Molecular profiling of serial liquid biopsy specimens utilizing cell free DNA and circulating tumor cells in TBCRC041: A phase II study of alisertib in endocrine resistant metastatic breast cancer

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Background

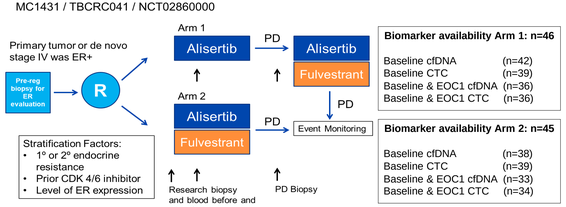
- In ER+ breast cancer models, Aurora A kinase (AURKA) activation is associated with expansion of CD44^{+}/CD24$^{low/-}$ tumor initiating cells, down-regulation of ER and endocrine therapy resistance. Alisertib, a selective AURKA inhibitor, can restore ER expression and endocrine sensitivity.
- In TBCRC041, a randomized phase 2 trial of alisertib ± fulvestrant in women with HR+/HER2- MBC demonstrated promising clinical activity for alisertib in patients with endocrine- and CDK 4/6 inhibitor-resistant MBC (JAMA Oncol. 2023;9:815).
- Here, we explore the association of cfDNA and CTCs with progression free survival in pretreatment baseline and end of cycle 1 (EOC1) biospecimens from TBCRC041 participants.

Methods

- Plasma cfDNA was sequenced using the Guardant INFINITY platform, which includes genomic and epigenomic analysis, reporting out genomic alterations and methylation tumor fraction (mTF). Pathogenic variants were determined using publicly available databases and Mayo Clinic annotation pipelines.
- CTCs were identified as nucleated, EpCAM+/ cytokeratin+/ CD45- cells and assessed for ER/HER2 staining (RareCyte, Seattle).
- PFS was defined as the time from randomization to progression or death.

Study Design

MC1431 / TBCRC041 / NCT02860000



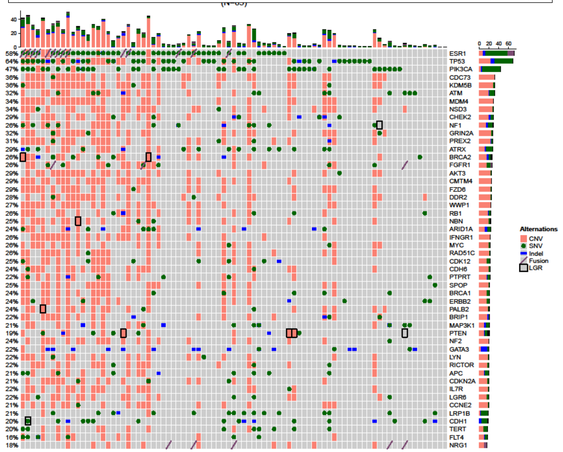
Demographics

Table 1. Patient Characteristics

	Arm 1 Alisertib (n=42)	Arm 2 Alisertib + Fulvestrant (n=38)
Age		
18-39	3 (7.1%)	4 (10.5%)
40-59	12 (28.6%)	17 (44.7%)
60-74	23 (54.8%)	15 (39.5%)
75 or older	4 (9.5%)	2 (5.3%)
Race		
White	37 (88.1%)	34 (89.5%)
Black/African American	3 (7.1%)	2 (5.3%)
Asian	1 (2.4%)	0
Not reported	1 (2.4%)	2 (5.3%)
Hispanic Ethnicity	1 (2.3%)	4 (10.5%)
ECOG Performance Status		
0	28 (66.7%)	21 (55.3%)
1	14 (33.3%)	16 (42.1%)
	0	1 (2.6%)
Any prior chemotherapy		
(Neo)adjuvant setting	25 (56.8%)	24 (57.1%)
Metastatic setting	21 (47.7%)	28 (66.7%)
Prior lines of chemotherapy in MBC		
0	23 (54.8%)	12 (31.6%)
1	7 (16.7%)	15 (39.5%)
2	12 (28.6%)	11 (28.9%)
Any prior endocrine therapy		
(Neo)adjuvant setting	27 (64.3%)	24 (63.1%)
Metastatic setting	42 (100%)	38 (100%)
Endocrine resistance		
Primary	9 (21.4%)	8 (21.1%)
Secondary	33 (78.6%)	30 (78.9%)
Metastatic tumor ERα expression		
Positive (≥ 10%)	30 (71.4%)	28 (73.7%)
Borderline (1 - 9.9%)	3 (7.1%)	3 (7.9%)
Negative	4 (9.5%)	2 (5.3%)
Insufficient tissue	5 (11.9%)	5 (13.2%)

Baseline landscape of somatic genetic alterations in ctDNA

Figure 1. OncoPrint of top 50 genes with somatic variants at baseline (n=80)



- Most patients had detectable ctDNA (78/80, 98%)
- *ESR1*, *PIK3CA*, and *TP53* were the most commonly altered genes
- High frequency of coamplification of *CDC73, KDM5B* (1q31.2) and *MDM4* (1q31.2) was observed.
- Fusions were detected in *ESR1, FGFR1,* and *NRG1*.

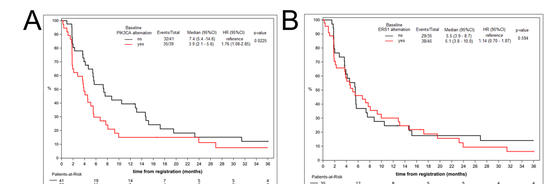
Baseline Somatic Variants (SNV and CNVs)

Table 2. Association of baseline somatic variants (SNV and CNVs) with PFS in specific genes of interest.

	percent positive (n=86)	results of conditional Cox model for progression-free survival		
		events	HR (95%CI)	p-value
ESR1	45 (56.3%)	67	1.14 (0.70 – 1.87)	0.5944
PIK3CA	39 (48.8%)	67	1.76 (1.08 – 2.85)	0.0225
PIK3CA +/- AKT1 +/- pTEN**	48 (60.0%)	67	2.08 (1.26 – 3.45)	0.0045
AKT1 pTEN	9 (11.3%) 13 (16.3%)			

**Among the 48 patients with a PIK3CA +/- AKT1 +/- pTEN mutation, only 9 did not have a PIK3CA mutation (4 had an AKT1 mutation and 5 a pTEN mutation). Univariate PFS analysis was performed for cohorts >10.

Figure 2. Baseline *PIK3CA* alteration was associated with shorter PFS with alisertib (A), while baseline *ESR1* alterations did not impact PFS (B).



Circulating tumor cells (CTCs)

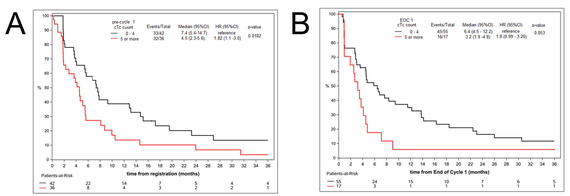
Median of 4 CTCs, 25th-75th IQR 0-36, Range of 0 – 83,298 in baseline samples.

Median of 2 CTCs, 25th-75th IQR 0-6, Range of 0 – 104,292 in EOC1 samples.

Table 3. Change in CTCs after treatment exposure.

		EOC1 CTC count (per 7.5 mL)			
		Arm 1 (n=36)		Arm 2 (n=34)	
		0 – 4	5 or more	0 – 4	5 or more
Baseline CTC count (per 7.5 mL)	0-4	16 (44.4%)	1 (2.8%)	19 (55.8%)	0
	5 or more	10 (27.8%)	9 (25.0%)	7 (20.6%)	8 (23.5%)

Figure 3: Baseline elevated CTCs was adversely associated with PFS (A). High CTCs at EOC1 associated with decreased PFS (B), though not statistically significant (p=0.53).



Methylated tumor fraction percentage in ctDNA (mTF%)

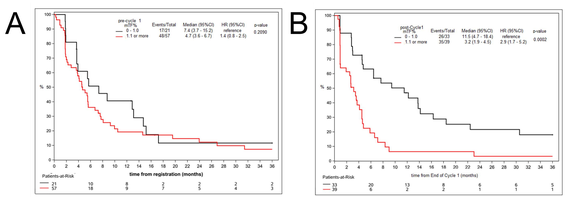
	EOC1 methylation percentage							
	Arm 1 (n=36 pairs)				Arm 2 (n=33 pairs)			
	0 – 1.0%	1.1-5.0%	5.1-10%	≥ 10%	0 – 1.0%	1.1-5.0%	5.1-10%	≥ 10%
0 – 1.0%	10 (27.8%)	1 (2.8%)	0	0	7 (21.2%)	1 (3.0%)	1 (3.0%)	
1.1-5.0%	3 (8.3%)	2 (5.6%)	1 (2.8%)	0	3 (9.1%)	2 (6.1%)	0	1 (3.0%)
5.1-10%	2 (5.6%)	2 (5.6%)	0	1 (2.8%)	2 (6.1%)	2 (6.1%)	0	1 (3.0%)
≥ 10%	1 (2.8%)	5 (13.9%)	1 (2.8%)	7 (19.4%)	0	5 (15.2%)	2 (6.1%)	6 (18.2%)

Baseline mTF%

Table 4. Change in mTF after treatment exposure.

- The median mTF% at baseline was (7.1%), IQR 0.9 – 21.5%, range 0 to 92.4%.
- The median mTF% at EOC1 was (1.3%), IQR 0.2 – 6.3%, range 0 to 56.5%.

Figure 4. Baseline elevated mTF% above 1% was not prognostic (A), whereas EOC1 methylated tumor fraction of <1% is associated with improved PFS (B).



Conclusions

- Among patients receiving alisertib ±fulvestrant, PFS varied based on the pretreatment *PIK3CA* mutation status.
- CTC enumeration and methylated tumor fraction in ctDNA provided complementary prognostic information. Baseline elevation of CTCs and mTF of <1% at EOC1 were associated with significant differences in PFS.
- Further analysis in ctDNA and CTCs to identify predictors of alisertib response is ongoing.
- Further development and evaluation of alisertib in ER+/HER2- metastatic breast cancer is planned (NCT06369285).

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