

Impact of Nuclear Oestrogen Receptor Beta Expression in Breast Cancer Patients Undergoing Neoadjuvant Chemotherapy

Auswirkungen der nuklearen Östrogenrezeptor-Beta-Expression auf mit neoadjuvanter Chemotherapie behandelte Brustkrebspatientinnen



Authors

Florian Heitz^{1,9}, Sherko Kümmel², Bianca Lederer³, Christine Solbach⁴, Knut Engels⁵, Beyhan Ataseven^{1,12}, Bruno Sinn^{6,7}, Jens Uwe Blohmer⁹, Carsten Denkert^{6,7,8}, Jana Barinoff⁹, Annette Fisseler-Eckhoff¹⁰, Sibylle Loibl^{3,11}

Affiliations

- 1 Department of Gynaecology and Gynaecologic Oncology, Kliniken Essen-Mitte, Evangelische HuysSENS-Stiftung, Essen, Germany
- 2 Breast Unit, Kliniken Essen-Mitte, Evangelische HuysSENS-Stiftung, Essen, Germany
- 3 German Breast Group (GBG), Neu-Isenburg, Germany
- 4 Department of Gynaecology and Obstetrics, Goethe University, Frankfurt, Germany
- 5 Center for Pathology, Cytology and Molecular Pathology, Neuss, Germany
- 6 Institute of Pathology, Charité University, Berlin, Germany
- 7 German Cancer Consortium (DKTK), Berlin, Germany
- 8 Institut für Pathologie, UKGM – Universitätsklinikum Marburg, Philipps-Universität Marburg, Marburg, Germany
- 9 Department of Gynaecology and Gynaecologic Oncology, Charité University, Berlin, Germany
- 10 Department of Pathology, Dr. Horst Schmidt Kliniken Wiesbaden, Wiesbaden, Germany
- 11 Senologic Oncology, Düsseldorf, Germany
- 12 Department of Obstetrics and Gynaecology, University Hospital, LMU München, München, Germany

Key words

breast cancer, oestrogen receptor beta, breast cancer subtypes, neoadjuvant chemotherapy

Schlüsselwörter

Brustkrebs, Östrogenrezeptor beta, Brustkrebssubtypen, neoadjuvante Chemotherapie

received 23.3.2019

revised 16.7.2019

accepted 31.7.2019

Bibliography

DOI <https://doi.org/10.1055/a-0987-9898>
Geburtsh Frauenheilk 2019; 79: 1110–1117 © Georg Thieme Verlag KG Stuttgart · New York | ISSN 0016-5751

Correspondence

Florian Heitz, MD
Department of Gynaecology and Gynaecologic Oncology, Evangelische HuysSENS-Stiftung, Kliniken-Essen-Mitte Henricistraße 92, 45136 Essen, Germany
florian.heitz@gmx.net

Supporting Information:
<https://doi.org/10.1055/a-0987-9898>

ABSTRACT

Introduction Oestrogen receptor beta (ER- β) is abundantly expressed in breast cancer (BC), but its impact on neoadjuvant chemotherapy outcome is unknown.

Patients and Methods Patients treated in the neoadjuvant GeparTrio trial with available tissue for immunohistochemical analyses were included. Nuclear ER- β expression was correlated with clinico-pathologic characteristics. The impact of its expression on pathological complete response (pCR [ypT0/ypN0]) and survival was determined.

Results Samples of 570 patients were available. Low nuclear ER- β expression (IRS < 9) was observed in 48.4% of hormone receptor positive and 58.6% of hormone receptor negative tumours. Low nuclear ER- β expression was associated with higher pCR rates compared to high nuclear ER- β expression (16.1% vs. 4.7%, $p = 0.026$). Low ER- β expression was no independent predictor of pCR in multivariate analyses. Disease-free and overall survival were not statistically different between patients with high and low nuclear ER- β expression. Triple-negative BCs showed low nuclear ER- β expression in 57.7%, and pCR rates were 27.1% and 0% ($p = 0.23$) in low and high ER- β expressing tumours, respectively.

Conclusion Low ER- β expression is associated with improved pCR rates in univariate analyses. However multivariate analyses and survival analyses do not indicate an impact of ER- β on survival in patients undergoing neoadjuvant chemotherapy. Further examination of ER- β as predictor for endocrine therapy might be of value.

ZUSAMMENFASSUNG

Einleitung Bei Brustkrebs wird der Östrogenrezeptor beta (ER- β) reichlich exprimiert, aber die Auswirkungen dieser Expression auf das Outcome nach neoadjuvanter Chemotherapie sind noch unbekannt.

Patientinnen und Methoden In dieser Analyse wurden neoadjuvant behandelte Patientinnen mit Mammakarzinom aus der GeparTrio-Studie aufgenommen. Die jeweilige nukleare ER- β -Expression wurde mit den klinischen und histologischen Merkmalen der Patientinnen korreliert. Die Auswirkungen der ER- β -Expression auf die pathologische Komplettremission (pCR [ypT0/ypN0]) sowie die Überlebensraten wurden analysiert.

Ergebnisse Insgesamt standen Gewebeproben von 570 Brustkrebspatientinnen für die Analyse zur Verfügung. Eine niedrige nukleare ER- β -Expression (IRS <9) wurde bei

48,4% der hormonrezeptorpositiven und 58,6% der hormonrezeptornegativen Tumoren festgestellt. Verglichen mit einer hohen nuklearen ER- β -Expression war eine niedrige nukleare ER- β -Expression mit höheren pCR-Raten assoziiert (16,1 vs. 4,7%, $p = 0,026$). Allerdings zeigten multivariate Analysen, dass eine niedrige ER- β -Expression keinen unabhängigen Prädiktor für die pCR darstellte. Es gab keine statistischen Unterschiede zwischen den Raten beim krankheitsfreien Überleben und den Gesamtüberlebensraten von Patientinnen mit hoher und Patientinnen mit niedriger nuklearer ER- β -Expression. Bei 57,7% der Patientinnen mit triple-negativem Brustkrebs war die nukleare ER- β -Expression niedrig, und die pCR-Raten waren 27,1 resp. 0% ($p = 0,23$) für Tumoren mit niedriger bzw. hoher ER- β -Expression.

Schlussfolgerung Bei der statistisch-univariaten Analyse war eine niedrige ER- β -Expression mit besseren pCR-Raten assoziiert. Allerdings wiesen weder multivariate Analysen noch die Analyse der Überlebensraten darauf hin, dass die ER- β -Expression Auswirkungen auf das Überleben von mit neoadjuvanter Chemotherapie behandelten Patientinnen hat. Weitere Untersuchungen von ER- β als ein möglicher Prädiktor für die endokrine Therapie könnten von Nutzen sein.

Introduction

Breast cancer (BC) is recognized as a heterogeneous disease exhibiting substantial differences with regard to biological behaviour [1] and requiring distinct therapeutic interventions [2]. Expression of steroid hormone receptors (HR) such as the oestrogen receptor (ER- α) and progesterone receptor (PgR) in addition to ErbB-2/human epidermal growth factor receptor 2 (HER2) are determined in all BC specimens. Gene-expression profiles are well-established biomarkers indicating the likelihood of relapse and predicting the success of further treatment, using endocrine therapy in patients bearing HR expressing tumours [3] and HER2 inhibitors in patients with HER2 overexpressing tumours [4–6].

In addition to the routinely assessed expression of ER- α , a second ER isoform ER- β was discovered in the 1990s [7] and is expressed in both, normal and neoplastic human breast tissue [8–10]. ER- β is co-expressed with ER- α in ~60% of primary BC and it was shown that ER- β expression is apparent in 50–80% of all ER- α negative tumours [11–14] and in approximately 44–55% of triple-negative BC (TNBC) [15, 16]. Although conflicting results with respect to clinical importance have been reported [13], expression of ER- β is generally associated with good prognosis in ER- α expressing tumours [9, 17–20] as well as in TNBC, and it was correlated to activity of tamoxifen [12], thus being thought to be a tumour-suppressor. TNBC which accounts for 11–20% of all BCs is defined by lack of expression of ER- α and PgR as well as HER2 [21]. At present, chemotherapy remains the mainstay of treatment in TNBC which is associated with poor long-term outcome compared with other breast cancer subtypes, particularly in patients without pathological complete response (pCR) to neoadjuvant therapy [22–24].

The aim of this study was to evaluate the ER- β expression rate and its association with

1. clinico-pathologic variables,
2. pCR rate after neoadjuvant chemotherapy and
3. progression-free and overall survival in patients treated in the neoadjuvant German Breast Group (GBG) GEPARTRIO trial, with tissue available for ER- β expression analyses [25, 26].

Materials and Methods

Data and tissue for the present analyses were derived from patients treated in the GBG “GEPARTRIO pilot study” and the GBG “GEPARTRIO trial”. Details of the GEPARTRIO pilot study and of the GBG GeparTrio trial are described elsewhere [25, 26]. Briefly, before chemotherapy, BC diagnosis had to be confirmed histologically from a core biopsy specimen and those samples were collected prospectively for translational research. 2357 registered patients in GBG “GEPARTRIO pilot study” ($n = 285$) and the GBG “GEPARTRIO trial” ($n = 2072$) were available in the data pool, tissue was available in 570 patients (24.2%). Of the 108 participating study centres, 74 centres provided tumour samples. Tumour samples for this analysis were coming to equal proportions from patients who had a response to the first 2 cycles of TAC and patients who did not respond to the first two cycles of TAC chemotherapy ($p = 0,24$). Patients were scheduled to receive two cycles of neoadjuvant chemotherapy consisting of docetaxel (T) 75 mg/m², doxorubicin (A) 50 mg/m², and cyclophosphamide (C) 500 mg/m². In case of sonographic response, patients were classified as responders and TAC treatment continued for four or six additional cycles. Non-responders were randomly assigned to either four additional cycles of TAC or four cycles of vinorelbine 25 mg/m² on days 1 and

8 plus capecitabine 1000 mg/m² on days 1 to 14 of a three-week cycle. pCR was defined as no invasive residual tumour in the breast and axilla (ypT0/ypN0) [27]. Patients received adjuvant endocrine therapy and radiotherapy according to the current national guidelines [28]. The GEPARTRIO protocol did not include trastuzumab as it was not standard-of-care at this time.

Histopathological examination

Primary diagnosis including tumour type and tumour grade were extracted from pathology reports, which were collected in the clinical study database. Tumours were graded according to the Bloom-Richardson grading modified by Elston and Ellis [29]. Lymph node status was assessed clinically and histopathologically at primary diagnosis.

Construction of tissue microarrays (TMA)

All BC core biopsies were histopathologically reviewed on hematoxylin and eosin (H&E) stained sections and representative tumour areas were selected for TMA construction. The TMA was constructed using a tissue micro-arrayer (Beecher Instruments; Woodland, USA). Pre-surgical core biopsies were placed vertically in the TMA acceptor block.

Immunohistochemical staining and interpretation

Immunohistochemical staining for the ER- β antibody (clone: 14C8; BioGenex; dilution 1 : 150) was performed using the peroxidase/DAB detection system as secondary antibody and for colour developing (Dako REAL™ Detection System, Peroxidase/DAB+, rabbit/mouse; Dako, Glostrup, Denmark). 14C8 is raised against the N-terminus of ER- β and has been shown to produce a consistently specific, strong nuclear expression like the C-terminal recognising antibody PPG5/10, but is capable to detect all ER- β isoforms [14]. Control tissue was included on the TMAs and was used for all staining runs. Immunohistochemical staining was evaluated by a board-certified pathologist (BS). TMAs were evaluated as virtual slides using the VMScope Slide explorer (VMScope, Berlin, Germany). For evaluation, an immune-reactivity scoring system (IRS) was used. The percentage of stained tumour cells was divided into five classes: 0 = 0% positive tumour cells, 1 = 1–10% positive tumour cells, 2 = 11–50% positive tumour cells, 3 = 51–80% positive tumour cells, 4 = > 80% positive tumour cells. The intensity of staining was scored as follows: 0 = negative, 1 = weak, 2 = moderate, 3 = strong. Both values were multiplied resulting in an IRS between 0 and 12 which was used for final analysis [30].

Subtyping was performed using ER- α and PgR at the Institute of Pathology, Charité University Hospital, Berlin, Germany; and HER2, which was tested centrally in all cases. HER2 overexpression required either immunohistochemical staining of 3+ or positivity by fluorescence in situ hybridization (FISH) technique. In case of an IHC2+ score confirmatory FISH testing was required. The following antibodies were used: rabbit monoclonal antibody against human ER- α (clone SP1, Neomarkers, 1 : 50); mouse monoclonal antibody against human progesterone PgR (clone PgR 636, Dako, 1 : 50); rabbit polyclonal antibody against human HER2 (Hercept-Test™ antibody, Dako, 1 : 500); ER- α and PgR immunohistochemistry was scored positive if at least 10% of tumour cell nuclei showed a staining signal. In case of conflicting results, the central

measurement was used. HR positivity was defined as ER- α and/or PgR positive. For this study, four patient groups based on the following subtypes were formed:

1. TNBC: ER- α -, PgR- and HER2-negative (TNBC);
2. HR+/HER2+: ER- α -positive and HER2-positive,
3. HR-/HER2+: ER- α -negative, any PgR and HER2-positive and
4. HR+/HER2-: ER- α - and/or PgR-positive and HER2-negative BC.

Statistics

To obtain a higher degree of objectivity, cut-off point determinations of the ordinaly assessed ER- β expression were conducted using the publicly available cut-off finder software [31]. The outcome for cut-off optimisation was pCR (ypT0/ypN0). For baseline characteristics, descriptive statistics were used. The correlation between ER- β expression and pCR rates in different subtypes were calculated using χ^2 test. Multivariate logistic regression models were used to determine the impact of ER- β expression on pCR-rates and were adjusted for age (median split: 51 years), clinical tumour stage (cT1–3 vs. > 3), clinical nodal status (cN > 0 vs. cN0), grade (1 + 2 vs. 3), histology (lobular/others vs. ductal) and for molecular subtypes (HR+/HER2- vs. HR+/HER2+ vs. HR-/HER2+ vs. TNBC). Disease-free survival was calculated in months from the date of diagnosis until the date of first relapse or death for each patient. Disease-free survival (DFS) time was censored at the date of last follow-up if no recurrence or death was observed. Overall survival (OS) time was censored at the date of last follow-up if no death was observed. DFS and OS survival probabilities were estimated using the Kaplan-Meier product limit method. Log-rank tests were used to calculate the survival functions. Cox proportional hazards models used for uni- and multivariate analyses adjusting for age, clinical tumour stage, clinical nodal status, grade, histology, breast-cancer subtypes and ER- β expression. P-values ≤ 0.05 were considered statistically significant.

For statistical analysis of data, the software package SPSS 22.0 was used. All tests were two-sided.

Results

Overall, 2357 patients were included into the GEPARTRIO study and samples for immunohistochemical analyses were available in 570 patients (24.2%). Differences between the baseline characteristics of patients included to the GEPARTRIO trial and the subset of patients for whom samples for immunohistochemistry were available, are presented in Supplement Table S1. Median age of patients included to the present analyses was 51 years, 66.7% of patients had cT2 tumours and 45.4% no lymph node involvement. With respect to the predefined BC subtypes, 57.3% of patients were HR+/HER2-, 14.8% HR+/HER2+, 8.4% HR-/HER2+ and 19.5% had TNBC.

Immunohistochemical determination of ER- β and cut-off definition

Representative pictures of nuclear ER- β staining specimens are presented in Supplement Figure S1. Since cytoplasmic staining for ER- β was generally weak and no cut-off for expression predicted pCR, we focused exclusively on nuclear staining. As shown in Supplement Figure S2 the distribution of nuclear ER- β staining

► **Table 1** High versus low nuclear ER-β expression in breast cancer subtypes and pCR rates depending on nuclear ER-β expression in different breast cancer subtypes.

		Overall n = 566	HR+/HER2- n = 324	HR+/HER2+ n = 84	HR-/HER2+ n = 47	TNBC n = 111	p-value
ER-β	low	511 (89.7%)	278 (85.8%)	68 (81.0%)	45 (95.7%)	107 (96.4%)	0.001
	high	55 (10.3%)	46 (14.2%)	16 (19.0%)	2 (4.3%)	4 (3.6%)	
pCR low ER-β	yes	111 (19.5%)	27 (8.3%)	9 (9.6%)	16 (34.0%)	29 (26.1%)	0.026
	no	395 (69.8%)	251 (77.5%)	59 (70.2%)	29 (61.4%)	74 (70.3%)	
pCR high ER-β	yes	6 (1.2%)	1 (0.3%)	2 (2.4%)	1 (2.1%)	0	0.092
	no	54 (9.5%)	45 (13.9%)	14 (16.7%)	1 (2.1%)	8 (3.6%)	

TNBC: tumours negative for ER-α, PgR and HER2; HR+/HER2+: ER-α-positive/HER2-positive; HR-/HER2+: ER-α-negative and/or PR negative/HER2-positive; HR+/HER2-: ER-α and/or PR positive and HER2-negative

was relatively homogenous between the distinct BC subtypes. When including all BC subtypes in the analysis, cut-off-finder software [31] provided an IRS 9 for nuclear ER-β staining as optimal cut-off to predict pCR (OR = 0.94, 95% CI 0.87–1.01, $p = 0.028$; Supplement Figure S3). As a special focus was shed on TNBC, a separate cut-off was generated with the cut-off-finder software for this group and provided IRS 5 for nuclear ER-β staining (no further data on the cut-off determination are shown).

Correlation between BC subtypes and ER-β expression

Using the above-determined cut-off value of IRS 9, there were substantial differences between BC subtypes and levels of ER-β expression (► **Table 1**). High ER-β expression was more frequent in the hormone receptor positive subtypes (14.2% in HR+/HER2- and 19.1% in HR+/HER2+), compared to hormone receptor negative subtypes (4.3% in HR-/HER2+ and 3.6% in TNBC) ($p = 0.001$), respectively, indicating a positive correlation between ER-α and ER-β. In patients with invasive ductal carcinoma and HR+/HER2- ($n = 259$), HR+/HER2+ ($n = 73$), HR-/HER2+ ($n = 42$) and TNBC ($n = 91$), high ER-β expression was found in 12.7, 15.1, 4.8 and 3.3%. That finding contrasts to high ER-β expression in patients with invasive lobular carcinoma, which was found in 29.5% of HR+/HER2- ($n = 44$); $p = 0.002$, 61.4% of HR+/HER2- ($n = 7$); $p = 0.001$, 0% of HR-/HER2+ ($n = 2$); $p = 0.883$ and 0% of TNBC ($n = 3$); $p = 0.822$, respectively. Further analyses were conducted in TNBC with the predefined cut-off value of IRS 5, leaving 48 tumours (43.2%) with low and 63 tumours (56.8%) with high ER-β expression. As shown in Supplement Table S2 no significant associations between low and high ER-β expression were found with respect to patient and tumour characteristics. Using the cut-off value of IRS 5, lower frequency of high ER-β expression (56.8%) was observed in TNBC compared to non-TNBC (67.5%), $p = 0.033$.

Predictive impact of ER-β expression on pCR

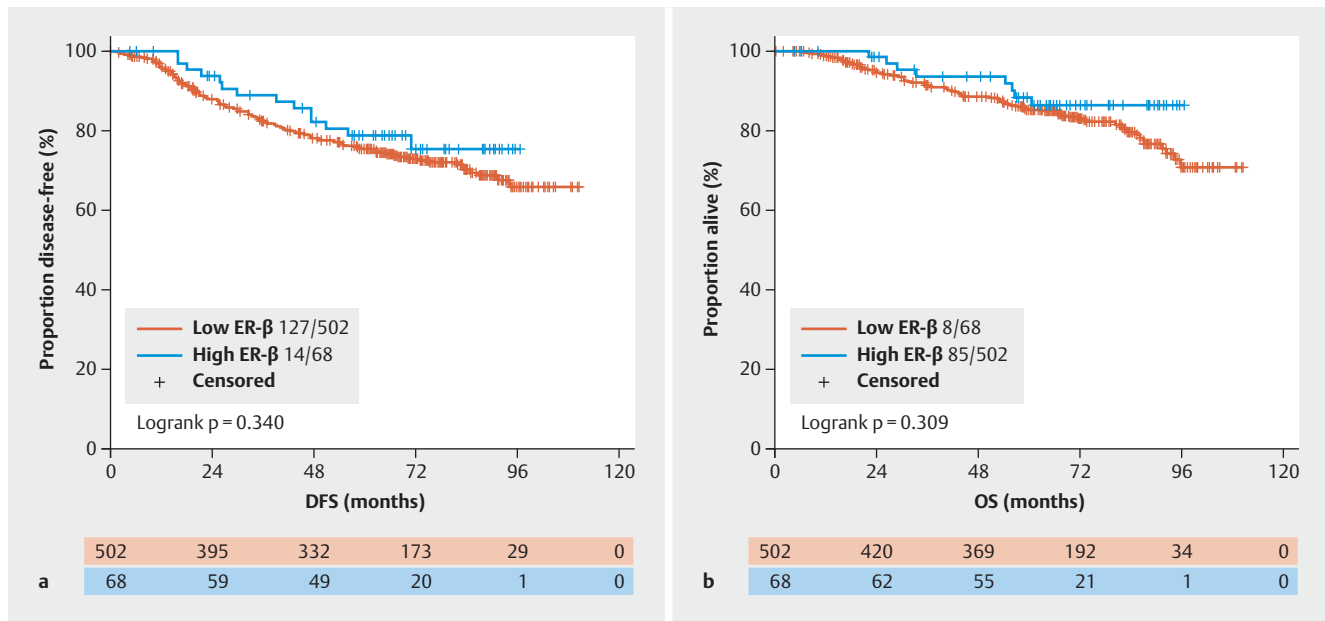
As shown in ► **Table 1**, low nuclear ER-β expression was associated with higher rates of pCR, compared to high nuclear ER-β expression (16.1 vs. 4.7%, $p = 0.026$). Within the subtypes, patients with HR+/HER2- and low ER-β expression were more likely to achieve pCR compared to high ER-β expression (9.7 vs. 2.2%, $p = 0.092$), although this was not statistically different. Within the other BC

subtypes, no significant differences were observed with respect to pCR rates. ► **Table 2** displays the multivariate model of categorized patient and tumour characteristics, showing that low ER-β expression has no independent predictive value for pCR, when including patients and tumour characteristics and especially all breast cancer subtypes (HR+/HER2-, HR+/HER2+, HR-/HER2+, TNBC). In subtype specific analyses, using the specifically defined cut-off value of IRS 5, comparing TNBC patients with non-TNBC patients, low and high ER-β expression led to pCR rates of 33.3

► **Table 2** Multivariate logistic regression model predicting pCR (ypT0/ypN0) based on clinicopathologic variables and immunohistochemistry (IHC).

	Parameter	ypT0/ypN0 OR (95% CI; p-value)
Age (years)	≤ 51	1
	> 51	0.48 (0.28–0.84; 0.011)
Clinical tumour stage	1–3	1
	> 3	1.15 (0.50–2.63; 0.747)
Clinical nodal stage	LN 0	1
	LN > 0	1.26 (0.72–2.20; 0.413)
Grade	1/2	1
	3	2.71 (1.54–4.74; 0.001)
Histology	ductal invasive	1
	lobular invasive/other	0.90 (0.42–1.94; 0.0790)
Breast cancer subtype	HR+/HER2-	1
	HR+/HER2+	1.03 (0.40–2.67; 0.951)
	HR-/HER2+	4.71 (2.11–10.50; < 0.001)
	TNBC	3.05 (1.60–5.80; 0.001)
IHC cut-off for nuclear ER-β expression	< IRS 9	1
	≥ IRS 9	0.34 (0.10–1.18; 0.090)

LN: lymph nodes; OR: odds ratio; CI: confidence interval; IRS: immune reactivity score; ypT0/ypN0: pathological complete response



► **Fig. 1** Disease free (a) and overall survival (b) of all patients evaluated for nuclear ER- β expression separated by cutoff of IRS 9.

and 20.6% in TNBC ($p=0.19$) and 14.9 and 11.1% ($p=0.29$) in non-TNBC, respectively. In a further multivariate logistic regression model including patients with TNBC exclusively, no patient or tumour characteristics were found to be significantly associated with pCR (data not shown).

Prognostic impact of ER- β expression

After a median follow-up of 66.7 months (range 66.1–67.4) 141 patients had a DFS event and 93 patients had died. No correlation of ER- β expression and DFS or OS was observed overall (► **Fig. 1**) or stratified by pCR (Supplement Figure S4). These data were confirmed in multivariate Cox regression analyses (► **Table 3**). In further univariate analyses determining the impact of high vs. low nuclear ER- β expression in subtypes stratified by pCR, again no significant prognostic effect was found neither on DFS nor on OS (data not shown).

Regarding DFS and OS analysis in TNBC (using the cut-off value of IRS 5) it was shown that there were no significant differences between low and high ER- β expression. In addition, there were no differences in OS and DFS comparing low and high ER- β expression in TNBC patients with pCR or without pCR (data not shown).

Discussion

The predictive and prognostic impact of nuclear ER- β expression in primary breast cancer (BC) of patients receiving neoadjuvant chemotherapy in a phase III trial was analysed in the present study. It was shown that low nuclear ER- β expression was generally associated with higher rates of pCR compared to high nuclear ER- β expression. However, low nuclear ER- β expression was a non-significant predictor of pCR in multivariate analyses and different levels of nuclear ER- β expression did not have any prognostic im-

act neither in the whole cohort, nor in any of the analysed subgroups.

Consistent with earlier reports, cytoplasmatic staining of ER- β using the 14C8 antibody [14] was generally weak, thus further analyses were restricted to nuclear expression. The observed level of nuclear ER- β expression in our study was comparatively high [15, 32], but does not contrast to other reports [33–36]. Basically, nuclear ER- β expression was homogeneously distributed in the different BC subtypes, which is in line with earlier reports [15, 32]. Nevertheless, ER- β seems to be co-expressed more frequently with ER- α [35, 37] and we found a 10% difference in the proportion of ER- β expression in favour of ER- α positive vs. ER- α negative tumours. No correlation was found between HER2 and ER- β expression, which has been described earlier [38]. ER- β has an anti-proliferative function in ER- α positive disease with improved response to tamoxifen treatment and anti-proliferative effects in vitro [32]. Those data are supported by the fact that in ER- α positive, high-grade tumours (G3) were more likely to show low ER- β expression, whereas tumours with G1 and G2 showed high ER- β expression [38, 39]. Patients with low ER- β expression, especially in ER- α and/or PgR positive and HER2-negative subtype, showed a trend for higher rates of pCR after chemotherapy, which might indicate a proliferative profile and thus higher susceptibility for chemotherapy. As a result, ER- β expression in ER- α and/or PgR positive and HER2-negative disease might help to predict for pCR or to stratify patients in future clinical trials. As expected, pCR rates were high in ER- α -negative and TNBC [23] and were even higher in tumours with low ER- β expression. Approximately 40% of patients with hormone receptor negative BC had high ER- β expression, which was associated with a lower probability of pCR. Noteworthy, none of the four patients with TNBC and high ER- β expression (3.6% of all TNBC) showed a pCR. The latter both groups might be target populations for further research of agents

► **Table 3** Multivariate Cox regression model with prognostic information adjusted for clinicopathologic variables and immunohistochemistry (IHC).

	Parameter	DFS HR (95% CI; p-value)	OS HR (95% CI; p-value)
Age (years)	≤ 51	1	1
	> 51	1.04 (0.74–1.46; 0.838)	0.99 (0.65–1.52; 0.968)
Clinical tumour stage	1–3	1	1
	> 3	1.89 (1.21–2.96; 0.005)	1.70 (0.99–2.93; 0.054)
Clinical nodal stage	LN 0	1	1
	LN > 0	1.46 (1.01–2.10; 0.043)	1.76 (1.11–2.80; 0.017)
Grade	1/2	1	1
	3	1.55 (1.08–2.22; 0.018)	1.25 (0.79–1.95; 0.339)
Histology	ductal invasive	1	1
	lobular invasive/other	1.50 (0.97–2.33; 0.069)	1.39 (0.81–2.38; 0.232)
Breast cancer subtype	HR+/HER2–	1	1
	HR+/HER2+	1.95 (1.22–3.11; 0.005)	1.16 (0.60–2.22; 0.666)
	HR–/HER2+	1.81 (1.00–3.30; 0.052)	1.79 (0.87–3.68; 0.117)
	TNBC	1.64 (1.05–2.55; 0.028)	1.97 (1.16–3.35; 0.012)
IHC cutoff for nuclear ER beta expression	< IRS 9	1	1
	≥ IRS 9	0.77 (0.43–1.39; 0.387)	0.73 (0.33–1.59; 0.423)

IRS: immune-reactivity scoring; LN: lymph nodes; HR: hazard ratio; CI: confidence interval; TNBC: tumours negative for ER- α , PR and HER2

targeting ER- β exclusively. Preclinical data suggest that estradiol reduces the activity of ER- β [40] and clinical data emphasise that ER- β predicts tamoxifen benefit in ER- α negative tumours [12, 41]. Survival outcome was not affected by ER- β expression independent of the pCR status in BC subtypes. This observation seems to be contradictory to the existing literature, as pCR is significantly associated with good prognosis, mainly in highly proliferating tumours [24]. Nevertheless, data shown here are in line with an earlier result derived from the same population indicating that patients with low androgen receptor expressing tumours had a higher chance of achieving a pCR compared to patients with high androgen receptor expressing tumours. In contrast to the recent findings that ER- β expression had no impact on prognosis, survival was better in patients with no pCR and high androgen receptor [42].

Even though ER- β expression analyses did not provide any striking prognostic or predictive information in our cohort of breast cancer patients, it remains unclear whether ER- β might serve as target for the treatment of breast cancer, mainly TNBC. In cell culture of an androgen receptor expressing TNBC cell line, the transfection of ER- β led to reduced cell proliferation, reduced metastatic potential and increased apoptosis. When treating these cell lines with enzalutamide, a more potent anti-androgen, the anti-proliferative effect of ER- β was increased [43]. In another paper it was shown that using a specific ER β antagonist in TNBC breast cancer cell lines lead to decreased IGF2 secretion and proliferation, possibly due to the suppression of the MAPK/PI3K/AKT pathways and IGF2 activation. Drugs specifically targeting ER β and/or MAPK/PI3K/AKT pathway might be possible candidates to treat

TNBC [44]. Moreover, a recent paper described the interplay between ER- β and TP53 and it was shown that tamoxifen enhanced the interaction between mutant TP53 and ER- β , which led to increased apoptosis [45]. Whether these approaches will be of benefit in patients with TNBC is speculative. However, it demonstrates that ER- β is an active compound in breast cancer cells and it is of relevance to further investigate the role of targeting ER- β .

The present study has some limitations. While the dichotomized nuclear expression of ER- β showed promising results when looking at achievement of pCR after NACT, it did not sustain as a predictive factor in all multivariate models. Moreover, the immunohistochemical analyses of a TMA might bear the risk of false negative results compared to the complete core section. However, previous publications had already demonstrated the method to be appropriate [46]. Even though the overall number of tissue samples was relatively high, the breakdown into four BC subtypes lead to small subgroups, limiting the power of the analyses. The information on Ki67 was not collected at the time which could allow for a better classification of BC subtypes. In addition, subtyping based on ER, PgR and HER2 is not 100% concordant with the gene expression profiling; nevertheless, it was shown that an immunohistochemical classification based on conventional markers is clinically relevant and supported by the recent St. Gallen breast conference panel [2]. The strengths of this study were the centrally determined immunohistochemical analyses for ER- α , ER- β , PgR and HER2, and the homogeneously treated population from a prospectively conducted phase III clinical trial.

In conclusion, our study showed that nuclear ER- β expression was homogeneously distributed in different BC subtypes. ER- β ex-

pression was not independently associated with pCR, DFS or OS in any BC subtype. Further examination of the predictive and prognostic role of ER- β in the endocrine treatment of patients with breast cancer might be warranted.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

- [1] Sorlie T, Perou CM, Tibshirani R et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001; 98: 10869–10874
- [2] Coates AS, Winer EP, Goldhirsch A et al. Tailoring therapies—improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. *Ann Oncol* 2015; 26: 1533–1546. doi:10.1093/annonc/mdv221
- [3] Cardoso F, van't Veer LJ, Bogaerts J et al. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *N Engl J Med* 2016; 375: 717–729. doi:10.1056/NEJMoa1602253
- [4] Piccart-Gebhart MJ, Procter M, Leyland-Jones B et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005; 353: 1659–1672
- [5] Goldhirsch A, Wood WC, Gelber RD et al. Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. *Ann Oncol* 2007; 18: 1133–1144
- [6] Romond EH, Perez EA, Bryant J et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005; 353: 1673–1684
- [7] Kuiper GG, Enmark E, Peltö-Huikko M et al. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci U S A* 1996; 93: 5925–5930
- [8] Leygue E, Dotzlaw H, Watson PH et al. Altered estrogen receptor alpha and beta messenger RNA expression during human breast tumorigenesis. *Cancer Res* 1998; 58: 3197–3201
- [9] Mann S, Laucirica R, Carlson N et al. Estrogen receptor beta expression in invasive breast cancer. *Hum Pathol* 2001; 32: 113–118
- [10] Skliris GP, Munot K, Bell SM et al. Reduced expression of oestrogen receptor beta in invasive breast cancer and its re-expression using DNA methyl transferase inhibitors in a cell line model. *J Pathol* 2003; 201: 213–220
- [11] Skliris GP, Leygue E, Curtis-Snell L et al. Expression of oestrogen receptor-beta in oestrogen receptor-alpha negative human breast tumours. *Br J Cancer* 2006; 95: 616–626
- [12] Honma N, Horii R, Iwase T et al. Clinical importance of estrogen receptor-beta evaluation in breast cancer patients treated with adjuvant tamoxifen therapy. *J Clin Oncol* 2008; 26: 3727–3734
- [13] Murphy LC, Watson PH. Is oestrogen receptor-beta a predictor of endocrine therapy responsiveness in human breast cancer? *Endocr Relat Cancer* 2006; 13: 327–334
- [14] Skliris GP, Parkes AT, Limer JL et al. Evaluation of seven oestrogen receptor beta antibodies for immunohistochemistry, western blotting, and flow cytometry in human breast tissue. *J Pathol* 2002; 197: 155–162. doi:10.1002/path.1077
- [15] Novelli F, Milella M, Melucci E et al. A divergent role for estrogen receptor-beta in node-positive and node-negative breast cancer classified according to molecular subtypes: an observational prospective study. *Breast Cancer Res* 2008; 10: R74
- [16] Litwiniuk MM, Roznowski K, Filas V et al. Expression of estrogen receptor beta in the breast carcinoma of BRCA1 mutation carriers. *BMC Cancer* 2008; 8: 100
- [17] Omoto Y, Inoue S, Ogawa S et al. Clinical value of the wild-type estrogen receptor beta expression in breast cancer. *Cancer Lett* 2001; 163: 207–212
- [18] Murphy LC, Leygue E, Niu Y et al. Relationship of coregulator and oestrogen receptor isoform expression to de novo tamoxifen resistance in human breast cancer. *Br J Cancer* 2002; 87: 1411–1416
- [19] Hopp TA, Weiss HL, Parra IS et al. Low levels of estrogen receptor beta protein predict resistance to tamoxifen therapy in breast cancer. *Clin Cancer Res* 2004; 10: 7490–7499
- [20] Myers E, Fleming FJ, Crotty TB et al. Inverse relationship between ER-beta and SRC-1 predicts outcome in endocrine-resistant breast cancer. *Br J Cancer* 2004; 91: 1687–1693
- [21] Gluz O, Liedtke C, Gottschalk N et al. Triple-negative breast cancer—current status and future directions. *Ann Oncol* 2009; 20: 1913–1927
- [22] Dent R, Trudeau M, Pritchard KI et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 2007; 13: 4429–4434
- [23] Cortazar P, Zhang L, Untch M et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet* 2014; 384: 164–172. doi:10.1016/S0140-6736(13)62422-8
- [24] von Minckwitz G, Untch M, Blohmer JU 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: 1796–1804. doi:10.1200/JCO.2011.38.8595
- [25] von Minckwitz G, Kummel S, Vogel P et al. Intensified neoadjuvant chemotherapy in early-responding breast cancer: phase III randomized GeparTrio study. *J Natl Cancer Inst* 2008; 100: 552–562
- [26] von Minckwitz G, Kummel S, Vogel P et al. Neoadjuvant vinorelbine-capecitabine versus docetaxel-doxorubicin-cyclophosphamide in early nonresponsive breast cancer: phase III randomized GeparTrio trial. *J Natl Cancer Inst* 2008; 100: 542–551
- [27] Huober J, von Minckwitz G, Denkert C et al. Effect of neoadjuvant anthracycline-taxane-based chemotherapy in different biological breast cancer phenotypes: overall results from the GeparTrio study. *Breast Cancer Res Treat* 2010; 124: 133–140. doi:10.1007/s10549-010-1103-9
- [28] Wockel A, Kreienberg R. First Revision of the German S3 Guideline 'Diagnosis, Therapy, and Follow-Up of Breast Cancer'. *Breast Care* 2008; 3: 82–86. doi:10.1159/000127509
- [29] Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 2002; 41: 154–161
- [30] Remmele W, Stegner HE. [Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue]. *Pathologe* 1987; 8: 138–140
- [31] Budczies J, Klauschen F, Sinn BV et al. Cutoff Finder: a comprehensive and straightforward Web application enabling rapid biomarker cutoff optimization. *PLoS One* 2012; 7: e51862. doi:10.1371/journal.pone.0051862
- [32] Reese JM, Suman VJ, Subramaniam M et al. ER β 1: characterization, prognosis, and evaluation of treatment strategies in ER α -positive and -negative breast cancer. *BMC Cancer* 2014; 14: 749. doi:10.1186/1471-2407-14-749
- [33] Saunders PT, Millar MR, Williams K et al. Expression of oestrogen receptor beta (ERbeta1) protein in human breast cancer biopsies. *Br J Cancer* 2002; 86: 250–256. doi:10.1038/sj.bjc.6600035
- [34] O'Neill PA, Davies MP, Shaaban AM et al. Wild-type oestrogen receptor beta (ERbeta1) mRNA and protein expression in Tamoxifen-treated post-menopausal breast cancers. *Br J Cancer* 2004; 91: 1694–1702. doi:10.1038/sj.bjc.6602183
- [35] Marotti JD, Collins LC, Hu R et al. Estrogen receptor-beta expression in invasive breast cancer in relation to molecular phenotype: results from the Nurses' Health Study. *Mod Pathol* 2010; 23: 197–204. doi:10.1038/modpathol.2009.158

- [36] Rosin G, de Boniface J, Karthik GM et al. Oestrogen receptors $\beta 1$ and $\beta 2$ have divergent roles in breast cancer survival and lymph node metastasis. *Br J Cancer* 2014; 111: 918–926. doi:10.1038/bjc.2014.398
- [37] Fuqua SA, Schiff R, Parra I et al. Estrogen receptor beta protein in human breast cancer: correlation with clinical tumor parameters. *Cancer Res* 2003; 63: 2434–2439
- [38] Huang B, Omoto Y, Iwase H et al. Differential expression of estrogen receptor α , $\beta 1$, and $\beta 2$ in lobular and ductal breast cancer. *Proc Natl Acad Sci U S A* 2014; 111: 1933–1938. doi:10.1073/pnas.1323719111
- [39] Haldosén LA, Zhao C, Dahlman-Wright K. Estrogen receptor beta in breast cancer. *Mol Cell Endocrinol* 2014; 382: 665–672. doi:10.1016/j.mce.2013.08.005
- [40] Esslimani-Sahla M, Simony-Lafontaine J, Kramar A et al. Estrogen receptor beta (ER beta) level but not its ER beta cx variant helps to predict tamoxifen resistance in breast cancer. *Clin Cancer Res* 2004; 10: 5769–5776. doi:10.1158/1078-0432.CCR-04-0389
- [41] Yan Y, Li X, Blanchard A et al. Expression of both estrogen receptor-beta 1 (ER- $\beta 1$) and its co-regulator steroid receptor RNA activator protein (SRAP) are predictive for benefit from tamoxifen therapy in patients with estrogen receptor-alpha (ER- α)-negative early breast cancer (EBC). *Ann Oncol* 2013; 24: 1986–1993. doi:10.1093/annonc/mdt132
- [42] Loibl S, Muller BM, von Minckwitz G et al. Androgen receptor expression in primary breast cancer and its predictive and prognostic value in patients treated with neoadjuvant chemotherapy. *Breast Cancer Res Treat* 2011; 130: 477–487. doi:10.1007/s10549-011-1715-8
- [43] Anestis A, Sarantis P, Theocharis S et al. Estrogen receptor beta increases sensitivity to enzalutamide in androgen receptor-positive triple-negative breast cancer. *J Cancer Res Clin Oncol* 2019; 145: 1221–1233. doi:10.1007/s00432-019-02872-9
- [44] Austin D, Hamilton N, Elshimali Y et al. Estrogen receptor-beta is a potential target for triple negative breast cancer treatment. *Oncotarget* 2018; 9: 33912–33930. doi:10.18632/oncotarget.26089
- [45] Mukhopadhyay UK, Oturkar CC, Adams C et al. TP53 Status as a Determinant of Pro- versus Anti-tumorigenic Effects of Estrogen Receptor-beta in Breast Cancer. *J Natl Cancer Inst* 2019. doi:10.1093/jnci/djz051
- [46] Sapino A, Marchio C, Senetta R et al. Routine assessment of prognostic factors in breast cancer using a multicore tissue microarray procedure. *Virchows Arch* 2006; 449: 288–296. doi:10.1007/s00428-006-0233-2