Defining transcriptomic profiles of early-stage Mucinous Breast Cancers – A FLEX sub study

Abirami Sivapiragasam[1], Ryan Christopher Denley[1], Adam Brufsky[2], Esther Hoogland Rehmus[3], Joyce O'Shaughnessy[4], Jennifer A. Crozier[5], Sami Diab[6], Julie Barone[7], Jill Yeager[1], Priya Menon[8], Midas M. Kuilman[9], Lavanya Samraj[10], Lisa Blumencranz[10], M. William Audeh[10], FLEX Investigators' Group

1. SUNY Upstate Medical University, Syracuse,NY; 2. Magee-Womens Hospital and the Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, PA; 3.Akron General Med Ctr, Akron, OH; 4. Baylor University Medical Center, Texas Oncology, US Oncology, Dallas, TX; 5. Baptist MD Anderson Cancer Center in Jacksonville, FL; 6. Colorado Integrative Cancer Center, Greenwood Village, CO; 7.Vail Health Shaw Regional Cancer Center, Edwards, CO; 8. Henry Ford health Jackson, MI 9. Research and Development, Agendia NV, Amsterdam, Netherlands;10. Medical Affairs, Agendia Inc., Irvine.

ASCO 2022 Abstract #3134

Background

Mucinous breast cancer is a rare histological subtype of invasive breast carcinoma (IBC) that accounts for 1-7% of all breast cancers and is associated with a favorable prognosis.[1,2]

The representation of Mucinous tumors is generally rare in clinical trials.[1] NCCN treatment guidelines for Mucinous tumors are based on tumor size and lymph node status, due to limited prospective data on systemic adjuvant therapies in Mucinous tumors.[3] To provide a better understanding of Mucinous breast cancers and factors contributing to their clinical behavior, we examined the transcriptomic profiles of Mucinous tumors in our FLEX study based on MammaPrint and BluePrint distribution.

MammaPrint (**MP**) stratified tumors by risk of distant metastasis [High (**HR**), Low (**LR**), Ultra-Low (UL) Risk], High Risk tumors were further classified into High 1 (H1) or High 2 (H2). MP with BluePrint (**BP**), a molecular subtyping assay, categorize tumors as Luminal A (MP LR), Luminal B (MP HR), HER-2, or Basal-type.[4,5]

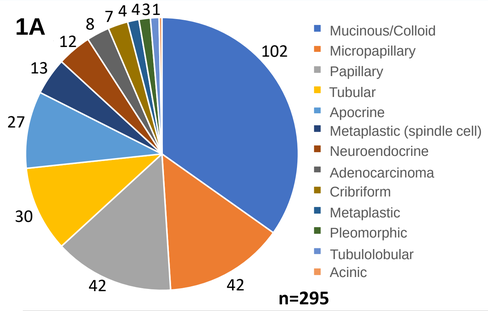
Methods

FLEX Study: The FLEX study (NCT03053193) enrolls patients with early breast cancer who undergo standard of care MP and BP testing, and consent to clinically annotated full transcriptome data collection.

Patient cohort: For this study, histologically confirmed early-stage Mucinous tumors (n=102) in the FLEX study database were included. All patients examined were ER-Positive/HER-2-Negative by IHC and Luminal A (n=56) or Luminal B (n=46) by BP. Mucinous tumors were compared with IDC-No special type tumors, matched for age, MP, and BP index (n=97).

Whole Transcriptome analysis: Differential gene expression analysis (**DGEA**) was performed using Limma R package. Significant Differentially Expressed Genes (**DEGs**) had an adjusted p-value <0.05 and absolute fold change >2. Gene Set Enrichment Analysis (**GSEA**) was done using fgsea' package and genes were ranked based on effect size.

Results

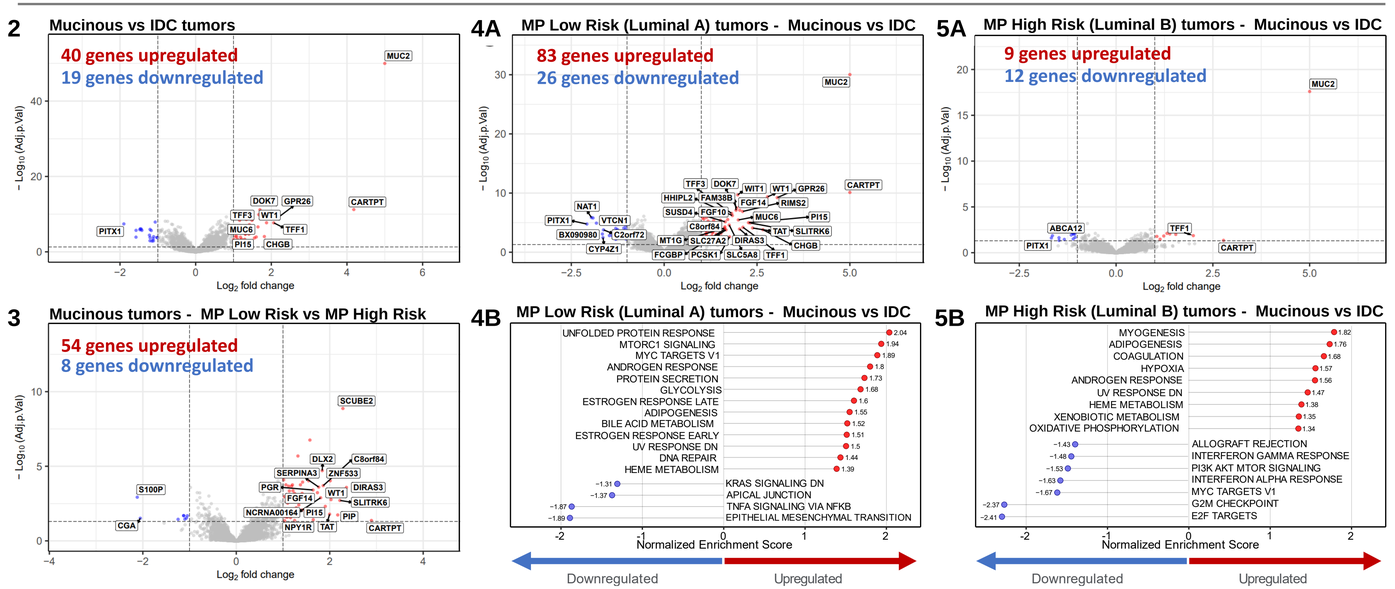


1B

	MammaPrint						BluePrint		
	High1	High2	MP index (HR)	Low	Ultra-Low	MP index (LR)	Luminal A	Luminal B	Total
IDC	4	40	-0.3 (-0.73, -0.02)	46	7	0.14 (0.01, 0.54)	53	44	97
mucinous	4	42	-0.22 (-0.87, -0.0)	47	9	0.19 (0, 0.72)	56	46	102

Boxplots: [I] Shows distribution of MammaPrint high risk. [II] Shows distribution of MammaPrint low risk.

Figure 1: A) Distribution of patients with rare histological subtypes (n= 295) within FLEX. B) Distribution of matched mucinous and IDC tumors by MP Risk and BP subtypes [Only BP Luminal subtypes are included]



DGEA in Fig 2) Mucinous vs IDC tumors, Fig 3) within Mucinous tumors, MP LR was compared with MP HR, Fig 4A) within Luminal A tumors, Mucinous tumors were compared with IDC, and Fig 5A) within Luminal B tumors, Mucinous tumors were compared with IDC. Significant DEGs are indicated by color: upregulated genes are red and downregulated genes are blue. GSEA within 4B) Luminal A tumors and 5B) Luminal B tumors.

- The distribution of rare histological subtypes in FLEX study is shown in **Figure 1A**. The prominent subtype, Mucinous, accounts for 1.2% of the total FLEX study population (N=8583). Of the 102 Mucinous tumors, 45% were MP High Risk and 55% were MP Low Risk including 9% MP Ultra-Low Risk tumors (**Figure 1B**).
- DGEA comparing Mucinous (n=102) with IDC (n=97) tumors revealed 59 DEGs, regardless of the genomic risk. Genes associated with Mucinous tumors, such as MUC2, TFF1, CARTPT were indeed among the upregulated genes[6] (**Figure 2**).
- DGEA comparing Mucinous MP LR vs HR tumors resulted in 62 DEGs, indicating LR and HR Mucinous tumors are biologically distinct types. LR Mucinous tumors showed upregulation of 54 genes in comparison to HR Mucinous tumors. Overexpression of SCUBE2 is associated with good clinical outcomes and is upregulated in LR mucinous tumors[7] (**Figure 3**).
- Comparison of Mucinous vs IDC tumors within Luminal A revealed 109 DEGs (**Figure 4A**). GSEA showed upregulation of pathways involved in estrogen response (early & late) and androgen response, unfolded protein response (UPR) and downregulation of Epithelial Mesenchymal Transition (EMT) and TNF-alpha signaling pathways in LR Mucinous tumors compared to LR IDC (**Figure 4B**).
- DGEA between HR Mucinous and HR IDC revealed 21 DEGs with immune pathways and E2F targets being downregulated in HR Mucinous tumors compared to HR IDC (**Figure 5A & 5B**).

Conclusions

- This study revealed genomic diversity in Mucinous tumors, where half of the Mucinous tumors examined were MP High Risk (Luminal B).
- MP Low Risk Mucinous tumors are biologically distinct from MP Low Risk IDC, even though both are genomically Low Risk. It remains to be determined whether these biological differences lead to a favorable prognosis in LR mucinous tumors.
- MP High Risk Mucinous tumors showed limited DEGs compared to High Risk IDCs, indicating these tumor types are genomically similar and may benefit from chemotherapy. This study highlights the importance of MP and BP testing in Mucinous tumors to identify the High Risk patients.

References

1. Di Saverio S et al. Breast Cancer Res Treat, 2008
2. Lyon et al. IARC 2019
3. NCCN Evidence blocks, version 2 2022
4. L. van 't Veer et al. 2018 EORTC-NCI-AACR Symposium
5. 5. D.M. Wolf et al. 2017 NPJ Breast Cancer
6. Kebin Lu et al. The Breast 53, 2020
7. Yuh-Charn Lin et al. Journal of Cell Science, 2014