Supplemental Tables

Table S1. Clinical characteristics of patients with metastatic breast cancer undergoing cfDNA testing (N=215).

Clinical Variable	***BRCA1/2* mutation absent (*BRCA* wild-type/WT) (N=186)**	***BRCA1/2* mutation present (*BRCA* mutant) (N=29)**	**p value for difference between *BRCA* WT and *BRCA* mutant**
Median age at metastatic breast cancer diagnosis	57 (48-65)	53 (50-64)	0.82
Tumor Subtype			0.48
HER2+	11 (5.9%)	3 (10.3%)	
HR+	134 (72%)	18 (62.1%)	
TNBC	24 (12.9%)	5 (17.2%)	
Unknown	17 (9.1%)	3 (10.3%)	
Number of prior lines of chemotherapy			0.18
0-1	124 (66.7%)	23 (79.3%)	
≥2	61 (32.8%)	6 (20.7%)	
Unknown	1 (0.5%)	0 (0%)	
First therapy post-cfDNA testing			0.73
Endocrine	57 (30.6%)	10 (34.5%)	
HER2 therapy	13 (7.0%)	2 (6.9%)	
Immunotherapy	14 (7.5%)	4 (13.8%)	
Chemotherapy	49 (26.3%)	6 (20.7%)	
Other	36 (19.4%)	3 (10.3%)	
None	13 (7.0%)	2 (6.9%)	
Unknown	4 (2.2%)	2 (6.9%)	

Table S2. Detailed clinical characteristics and classification of *BRCA1/2* mutations detectable by cfDNA.

Patient ID	Age at primary breast cancer diagnosis	Age at MBC diagnosis	Presence of visceral metastases at time of cfDNA testing? Y=yes, N=no	MBC therapies prior to cfDNA testing#	cfDNA *BRCA1/2* result	Specific cfDNA *BRCA1/2* alteration	Type of *BRCA1/2* cfDNA mutation	Previous known germline-pathogenic versus novel variant*	1st therapy post cfDNA testing	Time interval between tumor tissue genotyping and cfDNA specimens (days); F=tumor tissue genotyping test failure, NS=tumor tissue genotyping not sent+
2	75	83	Y	None	*BRCA1*	*BRCA1 V1590A*	SNV, missense	Novel Variant	AI	28
3	38	61	Y	Tamoxifen, SERD, SERD	*BRCA1*	*BRCA1 S681R*	SNV, missense	Novel Variant	SERD/CDK 4/6 inh	778
4	46	65	Y	None	*BRCA2*	*BRCA2 R2520*	SNV, nonsense	Known Germline-Pathogenic	SERD/CDK 4/6 inh	NS
5	27	36	Y	PI3K inh	*BRCA1*	*BRCA1 Q1240E*	SNV, missense	Known Germline-Pathogenic	Novel ADC	-71
6	45	47	Y	CDK 4/6 inh/AI	*BRCA2*	*BRCA2 p.Thr3033fs*	indel	Known Germline-Pathogenic	None	400
					BRCA1	*BRCA1 V627I*	SNV, missense	Novel Variant		

7	47	47	N	None	*BRCA1*	*BRCA1 W1712*	SNV, nonsense	Known Germline-Pathogenic	None	29
8	47	52	N	SERD, AI/mTOR, SERD, Capecitabine	*BRCA1*	*BRCA1 I1766M*	SNV, missense	Novel Variant	None	3693
9	63	64	N	None	*BRCA2*	*BRCA2 E2364Q*	SNV, missense	Novel Variant	Capecitabine	288
10	60	67	Y	None	*BRCA2*	*BRCA2 K1367T*	SNV, missense	Novel Variant	CDK 4/6 inh/AI	NS
11	58	67	N	SERD, Novel ADC	*BRCA1*	*BRCA1 E1033Q*	SNV, missense	Novel Variant	Capecitabine	789
					BRCA2	*BRCA2 Q684K*	SNV, missense	Novel Variant		
					BRCA1	*BRCA1 E761K*	SNV, missense	Novel Variant		
12	53	53	Y	None	*BRCA2*	*BRCA2 H2365Y*	SNV, missense	Novel Variant	Herceptin/AI	F
13	44	45	Y	Cisplatin, Vinorelbine	*BRCA2*	*BRCA2 E471*	SNV, nonsense	Known Germline-Pathogenic	Novel ADC	-327
14	68	70	Y	SERD	*BRCA1*	*BRCA1 G275D*	SNV, missense	Novel Variant	CDK 4/6 inh/AI	NS
15	44	51	Y	None	*BRCA2*	*BRCA2 Q548H*	SNV, missense	Novel Variant	SERD	2560
16	51	51	Y	None	*BRCA1*	*BRCA1 Splice Site SNV*	SNV, splice	Known Germline-Pathogenic	Carboplatin	NS

17	39	47	Y	Capecitabine, clinical trial, Carboplatin, Cisplatin, Doxil	*BRCA1*	*BRCA1 Exon 10 Deletion*	indel	Known Germline-Pathogenic	Glutaminase inhibitor/Paclitaxel	F
18	40	40	N	Tamoxifen, AI, SERD/CDK 4/6 inh	*BRCA1*	*BRCA1 R466W*	SNV, missense	Novel Variant	Capecitabine	703
19	50	54	Y	AI, AI/SERD, AI/mTOR inh, Capecitabine, AI/CDK 4/6 inh, Nab-paclitaxel, Eribulin, Paclitaxel + RAF inh	*BRCA2*	*BRCA2 E2947K*	SNV, missense	Novel Variant	Paclitaxel + HDAC inh	4
20	49	57	Y	SERD/CDK 4/6 inh	*BRCA2*	*BRCA2 E2391K*	SNV, missense	Novel Variant	CDK 4/6 inh/AI/mTOR	6
21	48	50	Y	None	*BRCA2*	*BRCA2 Exon 11 Deletion*	indel	Known Germline-Pathogenic	None	NS
23	46	46	Y	Paclitaxel, Carboplatin, Novel ADC	*BRCA1*	*BRCA1 Splice Site SNV*	SNV, splice	Known Germline-Pathogenic	HDAC inh + PD-1 inh	169
24	46	53	N	None	*BRCA2*	*BRCA2 E2081Q*	SNV, missense	Novel Variant	T-DM1	47

25	48	50	Y	AI, SERD/PI3K inh, SERD, CDK 4/6inh/AI/mTOR inh, Capecitabine, Novel ADC	*BRCA1*	*BRCA1 S864L*	SNV, missense	Novel Variant	None	1380
27	48	52	Y	None	*BRCA2*	*BRCA2 E1021K*	SNV, missense	Novel Variant	Trastuzumab/ Pertuzumab/ Docetaxel	NS
29	57	61	Y	SERD/CDK 4/6 inh, Capecitabine, Eribulin, Vinorelbine	*BRCA1*	*BRCA1 R1076T*	SNV, missense	Novel Variant	Novel ADC	NS
Synonymous Mutations										
1	48	50	Y	CDK 4/6 inh/AI, CDK 4/6 inh/AI/mTOR inh	*BRCA1*	*BRCA1 L1664L*	SNV, synonymous	Novel Variant	Novel ADC	574
5	27	36	Y	PI3K inh	*BRCA1*	*BRCA1 F1226F*	SNV, synonymous	Novel Variant	Novel ADC	-71
9	63	64	N	None	*BRCA1*	*BRCA1 P364P*	SNV, synonymous	Novel Variant	Capecitabine	288

11	58	67	N	SERD, novel ADC	*BRCA2*	*BRCA2 Q1138Q*	SNV, synonymous	Novel Variant	Capecitabine	789
22	55	79	N	None	*BRCA2*	*BRCA2 D3095D*	SNV, synonymous	Novel Variant	AI	39
26	80	83	Y	Capecitabine, AI/mTOR inh	*BRCA1*	*BRCA1 G1543G*	SNV, synonymous	Novel Variant	None	NS
28	47	55	Y	AI/CDK 4/6 inh	*BRCA1*	*BRCA1 S1377S*	SNV, synonymous	Novel Variant	None	578

#Therapies abbreviated as follows. *Inh* inhibitor, *CDK* cyclin dependent kinase, *SERD* selective estrogen receptor degrader, *AI* aromatase inhibitor, *PI3K* phosphoinositide 3-kinase, *mTOR* mammalian target of rapamycin, *RAF* rapidly accelerated fibrosarcoma, *ADC* Antibody Drug Conjugate.

*Classification by certified genetic counselors at our institution, based on ClinVar[18] and reputable genetic testing laboratories. Previously known germline-pathogenic variants have been denoted as "known germline-pathogenic," and rest as "novel variant."

+Negative value indicates cfDNA being sent prior to tumor tissue genotyping.

Supplemental Information- Results

Patient Demographics

Somatic *BRCA1/2* mutations were seen across breast cancer subtypes, including hormone receptor (HR) positive disease (8.4% of total population), triple-negative breast cancer (TNBC) (2.3%), and human epidermal growth factor receptor 2 (HER2) positive disease (1.4%), and unknown subtype (1.4%).

Seventy-six percent of patients with somatic *BRCA1/2* mutations had visceral disease, while the rest (24%) had non-visceral disease.

Of the 29 patients with somatic *BRCA1* or *BRCA2* mutations, 21 of 29 had genotyping of archival tumor conducted, of which 52.4% had tumor tissue genotyping on a metastatic lesion at the time of MBC diagnosis, 33.3% on a metastatic lesion post MBC diagnosis, and 14.3% on a primary tumor specimen. Test failure occurred in 9.5%.

Characteristics of cfDNA BRCA1/2 mutations

A significant portion of patients had documented negative germline *BRCA1/2* results, but these were not available for all patients. Of the 28 patients without a known germline *BRCA1/2* mutation, 12 (42.9%) had documented negative germline testing, and the remaining 16 (57.1%) did not have a family history consistent with a *BRCA1/2* phenotype although they did not have documented germline *BRCA1/2* testing results available, based on review of records. While the Guardant360® platform was previously not reporting germline *BRCA1/2* mutations detected in cfDNA, we conducted a post hoc analysis in collaboration with Guardant360® to confirm that the majority of patients did not have germline *BRCA1/2* mutations, based on

cfDNA analysis which can detect putative germline mutations [46]. Patient ID # 19 was found to have a cfDNA *BRCA1* c.2239 C>T mutation, which appeared to be a germline variant of uncertain significance, but no other germline *BRCA1/2* mutations were noted.

The *BRCA1* variants in 3 cases were identical in the blood and metastatic tumor tissue (patient ID # 7: BRCA1 ENSP00000350283.3:p.Trp1712Ter (ENST00000357654.3:c.5136G>A); specimens collected 29 days apart); patient ID # 23: BRCA1 splice donor variant (ENST00000357654.3:c.4986+1G>A; specimens collected 169 days apart). In the third case (patient ID # 19), there was a *BRCA1* mutation detected on tumor genotyping of a metastatic lesion, but a *BRCA2* mutation was identified by concurrent cfDNA analysis. On further discussion with Guardant360®, on a post hoc analysis, the *BRCA1* mutation noted in tissue was also identified in cfDNA as a germline variant of uncertain significance (but this result had been suppressed as the platform was initially not reporting germline variants). These specimens were collected within a few days of each other and may therefore represent spatial heterogeneity in tumor lesions and representation in cfDNA. Similarly, among 5 patients who had concurrent tumor tissue genotyping and cfDNA analysis (within a time interval of 40 days), only 2 of these cases demonstrated identical blood and tumor tissue *BRCA1* variants (patient ID #7 and #19), highlighting potential spatial heterogeneity in MBC.

We also observed that for 12/19 patients for whom tumor tissue genotyping results were available, the tumor tissue genotyping assay may not have covered the precise *BRCA1/2* mutation detected by cfDNA. However, in 5/7 cases where the tumor tissue genotyping assay did cover the specific identified *BRCA1/2* mutation seen by cfDNA, the tumor tissue genotyping assay did not identify the mutation seen in the blood.

Coexisting cfDNA mutations

In patients who had *BRCA1* cfDNA mutations alone (n=15), the most common coexisting cfDNA mutations were *TP53* (53.3%), *PIK3CA* (33.3%), *NF1* (20%), *EGFR* (20%), and *RHOA* (20%). In contrast, in patients who had *BRCA2* cfDNA mutations alone (n=11), the most common coexisting mutations were *PIK3CA* (63.6%), *TP53* (36.4%), *ERBB2* (27.3%), and *NF1* (27.3%).

Impact of cfDNA BRCA1 or BRCA2 mutation status on outcomes

We evaluated the impact of cfDNA *BRCA1* or *BRCA2* mutation status on PFS on the first therapy post-cfDNA testing (Supplemental Figure S2a) and OS (Supplemental Figure S2b), including the impact of known germline-pathogenic somatic *BRCA* mutations (n=9) on PFS on the first therapy post cfDNA testing (Supplemental Figure S2c) and OS (Supplemental Figure S2d); no significant impact of somatic *BRCA1/2* or known germline-pathogenic somatic *BRCA1/2* status, respectively, were seen in these analyses, but the small sample size precludes definitive conclusions.

For patients with somatic *BRCA1/2* mutant MBC, the median follow-up period was 4.7 months from the start of the first treatment post-testing and 18.7 months from the diagnosis of MBC. For patients with *BRCA1/2* WT MBC, the median follow-up period was 5.3 months from the start of the first treatment post-testing, and 22.1 months from the diagnosis of MBC. *BRCA1/2* cfDNA mutation status did not significantly impact PFS on the first therapy post-cfDNA testing (HR 1.07, 95% CI: 0.60-1.88, p=0.82) or OS (HR 1.14, 95% CI: 0.4-3.3, p=0.81). Overall, in a multivariate analysis adjusting for age and number of prior therapies, *BRCA1/2* cfDNA mutation status did not significantly affect PFS on the first therapy post-cfDNA testing or OS (data not shown).

In patients with cfDNA *BRCA1/2* mutations, there was a trend towards slightly improved median progression with receipt of chemotherapy compared with *BRCA1/2* WT patients (5.0 months vs 3.0 months), but similar median progression on hormone therapy (11.7 months vs 12.9 months), but given the small numbers, the results should be considered hypothesis generating and require validation in a larger dataset.

For patients with known germline-pathogenic somatic *BRCA1/2* mutations, the median follow up period was 5.1 months from the start of the first therapy post-testing, and 12.2 months from the diagnosis of MBC. *BRCA1/2* known germline-pathogenic somatic mutation status did not significantly impact PFS on the first therapy post cfDNA testing (HR 0.85, 95% CI: 0.31-2.33, p=0.76) or OS (HR 1.33, 95% CI: 0.18-10, p=0.78), compared to the aforementioned *BRCA1/2* WT population, though the results should be interpreted with caution given the relatively small numbers of patients in the known germline-pathogenic *BRCA1/2* mutant cohort limiting power to observe a statistically significant difference in outcomes. Similarly, a difference in outcomes between patients with known germline-pathogenic *BRCA1/2* mutations and those with novel variants could not be demonstrated given the small sample sizes of these cohorts, limiting the power to observe a statistically significant difference.

We observed the clinical response to a PARP inhibitor or platinum chemotherapy in 3 patients with known germline-pathogenic somatic *BRCA* mutations who received these treatments post cfDNA testing (remainder did not receive these treatments). The first patient (patient ID #16 from whom a CTC-culture line was developed as described in the manuscript) had a previously known germline-pathogenic *BRCA1* mutation in the absence of a germline *BRCA* mutation and derived therapeutic benefit with carboplatin (approximately 6 months), but not eribulin (disease progression within 3 months). A second patient (patient ID # 21) who similarly had a known germline-pathogenic *BRCA2* mutation in the absence of a germline *BRCA* mutation received the combination of carboplatin and paclitaxel for 16 months followed by carboplatin and liposomal doxorubicin for an additional 4 months. Finally, a third patient (patient ID # 17) with a known germline-pathogenic *BRCA1* mutation in the setting of a co-

existing known germline *BRCA* mutation received olaparib for 3.5 months with response. While definite conclusions cannot be drawn from this small sample of patients treated with platinum chemotherapy or a PARP inhibitor, these findings are hypothesis generating and should be evaluated further in prospective studies.