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Article

Predictive Role of *CD36* Expression in HER2-Positive Breast Cancer Patients Receiving Neoadjuvant Trastuzumab

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Abstract

Background: Despite huge efforts to identify biomarkers associated with long-term clinical outcomes in patients with early-stage HER2-positive breast cancer (HER2+ BC) treated with (neo)adjuvant anti-HER2 therapy, no reliable predictors have been identified so far. Fatty acid uptake, a process mediated by the transmembrane transporter CD36, has recently emerged as a potential determinant of resistance to anti-HER2 treatments in preclinical HER2+ BC models. **Methods:** Here, we investigated the association between baseline intratumor *CD36* gene expression and event-free survival in 180 patients enrolled in the phase III trial Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization (NeoALTTO), which randomly assigned stage II-III HER2+ BC patients to receive neoadjuvant lapatinib, trastuzumab, or lapatinib-trastuzumab in combination with chemotherapy. To this aim, we selected NeoALTTO trial patients for whom pretreatment whole transcriptomic data were available. The main study results were validated in an independent cohort of patients enrolled in the neoadjuvant phase II trial NeoSphere. **Results:** In 180 NeoALTTO patients, high intratumor *CD36* expression was independently associated with worse event-free survival in patients treated with trastuzumab-based therapy (hazard ratio [HR] = 1.72, 95% confidence interval [CI] = 1.20 to 2.46), but not with lapatinib-based (HR = 1.02, 95% CI = 0.68 to 1.53) or trastuzumab-lapatinib–based (HR =

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1.08, 95% CI = 0.60 to 1.94) therapy. Among 331 NeoSphere patients evaluated, high *CD36* expression was independently associated with worse patient disease-free survival in both the whole study cohort (HR = 1.197, 95% CI = 1.002 to 1.428) and patients receiving trastuzumab-based neoadjuvant therapy (HR = 1.282, 95% CI = 1.049 to 1.568). **Conclusions:** High *CD36* expression predicts worse clinical outcomes in early-stage HER2+ BC treated with trastuzumab-based neoadjuvant therapy.

In the phase III Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization (NeoALTTO) trial, neoadjuvant trastuzumab-lapatinib–based therapy increased pathologic complete response (pCR) rates compared with trastuzumab- or lapatinib-based treatment (1). In parallel, the phase II NeoSphere trial demonstrated increased pCR rates in patients treated with neoadjuvant trastuzumab-pertuzumab therapy compared with patients receiving trastuzumab- or pertuzumab-only treatment (2). Based on results of the NeoSphere trial as well as of other studies in which trastuzumab-pertuzumab–based neoadjuvant therapy was associated with excellent pCR rates and good safety profile (3-5), dual HER2 blockade has become the standard-of-care preoperative therapy for the majority of stage II-III HER2+ BC patients (6). However, dual HER2 blockade in the NeoALTTO and NeoSphere trials did not improve long-term clinical outcomes, such as event-free survival (EFS) or disease-free survival (DFS) (7,8). In the adjuvant setting, the ALTTO trial failed to show a survival advantage from adding lapatinib to trastuzumab-based therapy (9), whereas adding pertuzumab to adjuvant trastuzumab-based therapy in the Aphinity trial resulted in statistically significant improvement of invasive DFS in HER2+ BC patients with lymph node involvement (10).

Different tumor-related biomarkers, including *HER2* mRNA levels (11), HER2 protein levels (12), HER2 enrichment according to PAM50 classification (11), *ESR1* expression levels (11), exon 9 mutations of *PIK3CA* gene (12), tumor infiltrating lymphocytes (TILs) (13-15), and the 41-gene classifier TRAR (16), have been associated with the likelihood of achieving pCR in patients receiving neoadjuvant anti-HER2 therapies. However, except for TILs, no reliable predictors of long-term clinical outcomes in this setting have been identified so far.

HER2+ BC is a lipogenic malignancy (17), and HER2-induced enhancement of fatty acid (FA) biosynthesis sustains tumor cell growth, proliferation, and metastatization (18,19). More recently, FA uptake, as mediated by the transmembrane transporter CD36, has emerged as a metabolic process associated with acquired resistance to anti-HER2 agents in preclinical HER2+ BC models (20). Here, we investigated the association between *CD36* gene expression and the prognosis of HER2+ BC patients enrolled in the NeoALTTO trial, and we confirmed the main study findings in an independent cohort of patients enrolled in the NeoSphere trial.

Methods

Patient Population

The NeoALTTO trial (NCT00553358) is a multicenter, randomized, open-label, phase III study that investigated the antitumor activity of lapatinib, trastuzumab, or lapatinib-trastuzumab in combination with preoperative chemotherapy in stage II-III HER2+ BC patients (1,8). The NeoSphere trial (NCT00545688) is a multicenter, randomized, phase II study that investigated the antitumor activity of trastuzumab plus docetaxel, pertuzumab plus trastuzumab plus docetaxel, pertuzumab plus trastuzumab without chemotherapy, or pertuzumab plus docetaxel in stage II-III HER2+ BC (2,7). Details regarding the NeoALTTO and NeoSphere trials are reported in Supplementary Methods (available online).

Study Design

In the present analysis, we aimed to investigate the association between *CD36* expression, as evaluated from transcriptomic data, and EFS, defined as the time between patient random assignment and the occurrence of the first event (ie, breast cancer relapse after surgery, second primary malignancy, patient death, or failure to complete neoadjuvant therapy because of disease progression). As a secondary objective, we evaluated the association between *CD36* expression and pCR, defined as the absence of invasive tumor cells in the breast at time of surgery. The NeoSphere trial cohort was used as a validation cohort to confirm the association between *CD36* expression and DFS, defined as the time between surgery and the first documentation of progressive disease or death. pCR in the NeoSphere cohort was defined as the absence of invasive tumor cells in the breast and axilla at surgery.

Tumor Sample Processing and Gene Expression Analysis

Gene expression analysis in NeoALTTO tumor samples was performed through ClariomS platform. RNA concentration in each sample was determined by ND-1000 spectrophotometer (NanoDrop). RNA quality was checked using TapeStation 2200 (Agilent) and the RNA integrity number. For the analysis of samples from the NeoSphere trial, RNA was extracted from pretreatment formalin-fixed, paraffin-embedded (FFPE) core biopsies, and gene expression profiling was carried out with Affymetrix U133 Plus 2.0 gene chips, as previously described (21). Details are reported in the Supplementary Methods (available online).

Results

Study Population

The present analysis included 180 NeoALTTO patients (Figure 1). As reported in Table 1, this cohort was representative of the whole NeoALTTO study population in terms of clinically relevant patient- and tumor-related characteristics. As in the whole NeoALTTO cohort (8), patient EFS was similar in the 3 treatment arms (log-rank test $P = .41$; data not shown). The distribution of *CD36* expression levels in the whole study cohort and each treatment subcohort is reported in Supplementary Figure 1 (available online). Baseline *CD36* expression did not statistically significantly differ according to lymph node status, estrogen receptor (ER) status, tumor size, or pCR status at surgery in either the whole study cohort or individual treatment arms, whereas *CD36* expression was lower in patients older than 50 years in the trastuzumab-alone cohort (Supplementary Figure 2, available online).

Association Between *CD36* Gene Expression and EFS in Whole Patient Cohort

We assessed the association between *CD36* expression and patient EFS in a multivariable Cox regression model including clinically relevant patient-, tumor- and treatment-related variables

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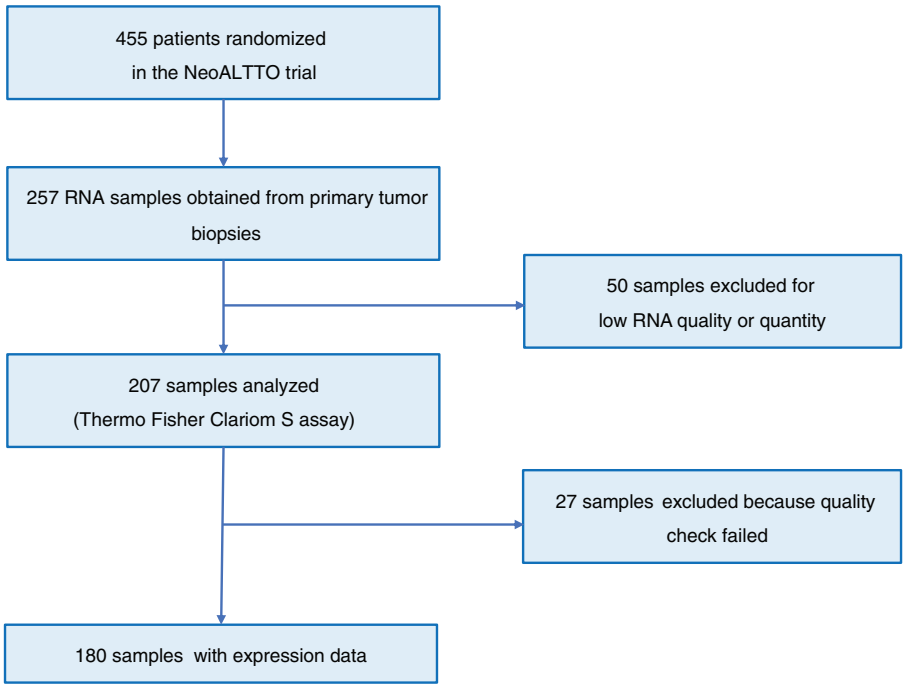

Figure 1. Study flow chart. Out of 455 patients randomly assigned in the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization (NeoALTTO) trial, tumor RNA was retrieved from 257 tumor biopsy specimens and was analyzed by Thermo Fisher Clariom S assay in 207 patients. After excluding 27 samples at quality check analysis, a total 180 RNA samples were used for this study.

(age at enrollment, tumor size, nodal involvement, ER status, pCR status at surgery, and treatment arm) (1). In this model, achieving pCR was associated with better EFS (hazard ratio [HR] = 0.35, 95% confidence interval [CI] = 0.16 to 0.76, $P = .01$) [in line with data from the whole NeoALTTO cohort (8)], whereas *CD36* expression was not statistically significantly associated with EFS (HR = 1.22, 95% CI = 0.95 to 1.57, $P = .12$) (data not shown).

Then, we hypothesized that *CD36* expression might be associated with clinical outcomes according to specific anti-HER2 treatments. To test this hypothesis, we fitted a multivariable Cox model that included the same clinic-pathological variables as well as the first-order interaction between baseline *CD36* levels and treatment arm. This analysis revealed an interaction between *CD36* expression and the type of treatment in explaining the observed EFS, with high *CD36* expression levels being independently associated with worse EFS only in patients receiving trastuzumab-based therapy (continuous scale, HR = 1.72, 95% CI = 1.20 to 2.46, $P = .003$) (Figure 2). This model also confirmed the well-established association between pCR and better EFS. The inclusion of *CD36* expression in the multivariable model improved its global predictive capability; indeed, the C-statistics value of a model including only clinical variables and treatment arm was 0.64 (95% CI = 0.56 to 0.71), which rose to 0.67 (95% CI = 0.61 to 0.74) after including *CD36* expression and its interaction with treatment arm.

Association Between *CD36* Expression and EFS in Individual Treatment Arms

Then, we performed additional analyses to explore the observed association between *CD36* expression and EFS in patients randomly assigned to individual treatment arms. At univariate analysis, high-baseline *CD36* expression was associated with statistically significantly worse EFS in patients receiving trastuzumab-based therapy (HR = 1.73, 95% CI = 1.20 to 2.49, $P = .03$) but not in patients treated with lapatinib (HR = 1.01, 95% CI = 0.69 to 1.47, $P = .98$) or lapatinib-trastuzumab (HR = 1.02, 95% CI = 0.54 to 1.95, $P = .94$). The predictive role of baseline *CD36* expression in trastuzumab-treated patients was confirmed by bivariate models adjusting the role of *CD36* for each of the following covariates: pCR status (HR = 1.69, 95% CI = 1.18 to 2.41, $P = .004$), ER status (HR = 1.75, 95% CI = 1.20 to 2.54, $P = .004$), tumor size (HR = 1.77, 95% CI = 1.20 to 2.60, $P = .004$), lymph node status (HR = 1.74, 95% CI = 1.20 to 2.51, $P = .003$), and age (HR = 1.78, 95% CI = 1.21 to 2.63, $P = .004$) (Table 2).

When *CD36* expression was dichotomized according to median *CD36* levels in the whole study cohort, we found a trend toward worse EFS in patients with high *CD36*-expressing tumors (Figure 3, A), and this difference reached statistical significance in the trastuzumab-alone arm (log-rank $P = .03$) (Figure 3, B). Based on the well-established association between pCR and better long-term clinical outcomes in early-stage HER2+ BC (8,22), as well as on our findings showing that both *CD36* expression and pCR status are independently associated with EFS (Figure 2), we divided patients into 2 groups according to *CD36* expression and pCR status: (group A) high *CD36*/no pCR (bad prognosis group), (group B) low *CD36*/any pCR status (yes or no), or high *CD36*/pCR (good prognosis group). We found statistically significantly lower 7-year EFS in group A compared with group B patients in both the whole patient cohort (53%, 95% CI = 40% to 65% vs 76%, 95% CI = 66% to 83%, respectively; log-rank test $P = .002$) and in the trastuzumab-alone cohort (47%, 95% CI = 26% to 66% vs 79%, 95% CI = 62% to 88%, respectively; log-rank test $P = .006$) (Figure 3, C and D). Notably, combining *CD36* expression and pCR status resulted in a better separation of Kaplan-Meier EFS curves compared with *CD36*-alone classification.

Interplay Between *CD36* Expression and Intratumor Immunity

TILs were available in 159 of 180 (88.3%) NeoALTTO patients included in our analysis. High-baseline TILs were associated with a lower risk of disease relapse (HR = 0.976, 95% CI = 0.957 to

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Table 1. Tumor and patient characteristics in the NeoALTTO study population and in this study cohort

Characteristic	NeoALTTO cohort, No. (%) (n = 455)	Study cohort, No. (%) (n = 180)	P
Age			
<50 y	222 (49	101 (56)	.10[a]
≥50 y	233 (51)	79 (44)	
Tumor size			
T2 (≤5 cm)	274 (60)	106 (59)	.76[a]
T3-T4 (>5 cm)	181 (40)	74 (41)	
Lymph node status			
N0	123 (27)	42 (23)	.34[a]
≥N1	332 (73)	138 (77)	
ER status			
Positive	216 (47)	91 (51)	.48[a]
Negative	239 (53)	89 (49)	
Planned conservative surgery			
Yes	130 (29)	45 (25)	.36[a]
No	325 (71)	135 (75)	
Treatment arm			
Trastuzumab	149 (33)	66 (37)	
Lapatinib	154 (34)	65 (36)	.31[a]
Combination	152 (33)	49 (27)	
pCR			
Yes	160 (35)	51 (28)	.10[a]
No	295 (65)	129 (72)	
Events	127 (28)	53 (29)	
Median follow-up (IQR), y	6.7 (5.7-6.8)	6.7 (6.1-6.8)	.32[c]
TILs median %, (range)[b]	12.5 (0-95)	12.5 (0-95)	

[a]The P value of the χ^2 test is indicated. ER = estrogen receptor; IQR = interquartile range; NeoALTTO = Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization; pCR = pathologic complete response; TILs = tumor-infiltrating lymphocytes.
[b]TIL assessment was available for 376 out of 455 and for 159 out of 180 patients in the NeoALTTO cohort and in this study cohort, respectively.
[c]P value of the Wilcoxon test.

0.995, $P = .01$), in line with previously published data (13). *CD36* expression did not correlate with TILs in either the whole study cohort or in individual treatment arms (Supplementary Figure 3, A, available online). Similarly, baseline *CD36* expression did not correlate with any of 14 tumor-infiltrating immune cells, including CD8+, regulatory T cells, or naïve or activated-memory CD4+ T cells, as estimated by transcriptomic data deconvolution analysis (Supplementary Figure 3, B, available online).

Then, we fitted a multivariable Cox regression model to investigate if the association between high *CD36* expression and worse EFS was independent of TILs and other relevant variables. In this model, high *CD36* expression maintained a statistically significant association with worse EFS, whereas high TILs were associated with better EFS (Supplementary Figure 4, available online). Bivariate analysis adjusting *CD36* expression for TILs in the trastuzumab-alone cohort confirmed an independent, bad predictive role of high *CD36* expression in this subcohort (HR = 1.61, 95% CI = 1.06 to 2.44, $P = .02$) (Table 2).

Validation of the Predictive Role of *CD36* Expression in the NeoSphere Trial

To confirm the role of high *CD36* expression in an independent cohort of HER2+ BC patients treated with neoadjuvant anti-HER2 therapy, we investigated the association between baseline intratumor *CD36* expression and DFS in 331 patients enrolled in the phase II NeoSphere trial. This clinical cohort was representative of the whole NeoSphere study population (Supplementary Table 1, available online). The distribution of *CD36* expression levels in our NeoSphere study cohort and in the "joined trastuzumab cohort," which included patients treated with neoadjuvant chemotherapy plus trastuzumab, chemotherapy plus trastuzumab plus pertuzumab, or trastuzumab plus pertuzumab, is reported in Supplementary Figure 5 (available online). Supplementary Table 2 (available online) summarizes similarities and differences between the NeoALTTO and the NeoSphere study cohorts in terms of patients included, clinical outcomes and methods used to quantify *CD36* expression.

At univariate analysis, high-baseline *CD36* expression showed a statistically significant association with worse DFS in the whole study cohort (HR = 1.192, 95% CI = 1.003 to 1.416, $P = .046$) and in the joined trastuzumab cohort (HR = 1.272, 95% CI = 1.047 to 1.546, $P = .02$). In individual treatment subcohorts, we observed similar trends, although statistical significance was not reached, likely due to low number of disease relapse events (Supplementary Table 3, available online).

Multivariable analysis adjusting the role of baseline *CD36* expression for clinically relevant variables (patient age, tumor size, lymph node status, ER status, and pCR status) revealed that *CD36* expression was the only covariate to be independently associated with patient DFS both in the whole study cohort (HR = 1.197, 95% CI = 1.002 to 1.428, $P = .047$) and in the joined trastuzumab cohort (HR = 1.282, 95% CI = 1.049 to 1.568, $P = .02$) (Supplementary Table 3, available online).

CD36 dichotomization based on its median value confirmed that patients with high-baseline intratumor *CD36* expression had statistically significantly worse DFS compared with patients with low *CD36* expression, and this was observed both in the whole study cohort and the joined trastuzumab cohort (Figure 4, A and B). As in the NeoALTTO cohort, *CD36* expression was not associated with the probability to achieve pCR (OR = 1.015, 95% CI = 0.983 to 1.048, $P = .38$). Patients with tumors expressing high *CD36* levels and failing to achieve pCR had worse DFS compared with the remaining patients in both the whole NeoSphere cohort and the joined trastuzumab cohort (Figure 4, C and D). However, differently from the NeoALTTO cohort, the NeoSphere cohort combining high *CD36* expression with the failure to achieve pCR did not result in a better separation of DFS curves compared with the evaluation of *CD36* alone.

Discussion

We reported an unfavorable and independent association between high intratumor *CD36* expression and worse EFS in HER2+ BC patients treated with neoadjuvant trastuzumab-based therapy in the context of the randomized, phase III trial NeoALTTO. The main study findings were validated in an independent cohort of HER2+ BC patients enrolled in the randomized, phase II trial NeoSphere. Our results consistently indicate a negative predictive role of a metabolic gene, namely *CD36*, which is not a classical downstream effector of the HER2 pathway, in HER2+ BC patients receiving trastuzumab-based neoadjuvant therapy.

In a recently published study, CD36 overexpression emerged as a mechanism of acquired resistance to lapatinib in preclinical models of human HER2+ BC, and high intratumor *CD36* expression was associated with worse overall survival in a cohort of HER2+ BC patients enrolled in the NeoALTTO trial (20). However, the clinical relevance of these results was limited by the following factors: 1) the association between *CD36*

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Variable	b	SE	*P* value	HR (95% CI)
Age (≥50 vs <50 years)	−0.13	0.31	.67	0.88 (0.48 to 1.61)
Tumor size (>5 cm vs ≤5 cm)	−0.03	0.30	.92	0.97 (0.54 to 1.74)
Lymph node status (≥N1 vs N0)	0.43	0.37	.24	1.53 (0.75 to 3.15)
ER status (negative vs positive)	0.27	0.31	.38	1.31 (0.72 to 2.39)
pCR (Yes vs No)	−1.08	0.41	.01	0.34 (0.15 to 0.75)
Single agent L vs single agent T	3.94	2.11	.06	-
Combination arm vs single agent T	3.45	2.61	.19	-
CD36	0.54	0.18	.003	-
*CD36**treatment arm	−0.52 −0.47	0.27 0.35	.06 .18	-
CD36 in single-agent L	-	-	-	1.02 (0.68 to 1.53)
CD36 in single-agent T	-	-	-	1.72 (1.20 to 2.46)
CD36 in combination arm	-	-	-	1.08 (0.60 to 1.94)

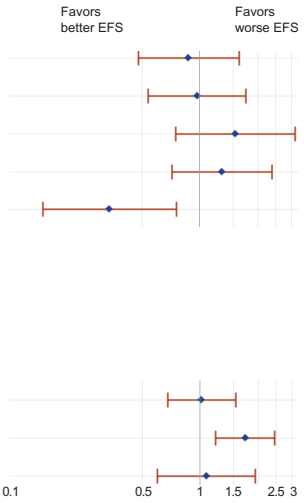

Figure 2. Multivariable penalized Cox regression model for event-free survival (EFS) in the whole study cohort. Hazard ratios (HR) and 95% confidence intervals (CIs) in relation to EFS are shown for each variable. A 30-week landmark analysis was performed, including 169 patients and 51 events. b = beta coefficient estimate; CD36*treatment arm = interaction term between CD36 expression and treatment arm; ER = estrogen receptor; L = lapatinib; pCR = pathologic complete response; SE = standard error; T = trastuzumab.

Table 2. Bivariate Cox regression models for EFS in patients randomly assigned to trastuzumab arm (n = 66 and 20 events)

Models	HR (95% CI)	*P*
Bivariate model[a] with pCR and *CD36*		
pCR (yes vs no)	0.44 (0.13 to 1.49)	.19
CD36	1.69 (1.18 to 2.41)	.004
Bivariate model with ER status and *CD36*		
ER status (neg vs pos)	1.16 (0.48 to 2.85)	.74
CD36	1.75 (1.20 to 2.54)	.004
Bivariate model with tumor size and *CD36*		
Tumor size (>5 cm vs ≤5 cm)	1.19 (0.45 to 3.16)	.72
CD36	1.77 (1.20 to 2.60)	.004
Bivariate model with lymph node status and *CD36*		
Lymph node status (≥N1 vs N0)	0.89 (0.34 to 2.34)	.82
CD36	1.74 (1.20 to 2.51)	.003
Bivariate model with age and *CD36*		
Age (≥50 vs <50 y)	1.25 (0.48 to 3.24)	.65
CD36	1.78 (1.21 to 2.63)	.004
Bivariate model with TILs and *CD36*		
TILs	0.98 (0.95 to 1.01)	.30
CD36	1.61 (1.06 to 2.44)	.02

[a]For the model including pCR and *CD36* as covariates, a 30-week landmark analysis was performed including 63 patients and 20 events. CI = confidence interval; ER = estrogen receptor; EFS = event-free survival; HR = hazard ratio; pCR = pathologic complete response; TILs = tumor-infiltrating lymphocytes.

expression and local or distant tumor relapse (eg, EFS or DFS) was not evaluated; 2) multivariable analysis adjusting for clinically or biologically relevant variables was not performed; 3) *CD36* was only analyzed as a dichotomous variable, not as a continuous one; and 4) the role of *CD36* in individual treatment arms was not investigated.

In our NeoALTTO trial cohort, high-baseline intratumor *CD36* expression, evaluated as a continuous variable, was independently associated with worse EFS in patients treated with trastuzumab-based therapy, but not in patients receiving lapatinib- or trastuzumab-lapatinib–based treatment. In the NeoSphere validation cohort, high-baseline *CD36* expression, evaluated as a continuous variable, was independently associated with worse DFS in the whole study cohort and the subset of patients treated with neoadjuvant trastuzumab-based therapy. The association between high *CD36* expression and worse EFS (NeoALTTO) or DFS (NeoSphere) in patients receiving neoadjuvant trastuzumab-based therapy was confirmed when *CD36* was evaluated as a dichotomous variable. Together, these findings indicate that *CD36* gene expression is a new and reliable biomarker that predicts poorer long-term clinical outcomes in early-stage HER2+ BC patients treated with neoadjuvant trastuzumab-based therapy.

In a recent study, CD36 expression in tumor-infiltrating $CD8^+$ T lymphocytes was shown to promote $CD8^+$ T-cell exhaustion, and it was associated with higher risk of tumor progression and lower survival of tumor-bearing mice and cancer patients (23). These results suggest that CD36 expression not only in cancer cells but also in specific intratumor immune cell subsets can affect tumor growth and progression and tumor-related outcomes. Because the antitumor activity of trastuzumab is in part mediated by the activation status of tumor-infiltrating immune cells (24,25), we hypothesized that the observed association between high *CD36* expression and worse clinical outcomes specifically in patients receiving neoadjuvant trastuzumab could result from hampered adaptive immunity-mediated antitumor activity of trastuzumab in CD36-overexpressing tumors. To study if intratumor *CD36* expression is associated with the presence or activation status of specific tumor-infiltrating immune cells, in the NeoALTTO cohort we investigated the correlation between *CD36* expression and TILs, or between *CD36* and the estimates of 14 tumor-infiltrating immune cells. However, we did not find a correlation between *CD36* expression and TILs or other immune cell subsets. Of note, *CD36* expression maintained an independent association (ie, regardless of TILs) with patient EFS in the whole study cohort and in patients treated with neoadjuvant trastuzumab-alone therapy. Although these results do not allow to definitely exclude a role of *CD36* expression in specific immune

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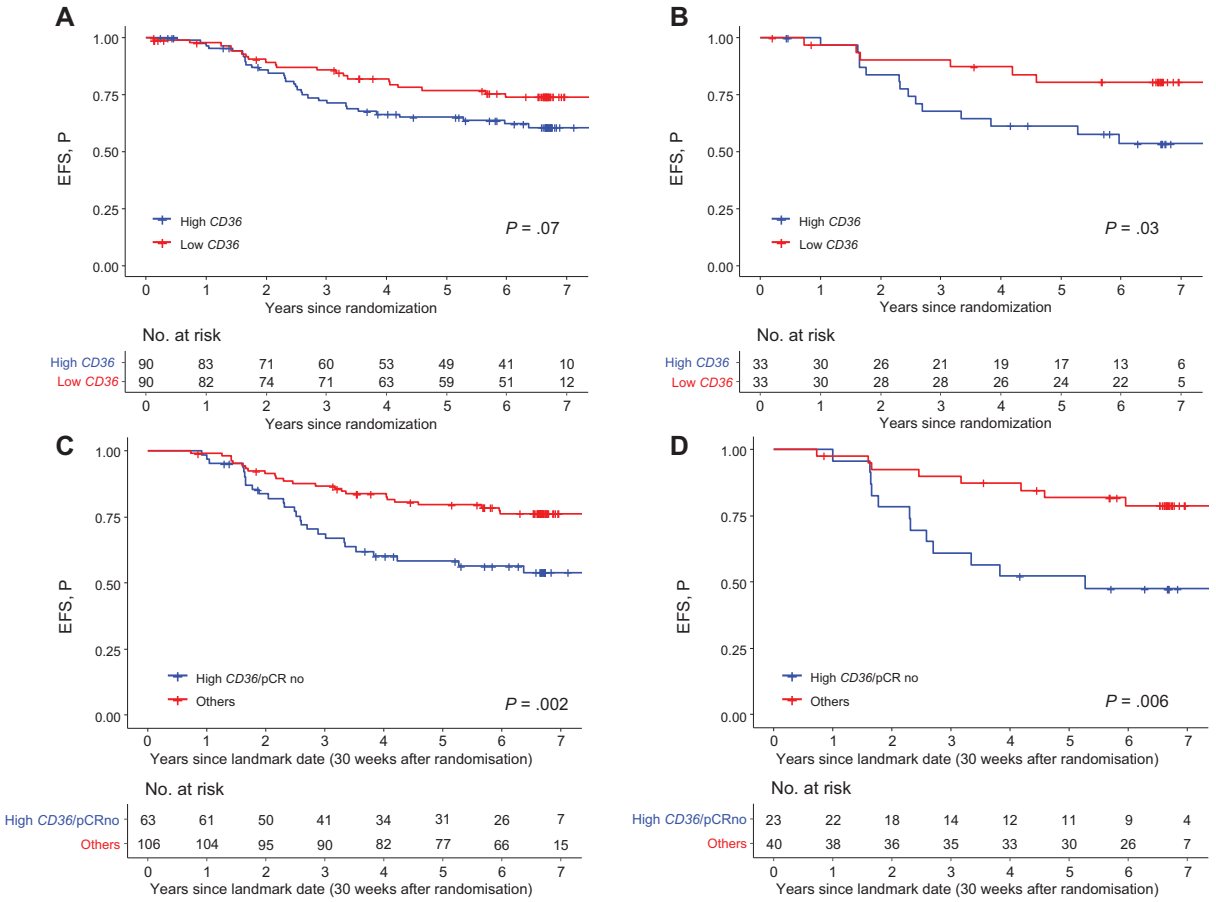

Figure 3. Event-free survival (EFS) probability according to baseline *CD36* expression and pathologic complete response (pCR) status. Kaplan-Meier curves representing patient EFS according to *CD36* expression, considered as a dichotomous variable, in the whole study cohort (**A**) (n = 180; 53 events) and in patients treated with trastuzumab-alone therapy (**B**) (n = 66; 20 events). Kaplan-Meier curves representing patient EFS according to both *CD36* expression, considered as dichotomous variable, and pCR status in the whole study cohort (**C**) (n = 180; 53 events) and in patients treated with trastuzumab-alone therapy (**D**) (n = 66; 20 events). In this analysis, patients were grouped according to both pCR status and *CD36* expression in the following 2 cohorts: high *CD36*/no pCR; others: low *CD36*/any pCR status (yes/no) or high *CD36*/pCR. A 30-week landmark analysis was performed, including 169 patients and 51 events. The *P* value of the log-rank test for the indicated comparisons is reported. Median *CD36* levels were used as a threshold for the definition of CD36 categories (low and high).

cell subsets, we speculate that the observed bad predictive role of high *CD36* expression might at least in part reflect CD36-mediated uptake of extracellular FA by cancer cells, consistent with recently published preclinical data (20). Future studies combining evaluations with single-cell resolution (eg, single-cell RNA-seq or immunohistochemistry analyses) will clarify if the expression of CD36 in specific tumor-infiltrating immune cell subsets, such as lymphocytes and macrophages, is associated with changes in their functional status.

Our findings could have implications in the context of the current debate on escalation or deescalation of anti-HER2 therapies in early-stage HER2+ BC patients, which is a highly relevant clinical issue in the perspective of treatment personalization (26). Indeed, high-baseline intratumor *CD36* levels identify patients who might benefit from more aggressive therapies, such as dual HER2 blockade in the context of (neo)adjuvant therapies or postneoadjuvant T-DM1 in patients with high-baseline *CD36* expression who fail to achieve pCR during neoadjuvant therapy. On the other hand, neoadjuvant dual HER2 blockade or postneoadjuvant T-DM1 could be less useful in patients with tumors expressing low CD36 levels. Prospective studies could be initiated to escalate or de-escalate (neo)adjuvant anti-HER2 treatments on the basis of high or low baseline *CD36* tumor expression.

The main limitation of this study is that we included only a subset of NeoALTTO and NeoSphere patients, that is, those patients for whom transcriptomic data from basal tumor biopsies were available. However, our study cohorts were representative of the NeoALTTO and NeoSphere population in terms of clinical characteristics (1,2,8). Another limitation is the fact that *CD36* gene expression was measured through different platforms in the 2 study cohorts, which implies that the *CD36* gene expression threshold used to discriminate patients with *CD36*-high vs *CD36*-low tumors in the NeoALTTO trial cannot be generalized to other clinical cohorts (including the NeoSphere cohort), in which *CD36* expression was quantified through different methods. Strengths of our study include 1) the novelty of the results, which establish high-*CD36* gene expression as a new biomarker that independently predicts worse clinical outcomes in early-stage HER2+ BC patients treated with trastuzumab-based neoadjuvant therapy; 2) the main study findings were obtained in a cohort of patients enrolled in a phase III randomized trial (NeoALTTO); and 3) NeoALTTO cohort results were validated in a cohort of patients enrolled in the randomized, phase II registration trial NeoSphere.

In conclusion, high-*CD36* gene expression is independently associated with worse clinical outcomes in early-stage HER2+ BC patients treated with neoadjuvant trastuzumab-based therapy. Along with the recently proposed mechanistic role of CD36

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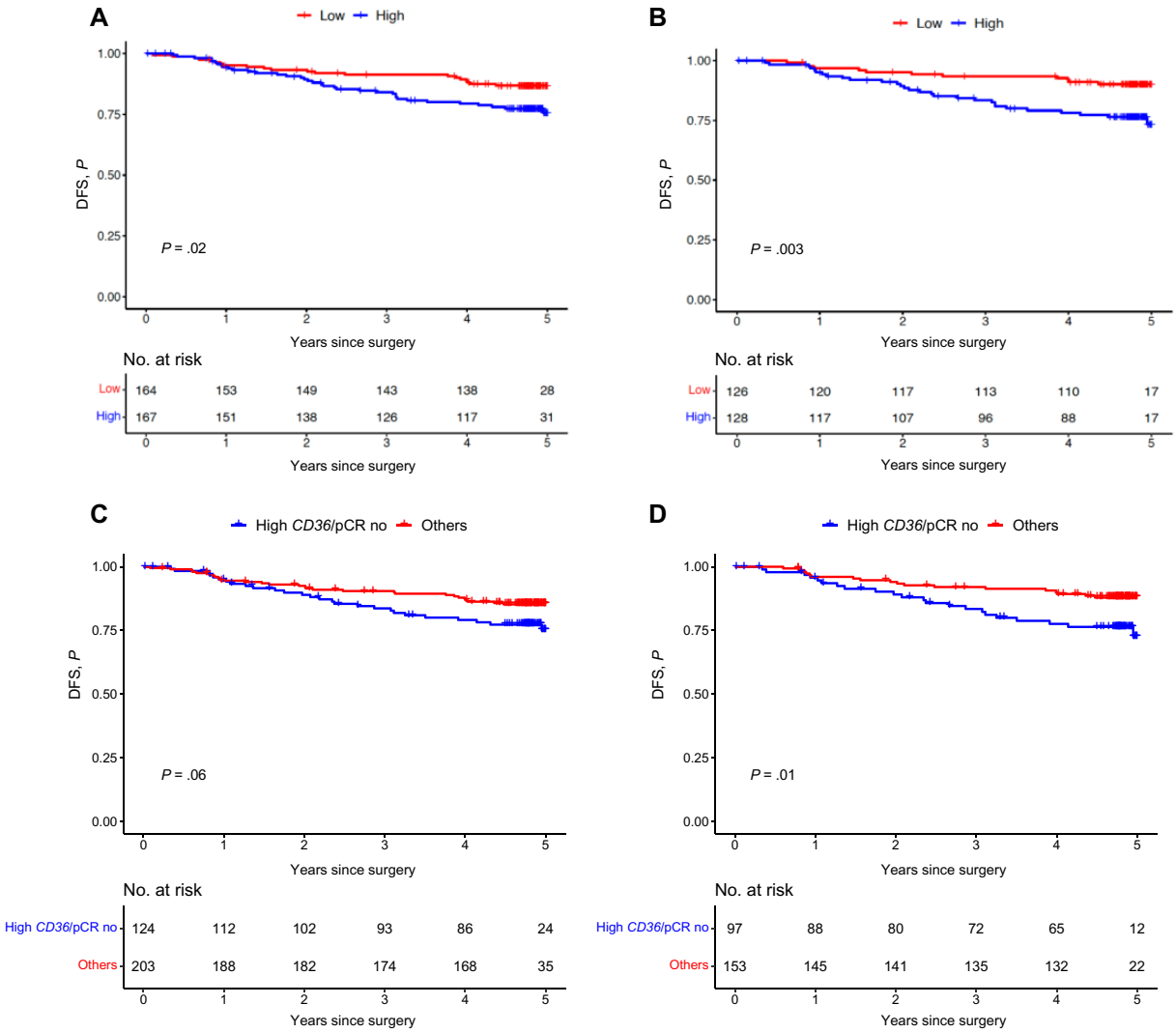

Figure 4. Disease-free survival (DFS) probability in NeoSphere patients according to baseline *CD36* expression. Kaplan-Meier curves representing patient DFS according to *CD36* expression, considered as a dichotomous variable, in the whole study cohort (**A**) and in patients in the joined trastuzumab cohorts (**B**). Kaplan-Meier curves representing patient DFS according to both *CD36* expression, considered as dichotomous variable, and pathologic complete response (pCR) status in whole study cohort (**C**) and in patients in the joined trastuzumab cohort (**D**). In this analysis, patients were grouped according to both pCR status and *CD36* expression in the following 2 cohorts: high *CD36*/no pCR; others: low *CD36*/any pCR status (yes/no) or high *CD36*/pCR. The *P* value of the log-rank test for the indicated comparisons is reported. Median *CD36* levels were used as a threshold for the definition of CD36 categories (low and high).

overexpression in mediating HER2+ BC resistance to anti-HER2 therapies, our findings point to CD36 as a new and promising target in HER2+ BC treatment.

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Data Availability

Restrictions apply to the availability of these data, which were used under license for this study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the TransALTTO committee and the Fondazione Michelangelo.

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