Infiltrating stromal immune cells in inflammatory breast cancer are associated with an improved outcome and increased PD-L1 expression

C. Van Berckelaer¹,²*, C. Rypens¹, P. van Dam², L. Pouillon², M. Parizel³, K. A. Schats⁴, M. Kockx⁵, W. A. A. Tjalma², P. Vermeulen¹,³, S. van Laere¹, F. Bertucci⁶, C. Colpaert⁴,⁷ and L. Dirix¹,³

Abstract

Background: Inflammatory breast cancer (IBC) is a rare and rapidly progressive form of invasive breast cancer. The aim of this study was to explore the clinical evolution, stromal tumour-infiltrating lymphocytes (sTIL) infiltration and programmed death-ligand 1 (PD-L1) expression in a large IBC cohort.

Patients and methods: Data were collected prospectively from patients with IBC as part of an international collaborative effort since 1996. In total, 143 patients with IBC starting treatment between June 1996 and December 2016 were included. Clinicopathological variables were collected, and sTIL were scored by two pathologists on standard H&E stained sections. PD-L1 expression was assessed using a validated PD-L1 (SP142) assay. A validation cohort of 64 patients with IBC was used to test our findings.

Results: Survival outcomes of IBC remained poor with a 5-year overall survival (OS) of 45.6%. OS was significantly better in patients with primary non-metastatic disease who received taxane-containing (neo)adjuvant therapy (P = 0.01), had a hormone receptor-positive tumour (P = 0.001) and had lower cN stage at diagnosis (P = 0.001). PD-L1 positivity on immune cells (42.9%) was higher in IBC than in non-IBC in both our patient samples and the validation cohort. Furthermore, PD-L1 expression predicted pCR (P = 0.002) and correlated with sTIL infiltration (P < 0.001). sTIL infiltration of more than 10% of the stroma was a significant predictor of improved OS (HR 0.47, 95% CI 0.27–0.81, P = 0.006) in a multivariate model.

Conclusions: IBC is characterised by poor survival and high PD-L1 immunoreactivity on sTIL. This suggests a role for PD1/PD-L1 inhibitors in the treatment of IBC. Furthermore, we showed that PD-L1 expression predicts response to neo-adjuvant therapy and that sTIL have prognostic significance in IBC.

Keywords: Inflammatory breast cancer (IBC), Immune response, Programmed death-ligand 1 (PD-L1), Stromal tumour-infiltrating lymphocytes (sTIL), Immune checkpoint modulators

* Correspondence: christophe.vanberckelaer@uantwerpen.be

¹Translational Cancer Research Unit, GZA Hospitals & CORE, MIPRO, University of Antwerp, Antwerp, Belgium
²Multidisciplinary Breast Clinic, Unit Gynaecologic Oncology, Antwerp University Hospital (UZA), Edegem, Belgium

Full list of author information is available at the end of the article.

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Background
Inflammatory breast cancer (IBC) is an uncommon and aggressive form of breast cancer, characterised by rapid progression and a high risk of lymph node involvement or distant metastasis at diagnosis [1]. IBC accounts for a disproportional fraction of breast cancer-related mortality and tends to affect mainly younger women. This results in a significant loss of life years [2]. Furthermore, the incidence appears to be increasing [3] and IBC-specific therapies are lacking.

An international expert panel on IBC agreed that the diagnosis of IBC remains based on clinical features (Additional file 1: Table S1), and all patients should be treated with neo-adjuvant anthracycline- and taxane-based chemotherapy (NACT), radical modified mastectomy in non-metastatic patients and radiotherapy including the supraclavicular regions and internal mammary lymph nodes [4]. Hormonal or targeted therapy based chemotherapy (NACT), radical modified mastectomy (NACT) and that this effect was enhanced compared to nIBC [15]. In a large IHC study, pCR was also more common in patients with PD-L1 expression (on tumour cells) and an association with lymph node status and CD20+ TILs was seen [16]. However, the prognostic effect of PD-L1 in IBC remains ambiguous as different studies generated conflicting results [15–19] (Table 1).

It is still unclear if and how the immune response is able to influence the IBC phenotype with its fulminant local progression, typical tumour emboli formation and contribute to the unique biological features associated with IBC [6, 7]. Other research groups have also underlined the importance of the tumour stroma in IBC [8, 9]. The invasion of the tumour stroma by stromal tumour-infiltrating lymphocytes (STIL) is often associated with a better prognosis in ER-negative, more proliferative subtypes of nIBC [10]. Since IBC is characterised by an increased mutational load, this might lead to increased tumour antigen-based attraction of cytotoxic T cells [11].

Programmed death-ligand 1 (PD-L1) is an immune checkpoint molecule that can be expressed on both infiltrating immune cells and tumour cells. While PD-L1 positivity in breast cancer was associated with worse survival and adverse clinicopathological features [11], an improved response to therapy and better outcome in triple-negative breast cancer (TNBC) was also seen [12, 13]. An upregulation of PD-L1, as a marker of active immune response against TNBC might be a possible explanation [14]. Bertucci et al. showed on mRNA level that PD-L1 expression in IBC was associated with an improved response to NACT and that this effect was enhanced compared to nIBC [15]. In a large IHC study, pCR was also more common in patients with PD-L1 expression (on tumour cells) and an association with lymph node status and CD20+ TILs was seen [16]. However, the prognostic effect of PD-L1 in IBC remains ambiguous as different studies generated conflicting results [15–19] (Table 1).

It is still unclear if and how the immune response is able to influence the IBC phenotype with its fulminant local progression, typical tumour emboli formation and

**Table 1 Overview of all PD-L1 studies in IBC and their key findings.**

| First author (year, country) | No. | Population | Detection method | Cutoff for PD-L1+ | Positivity rate | Associated clinical and pathological variables | Associated outcome variables |
|-------------------------------|-----|------------|------------------|------------------|----------------|-----------------------------------------------|-------------------------------|
| He et al. [17] (2018, USA)    | 68  | Post NACT TN: 19/65 | IHC TMA [3] | 28-8 ≥ 1% TC | 25/68 (36.8%) | / | Worse OS (P = 0.042) |
| Arias-Pulido et al. [16] (2018, Algeria) | 221 | Pre NACT TN: 44/221 | IHC TMA [2] | SP142 ≥ 5% TC and IC | TC: 18/221 (8.1%) IC: 146/221 (66.1%) | TC: N+, CD20+ TIL, pCR IC: grade 3, CD20+ TIL | IC: better DFS (P = 0.035) |
| Reddy et al. [18] (2017, USA) | 14  | Pre NACT TN: - | IHC Biopsy | NR | 3/14 (21.4%) | NR | NR |
| Hamm et al. [19] (2016, USA) | 12  | Pre NACT TN: - | IHC Biopsy | E1LN3 H-score (TC and IC) | TC: 4/12 (33.3%) IC: 8/12 (66.7%) | NR | NR |
| Bertucci et al. [15] (2015, France) | 112 | Pre NACT TN: 28/112 | mRNA | / | 42/112 (37.5%) | ER negativity, basal and HER2+ subtype, cytotoxic T cell response, pCR | / |
| Discovery cohort (Belgium) | 105 | Pre NACT TN: 23/105 | IHC Biopsy | SP142 ≥ 1% tumour area (TC and IC) | TC: 2/105 (1.9%) IC: 45/105 (42.9%) | IC: stIL, pCR | / |
| Validation cohort (France) | 62  | Pre NACT TN: 12/62 | IHC Biopsy | SP142 ≥ 1% tumour area (TC and IC) | TC: 0/62 (0%) IC: 24/62 (38.7%) | IC: stIL | / |
rapid metastasis. The aim of this study was to explore sTIL infiltration, PD-L1 expression and the clinical evolution in a cohort of 143 IBC patients diagnosed and treated in Antwerp, Belgium, and in a validation cohort of 64 patients from Marseille, France.

Methods

Patient selection
We analysed clinical data and tumour specimens from 143 consecutive patients with IBC who had their initial diagnosis and complete treatment at the GZA Hospital Sint-Augustinus or the Antwerp University Hospital between June 1, 1996, and December 31, 2016. All cases were diagnosed based on the clinical IBC definition (Additional file 1: Table S1) and were pathologically confirmed as invasive carcinoma. Clinicopathological variables of all patients were collected from in-hospital medical records. Estrogen (ER) and progesterone receptor (PgR) expression had been assessed in the pathology department using validated immunohistochemical tests and defined as positive if Allred score ≥ 3/8. Tumours were considered HER2-positive when a fluorescence in situ hybridisation (FISH) test documented amplification. The absence of residual invasive carcinoma in the resected breast specimen and in all sampled regional lymph nodes after completion of NACT was defined as pathological complete response (pCR). Pre-treatment tumour tissue samples (34% core needle and 66% open biopsy samples) were analysed for sTIL and PD-L1. Subtype-matched nIBC patients (N = 142), diagnosed in Sint-Augustinus in 2006, were selected as a control group for the comparison of sTIL and PD-L1 scoring between IBC and nIBC. An extra cohort of 64 IBC (M0—stage III) patients diagnosed and treated in Marseille, France (Institut Paoli-Calmettes), was used as a validation cohort.

Stromal tumour-infiltrating lymphocytes (sTIL)

TIL scoring was performed on haematoxylin and eosin (H&E) stained 5-µm sections of formalin-fixed paraffin-embedded (FFPE) pre-treatment tumour tissue by two different researchers (CC and MP) according to the recommendations by the International TILs Working Group [20]. Given the specific pathology of IBC with often small and dispersed tumour cell nests, TILs were reported for the stromal compartment (% stromal TILs, sTIL) in all areas containing invasive tumour cells on the H&E slide containing the most invasive tumour. The mean scores of both pathologists were used both as continuous and categorical variables: < 10% (category 1), ≥ 10–40% (category 2), and ≥ 40% (category 3). The inter-class correlation coefficient (ICC) (two-way, agreement model) was 0.738 (95% CI 0.628–0.819, P < 0.001) showing a substantial agreement.

PD-L1

PD-L1 expression was assessed on 5-µm FFPE slides using a validated PD-L1 assay (clone SP142, Ventana Benchmark) on the tumour (TC) and infiltrating immune cells (IC). The staining protocol was optimised independently and previously described [21]. Scoring was done by two different researchers (CC and CVB) and based on the percentage of the tumour area that was occupied by PD-L1+ immune cells or the percentage of PD-L1+ tumour cells. A score of 0 (PD-L1−), 1, 2 or 3 was assigned for < 1%, ≥ 1% but < 5%, ≥ 5% but < 10% or ≥ 10% PD-L1-positive cells per tumour area, respectively [22]. These cutoffs were used for both tumour and immune cells (ICC 0.854, 95%CI 0.792–0.819, P < 0.001), and in case of a discrepancy between the researchers, a consensus score was determined.

Statistical analysis

Data were analysed using R studio (Version 1.1.414). Cases with missing data were maintained in the database but excluded from the statistical analyses on a per test basis. Pearson chi² test (categorical variables) and ANOVA (continuous variables) were used to assess the relationship between the different parameters. Significant parameters were included in a multivariate regression model. Evaluated survival endpoints were recurrence-free survival (RFS), distant metastasis-free survival (DMFS) and overall survival (OS). Survival data were last updated on January 1, 2018. Survival curves were calculated with Kaplan-Meier estimates and compared using the log-rank test. Significant clinicopathological variables were included in a multivariate Cox proportional hazards model. P values were calculated two-sided and considered statistically significant when < 0.05.

Results

Clinicopathological characteristics

Overall, 143 patients were included and clinicopathological characteristics are provided in Table 2. Most tumours were invasive ductal adenocarcinomas (N = 134, 94.4%) and poorly differentiated (N = 94, 71.2%). Half of the patients presented with an ER-positive tumour (N = 74, 52.5%), and 61 (44.5%) patients had a HER2-positive tumour (Fig. 1a). At the time of diagnosis, 103 (72.0%) patients had node-positive disease and 40 (28.0%) patients presented with primary metastatic disease. From the patients without metastatic disease at diagnosis, 91 patients (88.3%) completed NACT. Most of these patients underwent radical mastectomy (83/91, 91.2%) followed by radiotherapy (84/91, 92.3%). Considering that systemic therapies have changed during this study time interval, only 49 out of 78 patients (62.8%) with HR-positive cancer completed adjuvant hormonal therapy (23 patients received tamoxifen and 26 patients an aromatase inhibitor).
Less than half of the patients with HER2+ cancer received targeted therapy (27/62, 43.5%). An anthracycline-based regimen (78/91, 85.7%) combined with a taxane (74/91, 81.3%) was the most common type of NACT (Additional file 1: Figure S1). Almost a quarter of the patients that underwent a mastectomy after completing NACT (20/82, 24.4%) achieved a pCR, but no clinicopathological variable predicted pCR.

**sTIL and PD-L1 data**

Mean sTIL infiltration was 17.63% (95%CI 15.00–20.26%) and median sTIL infiltration was 10.00% (Q1: 7.5%–Q3: 25.0%). Infiltration with sTIL was high in triple-negative IBC (median 17.5%, mean 22.8%, N = 23) compared to other IBC subtypes. However, only a trend towards significance (P = 0.078) was reached and no correlation with other clinicopathological parameters could be discovered. PD-L1 positivity on tumour cells was very uncommon. Only 2 out of 105 patients (1.9%) showed membranous tumour cell staining in more than 1% of the tumour cells. Immunoreactivity on the infiltrating immune cells was more frequent. In 45 out of 105 tissue samples (42.9%), at least 1% of the tumour area was occupied by PD-L1+ immune cells. There was a strong correlation between PD-L1 positivity and sTIL scores (P < 0.001) (Fig. 1b). A correlation between PD-L1 and differentiation (P = 0.01) was also observed, but unlike sTIL scores, a higher grade was not significant in the multivariate model (Additional file 1: Table S2). PD-L1 immunoreactivity of sTIL correlated with pCR (P = 0.002) (Fig. 2) and the mean sTIL score in patients that achieved pCR was significantly higher than in patients who did not (28.4% vs. 16.8%, P = 0.03). However, only PD-L1 expression remained significant in a multivariate model (OR 1.22, 95% CI 1.07–1.40, P = 0.005) (Additional file 1: Table S3).

The control population of nIBC patients was age- and subtype-matched (Additional file 1: Table S4) but, in accordance with IBC biology, differed significantly for nodal status (95.7 vs. 38.6%; P < 0.001) and presence of metastatic disease (28.0 vs. 0.0%, P < 0.001) at time of diagnosis. Infiltration with sTIL was comparable between IBC and nIBC (> 10% infiltration: 64.2% in IBC vs. 70.4% in nIBC, P = NS). However, in the HER2+ group, TILs were significantly higher in nIBC patients (Fig. 3a, b). PD-L1 expression on sTIL was significantly more frequent in IBC (PD-L1+ samples: 42.9% in IBC vs. 23.7% in nIBC, P = 0.006). This difference remained significant in all but the HER2+ molecular subtype (Fig. 3c, d). In a multivariate logistic regression model, using sTIL infiltration and molecular subtype as possible confounders, patients with IBC also showed significantly more PD-L1+ immune cells (OR 2.43, 95%CI 1.19–5.04, P = 0.01) compared to nIBC patients.

**Survival data**

The median time to disease recurrence in patients with initially localised disease was 3.50 years (95%CI 2.02–11.45 years) (Additional file 1: Figure S2A). Of the 52 patients that relapsed during the study interval, 71.2% (N = 42) presented with distant metastasis as the first site of relapse. Brain metastases occurred in 21 patients (40.4%) initially having local disease that developed distant disease (Additional file 1: Table S5). Brain-only relapse was observed in 9 patients (17.3%). In the total IBC population, the 5-year OS was 45.7%.

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**Table 2** Patient and tumour characteristics for the IBC series, (n) = number of patients. ER oestrogen receptor, PgR progesterone receptor, HR hormone receptor

| Characteristic                          | (n)   | Description                  |
|----------------------------------------|-------|------------------------------|
| Mean age (143)                         | 60.1  | years (25.7–91.2 years)      |
| Mean sTIL score (106)                  | 17.63 | %, 95% CI 15.00–20.26%       |
| Menopausal status (142)                |       |                              |
| Premenopausal                          | 42    | (29.4%)                      |
| Postmenopausal                         | 101   | (70.6%)                      |
| cN stage (142)                         |       |                              |
| 0                                      | 6     | (4.2%)                       |
| 1                                      | 53    | (37.9%)                      |
| 2                                      | 52    | (37.1%)                      |
| 3                                      | 29    | (20.7%)                      |
| cM stage (143)                         |       |                              |
| 0                                      | 103   | (72.0%)                      |
| 1                                      | 40    | (28.0%)                      |
| Pathological type (142)                |       |                              |
| Ductal                                 | 134   | (94.4%)                      |
| Lobular                                | 5     | (3.5%)                       |
| Mixed                                  | 3     | (2.1%)                       |
| Differentiation (133)                  |       |                              |
| Grade 1                                | 3     | (2.3%)                       |
| Grade 2                                | 35    | (26.3%)                      |
| Grade 3                                | 95    | (71.4%)                      |
| ER (141)                               |       |                              |
| Negative                               | 67    | (47.5%)                      |
| Positive                               | 74    | (52.5%)                      |
| PgR (141)                              |       |                              |
| Negative                               | 88    | (62.4%)                      |
| Positive                               | 53    | (37.6%)                      |
| HER2+ (139)                            |       |                              |
| Negative                               | 77    | (55.4%)                      |
| Positive                               | 62    | (44.6%)                      |
| Molecular subtype (138)                |       |                              |
| Luminal (HR+)                          | 76    | (55.1%)                      |
| HER2+ (HR-HER2+)                       | 30    | (21.7%)                      |
| TN (HR-HER2–)                          | 32    | (23.2%)                      |
| sTIL (106)                             |       |                              |
| < 10%                                  | 38    | (35.8%)                      |
| ≥ 10 to < 40%                          | 54    | (51.9%)                      |
| ≥ 40%                                  | 13    | (12.3%)                      |
| PD-L1 immune cells (105)               |       |                              |
| < 1%                                   | 60    | (57.1%)                      |
| ≥ 1 to < 5%                            | 28    | (26.7%)                      |
| ≥ 5 to < 10%                           | 13    | (12.4%)                      |
| ≥ 10%                                  | 4     | (3.8%)                       |
was 8.92 years (95%CI 4.79–13.03 years) in patients with initially localised disease and 2.38 years (95%CI 1.45–3.28 years) in patients with primary metastatic disease (Additional file 1: Figure S2B).

A number of clinicopathological parameters were associated with a better prognostic outcome. A summary and corresponding OS curves are shown in Fig. 4 (curves for DMFS and RFS can be found in Additional file 1: Figures S3 and S4). A positive HR status was prognostic for a better OS in the total study population ($P = 0.05$) and was also associated with a longer RFS ($P = 0.001$) and DMFS ($P = 0.004$). Patients with HER2-positive tumours showed no significant differences in OS, RFS and DMFS when compared to patients with HER2-negative tumours. However, our data suggest that treatment with trastuzumab is associated with an improved outcome ($N = 52$, 5-year OS in HER2+ patients: 66.2% (with therapy) vs. 43.9% (without therapy), $P = 0.04$, Additional file 1: Figure S5). Other prognostic parameters were nodal disease at the moment of diagnosis, receiving taxane-containing neo-adjuvant regimens and achieving pCR after NACT. Finally, sTIL infiltration above 10% (i.e., above the median sTIL score of 10%) was also a prognostic marker for OS ($P = 0.05$) in the total patient cohort. PD-L1 positivity on immune cells was significantly higher in HR-negative IBC (median 22.5%) versus HR-positive IBC (median 10.0%). Tissue samples with more than 1% PD-L1+ tumour cells were not observed, but 38.7% (24/62) of the samples showed more than 1% PD-L1+ immune cells. An association between PD-L1 expression and clinicopathological features was not found, and PD-L1 immunoreactivity did not significantly correlate with pCR. However, 52.9% of the patients with pCR showed PD-L1 immunoreactivity vs. 32.5% of the patients without pCR (Additional file 1: Figure S7). A negative HR status ($P = 0.04$) and higher sTIL score ($P = 0.01$) correlated with pCR. However, in a logistic multivariate model, only sTIL score remained significant (OR 1.24, 95%CI

Validation cohort

To independently test our findings, we analysed an additional group of 64 IBC patients with non-metastatic disease, displaying similar clinicopathological characteristics as the discovery cohort (Additional file 1: Table S6 and Figure S6). Most of these patients received NACT followed by a mastectomy (59/64) and 28.8% (17/59) of the patients achieved pCR. Mean sTIL infiltration was 18.5% (95%CI: 14.7–22.2%) and correlated with PD-L1 immunoreactivity ($P < 0.001$), comparable to the discovery cohort. Furthermore, sTIL were significantly higher ($P = 0.05$) in HR-negative IBC (median 22.5%) versus HR-positive IBC (median 10.0%).
1.04–1.47, \( P = 0.02 \). The median OS was 8.86 years (CI 4.69–13.47 years), but no clinicopathological parameters, including sTIL score, were associated with a better OS or DFS (Additional file 1: Figure S8).

**Discussion**

PD-L1 expression and sTIL infiltration were retrospectively analysed in a cohort of patients with IBC. We confirm the higher frequency of negative HR status in IBC tumours as compared to nIBC [23]. Almost half of the IBC patients (44.2%) presented with a HR-negative phenotype, while only 12% of all newly diagnosed breast cancers were HR-negative in our hospital (between 2010 and 2014) and SEER data reported that around 21% of the breast cancer patients have a HR-negative tumour type [24]. The lack of expression of hormone receptors has also been associated with a more aggressive clinical course in IBC [25]. In our study, patients with HR-positive tumours had a better RFS (HR 0.47, 95% CI 0.25–0.89, \( P = 0.02 \)). Likewise, we confirmed the increased incidence of HER2 overexpression among IBC tumours [26]. However, in contrast to a large case-only analysis of 2014 women with IBC [27], we could not demonstrate any prognostic difference between patients with HER2-positive disease as compared to patients with HER2-negative disease. The reason for this difference is probably the lack of trastuzumab therapy in the majority of HER2-positive patients in our cohort treated before 2005, since trastuzumab therapy did predict a better OS in HER2-positive patients (\( P = 0.04 \)).

Patients that received taxane-based NACT had a more favourable outcome in our cohort, and this confirms the findings made earlier by Cristofanilli et al [28]. We also confirm that patients with pCR after NACT have an improved outcome. However, despite the aggressive treatment, survival outcome is very poor with a 5-year OS of only 45.6%, similar to those reported by others [29, 30]. Additionally, relapse (either locally or distant metastasis) typically occurred early during follow-up with a median DFS of 3.5 years and brain-only relapse was seen in 17.3% of the patients. These data show that IBC is a clinically aggressive disease and that the underlying molecular determinants of the IBC phenotype will require more investigation.
Although breast cancer is considered moderately immunogenic, the presence of neo-antigens seems to elicit an immune response and infiltrating immune cells play an essential role in the host-defence mechanism against ER-negative, more proliferative subtypes of nIBC in both adjuvant and neo-adjuvant studies [31, 32]. In our IBC cohort, sTIL infiltration was higher in triple-negative IBC compared to other subtypes. The association
Fig. 4  

a Overview of all clinicopathological values with their $\chi^2$ value in the table. Compared using the log-rank test, with associated significance levels.  

b–d Kaplan-Meier curves of significant prognostic variables for OS.  

b Hormone receptor status, 5-year OS 51.6% (HR+) vs. 39.6% (HR−), $P=0.05$.  

c Clinical nodal status, 5-year OS 62.3% (N1) vs. 41.8% (N2) vs. 29.7% (N3), $P=0.003$.  

d sTIL score, 5-year OS 55.4% (> 10%) vs. 28.7% (< 10%), $P=0.05$.  

Van Berckelaer et al. Breast Cancer Research (2019) 21:28  
Page 8 of 12
Table 3 Cox proportional hazards models for RFS, DMFS and OS. All significant clinicopathological variables were included in a multivariate Cox proportional hazards model. Distant disease at diagnosis, nodal status and sTIL infiltration are significantly associated with OS. In the group with initially localised disease, nodal status was significantly associated with OS and both pCR after NACT and HR status were associated with RFS.

| Parameter | Hazard ratio | Lower 95% CI | Higher 95% CI | P value |
|-----------|--------------|--------------|---------------|---------|
| sTIL (> 10%) | 0.465 | 0.266 | 0.811 | 0.006 |
| cN stage | 1.635 | 1.137 | 2.353 | 0.008 |
| cM stage | 3.060 | 1.794 | 5.219 | < 0.001 |
| HR status | 0.631 | 0.357 | 1.114 | 0.11 |
| Taxane NACT | 0.934 | 0.424 | 2.055 | 0.86 |
| pCR | 0.406 | 0.166 | 0.929 | 0.05 |
| cN stage | 1.530 | 0.981 | 2.39 | 0.06 |
| HR status | 0.564 | 0.295 | 1.08 | 0.08 |
| Taxane NACT | 0.771 | 0.348 | 1.71 | 0.52 |
| pCR | 0.391 | 0.149 | 1.03 | 0.06 |
| cN stage | 1.652 | 1.020 | 2.674 | 0.04 |
| HR status | 0.453 | 0.224 | 0.918 | 0.03 |
| Taxane NACT | 0.672 | 0.296 | 1.524 | 0.34 |
| pCR | 0.368 | 0.126 | 1.075 | 0.07 |

between pCR and sTIL was only significant in the validation cohort. Using 142 nIBC controls, we also showed that the number of sTIL is comparable between IBC and nIBC. In the HER2+ subtype, the number of sTIL was significantly higher in the nIBC cohort; however, the median number of sTIL in IBC (luminal 10.0%, HER2+ 12.5%, TN 17.5%) was comparable with that reported in patients with metastatic TNBC (mTNBC). The Impassion130 trial, using the same assay, showed that Atezolizumab plus paclitaxel prolonged survival in patients with mTNBC and that this benefit was larger in PD-L1+ patients [36]. A recent meta-analysis of predominantly early-stage breast cancer studies showed that PD-L1 positivity (expression on the tumour and/or immune cells) was associated with a worse OS (HR 1.76, 95% CI 1.09–2.82, P = 0.02) and adverse clinicopathological features [11]. Interestingly, PD-L1 mRNA positivity was associated with a better prognosis and response to therapy in TNBC [12]. A better outcome was also observed in TNBC patients with PD-L1 expression on the infiltrating immune cells [13, 37]. This might indicate that the prognostic effect of PD-L1 depends on the underlying (subtype-specific) immune response. Data on PD-L1 expression in IBC are limited (Table 1). Furthermore, there are substantial differences in study population, sample type and used assays (used AB, scoring method, cutoff values, immune or tumour cell). This renders comparison of PD-L1 expression between different studies unreliable.

Immune cell positivity for PD-L1 was seen in 42.9% of the IBC patients, which is higher than previous studies have reported in unselected patients with breast cancer (6.0%) [12] and in patients with TNBC (31.6%) [32]. Although our results are in agreement with the high PD-L1 positivity rates that were observed in other IBC studies [16, 17], we are the first to demonstrate that, independent of molecular subtype, PD-L1 expression in IBC is more frequent than in nIBC (OR 2.43, P = 0.01). In a validation cohort, we showed a similar PD-L1 expression (38.6%, P = 0.8, Additional file 1: Table S5) that was also more frequent than in nIBC (P = 0.04).

Some studies have demonstrated a positive relationship between PD-L1 and a cytotoxic T cell response [15] or CD20+ TILs [16]. In our cohorts, there also was a strong correlation with sTIL score, suggesting that PD-L1 expression is related to immune cell activation (Fig. 5). Together with the frequent expression, this adaptive expression pattern of PD-L1 suggests a suppressed immune response phenotype in IBC. Immune checkpoint modulators might therefore be useful in the treatment of IBC patients.
Similar to the study of Arias-Pulido et al., the level of tumour staining was low in our cohorts [16]. This can be related to the used antibody, but a low constitutive expression of PD-L1 on IBC tumour cells might be another explanation. We could not confirm the reported association between PD-L1 and grade or other clinicopathological features [16]. There are however substantial clinical differences between both studies. Most importantly, populations with a different genetic background with different proportions of molecular subtypes are being analysed. The different scoring methodology and the use of a TMA might also have contributed to these discrepancies. At present, the prognostic role of PD-L1 expression in IBC remains unclear. We could not corroborate a beneficial effect of PD-L1 positivity, and others have even reported a worse OS in IBC patients with PD-L1-positive tumour cells [17]. Finally, we confirm a positive correlation between PD-L1 expression and pCR after NACT [16].

The strength of this study is the analysis of a large cohort and a validation cohort of patients with a rare form of breast cancer. We managed to explore disease characteristics, clinical evolution and immune parameters of IBC over a long period of time (1996–2018). PD-L1 antigenicity decreases over time, a problem inherent to all retrospective IHC studies. Although PD-L1 expression was reduced in the older FFPE blocks, the year of diagnosis did not affect PD-L1 expression on the sTIL in a multivariate model (Additional file 1: Figure S9). Other potential limitations of this study are its retrospective character, the double-centre design and the fact that there are some missing or incomplete data.

**Conclusions**

IBC is characterised by a higher frequency of more proliferative, HR-negative tumours. Similar to nIBC, patients with a HR-positive tumour have a better prognosis. PD-L1 immunoreactivity on immune cells was seen in 42.9% of our patients and in 38.6% of the patients in the validation cohort. This was significantly higher than the nIBC control group and suggests a possible role for immune checkpoint inhibitors in the treatment of IBC. Infiltration with lymphocytes (sTIL) was a significant predictor of OS (HR 0.47, 95%CI 0.27–0.81, P = 0.006). The prognostic role of sTIL, together with the high frequency of PD-L1-positive immune cells, is an indication that an activated but suppressed immune microenvironment contributes to the aggressive and unique biological features associated with IBC.
Additional file

**Additional file 1:** Table S1. Minimum criteria required for the diagnosis of IBC. Table S2. Correlation between PD-L1 and clinicopathological tumour characteristics. Table S3. A logistic multivariate model for PD-L1 expression on immune cells. Table S4. Patient and tumour characteristics of the nIBC control group. Table S5. Frequencies of organ-specific metastases. Table S6. Patient and tumour characteristics of the IBC validation cohort from Marseille. Figure S1. Neo-adjuvant chemotherapy regimens. Figure S2. Kaplan-Meier curves of DFS and OS in the total IBC population (143 patients). Figure S3. Kaplan-Meier curves of significant prognostic variables for DFS. Figure S4. Kaplan-Meier curves of significant prognostic variables for DMFS. Figure S5. Kaplan-Meier curve showing significant survival benefit for HER2-positive IBC patients that received trastuzumab. Figure S6. Clinicopathological characteristics of the validation cohort. Figure S7. PD-L1 immunoreactivity and pCR in the validation cohort. Figure S8. Prognostic clinicopathological variables in the validation cohort. Figure S9. PD-L1 antigenicity decreases over time. (DOCX 10547 kb)

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**Availability of data and materials**
The datasets generated during and/or analysed during the current study are not publicly available due individual patient privacy but are available from the corresponding author on reasonable request.

**Authors’ contributions**
CVB, PVD, S VL, CC and LD are responsible for the conception and design of this study. CVB and LP collected the patient data. CC, MP and CVB were responsible for the sTIL and PD-L1 scoring. Analysis and interpretation of this study. CVB and LP collected the patient data. CC, MP and CVB were responsible for the sTIL and PD-L1 scoring. Analysis and interpretation of this study. CVB, PVD, SVL, CC and LD have been involved in drafting the manuscript and revising it critically. All authors have given final approval of the final version to be published.

**Ethics approval**
This study is conducted in accordance with the ethical standards of the University of Antwerp and received ethical approval from the ethics committee (File number: 16/33/338).

**Consent for publication**
Not applicable.

**Competing interests**
KS is an employee of HistoGeneX NV. MK is the CEO of HistoGeneX NV, which performs immunohistochemistry and molecular testing for pharmaceutical companies as part of (pre)clinical studies that evaluate new anticancer drugs. Other authors declare that they have no competing interests.

**Abbreviations**
DMFS: Distant metastasis-free survival; ER: Estrogen receptor; FFPE: Formalin-fixed paraffin-embedded; H&E: Haematoxylin and eosin; HR-negative: Hormone receptor-negative; HR-positive: Hormone receptor-positive; IBC: Inflammatory breast cancer; ICC: Interclass correlation coefficient (ICC); mTNBC: Metastatic triple-negative breast cancer; NACT: Neo-adjuvant chemotherapy; nIBC: Non-inflammatory breast cancer; OS: Overall survival; pCR: Complete pathologic response; PD-L1: Programmed death-ligand 1; PgR: Progesteron receptor; DFS: Disease-free survival; sTIL: Stromal tumour-infiltrating lymphocytes; TILs: Tumour-infiltrating lymphocytes; TMA: Tissue microarray; TNBC: Triple-negative breast cancer; WIBCC: World IBC consortium

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**Author details**
1Translational Cancer Research Unit, GZA Hospitals & CORE, MIPRO, University of Antwerp, Antwerp, Belgium. 2Multidisciplinary Breast Clinic, Unit Gynaecologic Oncology, Antwerp University Hospital (UZA), Edegem, Belgium. 3Department of Medical Oncology, GZA Hospitals Sint-Augustinus, Antwerp, Belgium. 4Department of Pathology, Antwerp University Hospital (UZA), Edegem, Belgium. 5HistoGeneX, Antwerp, Belgium. 6Predictive Oncology team, Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm, CNRS, Aix-Marseille Université, Institut Paoli-Calmettes, Marseille, France. 7Department of Pathology, GZA Hospitals Sint-Augustinus, Antwerp, Belgium.

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**References**
1. Levine PH, Veneroso C. The epidemiology of inflammatory breast cancer. Semin Oncol. 2008;35(1):11–6.
2. Woodward WA. Inflammatory breast cancer: unique biological and therapeutic considerations. Lancet Oncol. 2015;16(15):e568–76.
3. Hance KW, Anderson WF, Devesa SS, Young HA, Levine PH. Trends in inflammatory breast carcinoma incidence and survival: the surveillance, epidemiology, and end results program at the National Cancer Institute. J Natl Cancer Inst. 2005;97(13):966–75.
4. Dawood S, Merajver SD, Viens P, Vermeulen PB, Svinain SM, Buchholz TA, et al. International expert panel on inflammatory breast cancer: consensus statement for standardized diagnosis and treatment. Ann Oncol. 2011;22(3):515–23.
5. Fouad TM, Barrera AMG, Reuben JM, Lucci A, Woodward WA, Stauder MC, et al. Inflammatory breast cancer: a proposed conceptual shift in the UICC-AJCC TNM staging system. Lancet Oncol. 2017;18(4):e228–e32.
6. Van Laere SJ, Ueno NT, Finetti P, Vermeulen P, Lucci A, Robertson FM, et al. Uncovering the molecular secrets of inflammatory breast cancer biology: an integrated analysis of three distinct affymetrix expression datasets. Clin Cancer Res. 2013;19(17):4685–96.
7. Bertucci F, Ueno NT, Finetti P, Vermeulen P, Lucci A, Robertson FM, et al. Gene expression profiles of inflammatory breast cancer: correlation with response to neoadjuvant chemotherapy and metastasis-free survival. Ann Oncol. 2014;25(2):358–65.
8. Boersma BJ, Reimers M, Yi M, Liu D, Lidwig JA, Luke BT, Stephens RM, et al. A stromal gene signature associated with inflammatory breast cancer. Int J Cancer. 2008;122(3):1324–32.
9. Chai F, Liang Y, Zhang F, Wang M, Zhong L, Jiang J. Systematically identify key genes in luminal A and non-inflammatory breast cancer. Gene. 2016;575(2P3):600–14.
10. Ahn SG, Jeong JH, Hong S, Jung WH. Current issues and clinical evidence in tumor-infiltrating lymphocytes in breast cancer. J Pathol Transl Med. 2015;49(5):355–63.
11. Zhang M, Sun H, Zhao S, Wang Y, Hu H, Wang Y, et al. Expression of PD-L1 and prognosis in breast cancer: a meta-analysis. Oncotarget. 2017;8(18):31347–54.
12. Sabatier R, Finetti P, Mamsseier E, Adelade J, Chaffantin M, Ali HR, et al. Prognostic and predictive value of PDL1 expression in breast cancer. Oncotarget. 2015;6(7):5449–64.
13. Zhao T, Li C, Wu Y, Li B, Zhang B. Prognostic value of PD-L1 expression in tumor infiltrating immune cells in cancers: a meta-analysis. PLoS One. 2017;12(4):e0176822.
14. Cimino-Mathews A, Thompson E, Taube JM, Ye X, Lu Y, Meerker A, et al. PD-L1 (B7-H1) expression and the immune tumour microenvironment in primary and metastatic breast carcinomas. Hum Pathol. 2016;47(1):52–63.
15. Bertucci F, Finetti P, Colpaert C, Mamsseier E, Panzel M, Dixir L, et al. PDL1 expression in inflammatory breast cancer is frequent and predicts for the pathological response to chemotherapy. Oncotarget. 2015;6(15):13506–19.
16. Arias-Pulido H, Cimino-Mathews A, Chaher N, Qualls C, Joste N, Colpaert C, et al. The combined presence of CD20+ B cells and PD-L1 + tumor-infiltrating lymphocytes in inflammatory breast cancer is prognostic of improved patient outcome. Breast Cancer Res Treat. 2018;171(2):273–82.
17. He J, Huo L, Ma J, Zhao J, Bassett RL, Sun X, et al. Expression of programmed death ligand 1 (PD-L1) in posttreatment primary inflammatory breast cancers and clinical implications. Ann J Clin Pathol. 2018;149(3):253–61.
18. Reddy SM, Wargo JA, Reuben A, Tetzlaff MT, Roszik J, Reuben JM, et al. Immune and molecular determinants of response to neoadjuvant chemotherapy in inflammatory breast cancer. J Clin Oncol. 2017;35(15_suppl):11501.

19. Hamm CA, Moran D, Rao K, Trusk PB, Pry K, Sausen M, et al. Genomic and immunological tumor profiling identifies targetable pathways and extensive CD8+PD-L1+ immune infiltration in inflammatory breast cancer tumors. Mol Cancer Ther. 2016;15(7):1746–56.

20. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group. Ann Oncol. 2014;25(2):259–71.

21. Schats KA, Van Vie EA, De Schepper S, Boeckx C, Schrijvers DM, Waelput W, et al. Validated programmed cell death ligand 1 immunohistochemistry assays (E1L3N and SP142) reveal similar immune cell staining patterns in melanoma when using the same sensitive detection system. Histopathology. 2017;70(2):253–63.

22. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563–7.

23. Robertson FM, Bondy M, Yang W, Yamauchi H, Wiggins S, Kamrudin S, et al. Inflammatory breast cancer: the disease, the biology, the treatment. CA Cancer J Clin. 2010;60(6):351–75.

24. Dunnwald LK, Rossing MA, Li CI. Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. Breast Cancer Res. 2007;9(1):R6.

25. Li J, Xia Y, Wu Q, Zhu S, Chen C, Yang W, et al. Outcomes of patients with inflammatory breast cancer by hormone receptor- and HER2-defined molecular subtypes: a population-based study from the SEER program. Oncotarget. 2017;8(30):49370–9.

26. Parton M, Dowsett M, Ashleigh S, Hills M, Low E, Smith IE. High incidence of HER-2 positivity in inflammatory breast cancer. Breast. 2004;13(2):97–103.

27. Zell JA, Tsang WP, Taylor TH, Mehta RS, Anton-Culver H. Prognostic impact of human epidermal growth factor-receptor 2 and hormone receptor status in inflammatory breast cancer (IBC): analysis of 2,014 IBC patient cases from the California Cancer Registry. Breast Cancer Res. 2009;11(1):R9.

28. Cristofanilli M, Gonzalez-Angulo AM, Buzdar AU, Kauf SW, Frye DK, Hortobagyi GN. Paclitaxel improves the prognosis in estrogen receptor negative inflammatory breast cancer: the M. D. Anderson Cancer Center experience. Clin Breast Cancer. 2004;4(6):415–9.

29. Dawood S, Ueno NT, Valero V, Woodward WA, Buchholz TA, Hortobagyi GN, et al. Differences in survival among women with stage III inflammatory and noninflammatory locally advanced breast cancer appear early: a large population-based study. Cancer. 2011;117(9):1819–26.

30. Ruth NM, Lin HY, Bedrosian I, Shaitelman SF, Ueno NT, Shen Y, et al. Underuse of trimodality treatment affects survival for patients with inflammatory breast cancer: an analysis of treatment and survival trends from the National Cancer Database. J Clin Oncol. 2014;32(19):2018–24.

31. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol. 2018;19(1):40–50.

32. Lox S, Sirtaine N, Piette F, Salgado R, Vale G, Van Eeck F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase II randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02–98. J Clin Oncol. 2013;31(7):860–7.

33. Solinas C, Carobottini L, De Silva P, Criccitelli C, Lamberti M. Tumor-infiltrating lymphocytes in breast cancer according to tumor subtype: current state of the art. Breast. 2017;35:142–50.

34. Harano K, Wang Y, Lim B, Seitz SR, Morris SW, Bailey DB, et al. Rates of immune cell infiltration in patients with triple-negative breast cancer by molecular type. PLoS One. 2018;13(10):e0204513.

35. Tsao MS, Kerr KM, Kock M, Beasley MB, Borczuk AC, Botling J, et al. PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of Blueprint phase 2 project. J Thorac Oncol. 2018;13(9):1302–11.

36. Schmid P, Adams S, Rugo HS, Scheweitss A, Barrios CH, Iwita H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med. 2018;379(22):2108–21.

37. Ali HR, Glont SE, Blows FM, Provenzano E, Dawson SJ, Liu B, et al. PD-L1 protein expression in breast cancer is rare, enriched in basal-like tumours and associated with infiltrating lymphocytes. Ann Oncol. 2015;26(7):1488–93.