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Differential effects of CD20+ B cells and PD-L1+ immune cells on pathologic complete response and outcome: comparison between inflammatory breast cancer and locally advanced breast cancer patients

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Ethical approval This observational retrospective study involving human participants was approved by the Committee for the Protection of Human Subjects at Dartmouth College (STUDY00029655). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Formal patient consent for studies using anonymous human specimens is not required.

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Abstract

Purpose—This study evaluated epidemiologic and immune factors associated with pathologic complete response (pCR), breast cancer-specific survival (BCSS) and disease-free survival (DFS) outcomes in inflammatory (IBC) and locally advanced breast cancer (LABC) patients.

Methods—Tumor-infiltrating lymphocytes (TILs) and CD20$^+$ B-cell frequencies (CD20$^+$), and PD-L1 expression on tumor (PD-L1$^+$carcinoma cells) and immune (PD-L1$^+$TILs) cells were analyzed by immunohistochemistry along with clinicopathologic factors as modifiers of pCR and outcomes in 221 IBC and 162 LABC patients. Analysis included Kaplan–Meier curves and Cox proportional hazard models.

Results—IBC and LABC display similar levels of TILs, CD20$^+$, and combined CD20$^+$ and PD-L1$^+$TILs (CD20$^+$PD-L1$^+$TILs), while LABC contained more PD-L1$^+$TILs and PD-L1$^+$ carcinoma cells. Absence of lymphovascular involvement, high TILs, PD-L1$^+$ carcinoma cells, and combined CD20$^+$ and PD-L1$^+$ carcinoma cells correlated with pCR in IBC and LABC patients. High PD-L1$^+$TILs correlated with pCR only in LABC; less lymph node involvement at diagnosis, CD20$^+$ and CD20$^+$PD-L1$^+$TILs correlated with pCR only in IBC ($P < 0.04$, all comparisons). Achievement of pCR in IBC and LABC patients correlated with BCSS and DFS ($P < 0.02$). In multivariate analyses, pCR remained an independent prognostic factor of improved DFS in IBC and LABC patients, but of BCSS in only LABC. CD20$^+$PD-L1$^+$TILs remained an independent prognostic factor of improved DFS and BCSS only in IBC.

Conclusion—CD20$^+$PD-L1$^+$TILs are an independent prognostic biomarker of improved outcomes in IBC, but not LABC. Selecting IBC patients by CD20 and PD-L1 status could stratify patients and potentially identify those in whom activating CD20 agents and anti-PD-1/PD-L1 therapy could be explored.

Keywords

Inflammatory breast cancer; Locally advanced breast cancer; Tumor-infiltrating lymphocytes; pCR; PD-L1; CD20; Immuno-oncology; Patient outcomes00B

Introduction

Inflammatory breast cancer (IBC) is a highly aggressive form of breast cancer accounting for less than 3% of all breast cancers, but responsible for ~ 10% of all breast cancer-related deaths in the USA [1]. IBC patients have a 43% increased risk of death from breast cancer compared with non-inflammatory locally advanced breast cancer (LABC) patients [2]. The introduction of systemic neoadjuvant chemotherapy (NACT) with targeted and endocrine therapy when appropriate, followed by loco-regional surgery and radiotherapy, has modestly improved survival of IBC patients [3, 4]. However, 5-year survival for IBC remains poor (~ 30%) [2, 5, 6].

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IBC is more frequent in Northern Africa, with a reported incidence between 5 and 11% of all breast cancer diagnoses [7–10]. Algeria is not an exception, with an incidence rate of ~ 5% (Chaher; unpublished data). Compared to other Northern African countries, the Algerian health system gives free access to the standard of care treatments to all breast cancer patients, providing a unique opportunity to compare IBC and LABC patients receiving similar treatments to identify clinicopathologic, epidemiological, and host immune factors associated with complete response (pCR). Achieving pCR following NACT is considered a surrogate marker of improved prognostic outcome [11], but the factors associated with pCR in IBC and LABC patients remain understudied.

Methods

Study design and participants

We identified 221 primary IBC and 162 non-inflammatory LABC patients with clinical stage IIIb disease diagnosed and treated at the Pierre et Marie Curie Cancer Center (PMCCC, Algiers, Algeria) between 2005 and 2009. Formalin-fixed paraffin embedded (FFPE) tumor samples were diagnostic surgical biopsies collected before initiation of neoadjuvant chemotherapy. In a previous study we reported 117 IBC and 59 non-IBC LABC cases [12] and more recently, 221 IBC cases were reported [13]. Clinical stage was classified according to the AJCC Staging Manual [14]. IBC is defined as T4d disease categorized at or greater than stage III. Ten stage IV IBC patients were excluded from the 221 IBC cohort. IBC was clinically defined according to the international consensus criteria [15]: Rapid onset (less than 6 months) of breast erythema, edema, and/or "peau d'orange," and/or warm breast, with or without an underlying palpable mass. The histological grading of the tumors was performed in accordance with the Bloom-Richardson classification. Pathologic Complete Response (pCR) was defined as the absence of any residual invasive cancer in the breast and the absence of any metastatic cells in the regional lymph nodes (ypT0/is, ypN0) following completion of NACT [16]. Standard hematoxylin and eosin-stained fullface sections of pre-treatment tumor tissue were used to evaluate the presence of overall TILs per international guidelines [17]. Briefly, stromal immune cell infiltration was defined as the percent of stromal areas containing mononuclear cells including lymphocytes, plasma cells and macrophages, and stratified using a median cut-point (with immune infiltration in 15% of tumor stroma area defined as high TILs). Women were classified as normal/lean if body mass index (BMI) was 25 kg/m^2 and obese if BMI was > 25 kg/m^2 [18]. Full demographic, clinical, and pathologic characteristics were extracted from all IBC and LABC patient medical records (Table 1 and supplementary Table S1).

Evaluation of ER, PR, and HER2 expression

FFPE tumor blocks were used to build tissue microarrays (two 1.5 mm cores per case) as described elsewhere [12]. ER, PR, and HER2 expression levels were evaluated using standard procedures with the modified avidin–biotin complex method on the Ventana XT Benchmark autostainer (Ventana Medical Systems, Inc., Tucson, AZ) using antibodies against ER (Thermo Scientific, Fremont, CA; clone RB-9016; dilution 1:100), PR (Dako; Carpinteria, CA; clone PgR 636; dilution 1:100), and HER2 (Ventana; clone 4B5) as

previously described [12]. Breast tissues were used as positive controls; the same tissues, incubated with an iso-type-matched antibody, were used as negative controls.

Detection of HER2 gene copy number by chromogenic in situ hybridization (CISH)

CISH was performed using the SPoT-Light_ HER2 CISH Kit (Zymed, Carlsbad, CA), according to the method provided by the manufacturer.

Scoring of IHC and CISH results

Positive status for ER and PR was defined as having nuclear staining in at least 1% of invasive tumor cells. HER2 protein staining of the membrane was set at four levels, according to the manufacturers' instructions (0, 1 +, 2 +, and 3 +). HER2 positive status was defined as an IHC score of 3 +. The tumors with an IHC score of 1 + or 2 + were confirmed by CISH. HER2 amplification was scored according to the Test Interpretation Guide provided by the manufacturer. Samples showing diploid and polysomy status were considered negative; samples showing low and high amplification were considered positive.

Evaluation and scoring of CD20 and PD-L1 expression

$CD20^{+}$ (mouse monoclonal antibody; clone L26 at a concentration of 0.16 μg/mL for 20 min at room temperature; Dako, Carpinteria, CA, USA), and PD-L1 (clone SP142 at a concentration of 0.096 μg/mL; Spring Bioscience, Pleasanton, CA) protein expression levels in both tumor cells ($PD\text{-}L1^{+}$ carcinoma cells) and immune cells ($PD\text{-}L1^{+}$ TILs) were evaluated as described in [13]. PD-L1 positivity was defined as 5% of TILs or tumor cells expressing PD-L1, and staining was scored as an average percentage across all tissue microarray spots. The 5% cut-off point has been reported to be associated with clinical response to anti-PD-1 therapy [19]. Membranous CD20 immunostaining in 1% TILs was considered positive; this cut-off point has been associated with patient outcome in breast cancer [20].

IHC assays and scoring of all biomarkers are reported following REMARK guidelines [21].

Chemotherapy, hormone therapy, and radiotherapy treatments

NACT of 3FAC3T (FAC: 500 mg/m^2 Fluorouracil, 50 mg/m^2 Adriamycin and 500 mg/m^2 Cyclophosphamide; 100 mg/m^2 Taxotere) was administered to 45% of IBC and 83% of LABC patients; 4FAC3T was given to 14% of IBC, and 4AC4T (60 mg/m^2 Adriamycin and 600 mg/m^2 Cyclophosphamide, and 100 mg/m^2 Taxotere) was administered to 12% of LABC patients; 6FAC3T was provided to 38% of IBC patients, and 6CMF (and 500 mg/m^2 Cyclophosphamide, Methotrexate 40 mg/m^2, and Fluorouracil, 500 mg/m^2) was given to 3% IBC and 5% LABC patients (Table S1). Anti-HER2 therapy was introduced in Algeria in 2008, but it was provided only to metastatic IBC patients; none of the IBC or LABC patients reported here receive anti-HER2 therapy. A combination of Tamoxifen and Goserelin was provided to 38% HR^{+} IBC and 38% HR^{+} LABC patients. Aromatase inhibitors were provided to 40% H R^{+} IBC and 37% HR^{+} LABC patients. All LABC patients and 99% of IBC patients underwent mastectomy, while the remaining 1% of IBC patients declined surgical treatment. Radiotherapy was provided to 88% and 85% of IBC and LABC patients, respectively (Table S1).

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Statistical analysis

Primary outcomes were pCR, BCSS and DFS. Patient outcome (BCSS and DFS) was analyzed with survival methods. The DFS interval was calculated from the date of diagnosis to development of first recurrence. Patients without recurrence were censored at the time of last follow-up or death. BCSS was calculated from the date of diagnosis with death from breast cancer scored as an event and censoring of other patients at the date of last follow-up or non-disease-related death. The Kaplan–Meier method with the log-rank test was used to estimate DFS and BCSS. Final multivariate models were obtained by a Cox stepwise procedure and verified by backward elimination to identify time-independent prognostic factors of outcome in IBC and LABC cohorts (13). Two-tailed P values less than 0.05 were considered statistically significant. Statistical analyses were carried out using SAS (version 9.3; Cary, NC, USA) and GraphPad Prism (version 7.02; San Diego, CA, USA) software. Further details are provided in Supplementary data (Statistical Methods).

Results

IBC is associated with aggressive risk factors and worse survival compared to LABC patients

Our cohort of IBC patients had high numbers of lymph nodes affected at diagnosis, increased rates of lymphovascular invasion (LVI), an absence of tumor masses, and high numbers of overweight/obese patients. Urban dwellers and people with medium to higher economic status were more often affected by IBC than LABC ($P < 0.002$ for all comparisons; Table 1). There were no differences between IBC and LABC patients by tumor grade, menopausal status, parity, histopathology, family history of cancer, recurrence rates, and tumor receptor status ($P > 0.05$ for all comparisons; Table S1).

During the evaluation period, 68% and 46% of IBC and LABC patients, respectively, died of cancer ($P < 0.0001$; Table 1). The median follow-up was 50 months (interquartile range (IQR), 31.7–69.2) and 66 months (IQR 33.2–86.6) in IBC and LABC, respectively. The overall survival (32% vs. 54%) as well as the 3-year (66% vs. 72%) and 5-year (37% vs. 55%) BCSS were worse in IBC than LABC patients (Fig. 1). Most of the characteristics observed in Algerian IBC patients are similar to other IBC cohorts described in North American [6, 22–24], European [25–29], and Northern African studies [7, 8, 10].

Higher numbers of CD20$^+$ and PD-L1$^+$ TILs cells positively correlate with outcome in IBC, but not LABC

There was no difference in overall TILs levels in IBC and LABC samples (Table 1). Higher numbers of TILs were observed in triple negative (TN) samples followed by lower levels in HER2$^+$, ER$^+$, and PR$^+$ samples in both IBC (Fig. 2a) and LABC (Fig. 2b) specimens. The presence of high TILs positively correlated with achievement of pCR, TN and ER$^-$ status, the presence of CD20$^+$ B cells, PD-L1$^+$ carcinoma cells, combined CD20$^+$ and PD-L1$^+$ carcinoma cells (CD20$^+$PD-L1$^+$carcinoma cells), and CD20$^+$PD-L1$^+$ TILs in both IBC and LABC patients ($P < 0.01$ for all comparisons; Table 2). The presence of high TILs correlated with the presence of PD-L1$^+$ TILs only in IBC patients ($P = 0.005$; Table 2). We recently demonstrated that CD20$^+$PD-L1$^+$ TILs was associated with both DFS and BCSS in the

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whole IBC cohort as well as in the TN IBC subtype [13]. $CD20^{+}PD\text{-}L1^{+}$ TILs were not associated with either DFS or BCSS in LABC patients (data not shown).

pCR is associated with improved outcome in IBC and LABC patients

pCR rates were significantly higher in LABC than in IBC patients (20% vs. 9%; $P = 0.005$; Table 1). Univariate analysis demonstrated a positive association between pCR and absence of recurrences, high survival rates, and absence of LVI in both IBC and LABC patients (Table 3; $P < 0.02$ for all comparisons). pCR rates were also higher in IBC patients with fewer involved lymph nodes, overweight, and HR-negative patients, and in older and post-menopausal LABC patients (Table 3; $P < 0.03$, for all associations). Higher pCR rates were associated with high overall numbers of TILs; low number of $PD\text{-}L1^{+}$ carcinoma cells and low number of $CD20^{+}PD\text{-}L1^{+}$carcinoma cells in both IBC and LABC; with high number of $CD20^{+}$ TILs and $CD20^{+}PD\text{-}L1^{+}$ TILs in IBC patients only; and with high $PD\text{-}L1^{+}$ TILs in LABC only ($P < 0.04$ for all associations; Table 3). While older LABC patients showed higher pCR rates, age was not associated with pCR in IBC patients (Table 3). On univariate analysis, patients who achieved pCR experienced improved BCSS and DFS in both IBC (Fig. 3a and b) and LABC (Fig. 3c and d), compared to patients who did not exhibit pCR ($P < 0.02$ for all associations).

Multivariate analysis reveals differential impact of pCR and immune cells on patient outcome for IBC and LABC

Multivariate analysis, using a stepwise evaluation and verified by backward elimination, revealed that the most significant favorable prognostic factors for DFS in the IBC cohort were pCR and $CD20^{+}PD\text{-}L1^{+}$ TILs, with the $CD20^{+}PD\text{-}L1^{+}$ TILs remaining significantly associated with improved DFS in TN IBC patients (Table 4). Similarly, multivariate analysis revealed that the most significant favorable prognostic factors for BCSS in the IBC cohort were $CD20^{+}PD\text{-}L1^{+}$ TILs and receipt of 3FAC3T, with $CD20^{+}PD\text{-}L1^{+}$ TILs remaining significantly associated with improved BCSS in TN IBC patients (Table 4). Multivariate analysis showed that pCR was a most significant favorable prognostic factor for DFS and BCSS in the LABC cohort, while the presence of LVI was associated with worse BCSS (Table 4). pCR remained positively associated with a favorable prognosis in TN LABC patients (Table 4).

Discussion

This large, retrospective, single-center study confirms the aggressive clinical features and adverse prognosis of IBC patients when compared with LABC patients, despite receiving a standardized, combined modality approach incorporating systemic NACT, surgery, and radiation therapy [5, 6]. The 5-year BCSS was worse in IBC than LABC patients (37% vs. 55%), similar to prior published studies [2, 6, 25, 30]. pCR rates were significantly lower in IBC (9%) than LABC patients (20%), as reported in other comparative studies where pCR rates ranged from 9 to 33% for IBC, and from 11 to 31% for LABC patients [27, 30–34]. pCR rates have been associated with improved IBC patient outcome in some studies [8, 26, 35–37], but not associated in other studies [27, 30, 38], and lack of association between pCR rates and clinicopathologic variables has also been reported [8, 28]. Here,

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univariate Kaplan–Meier analysis demonstrated improved DFS and BCSS for both IBC and LABC patients with higher pCR rates. Further, in multivariate analysis, high pCR rates were associated with improved DFS in IBC, LABC, and TN LABC patients, and with improved BCSS in LABC patients.

In a previous study, we reported that the presence of TILs was associated with increased rates of pCR following NACT and that immune infiltration by $CD20^+PDL1^+$TILs was an independent factor associated with long-term outcome in IBC patients. Our findings suggest that immune cells in the tumor microenvironment play a critical role in generating anti-tumor immune responses in IBC patients [13]. In this report, $CD20^+PDL1^+$TILs was not associated with outcome in LABC patients. The frequency of overall TILs, $CD20^+$, $CD20^+PDL1^+$TILs and $CD20^+PDL1^+$ carcinoma cells were similar in IBC and LABC, but the frequency of PD-$L1^+$ carcinoma cells and PD-$L1^+$ TILs were higher in LABC than in IBC without reaching significance. The lack of association of $CD20^+PDL1^+$TILs with outcome in LABC may be related to known differences in the TME in LABC compared to IBC, and the different functional roles of PD-L1 in the TME in these two tumor types [39, 40].

A recent study reported higher levels of PD-L1 protein expression in IBC than non-IBC samples (42.9% vs. 23.7%) and, as in our study, low PD-L1 + protein expression in cancer cells (~ 2%) [28]. Further, high PD-$L1^+$ TILs levels were associated with TILs and high pCR rates, as in our study, but in contrast to our study, they were not associated with outcome in IBC patients [28]. In another study in IBC patients, PD-L1 protein expression was higher in TILs (27%) than in the epithelial cancer cells (2%), and no association with outcome was observed [37]. It should be also noted there is a study reporting high levels of PD-$L1^+$ protein expression in carcinoma cells (~ 37%) without significant associations with clinicopathologic variables, but identifying PD-$L1^+$ carcinoma cells as an independent prognostic factor of worse overall survival in IBC patients [41]. Of note, PD-$L1^+$ TILs were not evaluated in that study. A recent meta-analysis of 19,400 breast cancer patients reported high PD-$L1^+$ expression in 74.3% in cancer cells; patients with high PD-$L1^+$ expression were more likely to achieve a pathological complete response after NACT, but overall survival was worse [42]. That study, however, evaluated PD-L1 expression as a whole, at the transcriptome and protein levels, and did not evaluate PD-L1 expression in stromal TILs. Interestingly, another meta-analysis of ~ 14,400 breast cancer patients evaluating PD-L1 expression by IHC reported the association of PD-L1 expression in cancer cells with poor prognosis, while PD-L1 + TILs was correlated with improved survival [43]. These data suggest that PD-L1 expression on tumor cells inhibits the recognition and elimination of tumor cells by cytotoxic T cells leading to an improper immune response and worse outcome; in contrast, expression of PD-L1 in TILs denotes the presence of a suppressed pre-existing immunity which can be released and/or re-invigorated by treatment and result in improved outcome.

The presence of high TILs and PD-$L1^+$TILs in both IBC and LABC would suggest similar patient outcomes, but IBC patients had worse overall clinical outcome than LABC patients. This may be related to other factors within the tumor microenvironment (TME) in IBC samples. While the simple measurement of PD-L1 expression identified it as a prognostic

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marker associated with outcome in IBC patients, a single measurement of PD-L1 expression does not capture the whole complexity of the TME in IBC, the different immune cell subtypes, as well as the spatial proximity of immune cell types to each other and to tumor cells within the TME. Supporting the importance of immune cells in IBC, it was demonstrated that in IBC patients who did not respond to NACT, mast cells were located within close proximity to CD8 + T cells, CD163 + macrophages, and tumor cells, suggesting mast cells may be exerting immunosuppressive effects by interacting with these cell types in particular [37]. Furthermore, a recent study reported that patients with $FOXP3^{+}$ Tregs clustered near $CD8^{+}$ cytotoxic T cells had a worse outcome, and pCR was achieved more often in patients with fewer Tregs near the tumor cells [44]. In addition, it has been demonstrated that activated immune cells induce the secretion of immune factors (TNF-α, IL-6, IL-1ß, TGF-β) associated with the EMT process, which can promote immune evasion and metastasis [45]. This could explain the aggressive metastatic behavior of IBC and poor outcomes. Collectively, a more detailed analysis of the composition and spatial location of immune cell within the TME to identify biomarkers of pCR and outcome in IBC is warranted. Similar studies in LABC will also provide a deeper knowledge of the TME in LABC patients and immune differences between IBC and LABC which could be of clinical value.

This study utilizes one of the largest and well annotated IBC and LABC cohorts analyzed to date, obtained from the main cancer center in Algeria, and is likely highly representative of the general population in Algeria. Due to socialized, free health care in Algeria, both IBC and LABC patients have access to standard of care treatments, which reduces treatment variability when analyzing clinical outcomes. Further, the diagnosis of IBC in all patients was made following the recommendations of the International IBC Expert Panel [15], and all biomarkers were characterized with validated antibodies. The absence of a validation cohort, lack of information about anti-HER2 therapy and other important known risk factors, and the use of TMAs represent limitations of our study. While anti-HER2 therapy in the NACT setting is associated with improved outcome [46], the lack of this therapy in the NACT in both IBC and LABC may have negatively affected patient outcomes. However, we reported DFS and BCSS outcomes in both IBC and LABC within the range of published IBC and LABC cohorts where anti-HER2 therapy was not available either as NACT or it was not provided to all HER2 + patients [8, 23, 25, 45, 47, 48].

Although comparison between TMAs and whole slide pathology scores revealed systematically higher values in full face slides [49], the scoring of PD-L1 by IHC assays widely varies even between TMAs or full face slides. Positive $PD\text{-}L1^{+}$ expression of 1.7%–60% was reported in ~ 6000 non-IBC patients using TMAs, and in the range of 21%–46% in ~ 900 patients. Variability was observed in cut-offs used (1% or 5%), antibodies used, and type of labeling (from membranous to cytoplasmic or both) [41]. The results we report in this study for PD-L1 expression using TMAs are within the range described in the previous studies. Variability was also found in the antibodies used for IHC to detect PD-L1, with Leica Bond Max (clone 22C3) and Ventana BenchMark Ultra platform being the most commonly used. Furthermore, while several tests or diagnostics assays for detecting PD-L1 exist [41–43], a recent expert report suggested that only the antibody anti-PD-L1

SP142 possesses proven diagnostic value for selecting metastatic TNBC patients eligible for atezolizumab immunotherapy [50].

Our study demonstrates the presence of an active pre-existing immune response, which could affect responses to NACT, and identifies $CD20^{+}PD\text{-}L1^{+}$ TILs as a predictive biomarker of pCR and outcome in IBC patients but not LABC patients. Given the high frequency of objective responses observed in $PD\text{-}L1^{+}$ metastatic TN breast cancer patients to immune therapy [51], selecting IBC patients by CD20 and PD-L1 status could potentially further identify or stratify IBC patients who would benefit from activating CD20 agents anti-PD-1/PD-L1 therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of interest

LAE receives honoraria and research funding to the institution from Aduro Biotech, Astrazeneca, Bristol Meyers Squibb, Corvus, EMD Serono, Genentech, HeritX, Inc., Maxcyte, Merck, Roche, Tempest; and royalties from Aduro Biotech. She has served on Consulting/Advisory boards for AbbVie, Amgen, Astrazeneca, Bayer, Bristol Meyers Squibb, Celgene, Chugai, eTHeRNA, Genentech, Gritstone, Medimmune, Molecuvax, Macrogenics, Novartis, Peregrine, Replimune, Roche, Silverback, Syndax, Vaccinex. ACM receives honoraria from Bristol-Myers Squibb and Roche and research funding to the institution from Bristol-Myers Squibb, Genentech, and HeritX, Inc. Dr. Fiering is a co-founder of and has a financial interest in Mosaic Immunoengineering Inc. The remaining authors declare no conflict of interest.

Abbreviations

BCSS	Breast cancer-specific survival
BMI	Body mass index
CI	Confidential interval
DFS	Disease-free survival
FFPETs	Formalin-fixed paraffin embedded tissues
HR	Hazard ratio

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IBC	Inflammatory breast cancer
LABC	Locally advanced breast cancer
LVI	Lymphovascular invasion
NACT	Neoadjuvant chemotherapy
pCR	Pathological complete response
PD-1	Programmed cell death 1
PD-L1	Programmed cell death ligand 1
TILs	Tumor infiltrating lymphocytes
TME	Tumor microenvironment
TN	Triple-negative

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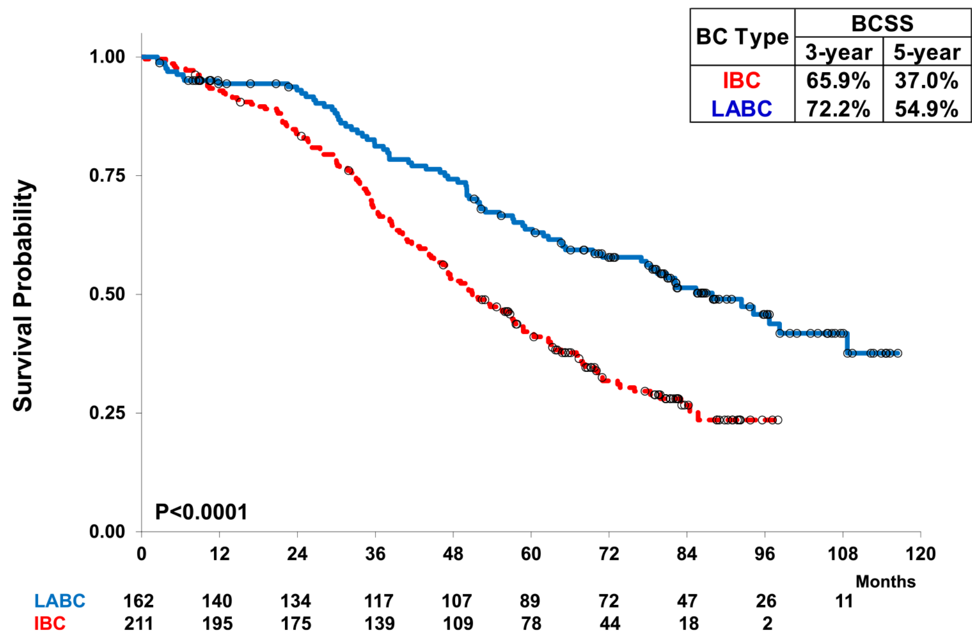

Fig. 1.

Breast cancer-specific survival (BCSS) is worse in IBC patients than in LABC. Kaplan–Meier survival estimates of BCSS in IBC (discontinuous red lines) and LABC (solid blue lines). The 3-and 5-year BCSS rates for IBC and LABC are shown in the inset. The number of patients at risk of relapse and/or death from IBC and LABC are shown below the x-axis at 0, 12, 24, 36, 48, 72, 84, 96, 108, and 120 months. Censored events are indicated by circles on each curve

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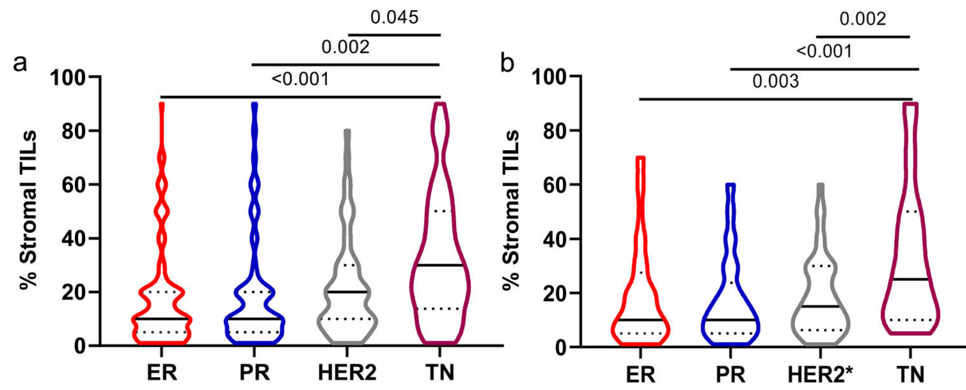

Fig. 2.

Tumor-infiltrating lymphocytes are higher in triple negative IBC and LABC. Violin plots show the high frequency of stromal TILs in TN followed by HER2, ER, and PR in **a** IBC and **b** LABC samples. The solid line in the violin plots indicate the mean and the dotted lines, the lower and upper quartiles

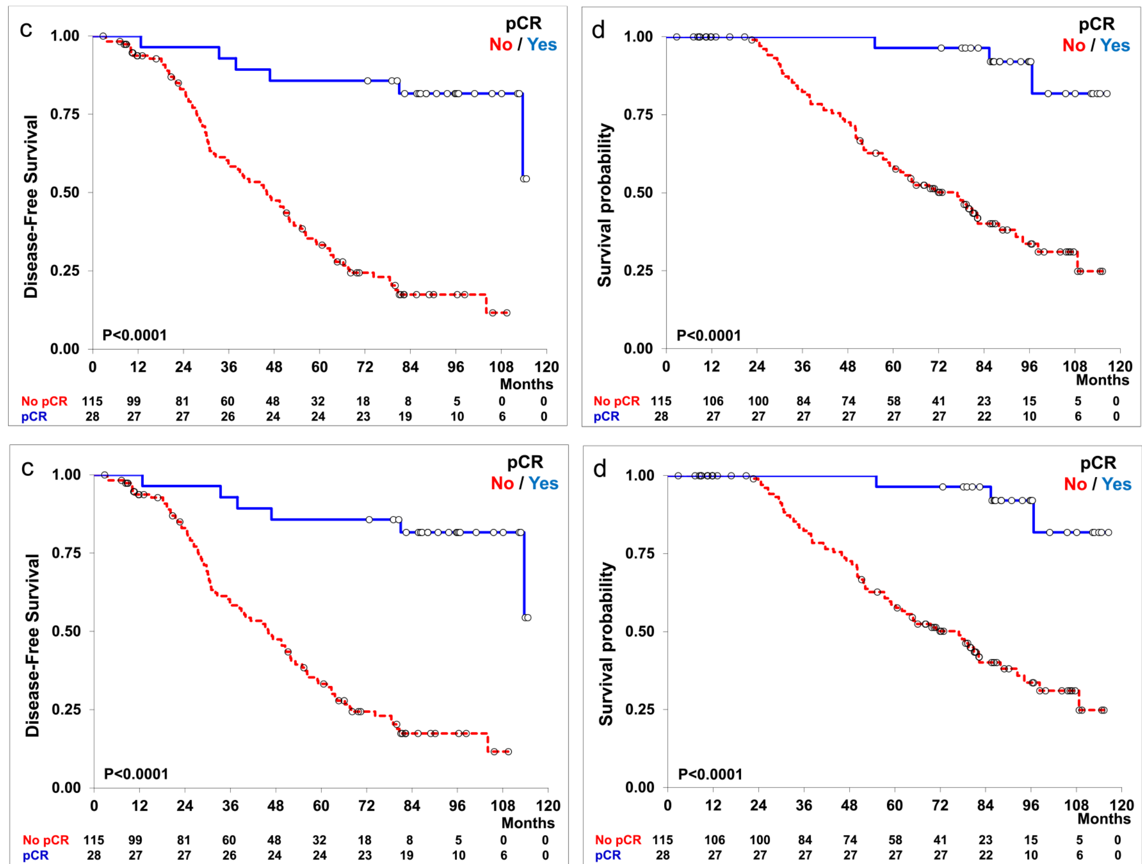

Fig. 3.

pCR rates are associated with improved outcome in IBC and LABC patients. Kaplan–Meier survival estimates of DFS (**a**, **d**) and BCSS (**b**, **c**) in IBC (**a**, **b**) and LABC (**c**, **d**) patients who showed pCR (solid blue lines) compared to those who did not (discontinuous red lines). The number of patients at risk of relapse and/or death from IBC and LABC are shown below the x-axis at 0, 12, 24, 36, 48, 72, 84, 96, 108, and 120 months. Censored events are indicated by circles on each curve

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Table 1

Demographic, clinicopathologic and molecular characteristics of IBC and LABC patients

Variable	IBC	LABC	*P*	Variable	IBC	LABC	*P*
Status at last follow-up				HR + HER2 +			
Alive	67 (31.7)	87 (53.7)		Negative	181 (85.8)	143 (88.3)	
Deceased	144 (68.3)	75 (46.3)	<0.0001	Positive	30 (14.2)	19 (11.7)	0.538
LN				HR-HER2 +			
3	68 (32.7)	77 (48.7)		Negative	186 (88.2)	144 (88.9)	
4	140 (67.3)	81 (51.3)	0.002	Positive	25 (11.8)	18 (11.1)	0.871
LVI				HR-HER2–			
Absent	47 (22.3)	143 (88.3)		Others	169 (80.1)	120 (74.1)	
Present	164 (77.7)	19 (11.7)	<0.0001	HR-HER2–	42 (19.9)	42 (25.9)	0.172
pCR				TILs			
No	177 (91.2)	115 (80.4)		Low (< 15%)	102 (48.3)	76 (47.2)	
Yes	17 (8.8)	28 (19.6)	0.006	High (15%)	109 (51.7)	85 (52.8)	0.835
Palpable masses				$CD20^+$			
No	138 (65.7)	0 (0.0)		< 1%	76 (36.9)	47 (30.3)	
Yes	72 (34.3)	162 (100.0)	<0.0001	1%	130 (63.1)	108 (69.7)	0.218
Residence				$PD\text{-}L1^+$ carcinoma cells			
Urban	140 (66.4)	63 (38.9)		< 5%	193 (91.5)	138 (85.2)	
Rural	71 (33.6)	99 (61.1)	<0.0001	5%	18 (8.5)	24 (14.8)	0.069
Body mass index				$PD\text{-}L1^+$ TILs			
Lean/normal	66 (46.5)	76 (46.9)		< 5%	72 (34.1)	41 (25.3)	
Overweight	143 (68.4)	86 (53.1)	0.004	5%	139 (65.9)	121 (74.7)	0.070
Socio-economic status				$CD20^+PDL1^+$ carcinoma cells			
Low	71 (33.6)	116 (71.6)		Others	195 (92.4)	142 (87.7)	
Medium	120 (56.9)	39 (24.1)		Positive	16 (7.6)	20 (12.3)	0.157
High	20 (9.5)	7 (4.3)	<0.0001	$CD20^+PDL1^+$ TILs			
HR + HER2–				Others	114 (54.0)	74 (45.7)	
Negative	97 (46.0)	79 (48.8)		Positive	97 (46.0)	88 (54.3)	0.118
Positive	114 (54.0)	83 (51.2)	0.603				

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LN lymph node involvement at diagnosis, *LVI* lymphovascular invasion, *pCR* pathological complete, *TILs* tumor-infiltrating lymphocytes

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Table 2

Associations of TILs with pathologic factors and immune cell subtypes in IBC and LABC patients

Variable	A. TILs in IBC patients			B. TILs in LABC patients		
	Low	High	P	Low	High	P
Pathologic complete response						
No	91 (96.8)	86 (86.0)		62 (89.9)	52 (71.2)	
Complete	3 (3.2)	14 (14.0)	0.010	7 (10.1)	21 (28.8)	0.006
HR-HER2–						
Others	92 (90.2)	77 (70.6)		64 (84.2)	55 (64.7)	
HR-HER2–	10 (9.8)	32 (29.4)	0.001	12 (15.8)	30 (35.3)	0.007
HR + HER2–						
Negative	35 (34.3)	62 (56.9)		29 (38.2)	49 (57.7)	
Positive	67 (65.7)	47 (43.1)	0.001	47 (61.8)	36 (42.3)	0.018
$CD20^{+}$						
< 1%	49 (49.5)	27 (25.2)		35 (46.7)	12 (15.2)	
1%	50 (50.5)	80 (74.8)	0.0005	40 (53.3)	67 (84.8)	< 0.0001
PD-$L1^{+}$ carcinoma cells						
< 5%	101 (99.0)	92 (84.4)		71 (93.4)	66 (77.7)	
5%	1 (1.0)	17 (15.6)	< 0.0001	5 (6.6)	19 (22.3)	0.007
PD-$L1^{+}$ TILs						
< 5%	44 (43.1)	28 (25.7)		21 (27.6)	19 (22.3)	
5%	58 (56.9)	81 (74.3)	0.009	55 (72.4)	66 (77.7)	0.469
$CD20^{+}PDL1^{+}$ carcinoma cells						
Others	101 (99.0)	94 (86.2)		73 (96.1)	68 (80.0)	
Positive	1 (1.0)	15 (13.8)	0.0004	3 (3.9)	17 (20.0)	0.003
$CD20^{+}PDL1^{+}$ TILs						
Others	69 (67.6)	45 (41.3)		46 (60.5)	27 (31.8)	
Positive	33 (32.4)	64 (58.7)	0.0002	30 (39.5)	58 (68.2)	0.0003

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Table 3

Factors associated with pathological complete response (pCR) in IBC and LABC

Variable	pCR_IBC			pCR_LABC		
	No	**Yes**	***P***	**No**	**Yes**	***P***
Recurrence						
Absent	58 (32.8)	11 (64.7)		33 (28.7)	22 (75.6)	
Present	119 (67.2)	6 (35.3)	0.015	82 (71.7)	6 (21.4)	< 0.0001
Survival						
Alive	51 (28.8)	11 (64.7)		53 (46.1)	25 (89.3)	
Deceased	126 (71.2)	6 (35.3)	0.005	62 (53.9)	3 (10.7)	< 0.0001
LVI						
Absent	32 (18.1)	9 (52.9)		98 (85.2)	28 (100.0)	
Present	145 (81.9)	8 (47.1)	0.002	17 (14.8)	0 (0.0)	0.025
LN						
3	53 (29.9)	10 (58.8)		54 (47.8)	13 (46.4)	
4	124 (70.1)	7 (41.2)	0.027	59 (52.2)	15 (53.6)	1.000
Body mass index						
Lean	62 (35.2)	1 (5.9)		53 (46.1)	14 (50.0)	
Overweight	114 (64.8)	16 (94.1)	0.013	62 (53.9)	14 (50.0)	0.833
HR + HER2−						
Negative	76 (42.9)	13 (76.5)		49 (42.6)	18 (64.3)	
Positive	101 (57.1)	4(23.5)	0.010	66 (57.4)	10 (35.7)	0.056
Age						
50	118 (66.7)	10 (58.8)		71 (61.7)	11 (39.3)	
> 50	59 (33.3)	7 (41.2)	0.594	44 (38.3)	17 (60.7)	0.035
Menopausal status at diagnose						
Premenopausal	119 (67.6)	9 (52.9)		76 (66.1)	11 (39.3)	
Postmenopausal	57 (32.4)	8 (47.1)	0.283	39 (33.9)	17 (60.7)	0.016
TILs						
Low (< 15%)	91 (51.4)	3 (17.6)		62 (54.4)	7 (25.0)	
High (15%)	86 (48.6)	14 (82.4)	0.010	52 (45.6)	21 (75.0)	0.006

Variable	pCR_IBC			pCR_LABC		
	No	Yes	*P*	No	Yes	*P*
PD-L1⁺ carcinoma cells						
< 5%	165 (93.2)	12 (70.6)		102 (88.7)	19 (67.9)	
5%	12 (6.8)	5 (29.4)	0.009	13 (11.3)	9 (32.1)	0.016
PD-L1⁺ TILs						
< 5%	62 (35.0)	4 (23.5)		36 (31.3)	1 (3.6)	
5%	115 (65.0)	13 (76.5)	0.428	79 (68.7)	27 (96.4)	0.001
CD20⁺						
< 1%	71 (40.8)	1 (5.9)		32 (29.4)	7 (25.0)	
1%	103 (59.2)	16 (94.1)	0.003	77 (70.6)	21 (75.0)	0.815
CD20 + PDL1⁺ carcinoma cells						
Others	167 (94.3)	12 (70.6)		104 (90.4)	20 (71.4)	
Positive	10 (5.7)	5 (29.4)	0.005	11 (9.6)	8 (28.6)	0.013
CD20 + PDL1⁺ TILs						
Others	100 (56.5)	5 (29.4)		56 (48.7)	8 (28.6)	
Positive	77 (43.5)	12 (70.6)	0.041	59 (51.3)	20 (71.4)	0.060

pCR pathological complete response, *LN* lymph node involvement at diagnosis, *LVI* lymphovascular invasion, *TILs* tumor-infiltrating lymphocytes

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Table 4

Multivariate analysis of factors associated with disease free survival and breast cancer-specific survival in IBC and LABC patients

Tumor type	Variable	HR	95%CI	*P* value
Disease-free survival				
Whole IBC cohort	pCR	0.40	0.16–0.99	0.049
	$CD20^{+}PDL1^{+}$ TILs	0.52	0.36–0.77	0.001
Triple-negative IBC	$CD20^{+}PDL1^{+}$ TILs	0.28	0.11–0.69	0.006
Whole LABC cohort	pCR	0.11	0.04–0.29	< 0.0001
Triple-negative LABC	pCR	0.09	0.01–0.71	0.022
Breast cancer-specific survival				
Whole IBC cohort	$CD20^{+}PDL1^{+}$ TILs	0.55	0.38–0.79	0.001
	3FAC3T	0.84	0.74–0.95	0.006
Triple-negative IBC	$CD20^{+}PDL1^{+}$ TILs	0.30	0.12–0.75	0.010
Whole LABC cohort	pCR	0.08	0.02–0.34	0.001
	LVI	2.04	1.04–4.00	0.039

HR hazard ratios, *CI* confidential intervals, *pCR* pathological complete response, *FACT, F* fluorouracil, *A* adriamycin, *C* cyclophosphamide, *T* Taxotere, *LVI* lymphovascular invasion