Supplementary Appendix

Supplement to: Modi S, Jacot W, Yamashita T, et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. N Engl J Med. DOI: 10.1056/NEJMoa2203690

This appendix has been provided by the authors to give readers additional information about the work.

SUPPLEMENTARY APPENDIX

Table of Contents

List of Investigators

Country, Site	Principal Investigator
Austria	
Klinikum Wels-Grieskirchen GmbH Abteilung fur Innere Medizin IV	Thomas Kühr
Kepler Universitatsklinikum Med Campus III Clinic of Internal Medicine 3 - Hematology and Oncology	Clemens Schmitt
Uniklinikum Salzburg, Landeskrankenhaus Universitatsklinik fur Innere Medizin III der PMU	Richard Greil
University Hospital Innsbruck	Daniel Egle
Belgium	
Universitaire Ziekenhuizen Leuven	Hans Wildiers
Institut Jules Bordet	Andrea Gombos
Grand Hôpital de Charleroi	Jean-Luc Canon
Cliniques Universitaires Saint-Luc	François Duhoux
Canada	
Tom Baker Cancer Center - Oshawa	Jan-Willem Henning
McGill University - Dept. Oncology Clinical Research Program	Jamil Asselah
China	
Cancer Hospital Chinese Academy of Medical Sciences	Binghe Xu
Fudan University Shanghai Cancer Center	Xichun Hu
Harbin Medical University Cancer Hospital	Qingyuan Zhang
Liaoning Cancer Hospital and Institute	Tao Sun
Sun Yat-sen Memorial Hospital, Sun Yat-sen University	Qiang Liu
The First Hospital of Jilin University	Wei Li
Zhejiang Cancer Hospital	Xiaojia Wang
Anhui Provincial Hospital	Yueyin Pan
Sir Run Run Shaw Hospital - Zhejiang University School of Medicine	Xian Wang
Hubei Cancer Hospital	Xinhong Wu
900TH Hospital of Joint Logistics Support Force	Xi Chen
West China Hospital, Sichuan University	Ting Luo
The Affiliated Drum Tower Hospital of Nanjing University	Wenjing Hu
Linyi Cancer Hospital	Jingfen Wang
Fujian Medical University Union Hospital	Xiaoyan Lin
France	
Institut Regional du Cancer de Montpellier - ICM Val d'Aurelle	William Jacot
Clinique Victor Hugo - Centre Jean Bernard	Hugues Bourgeois
Centre Eugene Marquis	Claudia Lefeuvre-Plesse
Centre CARIO-HPCA	Anne-Claire Hardy Bessard
CLCC - Institut de Cancerologie de l'Ouest (ICO) - Rene Gauducheau	Jean-Sebastien Frenel

Hôpital Tenon	Joseph Gligorov
Institut Curie	Jean-Yves Pierga
Centre Francois Baclesse	Christelle Levy
Institut Sainte Catherine	Julien Grenier
Hôpital d'Instruction des Armees Begin	Carole Helissey-Danis
Gustave Roussy	Fabrice André
Institut de Cancerologie de l'Ouest Site Paul Papin	Paule Augereau
Centre Hospitalier Valenciennes	Javed Sahir
L'Institut Paoli - Calmettes	Frederic Viret
CHU Brest - Hôpital Morvan	Helene Simon
Clinique Clementville	Cristian Villanueva
Greece	
Athens Medical Center	Marinos Tsiatas
Euromedica General Clinic Thessaloniki	Konstantinos Papazisis
University General Hospital of Heraklion	Dimitrios Mavroudis
General Hospital of Athens - Alexandra	Flora Zagouri
General Hospital of Thessaloniki -Papageorgiou	Christos Papandreou
Bioclinic of Thessaloniki	Ioannis Boukovinas
Interbalkan Medical Center of Thessaloniki	Sofia Baka
University General Hospital of Larisa	Athanasios Kotsakis
Hungary	
Orszagos Onkologiai Intezet	Gabor Rubovszky
SzSzB Megyei Korhazak es Egyetemi Oktatokorhaz	Klara Mezei
Israel	
Tel Aviv Sourasky Medical Center	Amir Sonnenblick
The Chaim Sheba Medical Center	Rinat Bernstein Molho
Rabin Medical Center - Beilinson Hospital	Rinat Yerushalmi
Rambam Medical Center	Ruth Perets
Kaplan Medical Center	Ella Evron
Hadassah University Hospital - Ein Kerem	Beatrice Uziely
Italy	
Ospedale degli Infermi - Rimini	Lorenzo Gianni
Azienda Ospedaliero Universitaria Policlinico Sant'Orsola Malpighi	Claudio Zamagni
Azienda Ospedaliero Universitaria Mater Domini-Campus Universitario	Pier Francesco Tassone
Azienda Ospedaliero Universitaria Policlinico - Vittorio Emanuele (Presidio Gaspare Rodolico)	Hector Soto Parra
Azienda Socio Sanitaria Territoriale di Monza (Presidio San Gerardo), U.O Oncologia Medica	Marina Elena Cazzaniga
Azienda Ospedaliero Universitaria Policlinico Tor Vergata	Ilaria Portarena

IEO Istituto Europeo di Oncologia	Marco Colleoni
Istituto Clinico Humanitas Rozzano, IRCCS	Giovanna Masci
Azienda Ospedaliera Card. G. Panico	Emiliano Tamburini
Istituto Nazionale Tumori IRCCS - Fondazione G. Pascale, Napoli	Michelino De Laurentiis
Azienda Socio Sanitaria Territoriale degli Spedali Civili di Brescia (Presidio Spedali Civili)	Rebecca Pedersini
Fondazione Policlinico Universitario Agostino Gemelli	Emilio Bria
IRCCS Ospedale Sacro Cuore Don Calabria	Stefania Gori
Japan	
National Cancer Center Hospital East	Yoichi Naito
National Cancer Center Hospital	Kan Yonemori
The Cancer Institute Hospital of Japanese Foundation for Cancer Research	Takayuki Kobayashi
Aichi Cancer Center Hospital	Hiroji Iwata
Kindai University Hospital	Tsutomu Iwasa
Hakuaikai Sagara Hospital	Yasuaki Sagara
Toranomon Hospital	Yuko Tanabe
Kanagawa Cancer Center	Toshinari Yamashita
NHO Shikoku Cancer Center	Kenjiro Aogi
National Hospital Organization Kyushu Cancer Center	Eriko Tokunaga
Shizuoka Cancer Center	Tomomi Hayashi
National Hospital Organization Hokkaido Cancer Center	Nobumoto Tomioka
NHO Osaka National Hospital	Hiroyuki Yasojima
Saitama Cancer Center	Kenichi Inoue
Hyogo College of Medicine Hospital	Yasuo Miyoshi
Local Independent Administrative Corporation Hiroshima City Hospital Organization Hiroshima City Hiroshima Citizens Hospital	Mitsuya Ito
Showa University Hospital	Junji Tsurutani
Tokai University Hospital	Naoki Niikura
Korea, Republic of	
Seoul National University Hospital	Seock-Ah Im
Samsung Medical Center	Yeon Hee Park
Seoul National University Bundang Hospital	Jee Hyun Kim
Asan Medical Center	Sung-Bae Kim
Severance Hospital, Yonsei University Health System	Joo Hyuk Sohn
National Cancer Center	Keun Seok Lee
Kyungpook National University Chilgok Hospital	Yee Soo Chae
Inha University Hospital	Moon-Hee Lee
Portugal	
Centro Hospitalar de Lisboa Norte, E.P.E. - Hospital de Santa Maria	Ana Rita Sousa

Unidade Local de Saude de Matosinhos, E.P.E. (Hospital Pedro Hispano)	Matilde Salgado
Centro Hospitalar de Entre o Douro e Vouga, E.P.E - Hospital de Sao Sebastiao	Ana Luisa Faria
Centro Hospitalar Vila Nova de Gaia/Espinho, E.P.E.	António Moreira Pinto
Centro Hospitalar de Tras-os-Montes e Alto Douro, EPE	Antonio Teira
Instituto Portugues de Oncologia do Porto Francisco Gentil, EPE	Ana Rodrigues
Hospital de Braga	Catarina Portela
Russian Federation	
FSBSI Russian Oncological Scientific Center n. a. NN Blokhin	Inna Ganshina
SBIH Moscow City Oncology Hospital No 62 of Moscow Healthcare Department	Daniil Stroyakovskiy
SBIH of Yaroslavl region Regional Clinical Oncological Hospital	Nikolay Kislov
Spain	
Hospital Ruber Internacional	Javier Cortes Castan
ICO l'Hospitalet - Hospital Duran i Reynals	Miguel Gil-Gil
Hospital Clinic de Barcelona	Maria Vidal
Complejo Hospitalario Universitario A Coruna	Silvia Antolin Novoa
Hospital Universitario Ramon y Cajal	Maria Fernandez Abad
Hospital Universitario Clinico San Carlos	Jose Angel Garcia Saenz
Hospital Vall Hebron	Cristina Saura Manich
Hospital del Mar	Sonia Servitja Tormo
Hospital Universitario Virgen Macarena	Esteban Nogales Fernandez
Hospital Clinico Universitario Virgen de la Victoria	Maria Jose Bermejo Perez
Hospital Universitario de Canarias	Josefina Cruz Jurado
Hospital Virgen del Rocio	Manuel Ruiz Borrego
Sweden	
Akademiska Sjukhuset	Henrik Lindman
Onkologkliniken, Sundsvall	Anna-Karin Wennstig
Karolinska Universitetssjukhuset - Solna	Kristina Weibring
Switzerland	
Kantonsspital Winterthur	Andreas Müller
Universitaetsspital Basel	Marcus Vetter
Kantonsspital St. Gallen	Jens Huober
Universitaetsspital Zuerich	Konstantin Dedes
Centre Hospitalier Universitaire Vaudois	Khalil Zaman
Kantonsspital Graubuenden	Michael Schwitter
Taiwan, Republic of China	
National Cheng Kung University Hospital	Wei-Pang Chung
National Taiwan University Hospital	Yen-Shen Lu

Kaohsiung Medical University Chung-Ho Memorial Hospital (KMUH)	Ming-Feng Hou
United Kingdom	
Queen Mary University of London	Peter Schmid
Royal Cornwall Hospitals NHS Trust	Duncan Wheatley
Nottingham University Hospitals City Campus, Nottingham	Srinivasan Madhusudan
United States	
Memorial Sloan Kettering Cancer Center	Shanu Modi
UCLA Hematology Oncology	Nicholas McAndrew
Ironwood Cancer & Research Centers - Scottsdale	Mikhail Shtivelband
The University of Texas MD Anderson Cancer Center	Naoto Ueno
Oregon Health & Science University	Zahi Mitri
St Francis Cancer Center	Stephen Dyar
Saint Barnabas Medical Center	Anna Litvak
Allegheny General Hospital	Jane Raymond
Southeastern Regional Medical Center	John McKnight
Midwestern Regional Medical Center	Eugene Ahn
Western Regional Medical Center, Inc.	Cynthia Lynch
UCSF	Hope Rugo
Dana-Farber Cancer Institute	Ian Krop
Torrance Memorial Physician Network	David Chan
The Cleveland Clinic Foundation	Halle Moore
Sylvester Comprehensive Cancer Center - Deerfield Beach	Reshma Mahtani
Houston Methodist Cancer Center	Polly Niravath
Banner MD Anderson Cancer Center	Shakeela Bahadur
Cancer Center of Kansas	Shaker Dakhil
Memorial Healthcare System	Adriana Milillo-Naraine
Stanford Cancer Institute	Fauzia Riaz
Brig Center for Cancer Care and Survivorship	David Schumaker
Baptist Cancer Center	Philip Lammers
Saint Luke's Hospital of Kansas City	Timothy Pluard
New York University Medical Center	Sylvia Adams
Moffitt Cancer Center	Hyo Han
Florida Cancer Specialists - South Region	Fadi Kayali
Tennessee Oncology - Skyline Satellite	Erika Hamilton

Additional Data Sharing Information

Data Sharing Questions	Answers
Will individual participant data be available (including data dictionaries)?	Yes
What data in particular will be shared?	Anonymized individual participant data (IPD) and applicable supporting clinical trial documents may be available upon request at (https://vivli.org). In cases where clinical trial data and supporting documents are provided pursuant to our company policies and procedures, Daiichi Sankyo Companies will continue to protect the privacy of the company and our clinical study patients. Details on data sharing criteria and the procedure for requesting access can be found at this web address: https://vivli.org/ourmember/daiichi-sankyo
What other documents will be available?	Clinical Trial Protocol, Statistical Analysis Plan, Informed Consent Form, and Clinical Study Report. In cases where clinical trial data and supporting documents are provided pursuant to our company policies and procedures, Daiichi Sankyo will continue to protect the privacy of our clinical trial participants.
When will data be available (start and end dates)?	Anonymized IPD will be available when the indication supported receives marketing approval and study results are published.
With whom?	Qualified science and medical researchers upon formal request and submission of research proposal detailing planned analyses.
For what types of analyses?	De-identified IPD and relevant clinical trial documents will be shared for the purpose of conducting legitimate research as specified in an approved formal research proposal.
By what mechanism will data be made available?	De-identified IPD may be available upon request at https://vivli.org/

Supplementary Methods

Patients

Demographic information was collected at study initiation. Age and sex were collected in an interactive web/voice response system. Age was determined as outlined in the statistical analysis protocol using the patient birth date as entered in the system, while sex was a binary selection (male or female). Ethnicity and race were self-reported by patients in the electronic case report form. Patients were asked about their ethnicity before being asked about their race. Available options for ethnicity included: Hispanic/Latino, Non-Hispanic/Non-Latino, Unknown, and Not Applicable. Hispanic or Latino is described as Hispanic or Latino: a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term, "Spanish origin," could be used in addition to "Hispanic or Latino." Available options for race included: American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, or Other. Patients could select multiple races. In countries that did not allow collection of this information, the patient's race and/or ethnicity was categorized as "Not Applicable."

Patients who had previously tested positive for human epidermal growth factor receptor 2 (HER2; immunohistochemistry [IHC] 3+ or IHC 2+/in situ hybridization [ISH]+) or had been treated with anti-HER2 therapy were excluded from this study. Patients were also excluded if they had spinal cord compression or clinically active central nervous system metastases (brain metastases) defined as untreated or symptomatic, or requiring therapy with corticosteroids or anticonvulsants to control associated symptoms. Patients with treated brain metastases that are no longer symptomatic and who require no treatment with corticosteroids or anticonvulsants could be included in the study if they have recovered from the acute toxic effect of radiotherapy. A minimum of 2 weeks must have elapsed between the end of whole-brain radiotherapy and study enrollment.

Dose Reductions

Two dose level reductions were allowed if needed for toxicity management. No primary prophylaxis for nausea or vomiting was mandated by the protocol and the premedication used was at investigator discretion.

HER2-Low Diagnostic Testing

As part of patient screening for DESTINY-Breast04 enrollment, archived or recent tumor biopsy specimens from potential participants in the study (who were required to have historically HER2-low breast cancer) were tested for HER2-low status at central pathology laboratories. HER2 IHC scores were

determined using the investigational VENTANA HER2/neu (4B5) IUO Assay system (hereafter referred to as the "VENTANA HER2 [4B5] assay"), which is identical in its formulation and automated staining process to the on-market VENTANA/PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody assay but scored using an algorithm adapted from the 2018 American Society of Clinical Oncology/College of American Pathologists testing guidelines.[1] When applicable (i.e., for specimens yielding HER2 IHC scores of 2+), central HER2 ISH testing was performed using the INFORM HER2 Dual ISH DNA Probe Cocktail IUO Assay system (hereafter referred to as the "INFORM HER2 Dual ISH" assay), which is identical in formulation, automated staining process, and scoring to the on-market INFORM/VENTANA HER2 Dual ISH DNA Probe Cocktail assay.

Routinely processed, formalin-fixed, paraffin-embedded (FFPE) tissues were considered suitable for use with the VENTANA HER2 (4B5) and INFORM HER2 Dual ISH assays, however, because pre-analytical variables (eg, time and type of fixation) may affect the results for all receptor assays, including HER2-low results, to be eligible for HER2 testing, specimens had to have been fixed in 10% neutral buffered formalin (6 to 72 hours fixation recommended). Serial sections (approximately 4-µm thick) of the FFPE specimens were mounted on glass microscope slides. Unstained slides were stored at 2°C–8°C or room temperature (15°C–25°C) for no more than 4 months before staining with the VENTANA HER2 (4B5) assay or for no more than 18 months before staining with the INFORM HER2 Dual ISH assay. Central pathologist review of 1 slide stained with hematoxylin and eosin had to confirm that the specimen contained sufficient tumor tissue for interpretation, i.e., the stained area of the slide had to contain at least 50 viable tumor cells with associated stroma. Specimens lacking sufficient tumor tissue were excluded from testing. Fine needle aspirates and other cytological specimens were also excluded from testing, as were decalcified bone metastases.

Patient specimens were stained with the investigational IHC and ISH assays on VENTANA BenchMark ULTRA instruments using the staining protocols shown in **Table S6**.

For the VENTANA HER2 (4B5) assay, duplicate slides from each case were stained with the VENTANA HER2 (4B5) primary antibody and with CONFIRM Negative Control Rabbit Ig (a species-matched negative reagent control [NRC]), respectively, on a BenchMark ULTRA instrument using the *ultra*View Universal DAB Detection Kit with the corresponding instrument staining protocol shown in **Table S6**. A trained central laboratory pathologist evaluated the invasive breast cancer cells on the stained slides for presence or absence of a brown colored diaminobenzidine (DAB) signal indicating localization of the HER2 antigen. The NRC-stained slide was used to assess nonspecific background staining. After

confirming that the case tissue morphology and background staining on the VENTANA HER2 (4B5) antibody-stained slide were acceptable according to the criteria shown in **Table S7**, and that no staining artifacts interfered with interpretation, the pathologist assigned a HER2 IHC score to the case based on the extent and intensity of membranous HER2-specific staining observed and the HER2-low scoring algorithm. Slides exhibiting unacceptable staining were not scored.

A HER2 IHC score of 0 or 3+ disqualified the patient from enrollment in DESTINY-Breast04. Cases assigned a HER2 score of IHC 1+ were considered HER2-low and satisfied the HER2-low eligibility requirement for DESTINY-Breast04 enrollment. Cases assigned a HER2 score of IHC 2+ were reflexed to further testing with the INFORM HER2 Dual ISH assay as described below. Those then found to be ISH− were also considered HER2-low, whereas those found to be ISH+ were considered HER2-positive rather than HER2-low, disqualifying the patient from enrollment in DESTINY-Breast04. This scoring workflow is shown in **Figure S4.**

For the INFORM HER2 Dual ISH assay, a single slide from each case was stained with the VENTANA HER2 Dual ISH DNA probe cocktail on a BenchMark ULTRA instrument using VENTANA *ultra*View SISH DNP Detection Kit (which causes a black signal to localize at the *HER2* gene on chromosome 17 [Chr17]) and VENTANA *ultra*View Red ISH DIG Detection Kit (which causes a red signal to localize at the Chr17 centromere) with the corresponding instrument staining protocol shown in **Table S6**. A trained central laboratory pathologist evaluated the stained slides for presence or absence of an enumerable field containing at least 20 invasive breast cancer nuclei, each containing red and black signals as defined in **Table S7**. If the slide was enumerable, the pathologist then evaluated the stained tumor tissue for *HER2* gene amplification status based on the ratio formed by dividing the sum of *HER2* signals for all 20 tumor nuclei by the sum of Chr17 signals for the same 20 nuclei. The *HER2* gene was defined as amplified (ISH+) if the *HER2*:Chr17 ratio was greater than 2.2 and as nonamplified (ISH−) if the *HER2*:Chr17 ratio was less than 1.8. However, if the *HER2*:Chr17 ratio fell between 1.8 and 2.2 (inclusive), an additional 20 nuclei were enumerated, and a new ratio was calculated on the basis of all 40 nuclei. The *HER2* gene was then defined as amplified (ISH+) if the HER2:Chr17 ratio was 2.0 or greater and as nonamplified (ISH−) if the HER2:Chr17 ratio was less than 2.0. IHC 2+ cases found to be ISH+ were considered HER2-positive and therefore ineligible for DESTINY-Breast04 enrollment, whereas with those found to be ISH− were considered HER2-low, also satisfying the HER2-low requirement for DESTINY-Breast04 eligibility.

Safety

Safety parameters included the incidence of treatment-emergent adverse events and serious adverse events. Treatment-emergent adverse events were defined as adverse events that occurred or worsened in severity after initiating the study drug until 47 days after the last dose of the study drug. Serious adverse events with an onset or worsening ≥48 days after the last dose of the study drug, if considered related to the study treatment, were also considered treatment-emergent and were recorded.

Interstitial Lung Disease/Pneumonitis

An independent adjudication committee was responsible for reviewing all cases of potential interstitial lung disease/pneumonitis. Any reported cases of interstitial lung disease were identified for adjudication based on the current Medical Dictionary for Regulatory Activities (MedDRA) version for the narrow interstitial lung disease Standardized MedDRA Query (SMQ), selected terms from the broad interstitial lung disease SMQ, and the preferred terms, respiratory failure, and acute respiratory failure. Interstitial lung disease/pneumonitis was managed in accordance with the study protocol (**Supplementary Table 2**).

Statistical Analysis

The primary and key secondary efficacy endpoints were hierarchically tested in the following order: progression-free survival in the patients with hormone receptor (HR)–positive metastatic breast cancer, progression-free survival in all patients, overall survival in the HR–positive group until a positive read out, then overall survival in all patients. One analysis was planned for progression-free survival. Up to three analyses of overall survival were planned per group sequential design utilizing 3-look Lan-DeMets alpha spending function with O'Brien-Fleming type stop boundary: the first interim analysis at time of the analysis for progression-free survival (provided progression-free survival is significant in both HR-positive group and all patients), if the first interim analysis was not significant, a second interim analysis for overall survival is planned when approximately 233 overall survival events (70% information fraction) in HR-positive group have been documented. If the second overall survival interim analysis was not significant, a final analysis after approximately 333 overall survival events in HR-positive group have been documented.

The distribution of progression-free survival and overall survival between the two treatment groups' primary efficacy analysis were compared using stratified log-rank test, with stratification factors from interactive web/voice response system, at two-sided significance level of 0.05. The Kaplan–Meier method was used to estimate quartile event times for progression-free survival, and overall survival. The 2-sided CIs of quartile event times were calculated using the Brookmeyer and Crowley method. Safety analyses were descriptive and reported with appropriate summary statistics. The hazard ratio of PFS and its two-

sided 95% CI were estimated using a stratified Cox proportional hazards regression model with treatment group as the model factor and the stratification factors from the interactive web/voice response system as strata. Note that the reported 95% confidence intervals were not adjusted for multiplicity and thus not used to infer effects. The proportion hazard assumption for Cox proportional hazards regression analyses was examined through visual inspection of the graphs of the log of negative log of estimated survival functions, indicative of proportion hazard when the curves of different treatment are parallel.

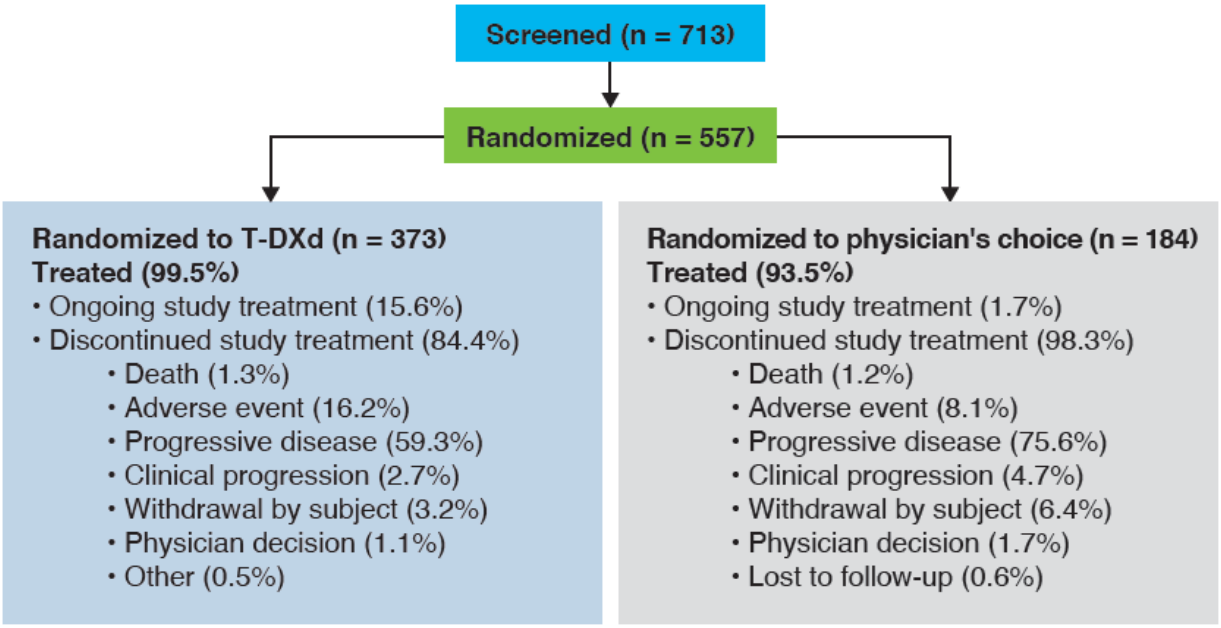
Every effort was made to collect tumor scans until disease progression, which was confirmed based on blinded independent central review. Patients without a PFS event were censored at the last adequate tumor assessment date. Patients with a PFS event after missing 2 or more consecutive tumor assessments were censored at the last adequate tumor assessment date. The primary PFS analysis does not censor for new anticancer therapy.

Contributors

SM, JT, NU, AP, DG, NH, and DC contributed to the conception and/or design of the study and the development of the study protocol. WJ, TY, JHS, MV, ET, YSC, KSL, NN, YHP, XW, MGG, WL, JYP, SAI, HM, HR, BX, RY, FZ, AG, SBK, QL, TL, CSM, PS, and TS were involved in acquisition, analysis, and interpretation of data. LY, YW, JS, PV, and GM were responsible for data analysis and contributed to its interpretation. All authors participated in the interpretation of data. All authors were involved in the drafting and revision of the manuscript, and all authors approved the final version of the manuscript for publication.

Supplementary Figures and Tables

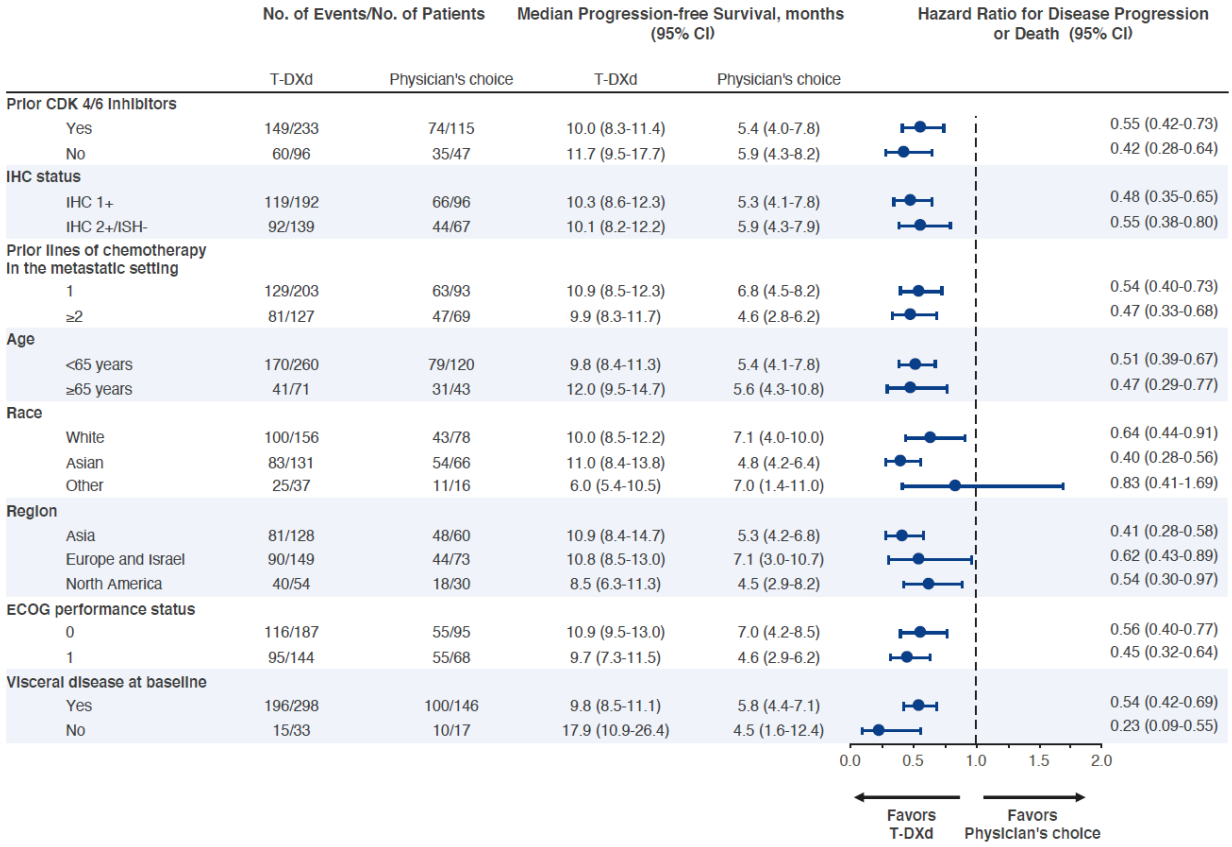
Figure S1. Patient Disposition.



An imbalance in randomization of patients (those not treated) occurred, primarily due to withdrawal of consent in the control arms prior to initiating treatment.

T-DXd, trastuzumab deruxtecan.

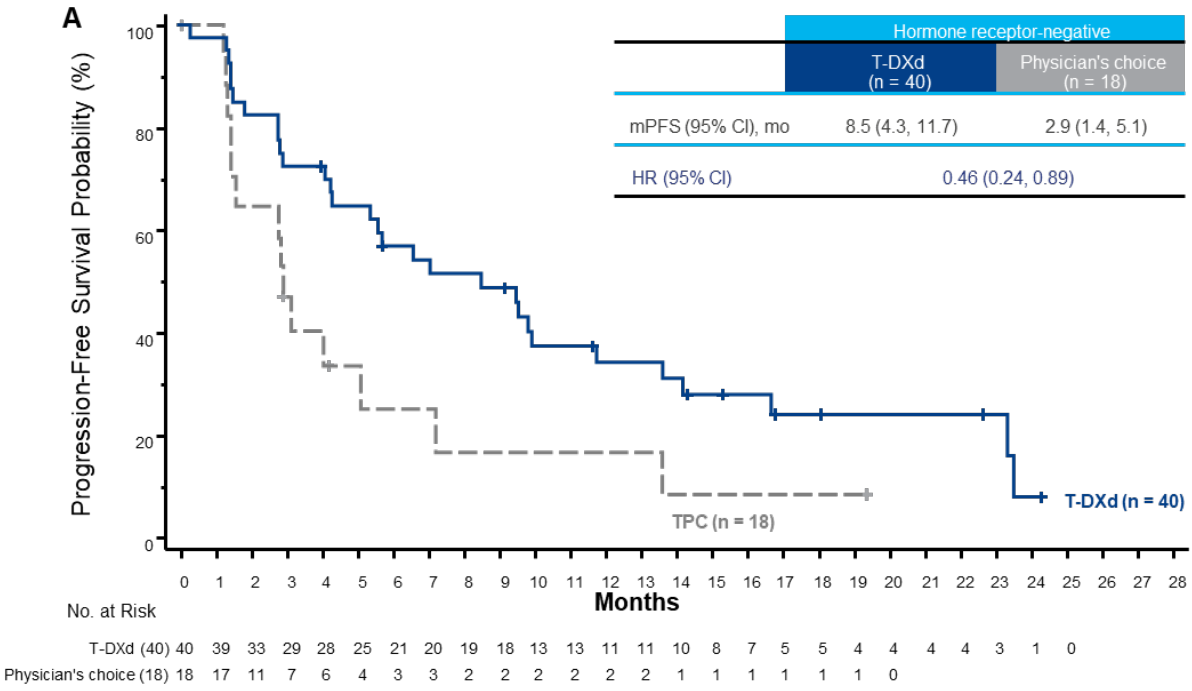
Figure S2. Subgroup Analysis of Progression-Free Survival in the Hormone Receptor–Positive Cohort.[a] Shown are progression-free survival, hazard ratios, and 95% confidence intervals in subgroups. The progression-free survival benefit of T-DXd over physician's choice was consistent across all subgroups.

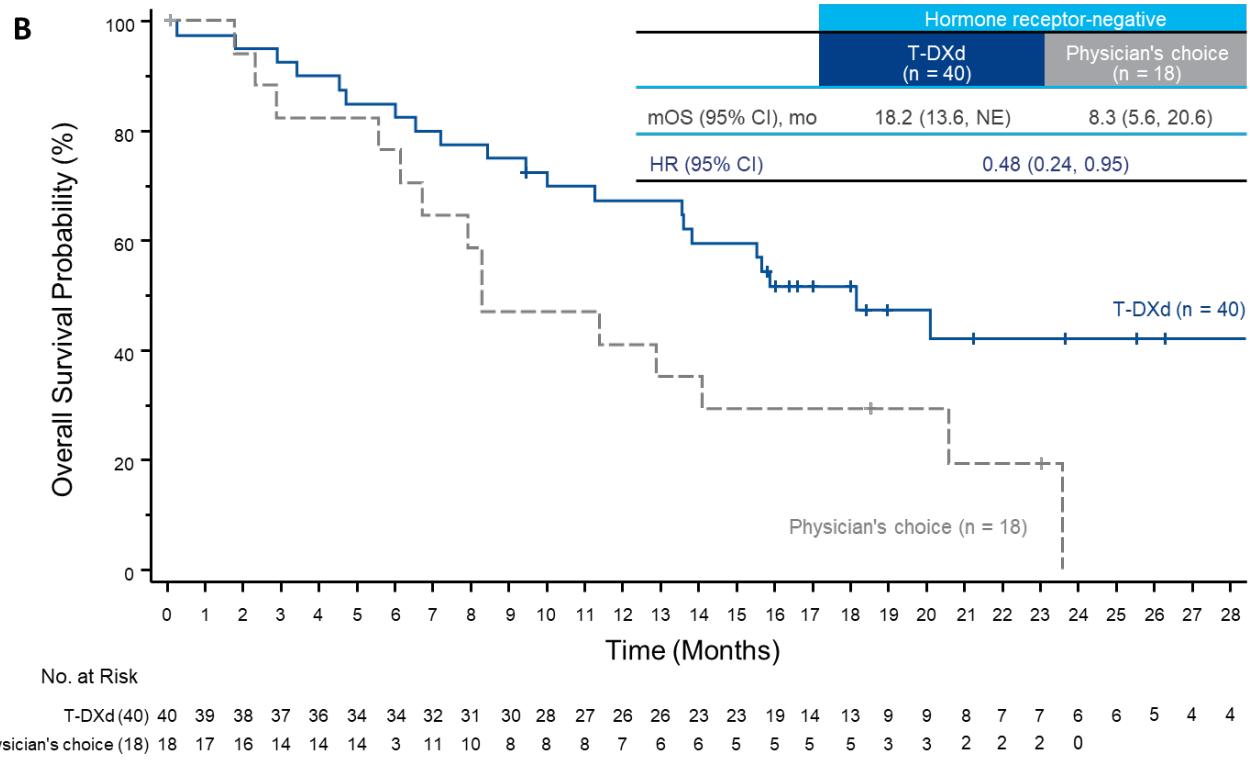


[a]Based on derived data, which includes protocol deviations.

CDK, cyclin-dependent kinase; CI, confidence interval; IHC, immunohistochemistry; ISH, in situ hybridization; PFS, progression-free survival; T-DXd, trastuzumab deruxtecan.

Figure S3. Kaplan–Meier Analysis of Progression-Free Survival and Overall Survival in the Hormone Receptor–Negative Cohort. Panel A shows the Kaplan–Meier analysis of progression-free survival, as assessed by blinded independent central review, in the hormone receptor–negative cohort as derived by the electronic data capture. Panel B shows Kaplan–Meier analysis of overall survival in the hormone receptor–negative cohort as derived by the electronic data capture.

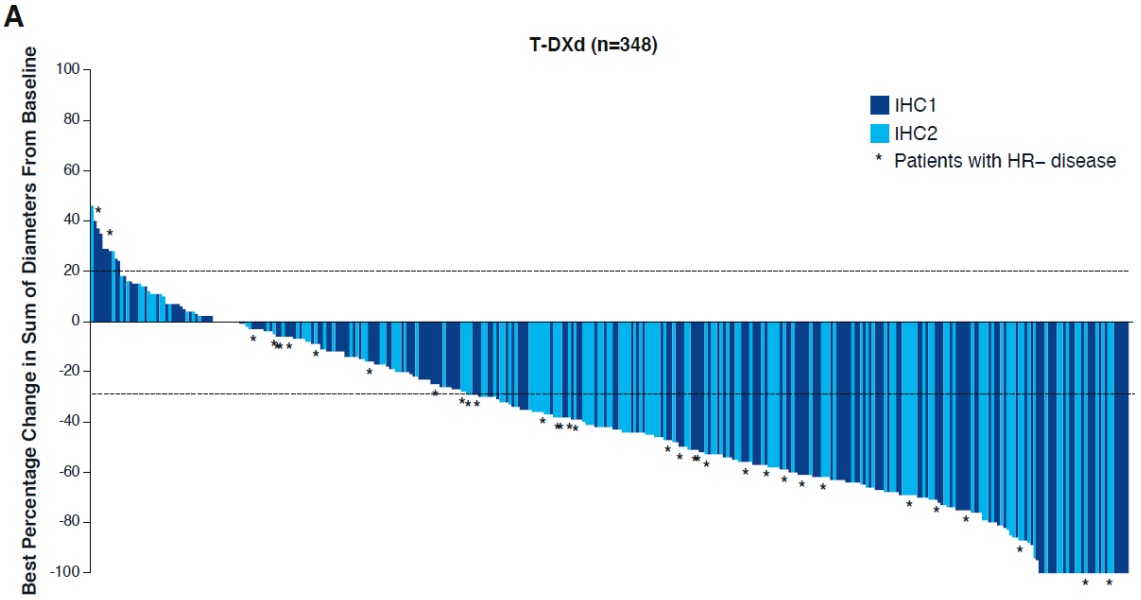




CI, confidence interval; HR, hazard ratio; mPFS, median progression-free survival; T-DXd, trastuzumab deruxtecan.

Figure S4. Antitumor Activity.

Shown are the best percentage changes from baseline in the sum of the largest diameters of measurable tumors in patients for whom data from both baseline and postbaseline assessments of target lesions by independent central review were available: 348 of 373 patients who received T-DXd (Panel A) and 156 of 184 patients who received physician's choice (Panel B). Patients with tumors categorized HER2 IHC1+ are shown in purple and HER2 IHC2+/ISH- is shown in teal. Patients with hormone receptor-negative tumors are designated with an asterisk. The upper dashed horizontal line indicates a 20% increase in tumor size in the patients who had disease progression, and the lower dashed line indicates a 30% decrease in tumor size (partial response).



B

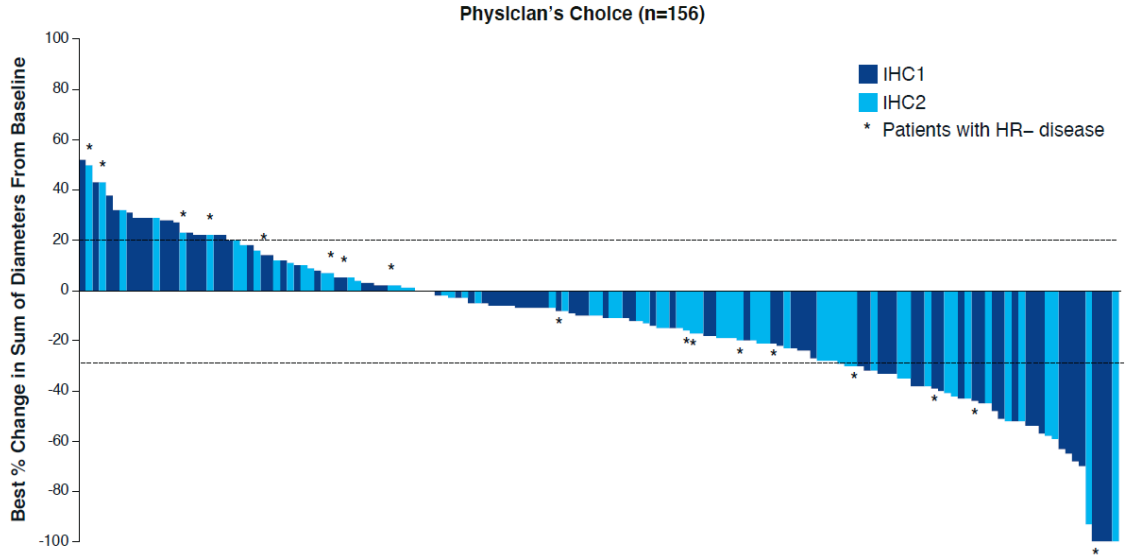
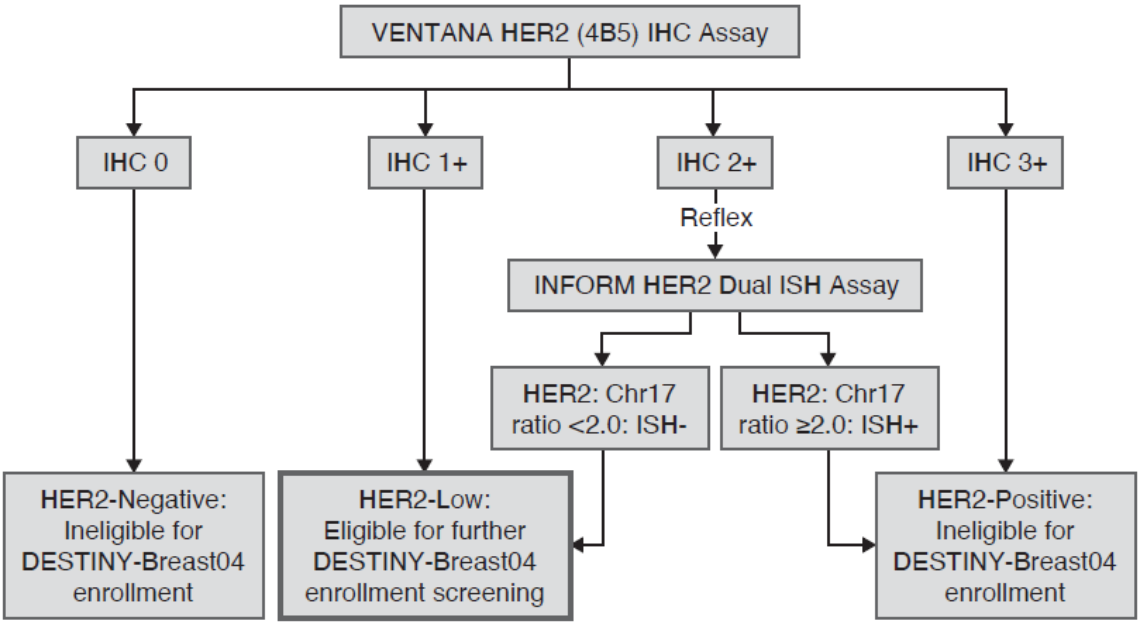

Physician's Choice (n=156)
Best % Change in Sum of Diameters From Baseline
100
80
60
40
20
0
-20
-40
-60
-80
-100
IHC1
IHC2
* Patients with HR– disease

Figure S5. HER2-Low Testing Using the VENTANA HER2 (4B5) IHC and INFORM HER2 Dual ISH Assays for DESTINY-Breast04 Enrollment Screening.



HER2, hormone epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

Table S1. HER2-Low Scoring Algorithm for Breast Cancer Tissue Stained With VENTANA HER2 (4B5) Antibody.

HER2-Low Scoring Algorithm for Breast Cancer		
Staining Pattern	**HER2-Low IHC Score**	**Clinical Implication**
No membrane staining is observed	HER2 IHC0	HER2-negative; ineligible
Faint, partial staining of the membrane in 10% or less of the cancer cells		
Faint, partial staining of the membrane in greater than 10% of the cancer cells	HER2 IHC1+	HER2-low: eligible
Weak-to-moderate complete staining of the membrane in greater than 10% of the cancer cells	HER2 IHC2+	Potentially eligible; reflex to HER2 ISH testing
Intense complete staining of the membrane in greater than 10% of the cancer cells	HER2 IHC3+	HER2-positive; ineligible

Samples with 10% of tumor cells or less demonstrating any membranous staining were scored as "0." Samples with partial, incomplete staining of the membrane in at least 10% of the tumor cells were scored as "1+." Samples with at least 10% of the tumor demonstrating weak staining of the entire membrane were scored as "2+," and samples with more than 10% of the tumor cells demonstrating strong staining of the entire membrane were scored as "3+." Borderline cases were examined at 40× or higher magnification to discriminate between "0", "1+," and "2+." When the signal was distributed heterogeneously, having more than one intensity level, throughout the tumor, the relative percentages of signal intensities were visually estimated and used to generate a diagnostic score. The final score was the highest numbered bin with 10% or more of the tumor staining. Only intact cells were used for interpretation of staining results, as necrotic or degenerated cells often stained nonspecifically. Background staining was defined as nonspecific staining of the FFPE tissue (as opposed to specific staining of lesional cells in the FFPE tissue). The pathologist also reviewed the HER2 (4B5) slide for the overall acceptability of staining.

FFPE, formalin-fixed, paraffin-embedded; HER2, hormone epidermal receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

Table S2. Management Guidelines for Pulmonary Toxicity.

CTCAE v5.0 Grade	Management Guidelines for T-DXd
	If a patient develops radiographic changes potentially consistent with ILD/pneumonitis or develops an acute onset of new or worsening pulmonary or other related signs/symptoms such as dyspnea, cough, or fever, rule out ILD/pneumonitis. If the AE is confirmed to have an etiology other than ILD/pneumonitis, follow the management guidance outlined in the "Other Non-Laboratory Adverse Events" in the dose modification section of the study protocol. If the AE is suspected to be ILD/pneumonitis, treatment with study drug should be interrupted pending further evaluation. Evaluations should include: • High-resolution CT • Pulmonologist consultation (infectious disease consultation as clinically indicated) • Blood culture and complete blood count (CBC). Other blood tests could be considered as needed • Consider bronchoscopy and bronchoalveolar lavage, if clinically indicated and feasible • Pulmonary function tests and pulse oximetry (SpO_2) • Arterial blood gases, if clinically indicated • One blood sample collection for PK analyses as soon as ILD/pneumonitis is suspected, if feasible Other tests could be considered, as needed. If the AE is confirmed to be ILD/pneumonitis, follow the ILD/pneumonitis management guidance as outlined below. All events of ILD/pneumonitis, regardless of severity or seriousness, will be followed until resolution including after drug discontinuation.
Grade 1	The administration of T-DXd must be interrupted for any ILD/pneumonitis events regardless of grade. • Monitor and closely follow-up in 2 to 7 days for onset of clinical symptoms and pulse oximetry • Consider follow-up imaging in 1 to 2 weeks (or as clinically indicated) • Consider starting systemic steroids (eg, at least 0.5 mg/kg/day prednisone or equivalent) until improvement, followed by gradual taper over at least 4 weeks • If worsening of diagnostic observations despite initiation of corticosteroids, then follow grade 2 guidelines[a] For grade 1 events, T-DXd can be restarted only if the event is fully resolved to grade 0:

	• If resolved in ≤ 28 days from day of onset, maintain dose • If resolved in > 28 days from day of onset, reduce dose one level However, if the event of Grade 1 ILD/pneumonitis occurs beyond Day 22 and has not resolved within 49 days from the last infusion, the study drug should be discontinued. [a]If patient is asymptomatic, then patient should still be considered as grade 1 even if steroid treatment is given.
Grade 2	Permanently discontinue patient from study treatment. • Promptly start and treat with systemic steroids (eg, at least 1 mg/kg/day prednisone or equivalent) for at least 14 days or until complete resolution of clinical and chest CT findings, then followed by a gradual taper over at least 4 weeks • Monitor symptoms closely • Re-image as clinically indicated • If worsening or no improvement in clinical or diagnostic observations in 5 days: - Consider increasing dose of steroids (eg, 2 mg/kg/day prednisone or equivalent) and administration may be switched to intravenous (eg, methylprednisolone) - Reconsider additional work-up for alternative etiologies as described above - Escalate care as clinically indicated
Grade 3 and 4	Permanently discontinue patient from study treatment. • Hospitalization required • Promptly initiate empiric high-dose methylprednisolone IV treatment (eg, 500–1000 mg/day for 3 days), followed by at least 1.0 mg/kg/day of prednisone (or equivalent) for at least 14 days or until complete resolution of clinical and chest CT findings, then followed by a gradual taper over at least 4 weeks • Re-image as clinically indicated • If still no improvement within 3 to 5 days: - Reconsider additional work-up for alternative etiologies as described above - Consider other immunosuppressants and/or treat per local practice

AE, adverse event; CT, computed tomography; CTCAE v5.0, Common Terminology Criteria for Adverse Events, version 5.0; ILD, interstitial lung disease; IV, intravenous; PK, pharmacokinetics.

Table S3. Representativeness of Study Participants.

Category	
Disease, problem, or condition under investigation	HER2-low (IHC1+, IHC2+/ISH-) breast cancer
Special considerations related to	
Sex and gender	Overall, breast cancer affects more women than men, with less than 1% of cases occurring in men.[2,3] In a large retrospective study involving 2203 cases of HER2-low breast cancer, about 0.7% were in men.[4]
Age	Overall, breast cancer incidence significantly increases with age and incidence rates are highest in those aged 65-74 years in the United States.[5] In the United States, only 10.3% of all breast cancers are diagnosed in patients under 45 years.[5] The median age of 685 evaluable patients with HER2-low breast cancer in a retrospective study was 59 years,[4] slightly lower than the median age of the overall breast cancer population – 62 years.[2]
Race or ethnic group	In the United States, hormone receptor–positive/HER2-negative breast cancers are the most common subtype in women of all ethnicities.[2] The proportion of cases with this subtype is highest in White women (76%), followed by Asian/Pacific Islander (71%), American Indian/Alaska Native (70%), Hispanic (69%), and Black (61%) women.[2] Of note, hormone receptor–negative/HER2-negative breast cancers occur at almost double the rate (21% of all cases) in Black women, compared with other ethnicities (range, 10%-12%).[2] Death rates for breast cancers overall are highest in Black women.[2]
Geography	Overall breast cancer incidence varies by geographic region and is higher in more developed countries.[5] Europe, North America, and Australia/New Zealand have the highest breast cancer incidence rates by region.[6]
Other considerations	For hormone receptor–positive/HER2-negative breast cancer, alcohol use, use of hormone replacement therapy, and older age have been identified as risk factors.[7] Hormone receptor–negative/HER2-negative (triple-negative) breast cancer is positively correlated with younger age and higher body mass index. Black women are disproportionately affected by triple-negative breast cancer

	and have higher mortality than women of other race/ethnicities; evidence suggests this disparity is due to both environmental and genetic factors.[7,8]
Overall representativeness of this trial	HER2 has been traditionally categorized on a binary measure of either HER2-positive or HER2-negative. However, approximately 60% of HER2-negative metastatic breast cancer patients express low levels of HER2 (immunohistochemistry [IHC] 1+, IHC2+/ISH negative)[9,10]; patients with HER2-low metastatic breast cancer make up the study population in DESTINY-Breast04. The representation of women and men in this trial reflects the expected representation of patients affected by HER2-low breast cancers. The age of trial participants was slightly lower than the median age of patients diagnosed with HER2-low breast cancer (55.9-57.5 years versus 59 years).[4] There was a higher number of patients from Europe/Israel (45%) relative to North America (16.7%) and Asia (38.2%). Only 1.8% of the overall population were Black or African American and were therefore underrepresented in this study.

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

Table S4. Overall Safety Summary.

Type of Adverse Events, n (%)	T-DXd (n = 371)	Physician's Choice (n = 172)
TEAEs	369 (99.5)	169 (98.3)
Grade ≥3	195 (52.6)	116 (67.4)
Serious TEAEs	103 (27.8)	43 (25.0)
TEAEs associated with dose discontinuations	60 (16.2)	14 (8.1)
Drug-related	56 (15.1)	12 (7.0)
TEAEs associated with dose interruptions	143 (38.5)	72 (41.9)
Drug-related	106 (28.6)	62 (36.0)
TEAEs associated with dose reductions	84 (22.6)	66 (38.4)
Drug-related	77 (20.8)	64 (37.2)
TEAEs associated with deaths	14 (3.8)	5 (2.9)
Drug-related	7 (1.9)	0

T-DXd, trastuzumab deruxtecan; TEAE, treatment-emergent adverse event.

Table S5. Exposure-Adjusted Incidence Rate (EAIR) of Treatment-Emergent Adverse Events.

Type of Adverse Events	T-DXd (n = 371)	Physician's Choice (n = 172)
Total Patient-Years of Exposure, years[a]	283.55	63.59
Any TEAEs, n (%)	369 (99.5)	169 (98.3)
EAIR per patient-year	1.30	2.66
Grade ≥3 TEAEs, n (%)	195 (52.6)	116 (67.4)
EAIR per patient-year	0.69	1.82
Serious TEAEs, n (%)	103 (27.8)	43 (25.0)
EAIR per patient-year	0.36	0.68
TEAEs associated with dose discontinuations, n (%)	60 (16.2)	14 (8.1)
EAIR per patient-year	0.21	0.22
TEAEs associated with dose interruptions, n (%)	143 (38.5)	72 (41.9)
EAIR per patient-year	0.50	1.13
TEAEs associated with dose reductions, n (%)	84 (22.6)	66 (38.4)
EAIR per patient-year	0.30	1.04
TEAEs associated with deaths, n (%)	14 (3.8)	5 (2.9)
EAIR per patient-year	0.05	0.08

[a]Patient-years of exposure are the treatment duration with year as unit.

T-DXd, trastuzumab deruxtecan; TEAE, treatment-emergent adverse event.

Table S6. Staining Protocol for Diagnostic Testing.

VENTANA HER2 (4B5) Assay Staining on the BenchMark ULTRA Instrument	
Selectable Procedure Step	**Method**
Deparaffinization	Selected
Cell conditioning (antigen unmasking)	ULTRA CC1, mild
Antibody (primary) VENTANA HER2/neu (4B5) IUO assay – *or* - CONFIRM negative control rabbit Ig	12 minutes, 36 ˚C
*ultra*Wash	Selected
Counterstain	Hematoxylin II, 4 minutes
After counterstain	Bluing, 4 minutes
INFORM HER2 Dual ISH Assay Staining on the BenchMark ULTRA Instrument	
Selectable Procedure Step	**Method**
Deparaffinization	Selected
Cell conditioning (CC2)	Selected CC2 standard
ISH-protease 3	20 min (tissue) 8 min (xenografts)
Denaturation	8 min
Hybridization	6 hours
Stringency wash	72°C
SISH multimer	Not selectable
Silver chromogen	8 min
Red ISH multimer	Not selectable
Red chromogen	8 min
Counterstain	Hematoxylin II - 8 min
After counterstain	Bluing reagent - 8 min

Each BenchMark ULTRA staining run contained one PATHWAY HER-2 4 in 1 Control Slide. This slide was stained with VENTANA HER2 (4B5) Assay primary antibody and reviewed by a pathologist to confirm the validity of the staining run. HER2 and chromosome 17 (Chr17) signals (1 to 2 copies per cell for each probe) in normal cells (e.g., stromal fibroblasts, endothelial cells, lymphocytes, and nonneoplastic cells) in and around the target carcinoma area were reviewed by a pathologist and used as internal positive controls for valid staining.

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

Table S7. Morphology and Background Staining Acceptability Criteria for Breast Cancer Tissue Stained With VENTANA HER2 (4B5) Antibody

	Acceptable	Unacceptable
Case tissue morphology	Cellular elements of interest are visualized, allowing clinical interpretation of the stain	Cellular elements of interest are not visualized, compromising clinical interpretation of the stain
Background staining	Background does not interfere with clinical interpretation of the stain	Background interferes with ability to interpret the stain

Supplementary Material References

References

1. Wolff AC, Hammond MEH, Allison KH, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol 2018;36:2105-22.
2. American Cancer Society. Breast Cancer Facts & Figures 2019-2020. Atlanta: American Cancer Society, Inc. 2019.
3. Ottini L, Capalbo C, Rizzolo P, et al. HER2-positive male breast cancer: an update. Breast Cancer (Dove Med Press). 2010;2:45-58.
4. Schettini F, Chic N, Brasó-Maristany F, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. NPJ Breast Cancer. 2021;7(1):1.
5. SEER Cancer Stat Facts: female breast cancer. National Cancer Institute, 2017. (Accessed November 4, 2021, at https://seer.cancer.gov/statfacts/html/breast.html.)
6. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. Breast Cancer (Dove Med Press). 2019;11:151-164.
7. BreastCancer.Org. Certain risk factors affect different types of breast cancer in different ways. (Accessed March 3, 2022, at https://www.breastcancer.org/research-news/20090522.)
8. Harbeck N, Penault-Llorca F, Cortes J, et al. Breast cancer. Nat Rev Dis Primers 2019;5:66.
9. Schettini F, Chic N, Brasó-Maristany F, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. NPJ breast cancer 2021;7:1.
10. Tarantino P, Hamilton E, Tolaney SM, et al. HER2-low breast cancer: Pathological and clinical landscape. J Clin Oncol 2020;38:1951-62.