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# Genomic risk classification and whole transcriptome analysis of HR+/HER2- Postpartum breast cancers - A FLEX sub study

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## BACKGROUND

Women diagnosed with breast cancer before the age 45 and within 5-10 years of a childbirth have increased risk of metastasis. These high-risk cancers, defined as postpartum breast cancer (PPBC), have significantly worse prognosis independent of clinical-pathologic features and demonstrate treatment resistance. We hypothesized that PPBC exhibits high risk molecular profiles and sought to define the genomic patterns of PPBC.

## METHODS

The prospective, observational FLEX Study (NCT03053193) includes stage I-III early BC patients who received MammaPrint®(MP) and/or BluePrint®(BP) testing and consented to full transcriptome and clinical data collection. We identified 377 HR positive and HER2-negative patients, age ≤50, grouped as PPBC 0-5 years (n=33); PPBC 6-10 years (n=51), PPBC beyond 10 years (n= 175) . Nulliparous (n=118) was used as the comparison group (p-values in Table 1). Median age, clinical characteristics, metabolic factors, and genomic test results (MP and BP) were compared between PPBC groups and nulliparous. Limma R package was used for differential gene expression analysis in age-matched cohorts. Using the genes with 2-fold change (FC) difference, pathway analysis was conducted using Metascape [1] and Reactome [2].

## RESULTS

**Table 1** shows patient tumor characteristics. Median age for PPBC in the first 5 years is 38 compared to 43 for nulliparous (p<0.001). No other clinical factors were significantly associated with any subgroup.

**Figure 1A** shows PPBC 0-5 had a significantly higher percentage of MP High 2 and lower percentage of MP Low Risk compared to nulliparous women (p < 0.05). Age matched cohorts showed similar trend in MP distribution.

**Figure 1B** shows that PPBC 0-5 had higher percentage of BluePrint Luminal B and Basal compared to nulliparous (not significant, p > 0.05).

**Figure 2** shows the volcano plot of whole transcriptome comparison of age-matched PPBC 0-5 and nulliparous. PPBC 0-5 cohort had 25 genes with 2-fold higher expression compared to nulliparous. Elevated expression of MUCL1 and CLEC3A is of particular interest (**Figure 3**):

- MUCL1 is associated with promoting the invasion and metastasis of breast cancer cells via promoting epithelial-mesenchymal transition [3].
- High CLEC3A expression was shown to correlate with metastatic potential and indicated a poor prognosis in breast cancer, particularly in ductal carcinoma [4].

**Figure 4** depicts the Reactome pathway analysis indicating enrichment of genes related to adaptive immune responses, humoral responses, and signal transduction in the PPBC 0-5 years group. Metascape analysis similarly showed enrichment of adaptive immune responses and immunoglobulin-mediated immune responses (not shown).

## RESULTS

**Table 1. Patient Tumor Characteristics**

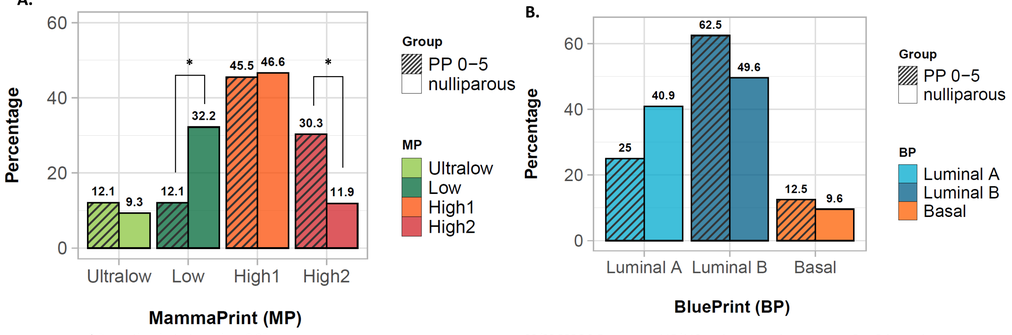
| | PPBC 0-5 years (n=33) | PPBC 0-10 years (n=84) | PPBC >10 years (n=175) | Nulliparous (n=118) | p-values compared to nulliparous: PPBC 0-5 | PPBC 0-10 | PPBC >10 |
|---|---|---|---|---|---|---|---|
| **Age years** | | | | | | | |
| Median | 38 | 41 | 47 | 43 | < 0.001 | 0.25 | < 0.001 |
| Range | 28.00 - 47.00 | 28.00 - 50.00 | 33.00 - 50.00 | 27.00 - 50.00 | | | |
| **T stage** | | | | | | | |
| T1 | 13 (48.1%) | 36 (56.2%) | 85 (66.4%) | 43 (53.8%) | 0.66 | 0.972 | 0.247 |
| T2 | 13 (48.1%) | 22 (34.4%) | 36 (28.1%) | 30 (37.5%) | | | |
| T3 | 1 (3.7%) | 4 (6.2%) | 6 (4.7%) | 5 (6.2%) | | | |
| T4 | 0 (0.0%) | 2 (3.1%) | 1 (0.8%) | 2 (2.5%) | | | |
| **Grade** | | | | | | | |
| G1 | 5 (15.6%) | 17 (20.7%) | 52 (30.4%) | 26 (22.8%) | 0.236 | 0.772 | 0.353 |
| G2 | 16 (50.0%) | 45 (54.9%) | 86 (50.3%) | 65 (57.0%) | | | |
| G3 | 11 (34.4%) | 20 (24.4%) | 33 (19.3%) | 23 (20.2%) | | | |
| **N stage** | | | | | | | |
| N0 | 21 (80.8%) | 50 (82.0%) | 101 (82.8%) | 66 (84.6%) | 0.76 | 0.819 | 0.846 |
| N1 | 5 (19.2%) | 11 (18.0%) | 21 (17.2%) | 12 (15.4%) | | | |
| **Histology** | | | | | | | |
| IDC | 31 (93.9%) | 71 (86.6%) | 151 (87.8%) | 106 (90.6%) | 0.866 | 0.62 | 0.763 |
| ILC | 1 (3.0%) | 8 (9.8%) | 14 (8.1%) | 8 (6.8%) | | | |
| MixedIDC&ILC | 1 (3.0%) | 3 (3.7%) | 7 (4.1%) | 3 (2.6%) | | | |
| **Menopausal** | | | | | | | |
| Pre-/Peri- | 31 (93.9%) | 77 (92.8%) | 129 (75.9%) | 96 (84.2%) | 0.247 | 0.08 | 0.102 |
| Post- | 2 (6.1%) | 6 (7.2%) | 41 (24.1%) | 18 (15.8%) | | | |
| **ki67** | | | | | | | |
| 0-10 | 3 (12.0%) | 16 (26.2%) | 24 (22.2%) | 25 (28.1%) | 0.098 | 0.837 | 0.432 |
| 11-20 | 3 (12.0%) | 11 (18.0%) | 31 (28.7%) | 19 (21.3%) | | | |
| >20 | 19 (76.0%) | 34 (55.7%) | 53 (49.1%) | 45 (50.6%) | | | |
| **Race/Ethnicity** | | | | | | | |
| White | 23 (79.3%) | 4 (5.3%) | 137 (87.3%) | 93 (83.8%) | 0.092 | 0.724 | 0.104 |
| Black | 6 (20.7%) | 9 (12.0%) | 17 (10.8%) | 10 (9.0%) | | | |
| AAPI | 0 (0.0%) | 62 (82.7%) | 3 (1.9%) | 8 (7.2%) | | | |

## CONCLUSION

In this study, PPBC 0-5 years of childbirth is associated with significantly higher risk of recurrence (MP High 2) and increased immune gene expression, other unique genes/pathways related to PPBC biology compared to BC in nulliparous women.

These findings are consistent with the poor clinical outcomes previously reported for PPBC. Increased frequency of MP High 2 tumors and immune biology indicate that PPBC might benefit from adding immunotherapy, to be explored in future trials.

**Figure 1. Barplots of percentages of MammaPrint and BluePrint results**




**MammaPrint (MP)**

* Proportional z-test, p-value <0.05

**BluePrint (BP)**

32/33 PPBC 0-5 years and 115/118 nulliparous patients also had BluePrint testing

**Figure 2. Volcano plot comparing age matched PPBC 0-5 and nulliparous**

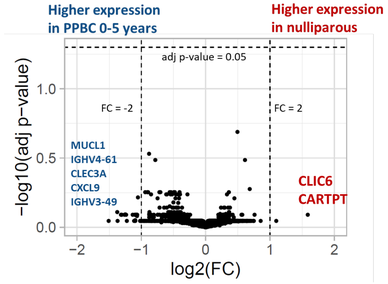
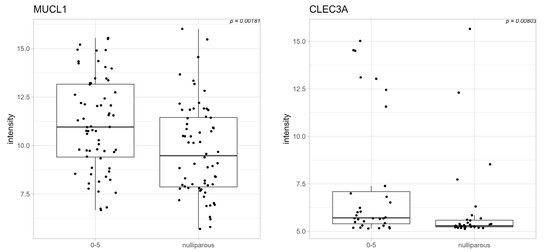
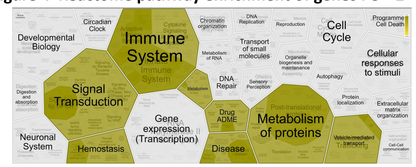



**Figure 3. Boxplots of MUCL1 and CLEC3A mRNA expression**




p-values are from Mann-Whitney test

**Figure 4 Reactome pathway enrichment of genes FC > 2**




Hierarchical representation of reactome pathways showing coverage of pathways genes among genes FC >2

**References**

1. Zhou et al., Nature Communication (2019), 10(1):1523
2. Fabregat et al. BMC Bioinformatics (2017), 18(1):142
3. Li et al. Oncol Rep. (2020), 44(2):509-518
4. Ni et al. Onco Targets Ther. (2018), 11:3303-3312

**Disclosures**

Virginia F. Borges: Nothing to declare