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Research Article

Clinical and Molecular Evaluation of HER2-Low and HER2-Ultralow Breast Cancer in the Penelope-B Clinical Trial Cohort

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ABSTRACT

The DestinyBreast (DB)04 and DB06 trials have shown clinical activity of trastuzumab-deruxtecan (T-DXd) in HER2-low and HER2-ultralow metastatic breast cancer. The identification of HER2-low and HER2-ultralow breast cancer is therefore essential for personalized therapy with T-DXd. We evaluated 723 residual tumors from the Penelope-B trial (NCT01864746) and correlated different

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levels of HER2 protein expression with prognosis and messenger RNA (mRNA) profiles, including HER2 transcripts. In Penelope-B, 57.68% (n = 417) of 723 residual tumors were HER2 low. The HER2-ultralow category was assigned to 109 (15.08%) tumors, and 197 (27.25%) tumors were completely HER2 negative (HER2 0). In Kaplan-Meier analysis, there were no survival differences among these 3 subgroups. There was no significant difference in HER2 mRNA expression between HER2-0 and HER2-ultralow tumors ($P = .08$). In contrast, there was a highly significant difference in HER2 mRNA expression between HER2-ultralow and HER2-low tumors ($P < .0001$) and between HER2-low and HER2-positive tumors ($P < .0001$). The extracellular protease cathepsin L, which has been suggested as a biomarker for extracellular cleavage of T-DXd, was detectable in all HER2-related subgroups and was a negative prognostic factor for invasive disease-free survival and overall survival ($P = .0001$) in preneoadjuvant core biopsies. In our study, we were able to characterize HER2 low as a clinically relevant and molecular defined tumor group with significantly increased HER2 expression. In contrast, for HER2 ultralow, we did not observe a defined molecular phenotype, despite the clinically relevant regulatory approval of T-DXd also in the ultralow subgroup. Additional investigations are needed to identify biomarkers beyond HER2 for T-DXd response as a basis for refined criteria for treatment eligibility.

Introduction

Since the 1990s, HER2 (*ERBB2*) has been the prototype of a predictive biomarker in breast cancer (BC),[1,2] and strong HER2 overexpression is required for trastuzumab response.[3] This established concept has been challenged by the development of anti-HER2 antibody-drug conjugates (ADCs). The DestinyBreast (DB)04 trial showed that patients with HER2-low tumors attain a significant benefit from trastuzumab-deruxtecan (T-DXd),[4] and subsequently, international HER2 reporting guidelines have been adapted to address this new tumor category.[5,6]

Whereas pathologists were still working on the characterization of HER2-low BC,[7,8] clinical trialists were already pushing the boundary to an even lower HER2 level, based on the phase 2 DAISY trial.[9] Recently, the DB06 trial[10] has shown a benefit from anti-HER2–ADC therapy also in patients with HER2-ultralow tumors. Consequently, the Food and Drug Administration (FDA) has approved T-DXd for therapy in unresectable or metastatic hormone receptor (HR)-positive BC with HER2-low or HER2-ultralow expression. The DB06 definition of HER2 ultralow as membrane expression in <10% of tumor cells is not covered by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines.[11]

From a biomarker perspective, this clinical trial result is challenging, and it is currently not clear how the existing international guidelines should be modified to include this new potential subgroup.[12] To provide a molecular basis for these biomarker decisions, we have evaluated the biological profile of HER2-low, HER2-ultralow, and HER2-0 BC in a large postneoadjuvant cohort from the phase III Penelope-B trial.[13] In this trial cohort, it was possible to evaluate a large cohort of patients with HR-positive, HER2-negative BC treated with high clinical risk after neoadjuvant chemotherapy (NACT). We focused on the research question whether HER2-low/-ultralow BC constitute biologically distinct molecular groups that could be characterized by gene expression.

Materials and Methods

Clinical Trial Cohort

The Penelope-B trial (NCT01864746; EudraCT 2013-001040-62) is a prospective, randomized, double-blind, placebo-controlled phase 3 trial in patients with HR-positive BC without pathological complete response to taxane-containing NACT. The aim of the trial was to investigate postneoadjuvant addition of the CDK4/6 inhibitor palbociclib for 1 year to standard adjuvant endocrine therapy. The trial was negative for the primary endpoint and did not show a significant benefit from postneoadjuvant palbociclib. Details of clinical parameters and results have been published.[14] HER2 was evaluated centrally before randomization by a combination of immunohistochemistry (IHC) and in situ hybridization (ISH), based on the ASCO/CAP recommendation,[11] including HER2 low (IHC 1+) as a separate category. For central IHC, the Ventana 4B5 antibody (Roche) was used, and ISH was performed with the Ventana HER2 dual SISH system (Roche). This central assessment was focused on residual tumors, which were also used for HER2-low and HER2-ultralow analysis. For HER2 ultralow, all cases that were IHC 0 in the central evaluation before randomization were reevaluated; HER2 ultralow was defined as any membranous staining in <10% of tumor cells. As suggested by the updated ASCO/CAP reporting recommendations, the evaluation included the intensity and the estimation of the percentage of positive cells; it was performed based on standard microscopy, without digital image analysis. Details of the histopathological analysis including example images and images with typical pitfalls for reporting have been published before.[15,16] The tumors without any staining were designated as HER2 0. In addition, we included a cohort of 50 residual tumors with HER2 overexpression from the GeparSixto, GeparSepto, GeparOcto, and GeparX neoadjuvant trials.

Gene Expression Analysis

Gene expression in postneoadjuvant tumors was analyzed with the HTG EdgeSeq Oncology Biomarker Panel (HTG Molecular Diagnostics Inc) targeting 2559 genes, as described before.[17] Based on 91 genes of this panel, Absolute Intrinsic Molecular Subtyping (AIMS) was calculated.[18] The AIMS approach provides standardized information about luminal A, luminal B, HER2-enriched, and basal-like subtypes based on gene expression, independent of the technical platform. Differences in gene expression among different groups were evaluated using LIMMA (v3.50.3) in R. Differential gene expression analysis involved

quantile normalization, followed by model fitting with lmfit and contrast matrices. Statistical testing was performed using eBayes, with adjusted *P* values for multiple comparisons. For analysis of cathepsin L (CTSL), messenger RNA (mRNA) expression data from n = 629 pretherapeutic core biopsies were evaluated as well.[17]

Statistical Evaluation

All statistical tests were performed 2 sided at a significance level of $P < .05$. The differences for invasive disease-free survival (iDFS) or overall survival (OS) were assessed using univariate Cox regression models and represented as hazard ratios with 95% CI. Statistical significance was assessed using Wald tests. Kaplan-Meier cumulative curves for iDFS and OS were drawn for patients with different HER2 expression and for AIMS subtypes. Differences between groups were analyzed using the log-rank test.

Results

Prevalence of HER2 Low/Ultralow in the Penelope-B Cohort

A total of 723 (57.84%) residual tumors with mRNA expression data were evaluated for HER2-low and HER2-ultralow status based on IHC (Fig. 1A). An overview of baseline parameters of the biomarker cohort compared with the complete Penelope-B cohort is shown in Supplementary Table S1. The definitions for HER2-related subgroups are shown in Figure 1B. Based on the ASCO/CAP guidelines from 2023[6] and 2018,[11] the classical HER2-positive and the HER2-low status (as defined in DB04) could be determined. The DB06 trial used the established ASCO/CAP guidelines to focus on an additional subgroup of HER2-ultralow tumors with incomplete and faint/barely perceptible membrane staining in ≤10% of tumor cells. This resulted in a remaining negative subgroup of HER2-0 tumors without any detectable membrane staining.

In the Penelope-B cohort, 57.68% (n = 417) of 723 residual tumors were HER2 low. The HER2-ultralow category was assigned to 109 (15.08%) tumors, and the remaining 197 (27.25%) tumors were completely negative for HER2 expression (HER2 0; Fig. 2A). Within the HER2-ultralow group, 50.46% had 1% positive cells and 92.66% had ≤5% positive cells (Fig. 2B). Staining of <1% was not observed in our cohort. In survival analysis, there was no difference in iDFS and OS in the 3 subgroups (Fig. 2C, D).

HER2-Low/-Ultralow Expression Is Not Related to Specific Absolute Intrinsic Molecular Subtypes

Based on mRNA expression of the post-NACT tumors, AIMS subtypes were determined.[18] As shown in Supplementary Figure S1, there was no systematic difference in AIMS subtypes in the 3 HER2 groups; the majority of tumors in each group were LumA, and the HER2-enriched AIMS subtype was only detected in a small subset of tumors. A combined survival analysis of AIMS subtypes and HER2-low versus HER2-IHC0 expression based on DB04 definition is shown in Supplementary Figure S2A, B. In high-risk AIMS subtypes (Basal/HER2E/LumB), the prognosis was statistically significantly improved in HER2-low tumors compared with HER2-IHC0 tumors (Supplementary Fig. S2E, F). In a combined analysis of subgroups based on DB06 (0, ultralow, and low; Supplementary Fig. S3A, B), a reduced prognosis was observed in the small group of tumors with HER2-ultralow and AIMS Basal/HER2E/LumB compared with other 2 groups (Supplementary Fig. S3E, F). For the low-risk AIMS subtypes, LumA/NormL, there was not survival difference in the different HER2-related groups, neither as defined in DB04 (Supplementary Fig. S2C, D) nor according to DB06 (Supplementary Fig. S3C, D). The interpretation of these combined analyses is limited by the small number of patients included in some of the subgroups.

Increased Expression of HER2 Messenger RNA in HER2-Low, but not HER2-Ultralow, Tumors

We evaluated HER2 mRNA expression in HER2-0, HER2-ultralow, and HER2-low residual tumors from Penelope-B and added a cohort of HER2-positive residual tumors (IHC3+ or 2+ with positive ISH) for further comparison (Fig. 3A). As shown in Figure 3A, there was no significant difference in HER2 mRNA expression between HER2-0 and HER2-ultralow tumors ($P = .08$). In contrast, there was a highly significant difference in HER2 mRNA expression between HER2-ultralow and HER2-low tumors ($P < .0001$) as well as between HER2-low and HER2-positive tumors ($P < .0001$). The differentially expressed genes in the different groups are shown in Figure 3B-E. Strong overexpression of the *ERBB2* gene was observed in HER2-low compared with HER2-IHC0 tumors (Fig. 3A); this overexpression was even stronger in the comparison of HER2-positive with HER2-low tumors (Fig. 3C). The comparison of HER2-0 versus HER2-ultralow tumors did not show a difference in HER2 expression (Fig. 3D), whereas HER2-ultralow versus HER2-low tumors showed clear overexpression of the *ERBB2* gene in the HER2-low subgroup (Fig. 3E). It should be noted that in classical HER2-positive BC, the HER2 mRNA expression is ~300% higher compared with HER2-low BC, whereas it is only ~41% higher in HER2-low tumors compared with HER2-IHC0 tumors. For HER2-ultralow tumors, there was no difference compared with HER2-0 tumors.

Cathepsin L Is Expressed in the Penelope-B Cohort Across All HER2-Related Subgroups

We evaluated the expression of CTSL mRNA, which had been identified as a regulator of extracellular T-DXd cleavage in functional assays and mouse experiments.[19] As shown in Figure 4A, CTSL was expressed across all HER2-related subgroups, with considerable heterogeneity and minimal differences in mean expression between subgroups. CTSL mRNA expression was detected as a negative prognostic marker in preneoadjuvant core biopsies (n = 629; Fig. 4B, C), but not in residual tumors (data not shown). The survival difference for CTSL4 was highly significant for iDFS (Fig. 4B; $P < .0001$) as well as OS (Fig. 4C; $P = .0001$).

Discussion

Based on the results of the DB06 trial, T-DXd has been approved in metastatic HR-positive BC with HER2-low or HER2-ultralow expression. These results are challenging the long-standing established principles of HER2 evaluation. We have characterized HER2-low and HER2-ultralow tumors in post-neoadjuvant tumors from the Penelope-B trial. HER2-low tumors were identified as a defined subgroup with significantly increased levels of HER2 gene expression. In contrast, in HER2-ultralow tumors, no differential expression of HER2 or other

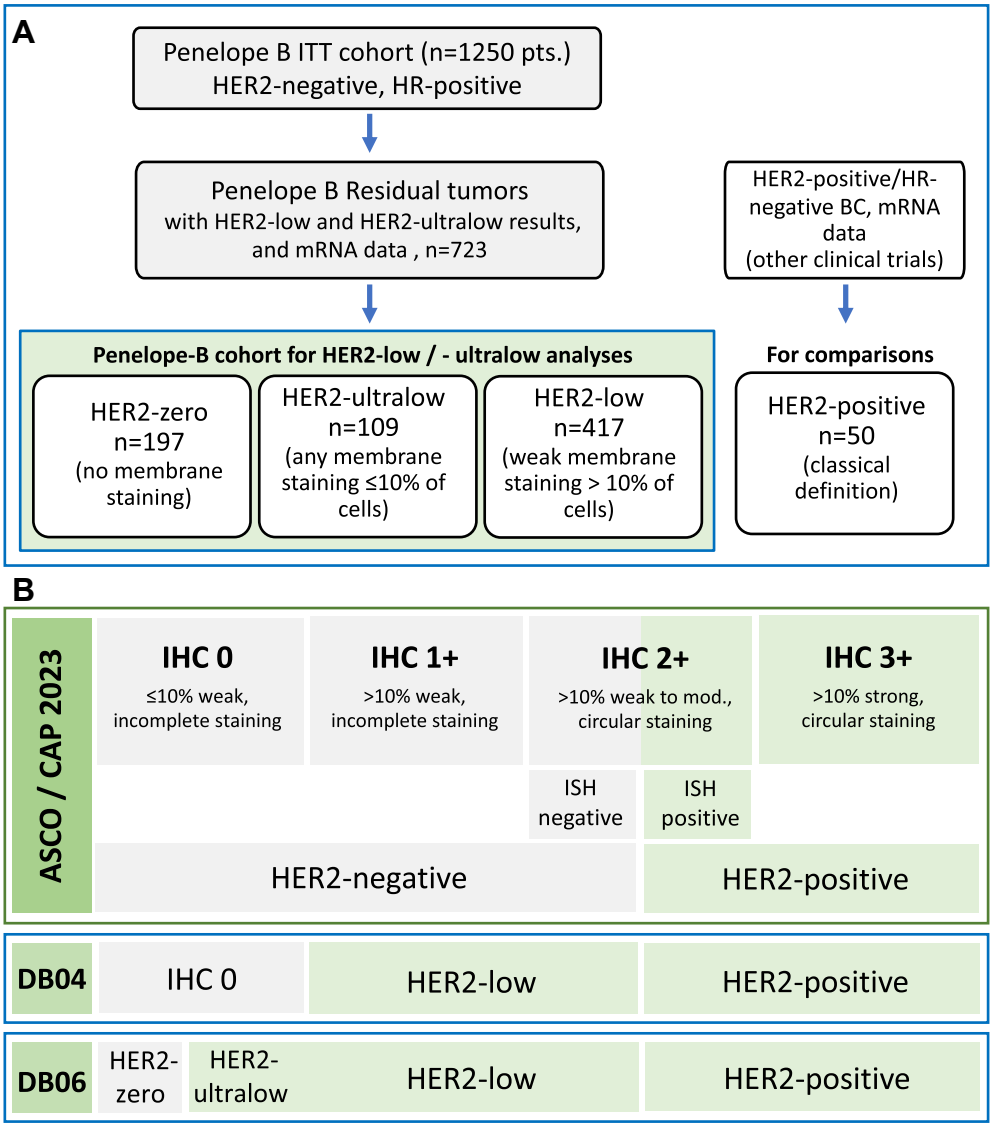

Figure 1.
Description of the Penelope-B residual tumor cohort as well as the different definitions for HER2 subgroups used in this study. (A) A cohort of 723 residual tumors from the Penelope-B trial with existing mRNA data are included; the cohort consists of 195 HER2-0 tumors (with no membrane staining), 109 HER2-ultralow tumors (with membrane staining in ≤10 of tumor cells), and 417 HER2-low tumors (weak membrane staining in >10% of cells). For selected comparison with classical HER2-positive tumors, we included a cohort of 50 residual tumors with HER2 overexpression from other neoadjuvant trials. (B) Overview of the definitions used in this study. The classical ASCO/CAP guidelines were recently updated, without a change compared with the previous version. The definitions used in the DB04 trial introduced the new group of HER2-low tumors, defined as HER2 IHC1+ or IHC2+ with negative ISH; for this definition, no change of the general ASCO/CAP evaluation scheme is necessary. In contrast, DB06 used the existing ASCO/CAP guideline scoring criteria with a focus on the additional group of HER2-ultralow tumors, defined as any membrane staining in ≤ 10% of tumor cells. Those tumors without any HER2 membrane staining are designated as the HER2-0 group. ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; BC, breast cancer; DB04, Destiny Breast04; DB06, Destiny Breast06; HR, hormone receptor; IHC, immunohistochemistry; ISH, in situ hybridization; ITT, intention to treat; mRNA, messenger RNA.

characteristic genes could be detected. Other reported candidate biomarkers for T-DXd, in particular the extracellular protease CTSL, were expressed in all subgroups. The results suggest that it is currently not necessary to further modify the ASCO/CAP HER2 guidelines, because HER2-ultralow tumors do not constitute a distinct biological tumor group. Research activities should be focused on biomarkers beyond HER2 for T-DXd response.

The results of the DB06 trial[10] have stimulated a discussion in both the pathology and the oncology communities.[12,20] For oncologists, the aim is to offer new therapeutic options to all patients who might derive a benefit based on the results of clinical trials. In the DB06 trial, the hazard ratio for improved survival with T-DXd was 0.62 (95% CI, 0.52-0.75; $P < .001$) in the HER2-low cohort and 0.78 (95% CI, 0.50-1.21; significance not tested) in the HER2-ultralow cohort. Because the overall effect in DB06 is consistent in the subgroups of patients with HER2-low as well as HER2-ultralow tumors, there is an obvious motivation to identify and treat both groups of patients also with T-DXd in clinical practice. It should be emphasized that in this situation, pathologists might decide to use provisional and pragmatic diagnostic criteria in routine diagnostic work to ensure that patients would have access to FDA-approved therapies.

In the long run, however, the central strategy is to adhere to reliable diagnostic categories, which are needed to ensure that all

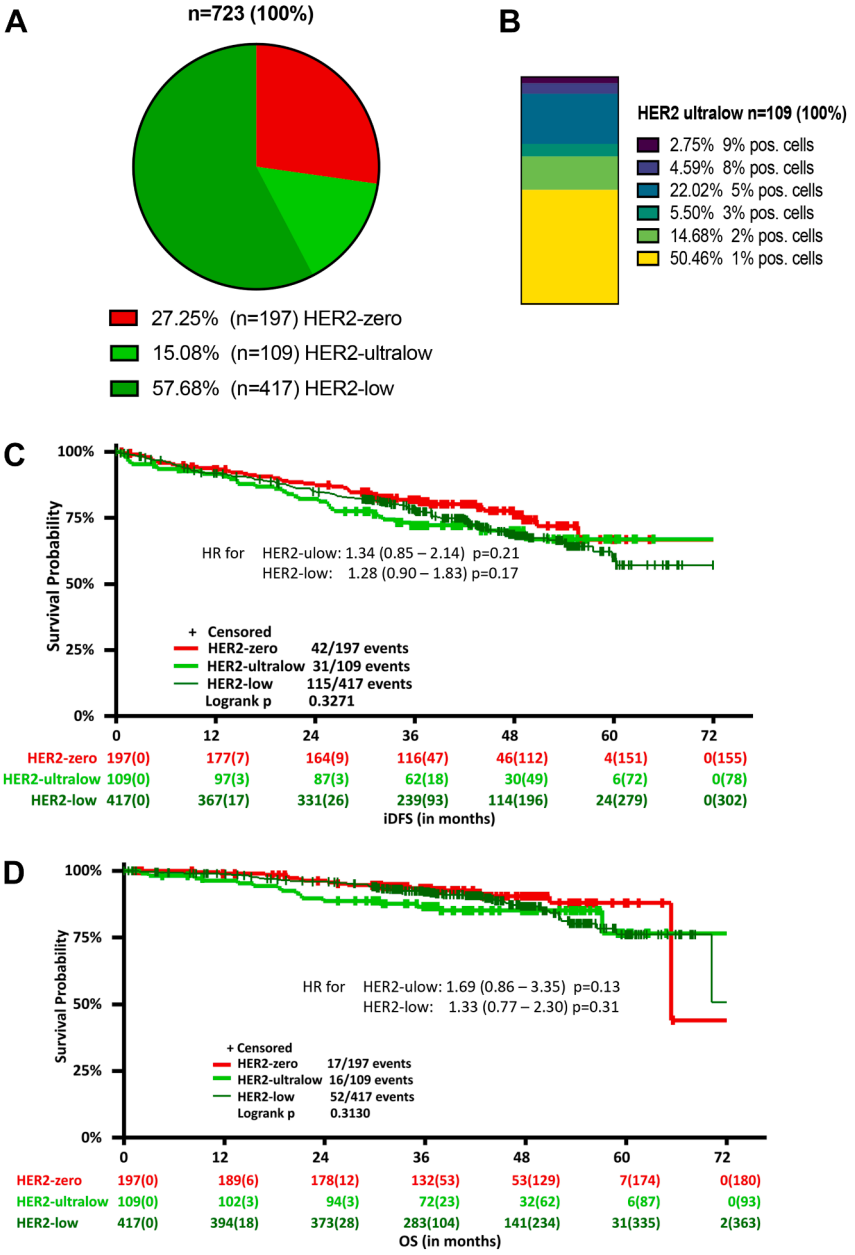

Figure 2.
Prevalence and survival analysis of different HER2-related groups in the Penelope-B residual tumor cohort. (A) Prevalence of HER2-0, HER2-ultralow, and HER2-low tumors in the residual tumor cohort. (B) Distribution of the percentage of positive cells within the HER2-ultralow subgroup. (C, D) Survival analysis of the 3 different groups in the Penelope-B residual tumor cohort; iDFS (C), OS (D). HR, hazard ratio; IDFS, invasive disease-free survival; OS, overall survival.

breast tumors worldwide are categorized based on identical diagnostic principles. For the pathologist community, our research results provide important information for diagnosis of tumors within the spectrum of low, ultralow, and 0/null IHC scores. Interestingly, as a positive result, the category of HER2-low tumors is a detectable biological subgroup in our evaluation. We showed that HER2-low tumors have significantly higher levels of HER2 mRNA expression, which could serve as an example that diagnostic identification of HER2-related tumor groups is possible also with HER2 expression levels below classical HER2 overexpression. In our mRNA panel, molecular differences between HER2-low and HER2-IHC0 tumors are limited, but HER2-low tumors show a significant increase of HER2 as a molecular alteration compared with HER2-ultralow as well as HER2-0 tumors. It should be noted that on an individual case level, mRNA assays are unlikely to be useful as a reflex test to distinguish tumors with very low levels of expression.

The new treatment options based on ADC therapy justify the use of HER2 low as a defined category for clinical diagnosis and treatment stratification. The HER2-low category is identical with the IHC1+/ISH-negative group already defined in the ASCO/CAP HER2 guidelines.[6]

In our study, we have not been able to identify molecular markers characteristic for HER2-ultralow tumors—not even increased HER2 mRNA levels, using the mRNA panel used in the Penelope-B cohort. Therefore, there is currently no molecular basis to characterize HER2 ultralow as a defined separate group.

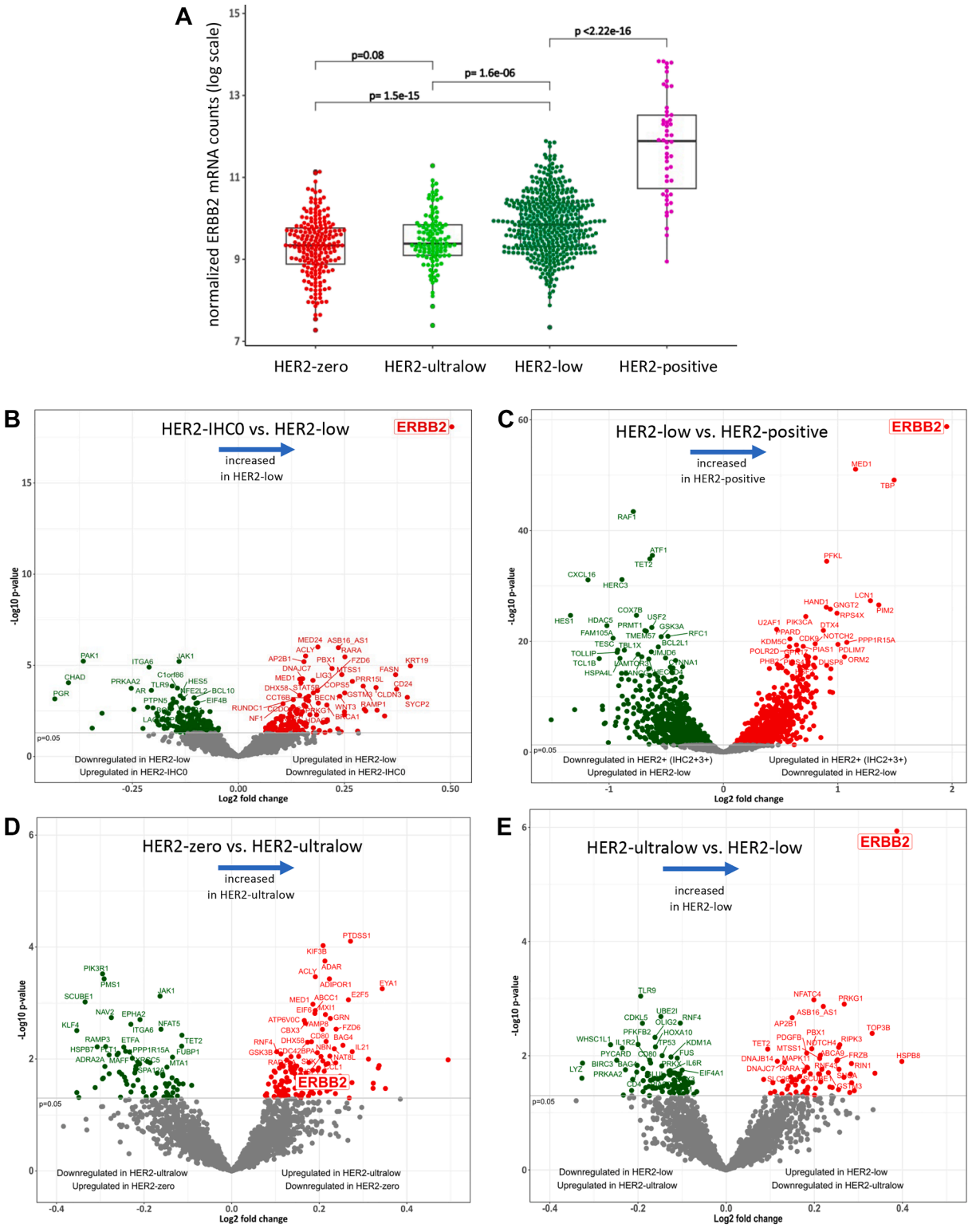

Figure 3.

Differential gene expression in different HER2-related subgroups as defined by the ASCO/CAP guidelines, in comparison with the definitions used in the DB04 and DB06 trials. (A) Comparison of expression levels of HER2 (*ERBB2*) mRNA HER2-0, HER2-ultralow, HER2-low, and HER2-positive tumors. Normalized expression of the *ERBB2* gene is shown on a log scale. (B, C) Comparison of HER2-related groups according to DB04 definition: HER2 IHC0 versus HER2 low, with strong overexpression of the HER2 (*ERBB2*) gene in HER2 low (B); HER2 low versus HER2 positive with even stronger overexpression of the HER2 gene in HER2 positive. (D, E) Comparison of HER2-related groups as defined in DB06: HER2 0 versus HER2 ultralow, without a difference in HER2 expression (D); HER2 ultralow versus HER2 low, with clear overexpression of the HER2 (*ERBB2*) gene in the HER2-low subgroup (E). It should be noted that the scale of the y-axis is identical in panels D and E, but different from panels B and C, with stronger overexpression of the HER2-positive group. ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; BC, breast cancer; DB04, Destiny Breast04; DB06, Destiny Breast06; IHC, immunohistochemistry; mRNA, messenger RNA.

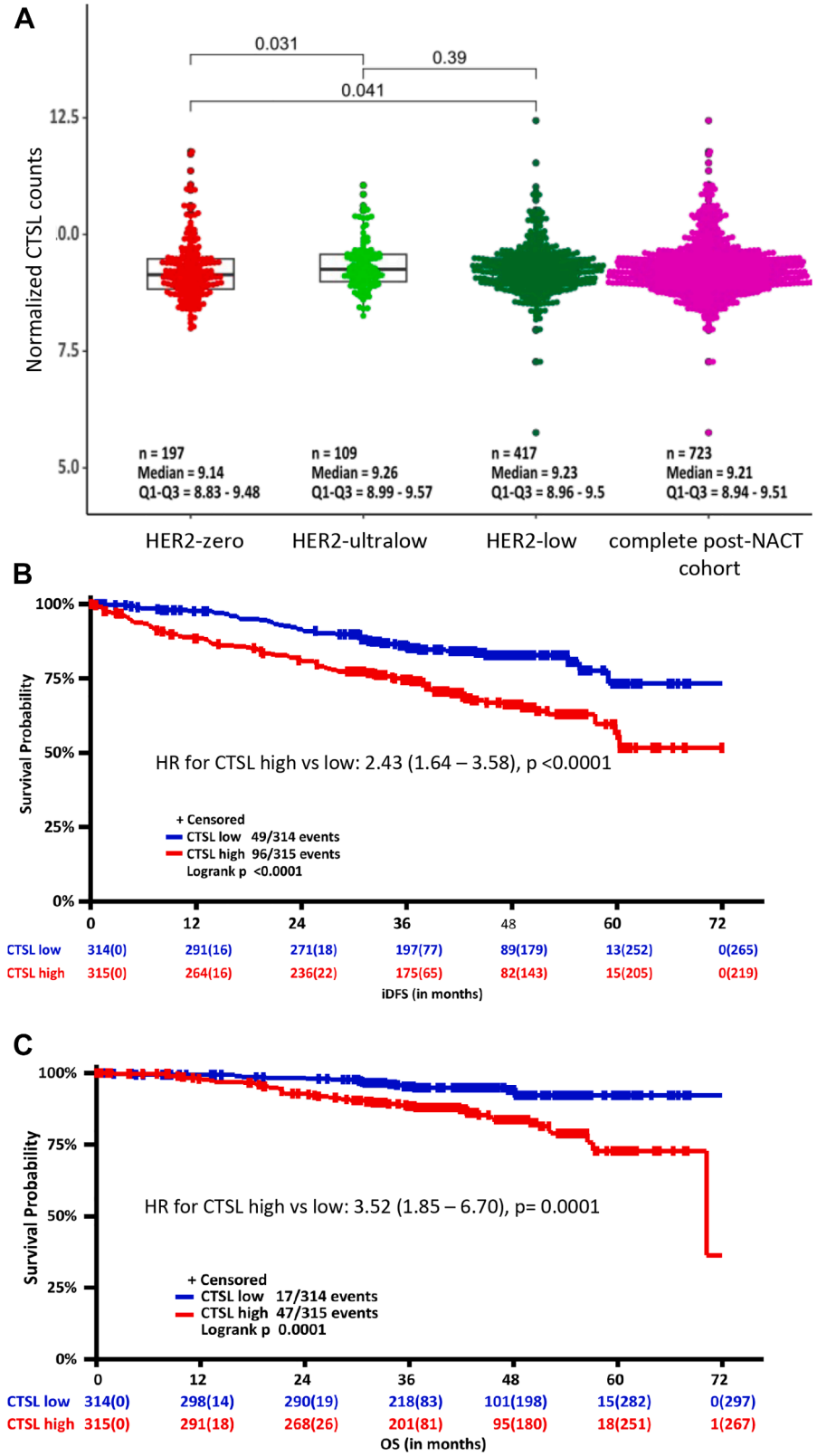

Figure 4.
Evaluation of Cathepsin L (CTSL) messenger RNA expression in the Penelope-B cohort. (A) Messenger RNA expression levels of CTSL in residual tumors from the Penelope-B group, in the different HER2-related subgroups as well as the complete cohort. (B) Prognostic relevance of CTSL in pretherapeutic core biopsies (n = 629) from the Penelope-B cohort for IDFS. (C) Prognostic relevance of CTSL in pretherapeutic core biopsies from the Penelope-B cohort for OS (cutpoint: median; *P* values: log-rank test). HR, hazard ratio; IDFS, invasive disease-free survival; NACT, neoadjuvant chemotherapy; OS, overall survival.

Low, ultralow, and 0/null IHC scores were not associated with differences in molecular subtypes or survival. A combination of AIMS subtypes and HER2-low status was relevant for prognosis in post-NACT samples, but in this combination, the prognostic differences were mainly derived from the established AIMS subtypes. We included a small group of classical HER2-positive tumors in our evaluation that was collected outside of the Penelope trial for comparisons with the HER2-low group. The expression levels of HER2 were considerably higher in this classical HER2-positive group, and increased HER2 mRNA expression was also identified as a main difference in this group of tumors.

Our results show that HER2 expression at both protein and mRNA levels is not a suitable parameter to identify HER2-ultralow tumors. Therefore, additional biomarkers to predict the response to T-DXd, in particular in the HER2-ultralow group, are urgently needed. Tsao et al[19] have systematically evaluated mechanisms of action of T-DXd in experimental systems. They have shown that ADC binding to HER2, followed by internalization and subsequent intracellular cleavage of the linker between trastuzumab and DXd, is a relevant mode of action in HER2-positive tumor cells, with extension of the chemotherapy effect to adjacent HER2-negative cells in a bystander effect. However, in HER2-negative tumors, they reported the additional option of extracellular cleavage of the linker between trastuzumab and DXd by tumor-specific cathepsin proteases, in particular, CTSL.

As a first step to further validate these findings, we have evaluated expression of CTSL in the Penelope-B cohort and found that it was independent of HER2 expression and could be detected in a subset of tumors across different HER2 levels, including HER2-0 tumors. In addition, we found a negative prognostic impact of CTSL expression in pretherapeutic core biopsies in the Penelope-B trial. CTSL has been described as a modulator of stroma reorganization in tumor tissue with relevance for increased tumor aggressiveness.[21,22]

From these observations, a possible complex future biomarker concept for T-DXd activity in tumors with different levels of HER2 expression is emerging, which combines extracellular and intracellular cleavage and payload release options, with different relevance of the intracellular versus extracellular release depending on HER2 expression levels. The predictive role of these markers can only be validated in clinical trial cohorts with T-DXd treatment, such as the DAISY and the DB06 trials, and these validations would be essential to improve current biomarkers for T-DXd. This extended focus for biomarker research would be more promising than trying to integrate HER2-ultralow tumors into the ASCO/CAP guidelines. It might be particularly relevant for the future interpretation of the results of the ongoing DB15 clinical trial. In DB15, patients with HER2-0 tumors (without any membrane staining) are included, as well. In this situation, additional biomarkers beyond HER2 would be needed to identify those HER2-0 tumors that would have the optimal benefit from T-DXd. The existing sample collections from the DAISY and the DB06 trials constitute an optimal basis for identification of these additional biomarkers, as also suggested by Wolff et al.[12] Based on existing preclinical results, a strong candidate for an additional biomarker would be CTSL. The results generated in DAISY and DB06 could then be validated in the DB15 trial cohort. This would be important to avoid the futile use of T-DXd, which might happen if further predictive biomarkers are not identified, resulting in unnecessary side effects for patients as well as financial burden to the health care systems.[23]

Our study is the first evaluation of HER2-low and HER2-ultralow tumors in a prospective postneoadjuvant clinical trial cohort of high-risk HR-positive BC. It should be noted as a limitation that triple-negative tumors were not included in Penelope-B and that we did not evaluate pretherapeutic core biopsies for HER2-ultralow expression. Furthermore, patients included in Penelope-B did not receive T-DXd treatment, and our survival analysis does not include effects related to the specific therapy. Therefore, additional studies in other cohorts are needed to validate our results. Furthermore, the analysis was based on a targeted RNA panel covering only a subset of the transcriptome, which does not rule out the possibility that additional markers could be identified using broader genomic, transcriptomic, or proteomic approaches.

In conclusion, our results show that HER2-low BC can be identified based on the classical ASCO/CAP guidelines, without adaptation of these guidelines. In contrast, with the approach used in our study, we could not detect any biological parameter that would characterize HER2-ultralow BC as distinct from HER2-0 BC, not even HER2 gene expression. These results suggest that the HER2-ultralow category might not be a defined molecular subgroup and an adaptation of the ASCO/CAP guidelines to fully integrate this subgroup is currently not justified. To ensure access of patients to FDA-approved T-DXd therapy in the metastatic setting, pragmatic diagnostic categories might be used by pathologists as an interim solution until additional data on predictive biomarkers are generated.

The results are relevant for postneoadjuvant clinical concepts that would add new ADC treatment options to therapy-resistant luminal BC. Additional translational investigations could be the basis for future biomarkers beyond HER2 that would reflect the different actions of anti-HER2 ADCs in the tumor microenvironment, with extracellular cleavage as a possible additional mechanism.

Author Contributions

C.D. performed conceptualization, data curation, funding acquisition, investigation, development of methodology, project administration, resource aquisition, supervision, validation, visualization, and writing the original draft. S.R. performed conceptualization, data curation, formal analysis, investigation, development of methodology, software acquisition, supervision, validation, visualization, and writing, review, and editing. A.P. performed conceptualization, data curation, investigation, development of methodology, and writing, review, and editing. F.M. performed conceptualization, data curation, resource aquisition, supervision, and writing, review, and editing. M.M. performed conceptualization, data curation, resource aquisition, and writing, review, and editing. T.K. performed investigation, development of methodology, resource aquisition, supervision, and writing, review, and editing. M.U. performed conceptualization, data curation, formal analysis, resource aquisition, and writing, review, and editing. H. Bonnefoi performed conceptualization, data curation, formal analysis, resource aquisition, and writing, review, and editing. S.-B.K. performed conceptualization, data curation, formal analysis, resource aquisition, and writing, review, and editing. N.H. performed data curation, project administration, resource aquisition, and writing, review, and editing. H. Bear performed conceptualization, data curation, formal analysis, resource aquisition, and writing, review, and editing. A.K.W. performed conceptualization, data curation, formal analysis, resource acquisition, and writing, review, and editing. S.-A.I. performed conceptualization, data curation, formal analysis, resource aquisition, and writing, review, and editing. A.D. performed conceptualization, data curation, formal analysis, resource aquisition, and writing, review, and editing. L.V.V. performed conceptualization, data curation, formal

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Data Availability

Clinical data and biomarker data reported in this article are available for research groups who provide translational research proposals. Proposals should be approved by the Penelope-B Translational Board. Proposal forms can be requested from trafo@gbg.de. Once the application has been approved and a data transfer agreement has been signed, researchers will be given access to the data.

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Declaration of Competing Interest

C.D. reports receiving grants from EU-H2020, Federal Ministry of Education and Research, German Cancer Aid, and German Breast Group (GBG), during the conduct of the study; personal fees from Novartis, Roche, BioNTech, MSD Oncology, Daiichi-Sankyo, Merck, AstraZeneca, and MolecularHealth; and institutional grants from Myriad, outside the submitted work. C.D. has a patent VMscope digital pathology software with royalties paid, a patent application related to prognostic subtypes in high-risk luminal breast cancer pending, as well as patents WO2020109570A1 and WO2015114146A1 pending, and WO2010076322A1, issued. S.R. reports receiving institutional grants from Daiichi-Sankyo, Gilead, Novartis, Pfizer, Roche, and Seagen during the conduct of this work, and from AbbVie, AstraZeneca, BMS, Daiichi-Sankyo, MolecularHealth, Novartis, Roche, and Pfizer, outside this work. S.R. declares to be a GBG Forschungs GmbH employee; receiving royalties/patents (EP14153692.0, EP21152186.9, EP15702464.7, and EP19808852.8) from GBG Forschungs GmbH and VM Scope GmbH as well as a patent application related to prognostic subtypes in high-risk luminal breast cancer pending. T.K. declares a patent application related to prognostic subtypes in high-risk luminal breast cancer pending. M.U. reports consulting fees paid to the institution from AstraZeneca, Daiichi-Sankyo, Lilly, MSD Merck, Myriad Genetics, Novartis, Pierre Fabre, Pfizer, Gilead, Roche, Sanofi-Aventis, Seagen, Stemline, and CD Pharma and honoraria paid to the institution from Astra Zeneca, Daiichi-Sankyo, Lilly, Myriad Genetics, Novartis, Pierre Fabre, Pfizer, Roche, Sanofi-Aventis, and Stemline, all outside the submitted work. S.-B.K. reports receiving institutional grants from Novartis and Sanofi-Aventis, personal consulting fees from AstraZeneca, Beigene, DaeHwah Pharma, Daiichi-Sankyo, Ensol Bioscience Inc, ISU Abxis, Lilly, Novartis, and OBI Pharma, and stock/stock options from Genopeaks and Neogene TC, all outside this work. N.H. reports receiving institutional grants from Daiichi-Sankyo, Gilead, Novartis, Pfizer, Roche, and Seagen during the conduct of this work and from AbbVie, AstraZeneca, BMS, Daiichi-Sankyo, MolecularHealth, Novartis, Roche, and Pfizer, outside this work. He declares to be a GBG Forschungs GmbH employee, and reports receiving royalties/patents (EP14153692.0, EP21152186.9, EP15702464.7, EP19808852.8) assigned to GBG Forschungs GmbH, and VM Scope GmbH as well as a patent application related to prognostic subtypes in high-risk luminal breast cancer pending. H. Bear reports stock ownership from Pfizer, AbbVie, and Viatris and research support from Merck Sharp & Dohme. N.M. reports receiving travel support from Novartis, AstraZeneca, Gilead, and Merck and participation in data safety or advisory board from Novartis, Gilead, and Merck, outside the submitted work. P.J. reports receiving grants and travel expenses from Gilead Sciences GmbH outside the submitted work. K.A.G. reports working in advisory boards AstraZeneca, Pfizer, Novartis, Gilead, Celuity, City of Hope, and Merck. C.W. is supported by the postdoctoral lecture qualification program of the Anneliese Pohl Foundation, Marburg. C.M.K. reports receiving travel support from Novartis. T.R. reports receiving institutional grants from German Cancer Aid, Else Kroener Fresenius Foundation, and German Society of Senology,

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She declares to be an GBG Forschungs GmbH employee; receiving royalties/patents (EP14153692.0, EP21152186.9, EP15702464.7, EP19808852.8) from GBG Forschungs GmbH and VM Scope GmbH as well as a patent application related to prognostic subtypes in high-risk luminal breast cancer pending. V.N. reports receiving institutional grants from Daiichi-Sankyo, Gilead, Novartis, Pfizer, Roche, and Seagen, during the conduct of this work, and from AbbVie, AstraZeneca, BMS, Daiichi-Sankyo, MolecularHealth, Novartis, Roche, and Pfizer, outside this work. She declares to be a GBG Forschungs GmbH employee and reports receiving royalties/patents (EP14153692.0, EP21152186.9, EP15702464.7, EP19808852.8) from GBG Forschungs GmbH and VM Scope GmbH as well as a patent application related to prognostic subtypes in high-risk luminal breast cancer pending. 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S.L. reports receiving nonfinancial interest as an advisory role in AGO Kommission Mamma, as the principal investigator (Aphinity), as a member in AGO, ASCO, DKG, and ESMO, and other nonfinancial interest from AstraZeneca, Daiichi-Sankyo, immunomedica/Gilead, Novartis, Pfizer, Roche, and Seagen. A.P., F.M., M.M., H. Bonnefoi, A.K.W., S.-A.I., A.D., L.V., T.S., J.A.G.-S., N.F., and J.T.-S. report no conflict of interest.

Ethics Approval and Consent to Participate

All patients provided written informed consent for trial participation and biomaterial collection. The translational investigations were approved by the Ethics committee of the Philipps-University Marburg (Project 38/20).

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