European Journal of Cancer 136 (2020) 7–15

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Integration of tumour infiltrating lymphocytes, programmed cell-death ligand-1, CD8 and FOXP3 in prognostic models for triple-negative breast cancer: Analysis of 244 stage I–III patients treated with standard therapy

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Received 28 February 2020; received in revised form 6 May 2020; accepted 14 May 2020

KEYWORDS
Tumour infiltrating lymphocytes;
CD8;
FOXP3;
PD-L1;
Triple negative;
Early breast cancer

Abstract ***Background:*** Tumour infiltrating lymphocytes (TILs) are an established prognostic biomarker for triple-negative breast cancer (TNBC). We evaluated the role of programmed cell-death ligand-1 (PD-L1), CD8 and FOXP3 expression in refining a prognostic model for non-metastatic TNBC beyond classic factors and TILs.
Methods: Primary tumour samples from 244 early patients with TNBC, all treated with surgery and chemotherapy, were collected. Stromal TILs were evaluated on haematoxylin–eosin slides according to guidelines. PD-L1, CD8 and FOXP3 were assessed by immunohistochemistry and evaluated by digital pathology.
Results: TILs, PD-L1, CD8 and FOXP3 were positively correlated with each other ($P < 0.001$). TILs were confirmed as an independent prognostic factor. When PD-L1, CD8 and FOXP3 were added to multivariable models including classic factors (age, stage, histologic grade) and TILs, PD-L1 provided the largest amount of additional prognostic information: likelihood ratio χ^2 4.60, $P = 0.032$ (in a model including classic factors and TILs 10% increments) and likelihood

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https://doi.org/10.1016/j.ejca.2020.05.014

ratio χ^2 6.50, $P = 0.011$ (in a model including classic factors and TILs >30% versus <30%). In the subset of patients treated with neoadjuvant chemotherapy, FOXP3 provided further prognostic information beyond classic factors, TILs and pathological complete response (pCR) (likelihood ratio χ^2 5.01, $P = 0.025$). For patients who did not achieve a pCR, the expression of CD8 and PD-L1 was significantly increased from baseline to residual disease.

Conclusions: Beyond clinicopathological factors and TILs, other immune biomarkers may add prognostic information for early TNBC. The increased PD-L1 expression on residual disease after neoadjuvant chemotherapy strengthens the rationale of testing immune checkpoint inhibitors in the post-neoadjuvant setting.

1. Introduction

Triple-negative breast cancer (TNBC), defined by the lack of expression of hormone receptors and lack of overexpression/amplification of HER2, represents around 15% of all breast cancers. It is the most aggressive breast cancer subtype, being associated with an increased risk of distant relapse, most frequently occurring within the first 3 years from diagnosis [1].

The stage at diagnosis [2] and, for patients treated with neoadjuvant chemotherapy (NACT), the pathological response at surgery [3], are classic prognostic factors for non-metastatic TNBC. More recently, tumour infiltrating lymphocytes (TILs) have reached level of evidence 1b as a prognostic biomarker [4]. TIL assessment according to the International TIL Working Group consensus guidelines is validated, reproducible, simple and inexpensive [5,6]; therefore, the routine evaluation of this biomarker for TNBC is currently endorsed by international guidelines [7,8].

However, TILs may provide only rough information on the state of immune activation; therefore, research is moving towards a deeper characterisation of the tumour immune microenvironment to explore other potential biomarkers able to further ameliorate risk prediction. In this perspective, programmed cell-death ligand-1 (PD-L1) is one of the most studied immune biomarkers in solid tumours [9]. In addition to PD-L1, characterisation of the type of T lymphocytes (cytotoxic CD8+ or regulatory FOXP3+) infiltrating the tumour may also provide information on the polarisation of the tumour immune response.

In this study, we aimed to investigate the prognostic role of PD-L1, CD8 and FOXP3 beyond established prognostic factors and TILs in a large cohort of patients with non-metastatic TNBCs to develop an integrated model for risk stratification.

2. Methods

2.1. Patient cohort

We identified 314 patients with non-metastatic TNBC (oestrogen receptor and progesterone receptor <10%, HER2 0/1+ by immunohistochemistry and/or fluorescent in situ hybridization not amplified) diagnosed from 2000 to 2015 at IRCCS Istituto Oncologico Veneto (Padova, Italy). Patients who did not receive any chemotherapy for primary TNBC (n = 45) and those with unavailable/not sufficient tumour tissue (n = 25) were excluded, leaving 244 patients in the study cohort. Clinicopathological, treatment and follow-up data were collected. The study protocol was approved by the competent ethical committee. Written informed consent was obtained from patients.

2.2. Pathology assessments

TILs, PD-L1, CD8 and FOXP3 were assessed on the following formailin-fixed paraffin-embedded tumour samples: surgical samples for patients treated with primary surgery followed by adjuvant chemotherapy and diagnostic core biopsy for patients treated with NACT followed by surgery. The level of immune markers assessed on these samples was used for analyses in the whole study cohort.

For the subset of patients treated with NACT who showed residual invasive breast cancer on the surgical sample, the FFPE surgical tumour block was also assessed for immune markers. Specific analyses involving immune marker evaluation on the post-treatment sample are described in a dedicated paragraph in the Results section.

Stromal TILs were assessed on a single haematoxylin–eosin stained slide and scored according to predefined criteria [5,10].

Methods for immunohistochemistry staining and assessment by digital software are provided as Appendix A.

PD-L1 expression on tumour cells was calculated as the percentage of positive tumour cells over the total of tumour cells. PD-L1 expression on stroma cells was calculated as the percentage of positive stroma cells over the total of stroma cells. PD-L1 expression was higher on stroma versus tumour cells: median PD-L1 was 2.6% (Q1–Q3 0.7%–18.6%) on tumour cells and 5.1% (Q1–Q3 0.2%–24.0%) on stroma cells. Because PD-L1 expression on tumour and stroma cells was strongly correlated (Spearman's coefficient 0.948, $P < 0.001$) we

decided, for further analyses, to consider PD-L1 on stroma cells.

The density of CD8 and FOXP3 was calculated as the number of cells/mm^2 of stroma area.

2.3. Statistical analysis

Statistical analysis was carried out using IBM SPSS (version 25) and R project [11].

Descriptive statistics were performed for patients' characteristics. The χ^2 and the Mann-Whitney tests were used to study association between variables.

The median follow-up was 81.6 months (95% CI: 75.1–88.0). Disease-free survival (DFS) was calculated from diagnosis to invasive relapse (locoregional or distant) or death from any cause, whichever first. Cox regression models were used to calculate HR (hazard ratio) and 95% CI (confidence interval). For survival analyses, TILs were considered as semi-continuous (10% increments) and as categorical variables (cut-off >30%, as previously described [4]). PD-L1, CD8 and FOXP3 were initially considered as continuous variables. The Harrell's c-index [12] was used to determine the optimal prognostic cut-offs for PD-L1, CD8 and FOXP3 to be used in further survival analyses.

Multivariate Cox proportional hazard regression analyses were adjusted for relevant clinical covariates: age, stage at diagnosis, histologic grade and pathological complete response (pCR, when applicable). The likelihood ratio test was used to compare the different prognostic models. The Kaplan-Meier method was used to estimate survival curves, and the log-rank test was used to test difference between groups.

For NACT-treated patients, the pCR was defined as the absence of residual invasive cancer in the breast and axilla (ypT0/is, ypN0). Odds ratios and their 95% CI for the association between immune variables and pCR were calculated by logistic regression analysis. The Wilcoxon-rank sum test was used to compare the level of immune markers before and after NACT.

All P values are two sided, with a significance level set at $P < 0.05$.

3. Results

3.1. Patient characteristics and association with immune markers

Table 1 shows the characteristics of the 244 patients included in this cohort. One hundred forty-five (59%) patients received primary surgery followed by adjuvant chemotherapy, whereas 99 (41%) patients received NACT followed by surgery and additional adjuvant chemotherapy in 31% of cases. Median levels of immune markers were as follows: TILs 10% (Q1:Q3 3%:25%), PD-L1 5.1% (Q1:Q3 0.2%:24.0%), CD8 242 (Q1:Q3 108:566), FOXP3

Table 1
Patient characteristics and immune markers according to main clinicopathological factors.

Characteristic		*N* tot	%	TILs % median (Q1:Q3)	*P*	PD-L1% median (Q1:Q3)	*P*	CD8 density median (Q1:Q3)	*P*	FOXP3 density median (Q1:Q3)	*P*
Age, years	Median (range)	53 (25–84)		–	–	–	–	–	–	–	–
	≤50	106	43.4	12.5 (5.0:35.0)	0.001	9.9 (1.5:32.3)	0.014	325 (137:661)	0.003	70 (26:156)	0.023
	>50	138	56.6	5.0 (2:20)		3.1 (0.1:17.7)		191 (94:422)		49 (19:107)	
Histotype	Ductal/NOS	219	90.5	10.0 (3.0:25.0)	0.008	5.2 (0.4:25.3)	0.033	250 (112:585)	0.002	59 (23:134)	<0.001
	Lobular	8	3.3	1.0 (1.0:4.0)		0.1 (0.0:0.2)		52 (14:88)		14 (11:16)	
	Apocrine	8	3.3	3.5 (1.5:25.0)		0.4 (0.0:1.4)		99 (43:311)		13 (5:23)	
	Metaplastic	4	1.7	32.5 (3.0–70.0)		35.0 (10.3:53.5)		320 (44:660)		136 (32:332)	
	Medullary	3	1.2	65.0 (10.0:85.0)		35.6 (11.1:80.0)		569 (560:613)		67 (7:111)	
Stage at diagnosis	I	79	32.4	5.0 (2.0:20.0)	0.202	2.4 (0.1:13.3)	0.064	201 (109:428)	0.573	45 (19:123)	0.330
	II	120	49.2	10.0 (3.5–32.5)		5.9 (1.2:27.9)		276 (104:644)		65 (21:148)	
	III	45	18.4	10.0 (5.0:20.0)		5.2 (0.2:35.0)		242 (123:443)		54 (24:110)	
Histologic grade	G1-2	28	12.3	4.0 (1.0:5.0)	0.001	0.2 (0.0:8.0)	0.023	123 (46:248)	0.007	29 (11:70)	0.002
	G3	199	87.7	10.0 (4.0:30.0)		5.6 (0.6:28.0)		270 (112:585)		59 (22:139)	
Ki67	Median (range)	55% (3%–90%)		–	–	–	–	–	–	–	–
	<30%	37	15.5	5.0 (1.0:20.0)	0.046	0.2 (0.0:15.0)	0.002	152 (66:361)	0.027	23 (12:59)	0.003
	≥30%	201	84.5	10.0 (5.0:25.0)		5.6 (0.7:28.0)		270 (114:585)		67 (25:139)	

N, number; Q1, first quartile; Q3, third quartile; TILs, tumour infiltrating lymphocytes; *P*, p value; NOS, not otherwise specified; PD-L1, programmed cell-death ligand-1.

A

		TILs %	PD-L1 %	CD8	FOXP3
TILs %	Coefficient*	1.000	0.592	0.759	0.54
PD-L1 %	Coefficient*	0.592	1.000	0.626	0.585
CD8	Coefficient*	0.759	0.626	1.000	0.666
FOXP3	Coefficient*	0.54	0.585	0.666	1.000

*Spearman's coefficient (p<0.001 for all correlations)

B

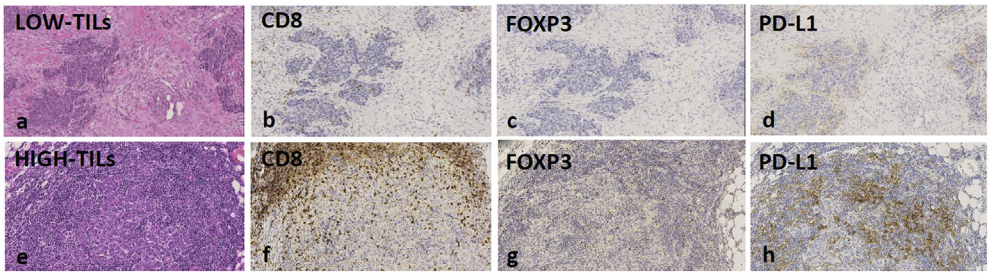

Fig. 1. Correlation between immune markers. A shows Spearman's correlation coefficients and *P* values. B shows representative pictures (10×) of cases with low (a) and high (e) TILs with matched CD8 (b,f), FOXP3 (c,g) and PD-L1 (d,h) immunohistochemical staining. TILs, tumour infiltrating lymphocytes; PD-L1, programmed cell-death ligand-1.

57 (Q1:Q3 21:130). Increased levels of TILs, PD-L1, CD8 and FOXP3 were significantly associated with age <50 years, ductal, metaplastic or medullary histotype, histologic grade 3 and higher Ki67.

3.2. Correlation between immunological markers

As shown in Fig. 1A, all immune markers were significantly positively correlated with each other ($P < 0.001$). TILs were strongly correlated with CD8 (Spearman's

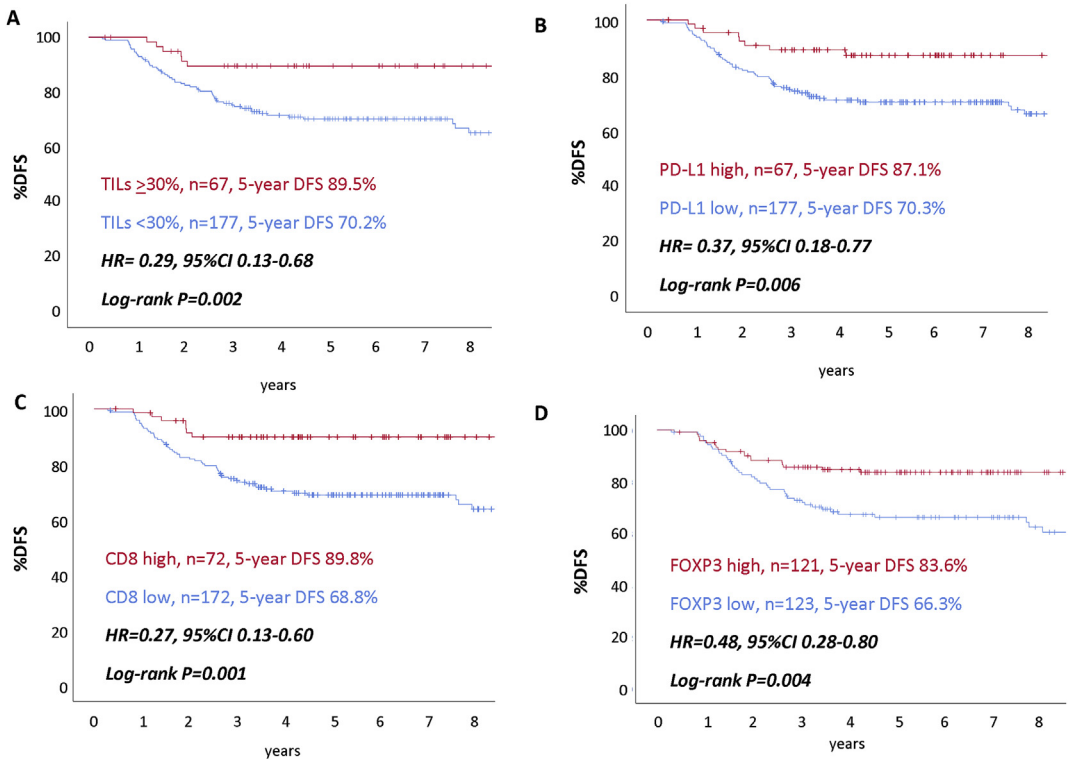

Fig. 2. DFS Kaplan–Meier curves according to immune variables: TILs (A), PD-L1 (B), CD8 (C) and FOXP3 (D). TILs, tumour infiltrating lymphocytes; PD-L1, programmed cell-death ligand-1; DFS, disease-free survival.

coefficient 0.759) and moderately correlated with PD-L1 (Spearman's coefficient 0.592) and FOXP3 (Spearman's coefficient 0.540). Fig. 1B shows pictures of samples with low and high TILs, with matched PD-L1, CD8 and FOXP3 staining.

3.3. Univariate survival analysis

TILs were significantly associated with improved DFS: HR = 0.81, 95% CI = 0.70–0.94 and $P = 0.006$ for each 10% TIL increment. Patients with TILs >30% showed a 5-year DFS rate of 89.5% versus 70% for patients with TILs ≤ 30% (log-rank $P = 0.002$, Fig. 2A).

We explored the association of PD-L1, CD8 and FOXP3 as continuous variables (1% increment for PD-L1, 1 unit increase for CD8 and FOXP3) with outcomes. In univariate analyses, all these parameters were significantly associated with DFS (HR = 0.980, 95% CI: 0.963–0.997, $P = 0.020$ for PD-L1; HR = 0.999, 95% CI: 0.998–1.000, $P = 0.004$ for CD8; HR = 0.995, 95% CI: 0.992–0.999, $P = 0.010$ for FOXP3). The Harrell's c-index for DFS for different cut-off points was calculated to determine the optimal prognostic cut-off for each variable. The cut-offs tested for PD-L1 corresponded to every 1% increase (i.e. 1%, 2%, to 100%). The cut-offs tested for CD8 and FOXP3 were those separating the variables in deciles. The cut-off points with the highest c-index were as follows: PD-L1 > 21% (c-index 0.574502, 28% of patients with high PD-L1), CD8 > 474 (c-index 0.585882, 30% of patients with high CD8) and FOXP3 > 57 (c-index 0.592259, 50% of patients with high FOXP3). Survival curves for DFS according to these cut-offs are shown in Fig. 2.

3.4. Multivariate survival analysis

Each of the immune variables was significantly associated with DFS in multivariate models including classic clinicopathologic factors (Appendix B, Table B1).

We then compared different prognostic models including clinicopathological factors (age, stage at diagnosis and histologic grade) and immune variables (Table 2). We confirmed that TILs provide significant additional prognostic information beyond classic covariates. PD-L1 conferred the largest amount of significant prognostic information beyond classic clinicopathological factors and TILs.

3.5. Analyses in the NACT cohort

Of 99 patients treated with NACT, information on pCR was available for 98 cases. Of those, 26.5% achieved a pCR. As expected, the achievement of pCR was associated with improved DFS (HR = 0.29, 95% CI: 0.10–0.82, $P = 0.019$).

Patients with pCR had significantly pre-treatment higher TIL and CD8 levels as compared with patients without pCR (Appendix C, Fig. C1). Logistic regression analysis confirmed a significant association of TILs and CD8 with pCR (Table 3). Cox regression showed that all pre-treatment immune markers were associated with improved DFS in univariate analysis (Table 3). TILs with the 30% cut-off added a significant prognostic value beyond clinicopathological features and pCR. Among PD-L1, CD8 and FOXP3, only the latter seemed to confer further prognostic information beyond clinicopathological features, TILs and pCR (Table 3).

Among the 72 patients without pCR after NACT, immune markers on post-NACT sample were available for n = 52. There was a significant increase in PD-L1 and CD8 levels in post-NACT versus pre-NACT samples (Fig. 3). Incremental TILs on residual disease were associated with improved DFS (HR = 0.58, 95% CI: 0.35–0.96, $P = 0.034$); PD-L1 on residual disease showed an association with improved DFS of borderline statistical significance (HR = 0.45, 95% CI: 0.19–1.07, $P = 0.069$, Appendix B, Table B2).

4. Discussion

TILs are an established prognostic factor for early TNBC [4]. Given the recognised role of immunity in TNBC and the need for further refinement of prognostic stratification, we assessed the prognostic value of PD-L1, CD8 and FOXP3 beyond established prognostic

Table 2
Additional (DFS) value of immune markers to prognostic multivariable models.

Model variables	Likelihood ratio χ^2	Likelihood ratio *P* value
CP + TILs 10% increment versus CP	17.08	<0.001
CP + TILs 10% increment + PD-L1 versus CP + TILs 10% increment	4.60	0.032
CP + TILs 10% increment + CD8 versus CP + TILs 10% increment	2.45	0.116
CP + TILs 10% increment + FOXP3 versus CP + TILs 10% increment	2.58	0.108
CP + TILs 30% cut-off versus CP	13.77	<0.001
CP + TILs 30% cut-off + PD-L1 versus CP + TILs 30% cut-off	6.50	0.011
CP + TILs 30% cut-off + CD8 versus CP + TILs 30% cut-off	5.89	0.015
CP + TILs 30% cut-off + FOXP3 versus CP + TILs 30% cut-off	3.95	0.047

P, p value; TILs, tumour infiltrating lymphocytes; PD-L1, programmed cell-death ligand-1; DFS, disease-free survival; CP, clinicopathological factors (age, stage at diagnosis, grade).

Table 3
Association between baseline immune markers with pCR and DFS in patients treated with neoadjuvant chemotherapy.

Univariate association between baseline immune biomarkers and pCR	OR	95% CI	*P*
TILs 10% increments	1.36	1.10–1.68	0.005
TILs 30% cut-off	2.95	0.95–9.18	0.062
PD-L1 high versus low	1.48	0.56–3.87	0.428
CD8 high versus low	3.88	1.48–10.90	0.010
FOXP3 high versus low	0.95	0.39–2.32	0.903
Univariate association between baseline immune biomarkers and DFS	**HR**	**95% CI**	***P***
TILs 10% increments	0.76	0.58–0.99	0.039
TILs 30% cut-off	0.12	0.02–0.88	0.037
PD-L1 high versus low	0.35	0.14–0.91	0.031
CD8 high versus low	0.18	0.04–0.76	0.020
FOXP3 high versus low	0.34	0.17–0.68	0.003
Added prognostic value beyond stage, TILs and pCR	**Likelihood ratio χ^2**	**Likelihood ratio *P* value**	
CP + pCR + TILs 10% increments versus CP + pCR	2.72	0.099	
CP + pCR + TILs 30% cut-off versus CP + pCR	5.16	0.023	
CP + pCR + TILs 30% cut-off + PD-L1 versus CP + pCR + TILs 30% cut-off	3.48	0.062	
CP + pCR + TILs 30% cut-off + CD8 versus CP + pCR + TILs 30% cut-off	1.79	0.181	
CP + pCR + TILs 30% cut-off + FOXP3 versus CP + pCR + TILs 30% cut-off	5.01	0.025	

P, p value; TILs, tumour infiltrating lymphocytes; PD-L1, programmed cell-death ligand-1; DFS, disease-free survival; pCR, pathological complete response.; CP, clinicopathological factors (age, stage at diagnosis, grade).

factors and TILs. Although other studies have previously evaluated the role of such biomarkers in TNBC [9,13–20], only few assessed their prognostic role in relation to TILs [13,17,20] without showing any added impact of these immune markers.

We evaluated a large cohort of patients with TNBC, all treated with surgery and chemotherapy. The vast majority (86%) of patients received anthracycline-containing treatment and a considerable proportion (72%) received anthracycline/taxane-based treatment.

We focused on TILs and on three immune biomarkers evaluated by digital pathology: PD-L1, CD8 and FOXP3. This is the first study to assess the prognostic role of PD-L1 as evaluated by the 73-10 assay. PD-L1 expression was more prevalent on stromal cells as compared with tumour cells, consistent with the typical expression pattern in breast cancer samples [9].

We described a positive association between TILs, PD-L1, CD8 and FOXP3. This is consistent with other studies [13,15,21–24] and with the assumption that PD-L1 expression and FOXP3+ T cells reflect, in TNBC, an ineffective/insufficient negative feedback in inflamed tumours. In a recent study, PD-L1, FOXP3 and other markers of inflammation were enriched in those triple

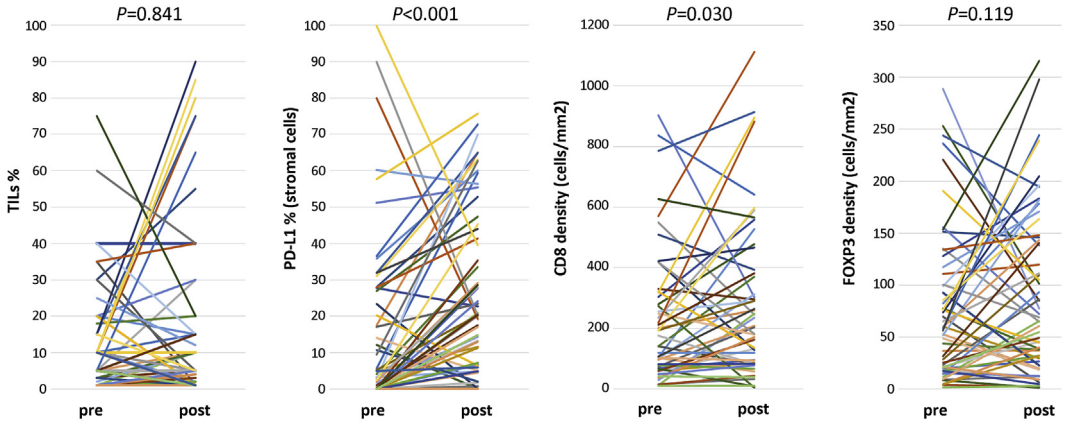

Fig. 3. Changes in the level of immune markers from pre-treatment from residual disease samples in patients treated with neoadjuvant chemotherapy who did not achieve a pathologic complete response.

negative tumours with a high infiltration of CD8+ lymphocytes in the tumour core [23]. To the opposite, 'cold' tumours are associated with low levels of PD-L1 and increased expression of the co-inhibitory molecule B7–H4, suggesting that this is the preferred immune escape mechanism in cold TNBC [23,25]. TILs showed a stronger correlation with CD8 and a modest correlation with FOXP3, corroborating the observation that, in TNBC, CD8+ cells are the main lymphocyte population of the immune infiltrate [26]. A modest correlation was observed between TILs and PD-L1, which is consistent with the type of stromal cells that express PD-L1 in breast cancer, that include TILs but also macrophages and morphologically fibroblast-like cells [17].

We confirmed the significant prognostic role of incremental TILs in TNBC, with results consistent with the recent pooled analysis [4]. PD-L1, CD8 and FOXP3 were also associated with improved DFS in univariate analyses. The favourable prognostic role of CD8+ T-cell infiltration had been previously demonstrated in oestrogen receptor–negative and TNBC disease [13,27]. With regards to FOXP3+ T cells, some reports have shown a positive association with improved prognosis in TNBC or basal-like oestrogen receptor–negative tumours [13,15,16,18], although this was not consistent with other studies [27]. In an analysis of FOXP3 RNA expression in samples from the FinHER trial, no correlation with outcomes was observed [14]. With regards to PD-L1, conflicting results have been reported in individual studies; however, the majority showed improved outcomes with increased PD-L1 levels in TNBC [9,28].

These data confirm that tumour inflammation is an important determinant of prognosis in TNBC. Because TILs are a rough method to recapitulate the level of inflammation in TNBC, the relevant question is whether the evaluation of additional biomarkers of immune activation could further refine prognostic models.

In our cohort, the assessment of a single immune marker, especially PD-L1, significantly added prognostic information beyond a model combining anatomical stage, grade, age and TILs. Very few other studies have attempted to answer this same question. In a series of 147 patients with TNBC, the favourable prognostic role of PD-L1 expression on immune cells that was observed in the whole cohort was not confirmed when the analysis was conducted separately in patients with low or high TILs [17]. Bottai et al. [13] analysed a cohort of 259 patients with TNBC, showing that CD8 and FOXP3 were not prognostic beyond TILs in multivariable models. In another study, the prognostic effect of FOXP3+ in oestrogen receptor–-negative basal tumours became insignificant when the CD8+ T-cell infiltration was taken into account [18]. A strength of our study is the use of a digital pathology software-assisted method for the evaluation of CD8, FOXP3 and PD-L1. As a potential limitation, spatial heterogeneity of immune cells in the tumour microenvironment, which contributes to determine the patient's prognosis [23], was not evaluated.

A more precise risk estimation is becoming more and more necessary for TNBC. Different treatment options for early TNBC are available, encompassing de-escalated and escalated systemic treatment strategies [8,29–32]. TILs have already shown their ability to inform on patients' prognosis, and recent evidence from a cohort of untreated patients with TNBC indicated an optimal outcome for patients with stage I and high TIL tumours [33]. With our data, we suggest that the inclusion of one additional immune biomarker may result in a finer risk stratification. Our data need validation in other studies to define the clinical utility of this multiple marker approach.

Another important opportunity for risk-based tailored treatment is offered by the post-neoadjuvant setting: pCR is a strong prognostic factor [3] and patients with residual disease after NACT may be candidates for further adjuvant chemotherapy or inclusion in clinical trials.

In our cohort, we confirmed the known correlation between baseline TILs and improved prognosis independently from pCR and other factors [34]. Furthermore, we suggest that refinement of prognostic models beyond stage, baseline TILs and pCR can be achieved by integrating an additional baseline immune marker such as FOXP3. Focussing only on patients not achieving a pCR after NACT, we also confirmed the prognostic role of TILs on residual disease [35,36].

Finally, we described a significant increase of PD-L1 and CD8 from baseline to residual disease after NACT, results that are consistent with available literature data on CD8 but not for PD-L1 [24,37,38]. Virtually all patients received anthracyclines as part of NACT, a class of drugs able to induce immunogenic cell death leading to an increased tumour inflammation; PD-L1 is a dynamic marker that may increase after chemotherapy exposure in parallel with the intensity of immune response activation [39].

5. Conclusions

In conclusion, we confirmed the outstanding prognostic role of markers of immune activation in TNBC, supporting the recommendation that TILs should be evaluated in routine clinical practice for this disease. We demonstrated for the first time that the assessment of an additional single immune biomarker among PD-L1, CD8 and FOXP3 provides relevant prognostic information able to refine the estimation of the patient's prognosis beyond classic factors and TILs. Finally, the finding of increased PD-L1 expression on residual disease after NACT strengthens the rationale of ongoing clinical trials evaluating the efficacy of adjuvant immune

checkpoint inhibitors in patients with TNBC not achieving a pCR (A-BRAVE NCT02926196; SWOG S1418 NCT02954874).

Conflict of interest statement

M.V.D. reports receiving personal fees from Genomic Health, Eli Lilly and Celgene outside the submitted work. V.G. reports receiving grants (to Institution) and personal fees from Roche and personal fees from Novartis and Eli Lilly, outside the submitted work. P.C. reports receiving grants (to Institution) from Agenzia Italiana del Farmaco AIFA and from Merck KGa during the conduct of the study; personal fees from Novartis, Eli Lilly, AstraZeneca, Tesaro, BMS and Roche and grants (to Institution) from Novartis, Roche and BMS, outside the submitted work. All other authors have nothing to disclose.

Funding

This work was supported by a grant from Merck KGaA, Darmstadt, Germany, and is part of an alliance between Merck KGaA, Darmstadt, Germany, and Pfizer. The funding source had no role in study design, data collection, analysis and interpretation, writing of the report and decision to submit the article for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2020.05.014.

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