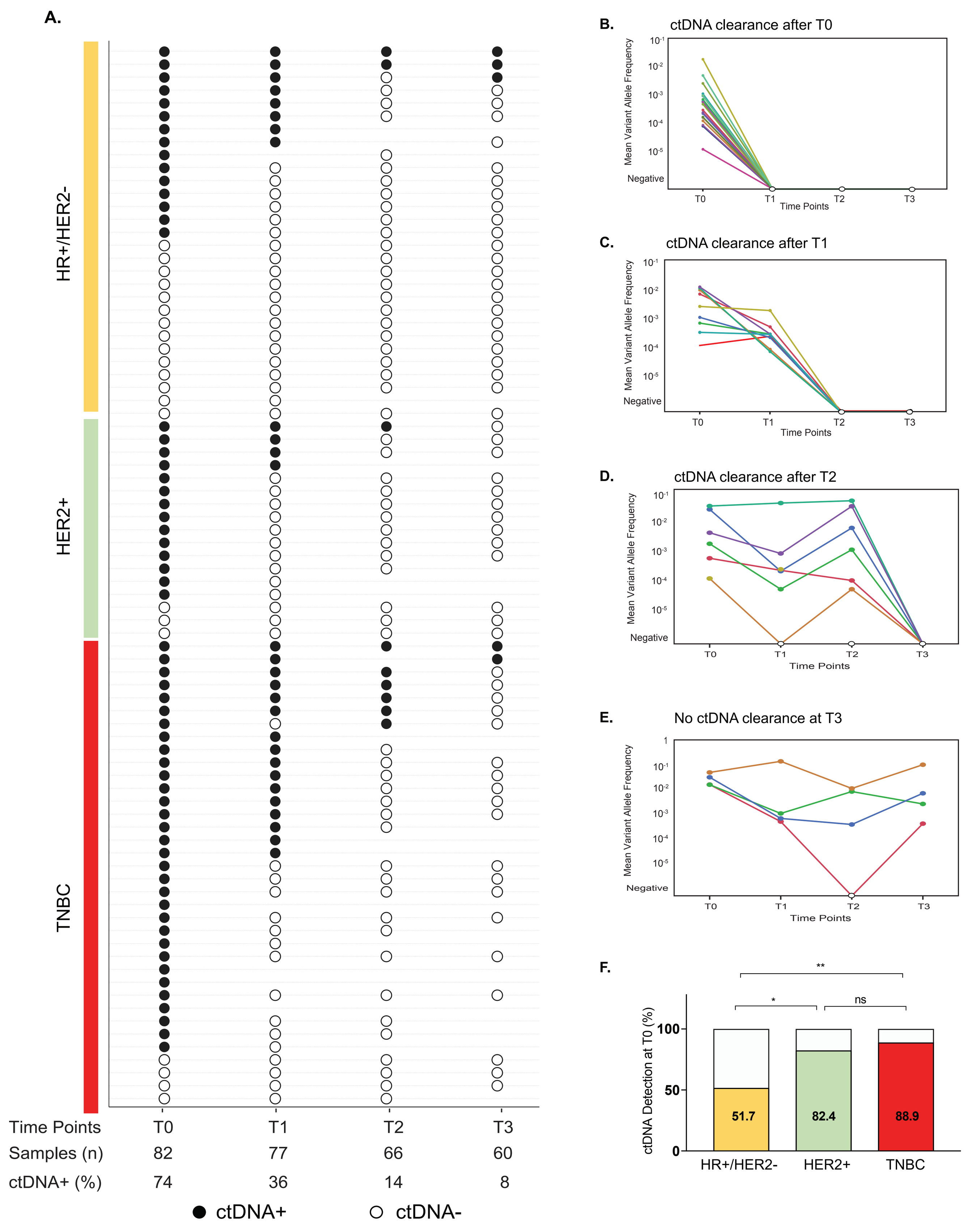
Results

Figure 2. Frequently Mutated Genes in Pretreatment Tumors Detected by Whole Exome Sequencing



The number of somatic mutations identified by whole exome sequencing (WES) at pretreatment time point (T0). Different colors indicate the synonymous and non-synonymous mutations called by WES (Top). The top 15 mutated genes and their corresponding frequencies are depicted (Bottom).

Figure 3. Overview of ctDNA Patterns in Serial Plasma Samples



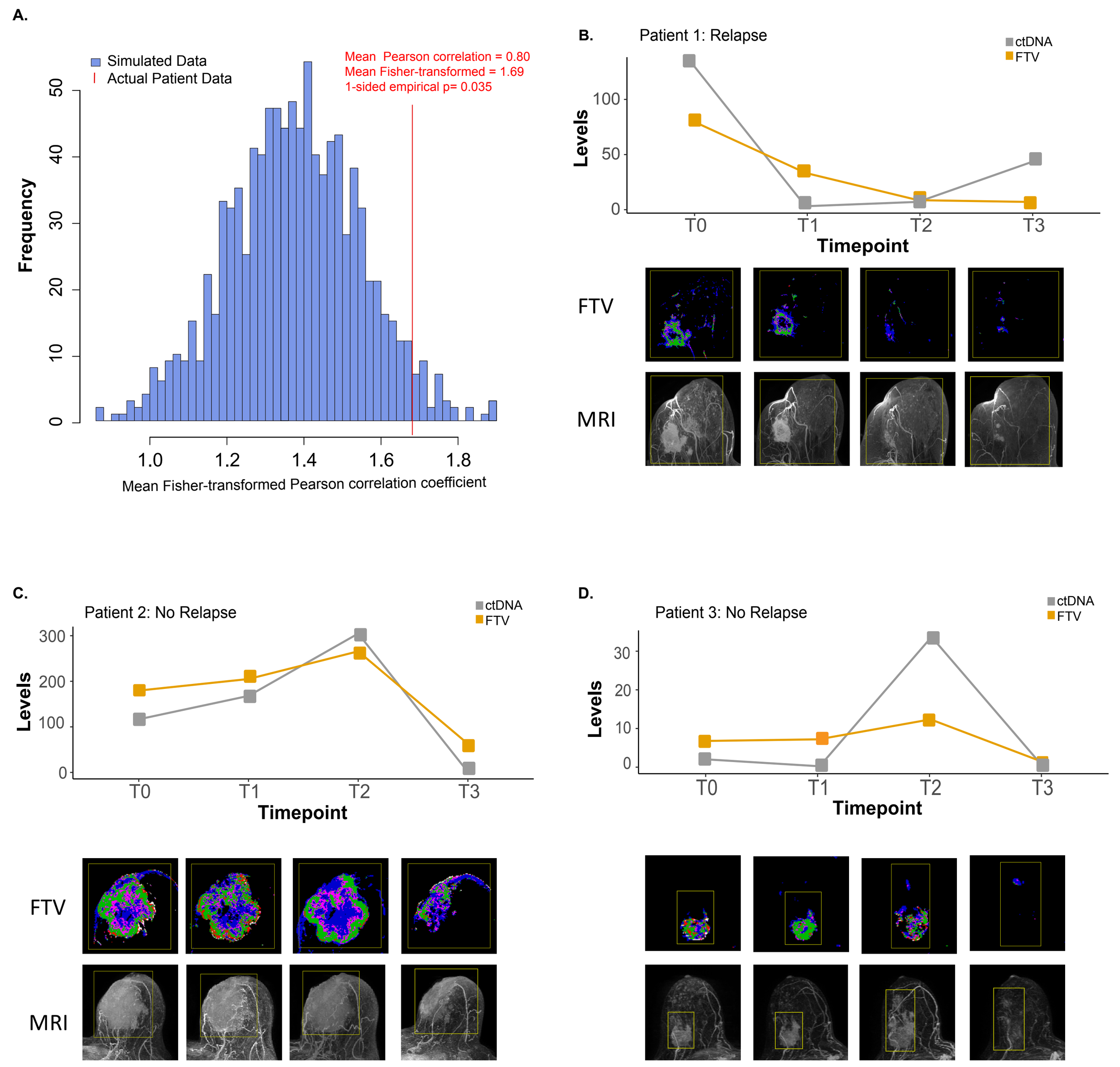
A. ctDNA detection in serial plasma samples from 82 patients. Samples with at least two detectable targets were considered ctDNA positive. **B-E.** ctDNA dynamics in patients with informative results for all time points (n=38). **F.** ctDNA detection rates at T0 by subtype. *p<0.05; **p<0.01; ns, non-significant

Table 1: ctDNA Positivity at T0 (Pretreatment) is Associated with Increased Tumor Burden and Aggressive Phenotype

	Total N (%)	ctDNA- n (%)	ctDNA+ n (%)	p-value (Fisher's test)
Subtype				0.0025
HER2+	17 (21)	3 (18)	14 (82)	
TNBC	36 (44)	4 (11)	32 (89)	
HR+/HER2-	29 (35)	14 (48)	15 (52)	
Clinical Stage (T)				0.0081
T1/T2	54 (71)	19 (35)	35 (65)	
T3/T4	22 (29)	1 (5)	21 (95)	
Clinical Stage (N)				0.0081
Negative	37 (49)	7 (19)	30 (81)	
Positive	39 (51)	13 (33)	26 (67)	
Grade				0.0048
II	20 (37)	9 (45)	11 (55)	
III	34 (63)	7 (21)	27 (79)	
Mammaprint				
Hi1	32 (39)	15 (47)	17 (53)	0.0006
Hi2	50 (61)	6 (12)	44 (88)	
		ctDNA- (Mean)	**ctDNA+ (Mean)**	**p-value (T test)**
FTV (cm^3); n=82		10.4	33.9	0.0011
LD by clinical exam (cm); n=78		4.2	5.2	0.0332
LD by MRI (cm); n=82		3.3	4.6	0.0022

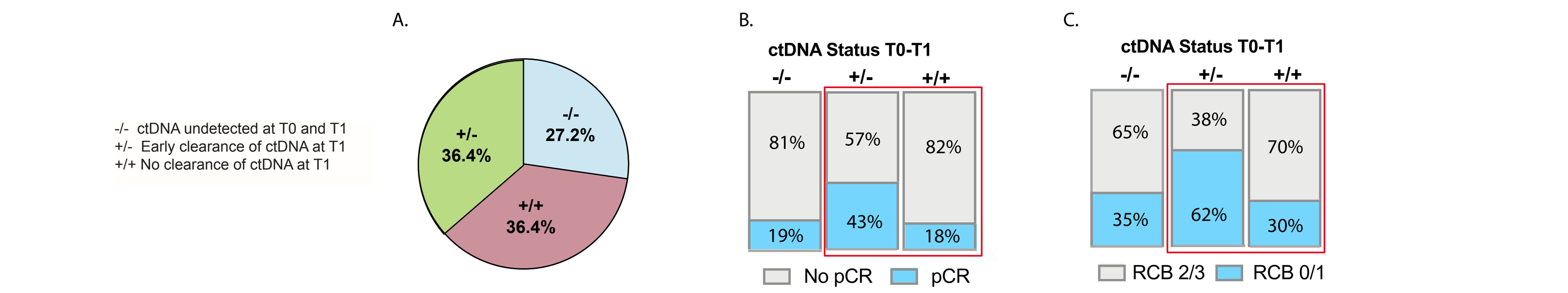
FTV, functional tumor volume; LD, longest diameter; MRI, magnetic resonance imaging.

Figure 4. Dynamics of ctDNA Levels During Neoadjuvant Therapy Reflects Changes in Tumor Response as Measured by MRI



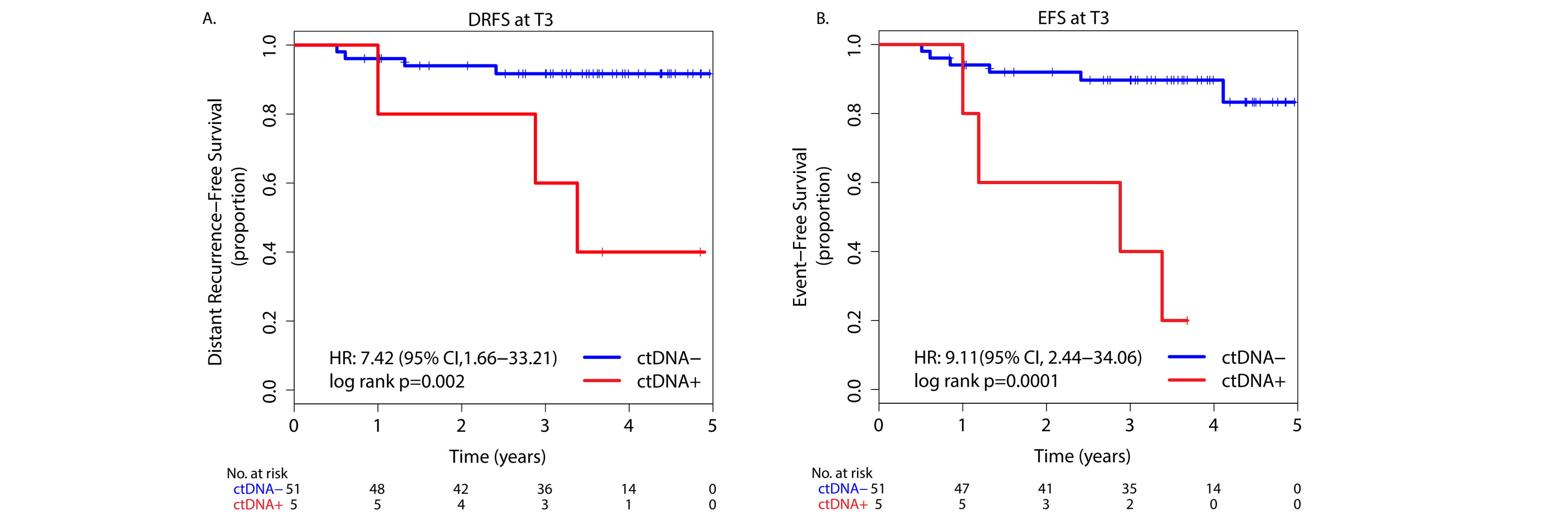
A. Distribution of mean Fisher-transformed Pearson correlation coefficients between serial measurements of ctDNA (mean VAF) and MRI (FTV, functional tumor volume). The Monte Carlo method was used to generate simulated data (1000 random permutations of sample labels). **B-D.** Representative cases showing similarities in longitudinal trajectories of ctDNA (number of mutant molecules per mL of plasma) and FTV (cm^3).

Figure 5. Early Clearance of ctDNA Predicts Response to Neoadjuvant Therapy



A. Proportion of patients according to early patterns of ctDNA dynamics between T0 and T1. **B-C.** For patients who are ctDNA+ at T0 (red box), early clearance of ctDNA predicted pCR (OR=3.38; LR p=0.040) and RCB 0/1 (OR=3.56; LR p=0.028).

Figure 6. Failure to Clear ctDNA After Neoadjuvant Therapy is Significantly Associated with Poor Clinical Outcomes



A. Distant recurrence-free survival (DRFS), **B.** Event-free survival (EFS) according to ctDNA detection after neoadjuvant therapy (T3).

Conclusions

- We demonstrate the feasibility of ctDNA detection in high-risk early stage breast cancer treated with neoadjuvant therapy using multiplex PCR/NGS analysis of 16 patient-specific targets.
- ctDNA positivity was significantly associated with increased tumor burden (by clinical and MRI examination), more aggressive tumor biology (as reflected in higher Mammaprint scores and grade) and subtype (HER2+ and TNBC).
- Dynamics of ctDNA levels during neoadjuvant therapy reflected changes in tumor responses as measured by MRI.
- Early clearance of ctDNA predicted response to neoadjuvant therapy.
- The presence of ctDNA after neoadjuvant therapy is significantly associated with poor distant recurrence-free survival (HR: 7.42; 95% CI,1.66–33.21; p=0.002) and event-free survival (HR: 9.11; 95% CI, 2.44–34.06; p=0.0001).

References

1. Abbosh C et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature*. 2017;545(7655):446-451.
2. Jamal-Hanjani M et al. Detection of ubiquitous and heterogeneous mutations in cell-free DNA from patients with early-stage non-small-cell lung cancer. Ann of Oncol. 2016;27(5):862-867.

Patient Advocate's Perspective

"Undergoing multiple core biopsies to determine if cancer has progressed in the neoadjuvant setting is concerning to patients due to pain during and post procedure, and time to heal the biopsy site. In addition, the biopsy site may not represent the diversity of the tumor burden so may not suffice as a predictor of the most appropriate therapeutic intervention. Breast cancer may also be spreading beyond local tumor tissue and invading other organs. Liquid biopsies (via blood draws) could be a less invasive, real time, alternate to multiple neoadjuvant biopsies while also identifying metastatic potential. ctDNA liquid biopsies will provide supplemental information to imaging and limit the number of pathological studies required to customize therapies." -Amy L. Delson, UCSF Breast Science Advocacy Core

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I-SPY2 Trial

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