A circulating tumor fraction DNA biomarker response stratified by *ESR1* mutation status correlates with overall survival in patients with ER+ HER2- metastatic breast cancer

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Published Abstract Number: 3676

INTRODUCTION

- *ESR1* mutations (*ESR1*m) are acquired resistance mutations that evolve during treatment with aromatase inhibitor (AI) therapy in approximately 40% of ER+ HER2- metastatic breast cancer (mBC) patients and are indicative of poor outcomes.
- Patients with *ESR1*m acquired on AI may experience improved treatment response if switched to selective estrogen receptor degraders (SERDs) like fulvestrant.
- Circulating tumor DNA (ctDNA) analysis can detect the emergence of *ESR1* mutations and simultaneously determine molecular response to therapy through changes in quantitative ctDNA tumor fraction (TF).
- We used real-world (RW) data to study the combined effect of *ESR1* mutation status and ctDNA TF dynamics as a prognostic for real-world overall survival in a cohort ER+ HER2- mBC patients.

METHODS

Selection of RW mBC cohort:

- Deidentified ER+, HER2- metastatic breast cancer patient records from the Tempus clinicogenomic database.
- Had a liquid biopsy up to one year prior to or 3 months post treatment initiation of the first AI-containing regimen (anastrozole, letrozole, or exemestane) a patient received.
- Second liquid biopsy ≥ 14 days after start of AI therapy (n=87 patients).

***xM used for Treatment Response Monitoring*:**

- ctDNA profiling was performed using the 105-gene Tempus liquid biopsy assay at two timepoints.
- TF was calculated using an ensemble algorithm that dynamically incorporates copy number variant (CNV) data, and somatic and germline VAFs to account for observed failure modes from single input methodologies and quantitative changes in TF across timepoints was calculated.
- Molecular Responder (MR): ≥50% reduction in TF from first to second test or TF was above the limit of blank (LOB) at the first test and below the limit of blank (b-LOB) in the second test.
- Molecular Non-responder (NR): < 50% reduction in TF from first to second test or TF was b-LOB at first test and above the LOB at the second test.
- Consistently b-LOB: TF was b-LOB at both tests.
- Patients were considered to be negative (WT) for *ESR1* mutations if no *ESR1* mutation was detected in either the first or second test.
- Patients were considered to be *ESR1* mutated (*ESR1m)* if an *ESR1* mutation was detected in the second test only, or if the summed variant allele frequency (VAF) of all *ESR1* variants detected increased from the first test to the second test.
- rwOS was defined as the interval from AI start to death, censored on the last known physician follow-up.
- Cox proportional hazards models were fitted to evaluate the relationship between *ESR1*and MR/NR status with rwOS using risk set adjustment.

SUMMARY

- xM used for treatment response monitoring (TRM) is a ctDNA algorithm that quantifies changes in ctDNA tumor fraction (molecular response) and simultaneously identifies *ESR1* resistance variants.
- In a heterogeneous, real-world cohort of metastatic ER+ HER2- breast cancers the combined effect of molecular response and *ESR1* mutation status was associated with rwOS outcomes.

RESULTS

Overview of ER+ HER2- RW cohort

Characteristic	Data available (N)	*ESR1*m N (%)	*ESR1* WT N (%)	p-value[2]
Total	87	25	62	
Female	87	25 (100%)	61 (98%)	>0.9
Age	87	58 (52, 66)[1]	60 (50, 67)[1]	0.6
Self-reported Race	75			0.2
American Indian or Alaska Native		0 (0%)	1	
Asian		1 (1.9%)	1 (4.8%)	
Black or African American		0 (0%)	7 (13%)	
Other/not stated		4 (16%)	9 (17%)	
White		16 (76%)	36 (67%)	
Self-reported Ethnicity	35			>0.9
Hispanic or Latino		1 (10%)	4 (16%	
Not Hispanic or Latino		9 (90%)	21 (84%)	
ECOG	46			
0-1		11 (100%)	34 (97%)	
2-4		0 (0%)	1 (2.9%)	
Menopause	45	10 (59%)	26 (78%)	>0.9
Line of AI therapy*	54			0.8
1-2		8 (53%)	22 (56%)	
3+		7 (47%)	17 (44%)	
TF Molecular Response	87			0.3
Molecular Responder		4 (16%)	18 (29%)	
Molecular Non-Responder		18 (72%)	33 (53%)	
Below limit of blank		3 (12%)	11 (18%)	

Table 1. Demographic and clinical characteristics of mBC ER+ HER2- RW cohort. [1]Age summarized as median (IQR); [2] p-values are based on Fisher's exact test, with the exception of age, which uses the Wilcoxon rank sum test. *Line of therapy of AI closest to first liquid biopsy.

Individual Patient Trajectories

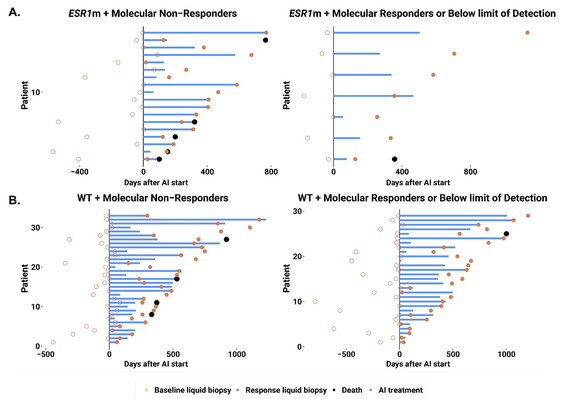

Figure 1. Swimmer plot showing timing of liquid biopsies, AI treatment duration, last known follow-up, and survival for (A.) patients with *ESR1* mutations and (B.) patients without *ESR1* mutations.

*ESR1*m patients had shorter rwOS compared to *ESR1* WT patients

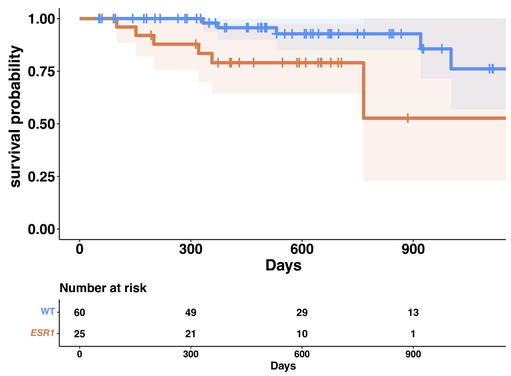

Figure 2. Kaplan-Meier analysis of rwOS stratified by *ESR1* mutation status. Patients with *ESR1*m experienced shorter rwOS than patients WT for *ESR1* across xM for TRM assays. Risk set adjusted Cox PH *ESR1*m **HR=4.11** (95% CI:1.19-14.17), **p-value=0.025**.

Patients with combined ESR1m + NR ΔctDNA TF experienced shorter rwOS compared to MR ΔctDNA TF patients with WT ESR1

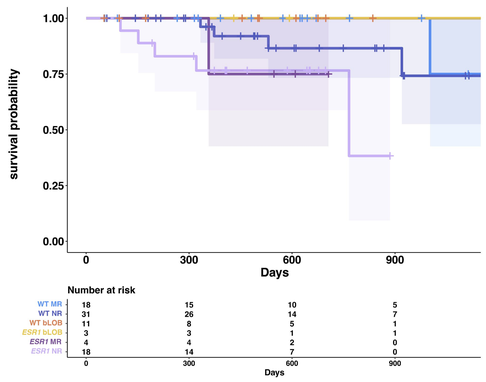

Figure 3. Kaplan-Meier analysis of rwOS stratified by a combined phenotype of *ESR1* mutation status and TF molecular response. Risk set adjusted Cox PH results (WT_MR as reference): for *ESR1* NR HR=11.95 (95% CI: 1.16-123.02), p-value=0.037; for *ESR1* MR (WT MR as reference) HR=9.06 (0.48-171.0), p-value = 0.142; for WT NR HR=2.67 (0.29-24.11), p-value = 0.383. Patients with TF consistently b-LOB (11 WT, 3 *ESR1*m) across tests have no recorded death events.

Limitations and Future Directions

- These analyses were performed in a small, real-world cohort that was heterogeneous with respect to timing of liquid biopsies relative to start and line of therapy of aromatase inhibitor treatment. In particular, given the wide range of time between liquid biopsies and incomplete longitudinal medication information, patients may have changed therapies between tests.
- A larger prospective clinical study is needed to validate these findings to assess if xM for TRM can identify early response to AI therapy.

ACKNOWLEDGMENTS

We thank Vanessa Nepomuceno from the Tempus Science Communications team for data visualization guidelines and poster review.

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