Supplemental File For: Circulating tumor DNA sequencing of gastroesophageal adenocarcinoma.

Supplemental Legend:

Supplemental Table S1. Description of cohorts and sub-cohorts utilized in this study.
Supplemental Table S2. Clinical information detailing patients in the "Baseline-cohort" ie untreated stage IV patients from UC and SMC who underwent ctDNA-NGS testing (n=144).
Supplemental Table S3. Peri-operative ctDNA samples and their timing relative to surgery, DFS, maxVAF at the time of draw, and staging.
Supplemental Table S4. Alterations detected in ctDNA in post-operative patients within 180 days of surgery.
Supplemental Table S5. Frequencies of **A)** genomic alterations, **B)** mutations, or **C)** amplifications in initial tests across the entire Global GEA (n=1627) and UC/SMC subsets as seen in Figure 3. **D)** GA frequencies in genes common to ctDNA-NGS, MSK-Impact, and TCGA reported as percentage altered.
Supplemental Table S6. Frequencies of GAs across the "Global-cohort" and UC/SMC subsets when narrowing patients from figure 3A to **A)** only initial tests with detectable non-synonymous alterations (n=1329) or **B**) only initial tests with maxVAF>0.5% (n=936), as seen in figure S3A-B. Similarly, initial tests from the "Global-cohort" were compared with MSK-Impact and TCGA using **C)** only ctDNA-NGS cases with non-synonymous alterations and **D)** ctDNA-NGS cases with maxVAF>0.5%.
Supplemental Table S7. Detailed molecular information regarding clinically *HER2*-positive patient cohort (n=58).
Supplemental Table S8. Alterations present upon progression of disease after 1L HER2-targeted therapy in patients with persistent *HER2* amplification.
Supplemental Table S9. Prognostic significance of alterations, mutations, and amplifications in potentially actionable genes.

Supplemental Figure S1. Relationship between tumor burden, number of alterations, and tumor mutation burden.
A) Amongst untreated stage IV GEA patients, the number of alterations detected (including synonymous) was directly related to the maxVAF (i.e. quantity of ctDNA). **B)** Calculated ctDNA tumor mutation burden correlated with the overall number of alterations detected and **C)** number of mutations detected (by excluding copy number alterations), as expected. **D)** When comparing TMB estimation by ctDNA-NGS with tissue-NGS, no clear correlation is evident. **E)** TMB correction by ctDNA-NGS and **F)** TMB correction by tisssue-NGS One were both independent of maxVAF, unlike the number of alterations detected.

Supplemental Figure S2. Number of detected genomic alterations by stage.
Distribution of genomic alterations (excluding synonymous mutations) per initial test by stage (black = mean, red = median) demonstrating that patients with stage IV disease have a trend towards higher number of alterations. Density represents the fraction of patients with a given number of GAs **A)** Stage I (n=9, mean=1.3, median 1) **B)** Stage II (n=22, mean=3.2, median 3) **C)** Stage III (n=59, mean=2.2, median 2) **D)** Stage IV (n=279, mean=4.2, median 3).

Supplemental Figure S3. Relative frequency of common (>5%) non-synonymous alterations between various ctDNA-NGS and tissue-NGS cohorts amongst patients with detectable alterations.
Analyses from Figure 3A and 3D were reproduced using the subsets of patients only with ctDNA alterations present and/or having a maxVAF of at least 0.5%; genes with alteration frequencies >5% are shown. A) Comparing incidences between the global versus UC and SMC cohort patients highlights that alteration frequencies equalized between UC and SMC when removing low-shedding ctDNA patients from the analysis. B) Similar results were seen when further filtered to patients with a maxVAF >0.5%. C) When comparing ctDNA-NGS with MSK-Impact and TCGA after filtering out non-altered ctDNA cases, alterations in EGFR and MET were still notably more common in ctDNA D) Differences seen in *TP53* mutation rate are no longer significant one patients are limited to those with maxVAF >0.5% - likely due to elimination of low-shedders, whereas lower *HER2* amplification in ctDNA remains significant, which presumably results from amplification loss after HER2-targeted therapy.

Supplemental Figure S4. **Distribution of oncogenic copy number with and without "adjustment" for maxVAF.**
A) The density distribution of amplifications in clinically relevant genes from 554 tests with insert image showing the lower density range, demonstrating predominantly low-level amplifications in *EGFR* and *MET*. B) Adjusted copy was obtained by normalizing copy number versus maxVAF, which further highlighted the predominantly low-level amplification of *MET* versus more pronounced tails for *FGFR2* and *HER2*.

Supplemental Figure S5.
Serial NGS of ctDNA and tissue highlight spatio-temporal heterogeneity in patients with RTK alterations treated with matched targeted therapies. A) Patient 1: Image indicating the presence or absence of *EGFR* and *FGFR2* amplifications over treatment course, analyzed by NGS in various tissue compartments (primary, colon, ascites, ctDNA) as well as FISH confirmatory analyses. *EGFR* amplification was present prior to treatment in the primary tumor and ctDNA, then eradicated in all compartments upon EGFR-targeting. **B) Patient 1:** Plot of *FGFR2* (red) and *EGFR* (green) amplifications over time, analyzed by NGS copy number and FISH ratios (*EGFR/CEN7*; *FGFR2/CEP10*). *FGFR2* amplification increased rapidly when the patient was on *EGFR*i + FOLFOX and FOLFIRI, but decreased to zero once *FGFR2i* was added. **C) Patient 2:** Image indicating the presence or absence of *HER2* amplification throughout time, analyzed by FISH, NGS, and Mass Spectrometry (MS) in various compartments (primary, liver, ctDNA). **D) Patient 2:** Plot of *HER2* amplification by FISH (green) and NGS (red) over time. Copy number is highly amplified at baseline, but decreases in both primary tissue and ctDNA when the patient is on *HER2*i. When the patient is taken off *HER2*i, its amplification level resurges. **E) Patient 3:** Image indicating the presence or absence of *MET* and *KRAS* amplifications throughout time, analyzed by NGS and MS in various compartments (primary, adrenal, ctDNA). **F) Patient 3:** Plot of *MET/KRAS* amplifications and *PIK3CA* mutation in the blood over time. While on MET inhibition and clearance of *MET* amplification in the blood, there is expansion of *KRAS* amplification and acquisition of a new *PIK3CA* mutation. **G) Patient 4:** Image indicating the presence or absence of PD-L1, *MYC/FGFR2/FGFR1* amplifications mutation in various compartments (primary, lymph node, liver, ctDNA) throughout time. **H) Patient 4:** Plot of *MYC* (red), *FGFR2* (green), and *FGFR1* (blue) amplifications over time using copy number. *FGFR2* amplification peaks at recurrence, at which time the patient is put on an *FGFR2* inhibitor, and *FGFR2* amplification subsequently decreased. The expansion in *MYC and FGFR1* amplification was observed at progression. **I) Patient 5:** Image indicating the presence or absence of *FGFR2* and *HER2* amplifications throughout time, analyzed by NGS and FISH in various compartments (primary, lymph node, ctDNA). **J) Patient 5:** Plot of *HER2/FGFR2* ctDNA amplifications and *ARID1A*/PIK3CA mutations in the blood over time. With resurgence of *FGFR2,* there is expansion of *ARID1A* mutation and acquisition of a new *PIK3CA* mutation.

Figure S1

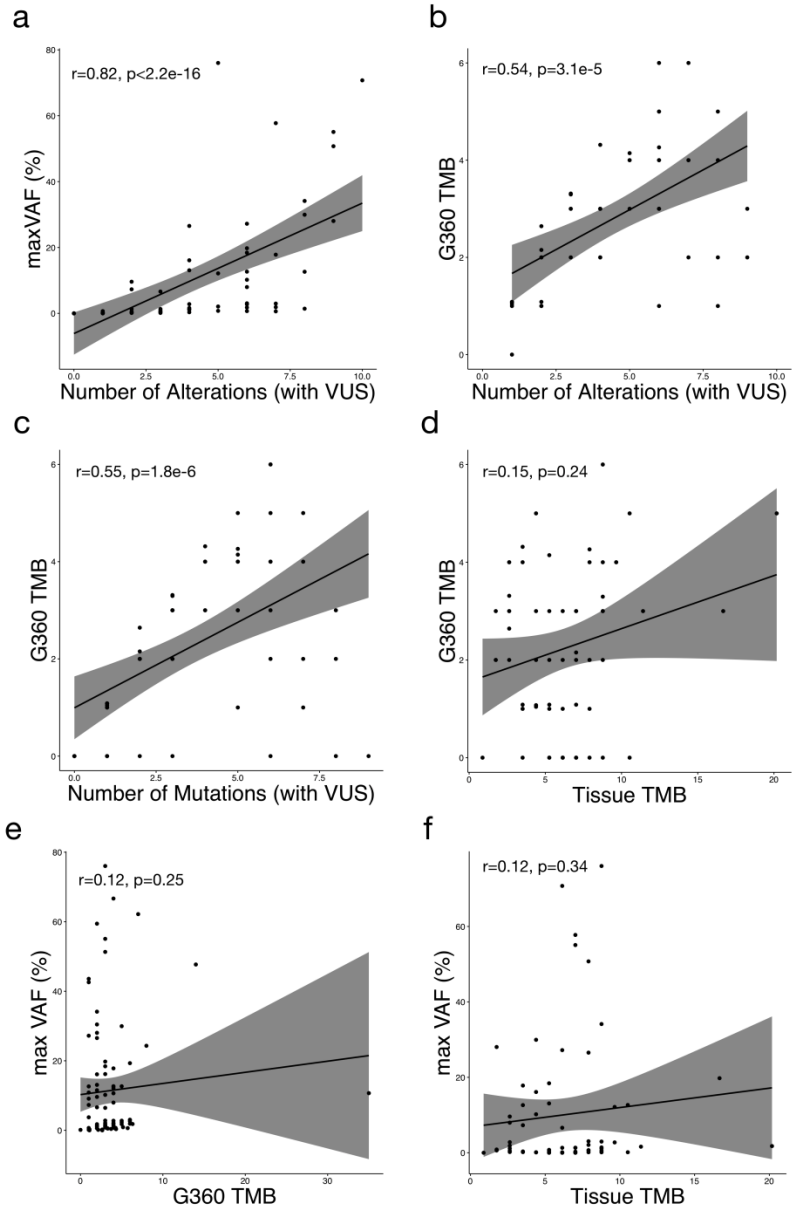

a
r=0.82, p<2.2e-16
maxVAF (%)
Number of Alterations (with VUS)
b
r=0.54, p=3.1e-5
G360 TMB
Number of Alterations (with VUS)
c
r=0.55, p=1.8e-6
G360 TMB
Number of Mutations (with VUS)
d
r=0.15, p=0.24
G360 TMB
Tissue TMB
e
r=0.12, p=0.25
max VAF (%)
G360 TMB
f
r=0.12, p=0.34
max VAF (%)
Tissue TMB

Figure S2

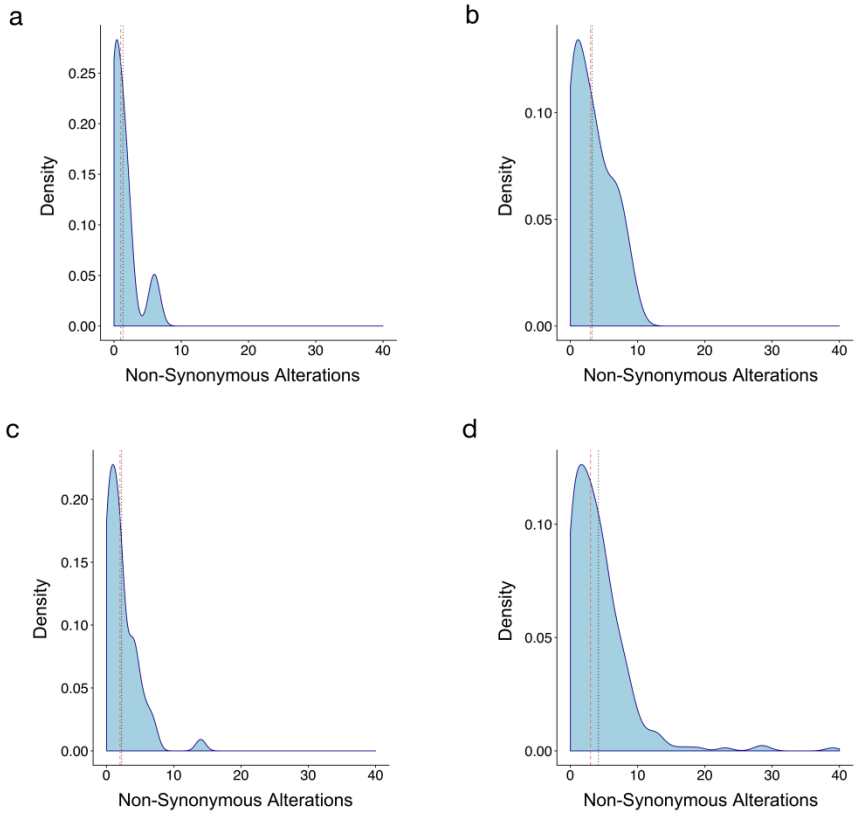

a
b
c
d
Density
Non-Synonymous Alterations
0.00
0.05
0.10
0.15
0.20
0.25
0
10
20
30
40

Figure S3

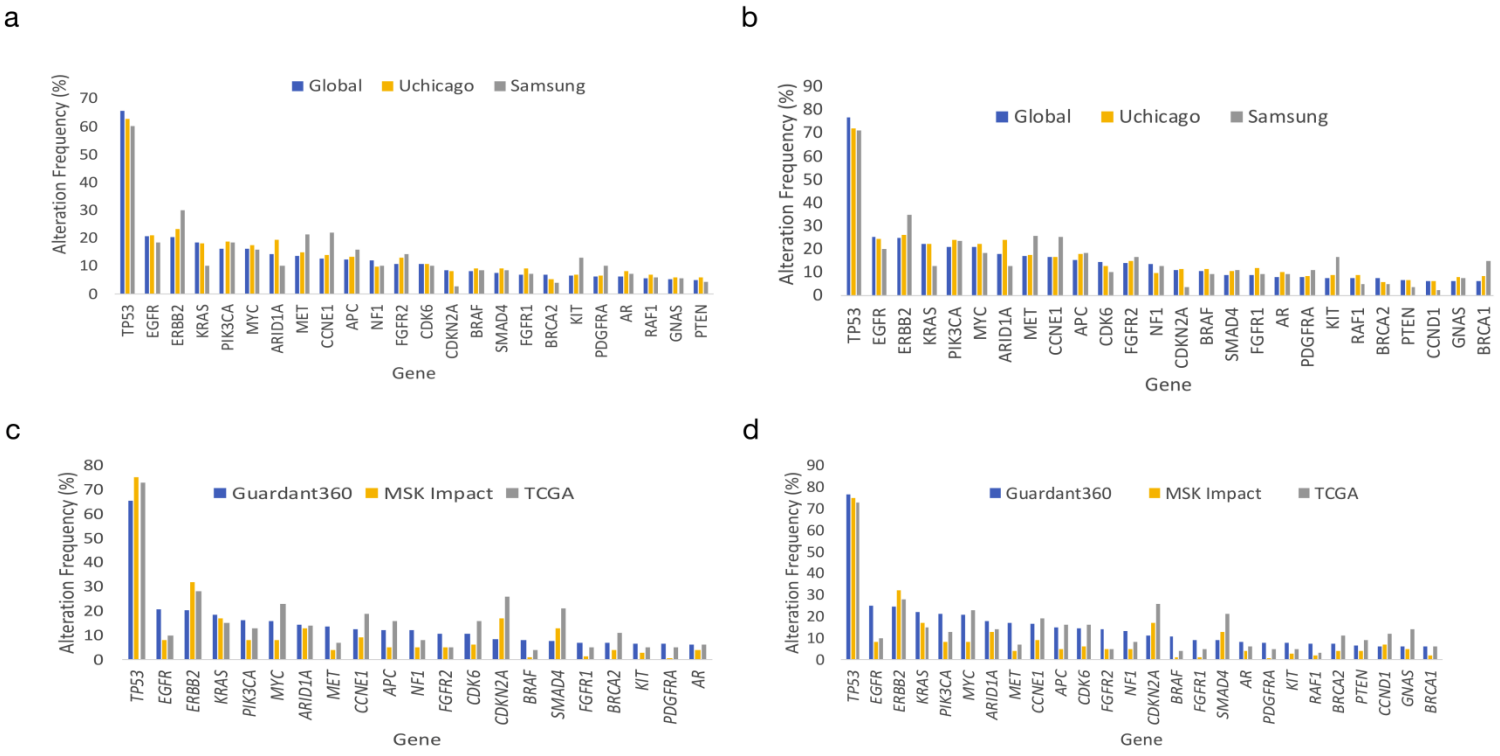

a
Global
Uchicago
Samsung
Alteration Frequency (%)
Gene
TP53 EGFR ERBB2 KRAS PIK3CA MYC ARID1A MET CCNE1 APC NF1 FGFR2 CDK6 CDKN2A BRAF SMAD4 FGFR1 BRCA2 KIT PDGFRA AR RAF1 GNAS PTEN
b
Global
Uchicago
Samsung
Alteration Frequency (%)
Gene
TP53 EGFR ERBB2 KRAS PIK3CA MYC ARID1A MET CCNE1 APC CDK6 FGFR2 NF1 CDKN2A BRAF SMAD4 FGFR1 AR PDGFRA KIT RAF1 BRCA2 PTEN CCND1 GNAS BRCA1
c
Guardant360
MSK Impact
TCGA
Alteration Frequency (%)
Gene
TP53 EGFR ERBB2 KRAS PIK3CA MYC ARID1A MET CCNE1 APC NF1 FGFR2 CDK6 CDKN2A BRAF SMAD4 FGFR1 BRCA2 KIT PDGFRA AR
d
Guardant360
MSK Impact
TCGA
Alteration Frequency (%)
Gene
TP53 EGFR ERBB2 KRAS PIK3CA MYC ARID1A MET CCNE1 APC CDK6 FGFR2 NF1 CDKN2A BRAF FGFR1 SMAD4 AR PDGFRA KIT RAF1 BRCA2 PTEN CCND1 GNAS BRCA1

Figure S4

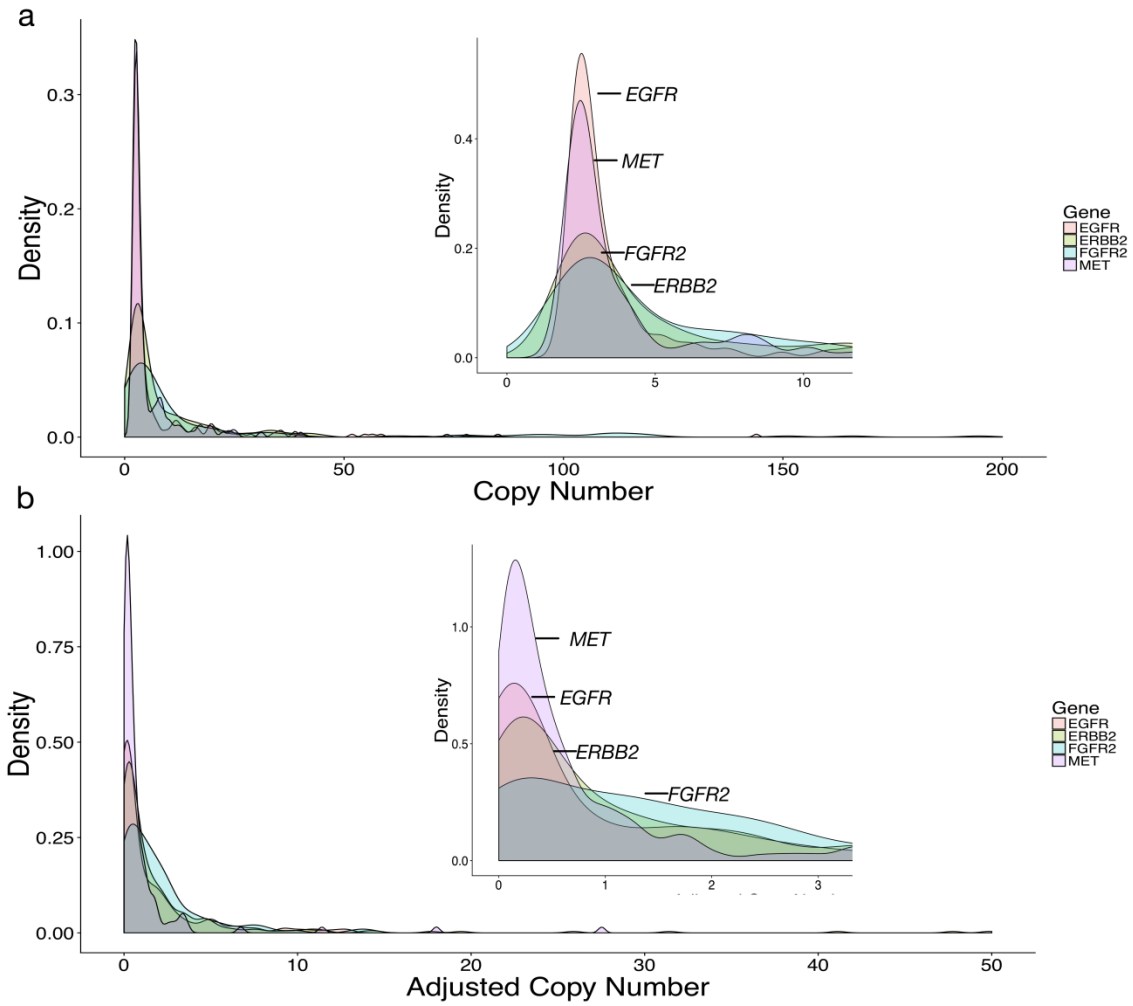
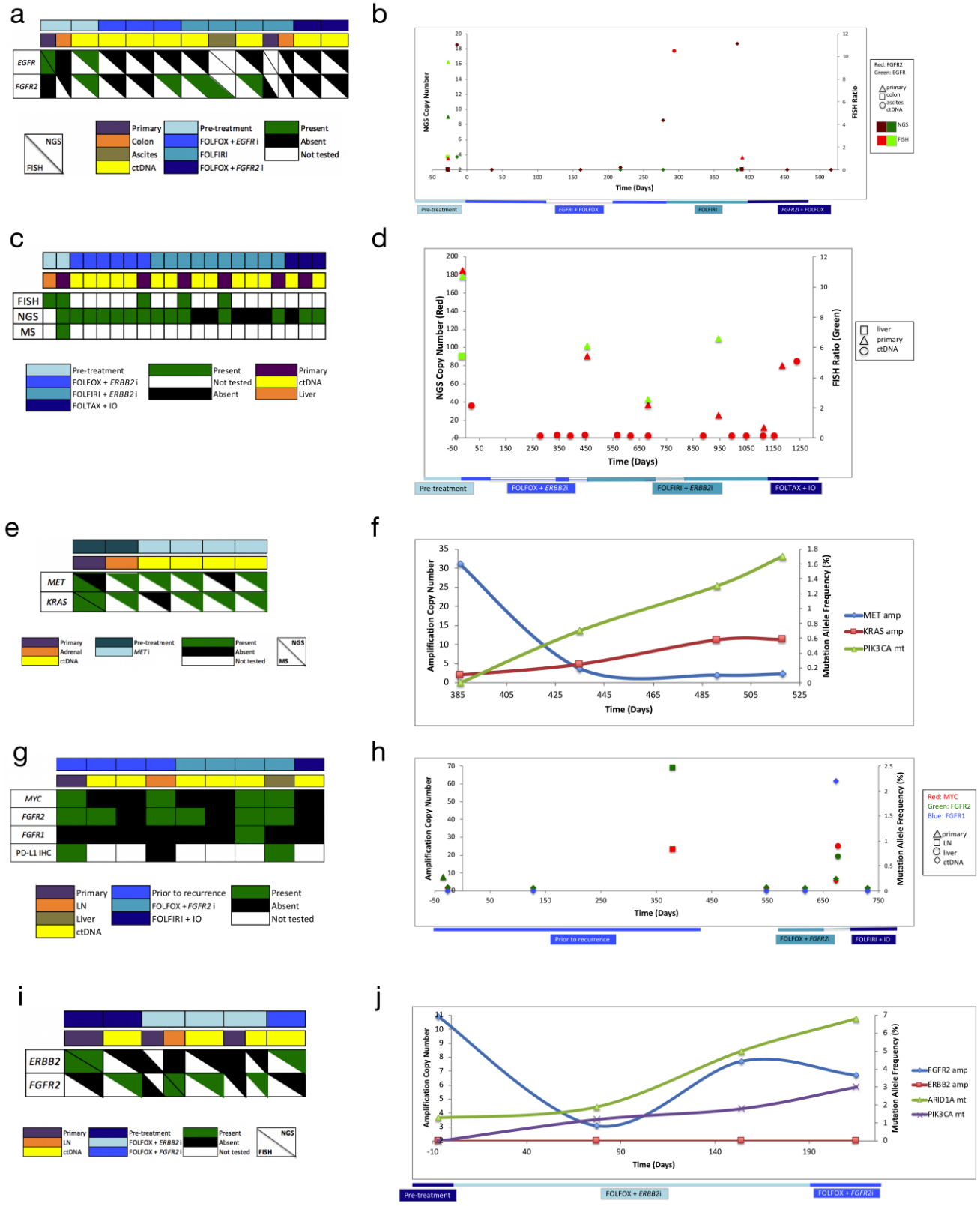

a
Density
Copy Number
EGFR
MET
FGFR2
ERBB2
Gene
EGFR
ERBB2
FGFR2
MET
b
Density
Adjusted Copy Number
MET
EGFR
ERBB2
FGFR2
Gene
EGFR
ERBB2
FGFR2
MET

Figure S5


a
EGFR
FGFR2
NGS
FISH
Primary
Colon
Ascites
ctDNA
Pre-treatment
FOLFOX + EGFR i
FOLFIRI
FOLFOX + FGFR2 i
Present
Absent
Not tested
b
NGS Copy Number
FISH Ratio
Time (Days)
Red: FGFR2
Green: EGFR
primary
colon
ascites
ctDNA
NGS
FISH
Pre-treatment
EGFRi + FOLFOX
FOLFIRI
FGFR2i + FOLFOX
c
FISH
NGS
MS
Pre-treatment
FOLFOX + ERBB2 i
FOLFIRI + ERBB2 i
FOLTAX + IO
Present
Not tested
Absent
Primary
ctDNA
Liver
d
NGS Copy Number (Red)
FISH Ratio (Green)
Time (Days)
liver
primary
ctDNA
Pre-treatment
FOLFOX + ERBB2i
FOLFIRI + ERBB2i
FOLTAX + IO
e
MET
KRAS
Primary
Adrenal
ctDNA
Pre-treatment
MET i
Present
Absent
Not tested
NGS
MS
f
Amplification Copy Number
Mutation Allele Frequency (%)
Time (Days)
MET amp
KRAS amp
PIK3CA mt
g
MYC
FGFR2
FGFR1
PD-L1 IHC
Primary
LN
Liver
ctDNA
Prior to recurrence
FOLFOX + FGFR2 i
FOLFIRI + IO
Present
Absent
Not tested
h
Amplification Copy Number
Mutation Allele Frequency (%)
Time (Days)
Red: MYC
Green: FGFR2
Blue: FGFR1
primary
LN
liver
ctDNA
Prior to recurrence
FOLFOX + FGFR2i
FOLFIRI + IO
i
ERBB2
FGFR2
Primary
LN
ctDNA
Pre-treatment
FOLFOX + ERBB2 i
FOLFOX + FGFR2 i
Present
Absent
Not tested
NGS
FISH
j
Amplification Copy Number
Mutation Allele Frequency (%)
Time (Days)
FGFR2 amp
ERBB2 amp
ARID1A mt
PIK3CA mt
Pre-treatment
FOLFOX + ERBB2i
FOLFOX + FGFR2i