Genomic landscape of ER-positive HER2-low early-stage breast cancers in the FLEX Study: MammaPrint, BluePrint and whole transcriptome analysis

Abirami Sivapiragasam[1], Adam Brufsky[2], Hannah Linden[3], Natasha Hunter[3], Cynthia Osborne[4], Joyce O'Shaughnessy[4], Sami Diab[5], Robert Maganini[6], Manojkumar Bupathi[7], Sung Ho Lee[8], Theodore Kim[9], Josien Haan[10], Lavanya Samraj[11], Katie Quinn[11], William Audeh[11], FLEX Investigators' Group

[1] SUNY Upstate Medical University, Syracuse, NY; [2]University of Pittsburgh Medical Center, Pittsburgh, PA; [3]University of Washington, Fred Hutchison Cancer Research Center, Seattle, WA; [4]Baylor University Medical Center, Texas Oncology Dallas, TX; [5]UCHealth Lone Tree Medical Center, Denver, CO; [6]Saint Alexius Medical Center, Chicago, IL, [7]Rocky Mountain Cancer Center, Aurora, CO; [8]Montefiore Nyack Hospital, Nyack, NY; [9]Katmai Oncology Group, Anchorage, AK; [10]Agendia, NV, Amsterdam, Netherlands; [11]Agendia Inc., Irvine, CA

Introduction

- Antibody-drug conjugates (ADCs) continue to emerge for the treatment of a new subset of patients with HER2-low breast cancer (1).
- There is limited evidence to demonstrate HER2-low tumors as a distinct biological subtype and why/if these tumors benefit from ADCs.
- To improve our understanding of this newly defined HER2 category of breast cancers, we evaluated clinical characteristics, MammaPrint (MP), BluePrint (BP), and the whole transcriptomic profile of HER2-low breast cancers in the FLEX study.

Methods

FLEX trial and genomic testing:

FLEX (NCT03053193) is a prospective, observational trial that includes stage I-III breast cancer patients who undergo MammaPrint (MP) testing (with or without BluePrint) as standard of care, and consent to full transcriptome and clinical data collection. MP classified tumors as Low Risk or High Risk (further stratified as High 1 and High 2). BluePrint (BP) is an 80-gene molecular subtyping signature, categorizes tumors as Luminal-, HER2- or Basal-Type. MP together with BP categorized tumors as Luminal A (MP Low Risk), Luminal B (MP High Risk), HER2 or Basal.

Study population:

In this study, clinically ER+/HER2- tumors were analyzed. The HER2-low cohort group (n=1698) was defined as HER2 IHC 1+ (ISH positive excluded) and IHC 2+, ISH Negative, and the HER2-0 group (n=1181) was defined as HER2 IHC 0.

Statistical analyses:

Two-tailed proportional z-test was used to compare clinical features and genomic subtypes of HER2-low vs. HER2-0 and the limma R package for differential gene expression analysis (DGEA). P-values were adjusted for multiple testing by the Benjamini-Hochberg procedure; significant differentially expressed genes (DEGs) had a p-value < 0.05 and a fold change >2.

Table 1. Comparison of clinical characteristics between HER2-low and HER2-Negative

Clinical characteristics		HER2-low	HER2-neg	p value
Menopausal status	Post	**1,208 (76.9%)**	**909 (83.1%)**	**<0.001**
	Pre/Peri	**363 (23.1%)**	**185 (16.9%)**	**<0.001**
N Stage	N0	954 (78.3%)	660 (79.6%)	0.496
	N1	248 (20.3%)	154 (18.6%)	0.351
	N2	9 (0.7%)	13 (1.6%)	0.116
	N3	8 (0.7%)	2 (0.2%)	0.317
T Stage	T1	828 (64.4%)	605 (69.0%)	0.031
	T2	382 (29.7%)	230 (26.2%)	0.084
	T3	60 (4.7%)	35 (4.0%)	0.516
	T4	15 (1.2%)	7 (0.8%)	0.534
Grade	**G1**	**459 (28.7%)**	**374 (33.8%)**	**0.006**
	G2	**895 (56.0%)**	**554 (50.0%)**	**0.003**
	G3	245 (15.3%)	179 (16.2%)	0.587

Table 2. Comparison of MammaPrint and BluePrint distribution between HER2-low and HER2-Negative

MP BP distribution		HER2-low	HER2-neg	p value
MP Result	HIGH RISK	787 (46.3%)	522 (44.2%)	0.271
	LOW RISK	911 (53.7%)	659 (55.8%)	0.271
MP categories	High 1	652 (38.4%)	416 (35.2%)	0.09
	High 2	135 (8.0%)	106 (9.0%)	0.364
	Low	**640 (37.7%)**	**494 (41.8%)**	**0.028**
	Ultralow	271 (16.0%)	165 (14.0%)	0.158
MP & BP Subtypes	Basal	**42 (2.5%)**	**53 (4.5%)**	**0.005**
	Luminal B	729 (43.5%)	469 (39.7%)	0.045
	Luminal A	903 (53.9%)	659 (55.8)	0.345

Fig 2: Multidimensional scaling plots– based on the top 500 genes with largest variance

Fig 2a: MDS plot, colored by IHC HER-0. 1+. 2+ (FISH-ve)

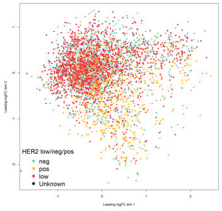
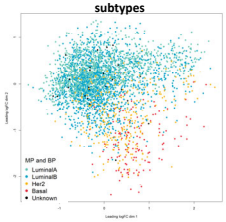
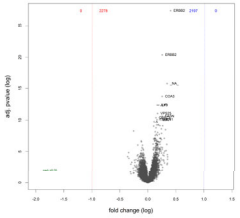


Fig 2b: MDS plot colored by MP BP subtypes



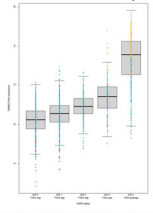
X-axis Principal Component 1 and on the y-axis Principal Component 2

Fig 3: DGEA comparing HER2-low with HER-0 samples



Differential gene expression analysis from whole transcriptome analysis, with log-fold change in x-axis and –log10 p-value in y axis

Fig 4: ERBB2 expression from whole transcriptome analysis based on HER2 IHC expression categories



HER2 mRNA expression in y axis, HER2 IHC categories s IHC-0,1 & 2 with FISH+ve, are shown in the x-axis, in comparison with positive controls HER2 IHC 3+ & 2+ FISH +ve

Results

- **Table 1:** Clinically ER+/HER2- tumors showed that the clinical characteristics between HER2-low and HER2-0 did not differ significantly except higher percentage of premenopausal within HER2-low (23% vs 17%, p < 0.01) and a higher percentage of grade 2 tumors in HER2-low.
- **Table 2:**MP and BP distributions were comparable between groups. Nearly half of the tumors in the HER2-Low were MP Low in both groups. Further stratifying the MP High risk tumors into High 1 and High 2, did not reveal difference in their distributions.
- BluePrint subtypes distribution revealed a lower proportion of ER+Basal in the HER2-low group vs HER2-neg.
- **Figure 2a & b:** Principal component analysis (PCA) of the 500 most variable genes did not reveal a separation of HER2-low and -0 tumors (fig 2a), but clustering was apparent when tumors were classified by BP (Fig 2b)
- **Figure 3:** Comparison of DEGs between HER2-low and HER2-0 showed 4475 DEGs. However, all DEGs were < 2-fold change. DGEA within Basal tumors revealed no DEGs. Within Luminal A tumors, more than 1800 DEGs were identified, and within Luminal B tumors, nearly 300 DEGs were identified, with less than 2-fold change: mean, max (1.09, 1.38) for Luminal A and (1.12, 1.44) for Luminal B
  [Fig not shown]
- **Figure 4:** We evaluated HER2 mRNA expression to compare with IHC expression. A significant difference (p<0.01) towards increased ERBB2 (HER2) expression was detected from HER2-0 to HER2-low, but there was a large overlap of expression between the 2 groups.

Conclusion

- The biological heterogeneity among IHC-defined HER2-negative tumors was better captured by MammaPrint and BluePrint than IHC/FISH. MammaPrint identified 53% of HER2-low tumors as Low Risk, a subgroup of patients known to have good outcomes without chemotherapy & a low risk of metastasis, Genomic testing of HER2-low tumors is important to spare the MP low risk tumors from the potential toxicities of ADCs.
- Future studies will investigate the utility of MammaPrint and BluePrint in predicting chemosensitivity and benefit from ADCs, such as T-DXd, in patients with HER2-low tumors.

References

1. Ferraro, E., Drago, J.Z. & Modi, S. Breast Cancer Res 23, 84 (2021).

Poster P. Presented at **San Antonio Breast Cancer Symposium**; December 5 – 9 2023; San Antonio, TX, USA.