Table S1. Prevalence by IHC category[a] in patients previously diagnosed with HER2-negative unresectable/metastatic BC

	HR positive, n/N (%)[b]				HR negative, n/N (%)[b]			
	IHC 0 with no staining	IHC 0 with staining	IHC 1+	IHC 2+/ISH−	IHC 0 with no staining	IHC 0 with staining	IHC 1+	IHC 2+/ISH−
All	97/554 (17.5)	63/554 (11.4)	298/554 (53.8)	96/554 (17.3)	36/159 (22.6)	39/159 (24.5)	72/159 (45.3)	12/159 (7.5)
Breast cancer type[c]								
Primary	28/156 (17.9)	17/156 (10.9)	80/156 (51.3)	31/156 (19.9)	6/32 (18.8)	10/32 (31.3)	15/32 (46.9)	1/32 (3.1)
Metastatic	69/395 (17.5)	46/395 (11.6)	216/395 (54.7)	64/395 (16.2)	30/127 (23.6)	29/127 (22.8)	57/127 (44.9)	11/127 (8.7)
Assay[d]								
Ventana 4B5	71/386 (18.4)	37/386 (9.6)	206/386 (53.4)	72/386 (18.7)	20/97 (20.6)	26/97 (26.8)	45/97 (46.4)	6/97 (6.2)
Non−Ventana 4B5[e]	25/152 (16.4)	24/152 (15.8)	82/152 (53.9)	21/152 (13.8)	15/58 (25.9)	12/58 (20.7)	25/58 (43.1)	6/58 (10.3)
Country								
Australia	2/22 (9.1)	6/22 (27.3)	12/22 (54.5)	2/22 (9.1)	2/5 (40.0)	1/5 (20.0)	2/5 (40.0)	0/5 (0)
Canada	10/55 (18.2)	7/55 (12.7)	27/55 (49.1)	11/55 (20.0)	7/24 (29.2)	4/24 (16.7)	10/24 (41.7)	3/24 (12.5)
Germany	7/85 (8.2)	8/85 (9.4)	56/85 (65.9)	14/85 (16.5)	3/26 (11.5)	3/26 (11.5)	17/26 (65.4)	3/26 (11.5)
France	17/70 (24.3)	0/70 (0)	43/70 (61.4)	10/70 (14.3)	1/2 (50.0)	0/2 (0)	1/2 (50.0)	0/2 (0)
Italy	14/88 (15.9)	15/88 (17.0)	52/88 (59.1)	7/88 (8.0)	10/36 (27.8)	7/36 (19.4)	16/36 (44.4)	3/36 (8.3)
Japan	17/120 (14.2)	21/120 (17.5)	55/120 (45.8)	27/120 (22.5)	6/35 (17.1)	16/35 (45.7)	12/35 (34.3)	1/35 (2.9)
South Korea	3/16 (18.8)	1/16 (6.3)	8/16 (50.0)	4/16 (25.0)	4/9 (44.4)	1/9 (11.1)	3/9 (33.3)	1/9 (11.1)
Portugal	21/48 (43.8)	0/48 (0)	20/48 (41.7)	7/48 (14.6)	1/2 (50.0)	0/2 (0)	1/2 (50.0)	0/2 (0)
United Kingdom	3/20 (15.0)	3/20 (15.0)	9/20 (45.0)	5/20 (25.0)	2/9 (22.2)	2/9 (22.2)	5/9 (55.6)	0/9 (0)
United States	3/30 (10.0)	2/30 (6.7)	16/30 (53.3)	9/30 (30.0)	0/11 (0)	5/11 (45.5)	5/11 (45.5)	1/11 (9.1)

BC, breast cancer; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IHC, immunohistochemistry; ISH, in situ hybridization. Percentages are calculated based on reported/non-missing data.

[a] IHC statuses were based on rescore results.

[b] Denominator is the number of patients with available HR status and HER2 category of respective assay, cancer type, or country.

[c] This subcategory only includes patients with a sample with an available biopsy date. Primary BC was defined as a rescored biopsy sample dated 30 days before the unresectable/metastatic BC diagnosis date. Metastatic was defined as a rescored biopsy sample dated on or after 30 days, prior to the unresectable/metastatic BC diagnosis date.

[d] Includes only known (non-missing) IHC tests.

[e] Non–Ventana 4B5 assays include HercepTest (Agilent), Bond Oracle HER2 IHC System (Leica), and unknown assays.

Table S2. Patient demographics by IHC category[a]

	HR-positive				HR-negative			
	IHC 0 with no staining (n=97)	IHC 0 with staining (n=63)	IHC 1+ (n=298)	IHC 2+/ISH– (n=96)	IHC 0 with no staining (n=36)	IHC 0 with staining (n=39)	IHC 1+ (n=72)	IHC 2+/ISH– (n=12)
Female, n (%)	96 (99.0)	63 (100.0)	298 (100.0)	96 (100.0)	36 (100.0)	39 (100.0)	72 (100.0)	11 (91.7)
Age at index date, median (range), years[b]	60 (28-90)	56 (29-77)	61 (31-93)	60 (32-97)	53 (35-78)	52 (35-92)	56 (31-80)	63 (38-78)
Age range, n (%)								
18-44 years	12 (12.4)	7 (11.1)	20 (6.7)	14 (14.6)	8 (22.2)	11 (28.2)	8 (11.1)	1 (8.3)
45-64 years	40 (41.2)	28 (44.4)	116 (38.9)	39 (40.6)	17 (47.2)	13 (33.3)	34 (47.2)	4 (33.3)
≥65 years	34 (35.1)	14 (22.2)	90 (30.2)	26 (27.1)	5 (13.9)	10 (25.6)	11 (15.3)	4 (33.3)
Age not reported/missing	11 (11.3)	14 (22.2)	72 (24.2)	17 (17.7)	6 (16.7)	5 (12.8)	19 (26.4)	3 (25.0)
Race, n (%)								
American Indian or Alaska Native	0	0	1 (0.3)	0	0	0	0	0
Asian	20 (20.6)	22 (34.9)	64 (21.5)	33 (34.4)	10 (27.8)	18 (46.2)	15 (20.8)	2 (16.7)
Black or African American	2 (2.1)	1 (1.6)	3 (1.0)	2 (2.1)	0	2 (5.1)	0	1 (8.3)
White	31 (32.0)	28 (44.4)	124 (41.6)	34 (35.4)	18 (50.0)	17 (43.6)	41 (56.9)	8 (66.7)
Other	0	0	4 (1.3)	0	0	0	0	0
Not reported/missing	44 (45.4)	12 (19.0)	102 (34.2)	27 (28.1)	8 (22.2)	2 (5.1)	16 (22.2)	1 (8.3)
Menopausal status, n (%)[c]								
Premenopausal	27 (27.8)	17 (27.0)	67 (22.5)	22 (22.9)	15 (41.7)	17 (43.6)	23 (31.9)	2 (16.7)
Postmenopausal	59 (60.8)	40 (63.5)	197 (66.1)	62 (64.6)	15 (41.7)	20 (51.3)	38 (52.8)	7 (58.3)
Not reported/missing	11 (11.3)	6 (9.5)	34 (11.4)	12 (12.5)	6 (16.7)	2 (5.1)	11 (15.3)	3 (25.0)

HR, hormone receptor; IHC, immunohistochemistry; ISH, in situ hybridization.
[a] IHC statuses were based on rescore results.
[b] Index date was defined as the date of human epidermal growth factor receptor 2–negative unresectable/metastatic breast cancer diagnosis.
[c] Status can be from records after the baseline period.

Table S3. Patient clinical characteristics by IHC category[a]								
	HR-positive, n (%)				HR-negative, n (%)			
	IHC 0 with no staining (n=97)	IHC 0 with staining (n=63)	IHC 1+ (n=298)	IHC 2+/ ISH− (n=96)	IHC 0 with no staining (n=36)	IHC 0 with staining (n=39)	IHC 1+ (n=72)	IHC 2+/ ISH− (n=12)
Time from initial BC diagnosis to metastatic BC diagnosis[b]								
<2 years	44 (45.4)	19 (30.2)	113 (37.9)	40 (41.7)	16 (44.4)	22 (56.4)	24 (33.3)	4 (33.3)
2-5 years	14 (14.4)	12 (19.0)	34 (11.4)	19 (19.8)	6 (16.7)	6 (15.4)	17 (23.6)	2 (16.7)
>5 years	28 (28.9)	17 (27.0)	78 (26.2)	20 (20.8)	8 (22.2)	6 (15.4)	12 (16.7)	3 (25.0)
Not reported/missing	11 (11.3)	15 (23.8)	73 (24.5)	17 (17.7)	6 (16.7)	5 (12.8)	19 (26.4)	3 (25.0)
Metastatic location[c]								
Bone only	17 (17.5)	14 (22.2)	69 (23.2)	21 (21.9)	2 (5.6)	2 (5.1)	4 (5.6)	1 (8.3)
Brain	4 (4.1)	4 (6.3)	10 (3.4)	4 (4.2)	2 (5.6)	2 (5.1)	7 (9.7)	2 (16.7)
Liver	33 (34.0)	18 (28.6)	88 (29.5)	23 (24.0)	12 (33.3)	5 (12.8)	26 (36.1)	6 (50.0)
Lung	22 (22.7)	12 (19.0)	70 (23.5)	24 (25.0)	11 (30.6)	13 (33.3)	21 (29.2)	3 (25.0)
Visceral	63 (64.9)	38 (60.3)	165 (55.4)	49 (51.0)	20 (55.6)	19 (48.7)	45 (62.5)	8 (66.7)
Number of metastatic locations[c]								
1	43 (44.3)	35 (55.6)	178 (59.7)	44 (45.8)	20 (55.6)	16 (41.0)	35 (48.6)	6 (50.0)
2	29 (29.9)	11 (17.5)	65 (21.8)	29 (30.2)	6 (16.7)	15 (38.5)	16 (22.2)	1 (8.3)
≥3	25 (25.8)	17 (27.0)	53 (17.8)	22 (22.9)	10 (27.8)	6 (15.4)	19 (26.4)	5 (41.7)
Metastatic or locally advanced at index date[b]								
Locally advanced	2 (2.1)	0	5 (1.7)	2 (2.1)	1 (2.8)	1 (2.6)	0	0
Metastatic	80 (82.5)	49 (77.8)	221 (74.2)	72 (75.0)	29 (80.6)	33 (84.6)	51 (70.8)	9 (75.0)
Both	6 (6.2)	0	4 (1.3)	6 (6.3)	1 (2.8)	1 (2.6)	2 (2.8)	0
Not reported/missing	9 (9.3)	14 (22.2)	68 (22.8)	16 (16.7)	5 (13.9)	4 (10.3)	19 (26.4)	3 (25.0)
Stage at initial BC diagnosis								
I	4 (4.1)	4 (6.3)	13 (4.4)	5 (5.2)	5 (13.9)	4 (10.3)	7 (9.7)	1 (8.3)
II	16 (16.5)	20 (31.7)	61 (20.5)	19 (19.8)	6 (16.7)	15 (38.5)	14 (19.4)	2 (16.7)
III	16 (16.5)	9 (14.3)	36 (12.1)	11 (11.5)	7 (19.4)	11 (28.2)	15 (20.8)	3 (25.0)
IV	20 (20.6)	8 (12.7)	32 (10.7)	21 (21.9)	4 (11.1)	5 (12.8)	1 (1.4)	1 (8.3)
Other/not reported/missing	41 (42.3)	22 (34.9)	156 (52.3)	40 (41.7)	14 (38.9)	4 (10.3)	35 (48.6)	5 (41.7)
HR, hormone receptor; IHC, immunohistochemistry; ISH, in situ hybridization.								

^a IHC statuses were based on rescore results.
^b Index date was defined as the date of human epidermal growth factor receptor 2–negative unresectable/metastatic breast cancer diagnosis.
^c Reported over the course of disease.

Table S4. Treatments received in the metastatic setting by IHC category							
	HR-positive, n (%)				HR-negative, n (%)		
	IHC 0 with no staining[a] (n=75)	IHC 0 with staining[a] (n=55)	IHC 1+[a] (n=238)	IHC 2+/ISH−[a] (n=80)	IHC 0 with no staining[a] (n=22)	IHC 0 with staining[a] (n=33)	IHC 1+[a] (n=60)	IHC 2+/ISH−[a] (n=11)
Overall							
Endocrine therapy	69/75 (92.0)	47/55 (85.5)	215/238 (90.3)	72/80 (90.0)	3/22 (13.6)	3/33 (9.1)	13/60 (21.7)	4/11 (36.4)
Chemotherapy	56/75 (74.7)	42/55 (76.4)	180/238 (75.6)	59/80 (73.8)	22/22 (100.0)	33/33 (100.0)	60/60 (100.0)	11/11 (100.0)
CDK4/6i	35/75 (46.7)	13/55 (23.6)	84/238 (35.3)	33/80 (41.3)	0/22 (0)	1/33 (3.0)	2/60 (3.3)	1/11 (9.1)
Other targeted therapies[b]	17/75 (22.7)	12/55 (21.8)	68/238 (28.6)	21/80 (26.3)	3/22 (13.6)	8/33 (24.2)	11/60 (18.3)	1/11 (9.1)
First treatment							
Endocrine monotherapy	35/75 (46.7)	27/55 (49.1)	98/238 (41.2)	39/80 (48.8)	1/22 (4.5)	0/33 (0)	2/60 (3.3)	0/11 (0)
Single-agent chemotherapy	12/75 (16.0)	11/55 (20.0)	49/238 (20.6)	13/80 (16.3)	8/22 (36.4)	15/33 (45.5)	31/60 (51.7)	5/11 (45.5)
CDK4/6i	0/75 (0)	0/55 (0)	0/238 (0)	1/80 (1.3)	0/22 (0)	0/33 (0)	0/60 (0)	0/11 (0)
Other targeted therapies[b]	0/75 (0)	0/55 (0)	0/238 (0)	0/80 (0)	0/22 (0)	0/33 (0)	0/60 (0)	0/11 (0)
Immunotherapy	0/75 (0)	0/55 (0)	0/238 (0)	0/80 (0)	0/22 (0)	1/33 (3.0)	2/60 (3.3)	0/11 (0)
Combination therapy (any)	28/75 (37.3)	17/55 (30.9)	91/238 (38.2)	27/80 (33.8)	13/22 (59.1)	17/33 (51.5)	25/60 (41.7)	6/11 (54.5)

CDK4/6i, cyclin-dependent kinase 4 and 6 inhibitors; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IHC, immunohistochemistry; ISH, in situ hybridization. Percentages were calculated based on reported/nonmissing data.

[a] IHC statuses are based on rescore results.

[b] Includes anti-HER2 agents, antiangiogenesis agents, poly (ADP-ribose) polymerase, phosphoinositide 3-kinase, and mechanistic target of rapamycin inhibitors.

Table S5. Clinical outcomes by IHC category[a]							
	HR positive, Kaplan-Meier estimate, median (95% CI), months				HR negative, Kaplan-Meier estimate, median (95% CI), months		
	IHC 0 with no staining (n=75)	IHC 0 with staining (n=55)	IHC 1+ (n=238)	IHC 2+/ISH– (n=80)	IHC 0 with no staining (n=22)	IHC 0 with staining (n=33)	IHC 1+ (n=60)	IHC 2+/ISH– (n=11)
Time to treatment failure	7.4 (5.1-10.0)	5.7 (3.3-7.1)	7.6 (5.8-9.8)	5.7 (4.3- 8.5)	4.8 (3.5- 10.7)	2.3 (1.7-3.5)	3.6 (2.7-4.4)	4.4 (0.0-11.0)
Time to first subsequent treatment	9.8 (7.1-14.5)	6.3 (4.0-8.2)	9.3 (8.0-12.8)	10.4 (6.3-15.3)	8.6 (4.4-11.8)	4.0 (2.8-5.4)	5.3 (3.4-7.6)	5.6 (0.3-11.2)
Overall survival	45.1 (35.6-54.2)	35.5 (26.0-53.7)	47.0 (40.7-53.2)	42.2 (31.1-48.3)	16.8 (10.7-30.8)	10.4 (7.7-27.5)	21.3 (14.1-29.4)	20.9 (3.5-NC)
HR, hormone receptor; IHC, immunohistochemistry; ISH, in situ hybridization, NC, not calculable. [a] IHC statuses were based on rescore results.								

Table S6. Concordance between historical and rescored HER2 slides					
	Agreement on IHC 0 (positive percent agreement[a]), n/N (%)	Agreement on IHC 1+ (positive percent agreement[a]), n/N (%)	Agreement on IHC 2+/ISH− (positive percent agreement[a]), n/N (%)	Agreement on all IHC categories (overall percent agreement[b]), n/N (%)	Kappa (95% CI)
All	193/276 (69.9)	232/303 (76.6)	100/207 (48.3)[c]	525/786 (66.8)	0.484 (0.434-0.535)
Assay[d]					
Ventana 4B5	146/215 (67.9)	171/216 (79.2)	72/136 (52.9)	389/567 (68.6)	0.511 (0.452-0.570)
Non–Ventana 4B5[e]	47/60 (78.3)	55/80 (68.8)	27/70 (38.6)	129/210 (61.4)	0.415 (0.318-0.512)
Country					
Australia	8/14 (57.1)	9/12 (75.0)	1/2 (50.0)	18/28 (64.3)	0.386 (0.091-0.681)
Canada	15/16 (93.8)	6/15 (40.0)	14/48 (29.2)	35/79 (44.3)	0.239 (0.113-0.365)
Germany	35/72 (48.6)	61/75 (81.3)	16/33 (48.5)	112/180 (62.2)	0.390 (0.280-0.500)
France	13/16 (81.3)	16/19 (84.2)	11/37 (29.7)	40/72 (55.6)	0.372 (0.229-0.515)
Italy	31/43 (72.1)	50/65 (76.9)	10/16 (62.5)	91/124 (73.4)	0.536 (0.397-0.675)
Japan	48/63 (76.2)	36/53 (67.9)	22/39 (56.4)	106/155 (68.4)	0.513 (0.401-0.625)
South Korea	6/6 (100.0)	6/10 (60.0)	4/9 (44.4)	16/25 (64.0)	0.459 (0.179-0.739)
Portugal	21/23 (91.3)	19/20 (95.0)	7/8 (87.5)	47/51 (92.2)	0.872 (0.752-0.993)
United Kingdom	8/13 (61.5)	10/13 (76.9)	5/5 (100.0)	23/31 (74.2)	0.585 (0.324-0.846)
United States	8/10 (80.0)	19/21 (90.5)	10/10 (100.0)	37/41 (90.2)	0.842 (0.694-0.990)

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization. Percentages were calculated based on reported/non-missing data.
[a] Positive percent agreement is the proportion of historical HER2-negative IHC scores and local laboratory rescores in the HER2-negative subgroupings (HER2 IHC 0, 1+, 2+) that were both positive.
[b] Overall percent agreement is the proportion of historical HER2-negative IHC scores and local laboratory rescores in the HER2-negative subgroupings (HER2 IHC 0, 1+, 2+) that were either in positive or negative agreement.

^c Of the 107 patients with historical IHC 2+ whose samples were rescored in a different IHC category, 100 were rescored as IHC 1+, and 7 were rescored as IHC 0.
^d Includes patients for whom information on the assay used was available.
^e Non–Ventana 4B5 assays included HercepTest (Agilent), Bond Oracle HER2 IHC System (Leica), and unknown assays.

Figure S1. Current HER2 scoring scheme. ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization. [a] HER2 IHC 0 with no staining was defined as no perceivable staining, and HER2 IHC 0 with staining was defined as faint or incomplete staining in <10% of cells per 2018 ASCO/CAP HER2 guidelines.[1]

HER2 Negative			HER2 Positive	
IHC 0	IHC 1+	IHC 2+ ISH-	IHC 2+ ISH+	IHC 3+
HER2 IHC 0	HER2-low			

IHC 0 with no staining[a]	IHC 0 with staining[a]