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ORIGINAL RESEARCH

Influence of HER2 expression on prognosis in metastatic triple-negative breast cancer—results from an international, multicenter analysis coordinated by the AGMT Study Group

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Background: Triple-negative breast cancer (TNBC) is associated with poor prognosis, and new treatment options are urgently needed. About 34%-39% of primary TNBCs show a low expression of human epidermal growth factor receptor 2 (HER2-low), which is a target for new anti-HER2 drugs. However, little is known about the frequency and the prognostic value of HER2-low in metastatic TNBC.

Patients and methods: We retrospectively included patients with TNBC from five European countries for this international, multicenter analysis. Triple-negativity had to be shown in a metastatic site or in the primary breast tumor diagnosed simultaneously or within 3 years before metastatic disease. HER2-low was defined as immunohistochemically (IHC) 1+ or 2+ without *ERBB2* gene amplification. Survival probabilities were calculated by the Kaplan—Meier method, and multivariable hazard ratios (HRs) were estimated by Cox regression models.

Results: In total, 691 patients, diagnosed between January 2006 and February 2021, were assessable. The incidence of HER2-low was 32.0% [95% confidence interval (CI) 28.5% to 35.5%], with similar proportions in metastases ($n = 265$; 29.8%) and primary tumors ($n = 425$; 33.4%; $P = 0.324$). The median overall survival (OS) in HER2-low and HER2-0 TNBC was 18.6 and 16.1 months, respectively (HR 1.00; 95% CI 0.83-1.19; $P = 0.969$). Similarly, in multivariable analysis, HER2-low had no significant impact on OS (HR 0.95; 95% CI 0.79-1.13; $P = 0.545$). No difference in prognosis was observed between HER2 IHC 0/1+ and IHC 2+ tumors (HR 0.89; 95% CI 0.69-1.17; $P = 0.414$).

Conclusions: In this large international dataset of metastatic TNBC, the frequency of HER2-low was 32.0%. Neither in univariable nor in multivariable analysis HER2-low showed any influence on OS.

Key words: triple-negative breast cancer, metastatic, HER2-low, OS, real-world data, prognosis

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INTRODUCTION

Triple-negative breast cancer (TNBC) is associated with poor prognosis, and new treatment options are urgently needed. About 34%-39% of triple-negative primary tumors show a low expression of the human epidermal growth factor receptor 2 (HER2-low) defined as immunohistochemically (IHC) 1+ or 2+ and lack of *ERBB2* gene amplification measured by *in situ* hybridization.[1-3] Generally, HER2-low tumors do not respond to trastuzumab[4] or T-DM1,[5] even if there seems to be a subgroup of patients—selected by a novel poly-ligand profiling technique—who might benefit from trastuzumab.[6] In contrast, new antibody—drug conjugates (ADCs) such as trastuzumab deruxtecan (T-DXd) and trastuzumab duocarmazine show activity in HER2-low tumors, because of their high drug-to-antibody ratio and their bystander killer effect.[7-9] The first results of the phase III trial DESTINY-Breast04 showed that in patients with pretreated HER2-low metastatic breast cancer, T-DXd induced significantly better progression-free survival and overall survival (OS) compared with physician's choice of chemotherapy.[10] In addition to ADCs, bispecific antibodies and HER2 vaccines are under investigation in HER2-low breast cancer.[2]

Regarding the prognostic significance of HER2-low in TNBC, conflicting results were reported in early-stage disease, with studies reporting no influence on the risk of recurrence or death,[11-13] a worse prognosis for HER2 IHC 2+ tumors,[14] or a better prognosis for HER2-low tumors,[3] respectively. A better prognosis was reported from a pooled analysis of four neoadjuvant trials of the German Breast Group, including 1162 patients with TNBC [multivariable disease-free survival (DFS) HR 0.64; 95% confidence interval (CI) 0.46-0.88; $P = 0.0066$], while no difference in the rate of pathological complete responses was found (48.0% versus 50.1%; $P = 0.21$).

Even less is known about the frequency and the prognostic value of low HER2 expression in metastatic TNBC. Because of the relatively low incidence of HER2-low TNBC, we pooled data from different registries and retrospective analyses conducted in five European countries.

PATIENTS AND METHODS

For this international, multicenter analysis, we retrospectively included patients with TNBC from five European countries (Austria, France, Italy, Portugal, and Spain). The project was approved by the Ethics Committee of the province Salzburg (IRB number: 415-E/1836/46-2021). For this project, data were collected from patients enrolled in institutional or national registries who either have given written informed consent approved by national and regional ethics committees or were already deceased before data entry. Data from patients recorded during daily routine were transmitted anonymously to the Austrian Group Medical Tumor Therapy (AGMT) in accordance with the General Data Protection Regulation and national data protection laws. Data were collected and managed using REDCap electronic data capture tools hosted by the AGMT.[15,16]

Triple-negativity had to be shown in a biopsy gained from a metastatic site. In case no biopsy was taken from a metastatic site, triple-negativity had to be shown in the primary breast tumor diagnosed simultaneously or within 3 years before metastatic disease. Patients with a history of breast cancer other than TNBC or history of other malignancies (except for cervical carcinoma *in situ*, basal cell carcinoma of the skin, or squamous cell carcinoma of the skin) and no available biopsy from a metastatic site showing TNBC were excluded from this analysis.

Estrogen receptor (ER) negativity was defined—according to the institutional standard—as either ER <10% or ER <1%. Because in several institutions the exact percentage of ER expression (below 10%) was not known, three categories of ER expression were made: <1%, <10%, and 1%-9%. HER2-low was defined as IHC 1+ or IHC 2+ and lack of *ERBB2* gene amplification measured by *in situ* hybridization. HER2-0 was defined as IHC 0+. No central HER2 or ER testing was carried out.

The primary endpoint was the observed frequency of low HER2 expression in metastatic TNBC. The main secondary endpoint was OS defined as the time from diagnosis of metastatic disease until death from any cause. Additional secondary endpoints were country-specific frequency of HER2-low and differences in HER2 expression between metastatic lesions versus primary breast tumors. Exploratory endpoints were OS in patients with HER2 IHC 0 versus IHC 1+ versus IHC 2+, OS in patients with HER2 IHC 0 or IHC 1+ versus IHC 2+, and the correlation of HER2-low with clinicopathological parameters.

Unadjusted survival probabilities were calculated by the Kaplan—Meier method and compared using the log-rank test. Univariable and multivariable hazard ratios (HRs) were estimated by Cox proportional hazards models. Covariable selection for the multivariable model was based on a stepwise selection method[17] including the following variables: age at diagnosis of metastatic disease (continuous), ER (<1% versus <10% versus 1%-9%), DFS (≤24 months versus >24 months versus *de novo* metastatic), T stage (1 versus 2 versus 3 versus 4), N stage (0 versus 1 versus 2 versus 3), American Joint Committee on Cancer stage (I versus II versus III versus IV), grade, visceral disease (yes versus no), and (neo)adjuvant chemotherapy (yes versus no). No imputation of missing values was used, because missing values do only affect the multivariate model, which is used here as a sensitivity analysis of the conclusions. An observed-cases approach was applied. As potential predictors for multivariable analysis, all parameters were used and their selection was done based on the stepwise selection method.[17] The model was re-fitted on selected predictors to decrease the number of missing to <2%. The HER2 status was forced into the model.

The software SAS version 9.4 (SAS Institute Inc., Cary, NC) was used for all statistical analysis. Summary statistics for discrete variables were expressed as frequency counts and percentages and for continuous variables as means and

standard deviations or medians and quartiles, where appropriate. Comparison of subgroups was carried out for the chi-square categorical parameters. For continuous variables, comparison of subgroups was carried out by the Wilcoxon rank sum test. For all hypotheses tested, a P value <0.05 indicated statistical significance. All tests were carried out as two-sided. No adjustment for multiple comparisons was carried out because only the primary endpoint was tested confirmatory.

RESULTS

In total, 691 assessable patients with metastatic TNBC diagnosed between January 2006 and February 2021 were included in this analysis: 294 (42.5%) from Austria, 173 (25.0%) from France, 161 (23.3%) from Italy, 33 (4.8%) from Spain, and 30 (4.3%) from Portugal (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2022.100747). All patients were female. The main patient characteristics and their association with HER2-low are summarized in Table 1. Additional characteristics are provided in Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2022.100747.

Frequency of HER2-low metastatic TNBC

A total of 221 patients (32.0%; 95% CI 28.5% to 35.5%) were classified as HER2-low and 470 (68.0%; 95% CI 64.5% to 71.5%) as HER2-0. HER2 status was mainly (425/691 = 61.5%) obtained from the primary tumor; however, the proportion of HER2-low was similar in primary tumors (33.4%; 95% CI 28.9% to 37.9%) and metastatic samples (29.8%; 95% CI 24.3% to 35.3%; $P = 0.324$; Figure 1). HER2-status from both primary tumor and metastasis was available in 500 patients. Changes from HER2-0 to HER2-low were seen in 24 patients (4.8%). Conversely, 35 patients (7.0%) had a primary tumor classified as HER2-low and a metastasis classified as HER2-0.

When comparing the frequency of HER2-low in the five different countries, we found no statistical difference ($P = 0.479$). The frequency for each country is provided in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2022.100747.

Impact of low HER2 expression on OS

The median OS in HER2-low and HER2-0 TNBC was 18.6 months (95% CI 16.5-20.3 months) and 16.1 months (95% CI 14.5-18.6 months), respectively, which was not statistically different (HR 1.00; 95% CI 0.83-1.19; $P = 0.969$; Figure 2).

Similarly, in multivariable analysis, low HER2 expression had no significant impact on prognosis compared to HER2-0 disease (HR 0.95; 95% CI 0.79-1.13; $P = 0.545$; Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2022.100747). Additionally, we did not identify a difference in OS between HER2 IHC 0, 1+, and 2+, respectively (Figure 3A). Furthermore, there was no difference in OS between patients with HER2 IHC 0/1+ tumors and IHC 2+ tumors (median OS: 16.8 versus 18.2 months; HR 0.89; 95% CI 0.69-1.17; $P = 0.412$; Figure 3B).

DISCUSSION

All international guidelines recommend testing for HER2 expression in invasive breast cancer, because the results significantly impact prognosis and therapeutic options. Until recently, only HER2 overexpression was of interest, since it is a known negative prognostic factor both in early and in advanced breast cancer and a positive predictive factor for HER2-targeting monoclonal antibodies, tyrosine kinase inhibitors, and ADCs. Even though a low HER2 expression (IHC 1+ or 2+ but negative *in situ* hybridization) was generally reported by pathologists, it did not have any influence on treatment recommendations. Now, the first positive results of ADCs in HER2-low metastatic breast cancer (MBC) are available[7,8,10] and have attracted the interest to this new breast cancer subtype. Several retrospective studies investigated the frequency and the prognostic value of HER2-low in early breast cancer reporting conflicting results concerning the influence of HER2-low on prognosis.[11-14] Recently, a monocentric study including 697 patients with early TNBC from the MD Anderson Cancer Center was reported. In this cohort, HER2-low did not have any impact on DFS, distant DFS, and OS either in TNBC or in HR+/HER2− early breast cancer.[11]

In contrast to early breast cancer, where generally larger datasets are available, little is known about the frequency of HER2-low expression in MBC, especially in the triple-negative subgroup. Recently, a subgroup analysis of the ASCENT phase III trial, which investigated the efficacy of sacituzuamb govitecan in the second-line or greater metastatic TNBC setting, was presented. In the patients with available HER2 IHC, 123/416 patients (29.5%) were HER2-low based on archivable tissue, which could be both breast primary or metastatic specimens.[18]

Here we provide, to our knowledge, the largest dataset of metastatic patients with TNBC to address the two important questions: what is the frequency of HER2-low in metastatic TNBC and does it influence prognosis? We identified a frequency for HER2-low of 32%—which seems to be slightly lower than that reported in early breast cancer (34%-39%)[1-3]—and did not find any influence on OS either in univariable (HR 1.00; 95% CI 0.83-1.19; $P = 0.969$) or in multivariable analysis (HR 0.95; 95% CI 0.79-1.13; $P = 0.545$). Similarly, there was no prognostic difference between HER2 IHC 2+ and IHC 0/1+ tumors.

Our results are well in line with those from other retrospective analyses, showing no difference in OS between patients with HER2-low metastatic TNBC compared to patients with HER2 0 (or 1+).[1,19] One explanation for these findings is that, in contrast to HER2 overexpression, low HER2 levels do not seem to influence disease biology. This assumption is supported by the results of Schettini et al., who did not find any differentially expressed genes according to low HER2 expression in TNBC.[1] Furthermore, only a small percentage (3%-4%) of HER2-low tumors were

Table 1. Patient characteristics and correlation of HER2-low with different clinicopathological parameters

	All (n = 691) N (%)	HER2-0 (n = 470) N (%)	HER2-low (n = 221) N (%)	P value
Median age[a] (range)	58 (25-103)	58 (26-102)	60 (25-103)	0.211[b]
AJCC stage at initial diagnosis				
Stage I-III	476 (68.9)	329 (70.0)	147 (66.5)	
I	42 (17.4)	28 (17.5)	14 (17.1)	0.431
II	67 (27.7)	40 (25.0)	27 (32.9)	
III	133 (55.0)	92 (57.5)	41 (50.0)	
Unknown (but not stage IV)	234 (49.2)	169 (51.4)	65 (44.2)	
Stage IV (*de novo* metastatic)[c]	212 (30.8)	141 (30.0)	71 (32.6)	0.572
Unknown	3 (0.4)	0 (0.0)	3 (1.4)	
DFS (excluding stage IV)				
DFS <24 months	301 (63.2)	204 (62.0)	97 (67.1)	0.405
DFS ≥24 months	175 (36.8)	125 (38.0)	50 (32.9)	
Grade primary tumor				
1	8 (1.4)	7 (1.8)	1 (0.5)	0.507
2	137 (23.9)	89 (23.0)	48 (25.7)	
3	429 (74.7)	291 (75.2)	138 (73.8)	
Unknown	117 (16.9)	83 (17.7)	34 (15.4)	
Number of metastatic sites[a]				
Mean/median (range)	1.82/1 (0-8)	1.82/1 (0-8)	1.81/1 (0-7)	
1	344 (49.8)	232 (49.4)	112 (50.7)	0.759
2-3	274 (39.7)	184 (39.2)	90 (40.7)	
≥4	61 (8.8)	44 (9.4)	17 (7.7)	
Metastatic sites[a]				
Visceral disease	416 (61.0)	278 (60.2)	138 (62.7)	0.327
Non-visceral disease only	266 (39.0)	184 (39.8)	82 (37.3)	
Unknown	9 (1.3)	8 (1.7)	1 (0.5)	
Histologic subtype				
No special type (NST)	431 (81.6)	287 (80.9)	144 (83.2)	0.750
Invasive lobular	25 (4.0)	15 (4.2)	10 (5.8)	
Other	72 (13.6)	53 (14.9)	19 (11.0)	
Unknown	163 (23.6)	115 (24.5)	48 (21.7)	
Estrogen receptor (ER) status				
ER <1%	454 (65.8)	299 (63.8)	155 (70.1)	0.144
ER 1%-9%	27 (3.9)	17 (3.6)	10 (4.5)	
ER <10% (not known if <1% or 1%-9%)	209 (30.3)	153 (32.6)	56 (25.3)	
Unknown	1 (0.1)	1 (0.2)	0 (0.0)	
HER2-status				
IHC0	470 (68.0)	470 (100.0)	0 (0.0)	NA
IHC1+	143 (20.7)	0 (0.0)	143 (64.7)	
IHC2+ and ISH negative	78 (11.3)	0 (0.0)	78 (35.3)	
Treatment for metastatic disease				
Chemotherapy	590 (90.2)	398 (90.3)	192 (90.1)	0.965
Chemotherapy unknown	37 (5.4)	29 (6.2)	8 (3.6)	
Anti-HER2 therapy	13 (2.4)	9 (2.4)	4 (2.4)	0.921
Anti-HER2 therapy Unknown	148 (21.4)	101 (21.5)	47 (21.3)	

AJCC, American Joint Committee on Cancer; DFS, disease-free survival; IHC, immunohistochemistry; ISH, *in situ* hybridization.
[a]At diagnosis of metastatic disease.
[b]Wilcoxon two-sample test, all others were chi-square tests.
[c]Including 40 patients with a history of non-triple-negative early breast cancer (25 in HER2-0, 15 in HER2-low).

HER2-enriched according to PAM50, which underlines the missing driver function of HER2-low.

One limitation of this analysis is that no central HER2 (and ER) testing was conducted, and the HER2-status was extracted from the available pathology report. The known interpathologist variability,[1,20] especially in the differentiation of HER2-0 and 1+, could have influenced our results. Another potential confounder is the fact that in our cohort HER2 testing was carried out in a timeframe of almost 20 years in which the standards of staining techniques and the guidelines for interpretation have slightly changed.[21] Nevertheless, the participating centers are generally experienced tertiary cancer centers with clearly standardized procedures for HER2 testing and reporting, which should minimize this potential bias. Furthermore, our analysis included ER-low-positive disease (1%-9%), which could have influenced the results, since ER-positive disease is known to have a higher frequency of HER2-low.[22] However, we could not detect any difference in OS for the three ER categories (<1%, 1%-10%, and <10%) in the multivariable model.

Conclusion

In this large international dataset of metastatic TNBC, the frequency of HER2-low was 32.0%. In contrast to HER2 positivity, HER2-low did not influence OS in metastatic TNBC.

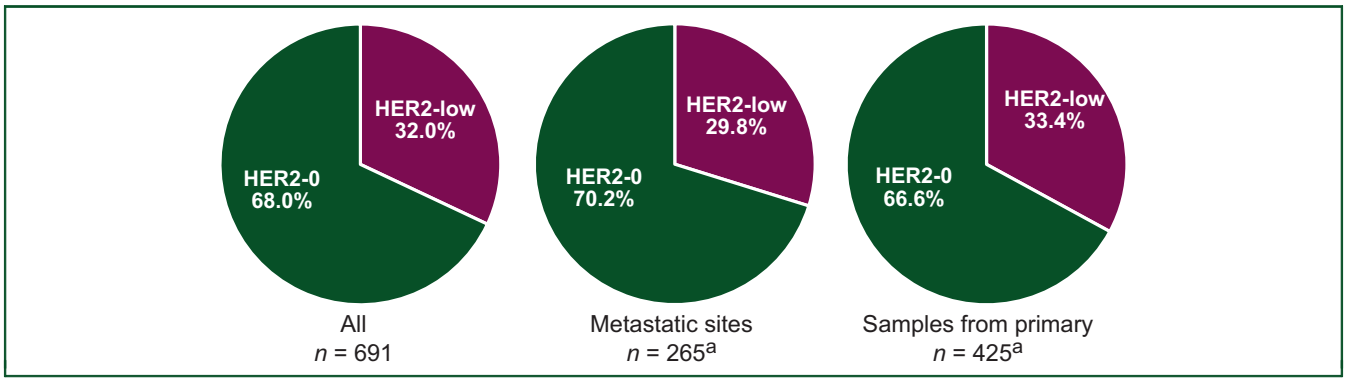

Figure 1. Frequency of low HER2 expression in metastatic patients with TNBC.
TNBC, triple-negative breast cancer.
[a]Origin of biopsy not known in one patient.

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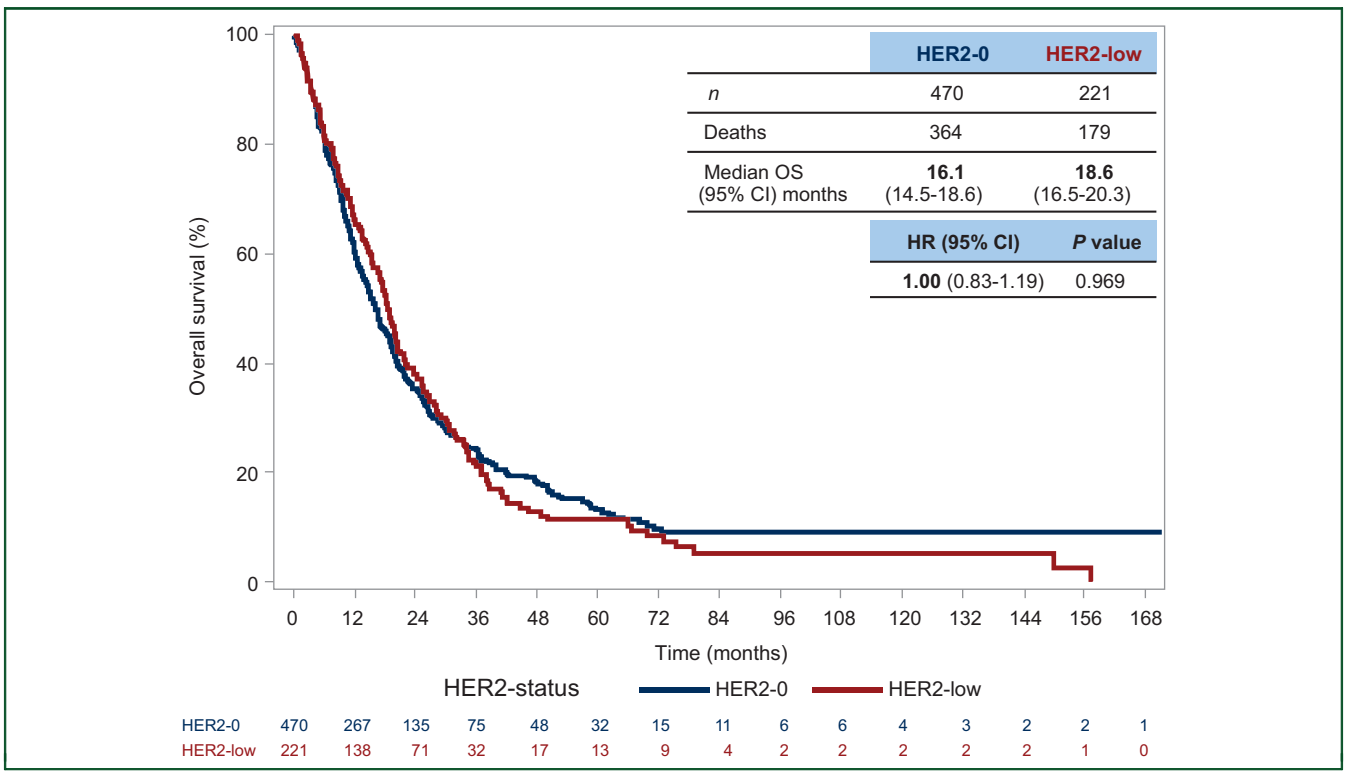

	HER2-0	HER2-low
n	470	221
Deaths	364	179
Median OS (95% CI) months	**16.1** (14.5-18.6)	**18.6** (16.5-20.3)

HR (95% CI)	*P* value
1.00 (0.83-1.19)	0.969

Figure 2. OS of patients with HER2-low versus HER2-0 metastatic TNBC.
The median progression-free survival values and the HR are the most important values and are indicated in bold.
CI, confidence interval; HR, hazard ratio; OS, overall survival; TNBC, triple-negative breast cancer.

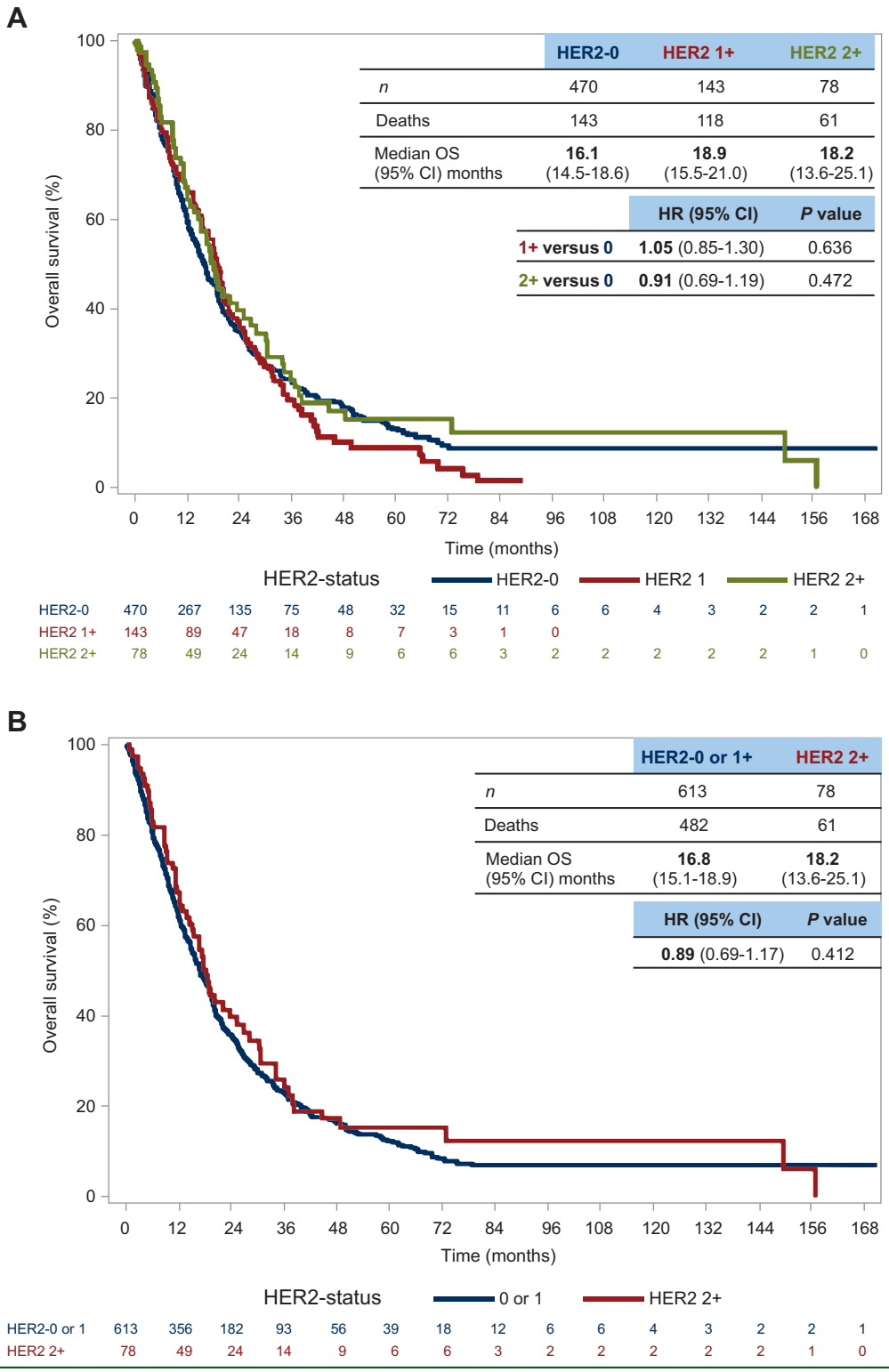

Figure 3. OS according to HER2-expression category.
The median progression-free survival values and the HR are the most important values and are indicated in bold.
(A) IHC 2+ versus IHC 1+ versus IHC 0; (B) IHC 2+ versus IHC 0/1+.
CI, confidence interval; HR, hazard ratio; IHC, immunohistochemistry; OS, overall survival.

DISCLOSURE

SPG: Invited speaker: Novartis, Roche, BMS, AstraZeneca, MSD, Pfizer, Lilly, Seagen, and Daiichi Sankyo; Advisory Board: Novartis, Roche, BMS, AstraZeneca, MSD, Pfizer, Lilly, Seagen, and Daiichi Sankyo; Research grant: Roche; Travel grant: Roche, Amgen, Shire, Novartis, Pfizer, Bayer, Celgene, and Daiichi Sankyo. VD: Honoraria: Roche and Daiichi Sankyo. ML: Advisory Board: Roche, AstraZeneca, Eli Lilly, Pfizer,

MSD, Seagen, Gilead, Exaxt Sciences, and Novartis; Honoraria: Roche, Sandoz, Takeda, Pfizer, Eli Lilly, Knight, Libbs, and Novartis. ADN: Research grant: Pfizer, Novartis, and Lilly; Travel grant: Amgen, Daiichi Sankyo. MM: Advisory Board: Roche, Lilly, Novartis, AstraZeneca, Daiichi Sankyo, and Pfizer; Honoraria: Roche, Eli Lilly, Novartis, AstraZeneca, Daiichi Sankyo, Pfizer, MSD, and Medmedia; Travel grant: Amgen, Roche, Novartis, Pierre Fabre, and Daiichi Sankyo. FLD: Advisory Board: Novartis, Roche, Daiichi Sankyo, Pfizer, Lilly, Seagen, and Sandoz; Honoraria: Novartis, Roche, Daiichi Sankyo, Pfizer, Lilly, Seagen, Sandoz, and Amgen; Travel grant: Novartis, Roche, Daiichi, Pfizer, Lilly, Seagen, Pierre Fabre, and Amgen. ACS: Advisory Board: Clovis, Lilly, Pfizer, GSK, Ferrer, and Roche; Research grant: Pfizer; Honoraria: GSK, AstraZeneca, Roche, MSD, and Esai; Travel grant: Roche, Daiichi Sankyo, and Pfizer. DAC: Advisory Board: Merck Sharp & Dohme, Nestlé, Novartis, and Pfizer; Research grant: Cuf Oncologia and AstraZeneca; Full or part-time Employment: Cuf Oncologia and NTT DATA; Honoraria: AstraZeneca, Roche, Merck KGaA, Novartis, NTT DATA, Pfizer, Roche, and Uriage; Travel grant: Daiichi Sankyo, Gilead, GSK, Merck KGaA, Merck Sharp & Dohme, Novartis, and OM Pharma. MVB: Advisory Board: Daiichi Sankyo; Honoraria: Novartis, Roche, Daiichi Sankyo. AP: Advisory Board: Novartis, Amgen, Celgene-BMS, Sandoz, Janssen, AstraZeneca, Abbvie, Takeda, Sanofi, Kite-Gilead, Roche, Pfizer, Saegen, and Daiichi Sankyo; Honoraria: Novartis, Amgen, Celgene-BMS, Sandoz, Janssen, AstraZeneca, Abbvie, Takeda, Sanofi, Kite-Gilead, Roche, Pfizer, Saegen, and Daiichi Sankyo. LDM: Honoraria: Roche, Novartis, Pfizer, Pierre Fabre, Seagen, Gilead, Daiichi-Sankyo, MSD, Exact sciences, Takeda, Ipsen, Eisai, Eli Lilly, and Celgene. FB: Research grant: Pfizer, Novartis, and Lilly. AG: Research grant: Pfizer, Novartis, and Lilly; Travel grant: Novartis. RB: Advisory Board: AstraZeneca, Lilly, Novartis, Pfizer, Pierre-Fabre, Roche, and Seagen; Research grant: Daiichi Sankyo, MSD, Novartis, and Roche; Honoraria: AstraZeneca, Daiichi Sankyo, Eisai, Lilly, MSD, Novartis, Pfizer, Pierre-Fabre, Puma, Roche, and Seagen; Travel grant: Roche, Daiichi Sankyo, Lilly, and Pfizer. CSS: Travel grant: Lilly, Pfizer. Invited speaker: Novartis. LP: Honoraria: Daiichi-Sankyo, SOTIO Biotech, and Beckman-Coulter. CS: Advisory Board: Roche, AstraZeneca, Novartis, Daiichi Sanyko, and Amgen; Research Grant: Amgen, Novartis, and AstraZeneca; Travel Grant: Pfizer, Amgen, Roche, AstraZeneca, and Lilly. NH: Honoraria: Astra Zeneca, Daiichi-Sankyo, Lilly, MSD, Novartis, Pierre Fabre, Pfizer, Roche, Sandoz/Hexal, and Seagen. GR: Advisory role: Roche, AstraZeneca, Daiichi Sankyo, Pfizer, Pierre Fabre, Eli Lilly, MSD, Novartis, Amgen, and Merck; Honoraria: Roche, Gilead, Pfizer, Eli Lilly, and Novartis. RG: Advisory role: Celgene, Novartis, Roche, BMS, Takeda, Abbvie, Astra Zeneca, Janssen C., MSD, Merck, Gilead, Daiichi Sankyo, Sanofi, and Pfizer; Honoraria: Celgene, Novartis, Roche, BMS, Takeda, Abbvie, Astra Zeneca, Janssen C., MSD, Merck, Gilead, Daiichi Sankyo, Sanofi, and Pfizer; Travel grant: Roche, Amgen, Janssen, and AstraZeneca. All other authors have declared no conflicts of interest.

DATA SHARING

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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