ORIGINAL RESEARCH

PTK7 as a potential prognostic and predictive marker of response to adjuvant chemotherapy in breast cancer patients, and resistance to anthracycline drugs

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Abstract: Biomarkers predicting resistance to particular chemotherapy regimens could play a key role in optimally individualized treatment concepts. PTK7 (protein tyrosine kinase 7) belongs to the receptor tyrosine kinase family involved in several physiological, but also malignant, cell behaviors. Recent studies in acute myeloid leukemia have associated PTK7 expression with resistance to anthracycline therapy. PTK7 mRNA expression in primary tumor tissue (PTT) and corresponding lymph node tissue (LNT) were retrospectively measured in 117 patients with early breast cancer; PTK7 expression was available in 103 PTT and 108 LNT samples. Median age was 60 years (range, 27–87 years). At a median follow-up of 28.5 months, 6 deaths and 16 recurrences had occurred. PTK7 expression correlations with clinicopathological features were computed and PTK7 expression effects on patient outcome were analyzed in three cohorts defined by adjuvant treatment: anthracycline-based treatment, other chemotherapy regimens (including taxane or other substances), or no chemotherapy. Association of PTK7 expression with clinicopathological features was seen only for age in PTT and nodal stage in LNT. High LN PTK7 was associated with poorer disease-free survival (DFS) in the total population (3-year DFS: low [81.7%] versus high [70.4%]; P=0.016) and in patients without adjuvant chemotherapy (3-year DFS: low [91.7%] versus high [22.3%]; $P<0.001$), but not in patients receiving adjuvant chemotherapy (P=0.552). DFS stratified by PTK7 expression was compared in treatment cohorts: In patients with low LN PTK7 expression, neither chemotherapy cohort showed significantly better survival than the no-chemotherapy cohort. In patients with high LN PTK7 expression, those receiving chemotherapy, including substances other than anthracyclines, but not those receiving only anthracycline-based chemotherapy, showed significantly better DFS than those receiving no chemotherapy (P=0.001). Our results support earlier findings that PTK7 may be a prognostic and predictive marker associated with resistance to anthracycline-based chemotherapy. Further investigations are needed to validate these findings in breast cancer.

Keywords: breast cancer, PTK7, protein tyrosine kinase 7, chemoresistance, prognostic factors, predictive factors

Introduction

Breast cancer (BC) is the most common malignancy for women in developed countries. During the last century, advances in screening, surgery, radiotherapy, and systemic therapy have led to high rates of local and distant disease control, resulting in survival improvement for patients with early-stage BC.[1–3] So far, it is accepted that BC is a systemic disease, with micrometastatic involvement at diagnosis in many patients. Therefore, during the last decades, systemic neoadjuvant/adjuvant therapy has been

used to eradicate potential micrometastatic disease in early treatment of BC patients. Adjuvant systemic therapies reduce risk for relapse and death, as shown in several meta-analyses.[4–6] In terms of targeted therapy, hormonal therapy in hormone receptor-positive[7–9] and trastuzumab therapy in human epidermal growth factor receptor 2 (HER2)-positive[10,11] patients are well established. However, chemotherapy remains an important backbone in systemic treatment, especially in patients with high relapse risk. Anthracycline-based poly-chemotherapy regimens are most widely used; the addition of taxanes into chemotherapy has improved survival benefits in the adjuvant setting.[5] Unfortunately, dose limiting and long-term adverse effects, as well as resistance, represent challenges to further improvement in the efficacy of adjuvant BC chemotherapy.[12] Therefore, it is very important to understand the molecular mechanism of resistance to chemotherapeutic drugs and spare the nonnegligible adverse effects for patients.

Protein tyrosine kinase 7 (PTK7), also known as colon carcinoma kinase, is an evolutionary, highly conserved cell surface planar cell polarity (PCP) receptor that belongs to the family of receptor tyrosine kinase. The PTK7 gene is located on chromosome 6 (6p21.1–p12.2),[13] and it encodes a transmembrane protein containing seven immunoglobulin-like loops, a transmembrane domain, and an inactive catalytic tyrosine kinase domain.[14,15] The ligand has not yet been identified. PTK7 has been shown to be highly involved in the canonical and noncanonical Wnt pathway.[16] It plays a role in multiple cellular processes including tube formation,[17] migration,[18] and invasion of endothelial cells and angiogenesis.[19] It is believed that PTK7 may affect cell clustering or cell contact persistence. Increased expression of PTK7 has been found in several human tumors, suggesting a role in human carcinogenesis.[20–25] However, the prognostic effect of PTK7 expression in cancer remains unclear. In a previous study, we identified PTK7 as a potential mediator of motility and invasiveness of BC cells.[26] A recent study indicates that PTK7 may induce resistance to anthracycline chemotherapy in human bone cancer cell lines and acute myeloid leukemia (AML) patients.[24] However, the role of PTK7 in early BC in the development of acquired chemoresistance against chemotherapeutic agents, including anthracyclines, has not yet been elucidated.

The aim of our analysis was to investigate messenger RNA (mRNA) expression of PTK7 by reverse transcription-polymerase chain reaction (RT-PCR) in early BC, to correlate PTK7 expression with clinicopathological features, to evaluate the effect of PTK7 expression on outcome in primary BC

patients, and to evaluate a possible predictive role of PTK7 expression regarding chemotherapy.

Material and methods
Patients and tissue collection
From 2006 to 2010, 117 BC patients (median age at diagnosis, 60 years; range, 27–87 years; 88/117 [75.2%] with infiltrated LN and 29/117 [24.8%] with noninfiltrated LN) were included from the Red Cross Women's Hospital Munich, Germany. This study was approved by Ethikkommission Ludwig-Maximilians-Universität ethics committee. Patients gave informed consent for the use of biological materials and relevant clinical data. Patients receiving neoadjuvant systemic therapy and those with primary distant metastases were excluded. Stage-adapted surgery was performed on breast and axillary LN. Primary tumor tissue (PTT) and lymph node tissue (LNT) were collected and stored immediately at −80°C from preparation. To ensure the histological status of harvested LN, the tissues were bisected, with half submitted for routine histology and the other half taken for examination by RT-PCR. Tumor grade; tumor, node, and metastasis classification; and histopathological tumor subtype were recorded. Hormonal receptor (estrogen receptor/progesterone receptor, immunoreactive score [IRS]) and HER2 status were assessed by immunohistochemistry and/or fluorescence in situ hybridization (all cases with HER2-HercepTest™ (Dako, Denmark) Score 2, according to American Society of Clinical Oncology/College of American Pathologists guidelines[27]). Patients were treated with radiotherapy according to current national guidelines. Clinicopathological features, type of chemotherapy, and follow-up were obtained from patient records.

Adjuvant systemic treatment was determined according to guidelines at the time of diagnosis. Of 117 patients, 69 (59%) received chemotherapy and 75 (64.1%) received hormonal therapy; 21 of 23 HER2-positive patients received combined chemotherapy/trastuzumab therapy; 45 patients did not receive chemotherapy because of patient preference, older age, or early disease stage. Typical chemotherapy regimens were either only anthracycline-based (doxorubicin/cyclophosphamide [AC]/epirubicin/cyclophosphamide [EC]/fluorouracil/doxorubicin/cyclophosphamide [FAC]/fluorouracil/epirubicin/cyclophosphamide [FEC], four to six cycles) or taxane-based (docetaxel/doxorubicin/cyclophosphamide [TAC]/fluorouracil/epirubicin/cyclophosphamide - docetaxel [FEC-Doc]/fluorouracil/epirubicin/cyclophosphamide - paclitaxel [FEC-Pac], four to six cycles, or six cycles of cyclophosphamide/methotrexate/fluorouracil [CMF]).

Total RNA isolation

Total RNA was isolated using the acid guanidine thiocyanate-phenol-chloroform extraction method.[28] After Na-acetate treatment, the total RNA was extracted with phenol-chloroform, precipitated with isopropanol, resuspended with lysis buffer, reprecipitated with isopropanol, washed with 80% ethanol, and finally dissolved in diethyl pyrocarbonate-treated H_2O. The total RNA concentration was determined by absorbance measurement (260/280 nm), and the quality of total RNA was verified by 1% agarose gel electrophoresis. Only samples with no evidence of DNA contamination and RNA degradation were used for cDNA synthesis.

cDNA synthesis

cDNA synthesis was performed using 5 µg total RNA in an oligo (desoxythymidine) primer mix (MWG Biotech, Ebersberg, Germany; total volume, 10 µL), which was heated at 70°C for 3 minutes and then cooled on ice. A master mix containing 1× reverse transcriptase buffer, 1 mmol/L deoxynucleotide triphosphates, 10 mmol/L dithiothreitol (DTT), 40 U RNase inhibitor (40 U/µL; Fermentas), and 50 U avian myeloblastosis virus reverse transcriptase (25 U/µL; Molecular Diagnostics, Roche) was added and subsequently incubated at 42°C for 2 hours. A stop reaction was performed with 80 µL Tris-ethylenediaminetetraacetic acid 10/0.1, followed by heating at 72°C for 7 minutes. The quality of the cDNA was verified using 1.5% agarose gel electrophoresis. Afterward, the cDNA probe was denatured with 10 µL and 1 N NaOH, pH neutralized with 5 µL 2 N HCl and 5 µL 2N Tris-HCl (pH, 7.5), and purified using the QIAquick PCR Purification kit (Quiagen, Hilden, Germany) according to the manufacturer's instructions.

RT-PCR

The PCR method was used to determine mRNA expression levels of PTK7 and α-tubulin (housekeeping gene) in fresh-frozen PTT and LNT samples. The following primer sequences were applied: PTK7-ex 7fwd, 5′-GGA AGC CAC ACT TCA CCT AGC AG-3′, PTK7-ex11rev, 5′-CTG CCA CAG TGA GCT GGA CAT GG-3′, α-TUB fwd, 5′-AAG TGA CAA GAC CAT TGG GGG AGG-3′, α-TUB rev, 5′ GGG CAT AGT TAT TGG CAG CAT-3′. All primers were synthesized by MWG Biotech. PCR was performed using 1 µL cDNA as a template in a master mix containing 1× PCR buffer, 1 mmol/L deoxynucleotide triphosphates, 1 pmol/µL of each specific primer, and 2.5 U Taq DNA polymerase (Sigma, Taufkirchen, Germany) for each sample and carried out in a thermal cycler (Eppendorf AG, Wessling-Berzdorf, Germany). PCR cycling conditions began with an initial DNA denaturation step at 94°C for 2 minutes, followed by 32 cycles with denaturation at 94°C for 30 seconds, primer annealing at the appropriate annealing temperature for 30 seconds, and extension at 72°C for 1 minute, followed by a final extension step at 72°C for 5 minutes. The amplified PCR products were analyzed by separation in a 2% agarose gel at 80 V for 30 minutes. The mRNA expression of the PCR products, with a size of approximately 100 bp to 1 kbp, was evaluated semiquantitatively, using Aida Image Software (Raytest, Straubenhardt, Germany).

Statistical analysis

Two-sample *t*-tests, one-way analysis of variance, or the Mann–Whitney *U* test were performed to test for associations between continuous and grouped variables; chi-square tests or Fisher's exact test were performed to evaluate associations between grouped variables.

Disease-free survival (DFS) and overall survival (OS) were defined in months from time of surgery. DFS end points were recurrent tumor and distant metastasis or death from BC. Survival estimates were obtained by the Kaplan–Meier method; the log-rank test was used to analyze for group differences. A binary PTK7 expression variable (high versus low) was defined using median expression as a cutoff. All reported *P*-values are two-tailed, with a nominal significance level of 5%. Statistical analysis was performed using SPSS (version 20, IBM Corporation, New York, NY, USA).

Results

Patient characteristics and outcome

PTK7 mRNA expression in PTT and corresponding LNT were retrospectively measured in 117 early BC patients. Median age at diagnosis was 60 years (range, 27–87 years). At a median follow-up period of 28.5 months, there were 16 recurrences (13 distant, 3 local) and 6 deaths. For analysis, we defined cohort A as patients receiving exclusively anthracycline-based chemotherapy, cohort B as those receiving chemotherapy including agents other than anthracyclines (eg, taxane-based and others), and cohort C as patients receiving no chemotherapy. Table 1 describes and compares key parameters by cohort: Patients receiving no chemotherapy (cohort C) were older (median age, 72 years) than in cohorts A and B (60 and 49 years, respectively). Significant differences among the three cohorts occurred in nodal status, histological type, and HER2 status. Neither in paired comparisons among the three treatment cohorts nor in comparisons of chemotherapy versus no chemotherapy (*P*=0.340; Figure 1A) were significant differences in DFS or OS observed.

Table 1 Comparing cohorts A through C

Parameter	Cohort A, exclusively anthracycline-based (n=19)	Cohort B, taxane-based/ others (n=50)	Cohort C, no chemotherapy (n=45)	Statistics, *P*-value
Median age in years, range	60 (44–73)	49 (27–75)	72 (36–87)	<0.001
Size, pT (tumor stage)				0.241
pT1	7 (36.8%)	17 (34.0%)	16 (35.5%)	
pT2	9 (47.4%)	28 (56.0%)	20 (44.4%)	
pT3	2 (20.0%)	5 (10.0%)	3 (6.7%)	
pT4	1 (14.3%)	0 (0%)	6 (13.3%)	
Histological type				0.017
Invasive ductal	16 (84.2%)	45 (90.0%)	32 (71.1%)	
Invasive lobular	3 (15.8%)	1 (2.0%)	11 (24.4%)	
Other	2 (4.4%)	4 (8.0%)	2 (33.3%)	
Pathologic nodal status (pN)				0.001
pN0	2 (42.2%)	8 (16.0%)	19 (42.2%)	
pN1	6 (13.3%)	27 (54.0%)	6 (13.3%)	
pN2	11 (24.4%)	10 (20.0%)	11 (24.4%)	
pN3	9 (20.0%)	5 (10.0%)	9 (20.0%)	
Pathologic grade				0.619
G1	0 (0%)	4 (8.0%)	5 (11.1%)	
G2	11 (57.9%)	30 (60.0%)	26 (57.8%)	
G3	8 (42.1%)	16 (32.0%)	14 (31.1%)	
Estrogen receptor status				0.245
Estrogen receptor positive	13 (68.4%)	36 (72.0%)	38 (84.4%)	
Estrogen receptor negative	6 (31.6%)	14 (28.0%)	7 (15.6%)	
Human epidermal growth factor receptor 2 status				<0.001
Human epidermal growth factor receptor 2 negative	18 (94.7%)	30 (61.2%)	41 (93.2%)	
Human epidermal growth factor receptor 2 positive	1 (5.3%)	19 (38.8%)	3 (6.8%)	

Notes: Cohort A, patients receiving exclusively anthracycline-based chemotherapy. Cohort B, patients receiving chemotherapy including agents other than anthracyclines (eg, taxane-based and others). Cohort C, patients receiving no chemotherapy.

PTK7 expression and association with clinicopathological features

PTK7 expression in PTT was distributed as follows: 1.72 (25th percentile), 3.04 (median), and 4.39 (75th percentile). PTK7 expression in LNT was distributed as follows: 0.58 (25th percentile), 1.68 (median), and 3.15 (75th percentile).

A moderate correlation of PTK7 levels in PTT and LNT was seen (Spearman-Rho, 0.432; *P*<0.001). The following associations of PTK7 expression in PTT and LNT with clinicopathological features (age; grade; tumor, node, and metastasis category; steroid hormonal receptor; and HER2 status) were seen (Table 2). High PTK7 expression in PTT occurred more frequently in younger patients; high PTK7 expression in LNT was significantly associated with higher pathologic nodal status. As a rough measure of association, LNT PTK7 level (but not PTT PTK7 level) treated as a continuous variable was correlated with nodal status (Spearman-Rho, 0.537; *P*<0.001).

Prognostic effect of PTK7 expression

The effects of PTK7 expression in PTT and in LNT on DFS and OS were first analyzed for the collective as a whole.

Although PTT PTK7 expression had no significant prognostic effect on DFS or OS, patients with low PTK7 expression in LNT had significantly better DFS than those with high PTK7 (*P*=0.016; 3-year DFS: low versus high, 81.7% versus 70.4%). In univariate Cox regression, high LNT PTK7 expression was associated with a hazard ratio of 3.76 (95% CI: 1.19 - 11.9). LNT PTK7 expression had no significant effect on OS.

PTK7 expression and chemotherapy benefit prediction

In view of the prognostic effect of PTK7 expression in LNT, but not PTT, we consider LNT PTK7 expression in the following predictive analysis.

The effect of PTK7 expression on DFS differed according to whether chemotherapy was administered at all. As illustrated in Figure 1B, patients in cohort C with high LNT PTK7 expression (n=14) showed significantly poorer DFS than those with low expression (n=21; 3-year DFS: 22.3% versus 91.7%; *P*>0.001); a corresponding difference was not seen in patients with chemotherapy (Figure 1C). These findings suggest a particular benefit from chemotherapy for patients with high LNT PTK7 expression.
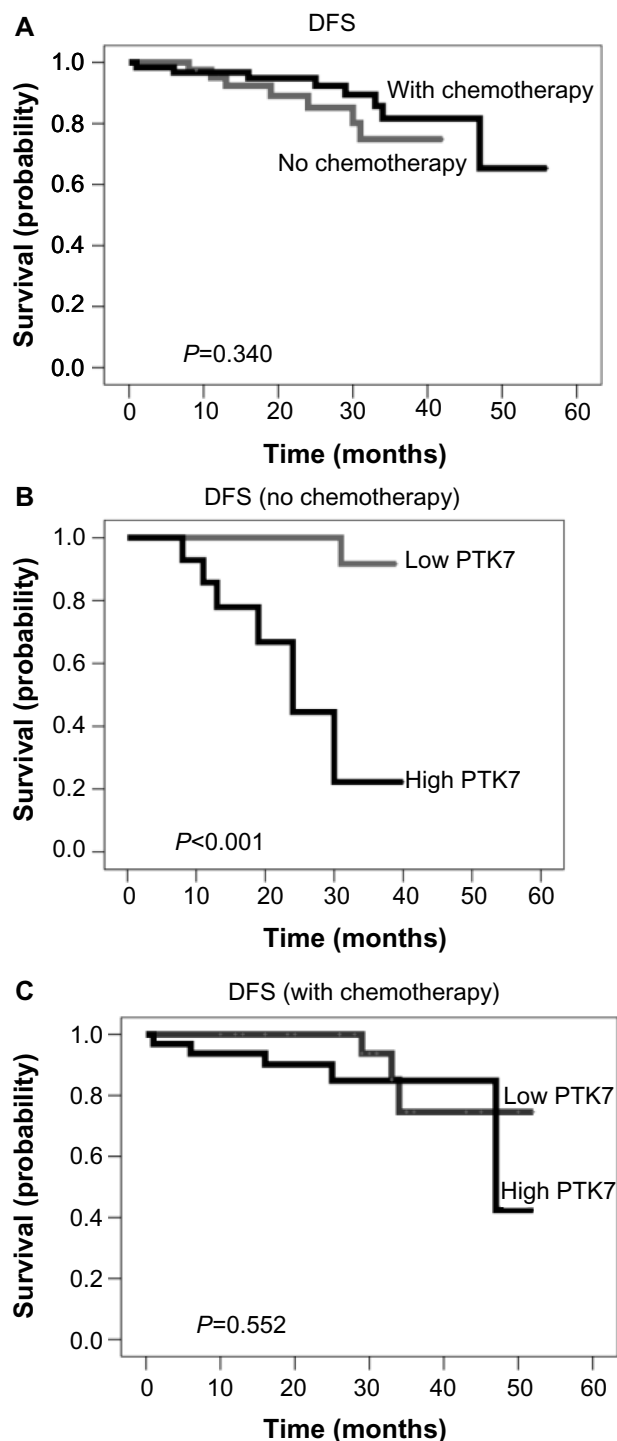
Figure 1 Kaplan–Meier curves for disease-free survival (DFS). (**A**) DFS in patients with and without adjuvant chemotherapy. (**B**) DFS in patients without adjuvant chemotherapy based on PTK7 expression. (**C**) DFS in patients with adjuvant chemotherapy based on PTK7 expression.

Taking into account the statistical power available, the hypothesis of an association of PTK7 with anthracycline resistance was investigated by evaluating the effect of PTK7 expression on DFS in the treatment cohorts defined earlier. For patients with low LNT PTK7 expression, DFS differences in A versus C and in B versus C were not significant

(Figure 2A and B). In patients with high PTK7 expression, the difference between cohort A and cohort C was borderline significant (P=0.060; Figure 2C), whereas a significant difference between cohorts B and C was observed (3-year DFS: 95.2% versus 20.8%; P=0.001; Figure 2D).

Chemotherapy selection could have been affected by differences in clinicopathological variables (Table 1; eg, more high-risk patients in cohort B). However, in multivariate analysis, hormone receptor status or histological type did not enter the models, so the large difference between cohorts B and C persisted in high-PTK7 patients.

Discussion
The current study of patients with primary BC is the first to investigate associations of PTK7 mRNA expression in both PTT and axillary LNT with clinicopathological parameters, as well as the effect of PTK7 mRNA expression on survival according to adjuvant chemotherapy subcohorts.

PTK7 is thought to play an important role in migration, invasion, angiogenesis, cell proliferation, and cell apoptosis.[19,29] In previous work, we showed that PTK7 is able to transform NIH3T3 cells and is a potential mediator of motility and invasiveness of BC cells.[26] Furthermore, we demonstrated that inhibition of endogenous PTK7 signaling in highly invasive BC cells by a dominant negative mutation or by siRNA silencing results in loss of capacity to invade the surrounding matrix and to migrate into the wound area. Our clinical data are consistent with this biological role, suggesting that the more PTK7 is expressed, the more aggressive the tumor cells are and the more they tend to migrate to the next destination (from PTT to LNT and from LNT to distant metastasis). PTK7 is upregulated in many human cancers (eg, lung cancer,[20] gastric cancer,[22] colon cancer,[23,25] AML,[24] and liposarcoma[21]); however, the prognostic significance of higher PTK7 expression may depend on cancer entity. In gastric cancer, for example, higher PTK7 expression has been linked to improved survival, whereas AML or liposarcoma patients with high tumor PTK7 had poorer outcomes.

This study detected a significant positive correlation between PTK7 expression in PTT and LNT, as well as a positive correlation between PTK7 expression level and nodal involvement (ie, tumor load). High LNT PTK7 expression was associated with substantially poorer DFS in the whole collective, with a hazard ratio of 3.76.

PTK7 expression is thought to be associated with resistance to anthracycline chemotherapy.[24] To investigate this question, our study has focused on survival, taking LNT PTK7 status and type of chemotherapy into account. PTK7 expression in LNT was associated with poorer DFS in

Table 2 Clinical parameters of patients with primary breast cancer tissue (PTT) and lymph node tissue (LNT) displaying low or high PTK7 expression (binary variable)

	PTK7 expression in PTT				PTK7 expression in LNT			
	Total	Low	High	P-value	Total	Low	High	P-value
Age, years								1.000
≤50	40	17 (42.5%)	23 (57.5%)		40	20 (50.0%)	20 (50.0%)	
>50	77	49 (63.6%)	28 (36.4%)	0.033	68	34 (50.0%)	34 (50.0%)	
Tumor size								0.640
pT1	40	21 (52.5%)	19 (47.5%)		36	21 (58.3%)	15 (41.7%)	
pT2	59	34 (57.6%)	25 (42.4%)		56	26 (46.4%)	30 (53.6%)	
pT3	11	7 (63.6%)	4 (36.4%)		10	4 (40.0%)	6 (60.00%)	
pT4	7	4 (57.1%)	3 (42.9%)	0.915	6	3 (50.0%)	3 (50.0%)	
Histological type								0.360
Invasive ductal	96	51 (53.1%)	45 (46.9%)		90	44 (48.9%)	46 (51.1%)	
Invasive lobular	15	11 (73.3%)	4 (26.7%)		12	8 (66.7%)	4 (33.3%)	
Other	6	4 (66.7%)	2 (33.3%)	0.297	6	2 (33.3%)	4 (66.7%)	
Lymph node status								<0.001
N0	29	18 (62.1%)	11 (37.9%)		28	20 (71.4%)	8 (28.6%)	
N1	44	23 (52.3%)	21 (47.7%)		41	25 (61.0%)	16 (39.0%)	
N2	27	14 (51.9%)	13 (48.1%)		23	8 (34.8%)	15 (65.2%)	
N3	17	11 (64.7%)	6 (35.3%)	0.708	16	1 (6.2%)	15 (93.8%)	
Estrogen receptor status								0.067
Positive	90	52 (57.8%)	38 (42.2%)		83	46 (55.4%)	37 (44.6%)	
Negative	27	14 (51.9%)	13 (48.1%)	0.660	25	8 (32.0%)	17 (68.0%)	
Human epidermal growth factor receptor 2								0.058
Negative	92	56 (60.9%)	36 (39.1%)		83	46 (55.4%)	37 (44.6%)	
Positive	23	10 (43.5%)	13 (56.5%)	0.160	23	7 (30.4%)	16 (69.6%)	
Histological grading								0.479
G1	9	5 (55.6%)	4 (44.4%)		9	6 (66.7%)	3 (33.3%)	
G2	70	41 (58.6%)	29 (41.4%)		65	33 (50.8%)	32 (49.2%)	
G3	38	20 (52.6%)	18 (47.4%)	0.837	34	15 (44.1%)	19 (55.9%)	
Chemotherapy type								0.348
Cohort A	19	12 (63.2%)	7 (36.8%)		17	6 (35.3%)	11 (64.7%)	
Cohort B	50	25 (50.5%)	25 (50.0%)		50	25 (50.0%)	25 (50.0%)	
Cohort C	45	26 (57.8%)	19 (36.8%)	0.561	39	22 (56.4%)	17 (43.6%)	

Notes: Cohort A: patients receiving exclusively anthracycline-based chemotherapy. Cohort B: patients receiving chemotherapy, including agents other than anthracyclines (eg, taxane-based and others). Cohort C: patients receiving no chemotherapy.

patients receiving no chemotherapy, but not in those receiving chemotherapy.

In our collective, high LNT PTK7 expression was associated with a significantly poorer DFS in patients receiving no chemotherapy, but not in those receiving chemotherapy.

In patients with high LNT PTK7 expression (but not in those with low LNT PTK7 expression), DFS in cohort A (anthracyclines only) was not significantly better than in the no-chemotherapy cohort (C) but was much better in cohort B (chemotherapy not limited to anthracyclines) than in cohort C. Taken together, these results are consistent with previous findings in AML, suggesting an association between high PTK7 and anthracycline resistance and with the interpretation that chemotherapy regimens including agents other

than anthracyclines are particularly effective in high-PTK7 patients. In a recent article,[30] we evaluated the effect of PTK7 expression measured by immunohistochemistry in triple-negative BC and observed a (nonsignificant) trend toward poorer survival of patients with PTK7-positive tumors treated with anthracycline-based chemotherapy (ie, consistent with the hypothesis of drug resistance).

To date, little progress has been made in the field of predictive biomarkers for chemotherapy benefit in BC. As a consequence, a vast majority of patients considered to be at moderate or high risk for relapse are treated with those cytotoxic agents viewed as the most active; namely, anthracyclines and taxanes, both of which are associated with significant adverse effects. Unfortunately, resistance remains a significant drawback to clinical success in chemotherapy, as in other
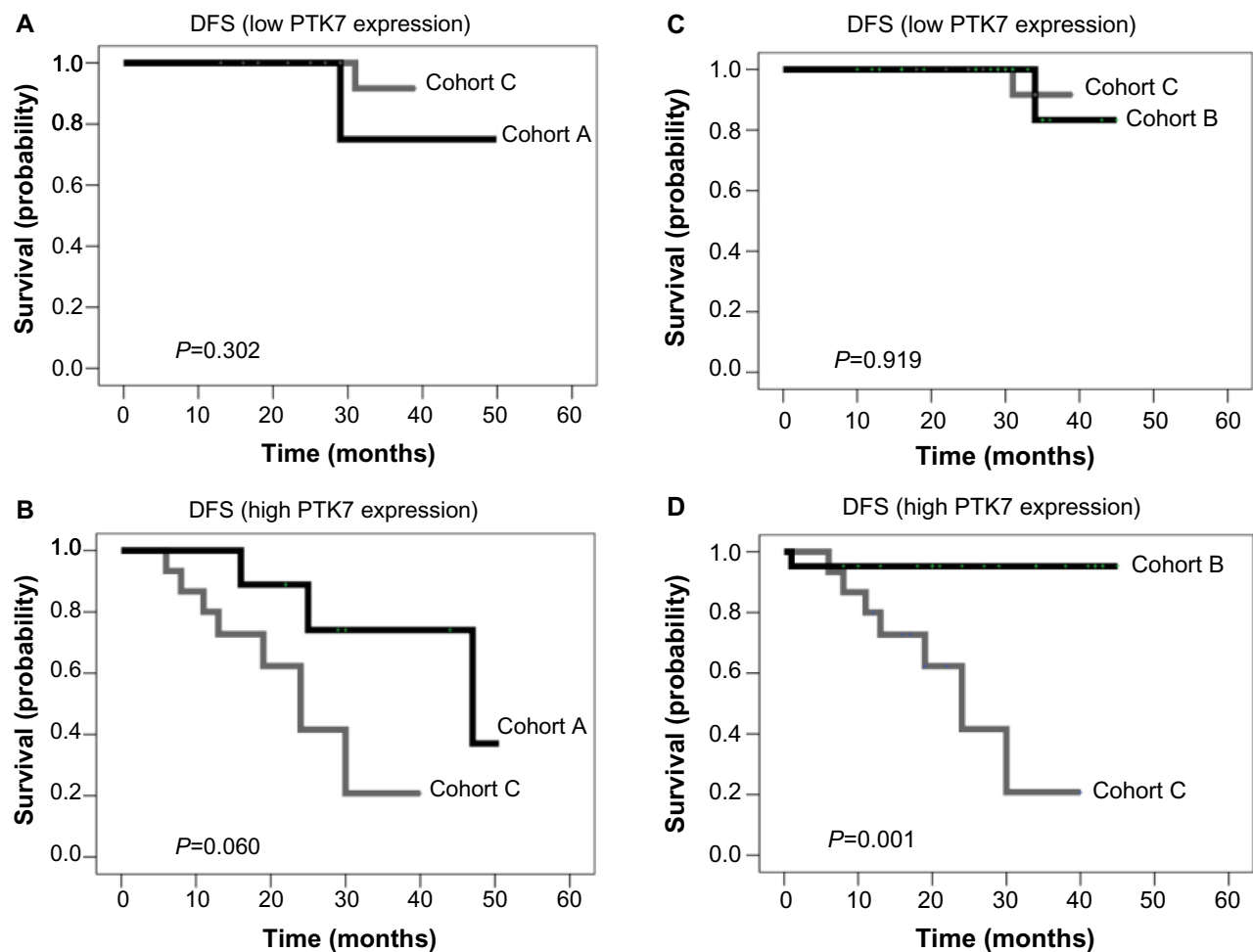
Figure 2 Kaplan–Meier curves for disease-free survival (DFS) distributed in therapy cohorts and protein tyrosine kinase (PTK) expression. (**A**) DFS in patients with low PTK7 expression treated with only anthracycline-based chemotherapy (cohort A) versus no chemotherapy (cohort C). (**B**) DFS in patients with high PTK7 expression treated with only anthracycline-based chemotherapy (cohort A) versus no chemotherapy (cohort C). (**C**) DFS in patients with low PTK7 expression treated with taxane-based/other chemotherapy (cohort B) versus no chemotherapy (cohort C). (**D**) DFS in patients with high PTK7 expression treated with taxane-based/other chemotherapy (cohort B) versus no chemotherapy (cohort C).

systemic therapy concepts. Therefore, identification of new markers with a predictive value regarding chemotherapy response can play a key role in the development of individualized treatment strategies to further improve efficacy and minimize adverse effects.

Conclusive recommendations from the current study are not yet possible because of weaknesses such as a nonstandardized detection assay, retrospective trial design, nonrandomized treatment groups, and limited sample size. In particular, the results should not be interpreted as a recommendation on which patients should receive adjuvant chemotherapy or a particular kind of chemotherapy. However, the results are certainly hypothesis-generating and warrant validation in a larger BC collective, particularly in view of the independent biological and medical evidence for a predictive role of PTK7 in chemotherapy response, and particularly in anthracycline resistance.

Conclusion

To the best of our knowledge, this is the first report using RT-PCR for investigating the relationship between PTK7 expression and outcome in BC specifically looking at anthracycline-based chemotherapy. High PTK7 expression in LNT is associated with high tumor load and with poorer DFS in patients with no chemotherapy or only-anthracycline-based adjuvant chemotherapy, but not in patients treated by taxane-based chemotherapy. Provided these findings can be independently validated, PTK7 may be an important prognostic and predictive biomarker. Further strategies for targeting PTK7 may thus offer novel therapeutic opportunities in BC treatments.

Author contributions

BA provided the concept and design; recruited patients and collected tissue; provided experimental and data analysis and interpretation, critical revision, and final approval; and wrote the first draft of the manuscript and provided comments. AG provided experimental and data analysis and interpretation, critical revision, and final approval and reviewed the draft and provided comments. WE provided the concept and design, recruited patients and collected tissue, and performed experimental and data analysis and interpretation, as well as critical revision, and provided comments. REK provided statistical analysis, data analysis and interpretation, critical revision, and review and comments on manuscript development, as well as final approval. BH provided experimental and data analysis and interpretation, critical revision, reviewed the draft, and provided comments. PK provided concept and design, experimental and data analysis and interpretation, critical revision, and final approval and reviewed the draft and provided comments. AU provided experimental and data analysis and interpretation, critical revision and final approval and reviewed the draft and provided comments. NH provided data analysis and interpretation and critical revision, reviewed and provided comments on manuscript development, and provided final approval.

Disclosure

PK and AU are listed as inventors on the European Patent Application no 10075169.2, "PTK-7 Protein Involved in Breast Cancer." The other authors report no conflicts of interest in this work.

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