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Original Research

Relevance of tumour-infiltrating lymphocytes, PD-1 and PD-L1 in patients with high-risk, nodal-metastasised breast cancer of the German Adjuvant Intergroup Node–positive study

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Abstract ***Background:*** Immune cell infiltration in breast cancer is important for the patient's prognosis and response to systemic therapies including immunotherapy. We sought to investigate the prevalence of tumour-infiltrating lymphocytes (TILs) and their association with immune checkpoints such as programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) in high-risk, node-positive breast cancer of the adjuvant German Adjuvant Intergroup Node–positive (GAIN-1) trial.
Patients and methods: We evaluated TILs by haematoxylin and eosin staining and PD-1 and PD-L1 (SP263 assay) expression by immunohistochemistry in 1318 formalin-fixed, paraffin-embedded breast carcinomas. The association of TILs with PD-1, PD-L1, molecular intrinsic subtypes, outcome and therapy regimens (dose-dense [dd] epirubicin, paclitaxel and cyclophosphamide [EPC] and dd epirubicin, cyclophosphamide, paclitaxel and capecitabine [ECPwX]) was statistically tested.
Results: Overall TILs density was significantly associated with the expression of PD-1 and PD-L1 in immune cells (each $p < 0.0001$) and PD-L1 in tumour cells ($p = 0.0051$). TILs were more common in triple-negative breast cancer (TNBC) and human epidermal growth factor receptor 2 (HER2)-positive tumours (each $p < 0.0001$). On multivariate Cox regression analyses, patients with breast cancer without TILs had an unfavourable disease-free survival (DFS) in the EPC arm compared with the EC-PwX arm (hazard ratio [HR] = 0.69 [0.44–1.06], $p = 0.0915$); but no differences were seen in tumours with TILs (HR = 1.24 [0.92–1.67], $p = 0.1566$, interaction $p = 0.0336$). PD-1–positive immune cells in TNBC were associated with a significantly better DFS (HR = 0.50 [0.25–0.99], $p = 0.0457$). PD-L1 expression had no impact on patient outcome.
Conclusions: TILs predict the benefit of intensified ddEPC compared with ddEC-PwX therapy in node-positive, high-risk breast cancer. TILs, PD-1 and PD-L1 are linked to each other indicating tumour immunogenicity. Moreover, PD-1–positive immune cells have a positive prognostic impact in TNBC.
Clinical trial: NCT00196872.

1. Introduction

Tumour-infiltrating lymphocytes (TILs) are present in all molecular subtypes of breast cancer (BC) but most common in triple-negative and non-luminal HER2-positive tumours [1–3]. Several studies have shown that TILs predict prognosis and response to systemic therapies in BC. Patients with triple-negative BC (TNBC) with increased TILs have a better overall survival (OS) in the adjuvant setting compared with patients without TILs [3–7]. In contrast, there was no prognostic significance for TILs in hormone receptor–positive, HER2-negative BC [3,4]. In neoadjuvant trials, increased TILs were associated with a higher pathological complete response (pCR) to systemic therapies, especially in TNBC and HER2-positive cancer [1,8,9]. Patients who achieve a pCR in these subtypes will have better survival [9–12].

Furthermore, it was recently demonstrated that TILs and PD-L1–positive immune cells may predict the response to immune checkpoint therapies with pembrolizumab and atezolizumab in BC [13–17]. So far, additional biomarkers for the identification of patients with BC that will have a benefit of such immuno-oncologic therapies have not been established. In other tumour entities, such as malignant melanoma and non–small cell lung cancer, testing of PD-1 and/or PD-L1 by immunohistochemistry (IHC) is an approach for patient selection [18–20]. The data on the prevalence of PD-1 and PD-L1 expression in BC are conflicting, and their relation to TILs and molecular subtypes is not well known, especially not in patient cohorts with defined treatment and reasonable follow-up. Previous studies have resulted in variable findings, probably also based on the use of different staining assays, definitions of positivity, and scoring criteria [21–23]. The objective of this work was to evaluate the clinical validity of TILs and the expression of immune checkpoints such as PD-1 and PD-L1 as prognostic and predictive biomarkers in a prospective cohort of central pathological validated, molecularly diverse BC samples of the adjuvant German Adjuvant Intergroup Node–positive (GAIN-1) trial. Because previous studies have suggested that TILs are related to the response of certain chemotherapies such as carboplatin and anthracycline [3,24], we also examined the impact of TILs in different chemotherapy regimens.

2. Patients and methods

2.1. Study population

The GAIN-1 study (ClinicalTRials.gov NCT00196872) was a prospective multicenter phase III trial to compare two dose-dense (dd) regimens, intensified dd epirubicin, paclitaxel and cyclophosphamide (EPC)

versus dd epirubicin, cyclophosphamide, paclitaxel and capecitabine (EC-PwX) and ibandronate versus observation in patients with high-risk, node-positive primary BC. In addition, radiotherapy, endocrine treatment and adjuvant trastuzumab (starting 05/2006) were given according to recommendations of the national 'Arbeitsgemeinschaft für Gynäkologische Onkologie (AGO)' guideline [25,26]. Patients with histologically confirmed, unilateral or bilateral primary node-positive BC were enrolled after providing written informed consent for clinical trial participation and use of biomaterials. Patients needed to have received adequate surgical treatment with histological complete resection (R0) of the primary tumour and ≥10 resected axillary nodes as per standard of care at the time of conducting the study. Overall, the trial recruited 3023 patients between 2004 and 2008, and 2994 patients were assigned for initial treatment. Clinicopathological data were extracted from the clinical study database. Survival data were available from all patients. Disease-free survival (DFS) was calculated as the time from study registration to any invasive recurrence (local, contralateral and distant), any second invasive cancer or death of any cause. Ethical committee approval from all centres participating in the clinical study and from the Institutional Review Board of Charité University Hospital Berlin (Germany) was obtained. This study was conducted adhering to the REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies) criteria [27].

2.2. Assessment of the molecular BC subtype

In total, 1371 FFPE samples were available for the construction of tissue microarrays (TMA). Tissue sections were stained with haematoxylin and eosin (H&E) for histological evaluation. Immunohistochemistry for oestrogen receptor alpha (SP1, Thermoscientific, 1:50), progesterone receptor (PgR 636, DAKO, Glostrup, Denmark, 1:50), HER2 (clone 4B5, Ventana Medical Systems, Tucson, AZ, USA) and Ki-67 (MIB1, DAKO, Glostrup, Denmark, 1:100) was performed using a Ventana Benchmark autostainer (Ventana, Tucson, AZ, USA). Hormone receptor positivity was defined as expression in at least 1% of the tumour cells [28]. Ki-67 staining was evaluated as recommended by the International Ki-67 in Breast Cancer Working Group [29]. HER2 status was assessed according to the American Society of Clinical Oncology (ASCO)/ College of American Pathologists (CAP) guidelines [30]. Equivocal HER2 cases were investigated by silver enhanced in situ hybridisation (SISH, ultraView SISH detection kit, Ventana Medical Systems, AZ, USA) carried out using the Ventana BenchMark ultra-automated staining system. Stained TMA sections were digitised and evaluated by a board-certified pathologist experienced in breast pathology (A.N.) using VM Slide Explorer 2.2 software (VMscope GmbH Berlin, Germany). For the classification of the so-called molecular intrinsic BC subtypes, the central pathologic assessment of the tumour biology was used. Subtypes were defined as follows: luminal A (oestrogen receptor [ER]− and/or progesterone receptor (PR) PR-positive, HER2-negative, Ki-67 low <20%), luminal B/HER2− (ER- and/or PR-positive, HER2-negative, Ki-67 high ≥21%), luminal B/HER2+ (ER- and/or PR-positive, HER2-positive, any Ki-67), non-luminal HER2-enriched (ER- and PR-negative, HER2-positive) and triple-negative (ER- and PR-negative, HER2-negative).

2.3. Evaluation of TILs

TILs were evaluated on H&E-stained sections following the recommendations of the International TILs working group [31]. As previously noticed, TILs were predominantly located within the stromal tissue of the tumour and therefore assessed in this compartment [8]. The mononuclear inflammatory infiltrate was assessed in predefined categories, no TILs (0%), 1−10%, 11−25%, 26−50% and >51%, by a board-certified pathologist (A.N.). Regions with non-invasive carcinoma, normal breast epithelium or necrosis were excluded from the evaluation.

2.4. Immunohistochemical evaluation of PD-1 and PD-L1

To examine the prevalence of PD-1 and PD-L1 expression, we applied an anti−PD-1 antibody (clone NAT 105, Cell Marque, 1:50) and an anti−PD-L1 antibody (clone SP263, Ventana Medical systems, Tucson, AZ, 1:100) using UltraView DAB detection on an automated staining system (Ventana BenchMark). We calibrated the PD-L1 antibody on a cell line microarray (Horizon Discovery) and applied the antibody in analogy to the respective clinical trial assay. Slides were digitised (AT2, Leica, Wetzlar, Germany), and images, analysed using VMscope software. The PD-L1 expression was scored in tumour and immune cells as previously described [32]. The percentage of PD-L1−positive tumour cells was proportionally evaluated in all tumour cells. PD-1 and PD-L1 immune cells were assessed relative to the whole tumour area.

2.5. Statistics

Pairs of binary variables were compared based on cross tables and Fisher's exact tests. Binary variables were compared with multicategorical variables by cross tables and χ^2 tests. The continuous clinical variable age was compared with a binary variable by a Wilcoxon test. DFS was the primary end-point and calculated as the time from study registration to any invasive recurrence (local, contralateral and distant), any second invasive cancer or death of any cause. OS is defined as the time from study registration to death from any cause. Cox regression models were used to examine the prognostic

effect of a biomarker variable or treatment: Regression models were constructed for the variable of interest only (univariate), with covariable treatment arm (bivariate) and with covariables, age, grade, tumour size, nodal status, histological type, central molecular subtype, treatment arm and type of surgery (multivariate). We used Kaplan–Meier curves to compare subgroups defined by biomarkers and/or treatment arms. Wherever two subgroups were compared, log-rank tests were performed. All reported p-values are two-sided, and $p \leq 0.05$ was considered statistically significant. No correction for multiple testing was applied. Confidence intervals symmetrically span 95%. Statistical calculations were performed using R 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria, 2016).

3. Results

3.1. Baseline data and central pathology of the study population

A subset of BC samples of the GAIN-1 cohort was available for the analysis of immune biomarkers. Clinicopathological parameters and treatment groups were equally distributed between the whole GAIN-1 cohort (n = 2994) and the study cohort (n = 1318), as shown in Table 1, confirming a representative selection. We found no significant differences in DFS and OS between patients included and those that were not included. As in the whole GAIN-1 cohort, we observed no significant differences in DFS and OS between the therapy arms in the analysis set.

In the original GAIN-1 cohort, local pathologists defined intrinsic molecular subtypes by the hormone receptor profile, HER2 status and tumour grade but not by using the Ki-67 proliferation index. We reassessed the hormone receptor expression and HER2 status in the study cohort (central pathology). To classify molecular subtypes of BC, we combined the centrally evaluated hormone receptor expression, HER2 status, and used the proliferation index (Ki-67) for categorisation of the luminal types (cut-off <20 vs. ≥21%). Owing to tissue shortage and quality, at least one central pathological parameter was determined in 1318 of 1371 BC samples; the complete central subtyping was feasible in 1131 cases. The majority was classified as luminal A (57.7%). The remaining cases (18.2%) were luminal B (7.5% HER2-negative and 10.7% HER2-positive), 7.2% were non-luminal HER2-positive and 16.9% were triple-negative. The progress of the sample set is displayed in Fig. 1.

Table 1
Comparison of the original (n = 2994) with the study cohort (n = 1318) using local pathology reports.

Parameter	GAIN-1 cohort	Study cohort	p-value
	n (%)	n (%)	
Age (years)			
Mean, range	50 (20–72)	50 (23–71)	0.1122
<40	438 (14.6)	194 (14.7)	0.2443
40–49	1057 (35.5)	488 (37.0)	
50–59	968 (32.3)	418 (31.7)	
≥60	531 (17.7)	218 (16.5)	
Surgery			0.2354
BCS	1672 (55.9)	753 (57.1)	
Mastectomy	1320 (44.1)	565 (42.9)	
Unknown	2	0	
Chemotherapy			0.7967
EPC	1500 (50.1)	664 (50.4)	
EC-PwX	1494 (49.9)	654 (49.6)	
Local ER			0.8345
Positive	2206 (73.7)	974 (73.9)	
Negative	787 (26.3)	344 (26.1)	
Unknown	1	0	
Local PR			0.5831
Positive	2011 (67.2)	893 (67.8)	
Negative	982 (32.8)	425 (32.2)	
Unknown	1	0	
Local HER2			0.7957
Positive	724 (24.8)	321 (25.1)	
Negative	2191 (75.2)	959 (74.9)	
Unknown	79	38	
Local subtypes			0.5372
Luminal A	1223 (41.8)	542 (42.0)	
Luminal B	1031 (35.2)	465 (36.0)	
ER-/PR-/HER2+	254 (8.7)	102 (7.9)	
TNBC	421 (14.4)	182 (14.1)	
Unknown	65	27	
Histologic type			0.2022
Ductal (NST)	2314 (77.3)	1023 (77.6)	
Lobular	374 (12.5)	151 (11.5)	
Other	306 (10.2)	144 (10.9)	
Tumour grade			0.5268
G1	96 (3.2)	44 (3.3)	
G2	1507 (50.4)	649 (49.3)	
G3	1385 (46.4)	624 (47.4)	
Unknown	6	1	
Tumour stage			0.9392
pT1	955 (32.0)	423 (32.2)	
pT2	1669 (55.9)	738 (56.2)	
pT3	305 (10.2)	130 (9.9)	
pT4	55 (1.8)	23 (1.8)	
Unknown	10	4	
Nodal stage			0.0273
pN1	1131 (37.8)	533 (40.4)	
pN2	1058 (35.3)	449 (34.1)	
pN3	805 (26.9)	336 (25.5)	

EC-PwX, epirubicin, cyclophosphamide, paclitaxel and capecitabine; EPC, epirubicin, paclitaxel and cyclophosphamide; TNBC, triple-negative breast cancer; ER, oestrogen receptor; GAIN, German Adjuvant Intergroup Node; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; BCS, breast conserving therapy.

3.2. Evaluation of TILs

Evaluation of TILs was feasible in 1271 BC samples. In 69% of the cases (n = 876), TILs (≥1%) were detectable. In 2.6% of the cases, high levels of TILs (n = 33; TILs >50%) were observed. Increased TILs (cut-off >1%) were associated with hormone receptor–negative BC ($p < 0.0001$), HER2 positivity ($p = 0.0051$), higher Ki-67 levels ($p < 0.0001$), high tumour grade ($p < 0.0001$),

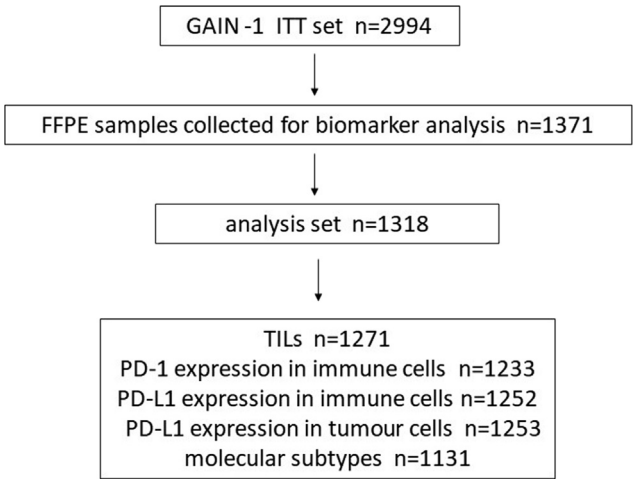


Fig. 1. Flow diagram showing the progress of the breast cancer samples. GAIN, German Adjuvant Intergroup Node; ITT, intention to treat; TILs, tumour-infiltrating lymphocytes.

invasive ductal (NST) histological type ($p < 0.0001$) and early pathological tumour stage (smaller tumour size) ($p = 0.0094$) but not with nodal stage ($p = 0.1678$) (Table 2). Similar results were obtained for alternative cut-offs >25% and >50%. Concerning the different molecular subtypes, highest levels of TILs occurred in TNBC and non-luminal HER2-positive BC ($p < 0.0001$).

3.3. Association of TILs with PD-1 and PD-L1 expression

Next, we compared the content of TILs with immunohistochemical expression levels of PD-1 and PD-L1. Both immune checkpoints are expressed in immune cells, whereas PD-L1 is also detectable in the membrane of tumour cells (Fig. 2). After scoring of PD-1 and PD-L1 expression, a cut-off of ≥1% was used for statistical tests [21]. BC with increased TILs showed a significant higher expression of PD-1 and PD-L1 in immune cells ($p < 0.0001$). In total, the presence of PD-L1 in tumour cells was very low but associated with TILs ($p = 0.0051$).

In correlation analyses (Fig. 3A), we investigated the relation between TILs, PD-1 and PD-L1 expression. We found a strong correlation between PD-1–positive and PD-L1–positive immune cells (Spearman's rank correlation coefficient [rho]: 0.536). We further noticed moderate correlations between TILs and PD-1–positive immune cells (r: 0.477), TILs and PD-L1–positive immune cells (r: 0.436) and PD-L1–positive immune cells and PD-L1–positive tumour cells (r: 0.323). Finally, we found modest correlations between TILs and PD-L1–positive tumour cells (r: 0.158) and PD-1–positive immune cells and PD-L1–positive tumour cells (r: 0.158).

Table 2
Association of TILs with clinicopathological parameters.

Parameter	No TILs	Any TILs	p-value
	n (%)	n (%)	
Age (years)			0.0049
<40	37 (9.4)	148 (16.9)	
40–59	258 (72.1)	592 (67,6)	
≥60	73 (18.5)	136 (15.5)	
Chemotherapy			0.5859
EPC	194 (49.1)	445 (50.8)	
EC-PwX	201 (50.9)	431 (49.2)	
Hormone receptors			<0.0001
ER/PR +	345 (90.3)	617 (72.7)	
ER/PR -	37 (9.7)	232 (27.3)	
HER2			0.0051
Positive	47 (12.3)	160 (18.7)	
Negative	335 (87.7)	696 (81.3)	
Missing	13	20	
Ki-67			<0.0001
1–15%	208 (61.2)	356 (45.5)	
16–20%	81 (23.8)	211 (27.0)	
≥21%	51 (15.0)	215 (27.5)	
Missing	55	94	
Molecular subtypes			<0.0001
Luminal A	233 (70.2)	414 (52.5)	
Luminal B/HER2-	26 (7.8)	59 (7.5)	
Luminal B/HER2+	36 (10.8)	83 (10.5)	
ER-/PR-/HER2+	9 (2.7)	71 (9.0)	
TNBC	28 (8.4)	161 (20.4)	
Missing	63	88	
Tumour grade			<0.0001
G1	16 (4.1)	26 (3.0)	
G2	237 (60.0)	388 (44.3)	
G3	142 (35.9)	461 (52.7)	
Unknown	0	1	
Histologic type			<0.0001
Ductal (NST)	276 (69.9)	710 (81.1)	
Lobular	69 (17.5)	76 (8.7)	
Other	50 (12.7)	90 (10.3)	
Tumour stage			0.0094
pT1	112 (28.4)	291 (33.4)	
pT2	220 (55.7)	495 (56.8)	
pT3+4	63 (15.9)	86 (9.9)	
Nodal stage			0.1678
pN1	150 (38.0)	364 (41.6)	
pN2	149 (37.7)	283 (32.3)	
pN3	96 (24.3)	229 (26.1)	

EC-PwX, epirubicin, cyclophosphamide, paclitaxel and capecitabine; EPC, epirubicin, paclitaxel and cyclophosphamide; ER, oestrogen receptor; TNBC, triple-negative breast cancer; TIL, tumour-infiltrating lymphocyte; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

3.4. Association of PD-1 and PD-L1 with clinicopathological factors

Expression of PD-1 in immune cells was significantly associated with poor tumour differentiation (high-grade), high Ki-67 levels, negativity of oestrogen and progesterone receptors and TNBC subtype (each $p < 0.0001$) and more common in NST (no special type) carcinomas compared with other types ($p = 0.0012$). No association with HER2 status ($p = 0.2412$),

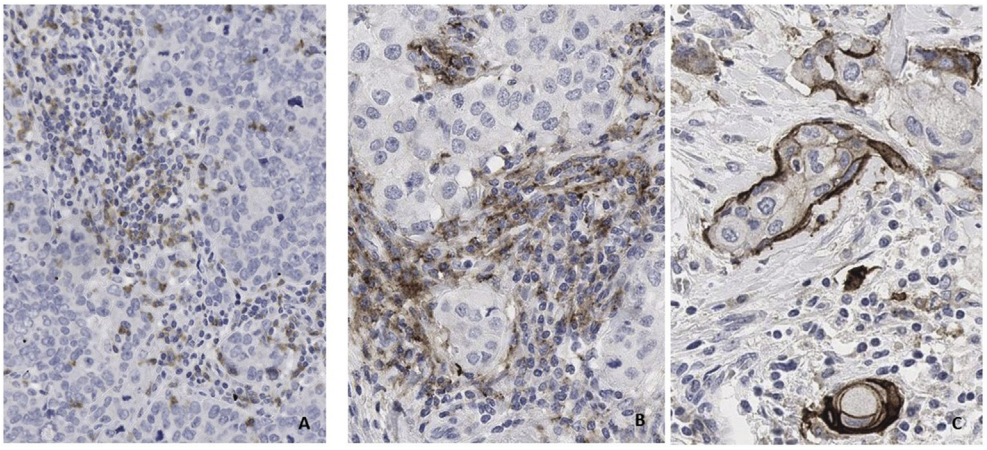


Fig. 2. Example of a high-grade, triple-negative breast carcinoma with increased (A) PD-1–positive and (B) PD-L1–positive immune cells as well as (C) PD-L1–positive tumour cells in immunohistochemistry.

pathological tumour stage ($p = 0.0858$) and nodal stage ($p = 0.9502$) was observed. As summarised in Table 3, the expression of PD-L1 in immune cells was significantly associated with high-grade BC, high Ki-67 levels, NST histology, negativity of oestrogen and progesterone receptors (each $p < 0.0001$), HER2 positivity ($p = 0.0036$), TNBC subtype ($p < 0.0001$) and early pathological tumour stage ($p = 0.0184$) but not with nodal stage ($p = 0.4739$). Expression of PD-L1 in tumour cells was significantly associated with high-grade BC ($p = 0.0011$), high Ki-67 levels, negative oestrogen and progesterone receptor expression, TNBC subtype (each $p < 0.0001$) and early nodal stage ($p = 0.0436$) but not with HER2 ($p = 0.4955$), histological type ($p = 0.2518$) and pathological tumour stage ($p = 0.7139$). The distribution of TILs and PD-1 and PD-L1 expression in the different molecular subtypes is given in Fig. 3B. In the subgroup of TNBC, PD-1– and PD-L1–positive immune cells were not associated with tumour or nodal stage.

3.5. Prognostic and predictive impact of TILs and immune checkpoint molecules

We further evaluated the prognostic and predictive value of TILs and immune checkpoint molecules. The median follow-up time was 74.3 months (range, 0.1–113.7). In the total study population, we observed no differences in DFS and OS in patients with breast carcinomas with and without TILs (cut-off ≥1%) or using TILs as a semicontinuous variable. In subgroup analyses, increased TILs (cut-off ≥1% or semi-continuous) in patients with TNBC showed a trend for better DFS and OS on univariate analysis and after adjustment by treatment arms (Fig. 4).

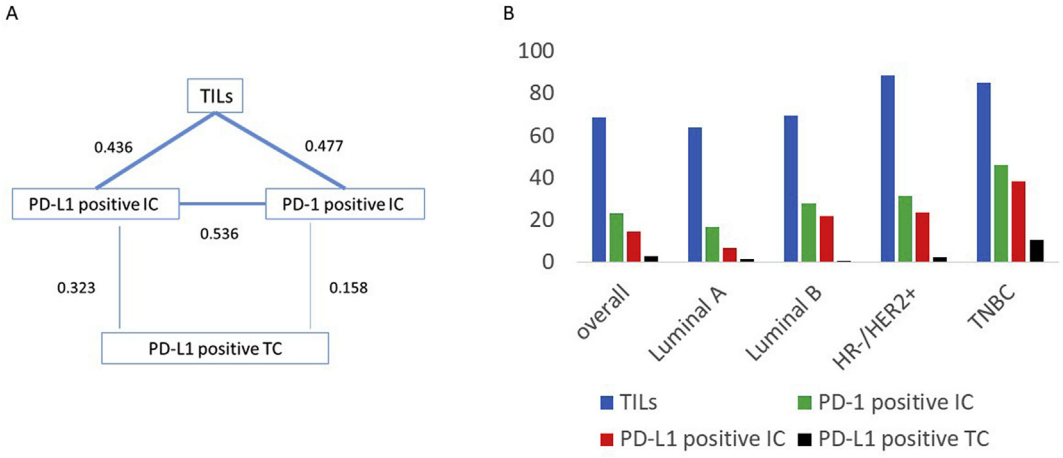


Fig. 3. (A) Correlation analysis between TILs, PD-1–positive immune cells (ICs), and PD-L1–positive immune and tumour cells (TCs). Values of the correlation coefficient (rho) are given. (B) Distribution of TILs, PD-1–positive immune cells, PD-L1–positive immune and tumour cells in %, in the global study cohort and different molecular subtypes (luminal A, luminal B, non-luminal HER2+ and TNBC). TNBC, triple-negative breast cancer; TIL, tumour-infiltrating lymphocyte.

Table 3
Association of PD-L1 with TILs, PD-1 and clinicopathological factors.

Parameter	PD-L1 in TILs			PD-L1 in tumour cells		
	Negative n (%)	Positive n (%)	p-value	Negative n (%)	Positive n (%)	p-value
TILs			<0.0001			<0.0001
<1%	370 (35.6)	6 (3.3)		376 (31.6)	1 (2.9)	
1–10%	465 (44.7)	38 (20.8)		494 (41.5)	9 (26.5)	
11–25%	176 (16.9)	83 (45.4)		243 (20.4)	16 (47.1)	
26–50%	22 (2.1)	34 (18.6)		50 (4.2)	6 (17.6)	
>51%	7 (0.7)	22 (12)		27 (2.3)	2 (5.8)	
PD-1			<0.0001			<0.0001
<1%	865 (85.6)	47 (26.1)		900 (77.7)	13 (39.4)	
1–5%	60 (5.9)	20 (11.1)		78 (6.7)	2 (6.1)	
6–10%	43 (4.3)	36 (20.0)		72 (6.2)	7 (21.2)	
11–24%	38 (3.8)	58 (32.2)		87 (7.5)	9 (27.3)	
>25%	4 (0.4)	19 (10.6)		21 (1.8)	2 (6.1)	
Chemotherapy			0.8116			0.1277
EPC	537 (50.3)	95 (51.4)		610 (50.1)	23 (63.9)	
EC-PwX	530 (49.7)	90 (48.6)		607 (49.9)	13 (36.1)	
Hormone receptors			<0.0001			<0.0001
ER/PR +	849 (82.9)	90 (50.0)		928 (79.2)	11 (33.3)	
ER/PR -	175 (17.1)	90 (50.0)		243 (20.8)	22 (66.7)	
HER2			0.0036			0.4955
Positive	160 (15.5)	45 (24.7)		201 (17.1)	4 (11.4)	
Negative	869 (84.5)	137 (75.3)		975 (82.9)	31 (88.6)	
Ki-67			<0.0001			<0.0001
1–15%	525 (56.9)	31 (17.4)		549 (51.4)	7 (21.2)	
16–20%	234 (25.4)	50 (28.1)		277 (25.9)	8 (24.2)	
>21%	163 (17.7)	97 (54.5)		242 (22.7)	18 (54.5)	
Molecular subtypes			<0.0001			<0.0001
Luminal A	589 (63.9)	44 (24.7)		624 (58.5)	9 (27.3)	
Luminal B/HER2-	64 (6.9)	20 (11.2)		83 (7.8)	1 (3.0)	
Luminal B/HER2+	94 (10.2)	24 (13.5)		117 (11.0)	1 (3.0)	
ER-/PR-/HER2+	61 (6.6)	19 (10.7)		78 (7.3)	2 (6.1)	
TNBC	114 (12.4)	71 (39.9)		165 (15.5)	20 (60.6)	
Histologic type			<0.0001			0.2518
Ductal (NST)	815 (76.4)	158 (85.4)		943 (77.5)	31 (86.1)	
Lobular	139 (13.0)	4 (2.2)		142 (11.7)	1 (2.8)	
Other	113 (10.6)	23 (12.4)		132 (10.8)	4 (11.1)	
Tumour grade			<0.0001			0.0011
G1	38 (3.6)	3 (1.6)		41 (3.4)	0 (0)	
G2	577 (54.1)	37 (20.1)		606 (49.8)	8 (22.2)	
G3	452 (42.4)	144 (78.3)		569 (46.8)	28 (77.8)	
Tumour stage			0.0184			0.6182
pT1	326 (30.6)	73 (39.9)		386 (31.8)	14 (38.9)	
PT2	608 (57.1)	97 (53.0)		686 (56.6)	19 (52.8)	
pT3+4	131 (12.3)	13 (7.1)		141 (11.6)	3 (8.3)	
Nodal stage			0.4739			0.0436
pN1	422 (39.6)	81 (43.8)		482 (39.6)	21 (58.3)	
pN2	365 (34.2)	62 (33.5)		417 (34.3)	11 (30.6)	
pN3	280 (26.2)	42 (22.7)		318 (26.1)	4 (11.1)	

EC-PwX, epirubicin, cyclophosphamide, paclitaxel and capecitabine; EPC, epirubicin, paclitaxel and cyclophosphamide; ER, oestrogen receptor; TNBC, triple-negative breast cancer; TIL, tumour-infiltrating lymphocyte.

Next, we investigated the relevance of TILs in the different chemotherapy arms. On multivariate Cox regression analyses, TILs had a significant positive prognostic impact on DFS in the EPC arm (hazard ratio [HR] = 0.57 [0.39–0.84], p = 0.0043) but not in the EC-PwX arm (HR = 1.26 [0.86–1.87], p = 0.2384, test for interaction p = 0.0336). Similarly, TILs as a semi-continuous variable had a prognostic impact on DFS in the EPC arm (HR = 0.78 [0.64–0.96], p = 0.0193) but not in the EC-PwX arm (HR = 0.96 [0.80–1.15], p = 0.6558). No differences were seen for the OS. Looking from another perspective, in patients with BC without TILs, we observed an unfavourable DFS in the EPC arm compared with the EC-PwX arm (HR = 0.69 [0.44–1.06], p = 0.0915); no differences in DFS between the treatment arms were found when TILs were present

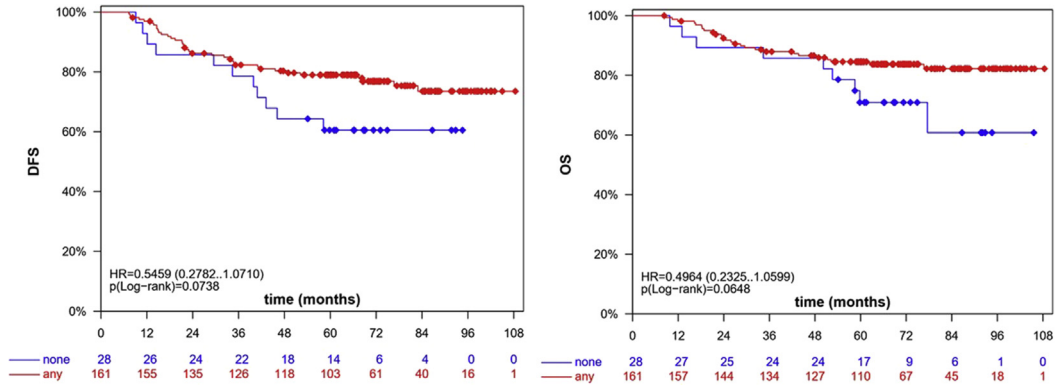


Fig. 4. Prognostic relevance of TILs in triple-negative breast cancer. Kaplan–Meier curves for estimated DFS and OS comparing tumours with and without TILs. DFS, disease-free survival; OS, overall survival; HR, hazard ratio; TILs, tumour-infiltrating lymphocytes.

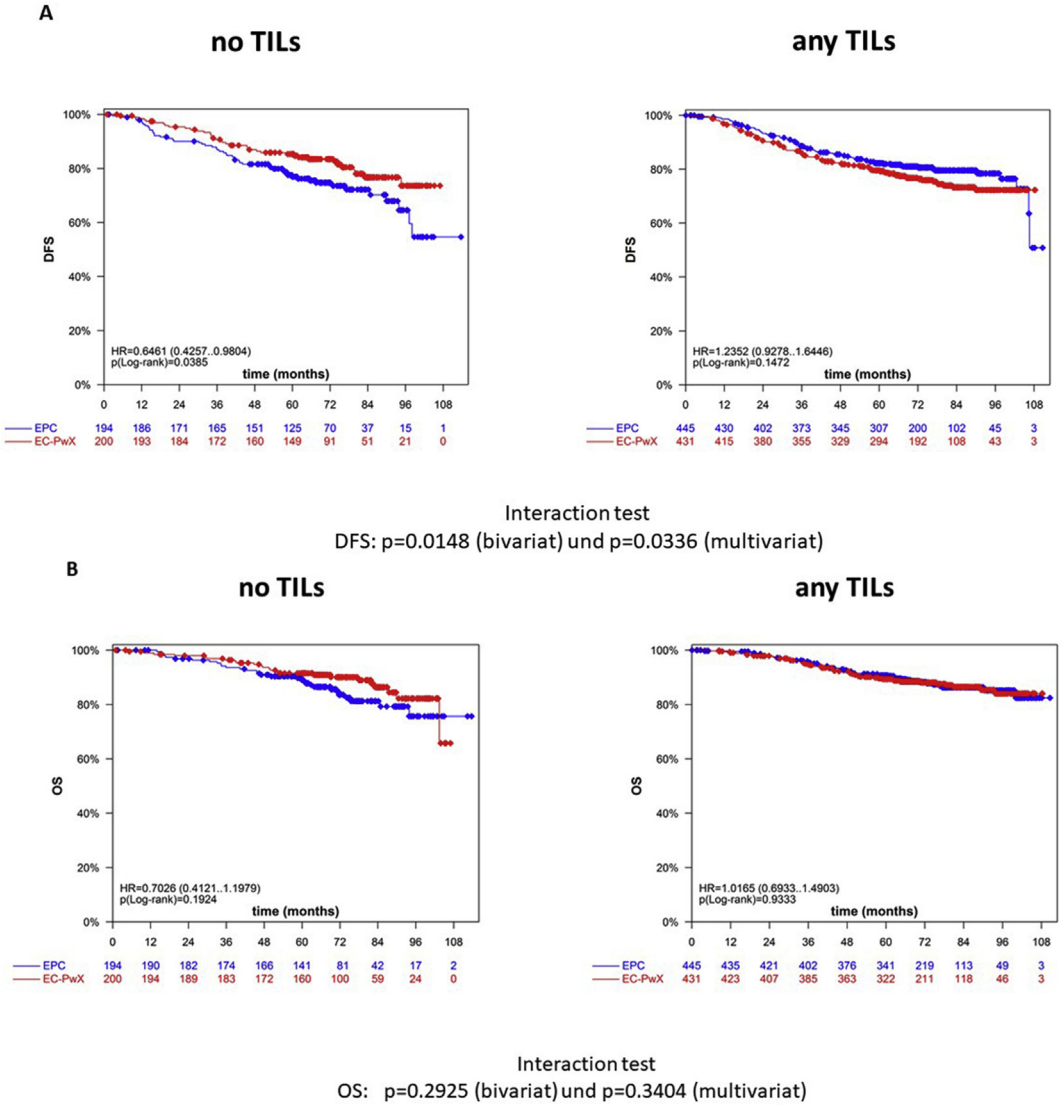
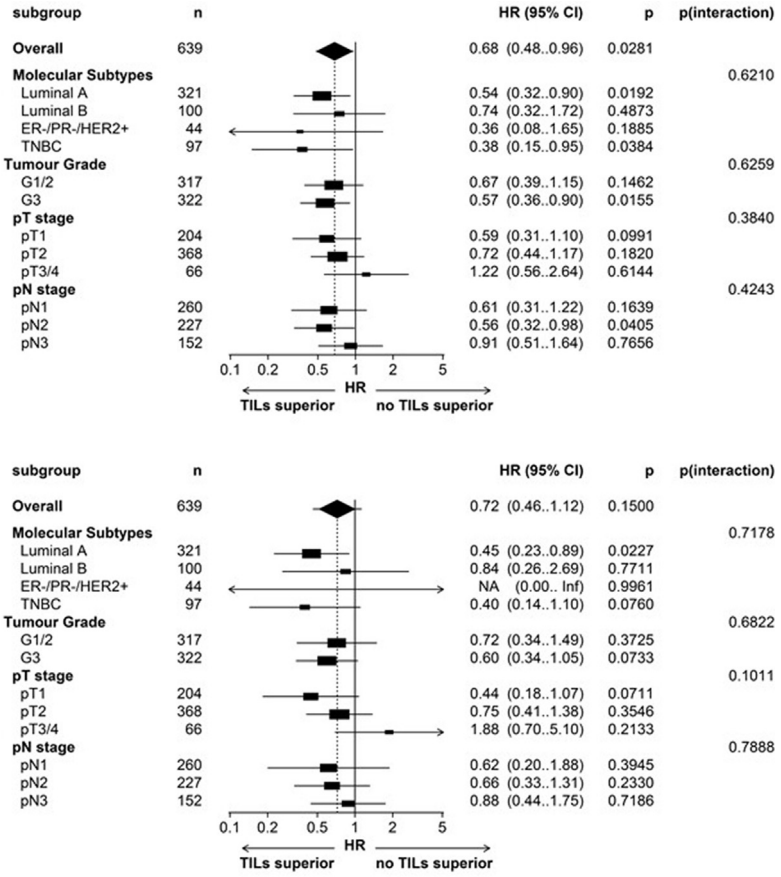
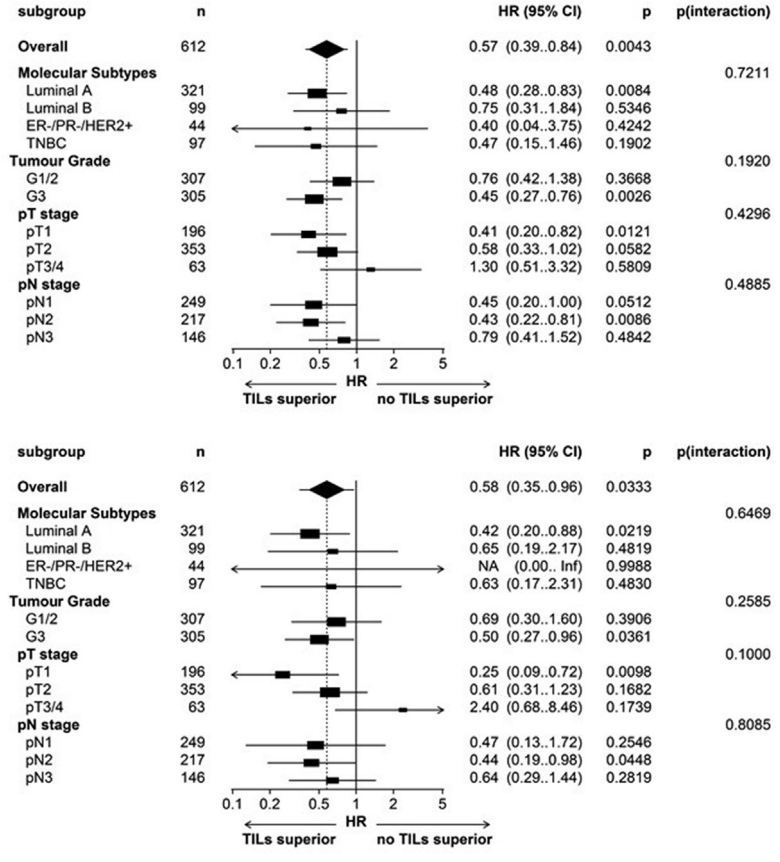


Fig. 5. Disease-free survival and overall survival of patients with breast cancer with or without any TILs in the different chemotherapy arms. (A) Kaplan–Meier curves for DFS in patients with TILs or without TILs by treatment arms. (B) Kaplan–Meier curves for OS in patients with TILs or without TILs by treatment arms. EPC, epirubicin, paclitaxel and cyclophosphamide; EC-PwX, epirubicin, cyclophosphamide, paclitaxel and capecitabine; DFS, disease-free survival; OS, overall survival; HR, hazard ratio; TILs, tumour-infiltrating lymphocytes.

A


subgroup n HR (95% CI) p p(interaction)
Overall 639 0.68 (0.48..0.96) 0.0281
Molecular Subtypes 0.6210
Luminal A 321 0.54 (0.32..0.90) 0.0192
Luminal B 100 0.74 (0.32..1.72) 0.4873
ER-/PR-/HER2+ 44 0.36 (0.08..1.65) 0.1885
TNBC 97 0.38 (0.15..0.95) 0.0384
Tumour Grade 0.6259
G1/2 317 0.67 (0.39..1.15) 0.1462
G3 322 0.57 (0.36..0.90) 0.0155
pT stage 0.3840
pT1 204 0.59 (0.31..1.10) 0.0991
pT2 368 0.72 (0.44..1.17) 0.1820
pT3/4 66 1.22 (0.56..2.64) 0.6144
pN stage 0.4243
pN1 260 0.61 (0.31..1.22) 0.1639
pN2 227 0.56 (0.32..0.98) 0.0405
pN3 152 0.91 (0.51..1.64) 0.7656
0.1 0.2 0.5 1 2 5
HR
TILs superior
no TILs superior
subgroup n HR (95% CI) p p(interaction)
Overall 639 0.72 (0.46..1.12) 0.1500
Molecular Subtypes 0.7178
Luminal A 321 0.45 (0.23..0.89) 0.0227
Luminal B 100 0.84 (0.26..2.69) 0.7711
ER-/PR-/HER2+ 44 NA (0.00.. Inf) 0.9961
TNBC 97 0.40 (0.14..1.10) 0.0760
Tumour Grade 0.6822
G1/2 317 0.72 (0.34..1.49) 0.3725
G3 322 0.60 (0.34..1.05) 0.0733
pT stage 0.1011
pT1 204 0.44 (0.18..1.07) 0.0711
pT2 368 0.75 (0.41..1.38) 0.3546
pT3/4 66 1.88 (0.70..5.10) 0.2133
pN stage 0.7888
pN1 260 0.62 (0.20..1.88) 0.3945
pN2 227 0.66 (0.33..1.31) 0.2330
pN3 152 0.88 (0.44..1.75) 0.7186
0.1 0.2 0.5 1 2 5
HR
TILs superior
no TILs superior

B


subgroup n HR (95% CI) p p(interaction)
Overall 612 0.57 (0.39..0.84) 0.0043
Molecular Subtypes 0.7211
Luminal A 321 0.48 (0.28..0.83) 0.0084
Luminal B 99 0.75 (0.31..1.84) 0.5346
ER-/PR-/HER2+ 44 0.40 (0.04..3.75) 0.4242
TNBC 97 0.47 (0.15..1.46) 0.1902
Tumour Grade 0.1920
G1/2 307 0.76 (0.42..1.38) 0.3668
G3 305 0.45 (0.27..0.76) 0.0026
pT stage 0.4296
pT1 196 0.41 (0.20..0.82) 0.0121
pT2 353 0.58 (0.33..1.02) 0.0582
pT3/4 63 1.30 (0.51..3.32) 0.5809
pN stage 0.4885
pN1 249 0.45 (0.20..1.00) 0.0512
pN2 217 0.43 (0.22..0.81) 0.0086
pN3 146 0.79 (0.41..1.52) 0.4842
0.1 0.2 0.5 1 2 5
HR
TILs superior
no TILs superior
subgroup n HR (95% CI) p p(interaction)
Overall 612 0.58 (0.35..0.96) 0.0333
Molecular Subtypes 0.6469
Luminal A 321 0.42 (0.20..0.88) 0.0219
Luminal B 99 0.65 (0.19..2.17) 0.4819
ER-/PR-/HER2+ 44 NA (0.00.. Inf) 0.9988
TNBC 97 0.63 (0.17..2.31) 0.4830
Tumour Grade 0.2585
G1/2 307 0.69 (0.30..1.60) 0.3906
G3 305 0.50 (0.27..0.96) 0.0361
pT stage 0.1000
pT1 196 0.25 (0.09..0.72) 0.0098
pT2 353 0.61 (0.31..1.23) 0.1682
pT3/4 63 2.40 (0.68..8.46) 0.1739
pN stage 0.8085
pN1 249 0.47 (0.13..1.72) 0.2546
pN2 217 0.44 (0.19..0.98) 0.0448
pN3 146 0.64 (0.29..1.44) 0.2819
0.1 0.2 0.5 1 2 5
HR
TILs superior
no TILs superior

(HR = 1.24 [0.92–1.67], p = 0.1566, test for interaction p = 0.0336). Regarding the OS, no differences were seen. Kaplan–Meier curves for patients with and without TILs by treatment arms are shown in Fig. 5 A and B.

In addition, we looked at the EPC arm and investigated the role of TILs in different subgroups such as molecular subtypes, tumour grade, tumour and nodal stage. According to the interaction tests, we did not find any significant differences in the subgroups on univariate and multivariate analyses (Fig. 6 A and B).

Finally, we observed that patients with PD-1–positive immune cells in TNBC had a significant better DFS (HR = 0.50 [0.25–0.99], p = 0.0457) than patients without PD-1–positive immune cells on multivariate analysis (Fig. 7A and B). PD-L1 did not show any significant impact on patient outcome.

4. Discussion

In this study, we investigated more than 1300 high-risk, node-positive BC samples prospectively collected from an adjuvant trial for TILs (H&E staining) and immune checkpoints, PD-1 and PD-L1 by IHC. Higher levels of TILs were significantly associated with triple-negative and non-luminal HER2-positive tumours as well as with high tumour grade, high proliferation and ductal type (NST), which is consistent with previous reports in the adjuvant setting [3,4]. We observed that TILs were more common in small tumours (early pT stage) but equally distributed among the pN stages. In a previous adjuvant study of node-positive BC, TILs were not associated with tumour size and lymph node involvement [3]. In contrast, higher levels of TILs were related to larger tumours and more involved lymph nodes in an adjuvant-treated cohort of node-positive and node-negative BC [4]. In TNBC, TILs were significantly lower with larger tumour size and more positive nodes [7]. These studies indicate an interaction between tumour load and TILs that seems to vary between different BC subtypes.

Here, we further observed that patients with TILs have a better DFS in the EPC arm compared with the EC-PwX arm. In the GAIN-1 trial, both dd chemotherapy regimens were compared, but no differences in DFS and OS were found [26]. When we looked at breast carcinomas without TILs, patients had an improved DFS in the EC-PwX arm compared with the EPC arm. It is possible that the addition of capecitabine may mediate an immune modulation.

Similarly, Loi et al [3] reported on an association between TILs and chemotherapy benefit in HER2-positive BC. In another previous neoadjuvant study, we have shown that TILs predict response to carboplatin in TNBC and HER2-positive BC [24]. These data indicate that TILs might have a predictive value for chemotherapy response. So far, TILs as a stratification factor for the selection of systemic treatment regimens is not yet ready to translate in clinical practice. Further prospective trials are needed to prove whether the analysis of TILs is reliable in adjuvant therapy prediction. Therefore, the evaluation of TILs should be included in the design of upcoming clinical trials.

The potential predictive role of TILs was also shown very recently for immune therapies with pembrolizumab and atezolizumab [13–15]. PD-1/PD-L1 blockade is now actively explored in BC, especially in TNBC, in the adjuvant and neoadjuvant setting. First results of clinical trials demonstrate that patients with PD-L1–positive immune cells and increased TILs (H&E staining) have a better response to these therapies [13,15,17,21]. The consideration of the expression status of PD-1/PD-L1 and the density of immune cells will be important for the selection of patients. Further studies are needed to explore whether TILs, IHC assays for immune checkpoints or a combination of both parameters is the best candidate for prediction of response in immune-modulating therapies.

In our analysis, we observed low expression levels of PD-L1 (SP263 assay) in tumour cells (3%), which is consistent with a recent study (1.7% positivity, E1L3N clone) [33] but opposite to other previous reports [21,23,34]. PD-L1 expression was more common in immune cells (15%) than in tumour cells, as in previous studies [33,34]. The prevalence of PD-1–positive immune cells was comparable with previous findings [22]. In the past, application of different PD-L1 IHC assays, scoring methods and evaluation of different tumour compartments led to variable results. Harmonisation among these tests are ongoing (comparison of clinical trial assays versus laboratory assays, refinement of evaluation algorithms and training to reduce interobserver variability), especially for patients with melanoma, non small cell lung cancer, head and neck and urothelial carcinoma, where the expression analysis of PD-L1 by IHC serves as a selection criterion [35,36]. Validated assays to determine the eligibility for immunotherapies is important. To our knowledge, we used for the first time in BC, a robust and validated PD-L1 assay (SP263, Ventana) that otherwise guides treatment decisions for checkpoint inhibitors in clinical trials for patients with non–small cell lung cancer and urothelial carcinoma under durvalumab therapy. Currently,

Fig. 6. Analysis of the relevance of TILs in different subgroups of the EPC treatment arm only. Univariate and multivariate Cox proportional hazards regressions for DFS and OS are shown as Forest plots. Hazard ratios (squares) and 95% confidence intervals (horizontal lines) are given for each parameter. Sizes of the squares are proportional to the number of patients. EPC, epirubicin, paclitaxel and cyclophosphamide; ER, oestrogen receptor; DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; TNBC, triple-negative breast cancer; TILs, tumour-infiltrating lymphocytes; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

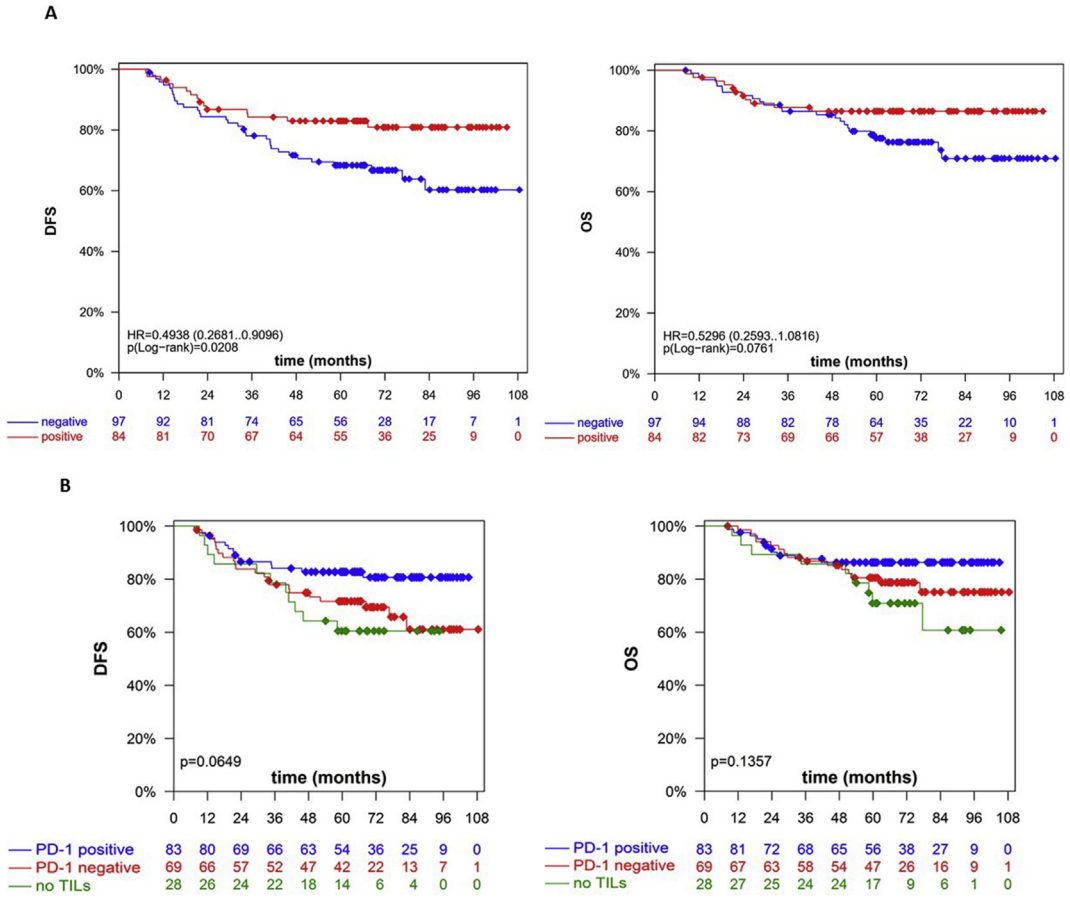


Fig. 7. Prognostic value of PD-1–positive immune cells in patients with triple-negative breast cancer (TNBC). (A) Kaplan–Meier curves comparing TNBC with PD-1–positive and PD-1–negative immune cells for DFS and OS. P values of the comparison of both groups are given. (B) Kaplan–Meier curves comparing TNBC with PD-1–positive immune cells, PD-1–negative immune cells and no inflammatory cells at all for DFS and OS with statistically no significant differences. HR, hazard ratio; DFS, disease-free survival; OS, overall survival.

a translational research programme in the randomised phase II trial (GeparNuevo, GBG) for the evaluation of durvalumab in combination with taxane–anthracycline therapy in patients with TNBC is ongoing. These investigations will show whether the prospective assessment of TILs allows a selection of patients for immune checkpoint inhibition.

As expected, overall TIL density is correlated with PD-1 and PD-L1 expression [33,37]. Highest levels of TILs, PD-1 and PD-L1 occurred in TNBC, confirming the inherent interaction with the immune system in this subtype. Thus, we found a strong trend for a better prognosis in patients with TNBC with TILs. This is in line with previous studies that have shown a strong prognostic role of TILs in early-stage TNBC, supporting to integrate TILs in a clinicopathological model for patients with TNBC [3,4,7]. We further observed that the presence of PD-1–positive immune cells in TNBC was significantly associated with a better DFS, whereas PD-L1 (both on immune cells and tumour cells) did not show any prognostic value. The prognostic relevance of PD-1/PD-L1 in BC and other tumour entities is controversial [22,34,38]. This can be explained by the heterogeneity of the studies, the variety of technical methods used for staining, scoring algorithms applied and composition as well as dynamics of the immune tumour environment.

In this study, we evaluated TILs and immune checkpoints on TMAs, where the entire tumour is not represented. We are aware that immune cell infiltration shows heterogeneity within a tumour that cannot be fully replicated in small tissue samples. However, our findings confirm previous data where full-face sections were examined [3–5]. On the other side, we believe that the important advantages of our study are the large sample size, tumour tissue of a clinical trial data set with long-term follow-up and experience in PD-1/PD-L1 assays [35,39].

In conclusion, we demonstrate that TILs predict the benefit from intensified ddEPC compared with ddEC-PwX treatment, in high-risk and nodal-metastasised BC. TILs correlate with PD-1 and PD-L1 and show the highest levels in TNBC. TILs and PD-1–positive immune cells show prognostic importance, whereas PD-L1 plays an inferior role with respect to the patient's prognosis.

Conflict of interest statement

V.M. received honoraria from and discloses a consulting or advisory role with Amgen, Celgene and Roche. K.W. is a shareholder and holds patents with Sividon Diagnostics. W.W. received research funding from Roche and discloses a consulting or advisory role with AZ, Roche, Takeda, Novartis, BMS and MSD. V.M. received honoraria from Amgen, AstraZeneca, Daiichi Sankyo, EISAI, Pfizer, Novartis, Roche and Teva, discloses a consulting or advisory role with Hexal, Roche, Pfizer, Amgen, Daiichi Sankyo, Nektar and EISAI and received travel and accommodation expenses from Roche and Pfizer. P.F. received research funding from Novartis and honoraria from Novartis, Roche, Amgen, Celgene and Pfizer and discloses a consulting or advisory role with Novartis, Roche, Amgen, Celgene and Pfizer. M.M. discloses a consulting or advisory role with Amgen. F.M. received honoraria from Roche, Amgen, AstraZeneca, EISAI, Celgene, Novartis, Pfizer and Genomic Health, discloses a consulting or advisory role with Roche, AstraZeneca and Novartis and received travel and accommodation expenses from Roche, Amgen, AstraZeneca, EISAI, Celgene, Novartis and Pfizer. W.D.S. received honoraria from AstraZeneca. E.S. received honoraria from Roche, Novartis and Pfizer, discloses a consulting or advisory role with Roche, Pfizer and Novartis and received travel and accommodation expenses from Roche and Novartis. S.L. received research funding from Abbvie, Amgen, AZ, Celgene, Novartis, Pfizer, Roche, Teva and Vifor, received honoraria for the institution (Pfizer and Roche) and discloses a consulting or advisory role for the institution (Novartis, Pfizer, Roche and SeaGen). C.D. is a shareholder with Sividon Diagnostics, received honoraria from Amgen, Novartis, Pfizer, Roche and Teva, discloses a consulting or advisory role with Amgen and MSD, holds patents for Predictive gene expression signature for therapy response and VMscope digital pathology software. All remaining authors (A.N., S.S., C.H.K., C.S., B.I.H., K.S., T.K. and C.S.) have declared no conflicts of interest.

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References

[1] 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;19:40–50.

[2] Stanton SE, Adams S, Disis ML. Variation in the incidence and magnitude of tumor-infiltrating lymphocytes in breast cancer subtypes: a systematic review. JAMA Oncol 2016;2:1354–60.

[3] Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III 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;31:860–7.

[4] Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol 2014;25:1544–50.

[5] Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol 2014;32:2959–66.

[6] Pruneri G, Gray KP, Vingiani A, Viale G, Curigliano G, Criscitiello C, et al. Tumor-infiltrating lymphocytes (TILs) are a powerful prognostic marker in patients with triple-negative breast cancer enrolled in the IBCSG phase III randomized clinical trial 22-00. Breast Canc Res Treat 2016;158:323–31.

[7] Loi S, Drubay D, Adams S, Pruneri G, Francis PA, Lacroix-Triki M, et al. Tumor-infiltrating lymphocytes and prognosis: a pooled individual patient Analysis of early-stage triple-negative breast cancers. J Clin Oncol 2019;37:559–69.

[8] Denkert C, Loibl S, Noske A, Roller M, Muller BM, Komor M, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 2010;28:105–13.

[9] Salgado R, Denkert C, Campbell C, Savas P, Nuciforo P, Aura C, et al. Tumor-infiltrating lymphocytes and associations with pathological complete response and event-free survival in HER2-positive early-stage breast cancer treated with lapatinib and trastuzumab: a secondary analysis of the NeoALTTO trial. JAMA Oncol 2015;1:448–54.

[10] von Minckwitz G, Untch M, JU Blohmer, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012;30:1796–804.

[11] Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 2014;384:164–72.

[12] Ingold Heppner B, Untch M, Denkert C, Pfitzner BM, Lederer B, Schmitt W, et al. Tumor-infiltrating lymphocytes: a predictive and prognostic biomarker in neoadjuvant-treated HER2-positive breast cancer. Clin Cancer Res 2016;22:5747–54.

[13] Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 2019;379(22):2108–21.

[14] Rugo HS, Delord JP, Im SA, Ott PA, Piha-Paul SA, Bedard PL, et al. Safety and antitumor activity of pembrolizumab in patients

with estrogen receptor-positive/human epidermal growth factor receptor 2-negative advanced breast cancer. Clin Cancer Res 2018;24:2804–11.
[15] Emens LA, Cruz C, Eder JP, Braiteh F, Chung C, Tolaney SM, et al. Long-term clinical outcomes and biomarker analyses of atezolizumab therapy for patients with metastatic triple-negative breast cancer: a phase 1 study. JAMA Oncol 2019;5(1):74–82.
[16] Emens LA. Breast cancer immunotherapy: facts and hopes. Clin Cancer Res 2018;24:511–20.
[17] Loi S, Adams S, Schmid P, Cortés J, Cescon DW, Winer EP, et al. LBA13Relationship between tumor infiltrating lymphocyte (TIL) levels and response to pembrolizumab (pembro) in metastatic triple-negative breast cancer (mTNBC): results from KEYNOTE-086. Ann Oncol 2017;28. mdx440.005-mdx440.005.
[18] Daud AI, Wolchok JD, Robert C, Hwu WJ, Weber JS, Ribas A, et al. Programmed death-ligand 1 expression and response to the anti-programmed death 1 antibody pembrolizumab in melanoma. J Clin Oncol 2016;34:4102–9.
[19] Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 2016;375:1823–33.
[20] Teixido C, Vilarino N, Reyes R, Reguart N. PD-L1 expression testing in non-small cell lung cancer. Ther Adv Med Oncol 2018;10. 1758835918763493.
[21] Nanda R, Chow LQ, Dees EC, Berger R, Gupta S, Geva R, et al. Pembrolizumab in patients with advanced triple-negative breast cancer: phase ib KEYNOTE-012 study. J Clin Oncol 2016;34:2460–7.
[22] Muenst S, Soysal SD, Gao F, Obermann EC, Oertli D, Gillanders WE. The presence of programmed death 1 (PD-1)-positive tumor-infiltrating lymphocytes is associated with poor prognosis in human breast cancer. Breast Canc Res Treat 2013;139:667–76.
[23] Mittendorf EA, Philips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, et al. PD-L1 expression in triple-negative breast cancer. Cancer Immunol Res 2014;2:361–70.
[24] Denkert C, von Minckwitz G, Brase JC, Sinn BV, Gade S, Kronenwett R, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. J Clin Oncol 2015;33:983–91.
[25] von Minckwitz G, Mobus V, Schneeweiss A, Huober J, Thomssen C, Untch M, et al. German adjuvant intergroup node-positive study: a phase III trial to compare oral ibandronate versus observation in patients with high-risk early breast cancer. J Clin Oncol 2013;31:3531–9.
[26] Mobus V, von Minckwitz G, Jackisch C, Luck HJ, Schneeweiss A, Tesch H, et al. German Adjuvant Intergroup Node-positive Study (GAIN): a phase III trial comparing two dose-dense regimens (iddEPC versus ddEC-PwX) in high-risk early breast cancer patients. Ann Oncol 2017;28:1803–10.
[27] McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, et al. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 2005;97:1180–4.
[28] Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med 2010;134:e48–72.
[29] Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in breast cancer: recommendations from the international Ki67 in breast cancer working group. J Natl Cancer Inst 2011;103:1656–64.
[30] Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 2013;31:3997–4013.
[31] 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 2014. Ann Oncol 2015;26:259–71.
[32] Scheel AH, Dietel M, Heukamp LC, Johrens K, Kirchner T, Reu S, et al. Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas. Mod Pathol 2016;29:1165–72.
[33] 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;26:1488–93.
[34] Muenst S, Schaerli AR, Gao F, Daster S, Trella E, Droeser RA, et al. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. Breast Canc Res Treat 2014;146:15–24.
[35] Scheel AH, Baenfer G, Baretton G, Dietel M, Diezko R, Henkel T, et al. Interlaboratory concordance of PD-L1 immunohistochemistry for non-small-cell lung cancer. Histopathology 2018;72:449–59.
[36] Eckstein M, Wirtz RM, Pfannstil C, Wach S, Stoehr R, Breyer J, et al. A multicenter round robin test of PD-L1 expression assessment in urothelial bladder cancer by immunohistochemistry and RT-qPCR with emphasis on prognosis prediction after radical cystectomy. Oncotarget 2018;9:15001–14.
[37] Kitano A, Ono M, Yoshida M, Noguchi E, Shimomura A, Shimoi T, et al. Tumour-infiltrating lymphocytes are correlated with higher expression levels of PD-1 and PD-L1 in early breast cancer. ESMO Open 2017;2:e000150.
[38] Brockhoff G, Seitz S, Weber F, Zeman F, Klinkhammer-Schalke M, Ortmann O, et al. The presence of PD-1 positive tumor infiltrating lymphocytes in triple negative breast cancers is associated with a favorable outcome of disease. Oncotarget 2018;9:6201–12.
[39] Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. CD274/PD-L1 gene amplification and PD-L1 protein expression are common events in squamous cell carcinoma of the oral cavity. Oncotarget 2016;7:12024–34.