

Supplemental Information for “Overall Survival with Circulating Tumor DNA-Guided Therapy in Advanced Non-Small Cell Lung Cancer”

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MSK-ACCESS Pipeline

MSK-ACCESS is a hybrid-capture, duplex barcoded sequencing panel that allows for two bioinformatic methods of calling mutations: (1) de novo base calling, requiring a given mutation to be present in at least three separate duplex consensus reads for recurrently observed “hotspot” mutations and at least five separate duplex consensus reads for non-hotspot mutations, and (2) “genotyping,” in which a variant previously observed in a given patient (in either MSK-IMPACT or MSK-ACCESS samples with de novo base calling) is called as a mutation with a lower threshold: at least one duplex consensus read or two simplex (single-stranded) consensus reads (see Brannon et al. Nat Comm 2021 for details). In this study, a total of four actionable mutations resulting in therapy matching were identified in MSK-ACCESS ctDNA samples using genotyping alone (i.e. these would not have been discovered, were they not previously discovered in a given patient’s tissue sample): one *EGFR* L858R mutation, one *EGFR* exon 19 deletion, and two *KRAS* G12C mutations. Because this is the default base calling algorithm for MSK-ACCESS, and because it is possible for driver mutation profiles to change across time independently of base calling methods (i.e. for biological reasons), these patients were treated as being matched to targeted therapy by ctDNA for the purposes of analysis.

ctDx Lung vs. MSK-ACCESS

Among 163 patients with ctDx Lung and MSK-ACCESS tests within 30 days of each other, 54 mutations were present on ctDx Lung testing only, while 55 mutations were present on MSK-ACCESS only. Of the 54 unique ctDx Lung mutations, 30 (56%) were filtered based on WBC sequencing (**eFigure 3**). The 24 (44%) remaining mutations were subthreshold or not detected at any frequency in MSK-ACCESS. A total of 216 mutations were detected on both assays. The concordance correlation coefficient of all mutations together was 0.98; the Pearson’s R for these same mutations was also 0.98, $p < 0.001$. Of 230 patients with both ctDx Lung and MSK-ACCESS testing, 13 were matched to targeted therapy based on a ctDNA alteration not detected on ctDx Lung testing, while three patients were matched based on a ctDNA alteration not detected on MSK-ACCESS.

Table S1. Regions included in the ctDx Lung Assay

SNV/Indel	Fusions	CNV
AKT1	ALK	B2M
ALK	EGFR	EGFR
B2M	FGFR2	ERBB2 (HER2)
BRAF	FGFR3	FGFR1
EGFR	NTRK1	KRAS
ERBB2 (HER2)	RET	MET
FGFR2	ROS1	MYC
FGFR3		NTRK1
KEAP1		PIK3CA
KRAS		PTEN
MAP2K1 (MEK1)		RICTOR
MET		STK11
NRAS		TP53
PIK3CA		
RET		
ROS1		
STK11		
TP53		

Table S2. Resistance alterations detected in ctDNA

	Prior targeted treatment (N=201) ^a	Prior nontargeted treatment (N=198) ^a	Treatment naïve (N=728) ^a	Total (N=1127) ^a
EGFR AMP	18	7	55	80
KRAS G12C	4	15	55	74
EGFR L858R	24	4	42	70
MET AMP	15	6	24	45
KRAS G12D	0	4	35	39
TP53 SPLICE	9	3	25	37
TP53 HOMDEL	6	9	22	37
EGFR exon 19 indel	15	2	19	36
RICTOR AMP	4	7	20	31
ALK fusion	6	3	21	30
ERBB2 AMP	5	6	16	27
EGFR T790M	19	0	4	23
KRAS G12V	0	2	19	21
MYC AMP	4	1	13	18
TP53 R175H	2	4	12	18
PIK3CA AMP	0	3	14	17
TP53 R273H	1	2	11	14
RET fusion	1	3	8	12
ROS1 fusion	4	2	5	11
KRAS G12A	0	1	10	11
NTRK1 AMP	1	3	6	10
KRAS G12S	2	2	6	10
Other TP53	49	69	274	392
Other EGFR	23	5	38	66
Other KRAS	4	5	29	38
Other STK11	3	8	24	35
Other KEAP1	2	4	18	24
Other PIK3CA	6	3	14	23
Other BRAF	1	5	16	22
Other ERBB2	4	2	14	20
Other PTEN	1	2	11	14
Other MET	0	1	11	12
Other NRAS	0	0	9	9
Other FGFR1	0	2	6	8
Other ALK	2	2	3	7
Other ROS1	4	2	1	7
Other B2M	2	0	2	4
Other FGFR3	2	0	1	3
Other AKT1	0	0	2	2
Other RET	1	0	0	1

^a Alterations not mutually exclusive

Table S3. Survival Summary for Figure 2b

Subcohort	ctDNA present		ctDNA absent	
	N	Median OS ^a (95%CI)	N	Median OS (95%CI)
All	722	13 (11-16)	380	32 (25-39)
MSK	654	13 (11-16)	323	31 (24-35)
Sydney	68	16 (10-22)	57	39 (21-90)
1 ctDNA alteration	317	16 (13-20)		
2 ctDNA alterations	212	12 (8-17)		
≥3 ctDNA alterations	193	12 (8-15)		
max VAF < median	354	19 (16-22)		
max VAF ≥ median	358	10 (8-11)	380	32 (25-39)
Incalculable CTF	291	25 (20-28)		
CTF < median	130	13 (8-22)		
CTF ≥ median	88	16 (1-30)		
VUS only	18	16 (1-30)		
Treatment naive	504	13 (11-17)	207	35 (24-56)
Previous treatment	218	15 (11-18)	173	31 (20-39)
EGFR	115	24 (18-28)	39	64 (37-118)
KRAS	109	10 (7-13)	24	31 (19-inf)
TP53	208	18 (13-22)	47	64 (31-64)

^a Median overall survival in months

Circulating tumor fraction (CTF); Memorial Sloan Kettering Cancer Center (MSK); overall survival (OS); variant allele frequency (VAF); circulating tumor DNA (ctDNA); variants of unknown significance (VUS).

Table S4. Patient characteristics among patients with time-matched PET imaging

Characteristic	N=457
Age, median (IQR), years	68 (58-75)
Sex, N (% total)	
Men	187 (41%)
Women	270 (59%)
Race, N (% total)	
White	277 (61%)
Asian	87 (19%)
Black	22 (5%)
Other	7 (2%)
Unknown	64 (14%)
Histology, N (% total)	
Adenocarcinoma	395 (86%)
Squamous	29 (6%)
Other ^a	33 (7%)
Smoking History, N (% total)	
Current/former	233 (51%)
Never	224 (49%)
Treatment History, N (% total)	
Treatment naïve	310 (68%)
Prior treatment	147 (32%)

Table S5. First Targeted Therapies Administered After Study Entry

Medication	MSK	Sydney	Total
Osimertinib	146	19	165
Crizotinib	43	4	47
Alectinib	28	4	32
Erlotinib	11	19	30
Sotorasib	17	0	17
Afatinib	7	6	13
Selpercatinib	11	2	13
Cabozantinib	10	1	11
Gefitinib	2	7	9
Lorlatinib	6	3	9
Trastuzumab_deruxtecan	9	0	9
Trastuzumab_emtansine	9	0	9
Repotrectinib	7	0	7
Dabrafenib	5	0	5
Trametinib	5	0	5
Brigatinib	3	2	5
Tepotinib	4	0	4
MRTX849	4	0	4
AP32788	4	0	4
RO5126766	4	0	4
Entrectinib	3	0	3
Capmatinib	2	0	2
TNO155	1	0	1
TAK-788	0	1	1
Neratinib	1	0	1
Pyrotinib	1	0	1
Pozotinib	0	1	1
Ponatinib	1	0	1
Patritumab_deruxtecan	1	0	1
Dacomitinib	1	0	1
CLN-081	1	0	1
Ceritinib	0	1	1
Ulixertinib	1	0	1

Table S6. Targets identified on ctDNA leading to targeted therapy

Alteration ^a	OncoKB Level of Evidence	MSK (N)	Sydney (N)	Total (N)
ALK fusion	1	25	4	29
BRAF G466V	2	1	0	1
BRAF L597Q	2	1	0	1
BRAF V600E	1	8	0	8
EGFR exon 19 del	1	72	11	83
EGFR G719C	1	1	1	2
EGFR L858R	1	42	12	54
EGFR L861Q	1	2	1	3
EGFR S768I	1	2	0	2
EGFR T790M	1	14	5	19
ERBB2 exon 20 indel	2	10	0	10
ERBB2 S310F	2	1	0	1
KRAS G12C	1	15	0	15
KRAS G12D	2	2	0	2
KRAS G12V	2	1	0	1
MET amplification	2	8	0	8
MET exon 14 splice	1	16	0	16
RET fusion	1	8	3	11
ROS1 fusion	1	8	2	10

^a Alterations not mutually exclusive

Table S7. Subgroup odds of matching to targeted therapy by ctDNA

Variable	N ctDNA matched/total (%)	p-value (Fisher's exact test)
Current/former smoker	99/595 (17%)	<0.001
Never smoker	157/532 (30%)	
Male	94/480 (20%)	0.09
Female	162/647 (25%)	
Adenocarcinoma	242/969 (25%)	<0.001
Non-adenocarcinoma	14/158 (9%)	
Prior treatment	80/399 (20%)	>0.1
Treatment naïve	176/728 (24%)	
Prior targeted therapy	54/201 (27%)	>0.1
No prior targeted therapy	202/926 (22%)	
Extrapulmonary lesion	106/335 (32%)	0.002
No extrapulmonary lesion	16/122 (13%)	
Sydney	36/125 (29%)	>0.1
MSK	220/1002 (22%)	

Table S8. Survival Summary for Fig. 4b

Subcohort	ctDNA-only detected		Tissue-matched ctDNA ^a	
	N	Median OS ^b (95%CI)	N	Median OS ^b (95%CI)
ctDNA-only (all)	109	10 (6-14)		
CNA	62	9 (5-17)		
Undetectable	33	7 (4-17)		
Subthreshold ^c	30	13 (3-21)	193	22 (18-28)
Mutation	66	11 (7-17)		
Undetectable	33	12 (6-24)		
Subthreshold ^c	35	13 (7-17)		
No overlap	54	13 (2-27)		

^a ctDNA alterations also found in tissue (i.e. ctDNA detected but no ctDNA-only alterations)

^b Median overall survival (OS) in months

^c Not reported with clinical pipeline but found retrospectively at sub-reporting levels in BAM files

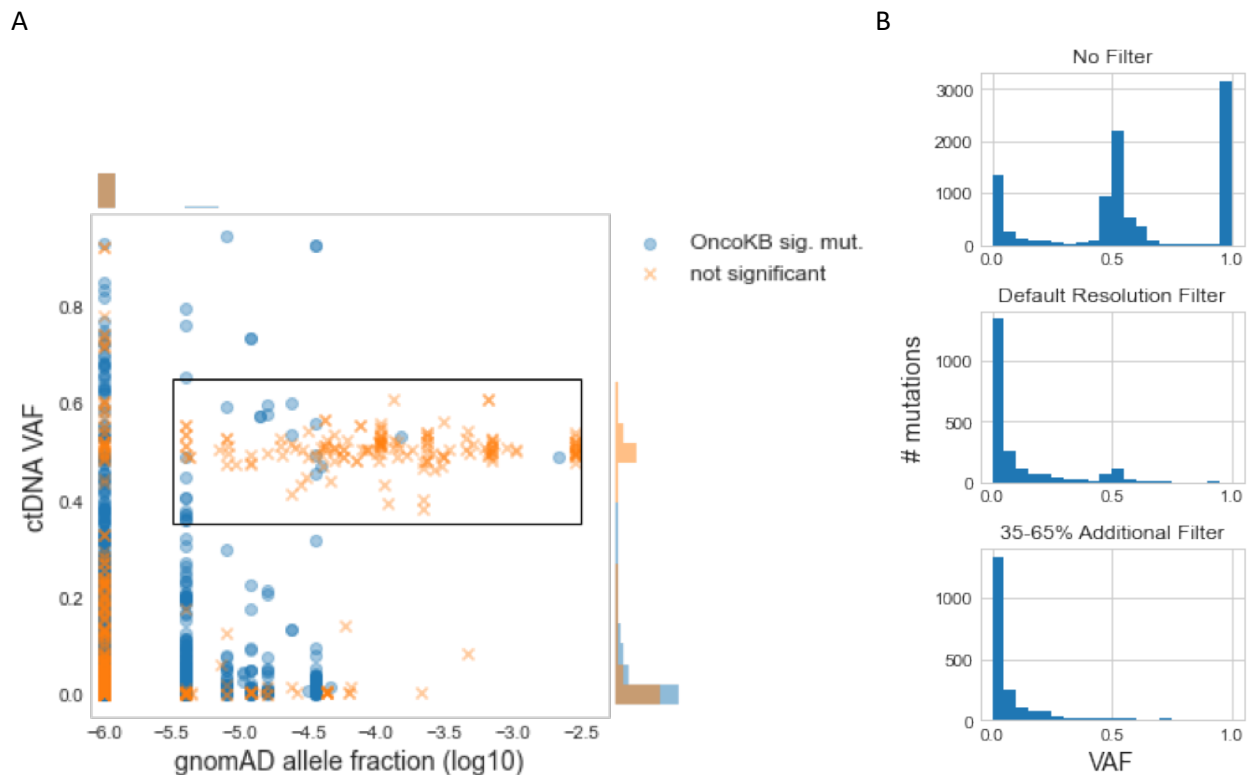


Fig. S1. Germline Filtering of Unmatched ctDNA.

A. Scatterplot of ctDNA variant allele frequency (VAF) vs. gnomAD allele fraction of all mutations in the study. Mutations not seen in gnomAD are set to the minimum value on the x-axis log scale. Histograms on the top and right show the relative densities of points on the respective axes. Mutations present in gnomAD with a VAF of 0.35-0.65 are highlighted by the box. B. Histogram of unfiltered VAFs (top), histogram of mutations with gnomAD allele frequency >0.5% excluded (middle), and histogram with non-functionally significant mutations with VAF 35-65% present in any allele frequency (x's in the box in A) excluded (bottom). Only mutations in the bottom panel were included in analysis.

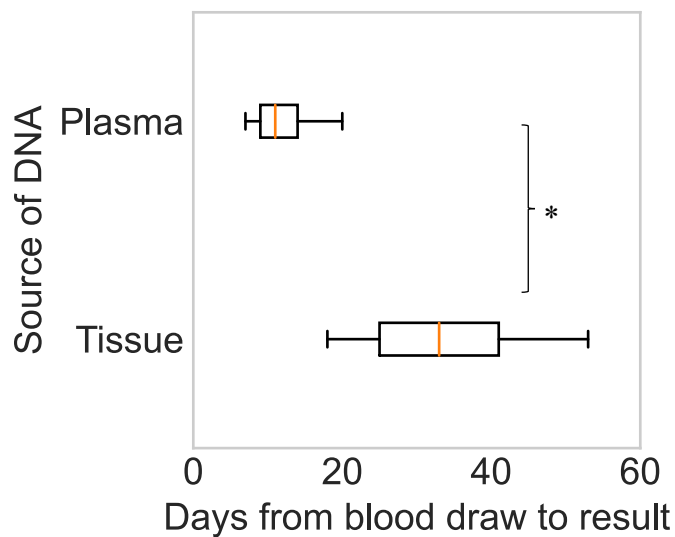


Fig. S2. Turnaround time with plasma ctDNA sequencing vs. tissue sequencing.

Boxplots showing median +/- IQR and 95%CI of turnaround time for liquid biopsy (MSK-ACCESS and ctDx Lung) and tissue (MSK-IMPACT) sequencing from date of blood collection. Tissue start time is the date of white blood cell control collection. The turnaround time for plasma ctDNA sequencing was significantly faster than for tissue sequencing (*Mann-Whitney U, $p < 0.001$).

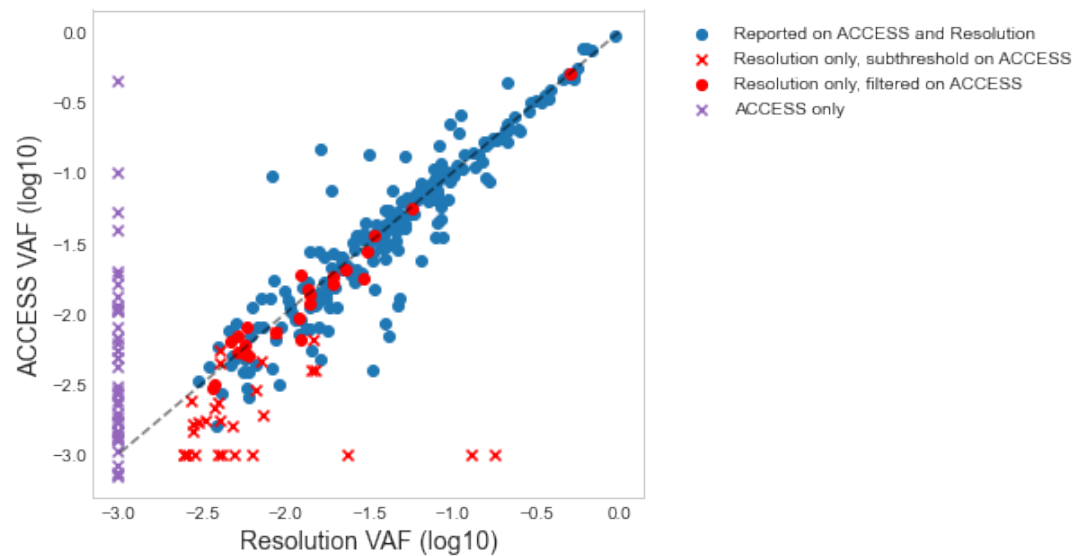


Fig. S3. Scatterplot of MSK-ACCESS vs. Resolution ctDNA assay variant allele fraction (VAF). Mutations that were subthreshold or filtered by white blood cell (WBC)-matched sequencing from MSK-ACCESS are shown in red. Values with VAF of zero are set to the minimum of their respective axes.

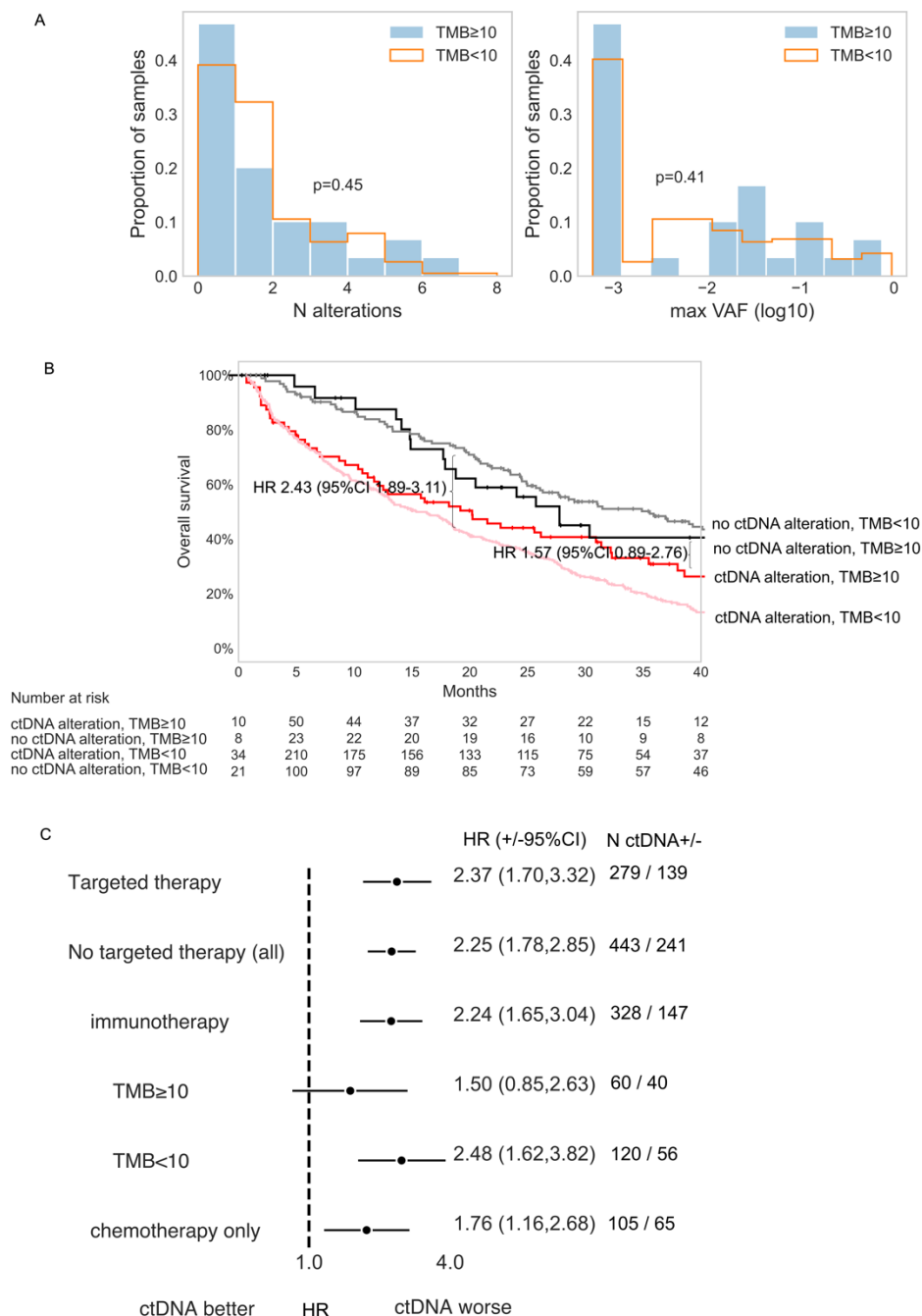


Fig. S4. ctDNA alterations and tumor mutational burden.

A. Histograms showing the number of alterations and maximum variant allele frequency (VAF) in patient samples sequenced with the ctDx Lung assay stratified by tumor mutational burden (TMB) greater or less than 10 mutations/megabase by MSK-IMPACT. P-values are from Mann-Whitney U tests. B. Kaplan-Meier curves showing the overall survival of patients in A and B stratified by whether ctDNA alterations were detected. Number at risk adjusted for study/variable entry time according to left truncation (see Methods). C. Forest plot showing the hazard ratios (HR) and 95% confidence intervals (CI) associated with ctDNA detection among patients treated with only the listed therapy classes. Targeted therapy ctDNA+ patients include all those treated with targeted therapy with any ctDNA alteration, not necessarily the matched alteration.

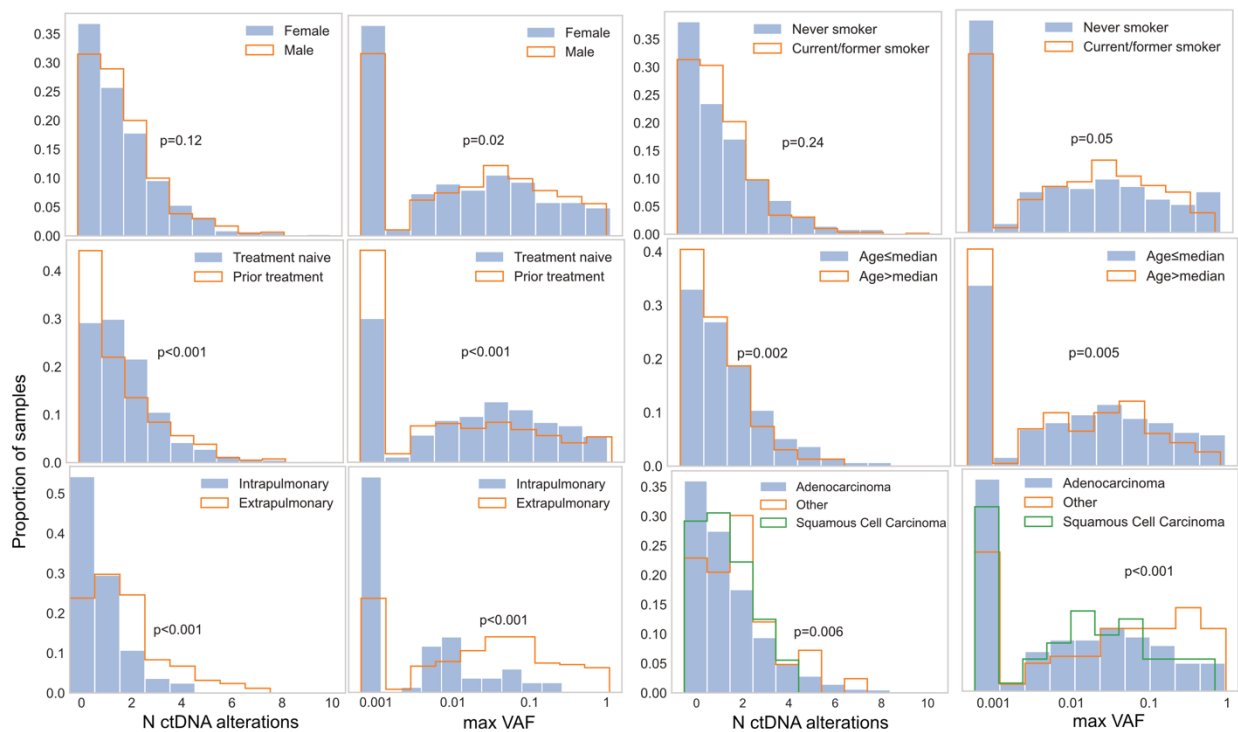


Fig. S5. Correlates of ctDNA alterations.

Histograms showing the proportion of patients with either number of ctDNA alterations or maximum mutation variant allele frequency (VAF) per sample. P-values are from Mann-Whitney U tests or Kruskal-Wallis tests for histologic subgroups. Raw VAFs of zero are set to the minimum value of the log axis.

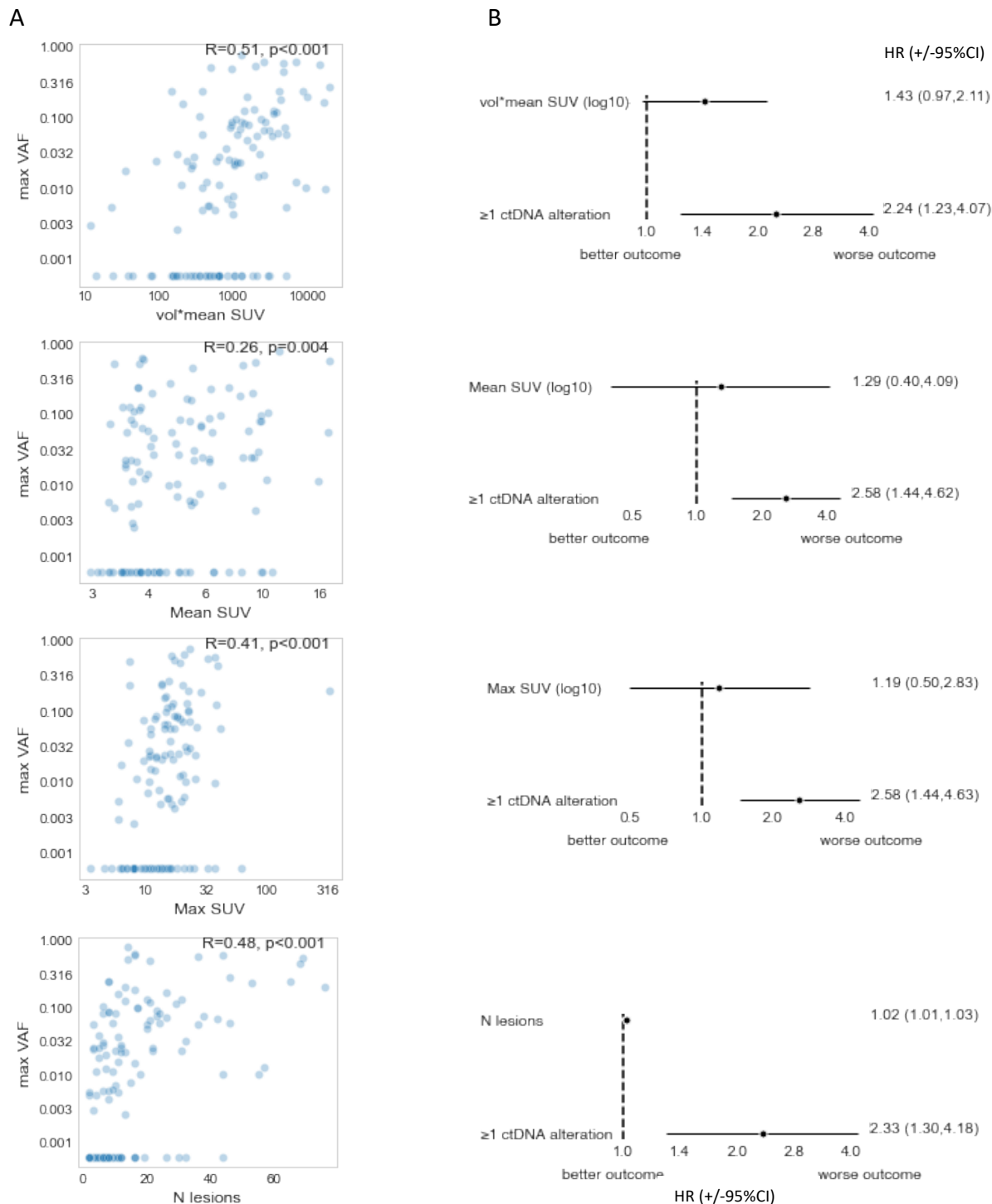
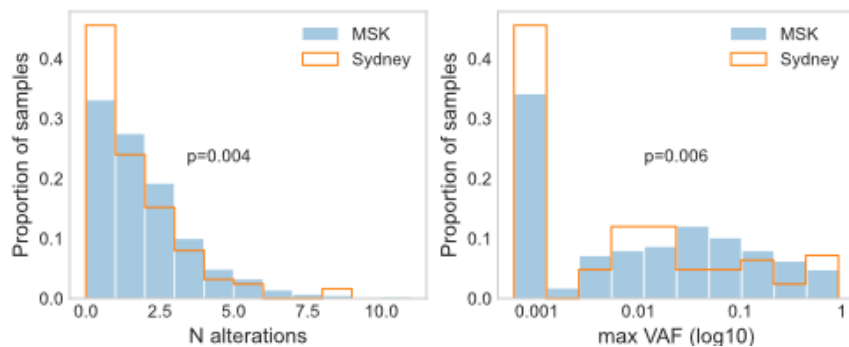


Fig. S6. PET associations of ctDNA mutations

Segmentation for radiomic analysis was performed for patients with treatment naïve adenocarcinoma with both PET scans and MSK-IMPACT within 30 days of ctDNA draw (N=128). A. Scatterplots showing relationships between radiomic parameters (x-axis) and maximum variant allele fraction (Max VAF) in ctDNA. Values of zero are set to the minimum of respective log axes. R and p from Spearman's correlation coefficient. B. Multivariate Cox proportional hazards models for the listed variables are shown. Radiomic variables are treated as continuous.

A



B

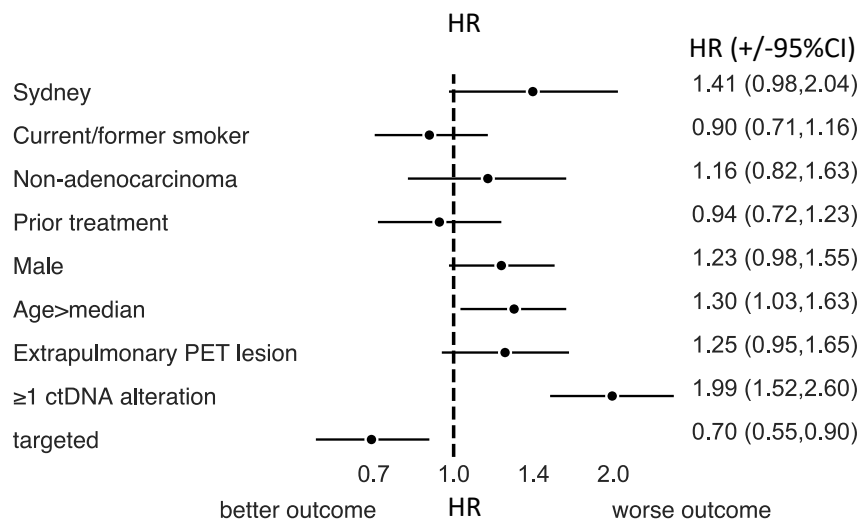


Fig. S7. Treatment site analyses.

A. Histograms showing the proportion of patients with either number of ctDNA alterations or maximum mutation variant allele frequency (VAF) per sample. P-values are from Mann-Whitney U tests or, in the case of histologic subgroups, Kruskal-Wallis. Raw VAFs of zero are set to the minimum value of the log axis. B. Multivariate Cox proportional hazards model including treatment site as an additional variable.

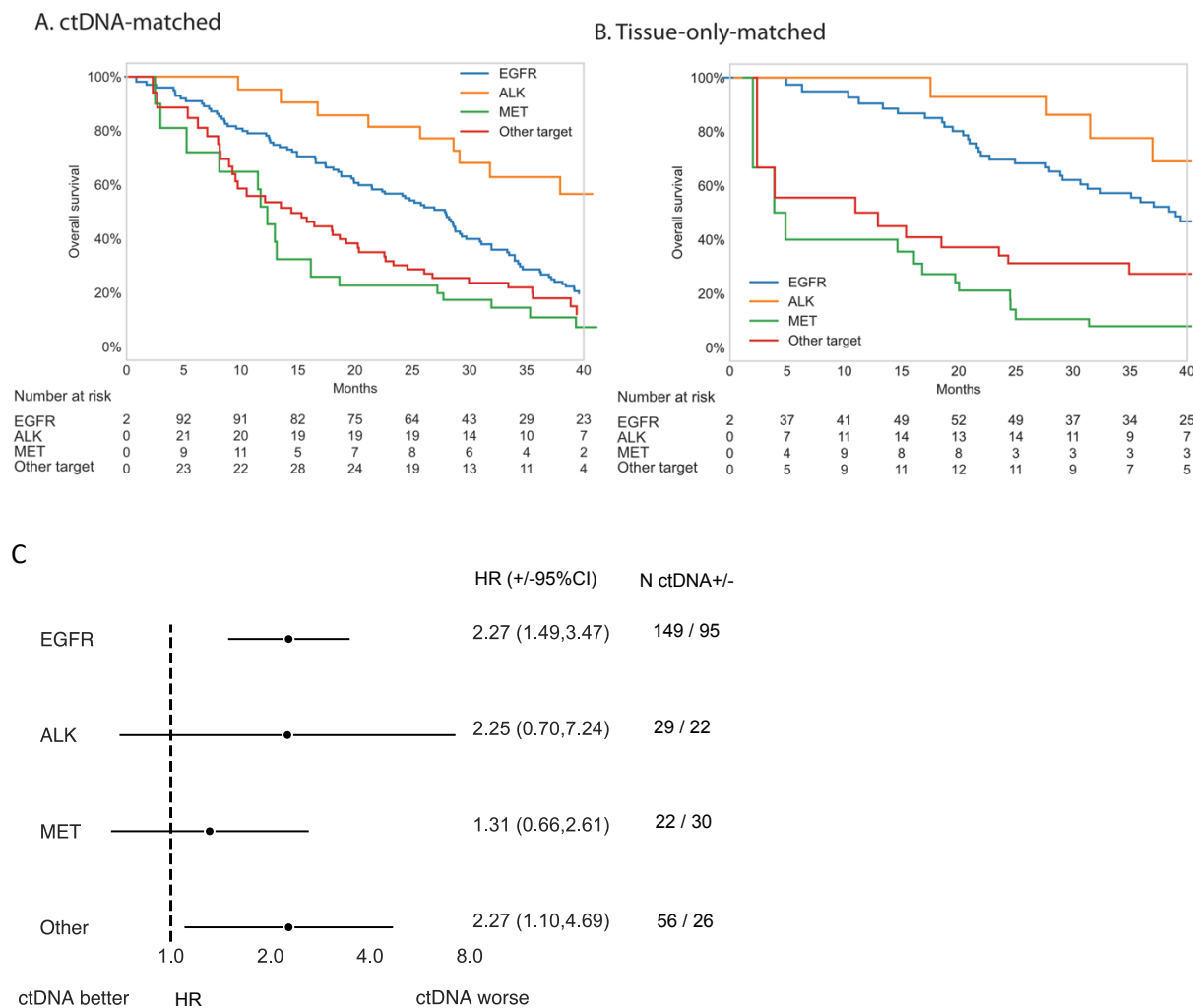


Fig. S8. Survival of Patients by Gene Target of Matched Therapies.

Kaplan-Meier survival curves for A. patients matched to targeted therapy by ctDNA, and B. patients matched to targeted therapy by tissue only vs. those not matched to targeted therapy. Number at risk in each category is adjusted for left truncation and time-dependent nature of targeted therapy variables. C. Forest plot showing hazard ratios and 95%CI comparing patients treated with ctDNA-matched therapy (“ctDNA”) to those treated with tissue-matched targeted therapy directed at the listed genes.

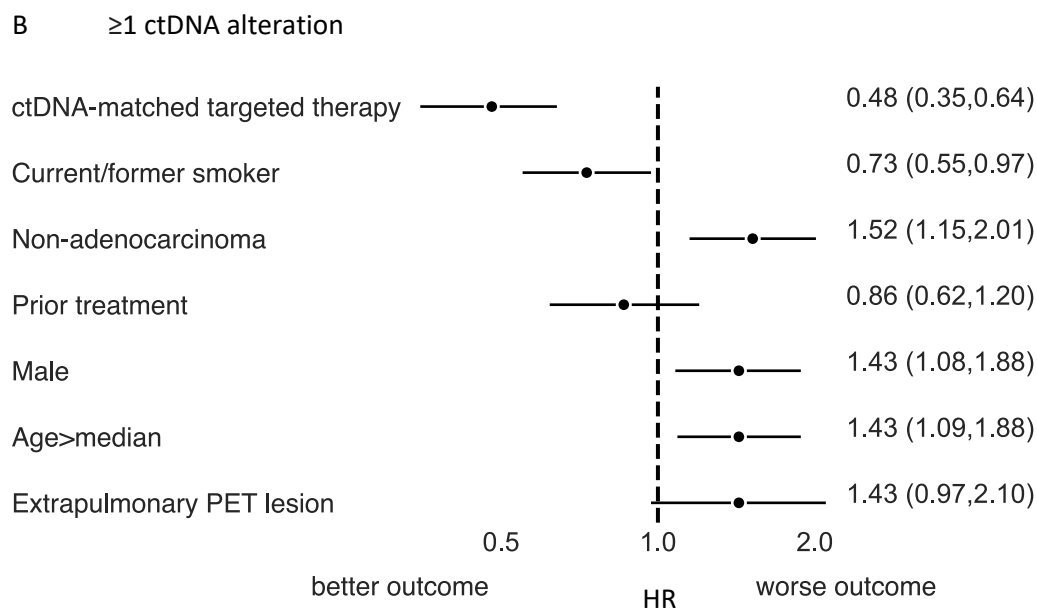
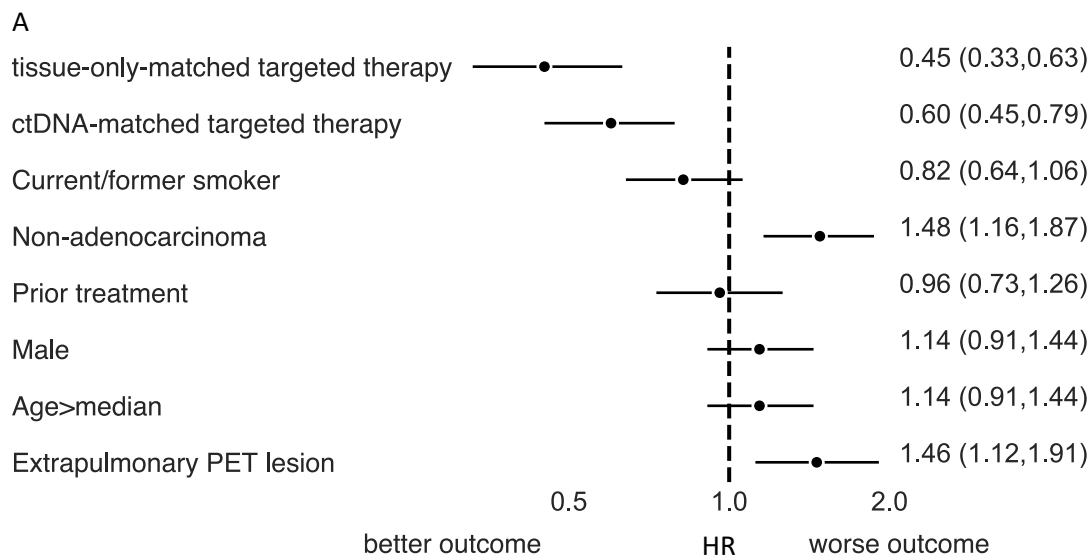


Fig. S9. Targeted therapy analysis corrected for multiple variables

Multivariate Cox Proportional Hazards models with the listed variables. A. ctDNA-matched targeted therapy appears to have a trend toward worse OS, although in a similar model restricted to only patients with at least one ctDNA alteration (B), the benefit from ctDNA-matched targeted therapy is comparable to that of tissue-only matched targeted therapy in the whole cohort.

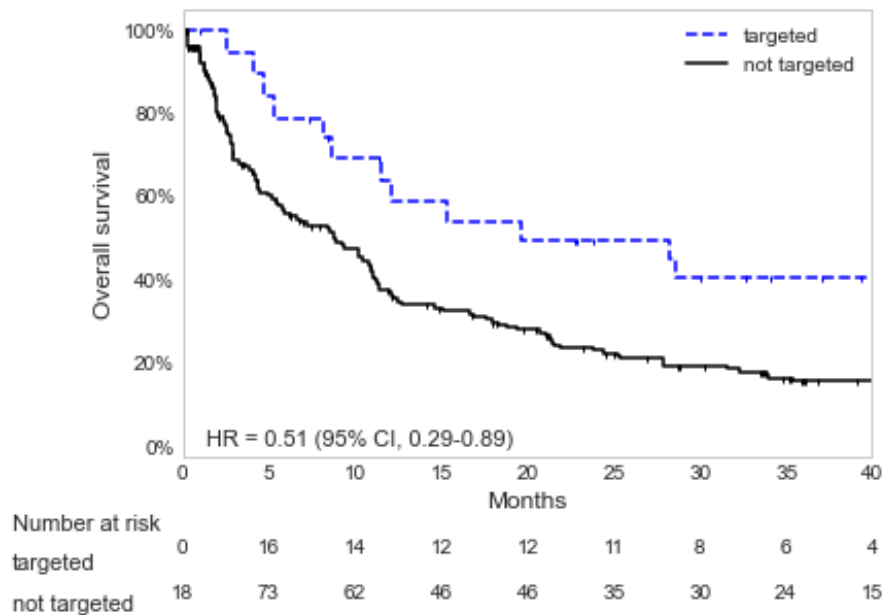


Fig. S10. Survival of patients without tissue sequencing matched to targeted therapy by ctDNA.

Kaplan-Meier survival curves for patients without tissue sequencing matched or not matched to targeted therapy. Number at risk in each category is adjusted for left truncation and time-dependent nature of targeted therapy variables.

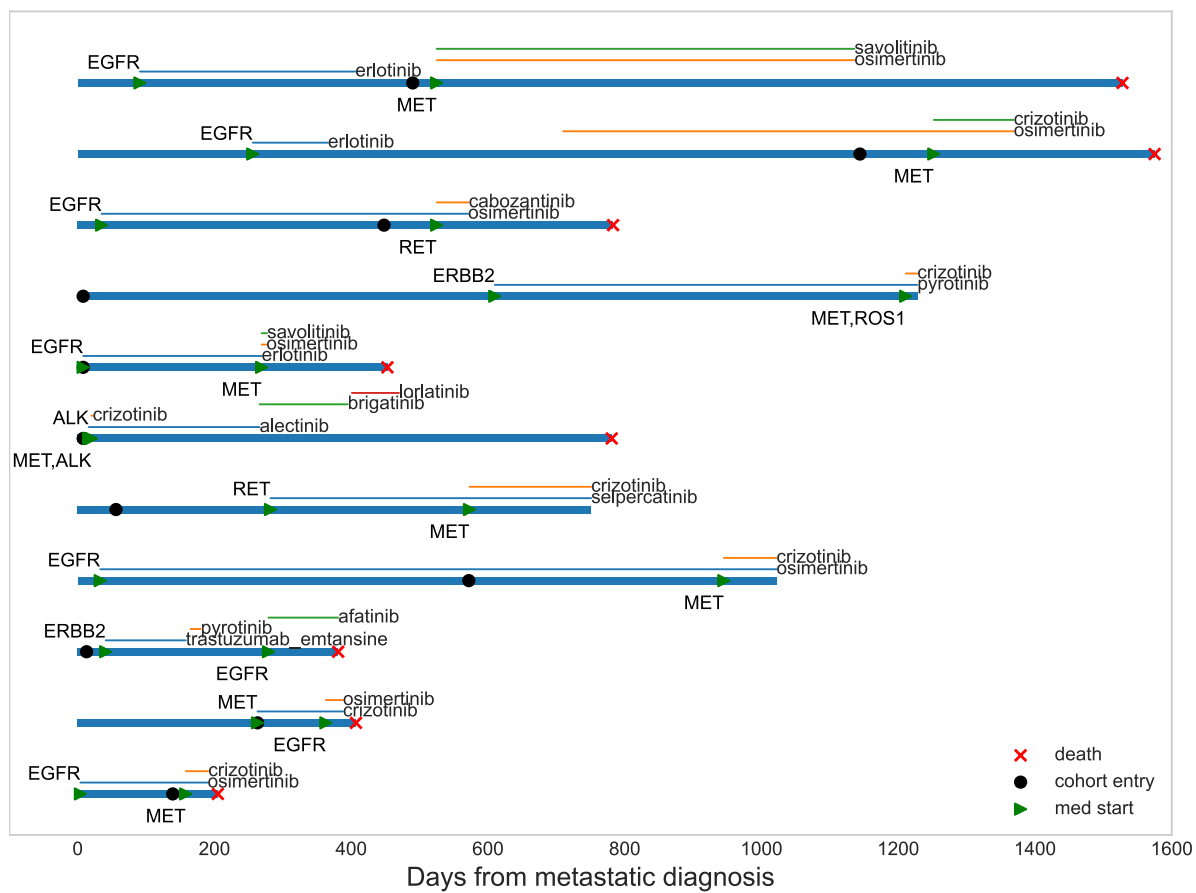


Fig. S11. Patients treated with multiple targeted therapies.

Swimmer plot of patients receiving targeted therapy to two distinct targets with at least one detected by ctDNA in the MSK cohort.

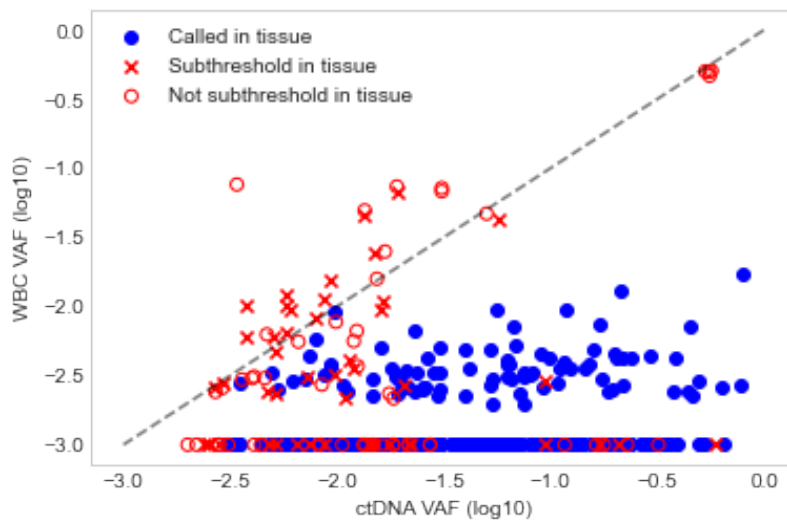


Fig. S12. Variant allele frequency (VAF) in MSK-IMPACT matched white blood cell (WBC) sequencing vs. circulating tumor (ct)DNA (plasma-only)

Dotted line corresponds to slope = 1. WBC mutations not detected are set to the minimum VAF for the purposes of graphing.

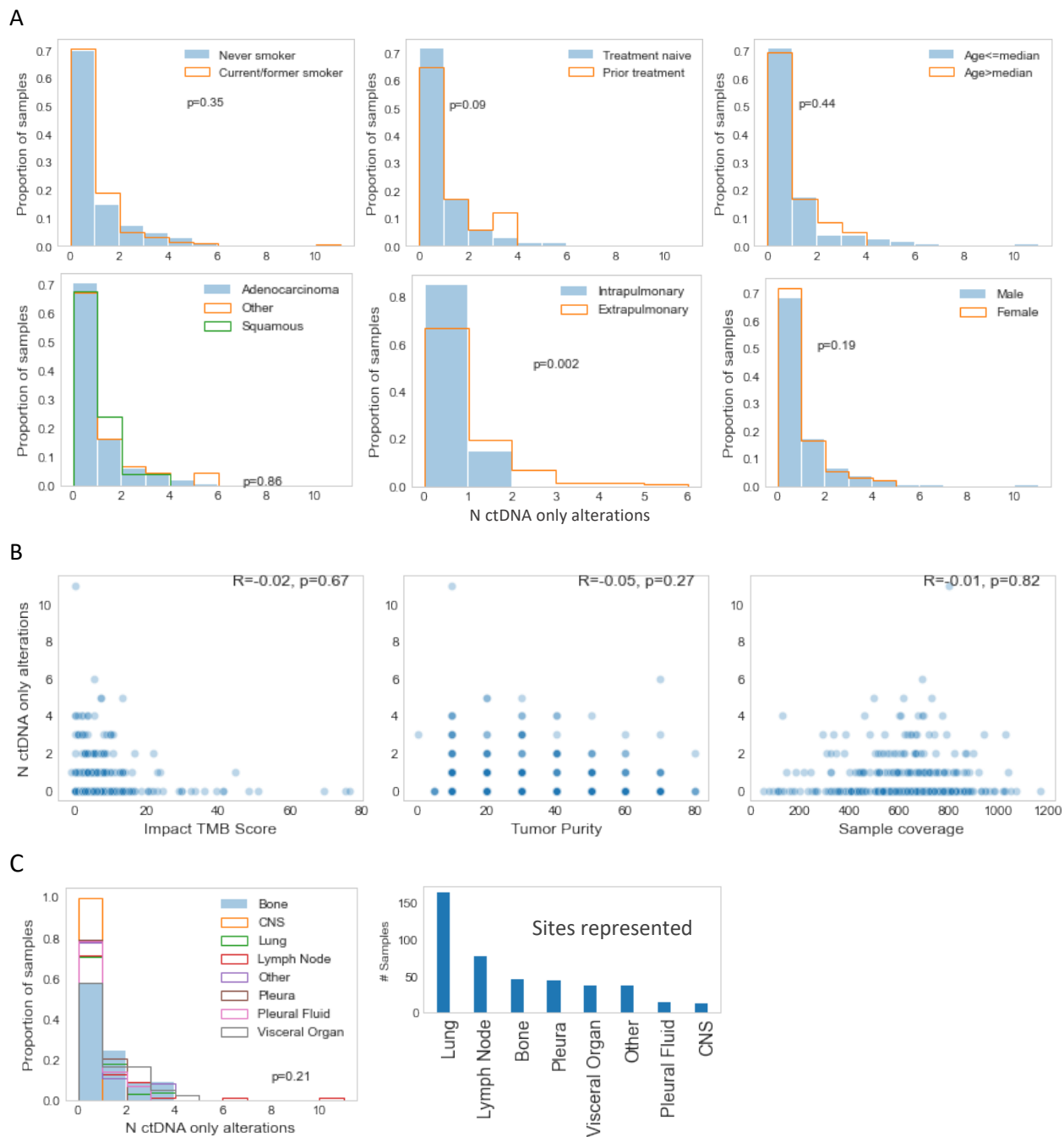


Fig. S13. Correlates of number of ctDNA-only alterations.

A. By clinical covariate. B. By properties of MSK-IMPACT sequencing. C. By site of MSK-IMPACT tissue sequencing. R values in scatterplots (B) are Spearman's correlation coefficients. P-values in histograms (A, C) are from Mann-Whitney U or Kruskal-Wallis tests as appropriate.

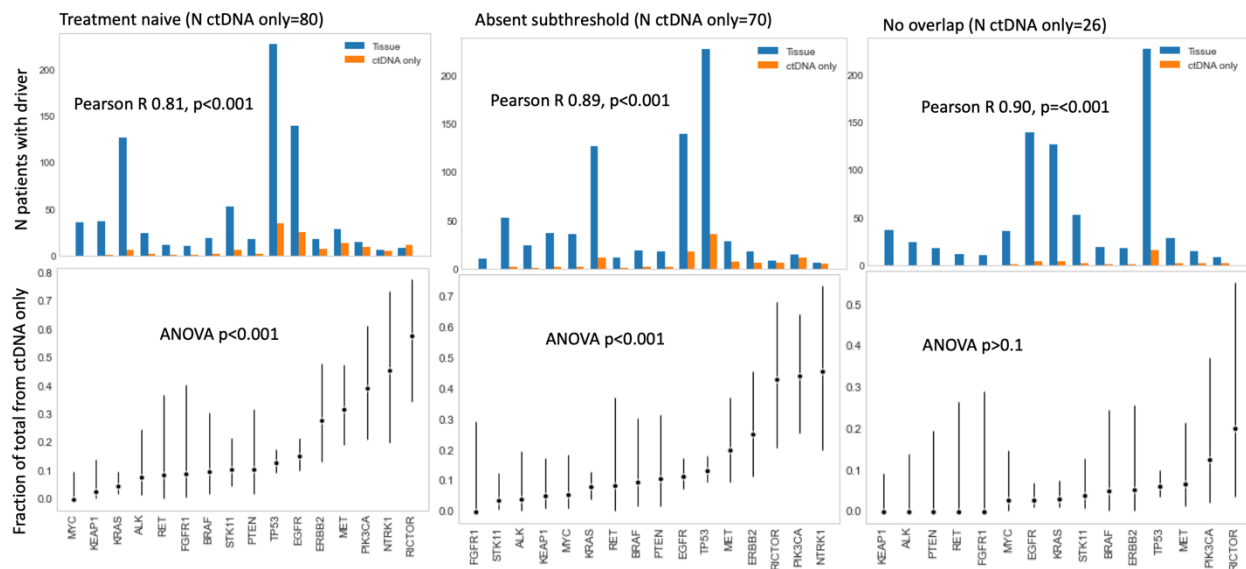


Fig. S14. Oncogenic driver representation in circulating tumor (ct)DNA-only vs. tissue

(top) Bars showing the number of patients with an oncogenic driver in a given gene as detected in either tissue (+/- ctDNA) or in ctDNA only (i.e. not in tissue). (bottom) The fraction of the total number of patients with an oncogenic driver in said gene detected only in ctDNA. Only genes with at least 10 patients possessing such a driver by either method are shown.

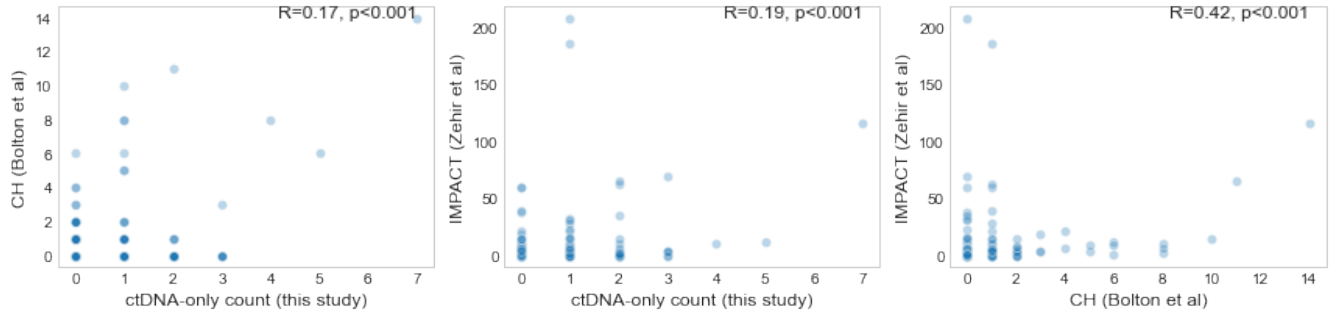
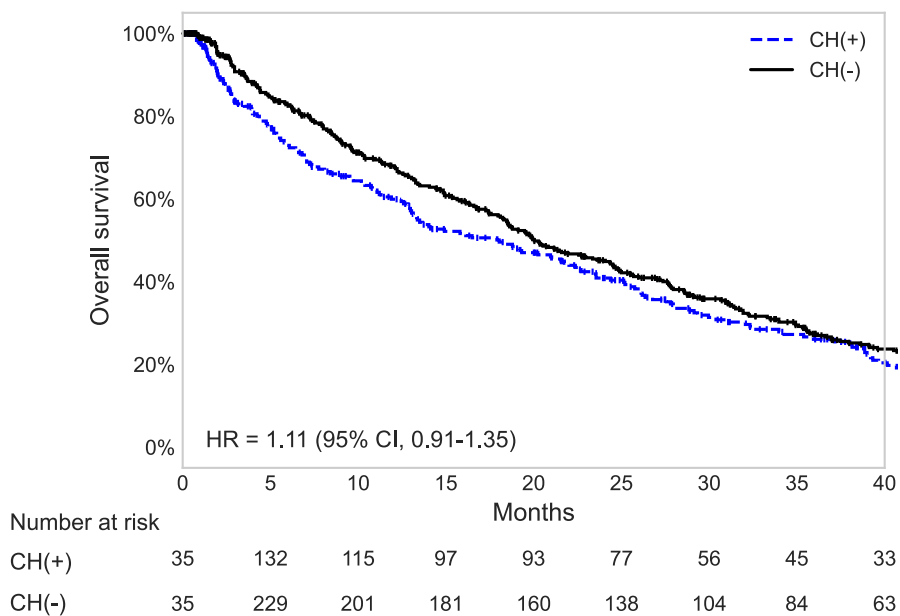


Fig. S15. Mutation prevalence in ctDNA vs. clonal hematopoietic (CH) or non-small cell lung cancer IMPACT databases.

Each point represents a specific mutation (ex: *EGFR* T790M). Axes show number of patients with a given specific mutation in the labeled dataset. R and p-values are from Spearman's correlation coefficient.

A



B

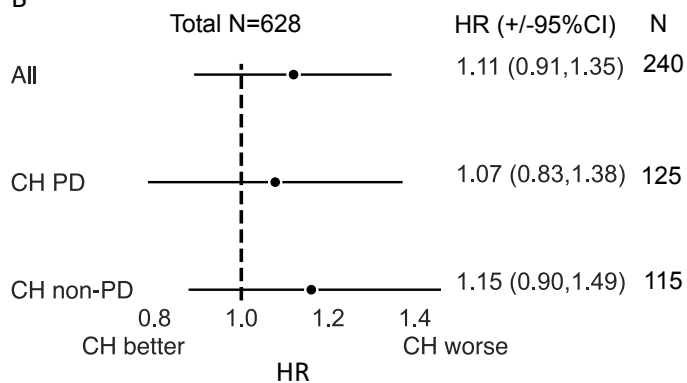


Fig. S16 Prognostic value of clonal hematopoiesis.

A. Kaplan-Meier curve for patients with vs. without clonal hematopoiesis (CH) from available CH-IMPACT calls. B. Forest plot showing hazard ratios and 95%CI for patients with CH vs. those without stratified by whether patients had putative driver (CH PD) mutations or not (CH non-PD). CH PD mutations are defined according to Bolton et al Nat Gen 2020.

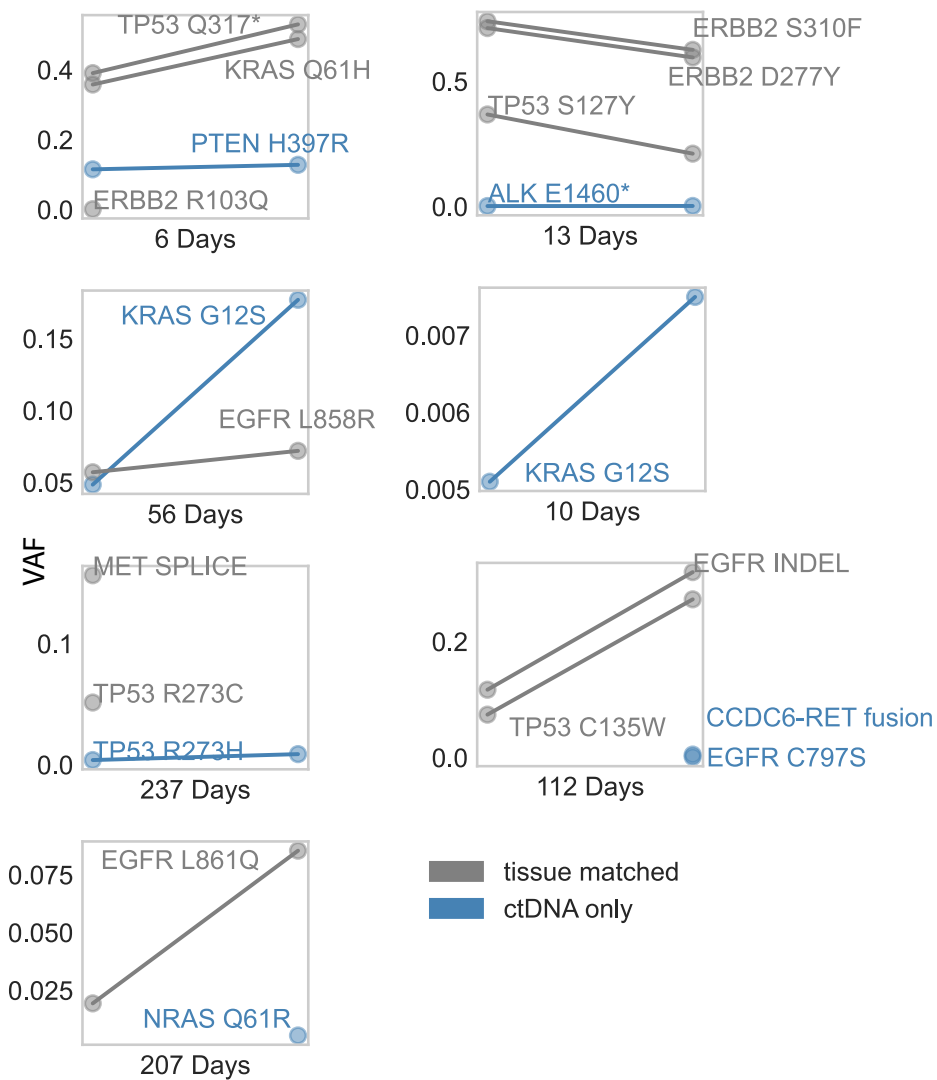


Fig. S17. Variant allele fractions (VAFs) of mutations in subsequent ctDNA draws in patients with mutations detected by ctDNA-only and multiple samples.

Time between draws shown on x axis.