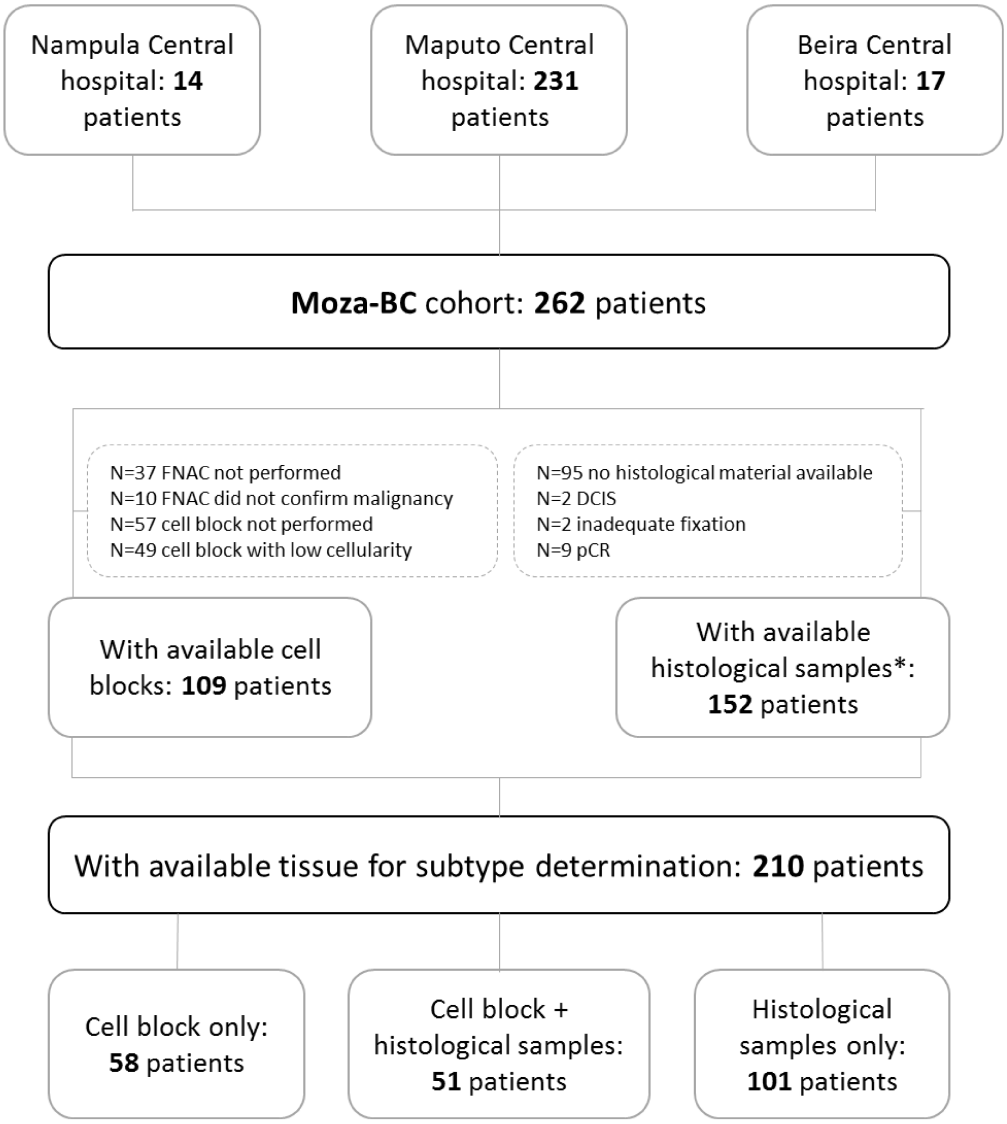
Supplementary material

"Breast cancer subtypes: implications for the treatment and survival of patients in Africa – a prospective cohort study from Mozambique"

Supplementary figure 1 – Participants of the Moza-BC cohort, according to the hospital from which they were included and according to tissue availability for the assessment of breast cancer subtypes



* 152 patients, with a total of three core needle biopsies, 13 incisional surgical biopsies, 22 excisional surgical biopsies, four tumorectomies and 128 mastectomies.

Supplementary table 1 – Comparison of baseline characteristics of patients included in the Moza-BC cohort with data regarding breast cancer subtypes who were treated at the Oncology Unit of the Maputo Central Hospital (MCH; n=175) *vs* those who were not treated at the Oncology Unit of the MCH (n=35)

	Followed at the MCH, n=175	Not followed at the MCH, n=35	P
Age in years (n, %)			.150
<40	44 (25.1)	8 (25.0)	
40-49	49 (28.0)	7 (21.9)	
50-59	42 (24.0)	4 (12.5)	
≥60	40 (22.9)	13 (40.6)	
Missing	0	3	
Race (n, %)			.585
Black	172 (98.3)	34 (100)	
Other[1]	3 (1.7)	0	
Missing	0	1	
Place of residence (n, %)			**<.001**
South (including Maputo)	150 (90.9)	20 (64.5)	
Center/North	15 (9.1)	11 (35.5)	
Missing	10	4	
Classic subtypes (n, %)			.303
ER-positive/HER2-negative	91 (52.0)	17 (48.6)	
HER2-positive	44 (25.1)	6 (17.1)	
TNBC	40 (22.9)	12 (34.3)	
Surrogate intrinsic subtypes (n, %)[2]			.491
Luminal A-like	25 (14.3)	3 (8.6)	
Luminal B-like	87 (49.7)	16 (45.7)	
HER2-enriched	23 (13.1)	4 (11.4)	
Basal-like	40 (22.9)	12 (34.3)	
Tumor characteristics (clinical staging) (n, %)			.293
cT1	6 (3.5)	1 (2.9)	
cT2	43 (24.9)	13 (37.1)	
cT3	52 (30.1)	12 (34.3)	
cT4	72 (41.6)	9 (25.7)	
Missing	2	0	
Lymph node status (clinical staging) (n, %)			.068
cN0	43 (28.1)	16 (45.7)	
cN+	110 (71.9)	19 (54.3)	
Missing	22	0	

[1]Includes: mixed and Indian race.

[2]Tumors were classified into surrogate intrinsic subtypes according to the St. Gallen classification as: Luminal A-like: estrogen receptor (ER)-positive, progesterone receptor (PR)-positivity≥20%, HER2-negative and Ki67≤29%; Luminal B-like: ER-positive/HER2-negative and either PR-positivity<20% or Ki67>29%, or ER-positive/HER2-positive; HER2-enriched: ER-negative/PR-negative/HER2-positive; and Basal-like: ER-negative/PR-negative/HER2-negative.

cN+: lymph node positive; ER: estrogen receptor; TNBC: triple-negative breast cancer.

Supplementary table 2 – Patients' baseline characteristics according to each breast cancer surrogate intrinsic subtype (St. Gallen classification)

	Luminal A-like N=28	Luminal B-like N=103	HER2-enriched n=27	Basal-like n=52	P
Age in years (n, %)					.769
<40	6 (22.2)	27 (26.7)	5 (18.5)	14 (26.9)	
40-49	7 (25.9)	31 (30.7)	6 (22.2)	12 (23.1)	
50-59	6 (22.2)	23 (22.8)	8 (29.6)	9 (17.3)	
≥60	8 (29.6)	20 (19.8)	8 (29.6)	17 (32.7)	
Missing	1	2	0	0	
Race (n, %)					**.044**
Black	25 (92.6)	102 (99.0)	27 (100)	52 (100)	
Other[1]	2 (7.4)	1 (1.0)	0	0	
Missing	1	0	0	0	
Education (n, %)					.532
0 years	7 (33.3)	17 (24.3)	3 (15.0)	5 (14.3)	
1-4 years	2 (9.5)	8 (11.4)	2 (10.0)	7 (20.0)	
>4 years	12 (57.1)	45 (64.3)	15 (75.0)	23 (65.7)	
Missing	7	33	7	17	
Place of residence (n, %)					.243
South (including Maputo)	22 (84.6)	89 (90.8)	22 (88.0)	37 (78.7)	
Center/North	4 (15.4)	9 (9.2)	3 (12.0)	10 (21.3)	
Missing	2	5	2	5	
Menopausal status (n, %)					.907
Pre-menopausal	13 (52.0)	48 (55.2)	11 (47.8)	20 (50.0)	
Post-menopausal	12 (48.0)	39 (44.8)	12 (52.2)	20 (50.0)	
Missing	3	16	4	12	
Body mass index (n, %)					.250
Under/normal weight (<25 kg/m^2)	9 (39.1)	36 (45.0)	6 (30.0)	10 (27.0)	
Overweight/obese (≥25 kg/m^2)	14 (60.9)	44 (55.0)	14 (70.0)	27 (73.0)	
Missing	5	23	7	15	
HIV status (n, %)[2]					.064
Negative/unknown	17 (68.0)	67 (77.0)	21 (91.3)	25 (62.5)	
Positive	8 (32.0)	20 (23.0)	2 (8.7)	15 (37.5)	
Missing	3	16	4	12	
Tumor characteristics (clinical staging) (n, %)					.065
cT1	3 (10.7)	1 (1.0)	2 (7.7)	1 (1.9)	
cT2	6 (21.4)	27 (26.5)	9 (34.6)	14 (26.9)	
cT3	6 (21.4)	29 (28.4)	11 (42.3)	18 (34.6)	
cT4	13 (46.4)	45 (44.1)	4 (15.4)	19 (36.5)	
Missing	0	1	1	0	
Lymph node status (clinical staging) (n, %)					.572
cN0	9 (36.0)	28 (29.8)	10 (41.7)	12 (26.7)	
cN+	16 (64.0)	66 (70.2)	14 (58.3)	33 (73.3)	
Missing	3	9	3	7	
Tumor characteristics (pathological staging) (n, %)					.101
(y)pT0/Tis	0	5 (6.5)	0	2 (5.9)	
(y)pT1	7 (33.3)	10 (13.0)	6 (27.3)	7 (20.6)	
(y)pT2	8 (38.1)	31 (40.3)	13 (59.1)	10 (29.4)	
(y)pT3	2 (9.5)	17 (22.1)	3 (13.6)	11 (32.4)	
(y)pT4	4 (19.0)	14 (18.2)	0	4 (11.8)	
Missing	7	26	5	8	
Lymph node status (pathological staging) (n, %)					.916
(y)pN0	4 (28.6)	15 (24.2)	5 (23.8)	9 (31.0)	
(y)pN1	7 (50.0)	22 (35.5)	7 (33.3)	11 (37.9)	
(y)pN2	2 (14.3)	16 (25.8)	6 (28.6)	4 (13.8)	
(y)pN3	1 (7.1)	9 (14.5)	3 (14.3)	5 (17.2)	
Missing	14	41	6	23	
Median tumor size at surgery in mm(median, range)	35 (12-120)	40 (0-180)	40 (2.5-80)	45 (0-180)	**.046**
Missing	8	26	5	18	

Multifocal tumors at surgery (n, %)					.933
No	15 (88.2)	66 (90.4)	18 (85.7)	28 (90.3)	
Yes	2 (11.8)	7 (9.6)	3 (14.3)	3 (9.7)	
Missing	11	30	6	21	
Lympho-vascular invasion at surgery (n, %)					.791
No	5 (35.7)	16 (24.2)	5 (23.8)	9 (30.0)	
Yes	9 (64.3)	50 (75.8)	16 (76.2)	21 (70.0)	
Missing	12	37	6	22	
Perineural invasion at surgery (n, %)					.803
No	11 (78.6)	49 (74.2)	16 (76.2)	25 (83.3)	
Yes	3 (21.4)	17 (25.8)	5 (23.8)	5 (16.7)	
Missing	12	37	6	22	
Histological grade at surgery (n, %)					**.038**
1	4 (20.0)	18 (25.0)	8 (38.1)	5 (16.1)	
2	11 (55.0)	37 (51.4)	7 (33.3)	9 (29.0)	
3	5 (25.0)	17 (23.6)	6 (28.6)	17 (54.8)	
Missing	8	31	6	21	
Histological subtype at surgery (n, %)					.661
No residual tumor/*in situ* carcinoma	0	5 (6.4)	0	2 (5.7)	
Invasive ductal carcinoma (NST)	18 (90.9)	63 (80.8)	19 (90.5)	31 (88.6)	
Other invasive subtypes[3]	2 (10.0)	10 (12.8)	2 (9.5)	2 (5.7)	
Missing	8	25	6	17	
Stage at diagnosis (n, %)					.285
I	0	1 (1.1)	1 (4.3)	1 (2.5)	
II	8 (32.0)	13 (14.9)	8 (34.8)	12 (30.0)	
III	13 (52.0)	56 (64.4)	12 (52.2)	18 (45.0)	
IV	4 (16.0)	17 (19.5)	2 (8.7)	9 (22.5)	
Missing[4]	3	16	4	12	

[1]Includes: mixed and Indian race.

[2]Seven patients had unknown HIV status; among HIV-positive patients, 31 (69%) had been previously diagnosed; the median time since HIV diagnosis was 3.93 years (range: 0.1-11.7); 41 (91%) patients were under anti-retroviral treatment (ART) when starting chemotherapy, mostly with the TDF+3TC+EFV regimen (28 patients); the median time under ART was 2 years (range 0.1-11.7); the median CD4+ cells count was 448 cells/µL (range 43-1104) and 39 (87%) patients had a CD4+ cells count > 200/µL.

[3]Includes: lobular, mixed, papillary, squamous cell carcinoma, metaplastic and mucinous breast cancer.

[4]Includes the 35 patients for whom there is available cT/N and/or (y)pT/N status, but without information regarding the presence of metastases.

NST: no special type.

Supplementary table 3 – Breast cancer clinical management according to each surrogate intrinsic subtype (St. Gallen classification)

	Luminal A-like N=28	Luminal B-like N=103	HER2-enriched N=27	Basal-like n=52	P
Time from first symptom to diagnosis (n, %)					.409
<180 days	5 (35.7)	31 (53.4)	7 (50.0)	13 (65.0)	
≥180 days	9 (64.3)	27 (46.6)	7 (50.0)	7 (35.0)	
Missing	14	45	13	32	
Timing of diagnosis (n, %)					.127
Pre-MTB implementation	10 (40.0)	50 (57.5)	8 (34.8)	23 (57.5)	
Post-MTB implementation	15 (60.0)	37 (42.5)	15 (65.2)	17 (42.5)	
Not applicable[1]	3	16	4	12	
Type of first treatment received (n, %)					.225
No treatment received	0	1 (1.2)	0	2 (5.0)	
Surgery	5 (20.0)	18 (20.9)	8 (34.8)	14 (35.0)	
Chemotherapy/Endocrine therapy[2]	20 (80.0)	67 (77.9)	15 (65.2)	24 (60.0)	
Missing	3	17	4	12	
Time from diagnosis until first treatment (n, %)					**.007**
<45 days	11 (44.0)	33 (38.8)	18 (78.3)	21 (55.3)	
≥45 days	14 (56.0)	52 (61.2)	5 (21.7)	17 (44.7)	
Missing	3	18	4	14	
Surgery (ever) (n, %)					.094
No	7 (26.9)	10 (11.4)	1 (4.0)	6 (15.0)	
Yes	19 (73.1)	78 (88.6)	24 (96.0)	34 (85.0)	
Missing	2	15	2	12	
Surgical intent (n, %)[3]					.562
Diagnostic	0	3 (3.8)	1 (4.2)	1 (2.9)	
Curative	17 (89.5)	61 (78.2)	20 (83.3)	28 (82.4)	
Palliative	0	11 (14.1)	1 (4.2)	2 (5.9)	
Unknown	2 (10.5)	3 (3.8)	2 (8.3)	3 (8.8)	
Type of breast surgery (n, %)[4]					.503
Total mastectomy	17 (89.5)	73 (93.6)	21 (87.5)	33 (97.1)	
Tumorectomy	2 (10.5)	5 (6.4)	3 (12.5)	1 (2.9)	
Status of surgical margins (n, %)					.521
Clean	16 (94.1)	64 (90.1)	20 (95.2)	26 (83.9)	
Positive	1 (5.9)	7 (9.9)	1 (4.8)	5 (16.1)	
Missing	2	7	3	3	
Axillary surgery – type (n, %)[5]					.799
Axillary dissection	16 (100)	68 (98.6)	22 (100)	31 (100)	
Sentinel lymph node biopsy	0	1 (1.4)	0	0	
Not done/missing	3	9	2	3	
Axillary surgery – completeness (n, %)[6]					.577
Not done/no isolated lymph nodes	3 (15.8)	9 (11.7)	2 (9.1)	3 (9.1)	
Incomplete	4 (21.1)	22 (28.6)	2 (9.1)	9 (27.3)	
Complete	12 (63.2)	46 (59.7)	18 (81.8)	21 (63.6)	
Missing	0	1	2	1	
Chemotherapy (ever received) (n, %)					.619
No	1 (4.0)	4 (4.6)	1 (4.3)	4 (10.0)	
Yes	24 (96.0)	83 (95.4)	22 (95.7)	36 (90.0)	
Missing	1	16	4	12	
Intent of first-line CT (n, %)[7]					.170
Neoadjuvant only	7 (29.2)	9 (10.8)	2 (9.1)	2 (5.6)	
Neoadjuvant + adjuvant	10 (41.7)	45 (54.2)	12 (54.5)	16 (44.4)	
Adjuvant only	3 (12.5)	13 (15.7)	6 (27.3)	10 (27.8)	
Palliative	4 (16.7)	16 (19.3)	2 (9.1)	8 (22.2)	
Neoadjuvant CT – outcome (n, %)					.174
Same stage	4 (23.5)	17 (31.5)	6 (42.9)	10 (55.6)	
Upstaging	1 (5.9)	10 (18.5)	2 (14.3)	1 (5.6)	
Downstaging	7 (41.2)	21 (38.9)	6 (42.9)	6 (33.3)	
Unknown[8]	1 (5.9)	6 (11.1)	0	1 (5.6)	

pCR rate after neoadjuvant CT (n, %)					.762
No pCR	17 (100)	50 (90.9)	14 (100)	16 (88.9)	
pCR only in the breast (ypT0/is)	0	3 (5.5)	0	1 (5.6)	
pCR in the breast and lymph nodes (ypT0/is, ypN0)	0	2 (3.6)	0	1 (5.6)	
Type of first-line CT regimen (n, %)[7]					.066
Anthracycline-based only	14 (58.3)	38 (45.8)	4 (18.2)	20 (55.6)	
Anthracyclines + taxanes based	10 (41.7)	41 (49.4)	17 (77.3)	15 (41.7)	
Other[9]	0	4 (4.8)	1 (4.5)	1 (2.8)	
First-line CT dose-intensity (n, %)[7]					.204
<85%	16 (69.6)	41 (51.9)	10 (50.0)	24 (68.6)	
≥85%	7 (30.4)	38 (48.1)	10 (50.0)	11 (31.4)	
Missing	1	4	2	1	
Cumulative dose of doxorubicin in mg/m^2 (median, range)	240 (180-360)	240 (60-420)	240 (180-360)	240 (120-360)	.588
Endocrine therapy (ever) (n, %)					**<.001**
No	9 (36.0)	28 (32.6)	12 (52.2)	31 (79.5)	
Yes	16 (64.0)	58 (67.4)	11 (47.8)	8 (20.5)	
Missing	1	17	4	13	
Radiotherapy (ever) (n, %)					.222
No	23 (92.0)	83 (96.5)	20 (87.0)	39 (97.5)	
Yes	2 (8.0)	3 (3.5)	3 (13.0)	1 (2.5)	
Missing	1	17	4	12	

[1]Not applicable as patients were not treated/followed at the Maputo Central Hospital and, therefore, were not discussed by the multidisciplinary tumor board.

[2]One patient received endocrine therapy as first treatment, who had a Luminal B-like tumor.

[3]Patients submitted to a surgical biopsy with diagnostic intent followed by a tumorectomy or a mastectomy with curative intent were included in the "Curative" intent group.

[4]Patients submitted to a tumorectomy followed by a mastectomy were included in the "Mastectomy" group.

[5]One patient received a sentinel lymph node biopsy followed by an axillary dissection and was, therefore, included in the “axillary dissection” group.

[6]Among patients receiving any type of breast surgery (n=155). Not done: not done or no isolated lymph nodes; incomplete: 1-5 isolated lymph nodes (in case of axillary lymph node dissection); complete: ≥6 isolated lymph nodes (in case of axillary lymph node dissection) or ≥1 isolated lymph nodes with ≤2 positive lymph nodes (in case of sentinel lymph node biopsy).

[7]First line of chemotherapy that the patient received includes neoadjuvant, adjuvant or palliative treatment. If the patient received part of chemotherapy as neoadjuvant (e.g. AC regimen), and another part as adjuvant chemotherapy (e.g. paclitaxel), the type of regimen, dose-intensity and abandonment refers to the entire scheme (neoadjuvant plus adjuvant).

[8]Cases in whom there was missing data regarding clinical staging or patient abandoned treatment.

[9]Includes: taxane-based CT (three patients), non-anthracycline/non-taxane-based CT (two patients) and unknown regimen (one patient). The preferred anthracycline-containing regimen was AC (cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2 every three weeks) and the preferred taxane used was paclitaxel (175 mg/m^2 every three weeks); dose-dense regimens were not used due to the unpredictable availability of granulocyte-colony stimulating factors.

CT: chemotherapy; ER: estrogen receptor; MTB: multidisciplinary tumor board; pCR: pathological complete response.

Supplementary Table 4 – Overall survival and disease-free survival estimates and multivariable analysis separately for stages I-II and III-IV, according to classic and surrogate intrinsic subtypes

	N	3-year overall survival % (95% CI)	P[1]	Adjusted HR (95% CI)[2]
Stages I-II				
Classic subtypes				
ER-positive/HER2-negative	15	77.0 (43.9-92.0)	0.228	1
HER2-positive	16	92.9 (59.1-99.0)		0.27 (0.03-2.65)
TNBC	13	57.0 (24.3-80.0)		1.13 (0.25-5.20)
Surrogate intrinsic subtypes				
Luminal A-like	8	75.0 (31.5-93.1)	0.201	1
Luminal B-like	14	84.6 (51.2-95.9)		0.66 (0.10-4.33)
HER2-Enriched	9	100		NC
Basal-like	13	57.0 (24.3-80.0)		1.00 (0.21-4.82)
Stages III-IV				
Classic subtypes				
ER-positive/HER2-negative	76	57.9 (45.1-68.7)	**<0.001**	1
HER2-positive	28	32.1 (14.9-50.8)		**2.16 (1.23-3.80)**
TNBC	27	21.0 (7.8-38.5)		**3.39 (1.91-6.01)**
Surrogate intrinsic subtypes				
Luminal A-like	17	57.0 (30.3-76.8)	**0.004**	1
Luminal B-like	73	51.7 (38.8-63.1)		0.82 (0.35-1.87)
HER2-Enriched	14	42.9 (17.7-66.0)		1.47 (0.52-4.12)
Basal-like	27	21.0 (7.8-38.5)		2.38 (0.99-5.68)
	N	**3-year disease-free survival % (95% CI)**	**P[1]**	**Adjusted HR (95% CI)[2]**
Stages I-II				
Classic subtypes				
ER-positive/HER2-negative	15	64.7 (34.4-83.7)	0.155	1
HER2-positive	16	92.9 (59.1-99.9)		0.21 (0.02-1.94)
TNBC	13	57.0 (24.3-80.0)		0.92 (0.22-3.90)
Surrogate intrinsic subtypes				
Luminal A-like	8	62.5 (22.9-86.1)	0.298	1
Luminal B-like	14	77.4 (4.9-92.1)		0.78 (0.15-4.17)
HER2-Enriched	9	100%		NC
Basal-like	13	57.0 (24.3-80.0)		0.91 (0.19-4.42)
Stage III				
Classic subtypes				
ER-positive/HER2-negative	58	50.0 (36.3-62.3)	**<0.001**	1
HER2-positive	23	23.2 (8.1-42.8)		**2.08 (1.15-3.79)**
TNBC	18	5.6 (0.4-22.4)		**3.52 (1.88-6.60)**
Surrogate intrinsic subtypes				
Luminal A-like	13	46.2 (19.2-69.6)	**0.005**	1
Luminal B-like	56	44.1 (30.5-57.0)		0.99 (0.42-2.31)
HER2-Enriched	12	33.3 (10.3-58.8)		1.90 (0.65-5.59)
Basal-like	18	5.6 (0.4-22.4)		**3.11 (1.25-7.70)**

[1]p value for the univariable survival analysis.

[2]Adjusted for age (<40 *vs* 40-49 *vs* 50-59 *vs* ≥60 years), HIV status (negative/unknown *vs* positive) and date of diagnosis (pre- *vs* post-multidisciplinary tumor board implementation).

ER: estrogen receptor; NC: non-calculable;

Supplementary table 5 – Concordance between cell blocks and histological samples for the estrogen receptor, the progesterone receptor, HER2 and Ki67 status in patients with both samples available (n=51)[1]

		Histological samples		Concordance (%)	κ
		ER-negative	ER-positive		
Cell blocks	ER-negative	20	3	88.2%	0.762
	ER-positive	3	25		
		PR-negative	PR-positive		
	PR-negative	28	3	80.4%	0.574
	PR-positive	7	13		
		HER2-negative	HER2-positive		
	HER2-negative	31	6	83.7%[2]	0.603
	HER2-positive	2	10		
		Ki67 low	Ki67 high		
	Ki67 low	31	6	76.1%[3]	0.271
	Ki67 high	5	4		

[1]Positive estrogen receptor (ER) and progesterone receptor (PR) status were defined with a ≥1% cut-off, and high Ki67 was defined as >29%. HER2-positivity was defined as an immunohistochemistry score of 3+ and/or a score of 2+ with an amplified silver *in situ* hybridization (SISH) test.

[2]Regarding HER2 evaluation, there were two surgical samples for which the immunohistochemistry score was 2+ (equivocal), but for which the SISH test was not successful.

[3]Regarding Ki67 evaluation, there were five samples (two cell blocks and three surgical samples) in which Ki67 was not tested.

Supplementary table 6 – Concordance for the estrogen receptor, the progesterone receptor, HER2 and Ki67 status in the 15 cell blocks submitted to re-staining and re-evaluation in Portugal by a third pathologist (quality control)[1]

		Portugal			Concordance (%)	κ
		ER-negative		ER-positive		
Mozambique	ER-negative	4		1	93.3%	0.842
	ER-positive	0		10		
		PR-		PR+		
	PR-negative	5		1	93.3%	0.857
	PR-positive	0		9		
		HER2 0/1+	2+	3+		
	HER2 0/1+	10	0	0	80.0%	0.541
	HER2 2+	1	1	0		
	HER2 3+	1	1	1		
		Ki67 low		Ki67 high		
	Ki67 low	6		4	50.0%[2]	0.248
	Ki67 high	3		1		

[1]Positive estrogen receptor (ER) and progesterone receptor (PR) status defined with a ≥1% cut-off and high Ki67 was defined as >29%.

[2]Regarding Ki67 evaluation, there was one sample in which Ki67 was not tested.