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Clearance of ctDNA in triple negative and Her2 positive breast cancer patients during neoadjuvant treatment is correlated with pathological complete responses

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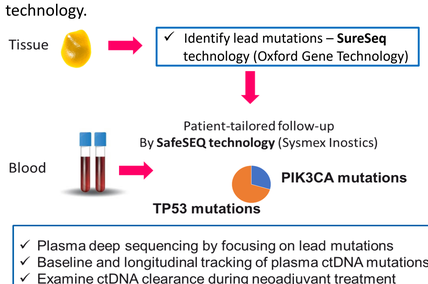
BACKGROUND

Neoadjuvant treatment (NAT) is being used widely to eliminate tumor burden in breast cancer (BC) patients; the primary goal of such treatments is to reduce the cancer prior to surgery. Although the standard of care is to perform surgery of primary BC after NAT, it is well known that for certain patients achieving clinical complete response (cCR) and pathological complete response (pCR), surgery following such treatment may be unnecessary.

Neoadjuvant therapy to shrink tumor — Surgical resection, local radiation — Adjuvant therapy to eradicate microscopic occult disease

However, "overtreatment" is common

Previous studies have shown that levels of circulating tumor DNA (ctDNA) during therapy and post-surgery can stratify patients that exhibit effective responses vs. those showing minimal residual disease. In this study, we performed longitudinal tracking of plasma TP53 and PIK3CA mutations pre-specified from NGS analysis of tumor tissue specimens from HER2-positive (HER2) and triple negative (TN) BC patients. The primary objective of this study was to assess ctDNA clearance during NAT as a correlate to effective response to treatment, as benchmarked by clinical complete response (cCR) and pathological complete response (pCR). To accomplish this, a prospective study was conducted to identify patient-specific PIK3CA and TP53 mutations in tissue using SureSeq NGS technology, which could then be used to track the presence/absence of mutations prior to, during, and following NAT using Sysmex SafeSEQ technology.



- ✓ Plasma deep sequencing by focusing on lead mutations
- ✓ Baseline and longitudinal tracking of plasma ctDNA mutations
- ✓ Examine ctDNA clearance during neoadjuvant treatment

PATIENTS AND METHODS

In total, 29 TN and HER2-positive BC patients were examined in this study. Tumor tissue samples from all patients were first examined for mutations in both the PIK3CA or TP53 genes using SureSeq technology (Oxford Gene Technology) after isolation of DNA using Qiasymphony DNA Tissue Kits. Individualized plasma ctDNA detection assays using SafeSEQ technology (Sysmex Inostics) were then applied to detect the specific mutations identified in tissue. Mutation detection was carried out in serial plasma samples in each BC patient at baseline prior to NAT, at treatment mid-point, and at post-treatment immediately prior to surgery. ctDNA analyses were performed only on patient samples in which a specific TP53 or PIK3CA mutation had been initially identified in that patient's tissue. All patients underwent a surgical excision after NAT. Patient characteristics are shown below in **Table 1**.

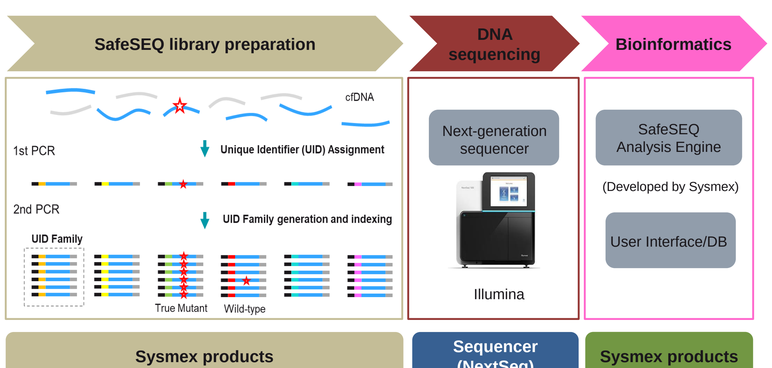
Patients characteristics

Median age 57y (31-88)

Clinical staging	N	%
T: cTx	1	3
cT1	3	11
cT2	18	64
cT3	3	11
cT4	3	11
N: cN0	12	43
cN1	9	32
cN2	1	3
cN3	6	22

Histological type	N	%
Inv. carcinoma NST	24	86
Inv. carc. w/ apocrine diff.	3	11
Inv. carc. w/ mucinous diff.	1	3
Tumor grade		
2	10	36
3	18	64
Molecular subtype		
Luminal B-HER2 positive	4	14
Triple negative	13	47
HER2-enriched	11	39

pCR	N	%
Yes	19	68
No	8	29
Pending	1	3
cCR		
Yes	16	57
No	12	43



RESULTS

- Of the 29 patients, 20 (69%) were found to have TP53 and/or PIK3CA mutations in tissue, as assessed by SureSeq.
- Of these 20 BC patients with mutations identified in tissue, 19 (95%) and 6 (30%) were found to have at least 1 TP53 or PIK3CA mutation, respectively, with 6 patients (30%) being positive for both TP53 and PIK3CA mutations. PIK3CA mutations were found predominantly in HER2-positive BC patients (37.5%), whereas PIK3CA mutations were found in only 7.7% of TN BC patients. In contrast, the overwhelming majority of TN BC patients (84.6%) showed at least one TP53 mutation.
- Of the 20 patients with TP53 and/or PIK3CA mutations identified in tissue, 17 (85%) showed detectable mutations using SafeSEQ in the plasma sample taken at baseline.
- The 17 patients having ctDNA detected at baseline were tracked longitudinally during NAT to correlate presence of ctDNA with efficacy of treatment (see **Table 2**). 12/17 patients attained clinical complete response (cCR) following NAT (8 TN BC; 4 HER2+), as indicated in green in **Table 2**. In contrast, 5 patients did not achieve cCR (3 TN BC; 2 HER2+), indicated in pink in **Table 2**.
- Of 15 patients with plasma samples available at mid-treatment, 5 (33%) showed detectable ctDNA (3 cCR patients vs 2 non-cCR patients).
- Immediately prior to surgery, 16/17 patient plasma samples were available for testing. Only 3 of these patients (18.8%) showed detectable ctDNA, and, importantly all 3 did NOT show cCR. Interestingly, none of the 11 cCR patients whose plasma was evaluated at surgery showed detectable ctDNA.
- Finally, in 37 plasma samples collected from the 17 patients over 3 time points post-surgery, no sample showed detectable ctDNA.

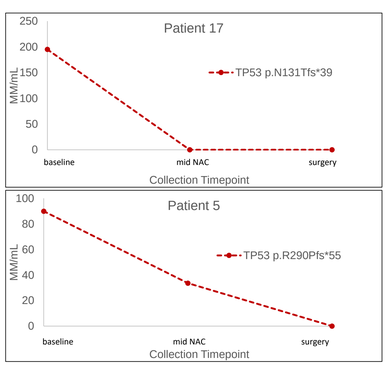


Figure 1. Representative plots of longitudinal ctDNA tracking. Two patients (Patient 17 and 5) show complete clearance of TP53 mutations in plasma before surgery, which is associated with cCR to NAT.
Mutant Molecules/mL (MM/mL)

Patient ID	Tumor type	cCR	pCR	ctDNA results: Baseline plasma	Mid-NAT plasma	Surgery plasma	10w post-surgery plasma	6m post-surgery plasma	12m post-surgery plasma
1	TNBC	yes	yes	TP53 p.R273P					
4	TNBC	yes	yes	TP53 p.R110Pfs*39; TP53 p.R175H					
5	HER2	yes	yes	TP53 p.R290Pfs*55	x				
6	TNBC	yes	yes	TP53 p.Q144*			NA		
9	TNBC	yes	yes	TP53 p.R273L	NA		NA		
12	HER2	yes	yes	TP53 p.M237I; PIK3CA p.E542K		NA			
14	TNBC	yes	yes	TP53 p.C176F	NA			NA	NA
11	TNBC	yes	yes	TP53 p.R175H	x				
17	TNBC	yes	yes	TP53 p.N131Tfs*39				NA	NA
30	HER2	yes	yes	TP53 p.R213*; PIK3CA p.H1047R					NA
28	TNBC	yes	yes	TP53 p.R273H					NA
29	HER2	yes	yes	TP53 p.K139_V143del	x				NA
10	HER2	no	no	TP53 p.V173L; PIK3CA p.H1047R					NA
7	TNBC	no	no	TP53 p.R110P		x			NA
19	TNBC	no	no	TP53 p.Y163*					NA
26	HER2	no	no	TP53 p.D281V; PIK3CA p.H1047R	x	x			NA
31	TNBC	no	no	TP53 p.S127F	x	x			NA

Table 2. Results of longitudinal ctDNA analysis. TP53 and PIK3CA mutations identified in plasma at baseline (pre-NAT) are listed under "baseline plasma" in the table above. These mutations were assessed again at multiple time-points: mid-NAT, post-NAT ("surgery"), and at 3 time-points post-surgery (10w, 6m, and 12m). Samples with ctDNA detected are indicated by an "x", whereas samples determined to be ctDNA negative are indicated by blank cells. "NA" denotes samples that were not available for analysis. *TNBC patients indicated in blue text; HER2+ patients indicated in black text. Green highlighted cells indicate patients with cCR; Pink highlighted cells indicate patients with NO cCR.*

CONCLUSIONS

- SafeSEQ NGS technology identified 17/20 (85%) BC patients with detectable mutations in plasma at baseline, enabling the vast majority of patients to be tracked via liquid biopsy during/following NAT.
- The absence of ctDNA following NAT was observed in 100% of cCR patients, indicating a favorable correlation with effective NAT response; moreover, the presence of ctDNA following NAT may associate with the lack of clinical/pathological complete response in BC patients.
- These findings prompt further studies/trials to investigate the value of ctDNA to predict clinical/pathological response that may aid in determining whether surgery is really necessary following neoadjuvant treatment.