# Immunological markers in patients with breast cancer occurring during pregnancy – results from GBG BCP study

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## Background

Breast cancer is one of the most common malignancies during pregnancy. Breast cancer in pregnancy (BCP) is still a rare event (1 in 3000 to 10000 pregnancies). The incidence is likely to increase as more women tend to delay childbearing into later life and the overall lifetime cancer risk increases with age.[1] Pregnancy presents a complex and unique immunological condition. Pregnant women are widely considered to be in a kind of immunosuppressed state, making them more susceptible to infectious diseases.[2]

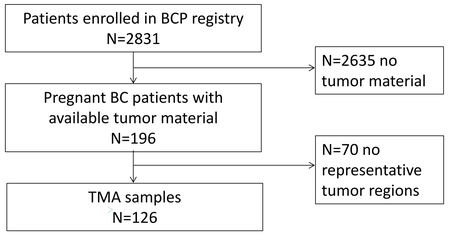
Recent studies have shown similarities between malignancies and the semi-allogenic fetus in terms of immune evasion strategies, for example upregulation of non-classical human leukocyte antigen G (HLA-G). The loss or downregulation of HLA (MHC class I) is also a way to escape anti-tumor immunity.[3] In addition, TIGIT (T cell immunoreceptor with Ig and ITIM domains) as well as PD-1/PD-L1 interactions are crucial in establishing immunotolerance in cancer, healthy adult tissue as well as the fetal-maternal interface.[4,5]

**The aim of this study was to investigate the tumor biology and immunology of pregnant breast cancer patients and the impact of pregnancy on the immunological characteristics of the breast cancer.**

## Patients and Methods

Tissue microarrays (TMAs) of formalin-fixed paraffin embedded core biopsies or surgical specimens from 126 pregnant breast cancer patients treated with neo-(adjuvant) chemotherapy were constructed. TMAs were stained via immunohistochemistry to assess estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), Ki-67 (<20% vs ≥20%), and immunomarkers HLA (≤5% vs >5%), HLA-G (≤5% vs >5%), PD-L1 (<1% vs. ≥1%), TIGIT and Nectin-4 as well as hematoxylin-eosin for the prevalence of tumor-infiltrating lymphocytes (TILs, ≤30% vs 31-60% vs >60%). PD-L1 expression using the 22C3 antibody (Abcam) was evaluated in tumour cells, immune cells, and in both tumor and immune cells.

### Figure 1. Flow diagram




## Results

### Table 1. Patient and tumor characteristics

| Parameter | Category | Overall N=126 N(%) |
|---|---|---|
| Age at diagnosis, years | 18-29 | 14 (11.1) |
| | 30-34 | 56 (44.4) |
| | 35-39 | 45 (35.7) |
| | ≥40 | 11 ( 8.7) |
| T stage* | T1 | 30 (23.8) |
| | T2 | 69 (54.8) |
| | T3 | 20 (15.9) |
| | T4 | 7 ( 5.6) |
| N stage* | N0 | 57 (45.2) |
| | N1 | 69 (54.8) |
| | N2 | 13 (10.3) |
| | N3 | 7 ( 5.6) |
| Tumor grading | G1 | 1 (0.8) |
| | G2 | 39 (31.0) |
| | G3 | 86 (68.3) |
| Histological tumor type | ductal or ductal-lobular invasive | 111 (89.5) |
| | lobular invasive | 7 ( 5.6) |
| | other | 6 ( 4.8) |
| HR status** | both ER and PgR negative | 53 (42.1) |
| | ER and/or PgR positive | 73 (57.9) |
| HER2 status** | negative | 95 (78.5) |
| | positive | 26 (21.5) |
| M status at diagnosis | M0 | 99 (83.9) |
| | M1 | 5 ( 4.2) |
| | MX | 14 (11.9) |
| Biological subtype** | TNBC | 42 (34.7) |
| | HER2+/HR- | 7 ( 5.8) |
| | HER2+/HR+ | 19 (15.7) |
| | HER2-/HR+ | 53 (43.8) |
| Ki-67 at diagnosis** | <20% | 56 (46.7) |
| | ≥20% | 64 (53.3) |
| TILs* | 0-30% | 58 (93.5) |
| | 31-60% | 3 ( 4.8) |
| | >60% | 1 ( 1.6) |

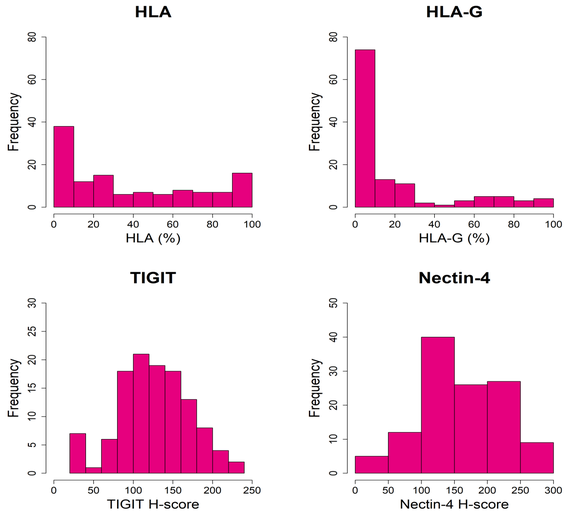
*maximum of cT and pT resp. cN and pN; **assessed from stained TMAs (via immunohistochemistry) by the central pathology, Marburg; Data are N (valid %)

### Table 2: PD-L1, HLA-A and HLA-G in the pregnant cohort

| Parameter | Category | Overall N=126 N(%) |
|---|---|---|
| **PD-L1 (immune cells)** | negative | 91 (76.5) |
| | positive | 28 (23.5) |
| **PD-L1 (tumor cells)** | negative | 108 (90.8) |
| | positive | 11 ( 9.2) |
| **PD-L1 (tumor and/or immune cells)** | negative | 91 (77.8) |
| | positive | 26 (22.2) |
| **HLA** | ≤5% | 26 (21.3) |
| | >5% | 96 (78.7) |
| **HLA-G** | ≤5% | 65 (53.7) |
| | >5% | 56 (46.3) |

Data are N (valid %)

### Figure 2: Distribution of biomarkers as continuous variables




- Median age of the patients was 34 (range 26-47) years. Among breast cancer subtypes, most patients had either TNBC (34.7%) or HER2-/HR+ (43.8%) tumors. 53.3% of patients had a high expression of Ki-67 (≥20%). TILs were detected in 62 out of 126 (49.2%) analyzed patients, of whom 93.5% (N=58) had a low expression of TILs (≤30%) (Table 1).
- HLA expression (≤5%) was downregulated in 21.3% of patients while 46.3% showed upregulation of HLA-G expression (>5%). Most tumors in our cohort were PD-L1 negative for both tumor cells (90.8%) and immune cells (76.5%) (Table 2).
- Overall, the median H-score was 127 (range 23.8-235) for TIGIT and 156 (range 4.9-288) for Nectin-4 (Figure 2).
- An increased but not significant median expression of HLA-G was observed in patients with T3/4 (N=27) compared to T1/2 (N=99) tumor stage (12.4% vs 3.4%, respectively, p=0.226) whereas the TIGIT median expression was significantly higher in patients with T1/2 compared to those with T3/4 tumor stage (135 vs. 116, respectively, p=0.02). There was no significant difference in the median expression of the other biomarkers HLA, PD-L1 and Nectin-4 in T1/2 vs. T3/4 subgroups. The median expression of all biomarkers did also not significantly differ in N0 vs. N+ subgroups.

## Conclusions

- **A heterogeneity of immunomarker expression was detected in the entire cohort of pregnant breast cancer patients.**
- **Subgroup analysis showed a significantly higher expression of TIGIT in patients with T1/2 tumor stage, which might be a sign of the initial anti-tumor response with activation of T- and NK-cells that decreases during tumor progression.**
- **Taken together, these findings suggest a heterogeneity of immunomarkers in tumor tissue, which might be related to the specific immunological situation during pregnancy. These results are hypothesis generating and further analyses are ongoing to evaluate the impact of this heterogeneity on non-pregnant patient cohort.**

## References

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