

Supplemental Table 1. Baseline clinicopathological characteristics of invasive breast cancer cases (n=34) within the "Discovery Cohort"

Variable	Level	Invasive BC (N=34)	M0 (N=17)	M1 (N=17)	% missing data
Age at diagnosis (years)	Median [IQR]	64 [54-72]	62 [54-71]	66 [58-72]	0%
Menopause - N (%)	No	8 (23.5)	4 (23.5)	4 (23.5)	0%
	Yes	26 (76.5)	13 (76.5)	13 (76.5)	
Tumor Histotype (N%)	IDC	34 (100)	17 (100)	17 (100)	0%
	ILC	0 (0.0)	0 (0.0)	0 (0.0)	
	IDC+ILC	0 (0.0)	0 (0.0)	0 (0.0)	
Site of onset - N (%)	Right	21 (61.8)	12 (70.6)	9 (52.9)	0%
	Left	13 (38.2)	5 (29.4)	8 (47.1)	
	Bilateral	0 (0.0)	0 (0.0)	0 (0.0)	
Tumor size (cm)	Median [IQR]	2.8 [1.9-4.7]	2.5 [1.9-3.0]	3.0 [2.3-4.7]	0%
T Classification - N (%)	T1c	10 (29.4)	6 (35.3)	4 (23.5)	0%
	T2	15 (44.1)	7 (41.1)	8 (47.1)	
	T3	2 (5.9)	2 (11.8)	0 (0.0)	
	T4	7 (20.6)	2 (11.8)	5 (29.4)	
N classification - N (%)	N0	18 (52.9)	10 (58.8)	8 (47.1)	0%
	N1	7 (20.6)	4 (23.5)	3 (17.6)	
	N2	2 (5.9)	0 (0.0)	2 (11.8)	
	N3	7 (20.6)	3 (17.7)	4 (23.5)	
M Classification - N (%)	M0	31 (91.2)	17 (100)	14 (82.4)	0%
	M1	3 (8.8)	0 (0.0)	3 (17.6)	
Lymph node status - N (%)	Negative	18 (52.9)	10 (58.8)	8 (47.1)	0%
	Positive	16 (47.1)	7 (41.2)	9 (52.9)	
Tumor stage - N (%)	1A	7 (20.6)	4 (23.5)	3 (17.6)	0%
	1B	1 (2.9)	1 (5.9)	0 (0.0)	
	2A	13 (38.2)	8 (47.1)	5 (29.4)	
	2B	2 (5.9)	0 (0.0)	2 (11.8)	
	3A	0 (0.0)	0 (0.0)	0 (0.0)	
	3B	2 (5.9)	1 (5.9)	1 (5.9)	
	3C	6 (17.7)	3 (17.6)	3 (17.6)	
	4	3 (8.8)	0 (0.0)	3 (17.6)	
Tumor Grade - N (%)	1	5 (14.7)	3 (17.6)	2 (11.8)	0%
	2	13 (38.2)	7 (41.2)	6 (35.3)	
	3	16 (47.1)	7 (41.2)	9 (52.9)	
Estrogen Receptor - N (%)	Negative	11 (32.4)	5 (29.4)	6 (35.3)	0%
	Positive	23 (67.6)	12 (70.6)	11 (64.7)	
Progesterone Receptor - N (%)	Negative	14 (41.2)	6 (35.3)	8 (47.1)	0%
	Positive	20 (58.8)	11 (64.7)	9 (52.9)	
HER2 - N (%)	HER2-amplified	11 (32.4)	2 (11.8)	9 (52.9)	0%
	HER2-not amplified	23 (67.6)	15 (88.2)	8 (47.1)	
KI67/Mib1	Median [IQR]	30 [26-50]	30 [21-37]	40 [30-57]	0%
Molecular Subtype	Luminal	23 (67.6)	12 (70.6)	11 (64.7)	0%
	HER2-amplified	4 (11.8)	0 (0.0)	4 (23.5)	
	Triple Negative	7 (20.6)	5 (29.4)	2 (11.8)	

Abbreviations - IQR: Interquartile Range (i.e. first-third quartiles)

Supplemental Table 2. Baseline clinico-pathological characteristics of the "Extended cohort" (n=223)

Variable	Level	All patients (N=223)	M0 (N=178)	M0>M1 (N=45)	% missing data
Age at diagnosis (years)	Median [IQR]	60.2 [47.9-70.7]	59.6 [47.7-70.1]	61.5 [48.2-71.7]	0%
Menopause - N (%)	No	69 (30.9)	57 (32.0)	12 (26.7)	0%
	Yes	154 (69.1)	121 (68.0)	33 (73.3)	
Tumor Histotype (N%)	IDC	206 (92.4)	168 (94.4)	38 (84.4)	0%
	ILC	13 (5.8)	6 (3.4)	7 (15.6)	
	IDC+ILC	4 (1.8)	4 (2.2)	0 (0.0)	
Site of onset - N (%)	Right	107 (48.0)	83 (46.6)	24 (53.3)	0%
	Left	115 (51.6)	95 (53.4)	20 (44.4)	
	Bilateral	1 (0.4)	0 (0)	1 (2.2)	
Tumor size (cm)	Median [IQR]	2.5 [1.9-3.4]	2.4 [1.8-3.0]	3.0 [2.5-4.5]	0%
T Classification - N (%)	T1c	61 (27.4)	55 (30.9)	6 (13.3)	0%
	T2	120 (53.8)	94 (52.8)	26 (57.8)	
	T3	14 (6.3)	11 (6.2)	3 (6.7)	
	T4	28 (12.6)	18 (10.1)	10 (22.2)	
N classification - N (%)	N0	94 (42.2)	82 (46.1)	12 (26.7)	0%
	N1	77 (34.5)	68 (38.2)	9 (20.0)	
	N2	23 (10.3)	17 (9.6)	6 (13.3)	
	N3	29 (13.0)	11 (6.2)	18 (40.0)	
M Classification - N (%)	M0	223 (100.0)	178 (100.0)	45 (100.0)	0%
	M1	0 (0.0)	0 (0.0)	0 (0.0)	
Lymph node status - N (%)	Negative	77 (34.5)	68 (38.2)	9 (20.0)	0%
	Positive	146 (65.5)	110 (61.8)	36 (80.0)	
Tumor stage - N (%)	1A	29 (13.0)	27 (15.2)	2 (4.4)	0%
	1B	7 (3.1)	7 (3.9)	0 (0.0)	
	2A	85 (38.1)	73 (41.0)	12 (26.7)	
	2B	34 (15.2)	29 (16.3)	5 (11.1)	
	3A	16 (7.2)	13 (7.3)	3 (6.7)	
	3B	23 (10.3)	18 (10.1)	5 (11.1)	
	3C	29 (13.0)	11 (6.2)	18 (40.0)	
Tumor Grade - N (%)	1	23 (10.7)	20 (11.6)	3 (7.0)	3,6%
	2	109 (50.7)	88 (51.2)	21 (48.8)	
	3	83 (38.6)	64 (37.2)	19 (44.2)	
Estrogen Receptor - N (%)	Negative	53 (23.8)	40 (22.5)	13 (28.9)	0%
	Positive	170 (76.2)	138 (77.5)	32 (71.1)	
Progesterone Receptor - N (%)	Negative	64 (28.7)	49 (27.5)	15 (33.3)	0%
	Positive	159 (71.3)	129 (72.5)	30 (66.7)	
HER2 - N (%)	HER2-amplified	53 (24.7)	38 (22.4)	15 (33.3)	3,6%
	HER2-not amplified	162 (75.3)	132 (77.6)	30 (66.7)	
Ki67/Mib1	Median [IQR]	30 [19-50]	25 [19-46.5]	32.5 [19-50]	3,6%
Molecular Subtype	Luminal	170 (77.6)	138 (79.3)	32 (71.1)	1,8%
	HER2-amplified	25 (11.4)	18 (10.3)	7 (15.6)	
	Triple Negative	24 (11.0)	18 (10.3)	6 (13.3)	

Abbreviations - IQR: Interquartile Range (i.e. first-third quartiles)

Supplemental Table 3. Clinico-pathological characteristics of the breast cancer cases (n=28) within the "plasma cohort"

Variable	Level	All BCs (N=28)	BC (N=13)	mBC(N=15)	% missing data
Age (years)	Median [IQR]	51.6 [44.3 - 61.7]	50.2 [44.4 - 55.8]	57.5 [43.6 - 64.9]	0%
Menopause - N (%)	No Yes	15 (53.6) 13 (46.4)	9 (69.2) 4 (30.8)	6 (40.0) 9 (60.0)	0%
Tumor Histotype (N%)	IDC ILC ILC+IDC	24 (85.7) 3 (10.7) 1 (3.6)	12 (92.3) 1 (7.7) 0 (0.0)	12 (80.0) 2 (13.3) 1 (6.7)	0%
Site of onset - N (%)	Right Left Bilateral	11 (39.3) 15 (53.6) 2 (7.1)	4 (30.8) 9 (69.2) 0 (0.0)	7 (46.7) 6 (40.0) 2 (13.3)	0%
Tumor size (cm)	Median [IQR]	1.6 [1.0 - 2.4]	1.1 [0.9 - 1.6]	2.0 [1.6 - 3.4]	3.6%
T Classification - N (%)	Tis T1 T2 T3 T4 ypT1c ypT2 ypTX	1 (3.6) 13 (46.4) 7 (25.0) 1 (3.6) 1 (3.6) 2 (7.1) 2 (7.1) 1 (3.6)	1 (7.7) 9 (69.2) 2 (15.4) 0 (0.0) 0 (0.0) 1 (7.7) 0 (0.0) 0 (0.0)	0 (0.0) 4 (26.7) 5 (33.3) 1 (6.7) 1 (6.7) 1 (6.7) 2 (13.3) 1 (6.7)	0%
N classification - N (%)	N0 N1 N2 N3 ypN1 ypN2	14 (50.0) 3 (10.7) 5 (17.9) 1 (3.6) 2 (7.1) 3 (10.7)	9 (69.2) 1 (7.7) 1 (7.7) 1 (7.7) 0 (0.0) 1 (7.7)	5 (33.3) 2 (13.3) 4 (26.7) 0 (0.0) 2 (13.3) 2 (13.3)	0%
Lymph node status - N (%)	N0 N1	14 (50.0) 14 (50.0)	9 (69.2) 4 (30.8)	5 (33.3) 10 (66.7)	0%
Tumor stage -at diagnosis N (%)	Not determined 1A 2A(2B 3A/3C	5 (17.9) 11 (39.3) 4 (14.3) 8 (28.5)	1 (7.7) 8 (61.5) 2 (15.4) 2 (15.4)	4 (26.7) 3 (20.0) 2 (13.3) 6 (40.0)	0%
Tumor Grade - N (%)	Not determined G1 G2 G3	4 (14.2) 1 (3.6) 14 (50.0) 9 (32.1)	2 (15.4) 1 (7.7) 5 (38.5) 5 (38.5)	2 (13.3) 0 (0.0) 9 (60.0) 4 (26.7)	0%
Estrogen Receptor - N (%)	Negative Positive	3 (10.7) 25 (89.3)	2 (15.4) 11 (84.6)	1 (6.7) 14 (93.3)	0%
Progesterone Receptor - N (%)	Negative Positive	6 (21.4) 22 (78.6)	3 (23.1) 10 (76.9)	3 (20.0) 12 (80.0)	0%
HER2 - N (%)	Not determined HER2-amplified HER2-not amplified	1 (3.6) 6 (22.2) 21 (77.8)	1 (7.7) 3 (23.1) 9 (69.2)	0 (0.0) 3 (20.0) 12 (80.0)	0%
Ki67/Mib1	Median [IQR]	18.0 [10.0 - 35.0]	15.0 [8.5 - 27.5]	20.0 [13.0 - 36.8]	10.7%
Molecular Subtypes	Luminal HER2-amplified Triple Negative	25 (89.3) 1 (3.6) 2 (7.1)	11 (84.6) 1 (7.7) 1 (7.7)	14 (93.3) 0 (0.0) 1 (6.7)	0%

Abbreviations - IQR: Interquartile Range (i.e. first-third quartiles)

Supplemental Table 4. The 8 differentially expressed miRNAs between non metastatic (M0) versus metastatic patient (M1) groups in the discovery cohort.

Transcript ID (Array design)	miRbase ID	TAQMAN ASSAY ID	M0 vs M1 (p-values)	M0 vs M1 (Fold-change)
hsa-miR-27a-5p	MI0000085	002445	0.0102033	3.62806
hsa-miR-29c-3p	MI0000087	000587	0.0394424	2.70565
hsa-miR-29c-5p	MI0000735	001818	0.0269752	2.17326
hsa-miR-3613-5p	MI0016003	463197 mat	0.0165238	-4.43844
hsa-miR-3916	MI0016422	464679 mat	0.0048431	2.10378
hsa-miR-422a	MI0001444	002297	0.0290374	3.67089
hsa-miR-4286	MI0015894	241500 mat	0.012723	8.69238
hsa-miR-504-5p	MI0003189	002084	0.0191389	2.21117

Supplemental Table 5. miR3613-5p and miR3916 expression in plasma EVs.

	Statistics	All BC (N=28)	Controls (N=11)	Effect size*	p-value [#]
miR-3613-5p	Median [IQR]	6.0 [4.7 - 16.2]	4.7 [1.4 - 6.2]	0,429	0,039
	Range (min, max)	0.0 - 41.0	0.0 - 9.5		
miR-3916	Median [IQR]	0.0 [0.0 - 3.1]	0.0 [0.0 - 0.0]	0,227	0.193
	Range (min, max)	0.0 - 22.0	0.0 - 5.6		

	Statistics	mBC (N=15)	BC (N=13)	Effect size*	p-value [#]
miR-3613-5p	Median [IQR]	5.9 [4.4 - 12.8]	6.2 [5.3 - 20.0]	-0,308	0,394
	Range (min, max)	0.0 - 41.0	0.0 - 32.0		
miR-3916	Median [IQR]	0.0 [0.0 - 0.0]	3.0 [0.0 - 5.1]	-0.456	0,020
	Range (min, max)	0.0 - 14.1	0.0 - 22.0		

Abbreviations - IQR: Interquartile Range (i.e. first-third quartiles)

*Glass rank-biserial correlation coefficient, is a measure of Effect Size (ES) that can be categorised as follows:

"Negligible ES" (differences cannot be discerned by the naked eye) for $ES < 0.1$ (absolute value)

"Small ES" for $0.1 \leq ES < 0.3$ (absolute value)

"Medium ES" for $0.3 \leq ES < 0.5$ (absolute value)

"Large ES" (differences can be seen by the naked eye) for $ES \geq 0.5$ (absolute value)

[#]p-value from Mann-Whitney U test