Early, On-Treatment Levels and Dynamic Changes of Genomic Instability in Circulating Tumor DNA Predict Response to Treatment and Outcome in Metastatic Breast Cancer Patients.

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**Supplementary data** 

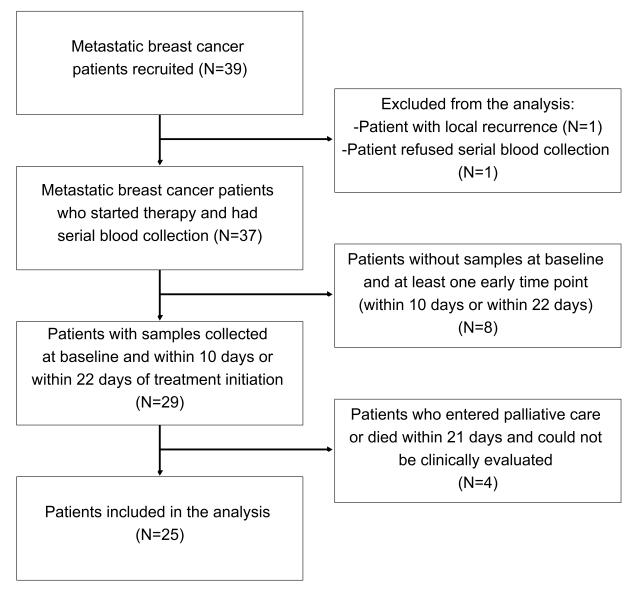
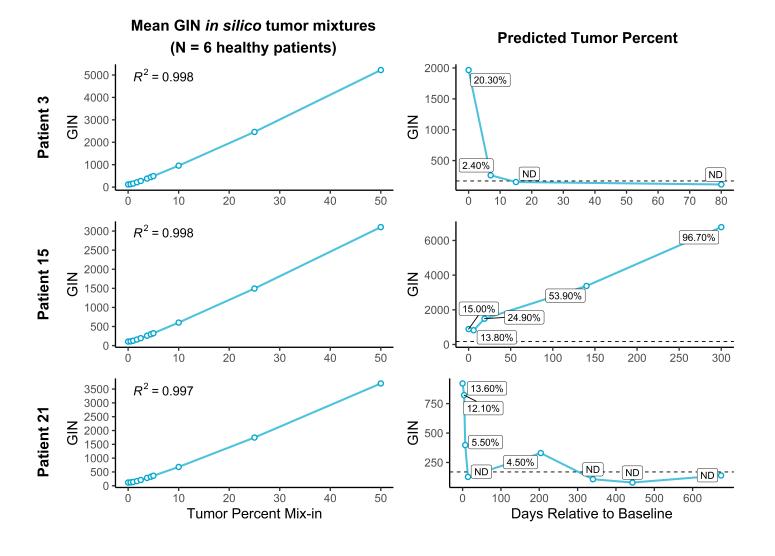


Figure S1. CONSORT flow diagram for the entire cohort of patients enrolled in this study.



**Figure S2.** Percentage of tumor DNA required for the detection of GIN values above the pre-defined threshold. On the left, the DNA extracted from the plasma of 3 patients with metastatic breast cancer (Patients 3, 15 and 21) is diluted with DNA from the plasma of a pool of 6 healthy patients. Loess Regression analysis is shown with the X-axis as the tumor percent mix-in and y-axis with the GIN value. As little as 1-2 % of tumor DNA is sufficient to reach a detectable (>170) GIN value in cfDNA. On the right, the serial GIN value curves are annotated with estimated % tumor DNA in the plasma sample in blood samples from the same 3 patients.

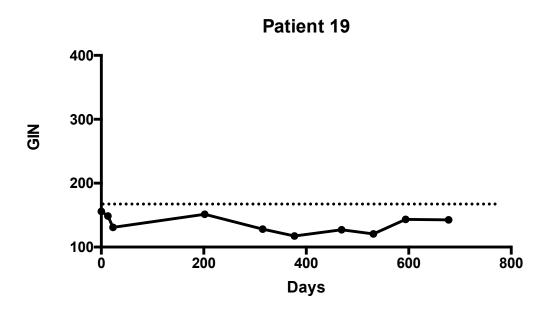
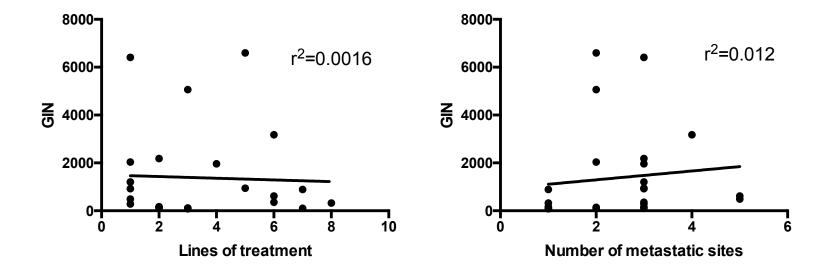
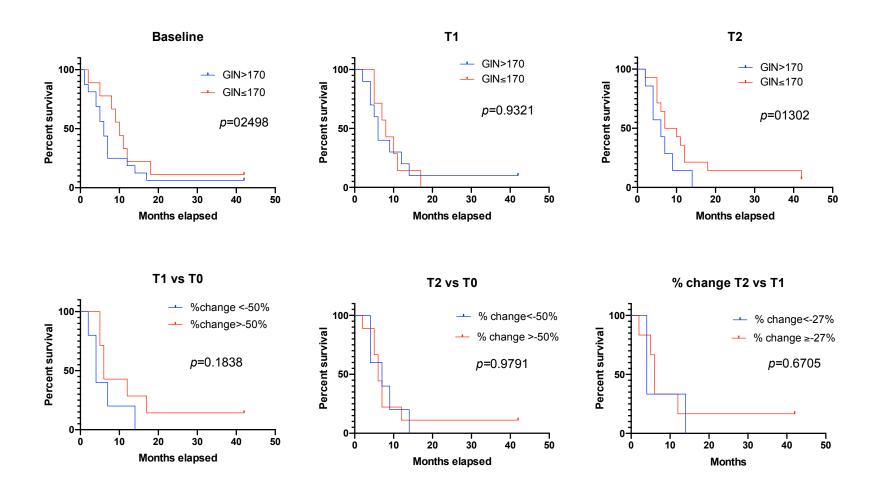


Figure S3. Undetectable GIN values in metastatic patient with no measurable disease (patient 19).

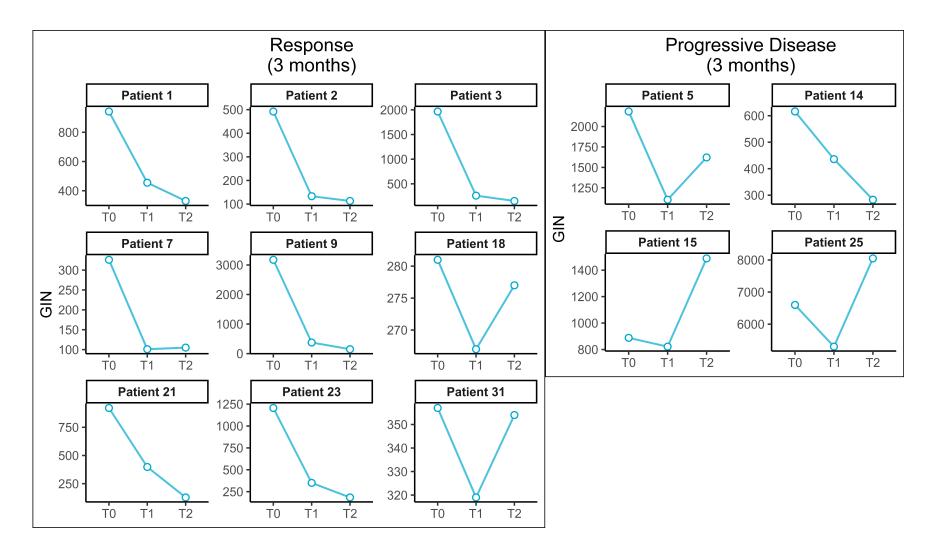
The patient had undergone surgery for brain metastasis and radiotherapy for bone metastasis before treatment, and at baseline, there was no active metastatic disease. During follow-up disease did not recur and GIN values remained below threshold for over 2 years of follow-up.



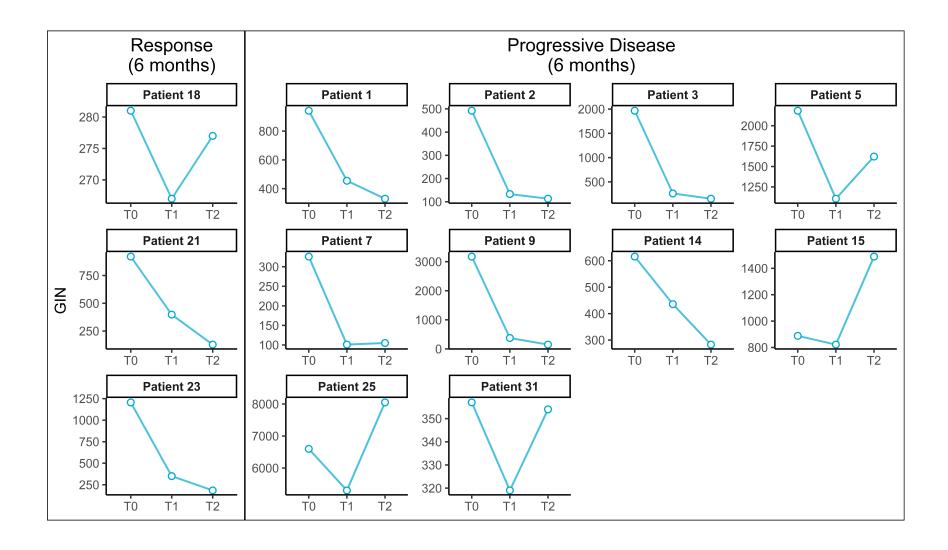
**Figure S4.** Correlation of baseline GIN values with number of lines of treatment (left) and number of metastatic sites at the time of treatment initiation (right). There appears to be no correlation of baseline GIN with either factor.



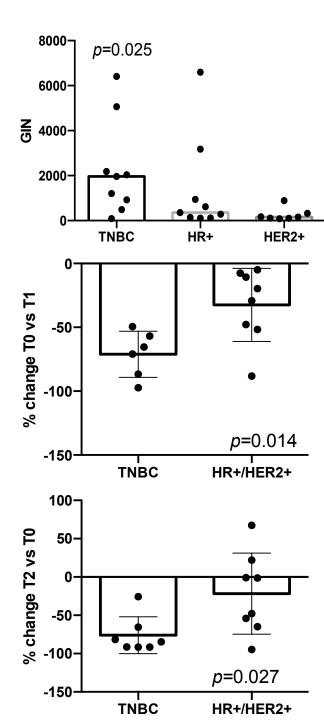
**Figure S5**. Progression free survival (PFS) and GIN detection and changes at different time points. Top: PFS of patients with plasma GIN values above and below pre-established threshold (170) at 3 different time points. Baseline (left); T1 (average 1 week post-treatment) (middle); T2 (average 2 weeks post-treatment) (right).Bottom: PFS of patients with plasma % change in GIN values above and below pre-established threshold (-50%) or the median (-27%). Left: % change T1 vs T0; middle: T2 vs T0; right: T2 vs T1.



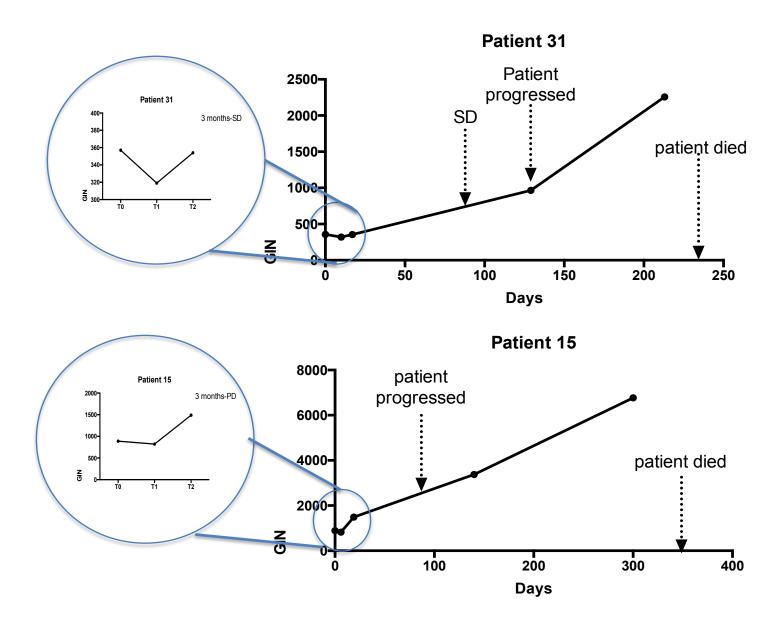
**Figure S6.** Patterns of dynamic changes in GIN values related to response at 3 months. The patterns of rise and fall of GIN values across the 3 week period after treatment initiation is displayed here. On the left are responding patients (partial response and stable disease) and the right, patient with progressive disease (PD) evaluated at 3 months post-treatment initiation. Note that in 3 of the 4 patients with PD, the GIN values rise from T1 to T2, whereas this is observed in only 2 of the 9 responding patients. Only 1 of the 4 patients with PD does not show a rise at T2, a pattern observed in 7 of the 9 responding patients.



**Figure S7.** Patterns of dynamic changes in GIN values related to response at 6 months. The patterns of rise and fall of GIN values across the 3 week period (T0, T1, T2) after treatment initiation is displayed here. On the left are responding patients (partial response and stable disease) and the right, patient with progressive disease (PD) evaluated at 6 months post-treatment initiation. Note that in 4 of the 10 patients with PD, the GIN values rise from T1 to T2, whereas this is observed in only 1 of the 3 responding patients.



**Figure S8.** GIN levels at baseline and changes at T1 and T2 time points for the 3 BC subtypes. Bar graphs and scatter plots showing GIN values at baseline (top), % change in GIN from T0 to T1 (middle) and % change in GIN from T2 to T0 (bottom). Error bars represent standard errors of the mean. TNBC: triple negative breast cancer; HR: hormone receptor; HER2: Human Epidermal growth factor Receptor 2. P values calculated using ANOVA.



**Figure S9.** GIN values in 2 patients with rise at T2 dynamic pattern with longer term follow-up. 2 of 5 patients with GIN rise at T2 relative to T1 showed a continuous increase in GIN over time until the time of progression and patient's death. SD: Stable disease; PD: progressive disease. Inset shows magnified GIN values and response at 3 months.

**Table S1.** Clinical characteristics

R+ NBC NBC R+ NBC NBC R+ NBC R+ NBC NBC R+ NBC R+ NBC NBC	Bone, liver, lung LN, bone, lung, brain, nervous Skin, LN, lung Bone Skin, LN, bone Lung LN, bone, liver, lung Bone, lung Skin Skin, LN, soft tissue, bone, brain Liver	3 5 3 1 3 1 4 2 1 5	Paclitaxel Carbo/Taxol Carbo/Gem Capecitabine Vinorelbine Eribulin Eribulin	5 1 4 3 2 8 6	942 492 1966 115 2184 326	7 7 7 8 6	455 133 262 240 1105	-52 -73* -87 NA	14 14 15	330 113 153	-65 -65* -91*	(%) -27 ND -35	SD PR PR	PD PD PD	5 5	16 30 9
NBC R+ NBC R+ NBC NBC NBC R+ er2+	Skin, LN, lung Bone Skin, LN, bone Lung LN, bone, liver, lung Bone, lung Skin Skin, LN, soft tissue, bone, brain	3 1 3 1 4 2	Carbo/Gem Capecitabine Vinorelbine Eribulin Eribulin PacliT	4 3 2 8 6	1966 115 2184 326	7 8 6	262 240	-87	15	153						
R+ NBC er2+ NBC NBC NBC R+ NBC LBC R+	Bone Skin, LN, bone Lung LN, bone, liver, lung Bone, lung Skin Skin, LN, soft tissue, bone, brain	1 3 1 4 2	Capecitabine Vinorelbine Eribulin Eribulin PacliT	3 2 8 6	115 2184 326	8	240				-91*	-35	PR	PD	5	0
NBC er2+ NBC NBC NBC R+ er2+ er2+	Skin, LN, bone Lung LN, bone, liver, lung Bone, lung Skin Skin, LN, soft tissue, bone, brain	3 1 4 2	Vinorelbine Eribulin Eribulin PacliT	2 8 6	2184 326	6		NA	14	4.10						9
R+ NBC NBC R+ er2+ er2+	Lung LN, bone, liver, lung Bone, lung Skin Skin, LN, soft tissue, bone, brain	1 4 2	Eribulin Eribulin PacliT	8	326		1105		17	448	NA	NA	SD	SD	9	12
R+ NBC NBC R+ er2+	LN, bone, liver, lung  Bone, lung  Skin  Skin, LN, soft tissue, bone, brain	4 2	Eribulin PacliT	6			1103	-49	19	1622	-26	47	PD#	PD#	0	1
NBC NBC R+ er2+	Bone, lung Skin Skin, LN, soft tissue, bone, brain	2	PacliT			6	101	-48*	13	105	-48*	ND	SD	PD	6	15
NBC R+ er2+ er2+	Skin Skin, LN, soft tissue, bone, brain	1		-	3176	6	376	-88	20	149	-95*	-55	PR	PD	6	26
R+ er2+ er2+	Skin, LN, soft tissue, bone, brain		0.1	3	5062	NA	NA	NA	14	428	-92	NA	PR	PD	6	24
er2+ er2+	brain	5	Caelyx	3	92	7	145	ND	14	110	ND	ND	PR	PD	5	$46^{\delta}$
er2+	Liver	Ĺ	PacliT	6	616	6	436	-29	13	283	-54	-35	PD	PD#	2	5
		1	Herceptin/Carbo	7	888	6	822	-8	19	1489	168	81	PD	PD	3	12
D .	Skin	1	PacliT/Herceptin/Perjeta	2	167	8	111	ND	15	104	ND	ND	PR	CR	11	33
R+	LN, lung	2	Carbo/Taxol	2	142	6	78	ND	13	120	ND	ND	PR	CR	10	41
R+	Bone, liver, lung	3	PacliT/Letrozole	1	281	5	267	-5	14	277	-1	4	PR	SD	14	30
er2+	Bone, brain, ovary	3	DoceT/Herceptin/Perjeta	2	156	NA	NA	NA	13	149	ND	NA	CR	CR	42 <sup>Ψ</sup>	42δ
er2+	Lung, brain, nervous spine	3	Carbo/Herceptin	7	103	NA	NA	NA	11	98	ND	NA	PD	PD	2	14
IBC	LN, chest wall, lung	3	Carbo/T	1	921	7	398	-57	14	128	-82*	-57	CR	CR	42 <sup>Ψ</sup>	42δ
er2+	Lung, brain	2	Carbo/Herceptin	3	108	9	129	ND	NA	NA	NA	NA	SD	SD	8	42δ
IBC	Bone, liver, omentum	3	Carbo/Cycloph.	1	1206	7	350	-71	21	184	-85	-47	SD	PR	12	21
R+	Bone, liver	2	Vinorelbine	5	6599	6	5300	-20	13	8050	22	52	PD#	PD#	0	1
IBC	LN, bone, lung	3	Abraxane/Atezolizumab	1	6413	7	107	-97*	NA	NA	NA	NA	PR	PR	17	30
1BC	Lung, brain	2	Abraxane/Atezolizumab	1	2035	NA	NA	NA	14	129	-92*	NA	SD	SD	7	20
R+	Liver, lung, omentum	3	Doxorubicin	6	357	6	319	-11	13	354	-1	11	SD	PD	4	8
er2+	Lung	1	T-DM1/Atezolizumab	3	80	NA	NA	NA	21	94	ND	NA	PR	PR	18	39δ
R+	Bone, Liver	2	Letrozole/Ribociclib	2	112	NA	NA	NA	14	148	ND	NA	SD	SD	12	28
	Average	2		3	1382	7	586	-49	1.5	655	-47	-6			11	23
SD 1															14	
Median 3			3	492	7	267	-51	14	149	-65	-27			7	24	
	Min 1			1	80	5	78	-97	11	94	-95	-57			0	1
		5		8	6599	9	5300	-5	21						42	46
1	R+ BC BC R+ r2+	R+ Bone, liver BC LN, bone, lung BC Lung, brain R+ Liver, lung, omentum r2+ Lung R+ Bone, Liver  Average SD Median	R+   Bone, liver   2     BC   LN, bone, lung   3     BC   Lung, brain   2     R+   Liver, lung, omentum   3     r2+   Lung   1     R+   Bone, Liver   2     Average   2     SD   1     Median   3     Min   1	R+ Bone, liver 2 Vinorelbine BC LN, bone, lung 3 Abraxane/Atezolizumab BC Lung, brain 2 Abraxane/Atezolizumab R+ Liver, lung, omentum 3 Doxorubicin r2+ Lung 1 T-DM1/Atezolizumab R+ Bone, Liver 2 Letrozole/Ribociclib  Average 2 SD 1 Median 3 Min 1	R+   Bone, liver   2   Vinorelbine   5     BC   LN, bone, lung   3   Abraxane/Atezolizumab   1     BC   Lung, brain   2   Abraxane/Atezolizumab   1     R+   Liver, lung, omentum   3   Doxorubicin   6     r2+   Lung   1   T-DM1/Atezolizumab   3     R+   Bone, Liver   2   Letrozole/Ribociclib   2	R+   Bone, liver   2   Vinorelbine   5   6599     BC   LN, bone, lung   3   Abraxane/Atezolizumab   1   6413     BC   Lung, brain   2   Abraxane/Atezolizumab   1   2035     R+   Liver, lung, omentum   3   Doxorubicin   6   357     r2+   Lung   1   T-DM1/Atezolizumab   3   80     R+   Bone, Liver   2   Letrozole/Ribociclib   2   112	R+   Bone, liver   2   Vinorelbine   5   6599   6     BC   LN, bone, lung   3   Abraxane/Atezolizumab   1   6413   7     BC   Lung, brain   2   Abraxane/Atezolizumab   1   2035   NA     R+   Liver, lung, omentum   3   Doxorubicin   6   357   6     r2+   Lung   1   T-DM1/Atezolizumab   3   80   NA     R+   Bone, Liver   2   Letrozole/Ribociclib   2   112   NA     Average   2   3   1382   7     SD   1   2   1942   1     Median   3   3   492   7     Min   1   1   80   5	R+   Bone, liver   2   Vinorelbine   5   6599   6   5300     BC   LN, bone, lung   3   Abraxane/Atezolizumab   1   6413   7   107     BC   Lung, brain   2   Abraxane/Atezolizumab   1   2035   NA   NA     R+   Liver, lung, omentum   3   Doxorubicin   6   357   6   319     r2+   Lung   1   T-DM1/Atezolizumab   3   80   NA   NA     R+   Bone, Liver   2   Letrozole/Ribociclib   2   112   NA   NA     Average   2   3   1382   7   586     SD   1   2   1942   1   1171     Median   3   3   492   7   267     Min   1   1   80   5   78	R+   Bone, liver   2   Vinorelbine   5   6599   6   5300   -20     BC   LN, bone, lung   3   Abraxane/Atezolizumab   1   6413   7   107   -97*     BC   Lung, brain   2   Abraxane/Atezolizumab   1   2035   NA   NA   NA     R+   Liver, lung, omentum   3   Doxorubicin   6   357   6   319   -11     r2+   Lung   1   T-DM1/Atezolizumab   3   80   NA   NA   NA     R+   Bone, Liver   2   Letrozole/Ribociclib   2   112   NA   NA   NA     Average   2   3   1382   7   586   -49     SD   1   2   1942   1   1171   31     Median   3   492   7   267   -51     Min   1   1   80   5   78   -97	R+   Bone, liver   2   Vinorelbine   5   6599   6   5300   -20   13     BC   LN, bone, lung   3   Abraxane/Atezolizumab   1   6413   7   107   -97*   NA     BC   Lung, brain   2   Abraxane/Atezolizumab   1   2035   NA   NA   NA   14     R+   Liver, lung, omentum   3   Doxorubicin   6   357   6   319   -11   13     r2+   Lung   1   T-DM1/Atezolizumab   3   80   NA   NA   NA   21     R+   Bone, Liver   2   Letrozole/Ribociclib   2   112   NA   NA   NA   14     Average   2   3   1382   7   586   -49   15     SD   1   2   1942   1   1171   31   3     Median   3   3   492   7   267   -51   14     Min   1   1   80   5   78   -97   11	R+   Bone, liver   2   Vinorelbine   5   6599   6   5300   -20   13   8050     BC   LN, bone, lung   3   Abraxane/Atezolizumab   1   6413   7   107   -97*   NA   NA     BC   Lung, brain   2   Abraxane/Atezolizumab   1   2035   NA   NA   NA   14   129     R+   Liver, lung, omentum   3   Doxorubicin   6   357   6   319   -11   13   354     r2+   Lung   1   T-DM1/Atezolizumab   3   80   NA   NA   NA   21   94     R+   Bone, Liver   2   Letrozole/Ribociclib   2   112   NA   NA   NA   14   148     Average   2   3   1382   7   586   -49   15   655     SD   1   2   1942   1   1171   31   3   1662     Median   3   492   7   267   -51   14   149     Min   1   1   80   5   78   -97   11   94     Min   1   1   80   5   78   -97   11   94     Average   2   3   180   5   78   -97   11   94     Min   1   1   80   5   78   -97   11   94     Min   1   Na   Na   Na   Na   Na   Na   Na	R+   Bone, liver   2   Vinorelbine   5   6599   6   5300   -20   13   8050   22     BC   LN, bone, lung   3   Abraxane/Atezolizumab   1   6413   7   107   -97*   NA   NA   NA     BC   Lung, brain   2   Abraxane/Atezolizumab   1   2035   NA   NA   NA   14   129   -92*     R+   Liver, lung, omentum   3   Doxorubicin   6   357   6   319   -11   13   354   -1     r2+   Lung   1   T-DM1/Atezolizumab   3   80   NA   NA   NA   21   94   ND     R+   Bone, Liver   2   Letrozole/Ribociclib   2   112   NA   NA   NA   14   148   ND      Average   2   3   1382   7   586   -49   15   655   -47     SD   1   2   1942   1   1171   31   3   1662   49     Median   3   3   492   7   267   -51   14   149   -65     Min   1   1   80   5   78   -97   11   94   -95     Min   1   1   80   5   78   -97   11   94   -95     Contact   Contact	R+   Bone, liver   2   Vinorelbine   5   6599   6   5300   -20   13   8050   22   52     BC   LN, bone, lung   3   Abraxane/Atezolizumab   1   6413   7   107   -97*   NA   NA   NA   NA     BC   Lung, brain   2   Abraxane/Atezolizumab   1   2035   NA   NA   NA   14   129   -92*   NA     R+   Liver, lung, omentum   3   Doxorubicin   6   357   6   319   -11   13   354   -1   11     R2+   Lung   1   T-DM1/Atezolizumab   3   80   NA   NA   NA   21   94   ND   NA     R+   Bone, Liver   2   Letrozole/Ribociclib   2   112   NA   NA   NA   14   148   ND   NA     Average   2   3   1382   7   586   -49   15   655   -47   -6     SD   1   2   1942   1   1171   31   3   1662   49   48     Median   3   3   492   7   267   -51   14   149   -65   -27     Min   1   1   80   5   78   -97   11   94   -95   -57     Min   1   1   80   5   78   -97   11   94   -95   -57	R+   Bone, liver   2   Vinorelbine   5   6599   6   5300   -20   13   8050   22   52   PD#     BC   LN, bone, lung   3   Abraxane/Atezolizumab   1   6413   7   107   -97*   NA   NA   NA   NA   NA   NA   PR     BC   Lung, brain   2   Abraxane/Atezolizumab   1   2035   NA   NA   NA   14   129   -92*   NA   SD     R+   Liver, lung, omentum   3   Doxorubicin   6   357   6   319   -11   13   354   -1   11   SD     R+   Lung   1   T-DM1/Atezolizumab   3   80   NA   NA   NA   21   94   ND   NA   PR     R+   Bone, Liver   2   Letrozole/Ribociclib   2   112   NA   NA   NA   14   148   ND   NA   SD      Average   2   3   1382   7   586   -49   15   655   -47   -6     SD   1   2   1942   1   1171   31   3   1662   49   48     Median   3   3   492   7   267   -51   14   149   -65   -27     Min   1   1   80   5   78   -97   11   94   -95   -57	R+   Bone, liver   2   Vinorelbine   5   6599   6   5300   -20   13   8050   22   52   PD#   PD#	R+   Bone, liver   2   Vinorelbine   5   6599   6   5300   -20   13   8050   22   52   PD#   PD#   0

Non-detectable GIN, \*difference calculated with 170 for ND values, \*patients who were dead at 3 or 6 months, \*patients with no evidence of disease, \*patients alive.