STUDY REPORT SYNOPSIS

D9673R00004 HER2-low Retrospective Epidemiology Study

A Multicenter Study to Estimate the Prevalence of HER2-low and Describe the SoC, Treatment Patterns, and Outcome in Real-world Practice among Unresectable and/or Metastatic Breast Cancer Patients with HER2-low Status – the RetroBC-HER2L Study

Milestones:	First patient in:	28 May 2021
	Preliminary data cut	12 Nov 2021
	Interim data cut	27 Jan 2022
	Final data cut	28 Apr 2022
	Final database lock	11 Jul 2022
	Final clinical study report	19 Dec 2022
Phase of development:	Retrospective Non-Interventional	
Sponsor:	AstraZeneca	

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This study was performed in compliance with Good Clinical Practice (GCP) and Good Pharmacoepidemiology Practice (GPP), including the archiving of essential documents.

Background/rationale: Breast cancers traditionally classified as human epidermal growth factor receptor 2 negative (HER2-neg) encompass ~ 80% of all breast cancers and represent a spectrum of human epidermal growth factor receptor 2 (HER2) expression. The validated HER2 immunohistochemistry (IHC) assays are optimized to select human epidermal growth factor receptor 2 positive (HER2-pos) patients suitable for traditional anti-HER2 antibody-based therapies, where HER2-neg definitions include both IHC 2+/in situ hybridization (ISH)- and IHC 1+ cancers (collectively referred to herein as human epidermal growth factor receptor 2 low [HER2-low]), and cancers with lower but detectable HER2 staining in less than 10% tumor cells (referred to herein as IHC > 0 < 1+), or no detectable staining (referred to herein as HER2-null). The latter 2 categories have been collectively defined as HER2 IHC zero by American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines 2018. Herein, HER2 IHC > 0 < 1+ (< 10% staining) and HER2-null (no staining) will be used to refer to ASCO/CAP definition of HER2 IHC zero. HER2-neg breast cancer subgroups that express low levels of HER2 have traditionally been defined as binary (HER2-pos/HER2-neg) and none of the targeted therapies tested in patients with lower HER2 expression, demonstrated efficacy, however, more recently HER2-low patients have been identified as a targetable patient population.

Treatment options for metastatic breast cancer depend on several factors including, but not limited to, the patient's overall health and the levels of hormone receptor (HR) and HER2 expression per IHC category in the tumor. For HER2-pos cancers, IHC score 3+ and 2+/ISH+, therapies that target HER2 combined with chemotherapy, endocrine therapy (ET), or other anti-HER2 agents can significantly improve survival. In a recently completed trial involving patients with previously treated HER2-low advanced metastatic breast cancer, trastuzumab deruxtecan (T-DXd), an antibody-drug conjugate, demonstrated significant antitumor activity and was approved in the US for the treatment of patients with metastatic HER2-low breast cancer (DESTINY-Breast04).

This worldwide (10 countries), multicenter, non-interventional, retrospective study aimed to describe the prevalence and clinical characteristics of metastatic HER2-low breast cancer by accurate rescoring of archived HER2 slides of HER2-neg patients and analyzing standard of care and clinical outcomes from medical chart abstraction or other means (eg, electronic health records [EHR], electronic medical records [EMR], and biobanks).

Objectives:

Primary objectives and hypotheses

- To describe the overall prevalence and disease burden of HER2-low (HER2 IHC score 1+, 2+/ISH-) among unresectable and/or metastatic breast cancer patients identified as HER2-neg, based on rescoring of historical HER2 fixed tissue IHC stained slides by Ventana 4B5 assay.
- To describe baseline patient characteristics, clinical presentation, treatment patterns, and clinical outcomes in HER2-low breast cancer patients (time to first subsequent treatment [TFST], time to treatment failure [TTF], overall survival [OS]), compared with the HER2 IHC zero patient population.

Secondary objectives and hypotheses

- To describe HER2-low disease by histopathological and clinicopathological characteristics compared with the HER2 IHC zero patient population.

- To characterize the concordance between historical HER2 IHC scores and local laboratory rescoring in the HER2-neg region (HER2 IHC scores 0, 1+, and 2+).
- To describe HER2-low prevalence among unresectable and/or metastatic breast cancer patients identified as HER2-neg based on other IHC assays, compared with Ventana 4B5 assay.
- To describe HER2-low prevalence in HR-positive (HR+) and HR-negative (HR-) population.

Exploratory objectives and hypotheses

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Study design: Worldwide, multicenter, non-interventional, retrospective study of patient medical records from metastatic breast cancer patients previously identified as HER2-neg, regardless of hormone status.

Data source: HER2 fixed tissue slide for rescoring and/or retesting, other biomarker testing results of archived tissue samples and curated patient level data derived from both medical chart abstraction, or other means including EHR/EMR/biobanks.

Study population: Patients with confirmed diagnosis of HER2-neg, unresectable and/or metastatic breast cancer regardless of hormone status dating back from 31 December 2017 - but not older than 01 January 2014 - who progressed on any systematic anti-cancer therapy (eg, ET, chemotherapy, cytokine-dependent kinase 4 and 6 inhibitor (CDK4/6i), targeted therapies other than anti-HER2, or immunotherapy).

Inclusion criteria:

Patients fulfilling all of the following criteria were eligible for this study:

- Men or women
  - ≥ 18 years of age when consent provided for future sample and clinical data use - applicable for all countries participating in the study except Japan.
  - ≥ 20 years of age when consent provided for future sample and clinical data use - applicable for Japan only.
- Had a histological or cytological confirmed diagnosis of unresectable or/and metastatic breast cancer between 01 January 2014 and 31 December 2017.

- Provided written consent allowing for data and samples to be used in this study and in the future. This will be covered by the consent for future use. If the patient is deceased, a waiver could be accepted.
- Diagnosed as HER2-neg (HER2 IHC scores 0, 1+, 2+/ISH-), regardless of hormone status.
- Progressed on any systemic anti-cancer therapy (eg, ET, chemotherapy, CDK4/6i, targeted therapies other than anti-HER2, or immunotherapy) in the metastatic setting.
- Had historical HER2 fixed tissue IHC stained slides (preferably stained using Ventana 4B5 assay) in acceptable quality for accurate rescoring.

Exclusion criteria:

Patients who met any of the following criteria were disqualified from entering the study:

- Had a history of other malignancies, other than basal cell carcinoma of the skin and squamous cell carcinoma of the skin.
- Patients with historical HER2 status of IHC 2+/ISH+ or 3+, or HER2 amplified.

Statistical methods: The patients identified for the current study were a sample of all patients in the relevant EHR/EMR databases and biobanks who met the inclusion and exclusion criteria; a sample size estimation was performed to achieve a 5% precision. It was planned to enroll a total of ~ 1000 HER2-neg metastatic breast cancer patients and aimed to capture ~ 400 HER2-low patients; the number of total HER2-neg metastatic breast cancer patients was subject to revision based on preliminary data analysis. Since over 60% of patients were rescored as HER2-low in the interim analysis, the sample size was reduced to 800 patients as it was considered very likely that overall, at least 400 patients would be HER2-low. Furthermore, assessments showed there would be no significant loss in precision for the primary and secondary outcomes by reducing the target from 1000 patients to 800 patients. Enrollment was closely followed to ensure that at least 400 patients were HER2-low, and at least 400 patients had their HER2 slide assessed using Ventana 4B5 assay.

Standard summary statistics were used for all descriptive variables, as appropriate, and are presented by HER2 and HR status. Continuous variables were summarized by the number of observations, mean, standard deviation, median, minimum, and maximum. Categorical variables were summarized by frequency counts and percentages for each category. Where appropriate, significant differences in characteristics and outcome measures of interest between HER2 groups were determined using chi-square test or Fisher's exact test. Digital scoring data analysis was performed as per a separate Statistical Analysis Plan (SAP) which has been appended to this Clinical Study Report (CSR). A preliminary analysis was conducted when ~ 233 eligible HER2-neg patients had been enrolled to assess the assumptions made when determining the sample size; an interim analysis was conducted based on the data collected for the first 400 HER2-neg patients enrolled.

Final Analyses Results:

For the purpose of consistent assessment of data collected during this study and presentation of results, HER2-low and HER2 IHC zero were compared, and defined as follows:

- HER2-low: comprising of HER2 IHC scores 1+, and 2+/ISH-.

- HER2 IHC zero: comprising of HER2-null (no staining) and IHC > 0 < 1+ (< 10% staining).

Analysis Sets and Patient Disposition

- Patients from 13 study sites in 10 countries were enrolled in the study.
- A total of 798 patients were enrolled in the study.
  - Of these, 563 patients were HR+, 161 patients were HR-, and 74 patients had missing HR status.
- Almost all enrolled patients (795 [99.6%] patients) were rescored as HER2-neg using Ventana 4B5 or other IHC assay. These patients comprised the Full Analysis Set (FAS). The remaining 3 of 798 patients were excluded from the FAS because they either had no rescore available or were HER2-pos.
- A total of 789 of 795 patients in the FAS adhered to the protocol and were included in the PP Set for analyses.
- A total of 635 of the 789 patients in the Per Protocol (PP) Set adhered to the protocol and had at least one metastatic treatment start date within 30 days prior to index date or 60 days after index date. These patients comprised the Evaluable Population (EP) Set. The decreased number compared to the PP set was due to patients without available dates. Index date was defined as the date of earliest metastatic breast cancer diagnosis identified during the patient selection period. For patients without metastasis during patient selection period, the earliest date of unresectable diagnosis during patient selection period was used as index date.
- A total of 297 of the 795 patients who were a subset from the FAS with available archived tissue samples and independent retesting of HER2 IHC, comprised the Archived Tissue Analysis (ARCH) Set.

HER2-low Prevalence Within the HER2-negative Population

- In this study, 789 patients were rescored as HER2-neg and were adherent to the protocol, of whom 787 (99.7%) patients had available rescores. The majority of HER2 slides were rescored by a Ventana 4B5 assay (556 [70.6%] HER2-neg patients).
- Overall, for all types of breast cancer, the prevalence of HER2-low was 67.2%.
  - There were no significant differences in HER2-low prevalence when rescores were assessed using a Ventana 4B5 (68.2%) and other assays (63.8%) ($p = 0.2528$).
- Overall, the HER2-low prevalence was statistically significantly greater ($p < 0.0001$) in the HR+ population versus HR- population (71.1% versus 52.8%, respectively).
- There were no numerical differences in the HER2-low prevalence between the patients in whom tissue samples were taken from the primary tumor site and patients in whom tissue samples were taken from a metastatic tumor site overall, or within each HR population.

Patient Demographic and Baseline Characteristics

- At index date, most patients (556 of 789 patients) were HR+, almost all patients (99.7%) were female. Most HER2-low metastatic breast cancer patients in this study were in the [redacted] to [redacted] year age group. Most of the patients were either White (46.4%) or Asian (23.4%). Most of the patients (43.5%) had never been smokers.
- There were no notable differences in demographic characteristics, co-morbidities, baseline or clinical characteristics between the HER2-low and HER2 IHC zero groups within both the HR- and HR+ populations.

Treatments Received

- Within the HR+ population, most patients received endocrine therapy (90.0%) followed by chemotherapy (75.3%).
  - No patients within the HR+ population were treated with immunotherapy.
  - There were no notable differences in the use of individual treatment agents between the HER2-low and HER2 IHC zero groups.
- Within the HR- population, all patients received chemotherapy.
  - In the HR- population, 18.3% patients received endocrine therapy and 18.3% patients received other targeted therapies. A total of 13.5% patients were treated with immunotherapy.
  - There were no notable differences in the use of individual treatment agents between the HER2-low and HER2 IHC zero groups.
- No meaningful differences in proportions were seen in the type of first treatment in metastatic setting across HER2-low and HER2 IHC zero groups within each HR population.
- No meaningful differences in proportions were seen in the type of monotherapy or combination therapy as first treatment in metastatic setting across HER2-low and HER2 IHC zero groups within each HR population.
- Limited data were available for lines of therapy because of limitations with the completeness of data sources.

Clinical Outcome Measures

- Overall, the clinical outcome median durations (TTF, TFST and OS) were generally similar between the HER2-low and HER2 IHC zero groups within the HR+ and HR- populations.
- Overall, patients in the HR+ population had a median OS of 43.89 months, and more than half of these patients (58.2%) were known to have died at the end of the study.
- Overall, patients in the HR- population had a median OS of 20.11 months, and most of these patients (81.0%) were known to have died at the end of the study.

Concordance of Historical HER2 IHC Score and Rescore in the HER2-neg Region

- The overall percentage agreement in historical and rescored HER2-neg IHC scores, irrespective of assay type, was 81.3% and the Kappa (95% confidence interval [CI]) between the historical and local laboratory rescores was 0.583 (0.523, 0.643), suggesting a moderate level of agreement between the historical HER2 IHC scores and the HER2-neg rescored values using any assay.
  - The interpretations were similar for Ventana 4B5 assay (81.8%) and non-Ventana assay (80.0%).
- A numerically greater proportion of patients scored historically as HER2-low were rescored the same (87.5%), compared to those scored historically as HER2 IHC zero score (69.9%).
  - This same interpretation can be made for Ventana 4B5 assay (90.3% versus 67.9%) but not non-Ventana assay, where the proportion of slides rescored the same was similar for HER2-low and HER2 IHC zero slides (80.7% versus 78.3%).

Concordance of Historical HER2 IHC score and Rescore in the HER2-neg Region (HER2 IHC scores zero, 1+, and 2+/ISH-)

- The overall percentage agreement in historical and rescored HER2-neg IHC scores, irrespective of assay type, was 66.8%, and the Kappa (95% CI) between the historical and local laboratory rescores was 0.484 (0.434, 0.535), suggesting a moderate level of agreement between the historical and rescored values using any assay.
  - The interpretations were similar for Ventana 4B5 and non-Ventana assays.

Histopathological Characteristics at Baseline

- The most common histopathological types of breast cancer overall were invasive ductal (44.1% patients), invasive carcinoma not otherwise specified (NOS) (14.6% patients) and invasive lobular (10.0% patients).
- Overall, there were no notable differences in the frequency of the histopathological types of breast cancer between the HER2-low and HER2 IHC zero groups, in the HR+ and HR- breast cancer populations.

Exploratory Endpoints

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Additional analysis

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Conclusion: This was a global, large sample size retrospective study of HER2-neg metastatic/unresectable breast cancer study with standardized training for rescoring and concordance analyses. However, results should be interpreted with caution due to the retrospective observational nature of the study.

- Sixty-seven percent (67%) of historically HER2-neg patients were HER2-low, suggesting that a majority of HER2-neg breast cancer patients may benefit from HER2-low targeted treatments.
- A moderate level of agreement was observed between historical and rescored HER2 slides (Kappa = 0.583, overall agreement 81.3%). This study provides evidence that

slides that were historically scored HER2-low are more likely to be rescored the same than those that were historically scored HER2 IHC zero using the Ventana 4B5 assay.

- No obvious differences were observed between HER2-low and HER2 IHC zero patients, irrespective of HR status in terms of demographics, clinical presentation, treatment patterns, clinical outcomes, histopathological and clinicopathological outcomes.
- The typical HER2-low metastatic breast cancer patient is female, aged 50+ years, and HR+.
- Seventy-five percent (75%) of HR+ patients and all HR- patients received chemotherapy during the observed period.
- Over half of the HR+ patients did not survive for 4 years from metastatic/unresectable breast cancer diagnosis, and over half of the HR- patients did not survive for 2 years, further demonstrating the unmet need for effective treatment options in this patient population.

Publications:

Spitzmüller A, Kapil A, Shumilov A, Chan J, Konstantinidou L, Hassan Z, et al. Computational pathology–based HER2 expression quantification in HER2-low breast cancer. Poster P6-04-03. San Antonio Breast Cancer Symposium. 2022;Dec 6-10.

Viale G, Basik M, Niikura N, Tokunaga E, Brucker S, Penault-Llorca F, et al. Retrospective study to estimate the prevalence and describe the clinicopathological characteristics, treatment patterns, and outcomes of HER2-low breast cancer. Poster HER2-15. San Antonio Breast Cancer Symposium. 2022;Dec 6-10.

Viale G, Niikura N, Tokunaga E, Aleynikova O, Hayashi N, Sohn J, et al. Retrospective study to estimate the prevalence of HER2-low breast cancer (BC) and describe its clinicopathological characteristics. Journal of Clinical Oncology. 2022; 40(16) suppl: 1087-1087.