Breast cancer patients in INFORM database receiving Guardant360 test from June 2014 to December 2023:
N=51,364

Excluded:
Patients without metastatic diagnoses: n=16,698

Had At least 2 metastatic diagnosis and 2 medical claims within 6 months prior to first metastatic diagnosis date:
N=34,666

Excluded:
Patients without treatment information or initiated more than 6 months after metastatic diagnosis
n=11,500

Initiated 1L treatment within 6 months of first metastatic diagnosis
N=23,166

Excluded:
Patients without CDK4/6 inhibitor treatment
n=11,466

Received at least one CDK4/6 inhibitor treatment
N=11,700

Excluded:
Patients with HER2 amplifications prior to CDK4/6 inhibitor treatment
n=15

Did not have HER amplifications prior to CDK4/6 inhibitor treatment
N=11,685

Excluded:
Patients with Guardant360 test outside of time window:
n=5,475

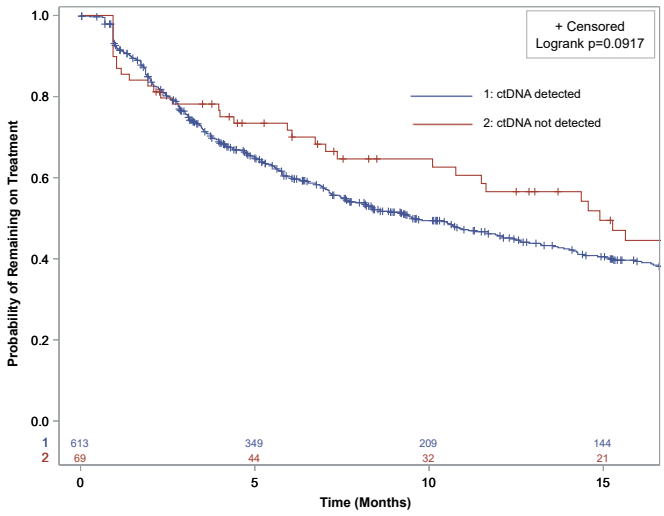
Received Guardant360 test within 90 days prior to CDK4/6 inhibitor therapy initiation
N=1,266

Received Guardant360 test within 90 days prior, and also during therapy or after end of therapy
N=207

Received Guardant360 test during therapy or within 90 days after end of CDK4/6 inhibitor therapy
N=4,737

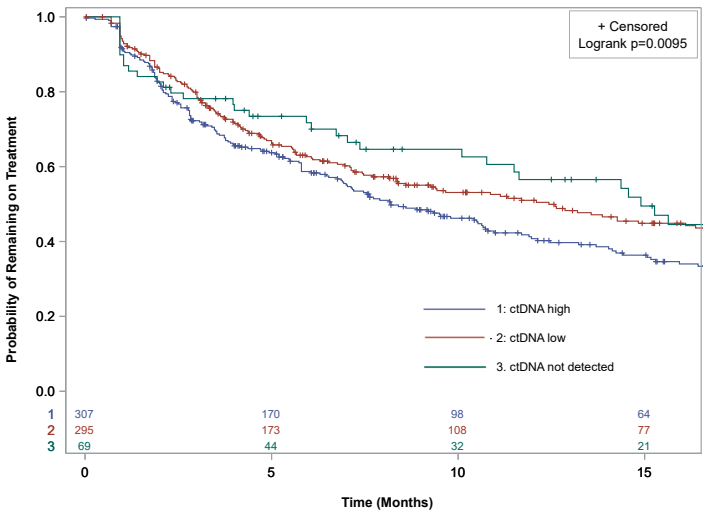
Supplemental Figure 1. Patient attrition table. Patients with mBC with ctDNA testing in the GuardantINFORM database from June 2014 to December 2023. Patients were excluded if they: 1) did not have >2 medical claims within 6 months prior to first metastatic diagnosis date (n=16,698), 2) did not initiate first-line treatment within 6 months of metastatic diagnosis, with at least one pharmacy claim and one medical claim within 6 months prior to first-line treatment initiation (n=11,500), 3) did not have claims for CDK4/6i treatment (n=11,466), 4) had HER2 amplification detected on ctDNA testing done prior to CDK4/6i treatment (n=15), or 5) had ctDNA testing that fell outside of the pre-CDK4/6i and/or post-CDK4/6i windows (n=5,475).

A



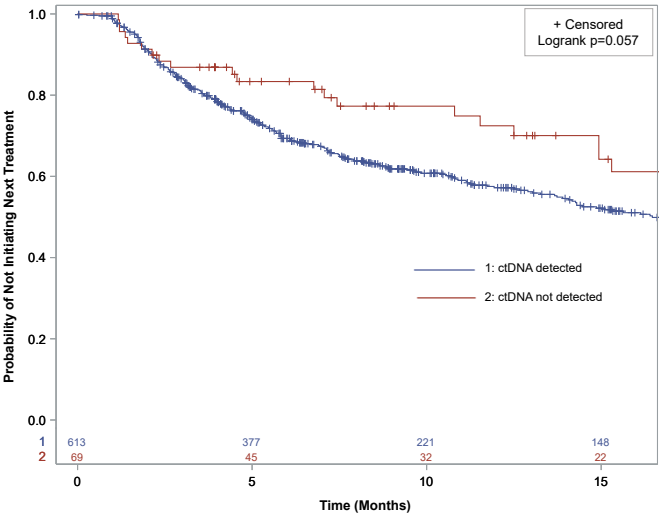
ctDNA detected		ctDNA not detected		Log-rank test p-value
Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)	
613	9.5 (7.7, 12.0)	69	14.9 (10.1, 34.8)	0.092

B



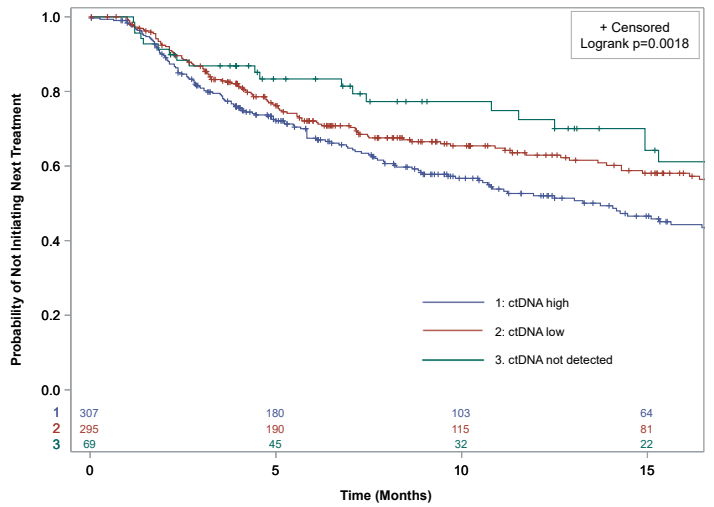
ctDNA not detected		ctDNA low (maxVAF ≤ 1.8%)		ctDNA high (maxVAF >1.8%)		Log-rank test p-value
Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)	
69	14.9 (10.1, 34.8)	295	12.4 (8.4, 16.4)	307	8.2 (6.9, 10.7)	0.010

C



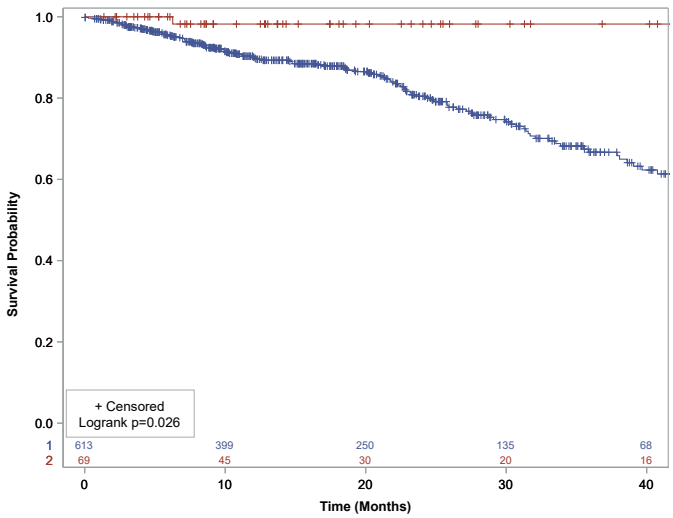
ctDNA detected		ctDNA not detected		Log-rank test p-value
Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)	
613	16.5 (13.9, 20.3)	69	37.8 (14.9, 52.1)	0.057

D



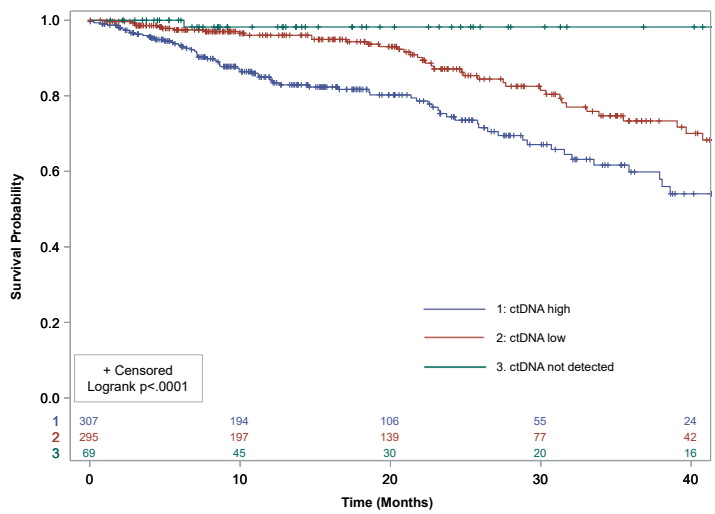
ctDNA not detected		ctDNA low (maxVAF ≤ 1.8%)		ctDNA high (maxVAF >1.8%)		Log-rank test p-value
Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)	
69	37.8 (14.9, 52.1)	295	22.8 (16.4, 37.2)	307	13.7 (10.5, 16.9)	0.002

E



ctDNA detected		ctDNA not detected		Log-rank test p-value
Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)	
613	59.6 (51.2, NR)	69	86.1 (54.4, NR)	0.003

F



ctDNA not detected		ctDNA low (maxVAF ≤ 1.8%)		ctDNA high (maxVAF >1.8%)		Log-rank test p-value
Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)	
69	86.1 (54.4, NR)	295	72.8 (51.2, NR)	307	59.6 (37.9, NR)	<0.0001

Supplemental Figure 2. Baseline ctDNA detection and impact on outcomes, first-line only. rwTTD (A), rwTTNT (B), and OS (C) between patients with ctDNA detected (blue, n = 613) or not detected (red, n = 69) at baseline. Statistical significance was determined using a log-rank test with p = 0.092 for rwTTD, p = 0.057 for rwTTNT, and p = 0.026 for OS. rwTTD (D), rwTTNT (E), and OS (F) between patients classified as ctDNA high (having a maxVAF above the median, blue, n =307), ctDNA low (having a maxVF below the median, red, n = 295), and ctDNA not detected (teal, n = 69). Log-rank test with p = 0.0095 for rwTTD, p = 0.002 for rwTTNT, and p < 0.0001 for OS.

Patients

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66

PIK3CA mut-1
PIK3CA mut-2
PIK3CA mut-3
PIK3CA mut-4
PIK3CA mut-5
PIK3CA mut-6
TP53 mut-1
TP53 mut-2
TP53 mut-3
TP53 mut-4
TP53 mut-5
AKT1
ALK
APC mut-1
APC mut-2
AR mut-1
AR mut-2
ARAF
ARID1A
ATM mut-1
ATM mut-2
BRAF
BRCA1 mut-1
BRCA1 mut-2
BRCA2 mut-1
BRCA2 mut-2
CCND1
CCND2
CCNE1
CDH1
CDK12
CDK4
CHEK2
CTNNB1
EGFR
ERBB2
ESR1 mut-1
ESR1 mut-2
ESR1 mut-3
ESR1 mut-4
EZH2
FGFR1
FGFR2
GATA3
GNAQ
GNAS
HNF1A
HRAS
KEAP1
KIT
KRAS mut-1
KRAS mut-2
KRAS mut-3
MAPK1
MAPK3
MET
MSH6
MTOR
NF1
NFE2L2
NOTCH1
NTRK1
NTRK3
PALB2
PDGFRA
PTEN
PTPN11
RAF1
RB1 mut-1
RB1 mut-2
RB1 mut-3
RB1 mut-4
RB1 mut-5
RB1 mut-6
RB1 mut-7
RB1 mut-8
RB1 mut-9
RB1 mut-10
RB1 mut-11
RB1 mut-12
RB1 mut-13
RET
RHOA
ROS1
SMAD4 mut-1
SMAD4 mut-2
SMAD4 mut-3
SMAD4 mut-4
SMAD4 mut-5
SMAD4 mut-6
SMAD4 mut-7
SMAD4 mut-8
SMAD4 mut-9
SMAD4 mut-10
SMAD4 mut-11
SMAD4 mut-12
SMAD4 mut-13
SMAD4 mut-14
SMAD4 mut-15
TERT
TSC1
VHL

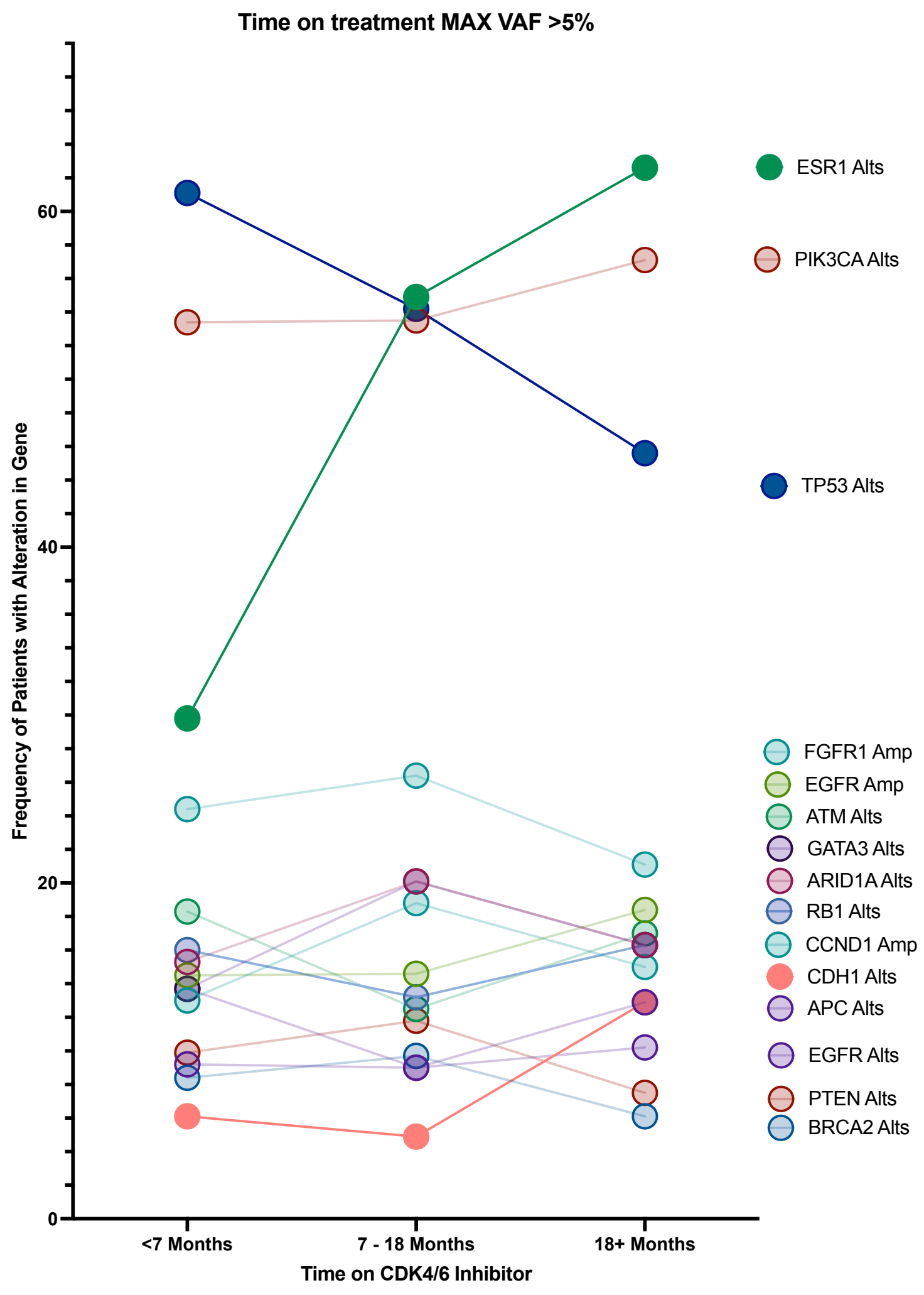
ΔVAF(Log_{10})

4
2
0
-2
-4

Clonal

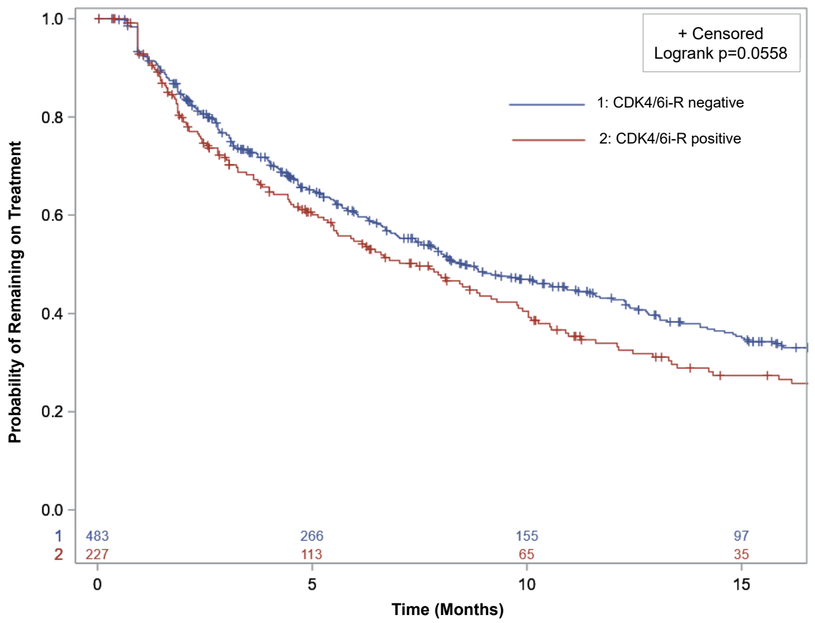
•

Supplemental Figure 3. Paired pre- and post-CDK4/6i changes in VAF. Heatmap of change in VAF comparing the pre- and post-CDK4/6i ctDNA from 66 patients with paired samples normalized according to the change in ctDNA fraction as a proxy of change in disease burden. Only patients with the same clonal alteration detected both pre- and post-CDK4/6i were included as the change in copy number adjusted VAF of this alteration was used as a proxy for tumor shed. The non-germline alteration in the sample with the highest VAF was treated as the clonal alteration and was used to estimate tumor fraction to generate a normalized change in VAF (deltaVAF) from pre- to post-CDK4/i. The color gradient of the boxes represents increase (red) or decrease (blue) in deltaVAF. Multiple mutations in the same gene are represented by separate rows.


Time on treatment MAX VAF >5%
Frequency of Patients with Alteration in Gene
0
20
40
60
<7 Months
7 - 18 Months
18+ Months
Time on CDK4/6 Inhibitor
ESR1 Alts
PIK3CA Alts
TP53 Alts
FGFR1 Amp
EGFR Amp
ATM Alts
GATA3 Alts
ARID1A Alts
RB1 Alts
CCND1 Amp
CDH1 Alts
APC Alts
EGFR Alts
PTEN Alts
BRCA2 Alts

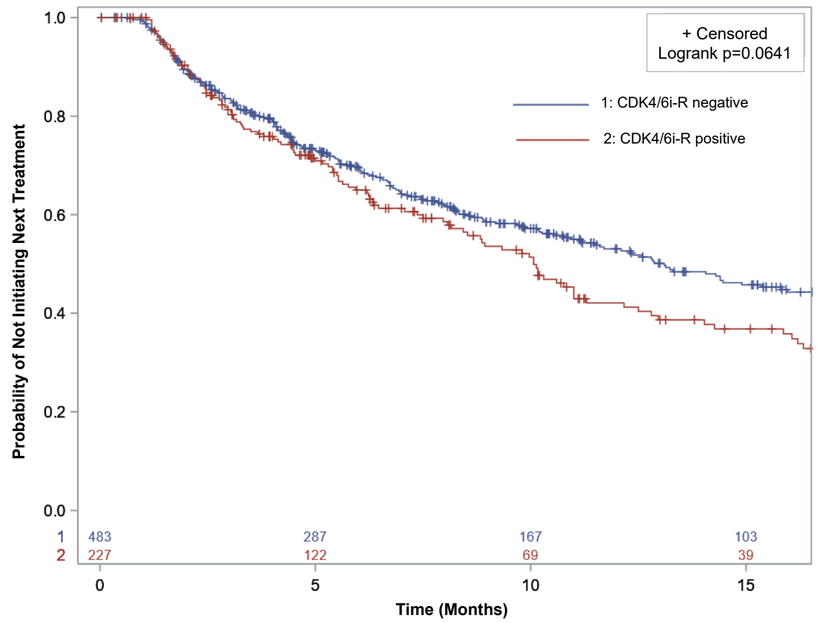
Supplemental Figure 4. Frequency of alterations by time on CDK4/6i treatment, limited to patients with ctDNA maxVAF >5%. Patients who remained on CDK4/6i therapy for <7 months were in the bottom tertile (n = 131), patients on therapy for 7-18 months were in the middle tertile (n = 144), and patients who remained on therapy for >18 months (n = 147) were in the upper tertile. A two-sided trend test based on the tertile group as a continuous variable and a two-sided ANOVA test based on tertile group as a categorical variable were conducted to identify any trend or difference in frequencies between tertiles, with *ESR1* having a trend test p-value <0.0001 and ANOVA test p-value of 0.000, *CDH1* having a trend test p-value of 0.032 and ANOVA test p-value of 0.025, and *TP53* having a trend test p-value of 0.010 and ANOVA test p-value of 0.034.

A



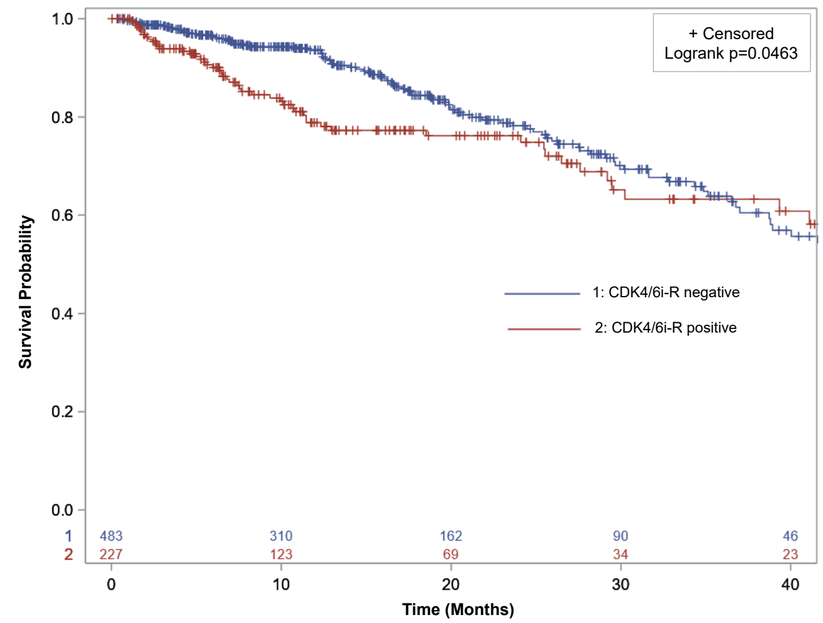
CDKi-R positive		CDKi-R negative		Log-rank test p-value	Adjusted hazard ratio (95% CI)	P-value
Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)			
227	7.4 (5.5, 9.2)	483	8.5 (7.4, 10.9)	0.0558	1.19 (0.98, 1.45)	0.082

B



CDKi-R positive		CDKi-R negative		Log-rank test p-value	Adjusted hazard ratio (95% CI)	P-value
Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)			
227	10.1 (8.2, 11.3)	483	13.1 (10.9, 16.0)	0.0641	1.19 (0.95, 1.48)	0.129

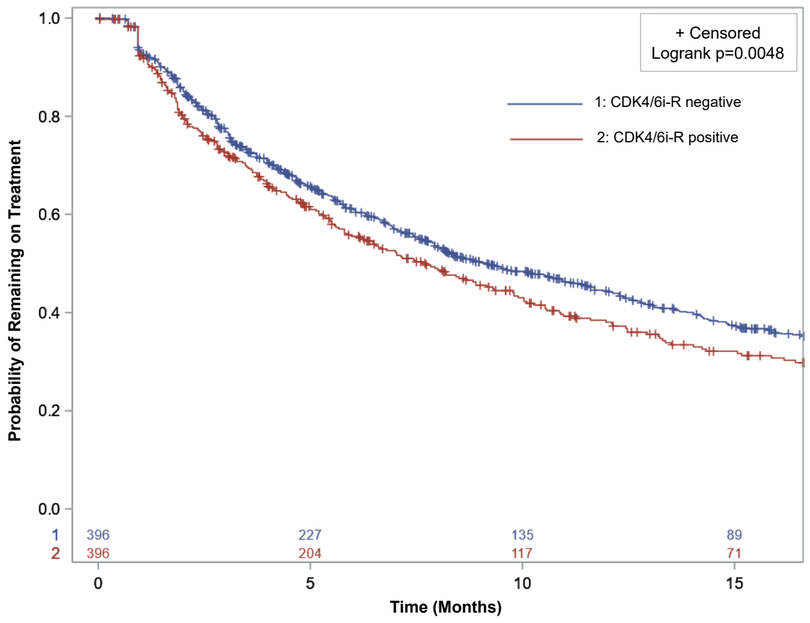
C



CDKi-R positive		CDKi-R negative		Log-rank test p-value	Adjusted hazard ratio (95% CI)	P-value
Total	Unadjusted median survival time in months (95% CI)	Total	Unadjusted median survival time in months (95% CI)			
227	51.3 (39.3, 65.7)	483	48.6 (38.8, NR)	0.0463	1.31 (0.93, 1.84)	0.128

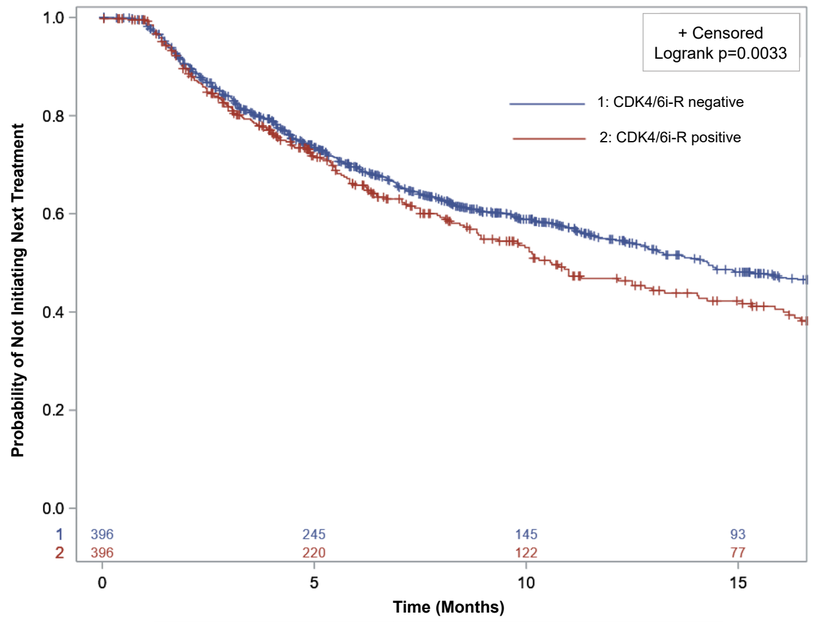
Supplemental Figure 5. Patient outcomes on second-line or later CDK4/6i therapy based pre-CDK4/6i ctDNA result. rwTTD (A), rwTTNT (B), and OS (C) for patients treated with CDK4/6i in the second or later line of therapy based on presence (red) or absence (blue) of putative CDK4/6i plus endocrine therapy resistance alterations (CDK4/6i-R) in their pre-CDK4/6i ctDNA across all lines of therapy. Statistical significance was determined using a log-rank test with $p = 0.0558$ for rwTTD, $p = 0.0641$ for rwTTNT, and $p = 0.0463$ for OS.

A



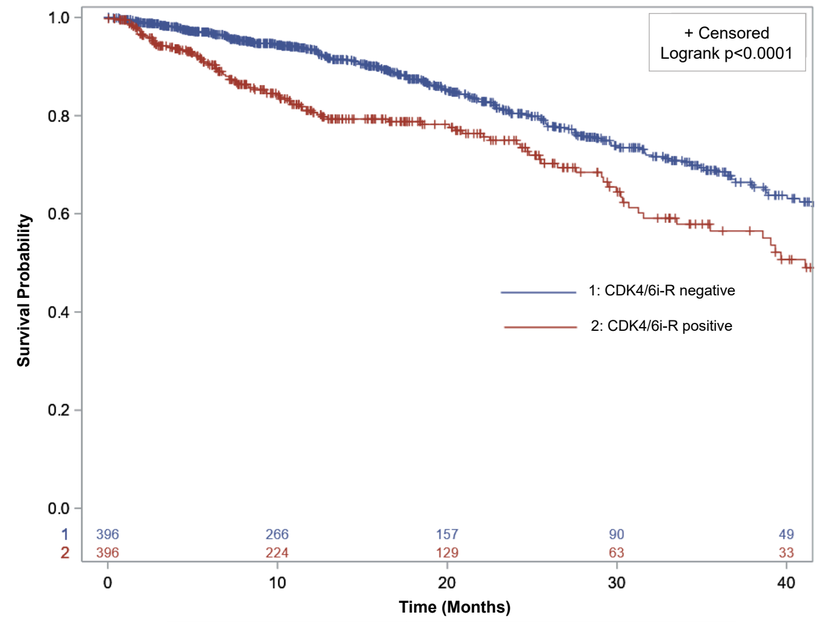
CDKi-R positive		CDKi-R negative		Weighted log-rank test p-value
Total	Weighted median survival time in months (95% CI)	Total	Weighted median survival time in months (95% CI)	
396	7.7 (6.2, 9.3)	396	9.2 (8.0, 11.0)	0.0048

B



CDKi-R positive		CDKi-R negative		Weighted log-rank test p-value
Total	Weighted median survival time in months (95% CI)	Total	Weighted median survival time in months (95% CI)	
396	10.6 (8.9, 13.3)	396	14.3 (12.6, 17.0)	0.0033

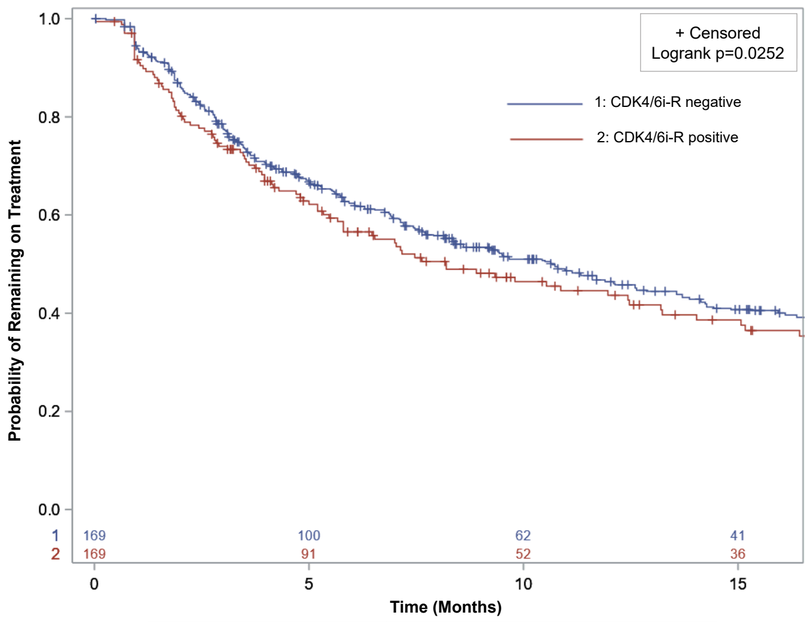
C



CDKi-R positive		CDKi-R negative		Weighted log-rank test p-value
Total	Weighted median survival time in months (95% CI)	Total	Weighted median survival time in months (95% CI)	
396	41.1 (33.5, 60.6)	396	59.6 (51.2, NR)	<0.0001

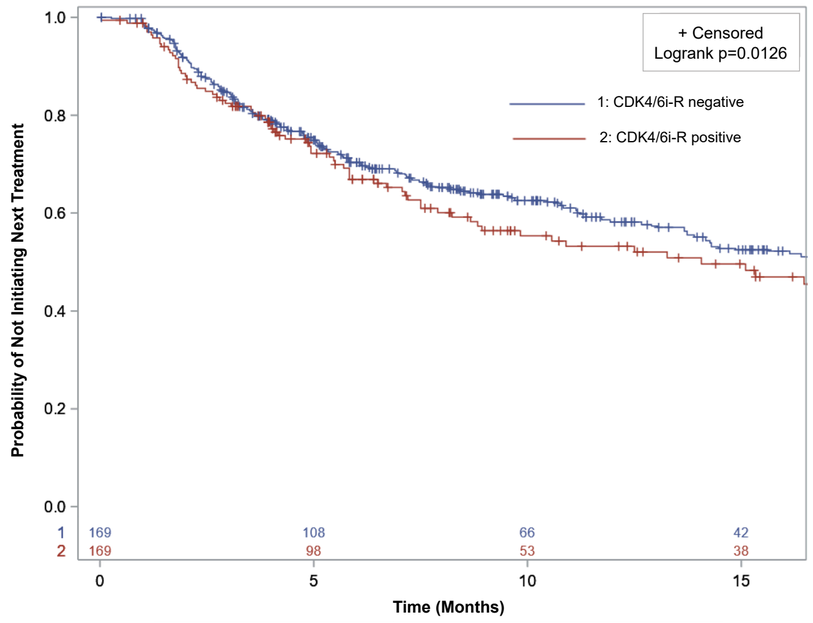
Supplemental Figure 6. Patient outcomes on all lines of CDK4/6i therapy based on pre-CDK4/6i ctDNA results with propensity score weighting. rwTTD (A), rwTTNT (B), and OS (C) comparison for patients with (red, n = 396) versus without (blue, n = 396) putative CDK4/6i plus endocrine therapy resistance alterations (CDK4/6i-R) in their pre-CDK4/6i ctDNA with propensity score weighting applied. Statistical significance was determined using a log-rank test with $p = 0.0048$ for rwTTD, $p = 0.0033$ for rwTTNT, and $p < 0.0001$ for OS.

A



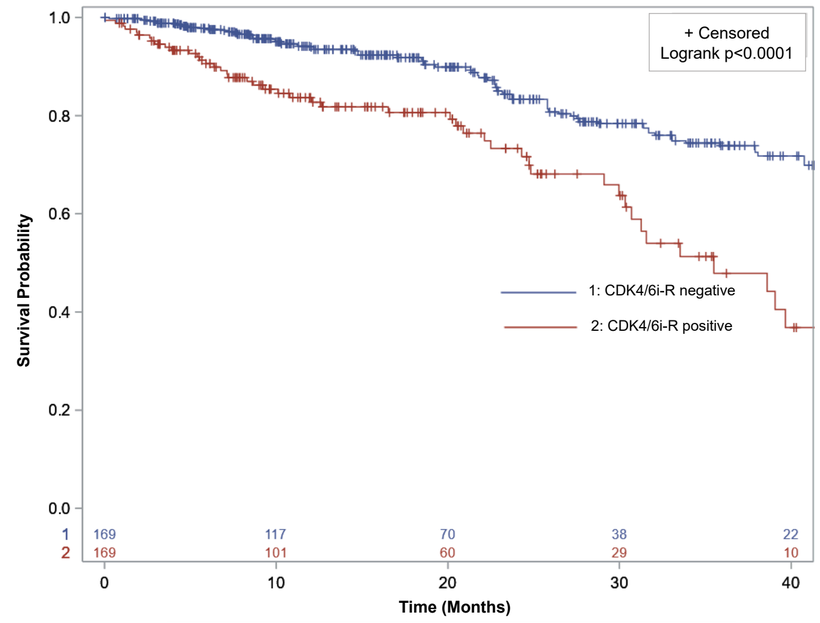
CDKi-R positive		CDKi-R negative		Weighted log-rank test p-value
Total	Weighted median survival time in months (95% CI)	Total	Weighted median survival time in months (95% CI)	
169	8.2 (5.8, 12.5)	169	10.7 (8.2, 12.9)	0.0252

B



CDKi-R positive		CDKi-R negative		Weighted log-rank test p-value
Total	Weighted median survival time in months (95% CI)	Total	Weighted median survival time in months (95% CI)	
169	14.1 (8.7, 18.4)	169	17.0 (13.7, 25.1)	0.0126

C

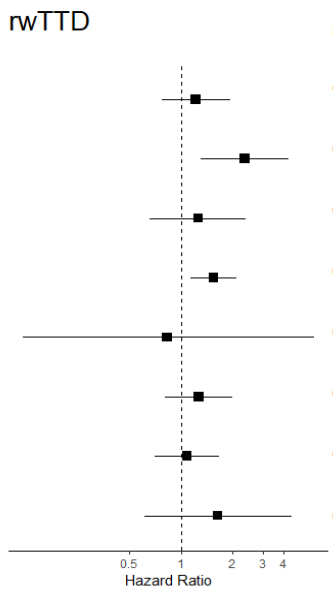


CDKi-R positive		CDKi-R negative		Weighted log-rank test p-value
Total	Weighted median survival time in months (95% CI)	Total	Weighted median survival time in months (95% CI)	
169	35.5 (30.3, NR)	169	72.8 (59.6, NR)	<0.0001

Supplemental Figure 7. Patient outcomes on first-line CDK4/6i therapy based pre-CDK4/6i ctDNA results with propensity score weighting. rwTTD (A), rwTTNT (B), and OS (C) comparison for patients with (red, n = 169) versus without (blue, n = 169) putative CDK4/6i plus endocrine therapy resistance alterations (CDK4/6i-R) in their pre-CDK4/6i ctDNA with propensity score weighting applied. Statistical significance was determined using a log-rank test with $p = 0.0252$ for rwTTD, $p = 0.0126$ for rwTTNT, and $p < 0.0001$ for OS.

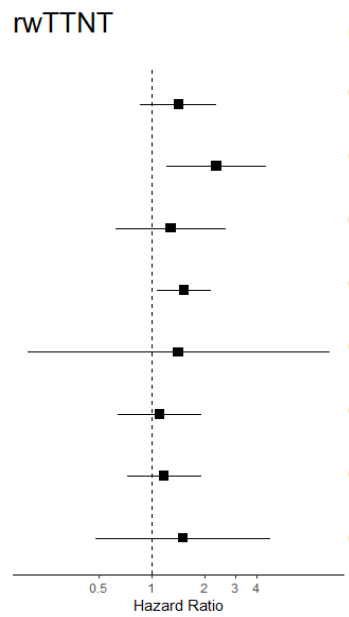
A

Gene	N	Hazard Ratio (95% CI)	p-value
AKT1	26	1.22 (0.77-1.94)	0.406
CCNE1	14	2.36 (1.3-4.27)	0.005
ERBB2	20	1.26 (0.66-2.39)	0.488
FGFR1	72	1.55 (1.14-2.12)	0.005
FGFR2	2	0.83 (0.12-6.01)	0.855
KRAS	30	1.27 (0.81-2)	0.304
PTEN	32	1.08 (0.7-1.68)	0.728
RB1	6	1.64 (0.61-4.45)	0.329



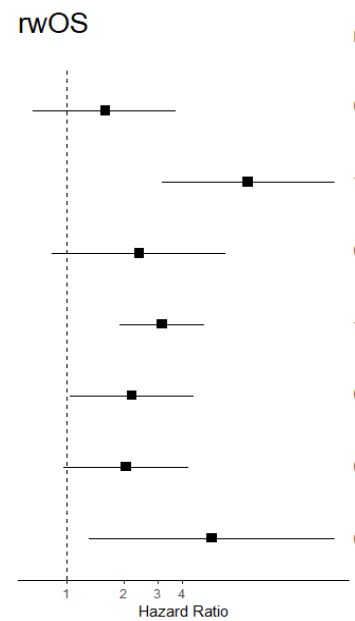
B

Gene	N	Hazard Ratio (95% CI)	p-value
AKT1	26	1.43 (0.87-2.35)	0.154
CCNE1	14	2.35 (1.22-4.53)	0.010
ERBB2	20	1.29 (0.63-2.66)	0.485
FGFR1	72	1.54 (1.08-2.19)	0.017
FGFR2	2	1.42 (0.2-10.35)	0.729
KRAS	30	1.12 (0.65-1.92)	0.679
PTEN	32	1.18 (0.73-1.93)	0.502
RB1	6	1.51 (0.48-4.76)	0.483



C

Gene	N	Hazard Ratio (95% CI)	p-value
AKT1	26	1.59 (0.67-3.73)	0.291
CCNE1	14	8.86 (3.14-25.026)	<.0001
ERBB2	20	2.39 (0.84-6.741)	0.101
FGFR1	72	3.15 (1.89-5.266)	<.0001
KRAS	30	2.19 (1.04-4.624)	0.040
PTEN	32	2.04 (0.96-4.331)	0.064
RB1	6	5.75 (1.31-25.182)	0.020



Supplemental Figure 8. Individual gene contributions to CDK4/6i outcomes. Individual gene impact on rwTTD (A), rwTTNT (B), and OS (C) as measured via individual Cox-Regression models with hazard ratios adjusted for patient age, gender, and year of ctDNA test and mapped to forest plots. Patients receiving first-line CDK4/6i with oncogenic alterations were compared to patients without any oncogenic alterations in the same gene. The individual contribution of each gene was assessed via eight separate Cox-Regression models with hazard ratios adjusted for patient age, gender, and year of ctDNA test and mapped to forest plots.

Supplemental Table 1. Guardant360 gene list.

SNVs and Indels (83)				CNA (19)	Fusions (11)
AKT1 ALK APC AR ARAF ARID1A ATM BRAF BRCA1 BRCA2 CCND1 CCND2 CCNE1 CDH1 CDK12 CDK4 CDK6 CDKN2A CHEK2 CTNNB1 DDR2 EGFR ERBB2	ESR1 EZH2 FANCA FBXW7 FGFR1 FGFR2 FGFR3 GATA3 GNA11 GNAQ GNAS HNF1A HRAS IDH1 IDH2 JAK2 JAK3 KEAP1 KIT KRAS MAP2K1 MAP2K2	MAPK1 MAPK3 MET MLH1 MPL MSH2 MSH6 MTOR MYC NF1 NFE2L2 NOTCH1 NPM1 NRAS NTRK1 NTRK2 NTRK3 PALB2 PDGFRA PIK3CA PMS2 PTEN PTPN11	RAD51D RAF1 RB1 RET RHEB RHOA RIT1 ROS1 SMAD4 SMO STK11 TERT TP53 TSC1 VHL	AR BRAF CCND1 CCND2 CCNE1 CDK4 CDK6 EGFR ERBB2 ESR1 FGFR1 FGFR2 KIT KRAS MET MYC PDGFRA PIK3CA RAF1	ALK BRAF EGFR FGFR1 FGFR2 FGFR3 MET NTRK1 NTRK2 NTRK3 RET ROS1
MSI – MSI-High					
TMB – Mutations per megabase					

Abbreviations: SNV, single nucleotide variants; indels, insertion/deletion alterations; CNA, copy number amplification; MSI, microsatellite instability; TMB, tumor mutation burden

Supplemental Table 2. Clonality analysis for *PIK3CA* and *ESR1* pre-versus post-CDK4/6i.

Gene	Cut-off	G360 Pre Trt		G360 Post Trt		Chi-square	Fisher Exact Test
		N	%	*N*	%		
PIK3CA							
Clonal or dominant subclone	>50%	192	85%	583	80%	0.053	0.053
Subclonal	<50%	33	15%	150	20%		
ESR1							
Clonal or dominant subclone	>50%	38	43%	268	45%	0.804	0.819
Subclonal	<50%	50	57%	333	55%		

Abbreviations: Trt, treatment

Supplemental Table 3. Singe versus multiple *PIK3CA* and *ESR1* alterations pre- vs post-CDK4/6.

Gene	Pre-treatment				Post-treatment				*P*-value
	Single		Multiple		Single		Multiple		
	N	%	*N*	%	*N*	%	*N*	%	
PIK3CA	444	87%	68	13%	1343	85%	238	15%	0.324
ESR1	138	71%	56	29%	810	63%	468	37%	0.036

Supplemental Table 4. Frequency of alterations by time on CDK4/6 treatment.

Gene Type	0-7m		7m-18m		18m+		Trend test *p*-value	Anova test *p*-value
	n	%	*n*	%	*n*	%		
APC SNV	20	9.0%	22	8.7%	24	9.9%	0.751	0.893
ARID1A SNV	34	15.4%	42	16.6%	32	13.2%	0.489	0.569
ATM SNV	41	18.6%	34	13.4%	44	18.2%	0.934	0.233
BRCA2 SNV	20	9.0%	25	9.9%	13	5.4%	0.134	0.151
CCND1 CNV	25	11.3%	33	13.0%	27	11.2%	0.930	0.778
CDH1 SNV	17	7.7%	17	6.7%	27	11.2%	0.176	0.185
EGFR CNV	20	9.0%	26	10.3%	27	11.2%	0.465	0.765
EGFR SNV	21	9.5%	24	9.5%	27	11.2%	0.558	0.788
ESR1 SNV	68	30.8%	130	51.4%	143	59.1%	<.0001	0.000
FGFR1 CNV	42	19.0%	49	19.4%	40	16.5%	0.468	0.674
GATA3 SNV	29	13.1%	41	16.2%	41	16.9%	0.270	0.502
PIK3CA SNV	108	48.9%	130	51.4%	123	50.8%	0.717	0.877
PTEN SNV	16	7.2%	28	11.1%	19	7.9%	0.862	0.283
RB1 SNV	25	11.3%	30	11.9%	30	12.4%	0.732	0.943
TP53 SNV	117	52.9%	124	49.0%	114	47.1%	0.195	0.415

Supplemental Table 5. Frequency of alterations by time on CDK4/6 treatment.

Gene Type	0-7m		7m-18m		18m+		Trend test *p*-value	Anova test *p*-value
	n	%	*n*	%	*n*	%		
APC SNV	12	9.2%	13	9.0%	19	12.9%	0.295	0.472
ARID1A SNV	20	15.3%	29	20.1%	24	16.3%	0.845	0.527
ATM SNV	24	18.3%	18	12.5%	25	17.0%	0.800	0.378
BRCA2 SNV	11	8.4%	14	9.7%	9	6.1%	0.468	0.523
CCND1 CNV	17	13.0%	27	18.8%	22	15.0%	0.680	0.406
CDH1 SNV	8	6.1%	7	4.9%	19	12.9%	0.032	0.025
EGFR CNV	19	14.5%	21	14.6%	27	18.4%	0.370	0.594
EGFR SNV	18	13.7%	13	9.0%	15	10.2%	0.361	0.433
ESR1 SNV	39	29.8%	79	54.9%	92	62.6%	<.0001	0.000
FGFR1 CNV	32	24.4%	38	26.4%	31	21.1%	0.497	0.565
GATA3 SNV	18	13.7%	29	20.1%	24	16.3%	0.596	0.361
PIK3CA SNV	70	53.4%	77	53.5%	84	57.1%	0.527	0.770
PTEN SNV	13	9.9%	17	11.8%	11	7.5%	0.471	0.461
RB1 SNV	21	16.0%	19	13.2%	24	16.3%	0.924	0.719
TP53 SNV	80	61.1%	78	54.2%	67	45.6%	0.010	0.034

Supplemental Table 6. Genes included in CDK4/6i-R resistance signature.

Gene and Alteration Type
RB1 loss of function SNVs/indels
PTEN loss of function SNVs/indels
AKT1 activating SNVs/indels
ERBB2 activating SNVs/indels
FGFR1 amplifications
FGFR2 amplifications
KRAS activating SNVs/indels/amplifications
CCNE1 amplifications
CCNE2 amplifications

Abbreviations: SNV, single nucleotide variants; indels, insertion/deletion alterations

Supplemental Table 7. rwTTD, rwTTNT and OS outcomes by CK4/6i-R status, with stratification for visceral metastases across all lines of CDK4/6i therapy.

Treatment Groups	CDKi-R Positive		CDKi-R Negative		Log Rank Test *P*-value	Adjusted Hazard Ratio (95% CI)	*P*-value	With Visceral Mets			Without Visceral Mets		
	Total	Unadjusted Median Survival Time in Months (95% CI)	Total	Unadjusted Median Survival Time in Months (95% CI)				*N*	Adjusted Hazard Ratio (95% CI)	*P*-value	*N*	Adjusted Hazard Ratio (95% CI)	*P*-value
TTD	396	7.7 (6.2, 9.3)	927	9.3 (8.1, 11)	0.003	1.24 (1.07, 1.43)	0.004	495	1.27 (1, 1.6)	0.048	828	1.21 (1, 1.46)	0.053
TTNT	396	10.6 (8.9, 13.3)	927	14.5 (12.9, 18.1)	0.001	1.29 (1.09, 1.52)	0.003	495	1.33 (1.02, 1.74)	0.032	828	1.21 (0.97, 1.51)	0.084
OS	396	41.1 (33.5, 60.6)	927	59.6 (54.1, NR)	<.0001	1.74 (1.35, 2.25)	<.0001	495	1.67 (1.11, 2.5)	0.014	828	1.82 (1.3, 2.54)	0.001

Supplemental Table 8. rwTTD, rwTTNT and OS outcomes by CK4/6i-R status, with stratification for visceral metastases for first-line CDK4/6i therapy.

Treatment Groups	CDKi-R Positive		CDKi-R Negative		Log Rank Test *P*-value	Adjusted Hazard Ratio (95% CI)	*P*-value	With Visceral Mets			Without Visceral Mets		
	Total	Unadjusted Median Survival Time in Months (95% CI)	Total	Unadjusted Median Survival Time in Months (95% CI)				*N*	Adjusted Hazard Ratio (95% CI)	*P*-value	*N*	Adjusted Hazard Ratio (95% CI)	*P*-value
TTD	169	8.2 (5.8, 12.5)	444	10.5 (8.2, 12.6)	0.035	1.31 (1.05, 1.64)	0.019	222	1.47 (1.02, 2.13)	0.040	391	1.27 (0.95, 1.7)	0.103
TTNT	169	14.1 (8.7, 18.4)	444	17.4 (14.2, 25.1)	0.010	1.38 (1.07, 1.78)	0.014	222	1.61 (1.06, 2.45)	0.027	391	1.26 (0.91, 1.75)	0.165
OS	169	35.5 (30.3, NR)	444	61.5 (57.9, NR)	<.0001	2.38 (1.62, 3.48)	<.0001	222	2.18 (1.16, 4.12)	0.016	391	2.44 (1.48, 4.03)	0.001

Supplemental Table 9. Summary of demographics for pre-matched population for CDKi-R for all lines of therapy.

Demographics	CDKi-R Positive		CDKi-R Negative		Absolute Standardized Mean Difference
	No./Mean	%/std	No./Mean	%/std	
Total N	396		927		
Age	60.5	12.1	62.7	12.2	0.18
Gender					
Female	395	100%	916	99%	0.11
Male	1	0%	11	1%	0.11
Year of Index ctDNA Test					
2015-2018	48	12%	86	9%	0.09
2019	37	9%	81	9%	0.02
2020	50	13%	111	12%	0.02
2021	55	14%	138	15%	0.03
2022	84	21%	207	22%	0.03
2023	122	31%	304	33%	0.04
Line of therapy					
1	169	43%	444	48%	0.10
2	113	29%	251	27%	0.03
3	55	14%	108	12%	0.07
4	59	15%	124	13%	0.04

Abbreviations: N, number; No., number; std, standard deviation; ctDNA, cell-free circulating tumor DNA

Supplemental Table 10. Summary of demographics for matched and weighted population for CDKi-R for all lines of therapy.

Demographics	CDKi-R Positive		CDKi-R Negative		Absolute Standardized Mean Difference
	No./Mean	%/std	No./Mean	%/std	
Total N	396		396		
Age	60.5	12.1	60.5	12.5	0.00
Gender					
Female	395	100%	395	100%	0.00
Male	1	0%	1	0%	0.00
Year of Index ctDNA Test					
2015-2018	48	12%	48	12%	0.00
2019	37	9%	36	9%	0.01
2020	50	13%	50	13%	0.00
2021	55	14%	55	14%	0.00
2022	84	21%	85	22%	0.01
2023	122	31%	123	31%	0.00
Line of therapy					
1	169	43%	169	43%	0.00
2	113	29%	114	29%	0.01
3	55	14%	55	14%	0.00
4	59	15%	59	15%	0.00

Abbreviations: N, number; No., number; std, standard deviation; ctDNA, cell-free circulating tumor DNA

Supplemental Table 11. Summary of demographics for pre-matched population for CDKi-R for first-line therapy only.

Demographics	CDKi-R Positive		CDKi-R Negative		Absolute Standardized Mean Difference
	No./Mean	%/std	No./Mean	%/std	
Total N	169		444		
Age	61.6	11.2	63.5	11.9	0.17
Gender					
Female	169	100%	437	98%	0.18
Male	0	0%	7	2%	0.18
Year of Index ctDNA Test					
2015-2018	22	13%	41	9%	0.12
2019	11	7%	43	10%	0.12
2020	21	12%	49	11%	0.04
2021	17	10%	72	16%	0.18
2022	48	28%	101	23%	0.13
2023	50	30%	138	31%	0.03

Abbreviations: N, number; No., number; std, standard deviation; ctDNA, cell-free circulating tumor DNA

Supplemental Table 12. Summary of demographics for matched and weighted population for CDKi-R for first-line therapy only.

Demographics	CDKi-R Positive		CDKi-R Negative		Absolute Standardized Mean Difference
	No./Mean	%/std	No./Mean	%/std	
Total N	169		169		
Age	61.6	11.1	61.5	12.3	0.01
Gender*					
Female	169	100%	167	99%	0.13
Male	0	0%	2	1%	0.13
Year of Index ctDNA Test					
2015-2018	22	13%	22	13%	0.00
2019	11	7%	11	7%	0.00
2020	21	12%	21	12%	0.00
2021	17	10%	17	10%	0.00
2022	48	28%	48	28%	0.00
2023	50	30%	50	30%	0.00

* Gender was not included in the propensity score model because of model convergence issues. Balance still summarized.

Abbreviations: N, number; No., number; std, standard deviation; ctDNA, cell-free circulating tumor DNA

Supplemental Table 13. Explanations for differences in gene frequency between ctDNA and tissue-based testing.

Gene(s)	Differences Between Cohorts	Potential Explanation
CDH1, GATA3	*CDH1* mutations were significantly more frequent in both tissue cohorts compared to ctDNA. *GATA3* mutations were significantly more frequent in the MSK cohort compared to both the ctDNA cohort and the DFCI cohort.	Historic versions of the ctDNA assay included in this study had targeted exon coverage of *CDH1* and *GATA3*, with later versions having more expanded coverage and higher frequencies detected. The frequency of *CDH1* alterations increased from 2% to 7% when comparing the historic ctDNA assay to the assay version with expanded exon coverage, and the frequency of *GATA3* alterations increased from 7% to 12%. Further differences between the *CDH1* frequency in ctDNA versus both tissue cohorts may be due to remaining differences in assay coverage (cannot speak to the specific coverage of the tissue assays as they are external).
BRCA2, AKT1, PTEN	*BRCA2* mutations were significantly more frequent in the DFCI cohort compared to both the ctDNA cohort and the MSK cohort. *AKT1* mutations were significantly more frequent in the MSK cohort compared to the ctDNA cohort. *PTEN* mutations were significantly more frequent in the DFCI cohort compared to the ctDNA cohort.	Differences in the frequencies may be due to assay differences (cannot speak to specifics of the tissue assays here as these are external assays) and/or differences between the populations.
CCND1, MYC	*CCND1* and *MYC* amplifications were significantly more frequent in both tissue cohorts compared to ctDNA. *CCND1* and *MYC* amplifications were also significantly more frequent in the MSK cohort versus the DFCI cohort.	Differences in copy number alterations compared to tissue-based assays may be due to challenges related to detecting copy number alterations via ctDNA, which is reliant on both level of the amplification and the degree of tumor shed. Differences in the frequencies between the two tissue assays may be due to assay differences (cannot speak to specifics here as these are external assays) and/or differences between the populations
EGFR	*EGFR* amplifications were significantly more frequent in ctDNA versus both tissue cohorts.	Historic versions of the ctDNA assay could not differentiate between focal *EGFR* amplifications and aneuploidy. As such, the higher *EGFR* amplification rates seen here may be due to aneuploidy rather than focal amplification.
TP53, ATM	*TP53* mutations were significantly more frequent in ctDNA compared to both tissue cohorts. *ATM* mutations were significantly more frequent in the ctDNA cohort compared to the MSK cohort. *ATM* mutations were also significantly more frequent in the DFCI cohort versus the MSK cohort.	The increased frequency of *TP53* and *ATM* alterations seen in the ctDNA cohort could reflect clonal hematopoiesis, though neither of these genes are currently associated with targeted therapies in mBC. Differences in the frequencies between the two tissue assays may be due to assay differences (cannot speak to specifics here as these are external assays) and/or differences between the populations
FGFR1	*FGFR1* amplifications were significantly more frequent in the MSK cohort versus the DFCI cohort.	Differences in the frequencies between the two tissue assays may be due to assay differences (cannot speak to specifics here as these are external assays) and/or differences between the populations.