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Use of Serial Multi-Template Liquid Biopsies in Triple Negative Breast Cancer Monitoring

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Background

- Triple negative breast cancer (TNBC) remains the most aggressive sub-type of breast cancer in which up to 1/3 of all patients will relapse early and distantly.
- There remains no proven method to monitor and detect early recurrence.
- This ongoing study examines the use of serial longitudinal liquid biopsies using a multi-template approach that identifies and analyzes cell-free DNA (cfDNA) and circulating tumor cells (CTCs) as a solution.

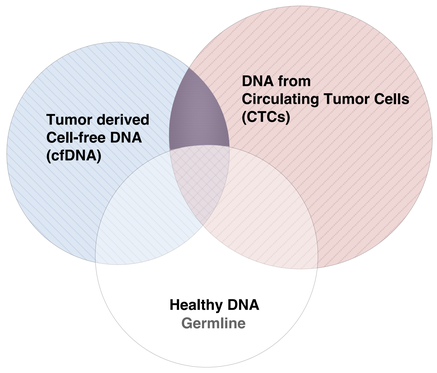


Figure 1.
Multi-template approach via ClearID liquid biopsy

ClearID was designed to analyze both circulating tumor cells (CTCs) and cfDNA alongside the patient's healthy DNA (germline) to provide a clear distinction between tumor and germline.

Results

163 patients (**77.25%**)	had evidence of mutations in at least one sample
48 patients (**24.3%**)	had **no** evidence of mutations in any sample
59 patients (**30.10%**)	had mutations on **two consecutive** blood draws
18 patients (**9.18%**)	had the **same** mutation on **two consecutive** blood draws
1 patient (**0.51%**)	had a mutation on **both** CTC and cfDNA on two consecutive draws

Results: Recurrences

Recurrences thus far	**11** patients (**5.68%**)
Types of recurrences	10 breast, 1 ovarian
Average time from treatment to time of recurrence	**21 months**

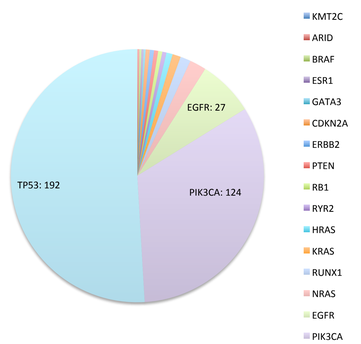
All recurrences to date have had at least two consecutive draws with mutations in either cfDNA, CTCs, or both.

36%	have had the same mutation on consecutive draws
18%	have had the same mutation on consecutive draws in both cfDNA and CTCs.

Total Number of Samples	**826**
% of cfDNA samples with mutations	22.84%
% of CTC samples with mutations	19.84%
% of samples with mutations on both cfDNA and CTCs	4.28%

Table 3. Sample collection characteristics

TNBC Mutation Frequencies as of May 15th, 2017 (excluding mutations expressing p.C1003* and p.L56S)



TP53 Mutation Expressed Protein Frequencies as of May 15th, 2017

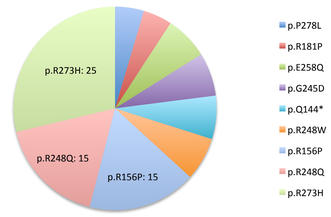


Fig 2 and 2a.
Most commonly seen mutations

Methods

- **211** patients with confirmed diagnosis of triple negative breast cancer and within 3 years of completion of therapy and in full remission were enrolled. **ClearID** liquid biopsies were performed every three months to look for cancer specific genomic mutations not seen in germline controls, but in cfDNA and CTCs using a custom 27-gene breast cancer panel.
- Reporting threshold was 1.0%.
- Prior validation of our gene panel demonstrated a false positive rate of 0.001 - 0.0007% in normal cfDNA and cell-based controls.

AKT1	3	*BRAF*	3	*EGFR*	12	*GATA3*	16	*KRAS*	4	*MAP2K4*	15	*NCOR1*	102	*PTEN*	15	*RYR2*	47
ARID1A	6	*CDH1*	45	*ERBB2*	7	*HRAS*	2	*LRP1*	68	*MAP3K1*	19	*NRAS*	2	*RB1*	35	*RYR3*	20
ATM	4	*CDKN2A*	6	*ESR1*	27	*KMT2C*	11	*LRP2*	101	*MED12*	7	*PIK3CA*	14	*RUNX1*	11	*TP53*	19

Table 1. Custom 27-gene panel showing number of amplicons per gene

- 621 amplicons targeting 27 genes
- Coverage of 52.5kb
- FFPE, CTC and cfDNA compatible
- Average size: 134bp
- Range size: 107-202bp

Patients (N)		**211**
Median Age (Years)		53 (28 -84)
Average time from Treatment		25 mos.
Stage	I	35.7%
	II	45.2%
	III	16.6%
	IV	0.01%
	NA	0.02%
Average # of blood draws		4

Table 2. Patient demographics

Conclusions

- **The majority (77.25%) of TNBC patients in our study post-treatment were found to have genomic mutations in at least one liquid biopsy sample - either cfDNA, CTC, or both.**
- **Of those patients found to have a mutation on any one sample, only 6.7% developed a recurrence and 93.3% remain NED.**
- **Unlike patients who remained NED, patients who recurred displayed persistent evidence of mutations in cfDNA, CTCs, or both in serial samples.**
- **When patients have any mutations on two successive draws, they are 2.7x's more likely to recur compared to those with only one positive sample.**
- **When patients have the same mutation on two successive samples, they are 3.3x's more likely to recur compared to those with only one positive sample.**
- **This study highlights the need for serial longitudinal monitoring using a multi-template approach to better fully understand patient specific tumor biology and kinetics.**
- **This study remains open as we anticipate several more recurrences in the coming months in patients currently exhibiting the same mutation in two consecutive samples who remain NED at this time.**

Presented by Paul Y. Song, MD