Whole Transcriptome Analysis of Breast Cancer Tumors with Discordant Onco*type* DX and MammaPrint Results in the FLEX trial

Matei Socoteanu[1], Joyce O'Shaughnessy[2], Kent Hoskins[3], Adam Brufsky[4], Cathy Graham[5], Svetislava Vukelja[6], Jamal Misleh[7], Karen Tedesco[8], Rakhshanda Layeequr-Rahman[9], June Lee[10], Julian Berrocal[11], Kamal Sharma[12], Albert Begas[13], Jennifer Crozier[14], Ian Grady[15], Nina D'Abreo[16], Midas Kuilman[17], Holly Nguyen[18], Lisa Blumencranz[18], William Audeh[18], and the FLEX Investigator Network

1. Texas Oncology - Longview, US Oncology, Longview, TX; 2. Texas Oncology-Baylor Sammons Cancer Center, US Oncology, Dallas, TX; 3. University of Illinois College of Medicine at Chicago; 4. Magee-Women's Hospital and the Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, PA; 5. Emory University School of Medicine, Atlanta, GA; 6. Texas Oncology - Tyler, US Oncology, Tyler, TX; 7. Medical Oncology Hematology Consultants PA, Newark, DE; 8. New York Oncology Hematology-Albany, US Oncology, Albany, NY; 9. Texas Tech University Health Sciences Center, Breast Center of Excellence, Amarillo, TX; 10. Breast Specialists of South Florida, Atlantis, FL; 11. Women's Health and Healing of the Palm Beaches, Palm Springs; 12. National Cancer Institute – National Institutes of Health, State College, PA; 13. Lynn Regional Cancer Center, Boca Raton, FL; 14. Baptist MD Anderson Cancer Center, Jacksonville, FL; 15. North Valley Breast Clinic, Redding, CA; 16. NYU Winthrop Hospital, Mineola, NY; 17. Research and Development, Agendia NV., Amsterdam, Netherlands; 18. Medical Affairs, Agendia Inc., Irvine, CA

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Contact: FLEX@agendia.com

BACKGROUND

Genomic tests, such as MammaPrint (MP) and Oncotype DX Breast Recurrence Score (RS), inform personalized treatment decision-making for patients with early breast cancer (EBC). Discordance among available genomic assays has been widely reported and may lead to uncertainty in treatment recommendations[1].

The RS is a genomic-based algorithm that guides adjuvant chemotherapy treatment decisions for women with (ER)-positive EBC, with reported age-related differences in chemotherapy benefit for women with intermediate Oncotype DX[2].

MINDACT[3] and MP observed a similar differential chemo effect based on age (≤ 50 vs > 50 years) in a genomic low risk group, however, further data provided insights into this response. Utilizing the FLEX database, the biological characteristics of breast cancer tumors between patients aged ≤ 50 years and > 50 years were compared. There were no substantial differences in gene expression between tumors, demonstrating 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[5].

Similarly, here, using FLEX whole transcriptome BP luminal-type database samples, we seek to examine differentially expressed genes among patients who received discordant RS and MP results and a biologic basis for these discordant results.

METHODS

FLEX Study: Patients with EBC enrolled in the FLEX study (NCT03053193) undergo standard of care MP and BluePrint (BP) tests, and consent to clinically annotated whole transcriptome data collection. This subanalysis was performed on patients who had received both MP and ODX within the FLEX Study.

Patient Cohort: This subanalysis was restricted to BP Luminal-type tumors (N = 704).

Gene Expression Analysis: Tumors with discordant MP and RS results were assessed by whole transcriptome analysis. Gene expression data were quantile normalized and analyzed using R package 'limma'. Differential gene expression (DEG) was considered significant with a 2-fold change and adjusted p-value < 0.05.

RESULTS

Figure 1. MP and ODx report different chemotherapy benefit

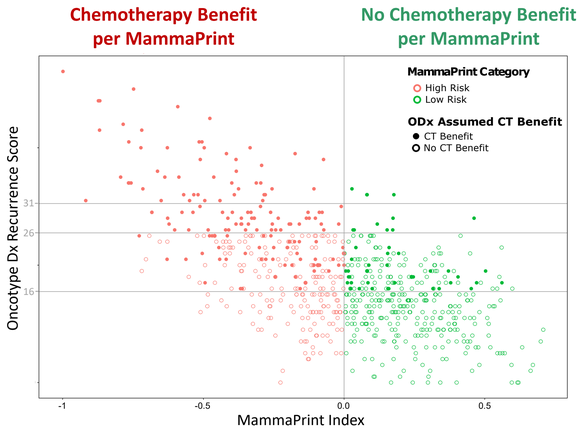

Table 1. Patient-Tumor Characteristics

	MP Low Risk	MP High Risk	P Value
Number of Patients	365	339	
ODx CT Report Recommendation			
no CT	320	195	<0.0001
CT	45	144	
ODx RS Category			
RS0-15	229	87	<0.0001
RS16-25	125	155	
RS26-30	7	47	
RS31-100	4	50	
Age			
≤50	89	93	0.3566
>50	276	246	
Menopausal status			
Pre or peri	79	80	0.6547
Post	282	259	

Chi-squared test for all statistical tests

Figure 2. Differential gene expression analysis and gene set enrichment analysis.

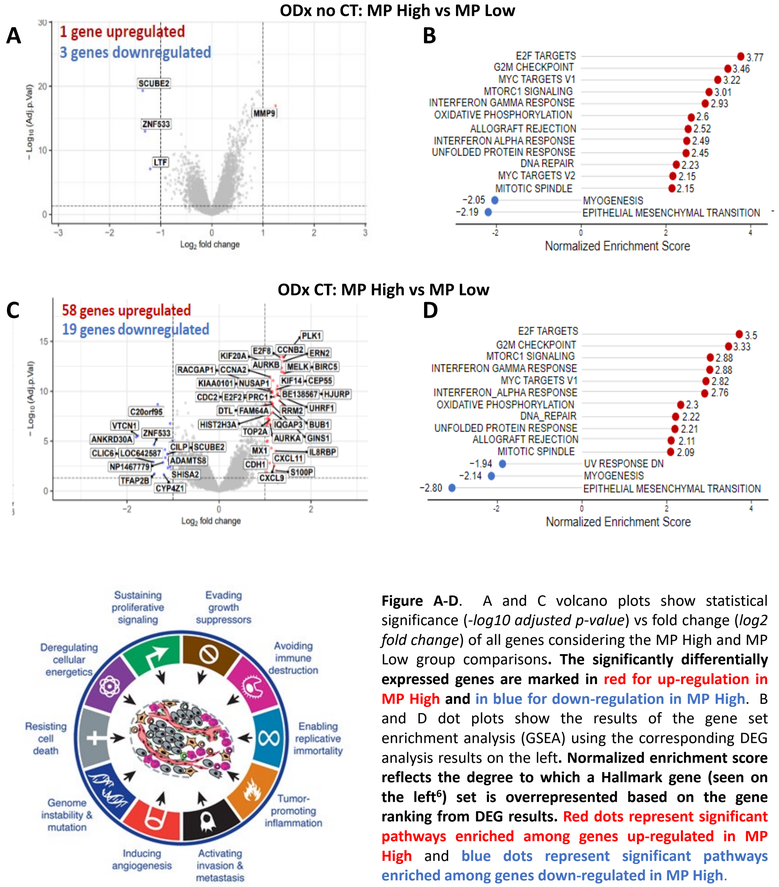

Figure A-D. A and C volcano plots show statistical significance (*-log10 adjusted p-value*) vs fold change (*log2 fold change*) of all genes considering the MP High and MP Low group comparisons. **The significantly differentially expressed genes are marked in red for up-regulation in MP High and in blue for down-regulation in MP High.** B and D dot plots show the results of the gene set enrichment analysis (GSEA) using the corresponding DEG analysis results on the left. **Normalized enrichment score reflects the degree to which a Hallmark gene (seen on the left[6]) set is overrepresented based on the gene ranking from DEG results. Red dots represent significant pathways enriched among genes up-regulated in MP High and blue dots represent significant pathways enriched among genes down-regulated in MP High.**

CONCLUSIONS

- MammaPrint and Oncotype Recurrence Score chemotherapy recommendations differ significantly, P <0.001 (Table 1).
- MP Low and High Risk patients are represented in every ODx RS category. While a trend is observable between MP and ODx score, there was no correlation. (Figure 1, Table 1).
- The B-20 Trial reported that ODx is predictive of CT benefit for patients with a RS >30[2]. This group only accounts for 7.7% of this analysis, suggestive of undertreatment for certain patient subsets. (Figure 2C)
- MP genes further differentiate the ODx no CT and CT groups into two distinct subgroups, highlighting heterogeneity within ODx populations (Figure 2A, 2C).
- 3 of the 16 ODx RS genes are differentially expressed within the ODx CT group: BIRC5, PGR, and SCUBE2. Yet these 3 genes do not partition the ODx CT group (Figure 2C).
- The MammaPrint High Risk category captures dysregulation of several hallmark pathways[5] including (Figure 2) :
  - sustained proliferative signaling
  - evading growth suppressors
  - avoiding immune destruction
  - tumor-promoting inflammation
  - genomic instability & mutation
  - activating invasion and metastasis
- **This analysis highlights the complexity of breast cancer and the ability of the 70 MP genes to identify the genomically high risk tumors and clarify who needs chemotherapy[5] (Figure 2).**

References

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