Supplementary figures

Title

Personalized ctDNA monitoring in metastatic HR+/HER2- breast cancer patients during endocrine and CDK4/6 inhibitor therapy

Authors

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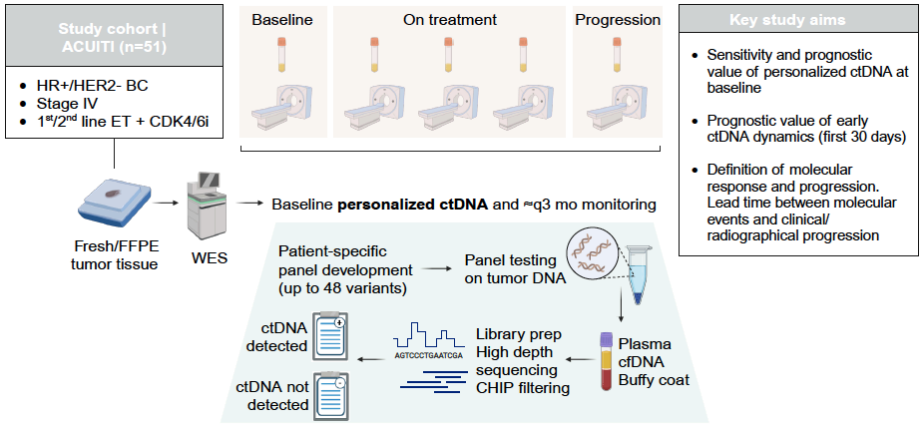
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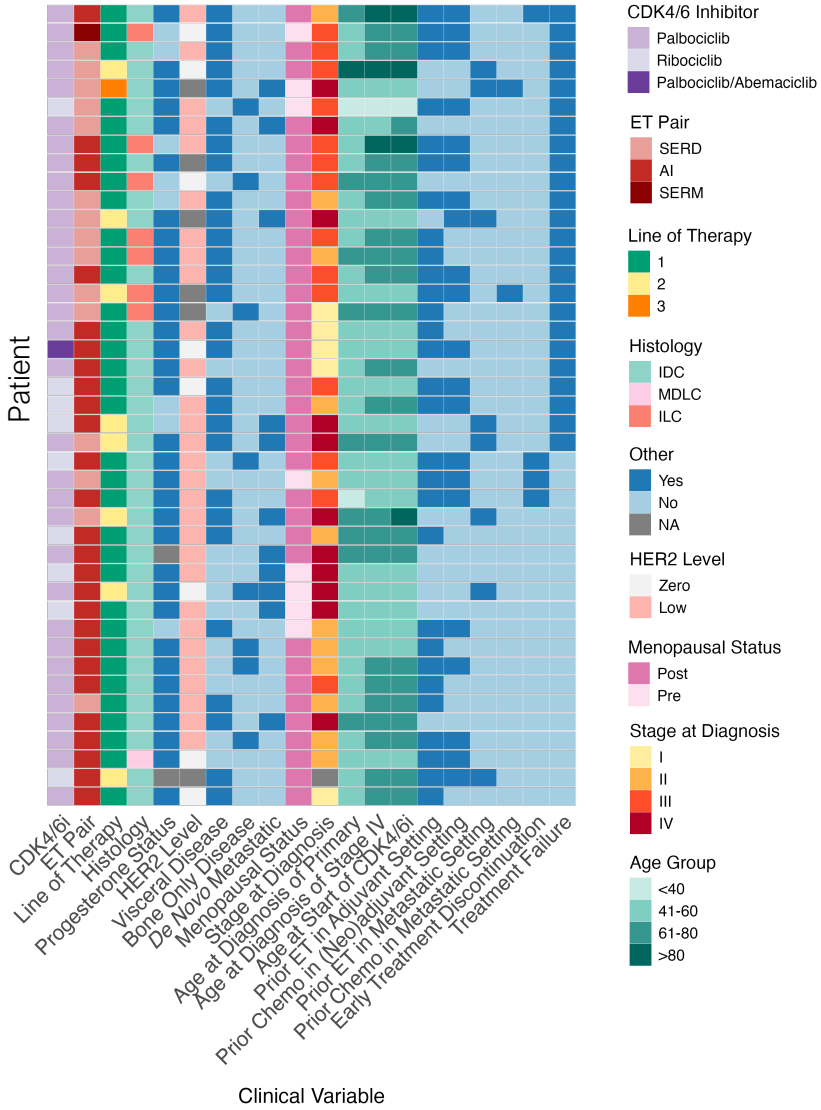
Running title: Personalized ctDNA tracking in HR+/HER2- metastatic breast cancer

Suplemmentary Figure 1. **(a)** Study design. Personalized assay generation worklow. **(b)** Oncoprint showcasing key clinicopathological and treatment-related variables for the 43 patients with successful tumor-informed panels. **(c)** Site of origin and timing of archival tissue for personalized assay generation. **(d)** ctDNA timepoints and tumor response by RECIST v1.1. and **(e)** radiologist assessment. Abbreviations: CDK4/6i, cyclin dependent kinase 4 and 6 inhibitor; ctDNA, circulating tumor DNA; ET, endocrine therapy; FFPE, formalin-fixed paraffin-embedded; HER2, human epidermal receptor 2; HR, hormone receptor; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; MDLC, mixed ductal lobular carcinoma; SERD, selective estrogen receptor degrader; SERM, selective estrogen receptor modulator; WES, whole exome sequencing.

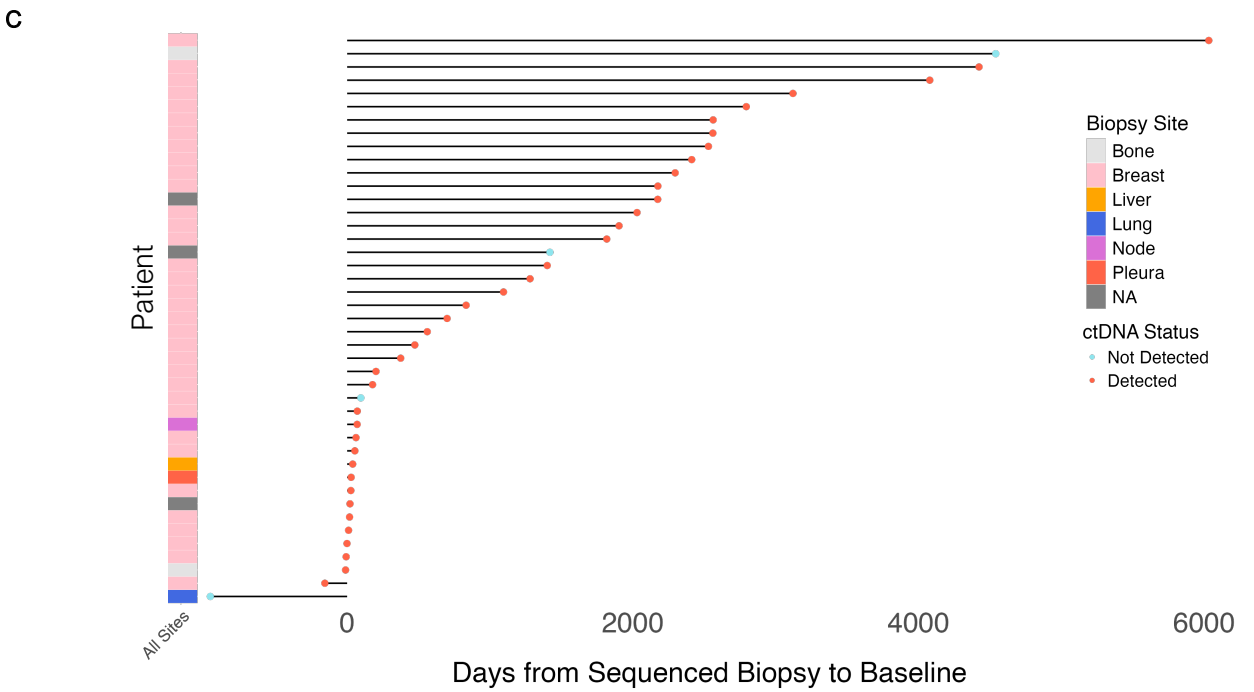
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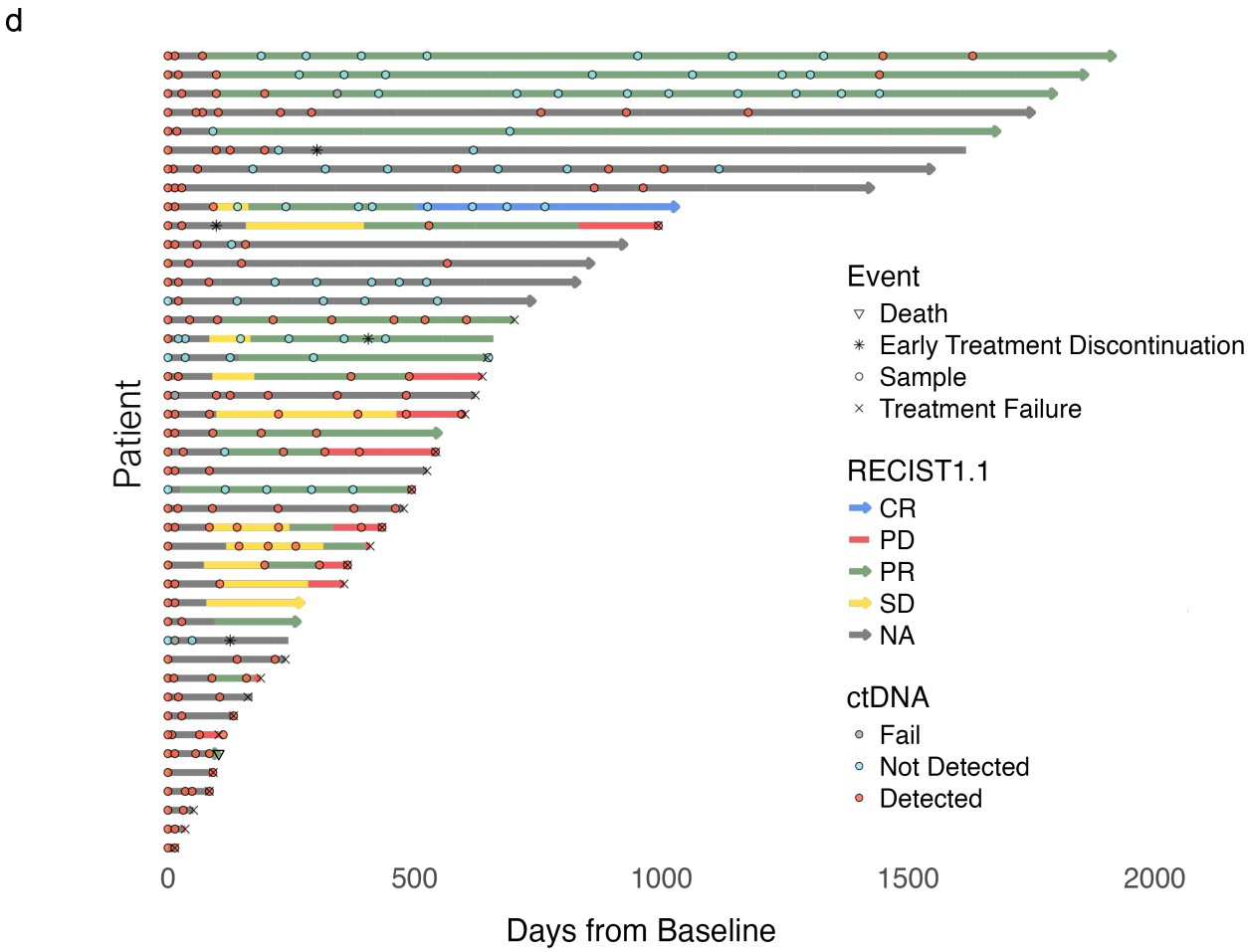


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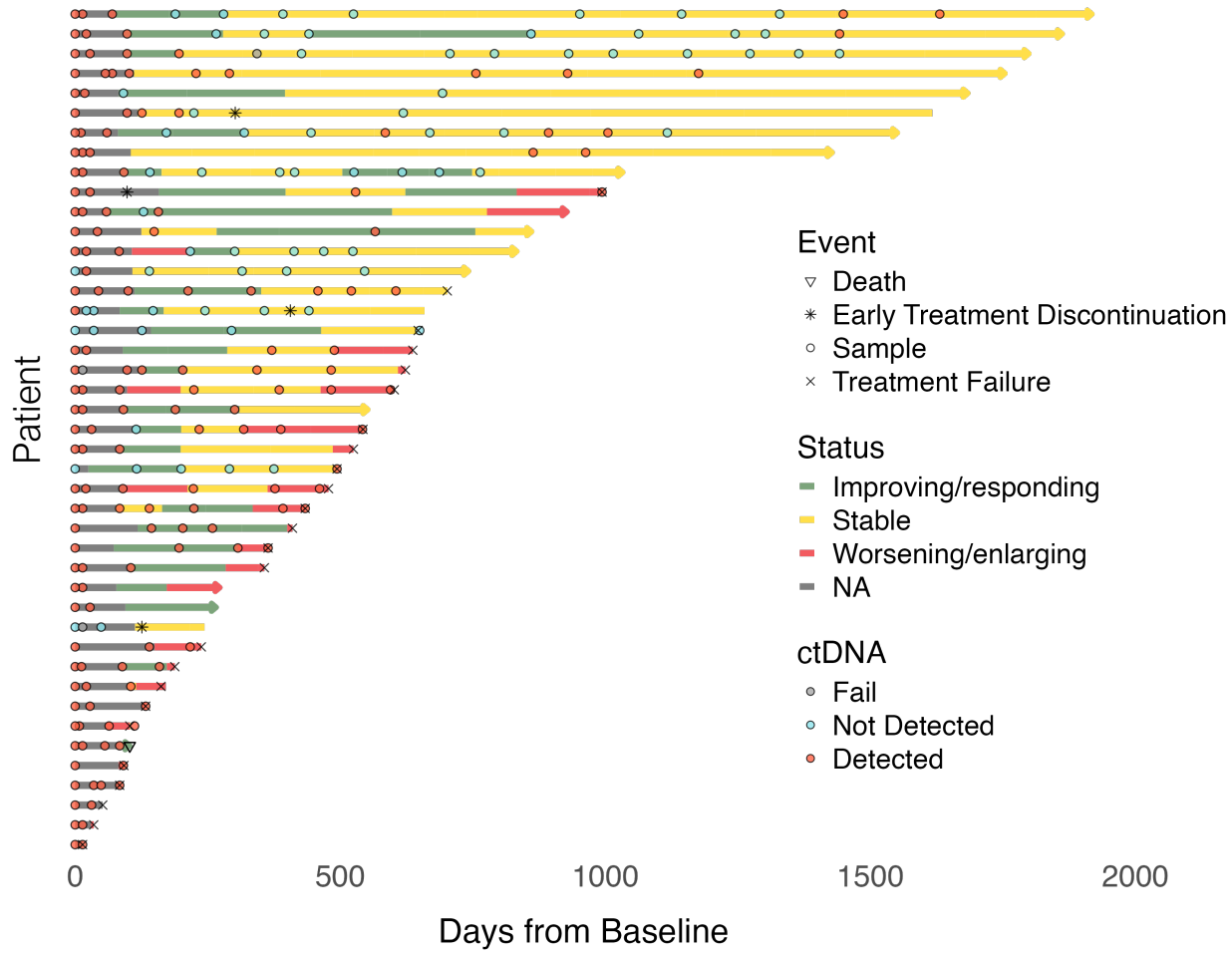
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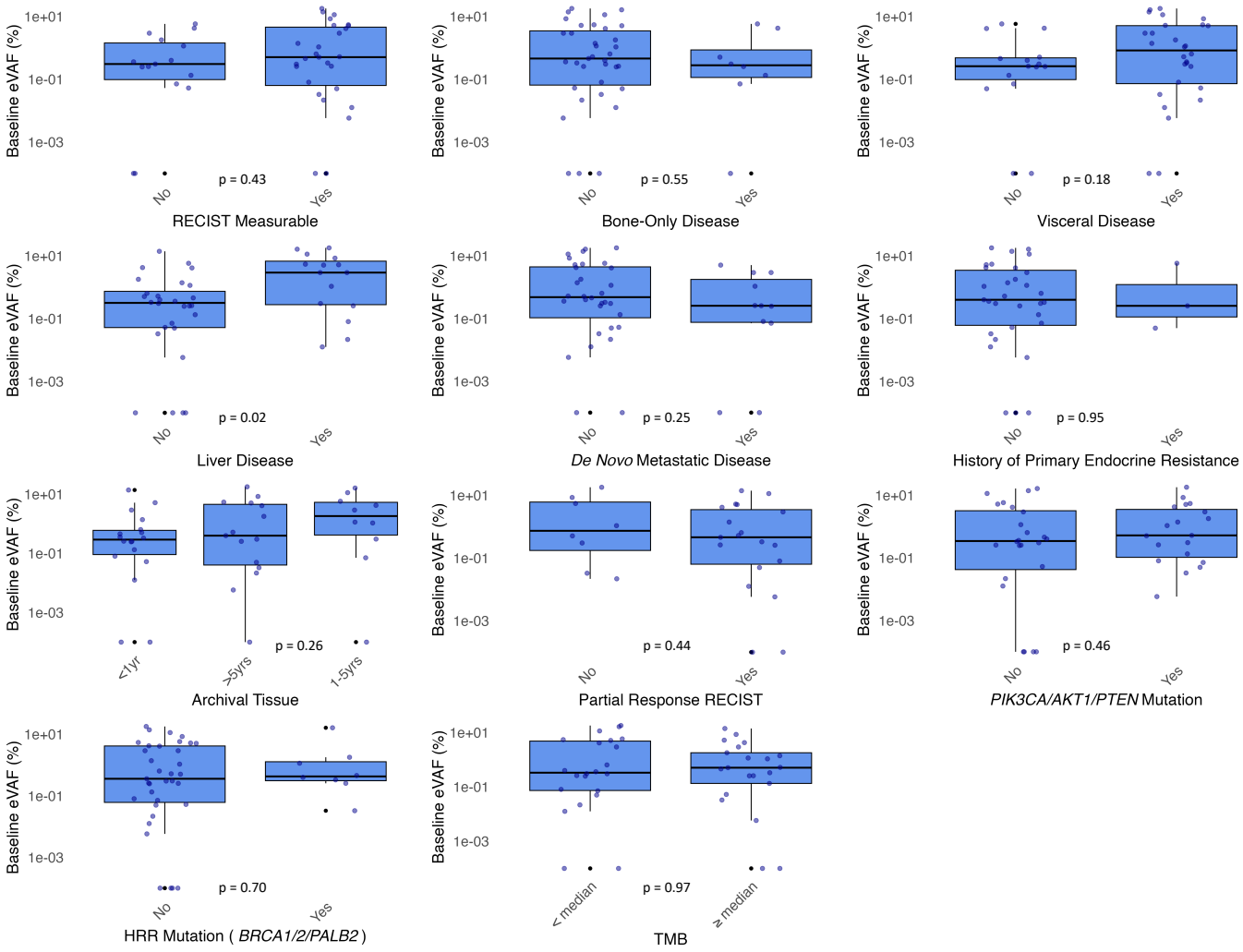
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e

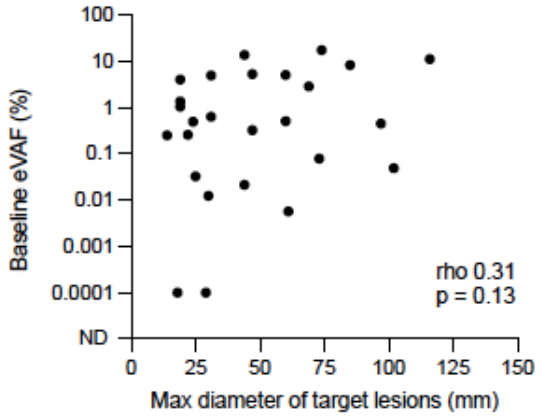


Supplementary Figure 2. **(a)** Clinicopathological and genomic associations with baseline eVAF, excluding failed samples. **(b)** Correlation of baseline eVAF with RECIST v1.1 target lesion measurements in the analytical cohort and **(c)** in patients with liver disease at baseline. **(d)** Time to treatment failure and **(e)** overall survival forest plots for univariable Cox models for baseline eVAF and key clinical variables including the presence of visceral disease, age at study entry, line of therapy, and ET pair on study. **(f)** Time to treatment failure and **(g)** overall survival forest plots for multivariable Cox models including baseline eVAF and the presence of visceral disease.

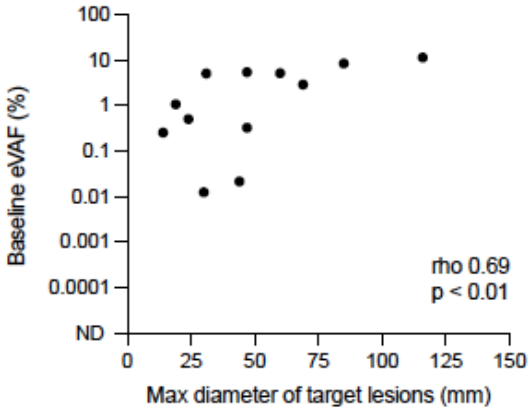
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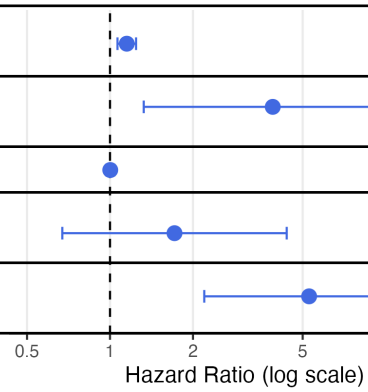


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d

Univariable models

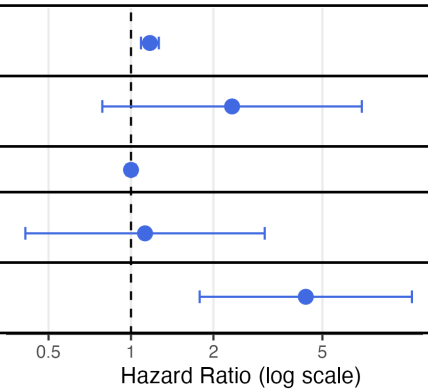
Variable	n = 43	HR (95% CI)	p-value
Baseline eVAF (continuous)	(n = 43)	1.15 (1.06 – 1.24)	<0.001
Visceral Disease	Yes (n = 28) No (n = 15)	3.89 (1.33 – 11.44)	0.013
Age (continuous)	(n =43)	1.00 (0.97 – 1.04)	0.906
Line of therapy	1 (n = 34) >1 (n = 9)	1.71 (0.67 – 4.38)	0.260
ET Pair	SERD/SERM (n = 16) AI (n = 27)	5.28 (2.20 – 12.67)	<0.001



e

Univariable models

Variable	n = 43	HR (95% CI)	p-value
Baseline eVAF (continuous)	(n = 43)	1.17 (1.09 – 1.26)	<0.001
Visceral Disease	Yes (n = 28) No (n = 15)	2.34 (0.79 – 6.96)	0.127
Age (continuous)	(n =43)	1.00 (0.96 – 1.04)	0.993
Line of therapy	1 (n = 34) >1 (n = 9)	1.13 (0.41 – 3.08)	0.818
ET Pair	SERD/SERM (n = 16) AI (n = 27)	4.35 (1.78 – 10.61)	0.001



f

Multivariable model

Variable	n = 43	HR (95% CI)	p-value
Baseline eVAF (continuous)	(n = 43)	1.13 (1.04 – 1.22)	0.003
Visceral Disease	Yes (n = 28) No (n = 15)	3.26 (1.08 – 9.81)	0.035



g

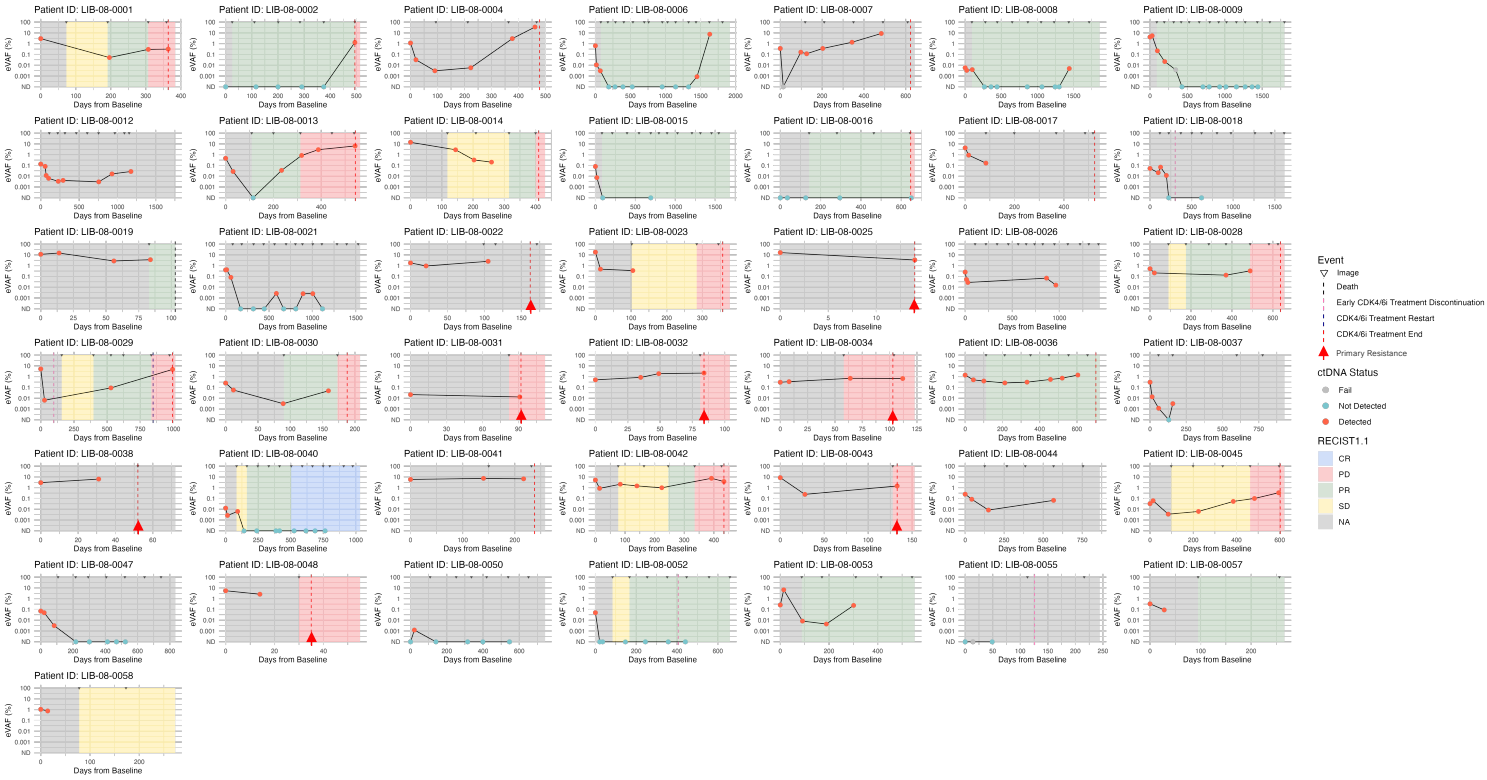
Multivariable model

Variable	n = 43	HR (95% CI)	p-value
Baseline eVAF (continuous)	(n = 43)	1.14 (1.05 – 1.23)	0.001
Visceral Disease	Yes (n = 28) No (n = 15)	2.13 (0.71 – 6.42)	0.178

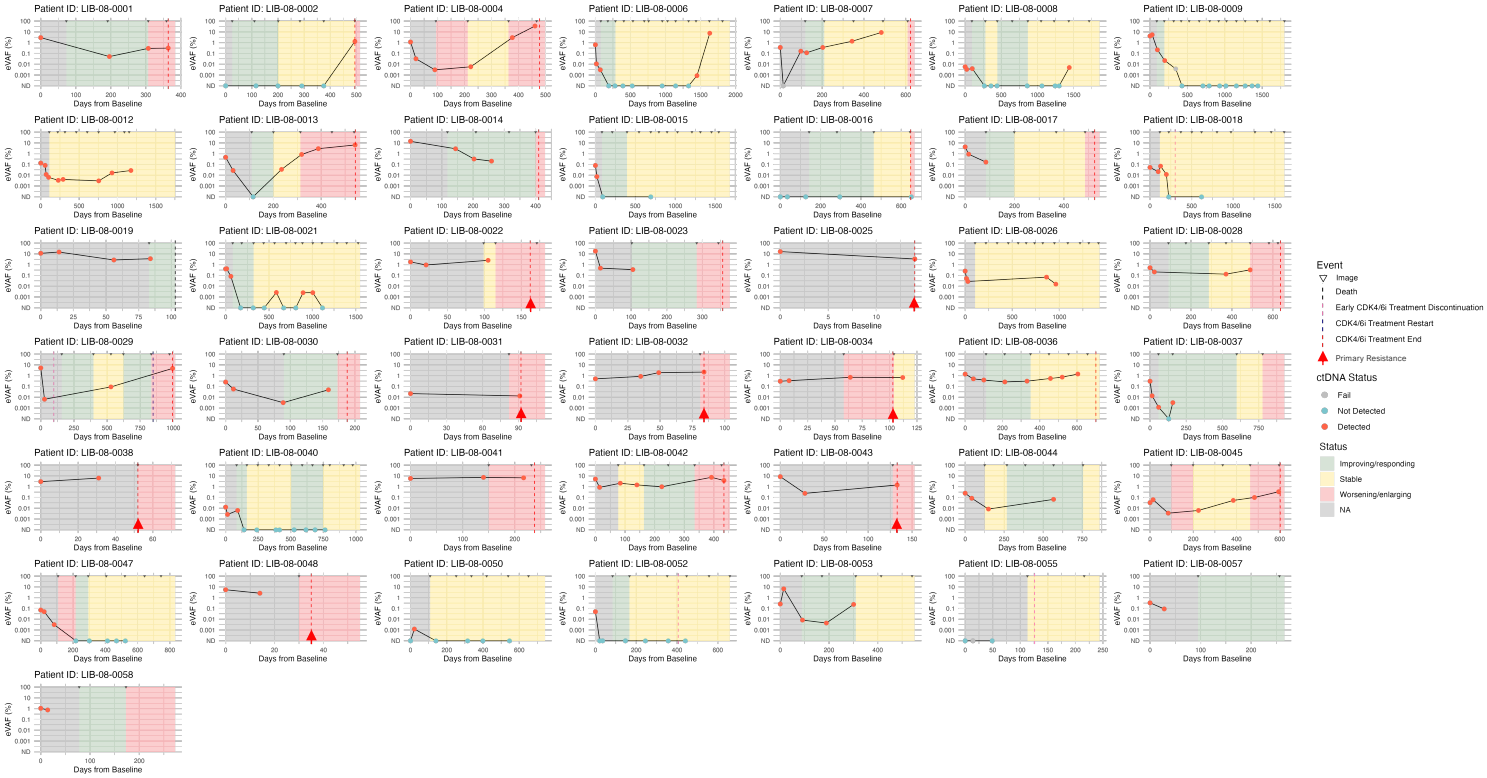


Supplementary Figure 3. **(a)** Individual plots of ctDNA kinetics until treatment failure or last follow-up. RECIST v1.1 response categories, time of radiological evaluations, ctDNA detection, and clinical outcomes are shown. Patients with primary resistance are indicated by a red arrow. **(b)** Individual plots of ctDNA kinetics until treatment failure or last follow-up with radiologist assessment shown. **(c)** Exploratory analysis of a patient with negative ctDNA samples using a refined calling pipeline to focus on 4 variants best supported by QC. **(d)** ctDNA kinetics during the first 90 days of treatment and RECIST v1.1 partial response. **(e)** Correlation of the changes in eVAF and RECIST v1.1 target lesion measurements at best response. **(f)** Case vignettes of patients with discordant changes in eVAF and RECIST v1.1 measurements at best response.

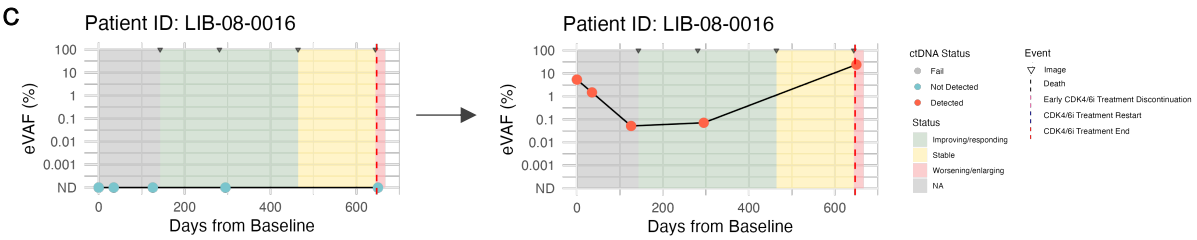
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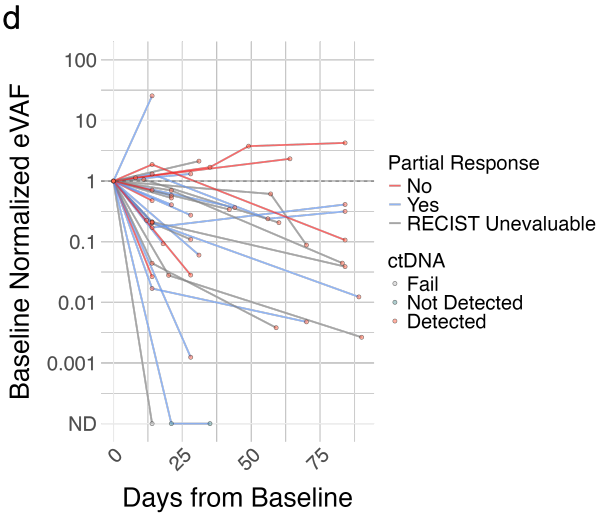


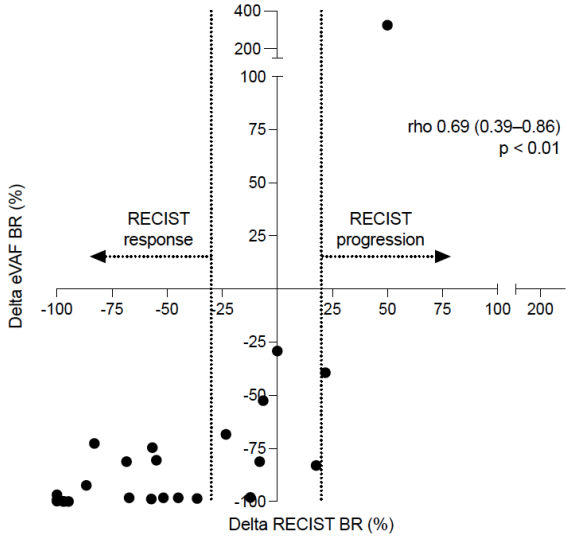
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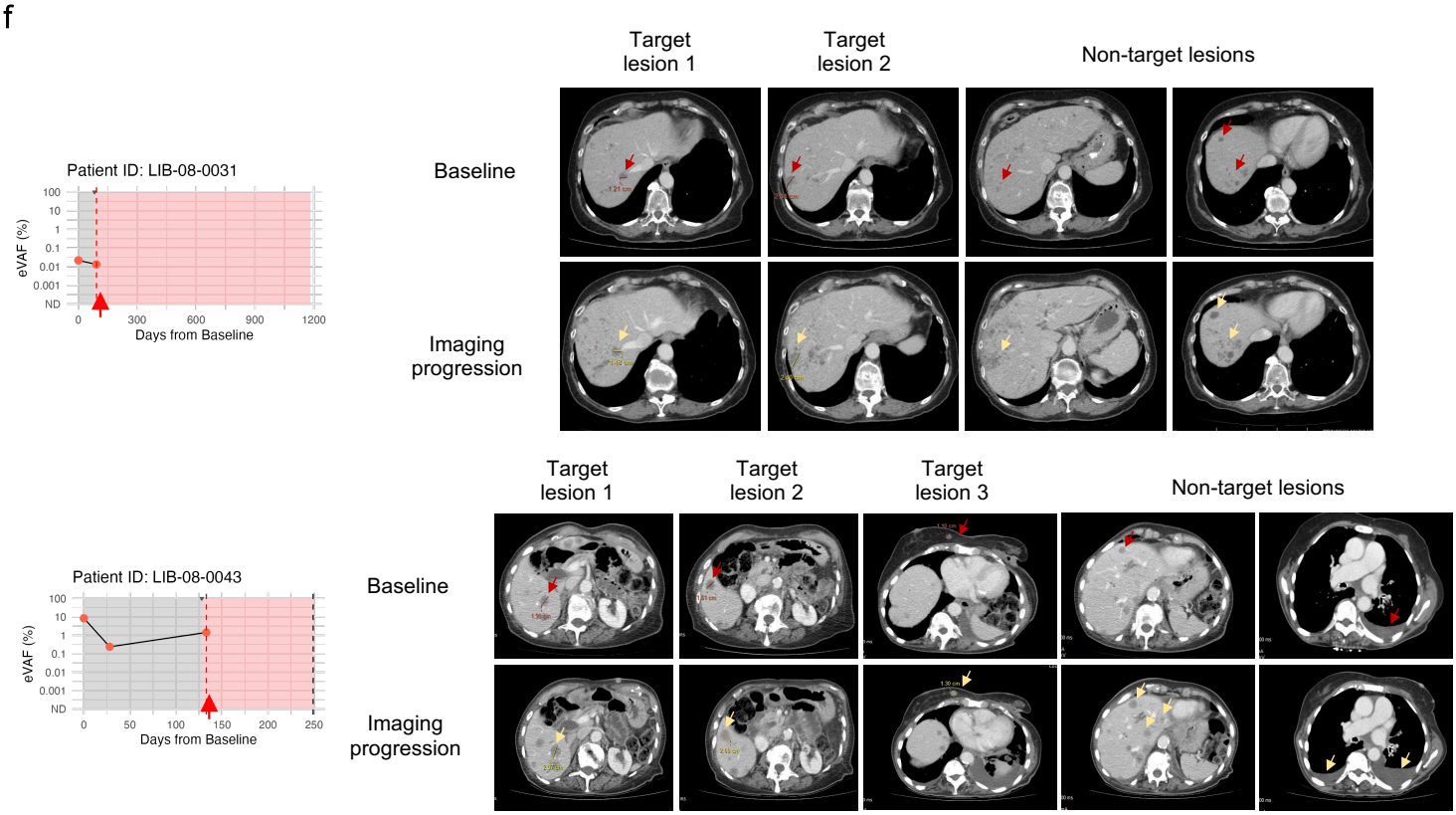


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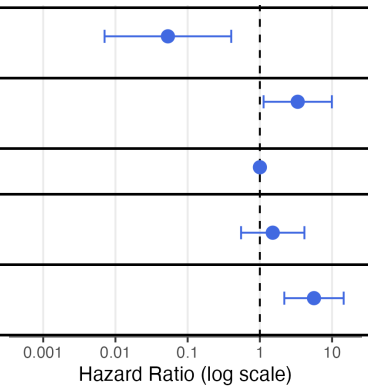


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a

Univariable models

Variable	n = 39	HR (95% CI)	p-value
Clearance*	Yes (n = 11) No (n = 28)	0.07 (0.01 – 0.52)	<0.001
Visceral Disease	Yes (n = 26) No (n = 13)	3.34 (1.13 – 9.93)	0.030
Age (continuous)	(n =39)	1.00 (0.97 – 1.04)	0.934
Line of therapy	1 (n = 31) >1 (n = 8)	1.51 (0.55 – 4.14)	0.425
ET Pair	SERD/SERM (n = 16) AI (n = 23)	5.62 (2.18 – 14.48)	<0.001

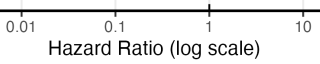


*Time-varying Cox model

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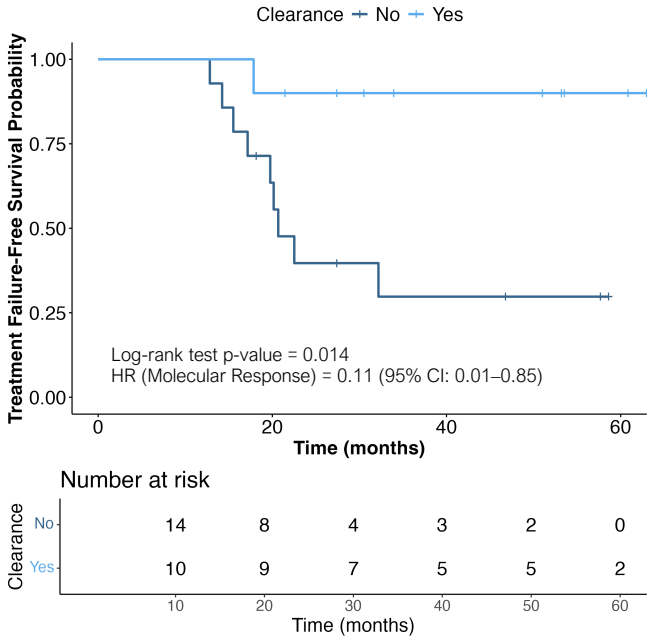
Univariable models

Variable	n = 39	HR (95% CI)	p-value
Clearance*	Yes (n = 11) No (n = 28)	0.07 (0.01 – 0.47)	<0.001
Visceral Disease	Yes (n = 26) No (n = 13)	2.34 (0.78 – 7.02)	0.129
Age (continuous)	(n =39)	0.99 (0.95 – 1.03)	0.648
Line of therapy	1 (n = 31) >1 (n = 8)	1.43 (0.52 – 3.95)	0.489
ET Pair	SERD/SERM (n = 16) AI (n = 23)	4.44 (1.76 – 11.24)	0.002

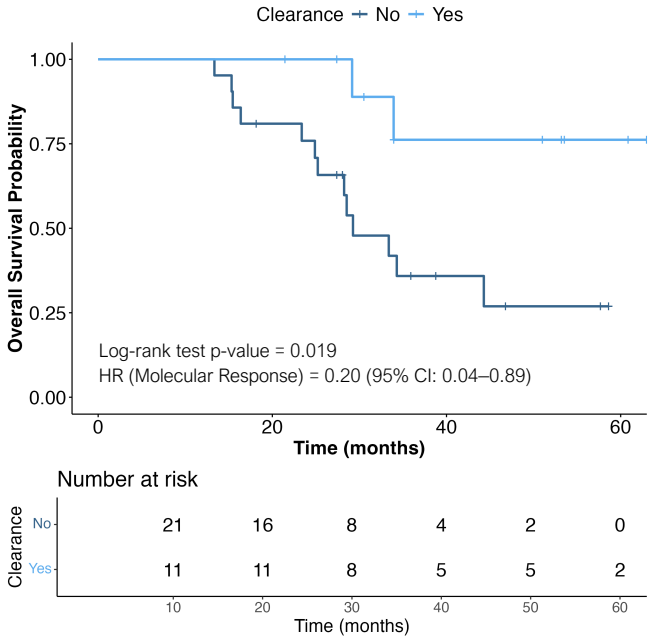


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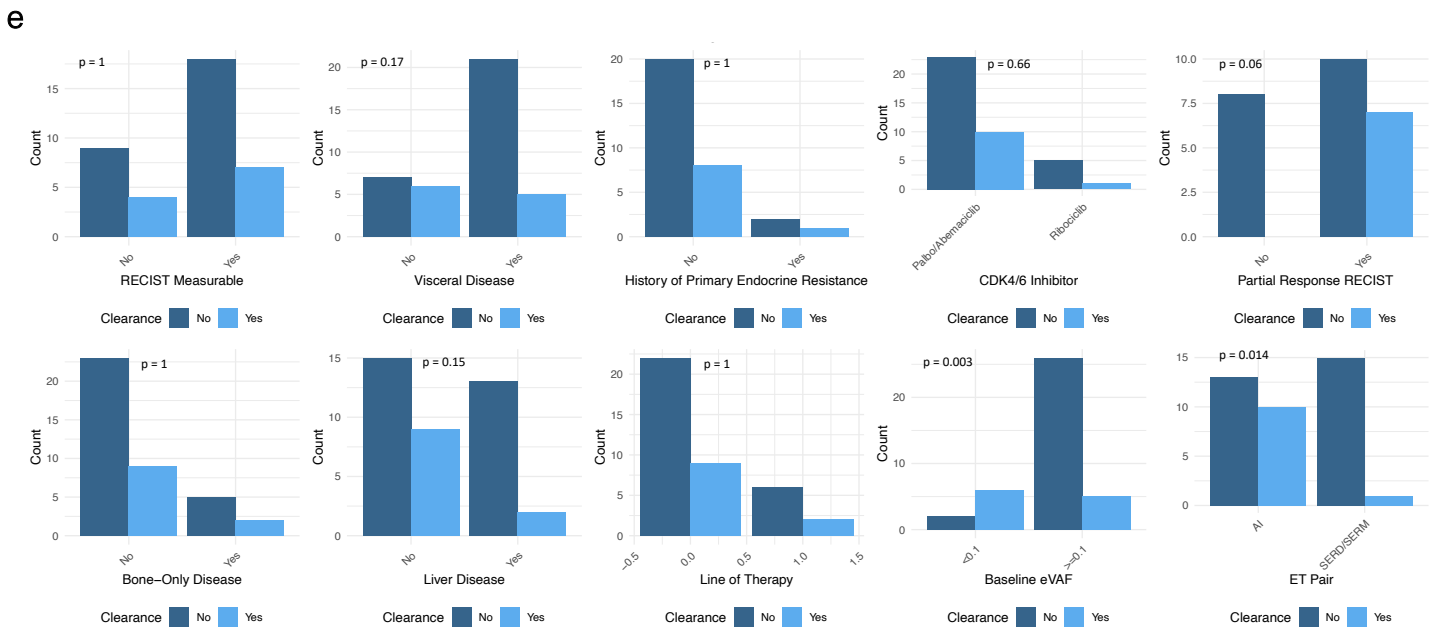
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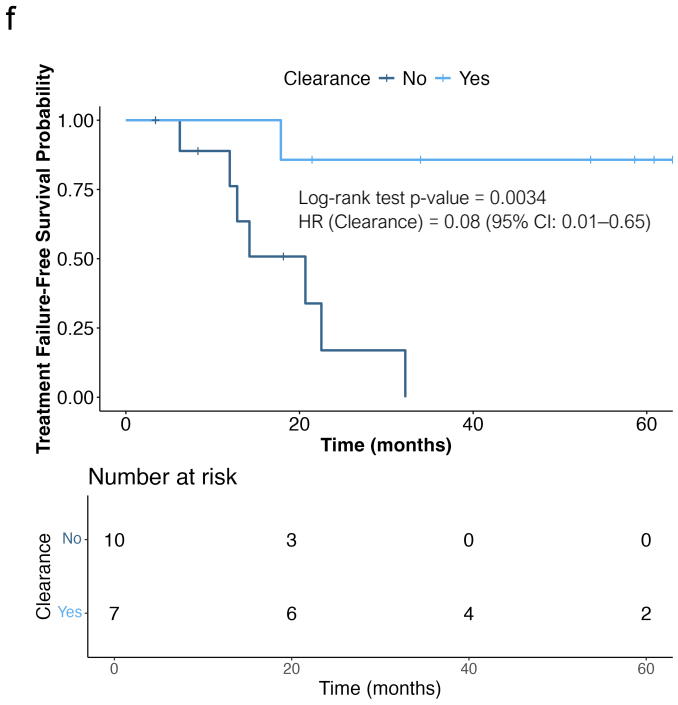


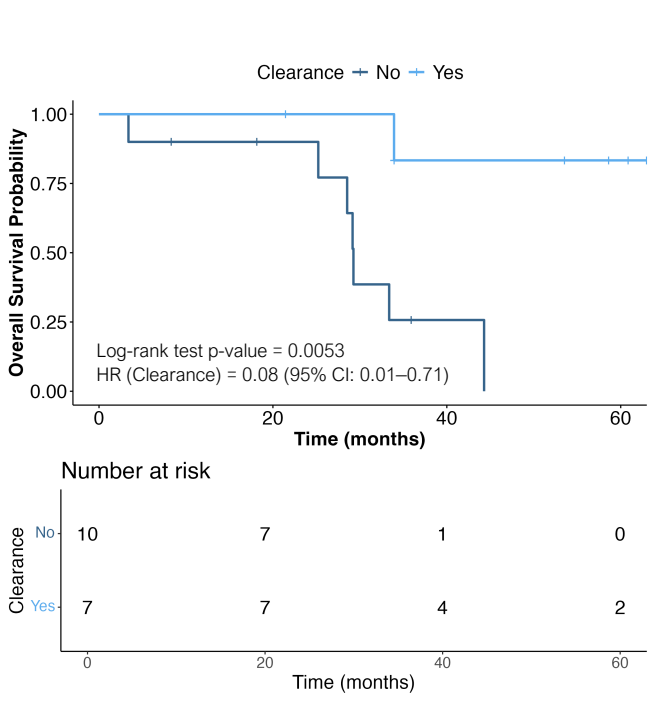
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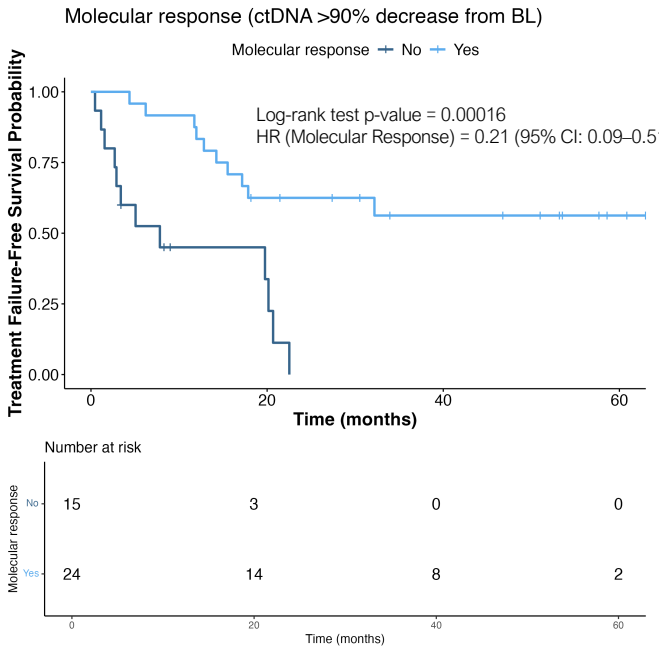




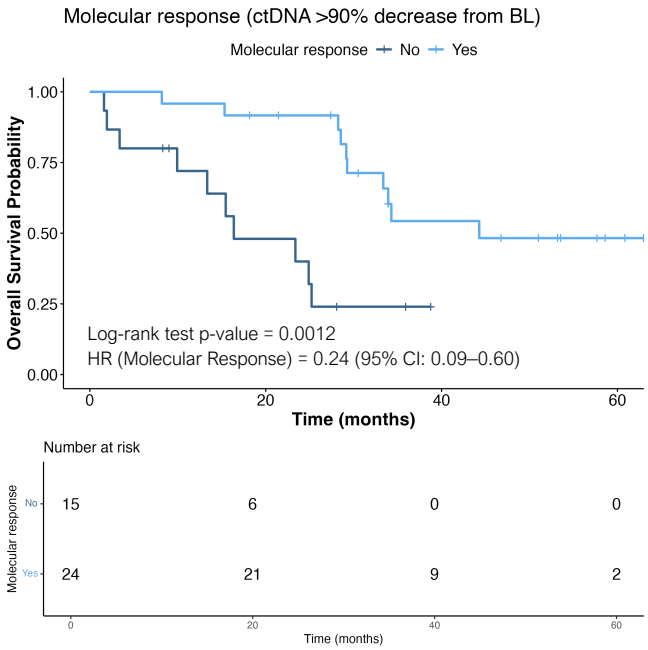


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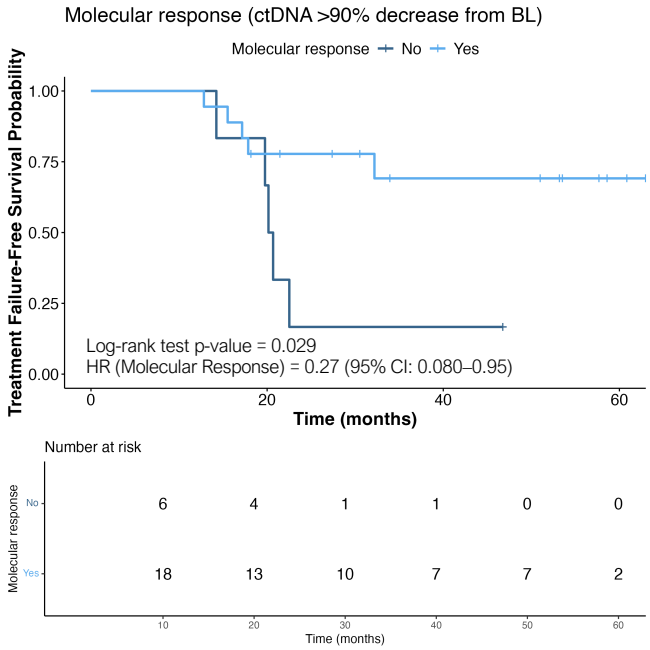
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