PD-L1 Expression in Triple Negative Breast Cancer: Insights from an African Ancestry-Enriched Multinational Cohort

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Abstract

Breast cancer incidence has been rising worldwide, increasing the urgency of understanding factors that drive aggressive subtypes and influence treatment response. Programmed death-ligand 1 (PD-L1) is an immune checkpoint protein expressed on tumor and immune cells that promotes immune evasion by inhibiting T-cell activation. In advanced triple-negative breast cancer (TNBC), a subtype with aggressive behavior and limited targeted treatment options, PD-L1 expression has emerged as a predictive biomarker for response to immune checkpoint inhibitors. However, most studies have focused on predominantly European-ancestry populations, leaving gaps in understanding PD-L1's expression and clinical relevance among women of African ancestry, who are disproportionately affected by TNBC. Indeed, data from the International Center for the Study of Breast Cancer Subtypes (ICSBCS) highlight a robust association between West Sub-Saharan African ancestry and elevated TNBC prevalence: ICSBCS analyses comparing Ghanaian (West African), Ethiopian (East African), African American, and European American patients found that over half of Ghanaian breast cancers are triple-negative, ~30% of African American breast cancer patients present as TNBC, with substantially lower frequencies in East African (15%) and European American (15.5%) cohorts.

A recent ICSBCS-affiliated Cancer Discovery (1) study identified hundreds of genes—and over 2,000 region-specific differences—associated with African ancestry in TNBC. The work also uncovered distinct immune signatures, including ancestry-linked variation in immune-cell infiltration, indicating that TNBC tumor immunology differs by African genetic ancestry. Taken together, these data underscore the critical need to examine PD-L1 expression in genetically diverse populations. Characterizing PD-L1 prevalence and patterns in women with African ancestry may reveal ancestry-specific tumor biology and inform equitable use of immunotherapies in populations disproportionately burdened by TNBC.

Methods and Materials

The International Center for the Study of Breast Cancer Subtypes (ICSBCS) biobank provides a unique ancestrally diverse tissue repository, enabling biomarker studies across historically underrepresented populations. Leveraging this resource, we are conducting a large-scale immunohistochemical (IHC) analysis of PD-L1 expression on formalin-fixed paraffin-embedded (FFPE) primary TNBC tumor tissue sections. The study aims to evaluate PD-L1 [SP142] of immune cells in 1,000 TNBC samples, equally distributed among Ghanaian, Ethiopian, African American (AA), and European American (EA) patients.

For assessment of % positive PD-L1 immune cell (IC) scoring in triple-negative breast cancer (TNBC) using the Ventana PD-L1 SP142 assay (REF 740-4859), tumor specimens were stained according to the manufacturer's instructions. PD-L1 expression was evaluated by measuring the proportion of tumor area occupied by PD-L1-expressing tumor-infiltrating immune cells (IC) of any intensity. According to the FDA-approved scoring algorithm, cases were designated "PD-L1 positive" if PD-L1 staining was present in ≥1% of the tumor area covered by immune cells.

Here, we report initial findings.

Methods and Materials

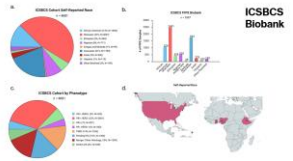


Figure 1. ICSBCS biobank supports ancestry-informed breast cancer research through multi-national informed consent and collection of biospecimens. (a) Self-reported ancestry distribution of ICSBCS-enrolled participants (n = 6841), with African, Ghanaian, Caucasian, Ethiopian, Nigerian, European, Asian, Hispanic, and Other/Declined represented by pie segments. (b) Histogram showing the number of participants enrolled per year from 2010 to 2024. (c) Distribution of enrolled participants by clinical breast cancer phenotypes, quantifying subtypes including ER+/HER2+, ER+/HER2−, ER−/HER2+, ER−/HER2− triple-negative (TNBC), DCIS/LCIS, benign, and pending IHC. (d) Geographic origins of enrolled participants, mapped across collection sites in the United States, Ghana, Nigeria, Ethiopia and the Caribbean, demonstrating the global scope and ancestral diversity of the biobank.

Results

To date, **500 cases** have been processed **(AA n = 77, Ethiopian n = 34, Ghanaian n = 350, EA n = 39).**The median patient age of diagnosis (min. - max.) AA 62yo (34-86), Ethiopian 57yo (28-75), Ghanaian 47yo (22-81), EA 56.5 (32-86). PD-L1 expression, measured as **percent positive immune cells by IHC**, showed means/median **(± SD, 95% CI)** of **4.50%/2% (± 1.30, 1.94–7.06)** in **Ethiopian**, **6.99%/1% (± 1.22, 4.59–9.38)** in **EA**, **4.97%/1% (± 0.87, 3.26–6.67)** in **AA**, and **1.33%/0% (± 0.41, 0.53–2.13)** in **Ghanaian** cases. **Tukey-Kramer Honestly Significant Difference (HSD) comparisons** revealed **significant differences** between **European American and Ghanaian (p < 0.0001)**, and between **African American and Ghanaian (p = 0.0009)**. No significant differences were observed between Ethiopian and Ghanaian (p = 0.0948), European American and Ethiopian (p = 0.5042), European American and African American (p = 0.5311), or African American and Ethiopian (p = 0.9908).
Corresponding **positive immune cell medians (IQR)** were **2% (8.0%)** for **Ethiopian**, **1% (4.5%)** for **EA**, **1% (6.0%)** for **AA,** and **0% (0.5%)** for **Ghanaian cases,** reflecting **heterogeneity** and **skewed PD-L1 expression**. Initial analyses suggest differences in PD-L1 expression across self-reported race and ancestry groups. Comprehensive clinical data and additional tumor microenvironment measures, including tumor-infiltrating lymphocyte (TIL) scores, are being collected for integrative assessment of PD-L1 and its clinical significance. Continued analysis will advance understanding of population-specific tumor immunobiology and inform equitable immunotherapy approaches in TNBC.
A contingency analysis revealed **a significant association between demographic group and PD-L1 immune cell status (Pearson $\chi^2(3) = 84.98$, $p < .0001$), indicating substantial variation in PD-L1 positivity across ancestries**. **Ethiopian** subjects showed the highest proportion of PD-L1 positive immune cell cases **(64.7%)**, followed by **European American (51.3%)** and **African American (50.7%)** cohorts, while **Ghanaian** cases had the lowest PD-L1 positivity (**14.9%**).
Mosaic plot visualization highlights these disparities, with disproportionate representation of negative PD-L1 cases in the Ghanaian cohort compared to other groups.

Results

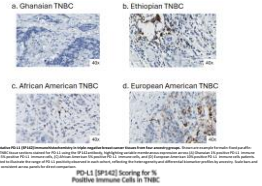


Figure 2. Representative PD-L1 [SP142] immunohistochemistry in triple-negative breast cancer tissues from four ancestral groups. Shown are examples from formalin-fixed paraffin-embedded (FFPE) TNBC tissue sections stained for PD-L1 using the SP142 antibody, highlighting variable membranous expression across (a) Ghanaian (1% positive PD-L1 immune cells, [B]) Ethiopian (51% positive PD-L1 immune cells, (c) African American (5% positive PD-L1 immune cells, and (d) European American (20% positive PD-L1 immune cells patients. Images were acquired at 40× magnification. Scale bars, 50 μm. Consistent staining protocols were used in each cohort, reflecting the heterogeneity and differential biomarker profiles by ancestry. Scale bars and magnifications are consistent across panels for direct comparisons.



Figure 3. Distribution of cases currently scored for PD-L1 [SP142] positive immune cells in TNBC.
Pie chart shows the proportional representation of Ghanaian (70%, n = 350), Ethiopian (7%, n = 34), African American (16%, n = 77), and European American (8%, n = 39) patients among the 500 cases with PD-L1 immunohistochemical scoring. This visual highlights the ancestry composition of the study cohort processed to date and underscores the emphasis on African ancestry representation in PD-L1 biomarker analysis.

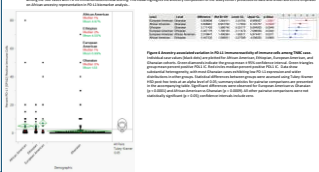


Figure 4. Ancestry-associated variation in PD-L1 immunoreactivity of immune cells among TNBC cases.
Individual case values (black dots) are plotted for African American, Ethiopian, European American, and Ghanaian patients. Boxplots show the median and interquartile range for each group. Ethiopian and European American patients exhibit the highest proportions positive PD-L1 IC. Pairwise median comparisons positive PD-L1 IC. Kate shows substantial heterogeneity, with most Ghanaian cases exhibiting low PD-L1 expression and wide interquartile ranges. Statistical significance was assessed using Tukey-Kramer Honestly Significant Difference (HSD) post-hoc tests at an alpha level of 0.05, correcting for multiple comparisons. Adjusted p-values are presented in the accompanying table. Significant differences were observed for European American vs Ghanaian (p < 0.001) and African American vs Ghanaian (p < 0.0009). All other pairwise comparisons were not statistically significant (p > 0.05); confidence intervals include zero.

Results



Figure 5. Distribution of PD-L1 immune cell scores across demographic groups. Mosaic plot illustrating the distribution of PD-L1 immune cell score categories (Positive, Negative) across four demographic cohorts (African American, Ethiopian, European American, Ghanaian, n = 500). The width represents sample size per group, while vertical proportions show the percentage PD-L1 positive and negative cases in each cohort. The accompanying contingency table summarizes counts, row percentages, and column test averages for each demographic group. Chi-square analysis (Likelihood Ratio and Pearson tests) indicates a statistically significant association between demographic group and PD-L1 immune cell status ($p < 0.0001$).

Count / Row % / Col %	Negative	Positive	Total
African American	38 / 49.35 / 7.60	39 / 50.65 / 11.01	77 / 15.40
Ethiopian	12 / 35.29 / 2.40	22 / 64.71 / 6.21	34 / 6.80
European American	19 / 48.72 / 3.80	20 / 51.28 / 5.65	39 / 7.80
Ghanaian	431 / 85.14 / 86.20	75 / 14.85 / 21.19	506 / 101.20
Total	500 / 100.00	354 / 70.80	500

Test	Chi-Square	Prob>ChiSq
Likelihood Ratio	80.182	<.0001*
Pearson	84.980	<.0001*

Discussion

These preliminary findings underscore the value of the **ICSBCS biobank** in **enabling ancestry-informed biomarker** research in **TNBC**. All samples evaluated in this study were derived from primary tumor sites. By systematically evaluating PD-L1 across Ghanaian, Ethiopian, AA, and EA populations, this study aims to uncover **differences in immune checkpoint activation** to inform tailored therapies. These data are preliminary, with limitations including small sample sizes in some ancestry groups, particularly Ethiopian and EA cohorts, which may affect precision. As analysis progresses toward the full 1,000-case cohort, continued integration of clinical, pathological, and molecular data will be essential for advancing **population-specific tumor immunobiology** and **improving equity in breast cancer outcomes**. These initial differences in PD-L1 expression among ancestry groups further emphasize the necessity of ancestry-informed biomarker research to optimize immunotherapy strategies for triple-negative breast cancer across diverse populations

Importantly, these preliminary findings are reassuring, suggesting that immunotherapeutic approaches targeting the PD-L/PD-L1 axis are likely to be similarly effective across diverse patient populations. This indicates that currently available immunotherapies may represent a promising strategy to mitigate breast cancer disparities in the United States, where African American women experience a higher frequency of TNBC compared to White American women. Furthermore, these results highlight the imperative for continued investment in international breast cancer research involving African patients, both to address the growing breast cancer burden on the continent and to provide a more comprehensive understanding of TNBC biology. Such efforts will be critical to guide equitable, ancestry-informed therapeutic strategies worldwide.

Contact

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