

Title: Methylation-based ctDNA monitoring in metastatic breast cancer during CDK4/6 inhibitor therapy

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One Sentence Summary: A blood test measuring methylation-based tumor DNA closely mirrors imaging results in metastatic breast cancer patients and detects signs of treatment failure a median of six months before clinical progression.

Supplementary Figure Legends:

Sup. Figure 1. Baseline Association. (A) Study design. Association between clinical variables of interest and baseline mTF: (B) CDK4/6-inhibitor, (C) Survival status, (D) endocrine therapy pair, (E) *de novo* metastatic disease diagnosed at baseline, (F) menopausal status, (G) previous (neo)adjuvant chemotherapy, (H) prior endocrine therapy, (I) prior endocrine therapy in metastatic setting, (J) RECIST-measurable disease at baseline, (K) visceral disease at baseline. Association between ctDNA detection at baseline using the maxVAF and (L) time to CDK4/6i discontinuation or (M) overall survival. Survival curves were estimated using the Kaplan-Meier method; shaded bands represent 95% confidence intervals. P-values were derived from two-sided log-rank tests; no adjustments were made for multiple comparisons. ET: endocrine therapy, Met: metastatic. Created in BioRender. Elliott, M. (2026)

<https://BioRender.com/ohqowvn>

Sup. Figure 2. Comparison Between Methylation Tumor Fraction and maxVAF. (A) Correlation between mTF and maxVAF across all tested timepoints. (B) Baseline maxVAF and association with RECIST-measurable disease status. (C) Association between maxVAF at baseline and clearance. (D) Longitudinal comparison between mTF and maxVAF detection at matched timepoints. Association between maxVAF clearance (E) time to CDK4/6i discontinuation or (F) overall survival. Survival curves were estimated using the Kaplan-Meier method; shaded bands represent 95% confidence intervals. P-values were derived from two-sided log-rank tests; no adjustments were made for multiple comparisons.

Supplementary Table 3. Association Between a $\geq 50\%$ Decrease in ctDNA and Clinical Outcome. Landmark analyses were performed at 2-4, 4-6, and 6-8 month intervals to evaluate whether a $\geq 50\%$ decline in mTF or maxVAF from baseline was associated with TTD and OS. Hazard ratios with 95% confidence intervals and p-values were derived from two-sided Cox proportional hazards models. No adjustments were made for multiple comparisons. Exact p-values are provided where possible. Patients were classified based on whether they achieved a $\geq 50\%$ decline or experienced any increase in mTF or maxVAF within each specified time window. mo.: months.

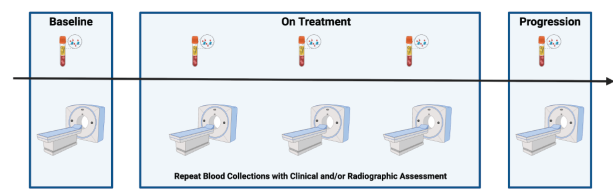
Sup. Figure 4. Defining Metrics of Response using maxVAF. (A) Consort diagram outlining participant numbers, radiographic restaging evaluations, and plasma timepoints in the study. (B) Exploratory definitions of molecular response and progression using changes in mTF and maxVAF over time with all matched (n=110) ctDNA and RECIST-evaluable restaging timepoints. mTF: Methylation-based tumor fraction, BL: baseline, NGS: next generation sequencing, maxVAF: maximum somatic variant allele fraction.

Sup. Table 1. Clinical Features of Participants without detectable ctDNA at baseline. Clinical features in patients without detectable ctDNA, as measured by mTF, at baseline.

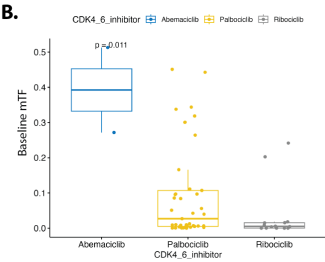
Sup. Table 2. Association Between a $\geq 50\%$ Decrease in ctDNA and Clinical Outcome. mo.: months

Sup. Figure 1

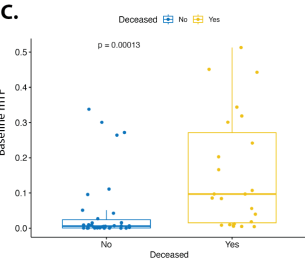
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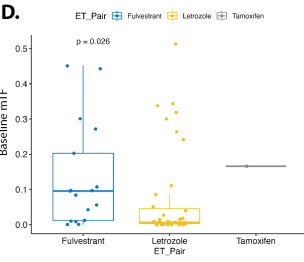
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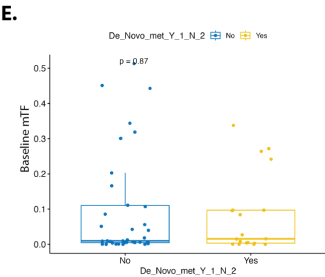
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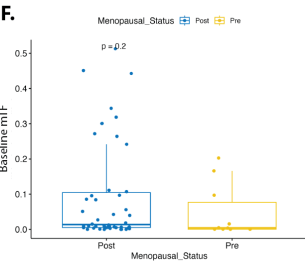
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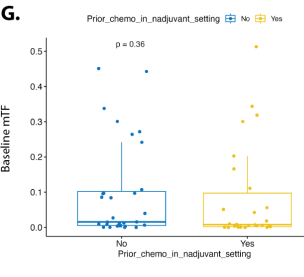
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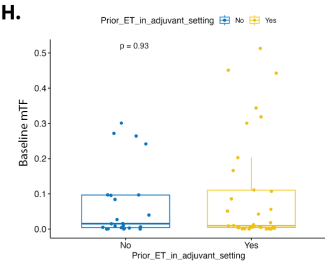
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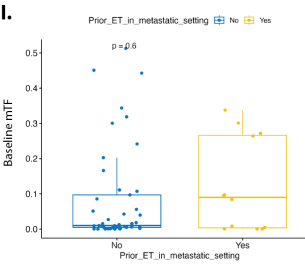
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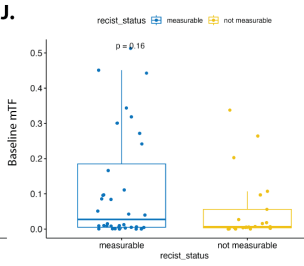
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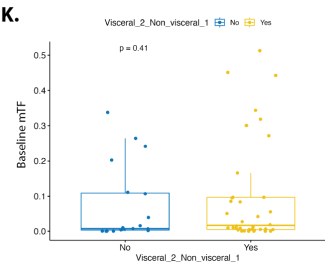
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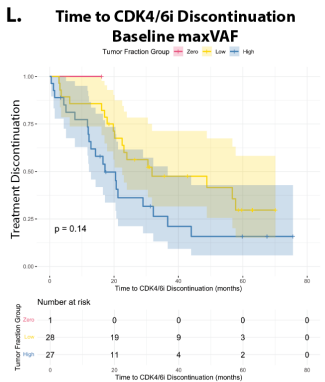
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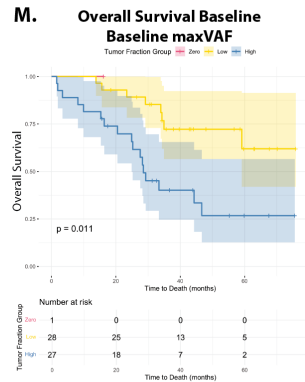
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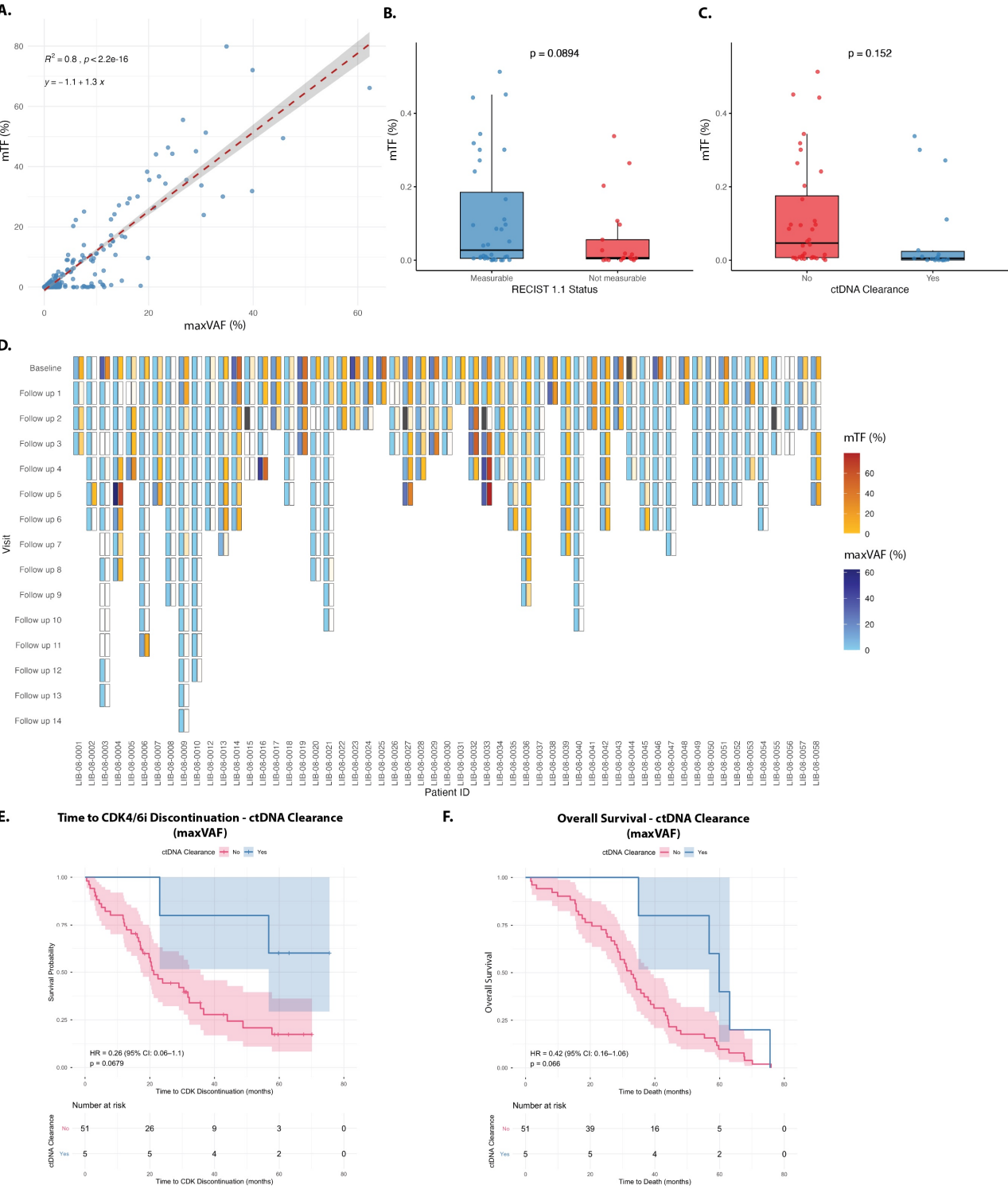
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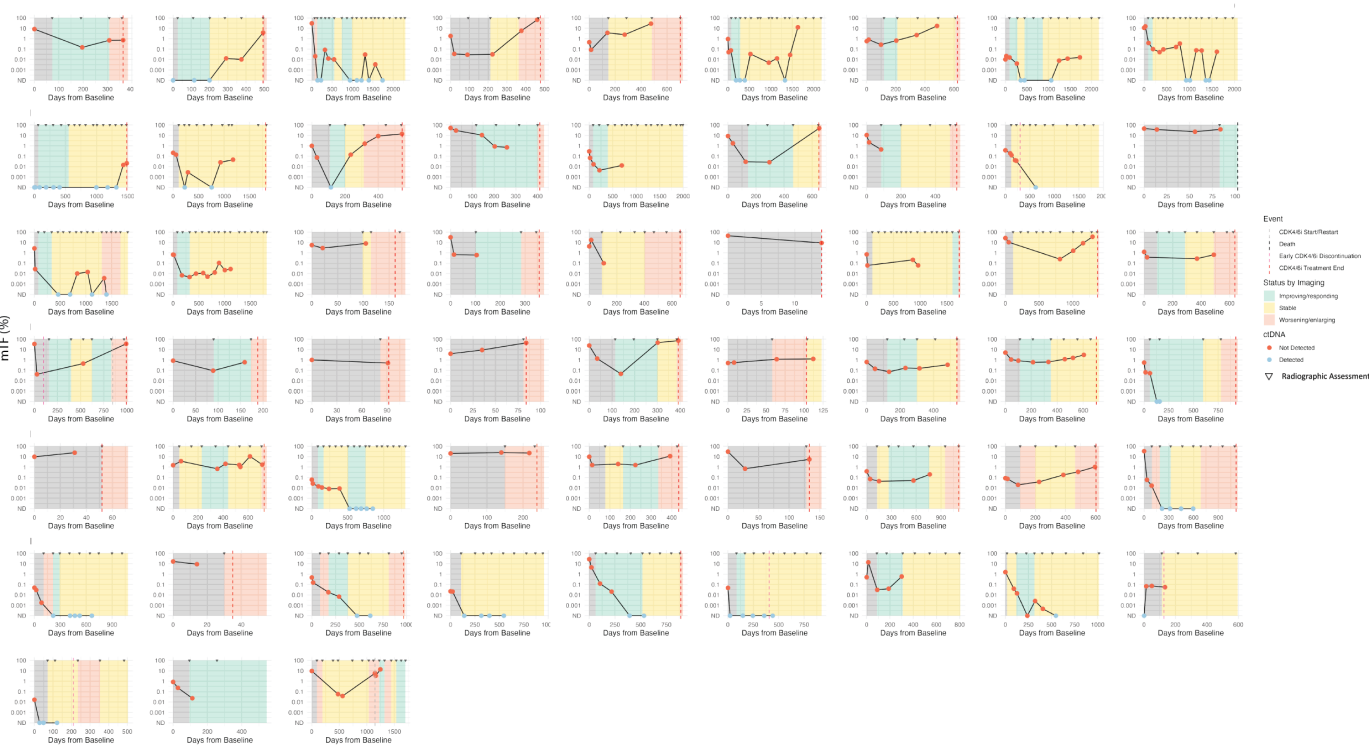


Sup. Figure 2

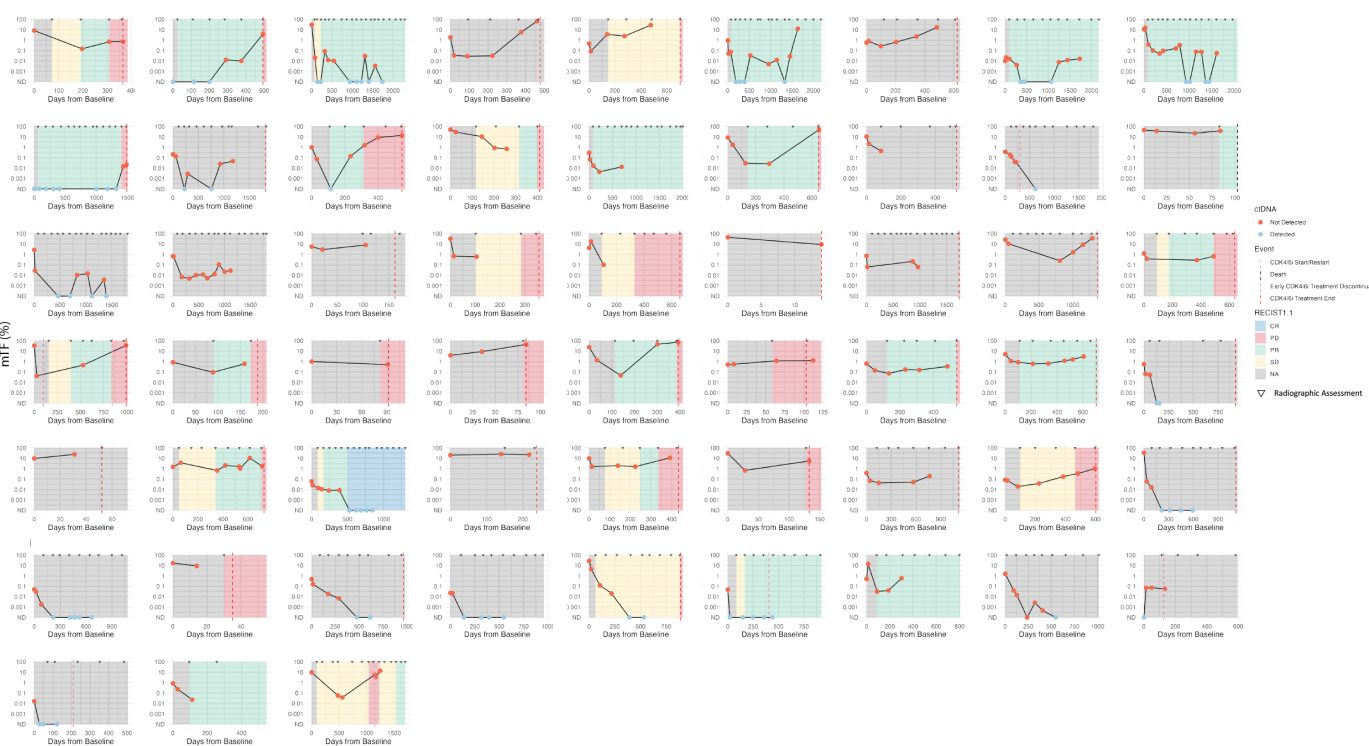


Sup. Figure 3

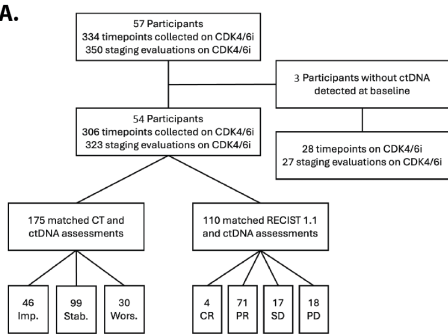
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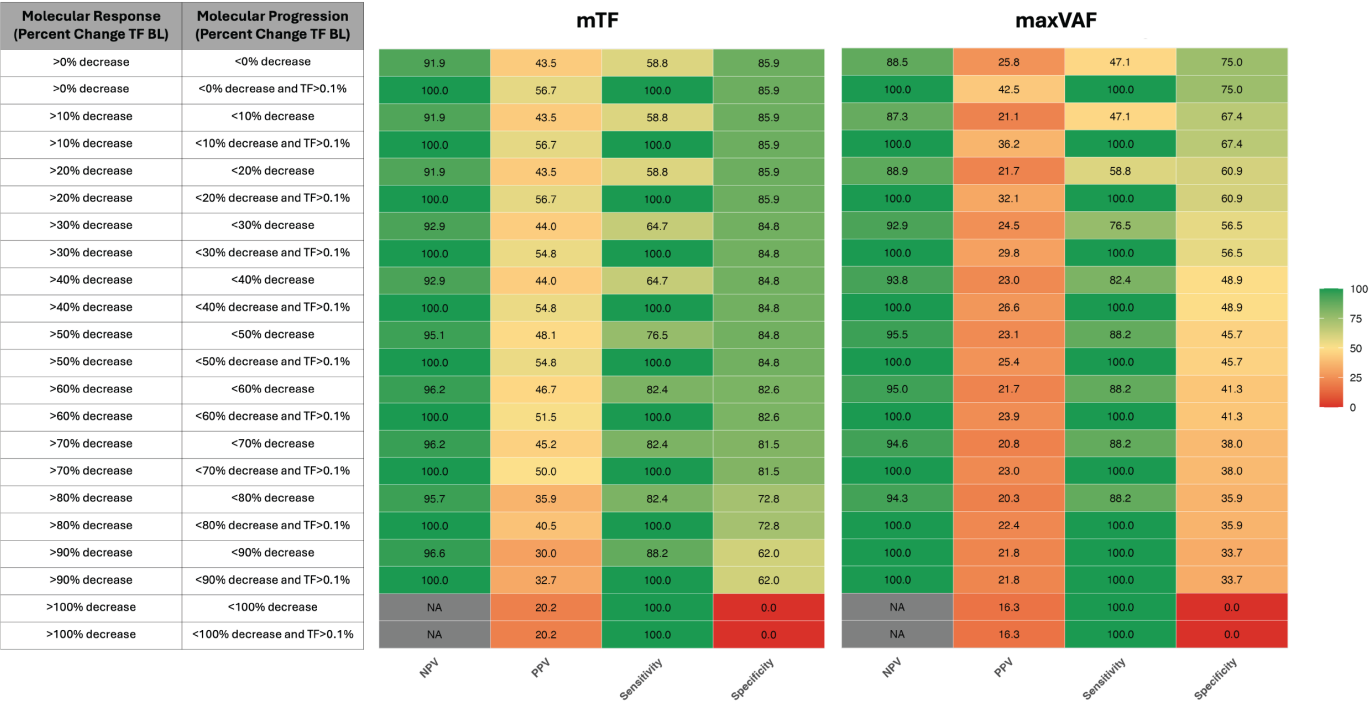
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Sup. Figure 4



B. RECIST v1.1 Evaluable Response or Disease Progression



Sup. Table 1

Participant ID	Baseline ctDNA outcome	Baseline clinical variables of interest	Clinical Progression in Follow Up	Future ctDNA detection
LIB-08-0002	Not Detected	Two 5 mm nodules, biopsy proven metastatic breast cancer	Yes	Yes
LIB-08-0010	Not Detected	Pleura, mediastinal lymph node, lung, liver. Grade 2	No	No
LIB-08-0055	Not Detected	Bone only (isolated lesion)	No	No

Sup. Table 2

mTF Decrease and Association with TTD							
Window	Comparison	HR (95% CI)	p-value	Group (n)	Refgroup (n)	Group median TTD (mo.)	Refgroup median TTD (mo.)
2-4mo.	Any increase @2-4mo. vs No increase	2.1 (0.71 to 6.4)	0.18	5	28	5.1	30.5
4-6mo.	Any increase @4-6mo. vs No increase	2.2 (0.90 to 5.5)	0.084	7	38	7.8	30.5
6-8mo.	Any increase @6-8mo. vs No increase	1.9 (0.76 to 4.6)	0.18	7	39	20.1	30.5
2-4mo.	≥50% drop @2-4mo. vs <50%	0.87 (0.31 to 2.4)	0.79	25	8	29	24
4-6mo.	≥50% drop @4-6mo. vs <50%	0.48 (0.21 to 1.1)	0.084	35	10	30.5	7.8
6-8mo.	≥50% drop @6-8mo. vs <50%	0.47 (0.20 to 1.1)	0.079	37	9	30.5	20.1
MaxVAF Decrease and Association with TTD							
Window	Comparison	HR (95% CI)	p-value	Group (n)	Refgroup (n)	Group median OS (mo.)	Refgroup median OS (mo.)
2-4mo.	Any increase @2-4mo. vs No increase	0.68 (0.27 to 1.7)	0.42	11	22	57.8	22.5
4-6mo.	Any increase @4-6mo. vs No increase	0.88 (0.39 to 2.0)	0.75	13	31	24	22.5
6-8mo.	Any increase @6-8mo. vs No increase	1.3 (0.62 to 2.9)	0.46	12	34	23	29
2-4mo.	≥50% drop @2-4mo. vs <50%	0.72 (0.28 to 1.8)	0.49	9	24	36.7	21.2
4-6mo.	≥50% drop @4-6mo. vs <50%	0.93 (0.43 to 2.0)	0.85	14	30	22.5	24
6-8mo.	≥50% drop @6-8mo. vs <50%	0.80 (0.38 to 1.7)	0.58	15	31	29	23
mTF Decrease and Association with OS							
Window	Comparison	HR (95% CI)	p-value	Group (n)	Refgroup (n)	Group median OS (mo.)	Refgroup median OS (mo.)
2-4mo.	Any increase @2-4mo. vs No increase	2.6 (0.70 to 9.7)	0.15	5	28	16.4	NR
4-6mo.	Any increase @4-6mo. vs No increase	2.6 (0.92 to 7.1)	0.073	7	38	24.9	59.1
6-8mo.	Any increase @6-8mo. vs No increase	2.4 (0.87 to 6.7)	0.09	7	39	24.9	59.1
2-4mo.	≥50% drop @2-4mo. vs <50%	0.39 (0.12 to 1.2)	0.11	25	8	NR	16.4
4-6mo.	≥50% drop @4-6mo. vs <50%	0.30 (0.12 to 0.76)	0.011	35	10	NR	24.9
6-8mo.	≥50% drop @6-8mo. vs <50%	0.30 (0.12 to 0.76)	0.011	37	9	59.1	24.9
MaxVAF Decrease and Association with OS							
Window	Comparison	HR (95% CI)	p-value	Group (n)	Refgroup (n)	Group median OS (mo.)	Refgroup median OS (mo.)
2-4mo.	Any increase @2-4mo. vs No increase	1.0 (0.31 to 3.4)	0.96	11	22	59.1	NR
4-6mo.	Any increase @4-6mo. vs No increase	1.6 (0.60 to 4.0)	0.37	13	31	59.1	NR
6-8mo.	Any increase @6-8mo. vs No increase	2.0 (0.77 to 5.0)	0.16	12	34	34.9	NR
2-4mo.	≥50% drop @2-4mo. vs <50%	0.50 (0.11 to 2.3)	0.37	9	24	NR	59.1
4-6mo.	≥50% drop @4-6mo. vs <50%	0.73 (0.26 to 2.1)	0.55	14	30	NR	59.1
6-8mo.	≥50% drop @6-8mo. vs <50%	0.81 (0.31 to 2.1)	0.66	15	31	NR	59.1