Supplementary Tables

Table S1. List of antibodies used for immunohistochemical analyses. The analyses were performed on a Dako Omnis staining platform using the polymer-based EnVision FLEX antigen retrieval system. PD-L1, programmed death-ligand 1; RTU, ready to use.

Marker	Clone	Dilution	Antigen Retrieval	Scoring
ER	EP1	RTU	High pH, 20'	ASCO/CAP and St Gallen guidelines; positive if ≥1% of tumor cell nuclei are immunoreactive.
PgR	PgR 636	1:100	High pH, 30′	ASCO/CAP and St Gallen guidelines; positive if ≥1% of tumor cell nuclei are immunoreactive.
Ki-67	MIB1	RTU	High pH, 30′	International Ki67 in Breast Cancer Working Group; high if ≥30% of tumor cell nuclei are immunoreactive
HER2	Polyclonal	1:400	Low pH, 30'	ASCO/CAP guidelines; 3+ if complete membrane staining that is intense and >10% of tumor cells; 2+ if weak to moderate complete membrane staining in >10% of tumor cells or complete membrane staining that is intense but within ≤10% of tumor cells; 1+ if incomplete membrane staining that is faint/barely perceptible and within >10% of tumor cells; 0 if no staining observed or membrane stating that is incomplete and is faint/barely perceptible and within ≤10% of tumor cells.
TILs	n/a	n/a	n/a	
CD4	4B12	RTU	Low pH, 30'	Negative if complete loss of membrane staining within the lymphocytes.
CD8	C8/144B	1:50	Low pH, 30'	Negative if complete loss of membrane staining within the lymphocytes; low, 1–30%; intermediate, 31–50%; high, >50%.
FOXP3	Polyclonal	1:200	Low pH, 30'	Negative if complete loss of membrane staining within the lymphocytes.
PD-L1	22C3	RTU	Low pH, 30'	Combined positive score (CPS), i.e. total number of PD-L1+ tumor cells, lymphocytes, and macrophages divided by the total number of viable tumor cells, multiplied by 100.

Table S2. Analysis of biomarker status in the study population and controls. Estrogen receptor (ER) and progesterone receptor (PR) expression. HR, hormone receptors. Significant correlations among the different subsets of patients are highlighted with a star (*).

	PrBC (*n* = 83)	EOBC (*n* = 89)	MSK (*n* = 438)	*p*-value
ER+, *n* (%)	46 (55.4)	65 (73.0)	395 (90.2)	<0.0001*
PgR+, *n* (%)	43 (51.8)	63 (70.8)	342 (78.1)	<0.0001*
Subtypes, *n* (%)				
HR+/HER2-	44 (53.0)	59 (66.3)	381 (86.9)	<0.0001*
HER2+	9 (10.8)	10 (11.2)	21 (4.8)	0.019*
HR-/HER2-	30 (36.1)	20 (22.5)	36 (8.2)	<0.0001*

Table S3. Therapeutic data of the PrBC and the EOBC. PrBC, breast cancer during pregnancy; EOBC, early-onset breast cancers; HR, hormone receptors; TILs, tumor-infiltrating lymphocytes; FOXP3, forkhead box P3; PD-L1, programmed death-ligand 1; CPS, combined positive score. Significant correlations are highlighted with a star (*).

	Endocrine Therapy					Chemotherapy				
	PrBC		EOBC		*p*-Value	PrBC		EOBC		*p*-Value
	Yes	No	Yes	No		Yes	No	Yes	No	
All cases with FU, *n* (%)	49 (62.0)	30 (38.0)	18 (39.1)	28 (60.9)	0.0133*	66 (83.5)	13 (16.5)	31 (67.4)	15 (32.6)	0.0367*
HR+HER2-	42 (97.7)	1 (2.3)	16 (51.6)	15 (48.4)	0.0001*	30 (71.4)	12 (28.6)	19 (61.3)	12 (38.7)	0.3620
HER2+	2 (22.2)	7 (77.8)	1 (25.0)	3 (75.0)	0.9126	9 (100.0)	0	3 (75.0)	1 (25.0)	0.2416
HR-/HER2-	5 (18.5)	22 (81.5)	1 (9.1)	10 (90.9)	0.4698	27 (96.4)	1 (3.6)	9 (81.8)	2 (18.2)	0.1233
Presence of TILs, *n* (%)	44 (62.9)	26 (37.1)	14 (35.0)	26 (65.0)	0.0048*	56 (81.2)	13 (18.8)	29 (72.5)	11 (27.5)	0.2929
Low	32 (65.3)	17 (34.7)	10 (37.0)	17 (63.0)	0.0176*	39 (81.3)	9 (18.7)	20 (74.1)	7 (25.9)	0.4625
Intermediate	7 (58.3)	5 (41.7)	2 (25.0)	6 (75.0)	0.1421	11 (91.7)	1 (8.3)	6 (75.0)	2 (25.0)	0.3064
High	5 (55.6)	4 (44.4)	2 (40.0)	3 (60.0)	0.5769	6 (66.7)	3 (33.3)	3 (60.0)	2 (40.0)	0.8030
CD8 TILs, *n* (%)	40 (61.5)	25 (38.5)	13 (36.1)	23 (63.9)	0.0125*	55 (85.9)	9 (14.1)	26 (72.2)	10 (27.8)	0.0933
Low	15 (68.2)	7 (31.8)	9 (45.0)	11 (55.0)	0.1294	19 (86.4)	3 (13.6)	13 (65.0)	7 (35.0)	0.1044
Intermediate	16 (59.3)	11 (40.7)	2 (22.2)	7 (77.8)	0.0542	20 (76.9)	6 (23.1)	8 (88.9)	1 (11.1)	0.4339
High	9 (56.3)	7 (43.7)	2 (28.6)	5 (71.4)	0.2214	16 (100.0)	0	5 (71.4)	2 (28.6)	0.2445
CD4 TILs, *n* (%)	15 (55.6)	12 (44.4)	11 (36.7)	19 (63.3)	0.1528	26 (96.3)	1 (3.7)	23 (76.7)	7 (23.3)	0.0331*
FOXP3, *n* (%)	15 (55.6)	12 (44.4)	7 (41.2)	10 (58.8)	0.3529	23 (85.2)	4 (14.8)	10 (58.8)	7 (41.2)	0.0492*
PD-L1 CPS ≥10, *n* (%)	-	-	3 (37.5)	5 (62.5)	-	-	-	5 (62.5)	3 (37.5)	-

Table S4. Disease progression and patients' death status both in PrBC and EOBC based on the administration of chemotherapy. PrBC, breast cancer during pregnancy; EOBC, early-onset breast cancers; HR, hormone receptors; TILs, tumor-infiltrating lymphocytes; FOXP3, forkhead box P3; PD-L1, programmed death-ligand 1; CPS, combined positive score. Significant correlations are highlighted with a star (*).

	Chemotherapy									
	Disease Recurrence					Died of Disease				
	PrBC		EOBC		*p*-Value	PrBC		EOBC		*p*-Value
	Yes	No	Yes	No		Yes	No	Yes	No	
All cases with FU, *n* (%)	31 (47.0)	35 (53.0)	3 (9.7)	28 (90.3)	0.0003*	16 (24.2)	50 (75.8)	0	31 (100)	0.0027*
HR+HER2-	13 (43.3)	17 (56.7)	3 (15.8)	16 (84.2)	0.0451*	6 (20.0)	24 (80.0)	0	19 (100)	0.0374*
HER2+	5 (55.6)	4 (44.4)	0	3 (100)	0.0909	2 (22.2)	7 (77.8)	0	3 (100)	0.3710
HR-/HER2-	13 (48.1)	14 (51.9)	0	9 (100)	0.0092*	8 (29.6)	19 (70.4)	0	9 (100)	0.0641
Presence of TILs, *n* (%)	26 (46.4)	30 (53.6)	2 (6.9)	27 (93.1)	0.0002*	13 (23.2)	43 (76.8)	0	29 (100)	0.0048*
Low	19 (48.7)	20 (51.3)	2 (10.0)	18 (90.0)	0.0032*	10 (25.6)	29 (74.4)	0	20 (100)	0.0129*
Intermediate	5 (45.4)	6 (54.5)	0	6 (100)	0.0493*	2 (18.2)	9 (81.8)	0	6 (100)	0.2662
High	2 (33.3)	4 (66.6)	0	3 (100)	0.2568	1 (16.6)	5 (83.3)	0	3 (100)	0.4532
CD8 TILs, *n* (%)	16 (40.0)	24 (60.0)	2 (7.7)	24 (92.3)	0.0039*	8 (20.0)	32 (80.0)	0	26 (100)	0.0149*
Low	13 (68.4)	6 (31.6)	1 (7.7)	12 (92.3)	0.0007*	8 (42.1)	11 (57.9)	0	13 (100)	0.0069*
Intermediate	6 (30.0)	14 (70.0)	1 (12.5)	7 (87.5)	0.3339	3 (15.0)	17 (85.0)	0	8 (100)	0.2463
High	7 (43.7)	9 (56.3)	0	5 (100)	0.0701	2 (12.5)	14 (87.5)	0	5 (100)	0.4058
CD4 TILs, *n* (%)	9 (34.6)	17 (65.4)	2 (8.7)	21 (91.3)	0.0299*	2 (7.7)	24 (92.3)	0	23 (100)	0.1744
FOXP3, *n* (%)	9 (39.1)	14 (60.9)	0	10 (100)	0.0204*	3 (13.0)	20 (87.0)	0	10 (100)	0.2309

	Chemotherapy								
	Disease Recurrence					**Died of Disease**			
	PrBC		**EOBC**		*p*-Value	**PrBC**		**EOBC**		*p*-Value
	Yes	**No**	**Yes**	**No**		**Yes**	**No**	**Yes**	**No**	
PD-L1 CPS ≥10, *n* (%)	1 (33.3)	2 (66.6)	0	5 (100)	0.1675	0	3 (100)	0	5 (100)	-

Table S5. Disease progression and patients' death status in the study and control groups based on the administration of endocrine therapy. PrBC, breast cancer during pregnancy; EOBC, early-onset breast cancers; HR, hormone receptors; TILs, tumor-infiltrating lymphocytes; FOXP3, forkhead box P3; PD-L1, programmed death-ligand 1; CPS, combined positive score. Significant correlations are highlighted with a star (*).

	Endocrine Therapy								
	Disease Recurrence					**Died of Disease**			
	PrBC		**EOBC**		*p*-Value	**PrBC**		**EOBC**		*p*-Value
	Yes	**No**	**Yes**	**No**		**Yes**	**No**	**Yes**	**No**	
All cases with FU, *n* (%)	20 (40.8)	29 (59.2)	3 (16.6)	15 (83.3)	0.0649	10 (20.4)	39 (79.6)	1 (5.6)	17 (94.6)	0.1457
HR+HER2-	16 (38.1)	26 (61.9)	3 (18.8)	13 (81.2)	0.1606	6 (14.3)	36 (85.7)	1 (6.3)	15 (93.7)	0.4011
HER2+	1 (50.0)	1 (50.0)	0	1 (100)	0.3864	1 (100)	1	0	1 (100)	0.3864
HR-/HER2-	3 (60.0)	2 (40.0)	0	1 (100)	0.2733	3 (60.0)	2 (40.0)	0	1 (100)	0.2733
Presence of TILs, *n* (%)	18 (40.9)	26 (59.1)	1 (7.1)	13 (92.9)	0.0190*	8 (18.2)	36 (81.8)	0	14 (100)	0.0857
Low	13 (40.6)	19 (59.4)	1 (10.0)	9 (90.0)	0.0729	6 (18.8)	26 (81.2)	0	10 (100)	0.1391
Intermediate	4 (57.1)	3 (42.9)	0	2 (100)	0.1515	1 (14.3)	6 (85.7)	0	2 (100)	0.5707
High	1 (20.0)	4 (80.0)	0	2 (100)	0.4945	1 (20.0)	4 (80.0)	0	2 (100)	0.4945
CD8 TILs, *n* (%)	16 (40.0)	24 (60.0)	1 (7.7)	12 (92.3)	0.0301*	8 (20.0)	32 (80.0)	0	13 (100)	0.0801
Low	9 (60.0)	6 (40.0)	1 (11.1)	8 (88.9)	0.0186*	5 (33.3)	10 (66.6)	0	9 (100)	0.0515
Intermediate	4 (25.0)	12 (75.0)	0	2 (100)	0.4226	2 (12.5)	14 (87.5)	0	2 (100)	0.5958
High	3 (33.3)	6 (66.6)	0	2 (100)	0.3383	1 (11.1)	8 (88.9)	0	2 (100)	0.6210
CD4 TILs, *n* (%)	4 (26.6)	11 (73.3)	1 (9.1)	10 (90.9)	0.2612	1 (6.66)	14 (93.3)	0	11 (100)	0.3825
FOXP3, *n* (%)	6 (40.0)	9 (60.0)	0	7 (100)	0.0497*	3 (20.0)	12 (80.0)	0	7 (100)	0.2029
PD-L1 CPS ≥10, *n* (%)	0	2 (100)	0	4 (100)	-	0	2 (100)	0	4 (100)	-