ASCO

Whole transcriptome analysis comparing HR+ HER2- breast cancer tumors from patients ≤ 50 years and > 50 years Cathy Graham¹, Douglas K. Marks², Nina D'Abreo², Sami Diab³, Vijayakrishna K. Gadi⁴, Midas M. Kuilman⁵, Andrea Menicucci⁶, Amy M. Truitt⁶, Shiyu Wang⁶, Patricia Dauer⁶, William Audeh⁶, FLEX Investigators' Group⁸

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BACKGROUND

- Recent prospective clinical trials have demonstrated a differential chemotherapy effect based on age (\leq 50 vs. > 50 years) or menopausal status (pre- vs. post-) in a genomic low risk group^{1,2}. However, it is unclear whether these differences are a direct anti-tumor effect of chemotherapy or a secondary ovarian suppression effect caused by chemotherapy.
- In the current study, we aimed to compare the biological characteristics of breast cancer tumors from patients aged \leq 50 years and from patients aged > 50 years using whole transcriptome analysis to provide insights into this differential chemotherapy response.

METHODS

FLEX Study: The FLEX study (NCT03053193) is an ongoing, prospective study of stage I-III breast cancer patients that receive the MammaPrint (MP) 70-gene signature test with or without the BluePrint (BP) 80-gene signature test and consent to clinically annotated gene expression data collection.

Patient Cohort: 3868 patients with HR+ HER2- tumors were evaluated, of whom 808 were aged \leq 50 years and 3060 were aged > 50 years. Clinical risk was assessed based on the MINDACT algorithm³. MP classified tumors as Low Risk (LR) or High Risk (HR). HR was stratified to H1 or H2; H2 exhibits a greater chemotherapy response^{4,5}. BP and MP classified tumors as a Luminal A-, Luminal B-, HER2-, or Basal-type.

Gene Expression Analysis: Differential gene expression analysis of microarray data was performed with the R package 'limma'. Older patients were randomly selected to obtain an equal sample size as younger patients. Differentially expressed genes (DEGs) were compared in five iterations in all samples, and in subgroups based on clinical risk, MP risk, and BP subtype. DEGs were considered significant if FDR < 0.05and fold change \geq 2.

Statistical Analysis: Differences in MP, BP, and clinical features were assessed by Chi-Squared, Fishers' exact test[†], or *t* test.

RESULTS

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BluePrint Image: Second system Secon	High 2, H2	106 (25%)	235 (18%)	(18%) p < 0.01	
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Luminal B-type 373 (46%) 1218 (40%) HER2-type 0 (0%) 5 (0.2%) Basal-type 52 (6%) 113 (3.7%)	Luminal A-type	383 (47%)	1724 (56%)		
HER2-type 0 (0%) 5 (0.2%) Basal-type 52 (6%) 113 (3.7%)	Luminal B-type	373 (46%)	1218 (40%)	n <0 001	
Basal-type 52 (6%) 113 (3.7%)	HER2-type	0 (0%)	5 (0.2%)	2%)	
	Basal-type	52 (6%) 113 (3.7%)			

Figure 1. Percentage of MammaPrint results and BluePrint subtypes by age group. (A) MP results as Low Risk versus High Risk. Within the High Risk group, women were categorized as H1 or H2 (B). (C) BP tumor subtypes in women ≤ 50 (left) and > 50 years (right).



Figure 2. Principal component analysis of the top 500 genes with the highest variance.





Gene	Function
AREG	An autocrine growth factor that interacts with EGF/TGF- α receptors to prom in invasive breast carcinomas.
CLIC6	A member of the chloride intracellular channel protein family, which regulate
CLEC3A	May play a role in cell adhesion, and promotes tumor progression and poor p
CYP4Z1	Part of the cluster of cytochrome P450 genes which are related to drug met which is regulated by glucocorticoid and progesterone receptors.
ESR1	Encodes estrogen receptor alpha (ER α), a transcription factor that is overexpr



Table 2. DEGs between \leq 50 versus > 50 in all samples, and in

subgroups based on clinical risk, MP risk, and BP subtype.

	Up/Down	Genes
	Upregulated	AREG, CLIC6
	Downregulated	CLEC3A, CYP4Z1, ESR1
k	Upregulated	CXCL13, AREG, CLIC6, PEG10
	Downregulated	CLEC3A, ESR1
k	Upregulated	CLIC6
	Downregulated	CLEC3A, CYP4Z1, ESR1
	Upregulated	AREG, CLIC6, CXCL13,
	Downregulated	CLEC3A, ESR1
Η1 d	Upregulated	AREG
	Downregulated	CLEC3A
e	Upregulated	AREG, CLIC6
	Downregulated	CLEC3A
	Upregulated	
	Downregulated	PDLIM3

note growth of normal epithelial cells. It is enriched

es chloride ion transport.

prognosis in breast invasive ductal cancer.

tabolism. It is overexpressed in breast cancer cells,

ressed in breast cancer.

RESULTS

- Approximately 81% of patients aged \leq 50 were pre- or perimenopausal, whereas 95% of patients aged > 50 were postmenopausal (Table 1).
- A higher proportion of patients aged \leq 50 had tumors of high clinical risk (54%) compared to patients aged > 50 (39%) (p < 0.0001) (**Table 1**).
- Approximately 53% of patients aged \leq 50 had a HR tumor, while patients aged > 50 had a lower frequency (44%) of HR tumors (p < 0.001) (**Table 1, Figure 1A**). A higher frequency of younger patients were classified as H2 (25%) compared to those > 50 (18%) (Table 1, Figure 1B).
- Younger patients had a higher proportion of tumors that classified as BP Luminal B- and Basal-type than older patients (p < 0.001) (**Table 1, Figure 1C**).
- Principle component analysis of the top 500 genes with the highest variance revealed no distinct clustering by age group (Figure 2).
- Five DEGs were detected in tumors from patients aged \leq 50, and fewer were detected when adjusted for MP risk and BP subtype (Table 2). Table 3 lists functions for the respective five DEGs.

CONCLUSIONS

- Whole transcriptome analysis identified no substantial differences in gene expression between tumors, including Low Risk Luminal-type tumors, from women aged \leq 50 (mostly pre- or peri-menopausal) and women aged > 50 (mostly post-menopausal).
- No clear biological distinction between age groups suggests that MP and BP provide consistent results and are not influenced by patient age.
- These data support the likely explanation that the observed age-dependent difference in chemotherapy benefit is not due to intrinsic biological differences in breast cancers due to age, but rather to differences in the effect of chemotherapy on the host.

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