PS2-11-03: FISH-Positive Circulating Tumor Cells Persist After Effective Therapy in ER+ Breast Cancer: Insights Into Genomic Mechanisms of Endocrine Resistance

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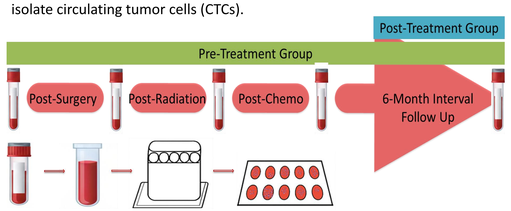
Introduction:

Estrogen receptor (ER) positive breast cancer is commonly treated with endocrine therapy, yet resistance to estrogen deprivation remains a significant clinical challenge. Using custom FISH probes designed to detect tumor-specific genomic alterations, we identified circulating tumor cells (CTCs) of true tumor origin in early-stage luminal breast cancer patients. Longitudinal analysis revealed that 80% of patients had FISH-positive CTCs after completing therapy and while on antiestrogen treatment, despite no clinical evidence of disease. This finding suggests that standard endocrine therapy is insufficient to eradicate all malignant clones, indicating the persistence of therapy-resistant tumor cells.

Methods:

Patient Selection Two ER+/HER2– breast cancer cohorts were enrolled under IRB approval. The Pre-Treatment (PrT) cohort included newly diagnosed stage I–III patients followed from diagnosis through therapy. The Post-Treatment (PoT) cohort included stage I–III survivors who had completed treatment 1–10 years prior. Use of anti-estrogen therapy at enrollment varied.

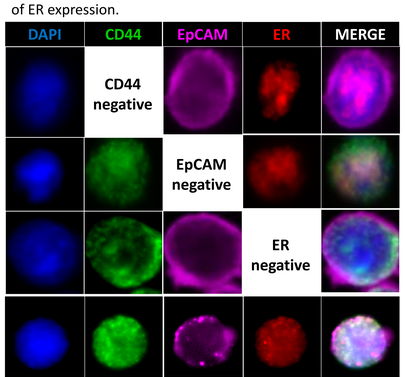
CTC Collection Whole blood samples were collected at defined intervals: pre-treatment, post-surgery, post-radiation, post-chemotherapy, or at 6-month follow-up for PoT patients. Size-based filtration of whole blood was used to isolate circulating tumor cells (CTCs).



Parent Tumor Genomic Profiling Primary tumor DNA underwent somatic copy-number analysis using the Affymetrix Oncoscan microarray platform (Mayo Clinic). Recurrent genomic alterations were categorized as proliferation drivers, established estrogen-resistance mechanisms, or regulators of ER transcription.

Custom FISH Probe Development Four to five tumor-specific FISH probes were designed from each patient's genomic alterations. Probes were applied to PrT and PoT blood samples. Cells with two probe copies were considered normal; copy-number gain or loss defined a CTC of tumor origin.

CTC Immunofluorescence Multiplex immunofluorescence staining included EpCAM, ER, and CD45 (leukocyte marker) or CD44 (mesenchymal marker). CTCs were identified as EpCAM$^+$/ER±/CD45$^-$ or EpCAM$^-$/CD44$^+$/ER±. Staining was used to assess epithelial and mesenchymal phenotype and heterogeneity of ER expression.



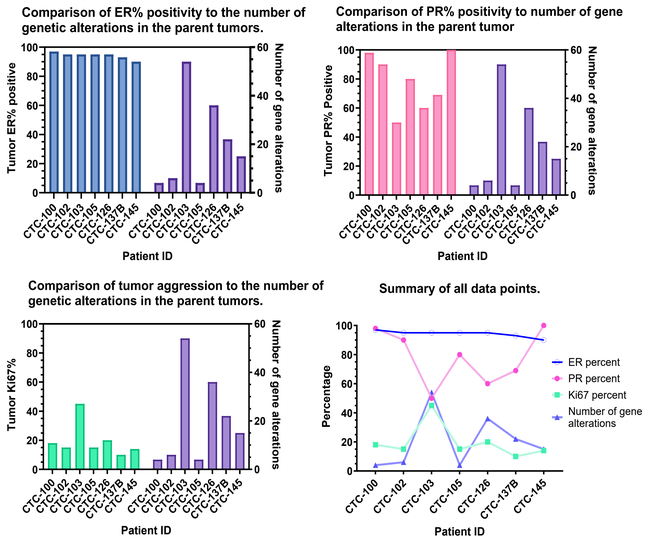
Results:

Figure 1. Patient demographics.

ID	Demographics		Parent Tumor Characteristics					Number of gene alterations in parent tumor
	Race	Age	AJCC 8th ed Cancer stage	Cancer subtype	% ER+	% PR+	% Ki67	
Luminal A								
CTC-100	AA	64	1A	Ductal	97	98	18	4
CTC-102	AA	59	1B	Mixed	95	90	15	6
CTC-105	C	54	1A	Ductal	95	80	15	4
CTC-137B	C	67	1B	Ductal	96	69	10	22
CTC-145	C	51	1A	Mixed	90	100	14	15
Luminal B								
CTC-103	AA	69	1A	Ductal	95	50	45	54
CTC-126	C	31	1A	Ductal	95	60	20	36

All enrolled patients had early-stage, ER+ parent tumors with variable degrees of gene alterations. Although the cohort size is small, the number of genomic alterations aligned with low-risk Luminal A tumors, while those with more numerous alterations corresponded to higher-risk Luminal B disease.

Figure 2. ER, PR, and Ki67 expression do not correlate with genomic alteration burden in parent tumors.



All parent tumors were strongly ER-positive; however, ER% showed no correlation with the number of gene alterations (Spearman r = –0.3479, p = 0.4571). Ki67% likewise showed no correlation (Spearman r = 0.3364, p = 0.4536). In contrast, PR% demonstrated a moderate inverse relationship with mutation burden (Spearman r = –0.7207), although this trend did not reach statistical significance (p = 0.0778).

These results indicate that high ER expression does not predict lower genomic instability nor avoidance of ER-resistance mechanisms. The observed trend suggests that PR loss—reflecting impaired ER signaling—may better identify tumors with a higher number of genomic alterations and a greater likelihood of harboring endocrine-resistant biology.

Figure 3. Immunofluorescence revealed heterogeneity in ER and EpCAM expression.

Patient ID	CTCs Present by FISH?	CTCs Present by IF?	ER- CTCs by IF	ER+ CTCs by IF	EpCAM- CTCs by IF
CTC-126	NA	Yes; 54 of 2978 (2%)	79.63%	3.70%	16.67%
CTC-137B	Yes; 5 of 247 (2%)	Yes; 62 of 5863 (1%)	93.55%	1.61%	4.84%
CTC-145	Yes; 15 of 383 (4%)	Yes; 28 of 553 (5%)	78.57%	7.14%	14.29%

Although all tumors were highly ER+, CTCs exhibited varying phenotypes; patients had both ER+ and ER- subpopulations, as well as CTCs without EpCAM. These findings not only support that underlying ER-resistance mechanisms may be at play but also allude to a mesenchymal phenotype which could correlate with more aggressive tumors.

Figure 4. Genomic Alterations Reveal Redundant ER-Resistance Pathways.

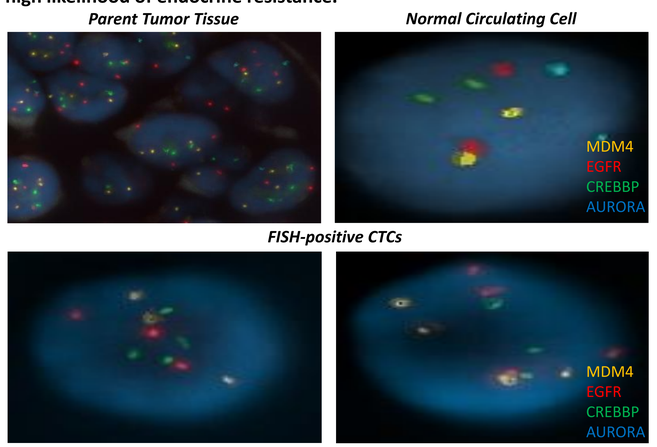
	Genes	CTC-100	CTC-102	CTC-103	CTC-105	CTC-126	CTC-137B	CTC-145
Proliferation drivers	*MCL1*						N	
	MDM4							
	NTRK1						N	
	SDHC							
	SDHD		N		N			N
	CHEK1		N	N	N			N
	BCL6	N	N		N		N	N
ER resistance genes	*ATM*		N		N			N
	PIK3CA	N						
	AKT3	N					N	
	EGFR	N	N		N	N		
	MTOR	N	N		N		N	N
ER transcriptional regulation genes	*ARID1A*	N	N		N		N	N
	CREBBP	N	N					
	SPEN	N	N		N		N	N

• N = normal copy number • Amplification • Loss • Mutation or Loss of Heterozygosity

Genomic profiling of parent tumors demonstrated that every tumor harbored more than one mechanism associated with ER resistance or proliferative escape, revealing extensive pathway redundancy. Alterations in **MDM4 and SDHC were present in 100% of tumors**. MDM4 suppresses p53-mediated cell-cycle arrest, allowing survival of genomically unstable, therapy-resistant clones. SDHC alterations disrupt mitochondrial metabolism, creating pseudohypoxia and metabolic reprogramming that reduce reliance on ER signaling and promote proliferation.

Additionally, **86%** of tumors showed alterations in **MCL1, NTRK1, and PIK3CA**, supporting anti-apoptotic signaling and ER-independent growth. Alterations in **ARID1A, SPEN, PIK3CA, and CREBBP** directly affected ER-regulated transcription through chromatin remodeling or co-activator dysfunction. **CREBBP** alterations were observed in **71%** of tumors, indicating widespread disruption of ER transcriptional control.

Figure 5. FISH analysis identifies heterogeneous, genomically altered CTCs with high likelihood of endocrine resistance.



Although parent tumors were strongly ER-positive, FISH revealed that they contained a heterogeneous mix of cells with multiple amplified genes associated with ER-resistance mechanisms. FISH analysis of blood samples detected CTCs in both pre-treatment and post-treatment patients, confirming that resistant clones circulate early and persist despite therapy. Importantly, CTCs displayed wide variability in the number and pattern of gene amplifications, with many showing multiple concurrent alterations. This marked genomic heterogeneity among CTCs indicates the presence of highly adaptable, endocrine-resistant subclones capable of surviving anti-estrogen therapy and potentially contributing to future recurrence.

Conclusion:

In this study of early-stage ER+/HER2– luminal breast cancers, parent tumors demonstrated extensive genomic heterogeneity, with every tumor harboring multiple proliferative drivers, redundant estrogen-resistance mechanisms, and disruptions in ER-regulatory pathways.

Hormone-receptor expression was uniformly high, yet ER% and Ki67% did not correlate with mutation burden, indicating that ER positivity does not reliably reflect underlying genomic instability or endocrine-resistant biology.

Custom FISH probes revealed that FISH-positive CTCs carried numerous, heterogeneous gene amplifications spanning multiple escape mechanisms, highlighting a circulating reservoir of highly adaptable, endocrine-resistant clones with proliferative potential.

Together, these findings demonstrate that endocrine therapy often fails to eradicate genomically diverse resistant populations and underscore the need for therapeutic strategies that target the multifaceted and overlapping mechanisms driving endocrine resistance and late recurrence in luminal breast cancer.

References

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