Lessons Learned from ctDNA NGS in 25,000 Advanced Cancer Patients in Clinical Practice

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Disclosure: Employee
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Outline/Key Points

1. Predictive Diagnostic – what are the critical performance characteristics?

   a) Why comprehensive genomic plasma testing “CGP testing” is critical?

   b) What sensitivity (limit of detection) is required for advance cancer patients?

2. Path to validate analytical claims when orthogonal reference methods for plasma do not exist

3. Clinical outcomes for different type of alterations and low MAF variants

Case 1: Multiple resistance mechanisms in *EGFR* mutant NSCLC

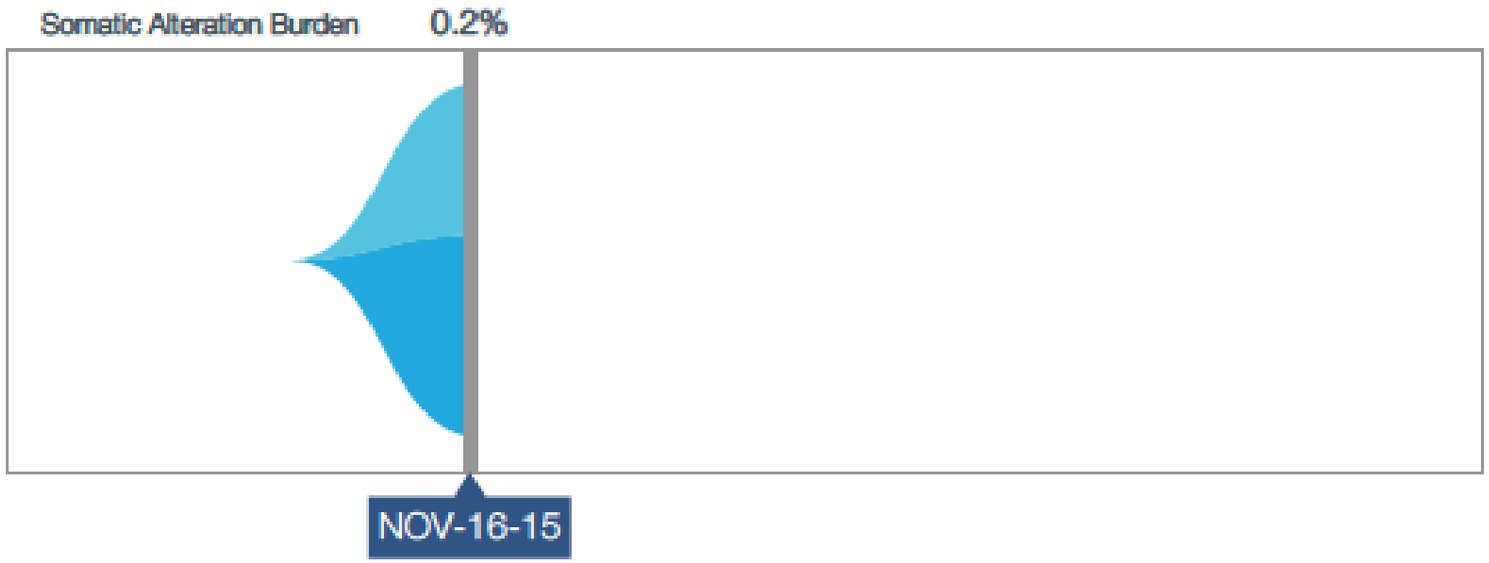
- 49 year old female with NSCLC diagnosed at age 46
- *EGFR* exon 19 deletion identified at diagnosis via tissue testing
- Progressing on afatinib after ~18 months
- Guardant360 performed to look for resistance mechanism without another biopsy

Courtesy of Dr. Young Chae, Northwestern University

EGFR T790M Drives Resistance in 50% of EGFR mutated NSCLC

Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the relative changes of observed cfDNA at different sample submission time points. The "Somatic Alteration Burden" value below refers to the maximum % cfDNA detected at each time point. Amplifications are not plotted.



2 Total Alteration(s) Detected

2 with Associated Therapy
1 Associated with Lack of Response
Multiple Clinical Trials Available

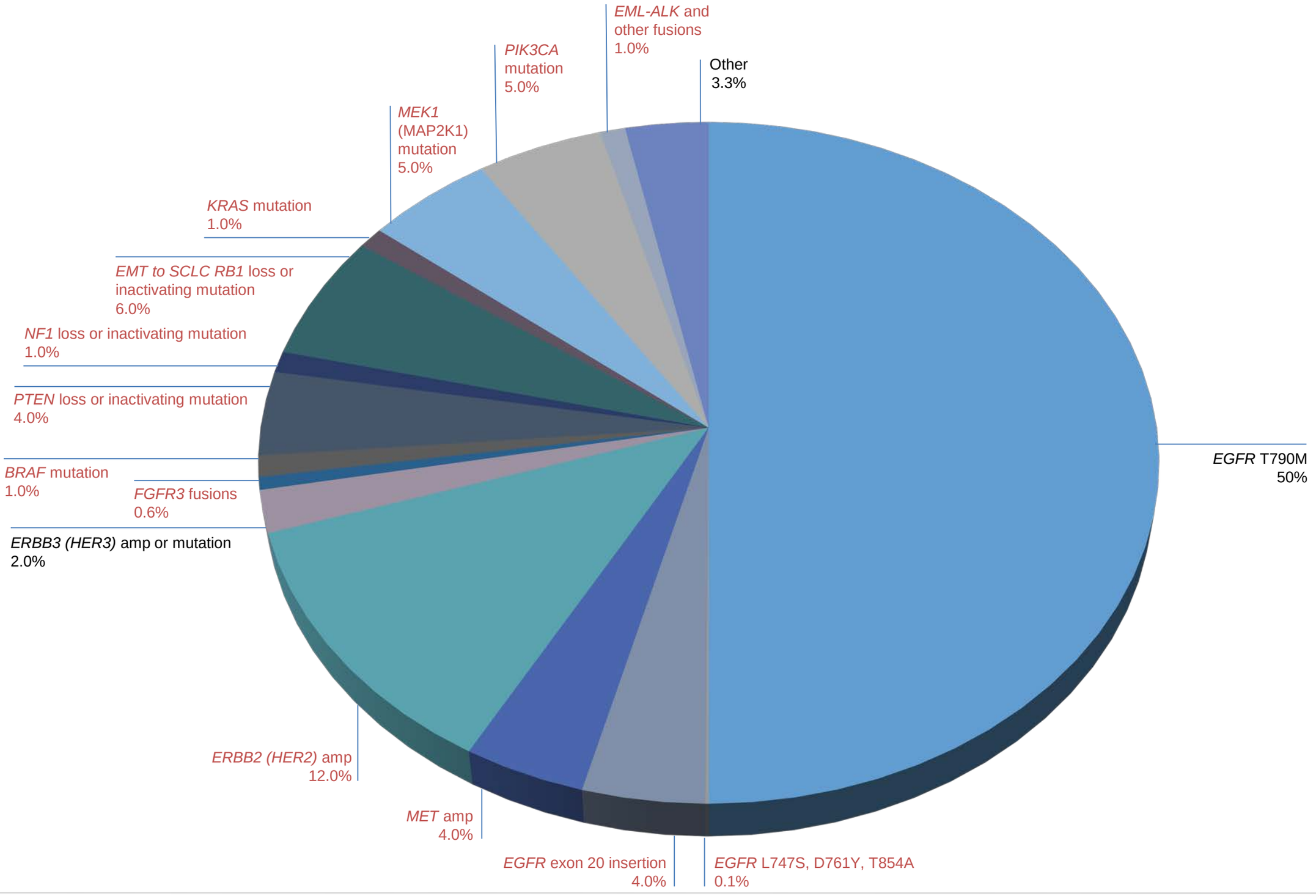
Summary of Alterations & Associated Treatment Options

The percentage, or allele frequency, of altered cell-free DNA (% cfDNA) circulating in blood is related to the unique tumor biology of this patient. Factors that may affect the amount/percentages of detected genomic alterations in circulating cell-free DNA in blood include tumor growth, turn-over, size, heterogeneity, vascularization, disease progression, or treatment.

Alteration		% cfDNA	cfDNA Amplification	FDA Approved in Indication	Available for Use in Other Indications	Clinical Drug Trials
EGFR	*E746_A750 Del*	0.2		Afatinib, Erlotinib, Gefitinib	Necitumumab, Panitumumab	Trials Available
	T790M	0.2		Osimertinib Lack of Response: Erlotinib, Gefitinib	Afatinib, Necitumumab, Panitumumab	Trials Available

Courtesy of Dr. Young Chae, Northwestern University

Beyond *EGFR* T790M – Genomic Mechanisms of Acquired Resistance



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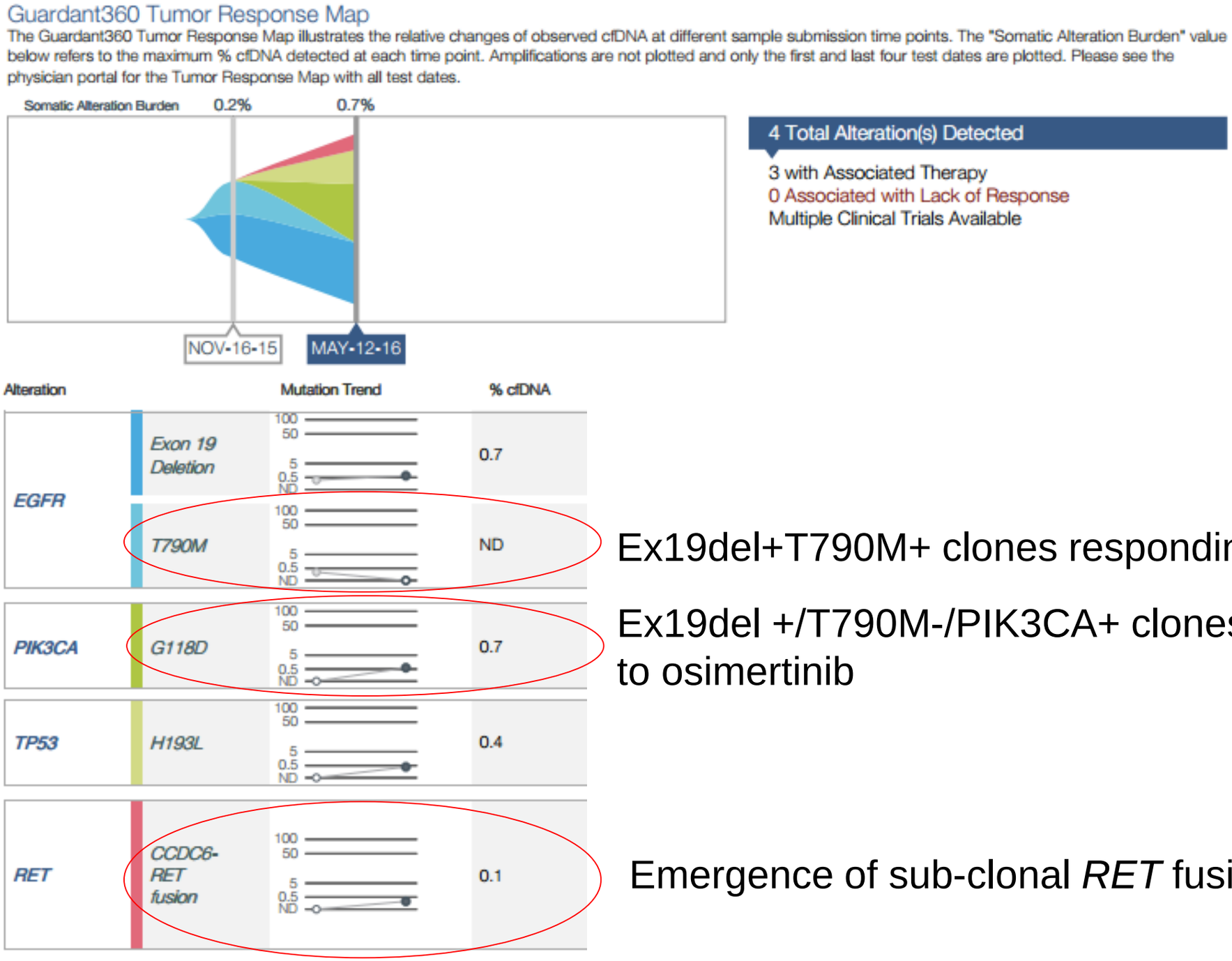
Osimertinib Worked for 6 Months – Monitoring Just for *EGFR* T790M Would Have Missed the Full Picture



Ex19del+T790M+ clones responding to osimertinib

Ex19del +/T790M-/PIK3CA+ clones not responding to osimertinib

Emergence of sub-clonal *RET* fusion

Courtesy of Dr. Young Chae, Northwestern University

NCCN Guideline Somatic Genomic Targets

Eleven somatic genomic targets in seven cancer types across all four major types of genomic alterations

Cancer Type	Targetable Genomic Alterations						
NSCLC	*EGFR* mt	*BRAF* mt	*ERBB2* mt	*ALK* fusion	*ROS1* fusion	*RET* fusion	*MET* amp and exon 14 skipping mt
Colorectal	*KRAS* mt exons 2,3,4	*NRAS* mt exons 2,3,4	*BRAF* mt	MSI (or MMR IHC) if ≤ 70 or older if relative with CRC ≤ 50 or 2 relatives with CRC			
Breast	*ERBB2 (HER2)* amp	*BRCA1/2* germline if: – early onset ≤ 45 – triple negative breast cancer ≤ 60 – male breast cancer at any age – dx at any age & family hx breast, ovarian, pancreas or prostate					
Gastric & Gastro-esophageal	*ERBB2* (HER2) amp						
Melanoma	*BRAF* mt	*KIT* mt					
GIST	*KIT* mt	*PDGFRA* mt	*BRAF* mt				

Guardant360 – No *A Priori* Knowledge Requirement

Critical exons completely sequenced and all four major classes of alterations

POINT MUTATIONS - ***Complete**** *or Critical Exon Coverage in 70 Genes*

AKT1	*ALK*	***APC***	***AR***	*ARAF*	***ARID1A***	*ATM*	***BRAF***	***BRCA1***	***BRCA2***
CCND1	***CCND2***	***CCNE1***	*CDH1*	***CDK4***	***CDK6***	***CDKN2A***	***CDKN2B***	*CTNNB1*	***EGFR***
ERBB2	*ESR1*	*EZH2*	*FBXW7*	***FGFR1***	***FGFR2***	*FGFR3*	*GATA3*	*GNA11*	*GNAQ*
GNAS	*HNF1A*	***HRAS***	*IDH1*	*IDH2*	*JAK2*	*JAK3*	***KIT***	***KRAS***	*MAP2K1*
MAP2K2	***MET***	*MLH1*	*MPL*	***MYC***	***NF1***	*NFE2L2*	*NOTCH1*	*NPM1*	***NRAS***
NTRK1	***PDGFRA***	***PIK3CA***	***PTEN***	*PTPN11*	***RAF1***	***RB1***	*RET*	*RHEB*	*RHOA*
RIT1	*ROS1*	*SMAD4*	*SMO*	*SRC*	*STK11*	*TERT*	***TP53***	*TSC1*	*VHL*

AMPLIFICATIONS

AR	***BRAF***	***CCND1***	***CCND2***	***CCNE1***	***CDK4***	***CDK6***	***EGFR***	***ERBB2***
FGFR1	***FGFR2***	***KIT***	***KRAS***	***MET***	***MYC***	***PDGFRA***	***PIK3CA***	***RAF1***

FUSIONS

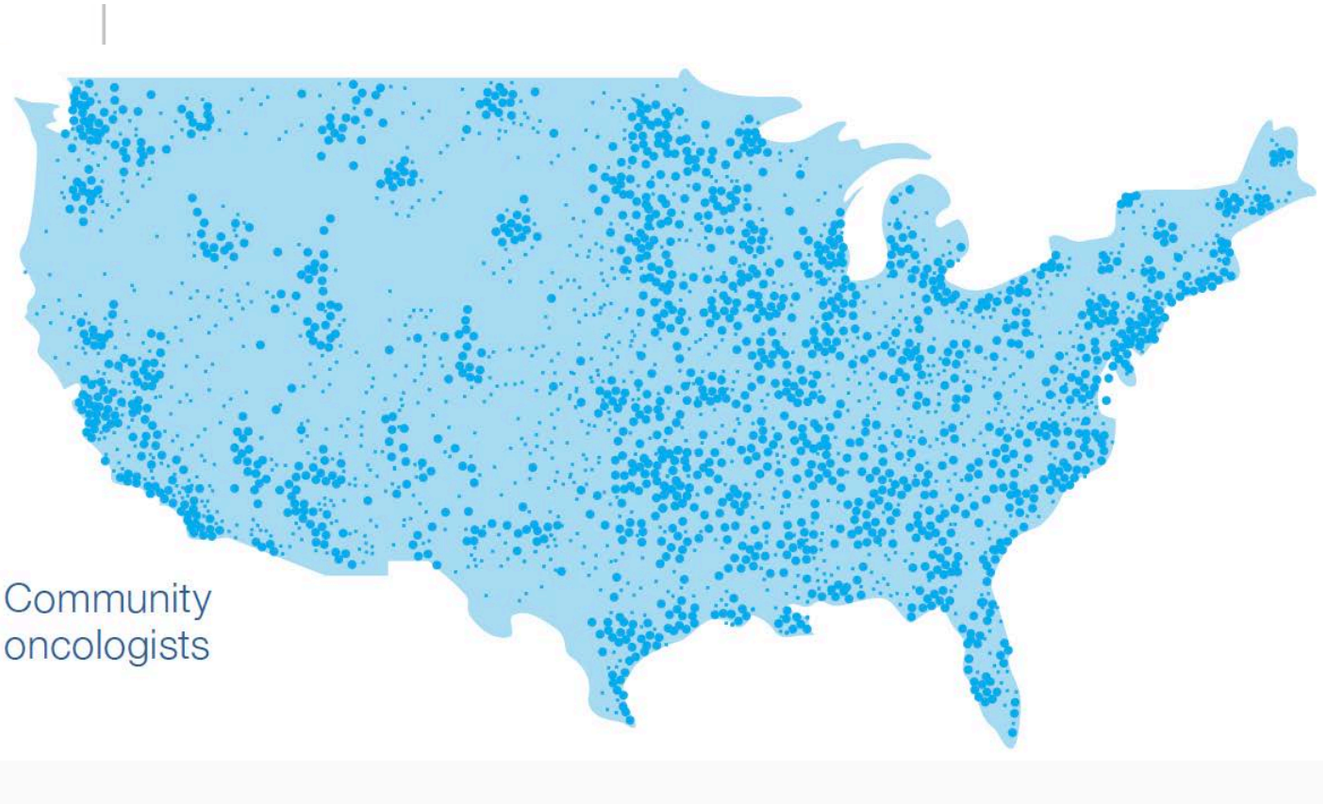
ALK	*FGFR2*	*FGFR3*	*RET*	*ROS1*	*NTRK1*

INDELS

EGFR exons 19/20	*ERBB2* exons 19/20	*MET* exon 14 skipping

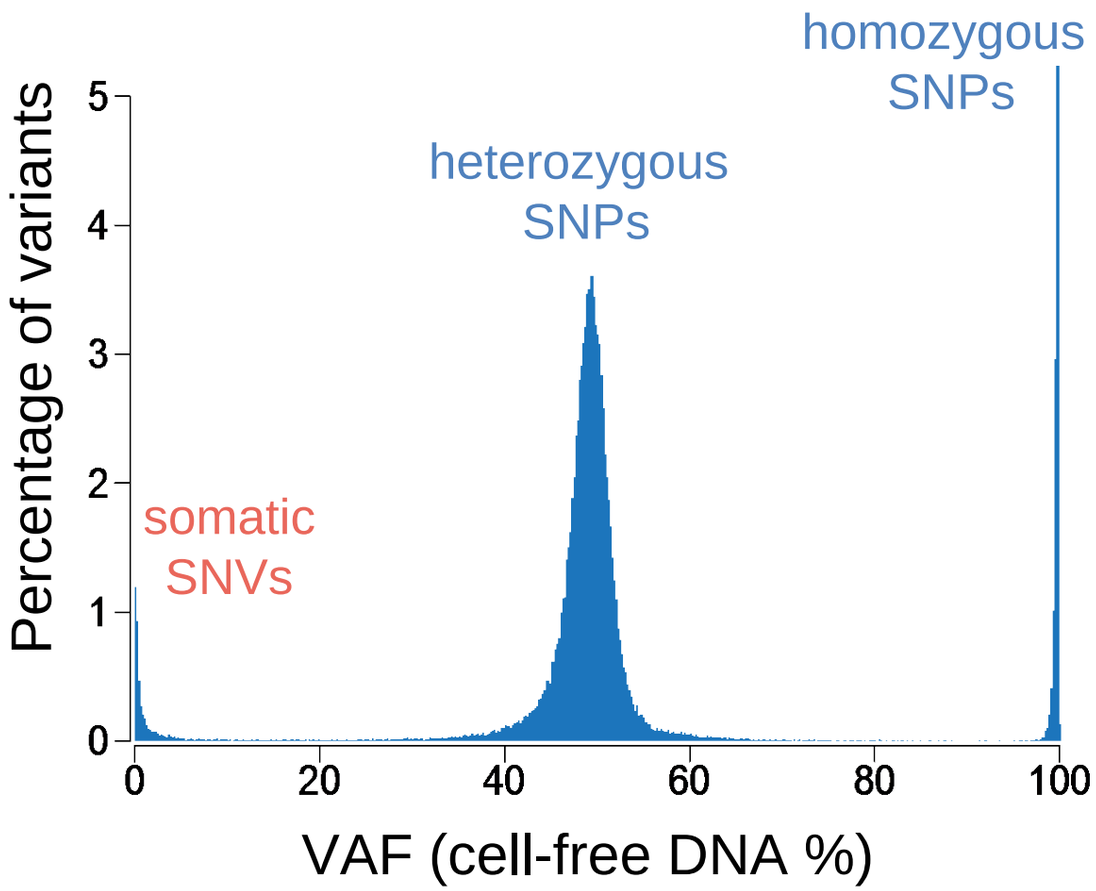
Guardant Health's experience in running G360 as an LDT

- Launched as an LDT in June 2014
- Leader in liquid biopsy market: processed >25,000 samples from >2,500 ordering physicians to date.

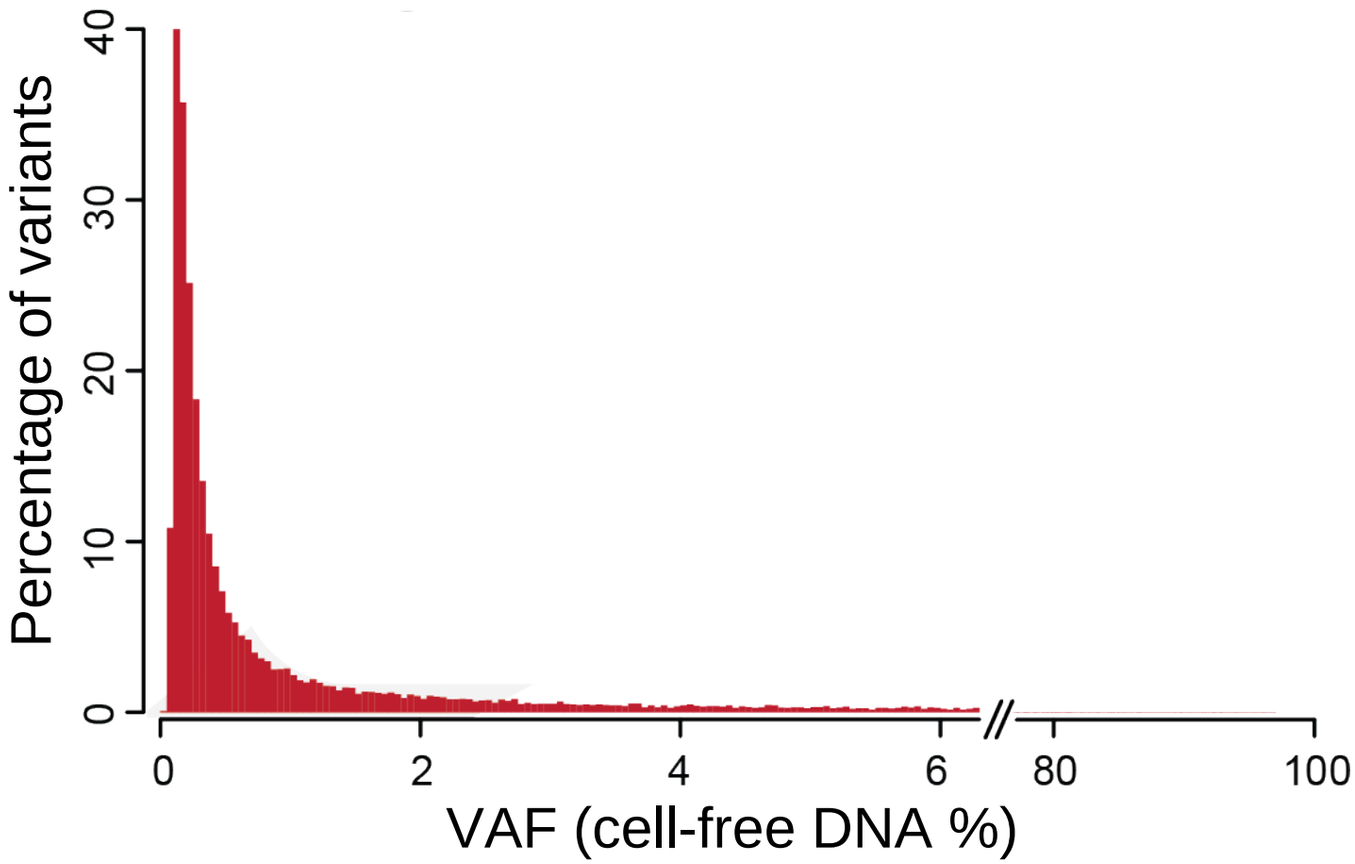


Half of reported variants occur below 0.4% Variant Allele Fraction (N=20,000)

All variants detected

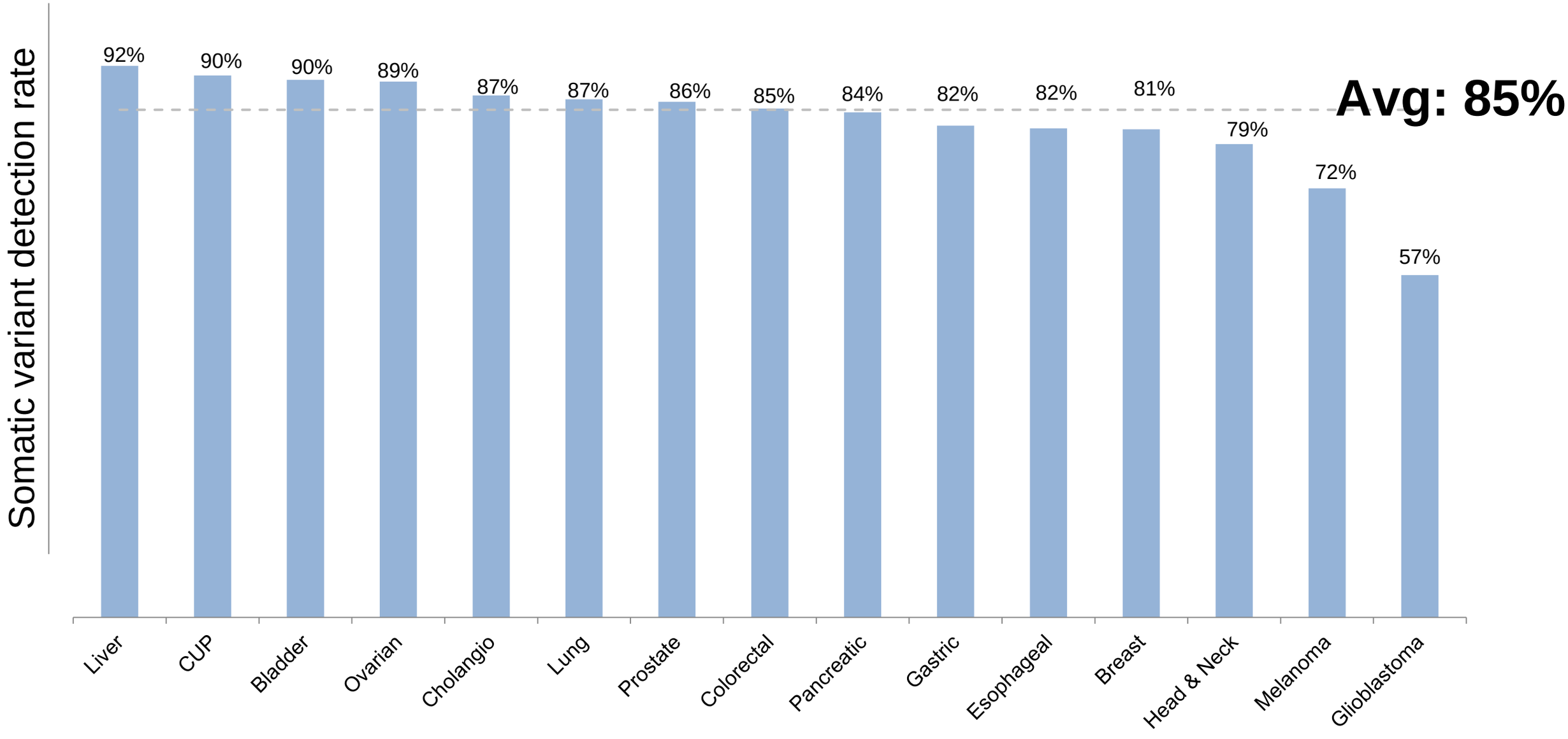


Reported somatic variants



Zill et al. 2016 ASCO Abstract J Clinical Oncology 34(18)_suppl

cancers



Zill et al. 2016 ASCO Abstract J Clinical Oncology 34(18)_suppl

Path for Validation of Analytical Claims

- Unlike sheared genomic DNA or synthetic material, cell-line derived cfDNA are well suited to be used as contrived samples.

  - We believe that the genomic profile of cell line-derived cell-free DNA (cfDNA) is highly similar to matched cell line-derived gDNA (specimen commutability).

  - Using titrated cell line-derived cfDNA, analytical performance of the assay can be studied.

Variant Type	Reportable Range	Unique Variants Tested	Sensitivity [95% CI]	PPV [95% CI]
SNVs	≥0.2%	90	98.9% [93.1-99.9]	100% [94.8-100%]
Indels	≥2 molecules	37	95.7% [84.0-98.7%]	100% [90.0-100%]
CNAs	≥2.20 copies	70	94.3% [85.3-98.2%]	93.0% [83.6-98.3%]
SVs	≥2 molecules	19	100% [79.1-100%]	100% [79.1-100%]

Analytic Specificity > 99.9999%

54-Gene Panel vs. Whole Exome Sequencing

A New Gold Standard for Specificity:
~1,560,000 base pairs sequenced -
(20 Samples x 78,000 BPs per sample)

	Reference		
G360	Positive	Negative	**Total**
Positive	365	1	366
Negative	0	1,559,634	1,559,634
Total	365	1,559,635	1,560,000

Performance		**(95% CI)**
Specificity	>99.9999%	(>99.9999%-100.0%)
Sensitivity	100%	(98.7%-100.0%)
Concordance	99.9999%	(99.9999%-100.0%)

The single putative false positive sample was sent to another outside reference lab (Stanford Protein and Nucleic Acid Facility) where Sanger Sequencing found it to be a true positive.

Lanman et al. 2015 PLOS One

High Analytic Specificity

68-Gene Panel vs. Whole Exome Sequencing

2nd Study Re-Confirms Near-Perfect Specificity:
~1,515,000 base pairs sequenced – 68-gene panel
(15 Samples x 101,000 BPs per sample)

	Reference		
G360	Positive	Negative	**Total**
Positive	215	3	218
Negative	0	1,514,785	1,514,785
Total	215	1,514,788	1,515,003

Performance		**(95% CI)**
Specificity	>99.9998%	(>99.9998%-100.0%)
Sensitivity	100%	(97.8%-100.0%)
Concordance	99.9999%	(99.9998%-100.0%)

The samples with the three putative false positives were sent to another outside reference lab (Stanford) where Sanger Sequencing found all three to be true positives.

Mortimer et al. 2016 *In Review*

ctDNA NGS to ctDNA ddPCR

Using highly-validated clinical ddPCR assays as reference, G360 demonstrated perfect qualitative and excellent quantitative accuracy even at low allelic fractions

EGFR p.L858R

	ddPCR +	ddPCR -
G360 +	58	0
G360 -	0	55

PPA = 100% [92.3-100%]
NPA = 100% [91.9-100%]
PPV = 100% [92.3-100%]
NPV = 100% [91.9-100%]

Ex19 deletions

	ddPCR +	ddPCR -
G360 +	25	0
G360 -	0	71

PPA = 100% [91.4-100%]
NPA = 100% [92.0-100%]
PPV = 100% [91.4-100%]
NPV = 100% [92.0-100%]

p.T790M

	ddPCR +	ddPCR -
G360 +	53	0
G360 -	0	52

PPA = 100% [91.6-100%]
NPA = 100% [91.4-100%]
PPV = 100% [91.6-100%]
NPV = 100% [91.4-100%]

KRAS p.G12any

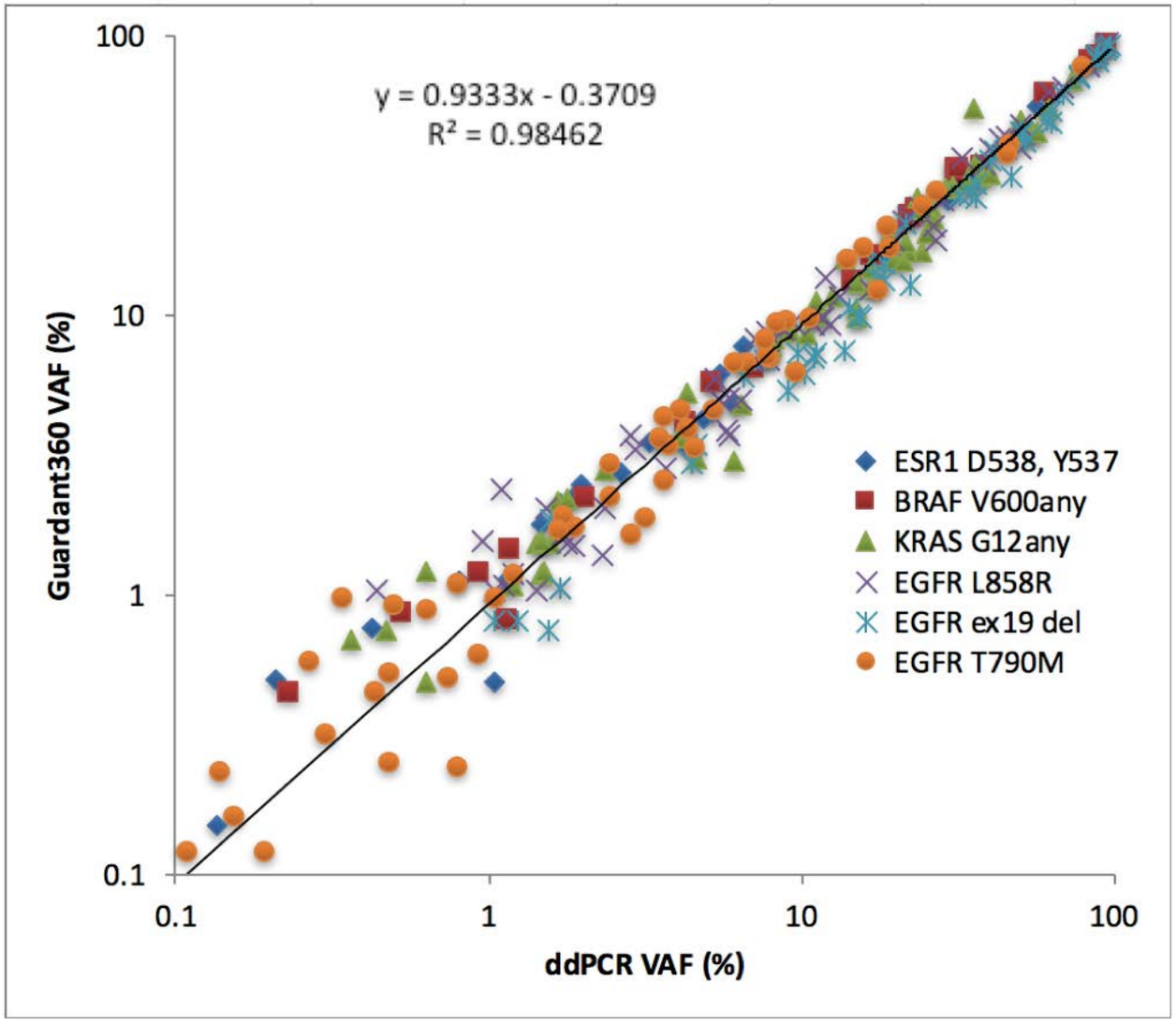
	ddPCR +	ddPCR -
G360 +	58	0
G360 -	0	18

PPA = 100% [92.3-100%]
NPA = 100% [78.1-100%]
PPV = 100% [92.3-100%]
NPV = 100% [78.1-100%]

BRAF p.V600any

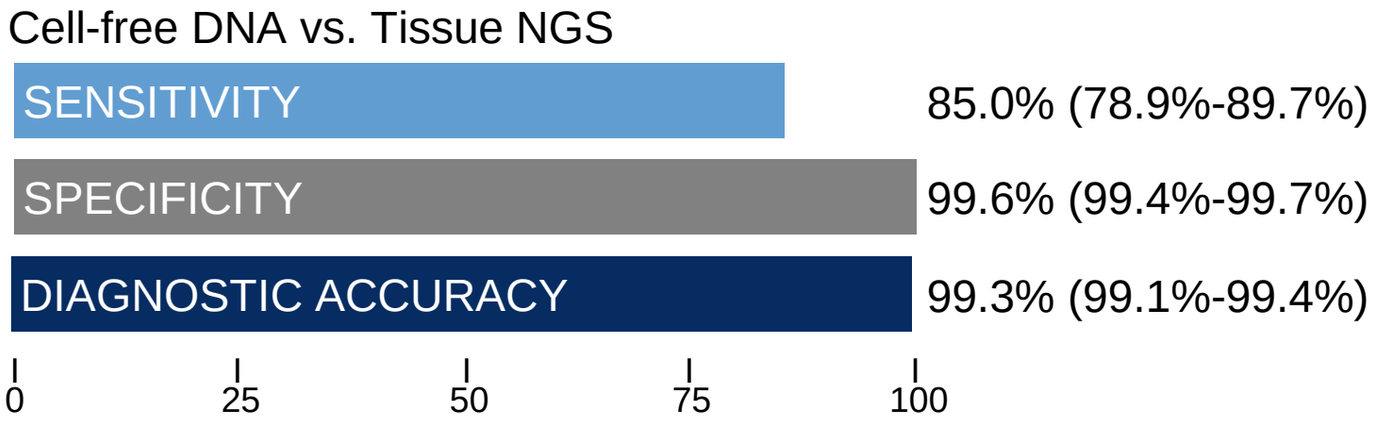
	ddPCR +	ddPCR -
G360 +	21	0
G360 -	0	55

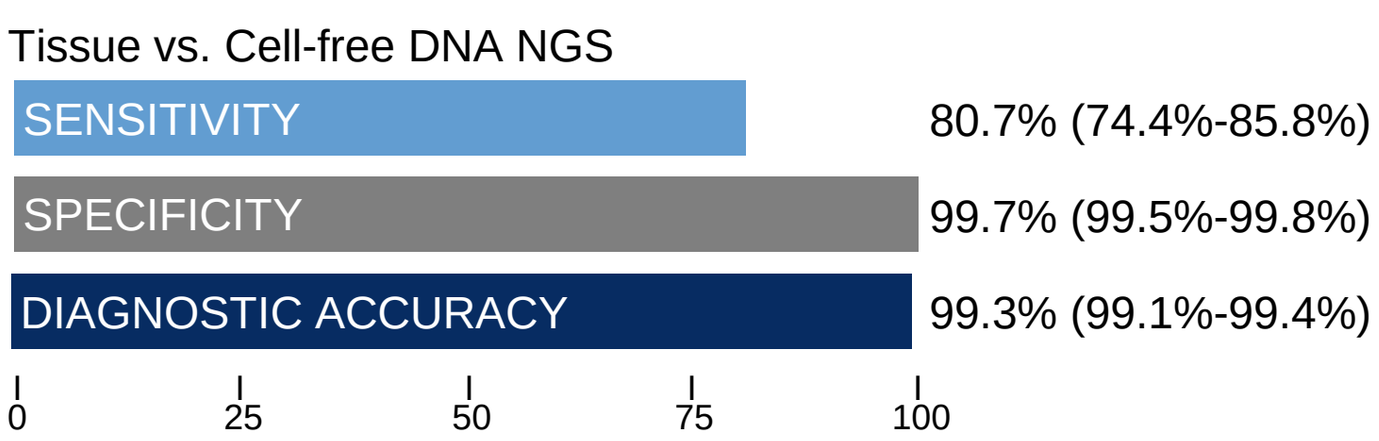
PPA = 100% [80.8-100%]
NPA = 100% [91.9-100%]
PPV = 100% [80.8-100%]
NPV = 100% [91.9-100%]



Quantitative Calibration Important for Relative Variant Allele Fractions

Tissue NGS vs. Plasma Cell-Free NGS on 165 Paired Samples from Five Centers

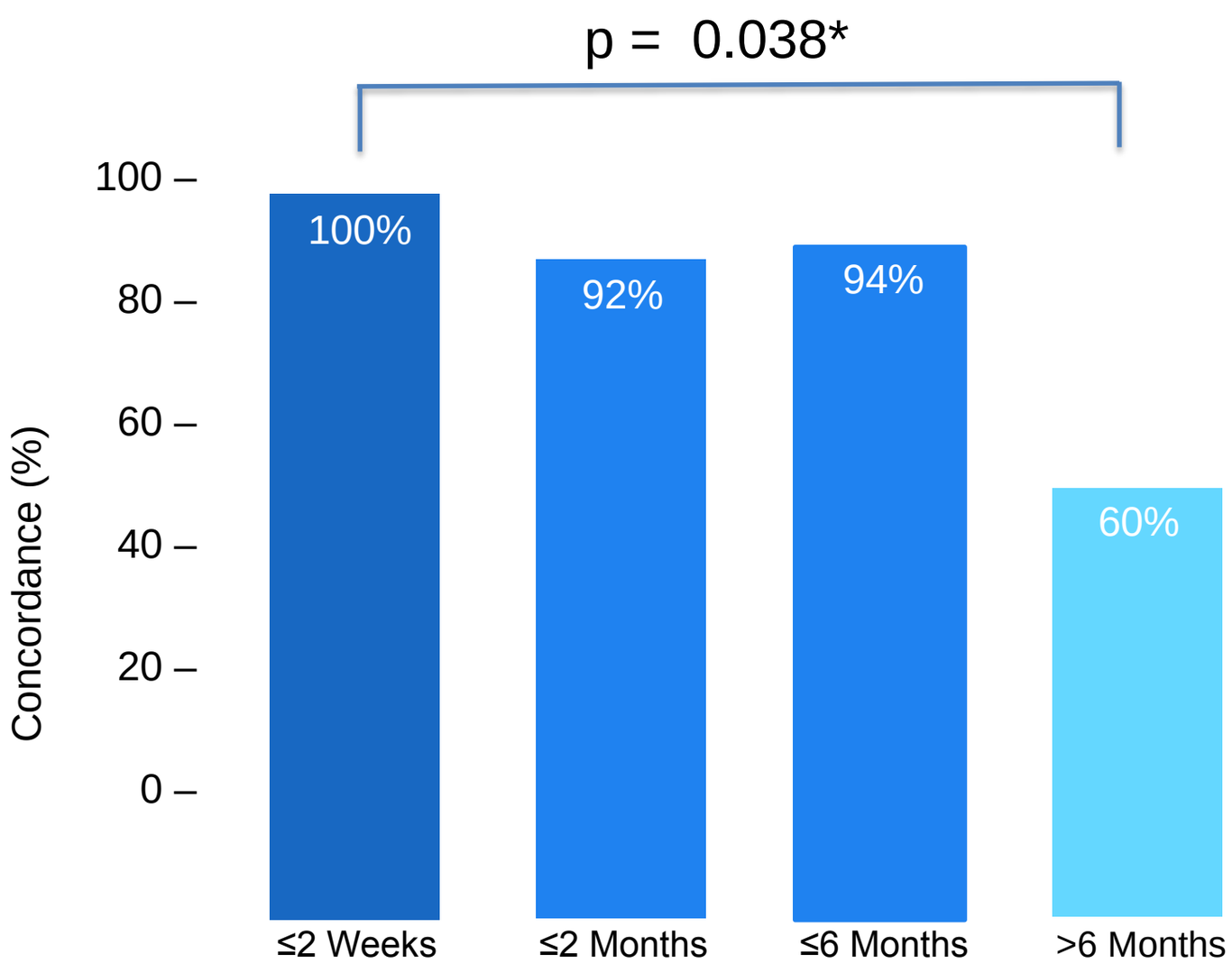




Cell-free DNA sensitivity may be limited when tumor DNA is not shed into circulation. Tissue DNA sensitivity may be limited because samples fail to capture tumor heterogeneity.

Lanman et al. 2015 *PLoS One*

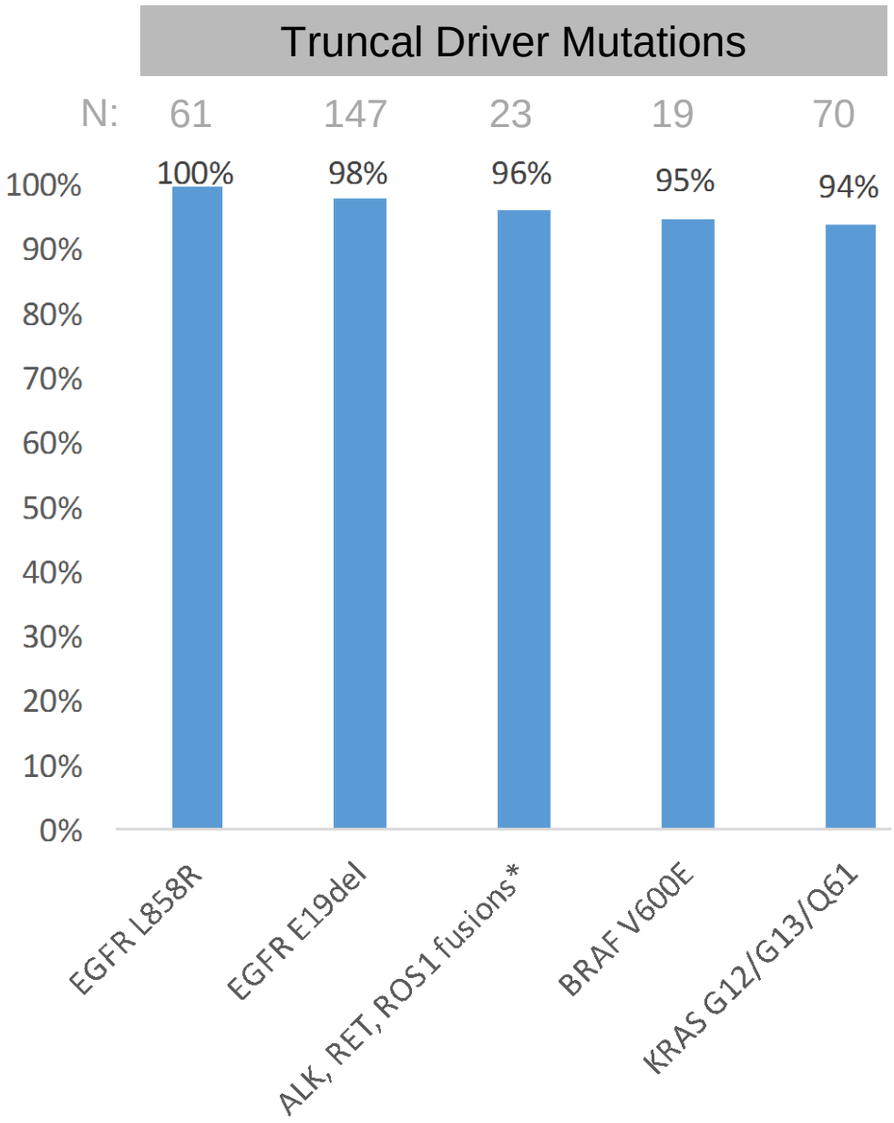
Temporal Heterogeneity Confounds Plasma to Tissue NGS Concordance: Blinded External Validation Study



Prospective study of 102 Consecutive NSCLC patients

Thompson et al. 2016 *Clinical Cancer Research*

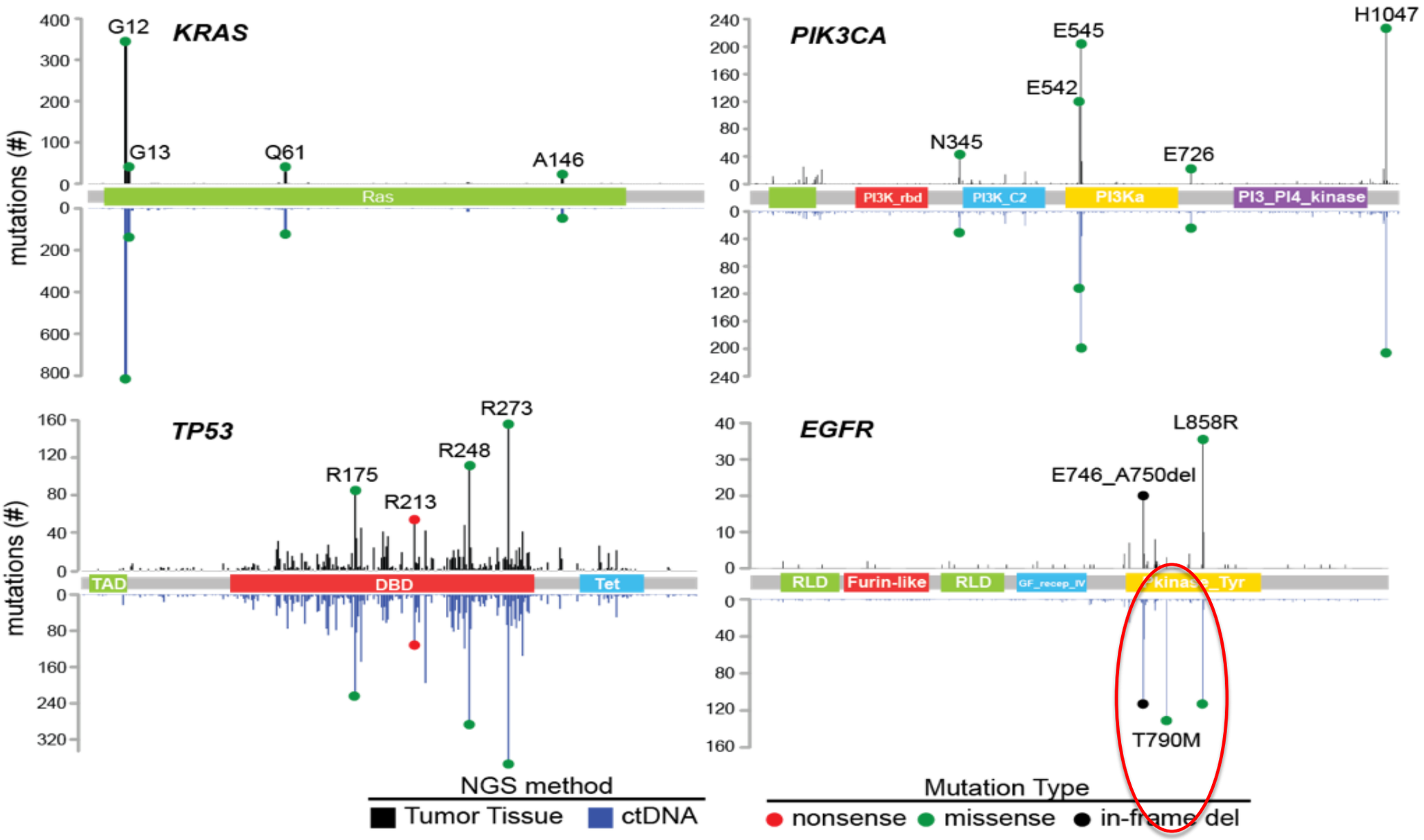
ctDNA PPV vs tissue biopsy
(NSCLC, CRC, Breast, Others)



- Truncal driver mutations
  - PPV remained high (94.5%, n=71) for low MAF truncal mutations (<0.5%)
  - * The single ctDNA-positive, tissue-negative *ALK* fusion responded to crizotinib
- Subclonal mutations indicate likely resistance
  - Discordant resistance cases likely reflect evolution on therapy after initial tissue biopsy

Zill et al. 2016 ASCO Abstract J Clinical Oncology 34(18)_suppl

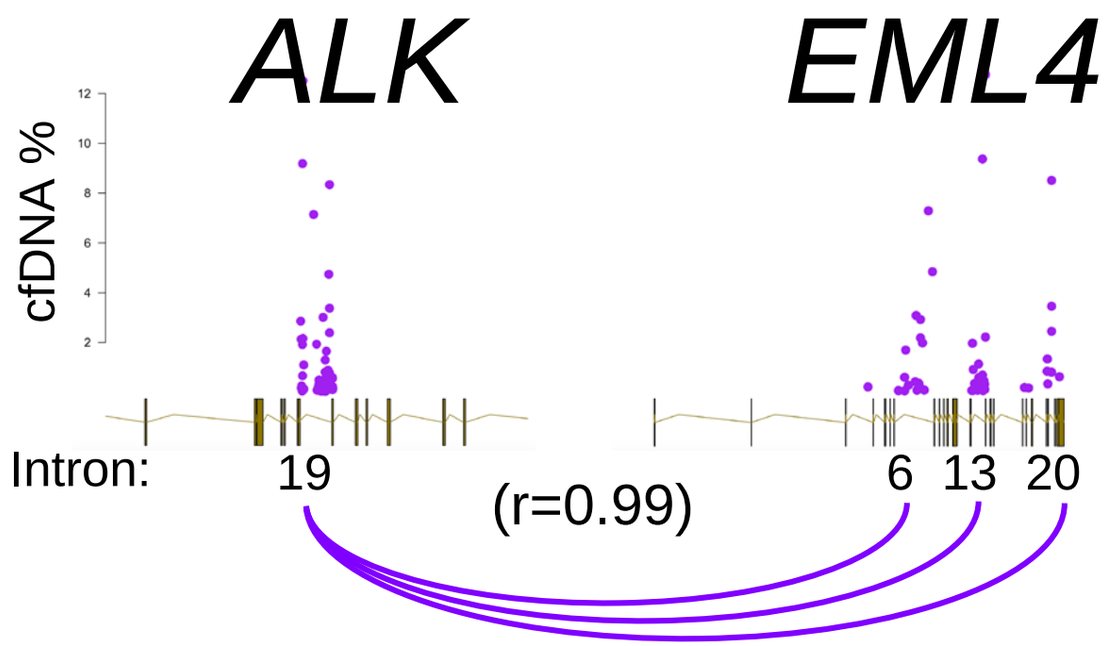
Mutations in First 20,000 Guardant360 Patients Reflect Prevalence in TCGA Tumor Tissue Compendia



The secondary resistance mechanism *EGFR* T790M frequently found in ctDNA cohort, but rare in TCGA (surgical/treatment naïve)

Zill et al. 2016 ASCO Abstract J Clinical Oncology 34(18)_suppl

ctDNA Fusion Breakpoint Patterns Mirror TCGA



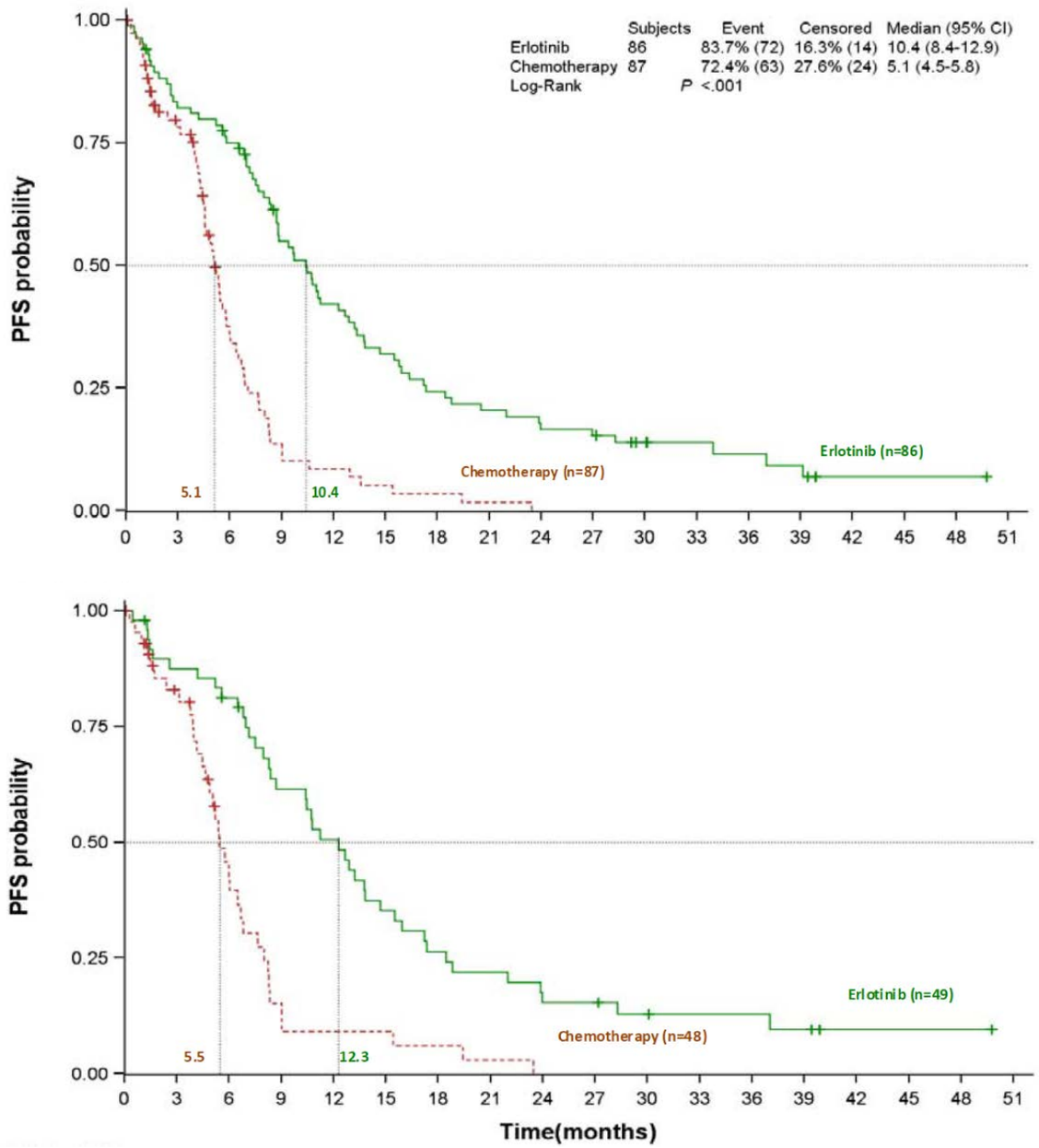
EML4 intron	Breakpoints in ctDNA (n=41)	Breakpoints in COSMIC (n=375)
13	46%	47%
6	37%	35%
20	5%	14%

Morán T, et al. 2013 *Transl Lung Cancer Res*

Zill et al. 2016 ASCO Abstract J Clinical Oncology 34(18)_suppl

Clinical Outcomes for Different Variant Types in CtDNA NGS, and Low Variant Allele Fraction Calls In Particular

EGFR Mutations Respond to TKI Whether Measured in Tissue (A) or Plasma (B)



Erlotinib

Carboplatin with docetaxel or gemcitabine

A - *EGFR* L858R or exon 19 Del Measured in Tissue (N = 86)
Median PFS (95% CI):
Erlotinib arm 10.4 mos (8.4 – 12.9)
Chemotx arm 5.1 mos (4.5 – 5.8)

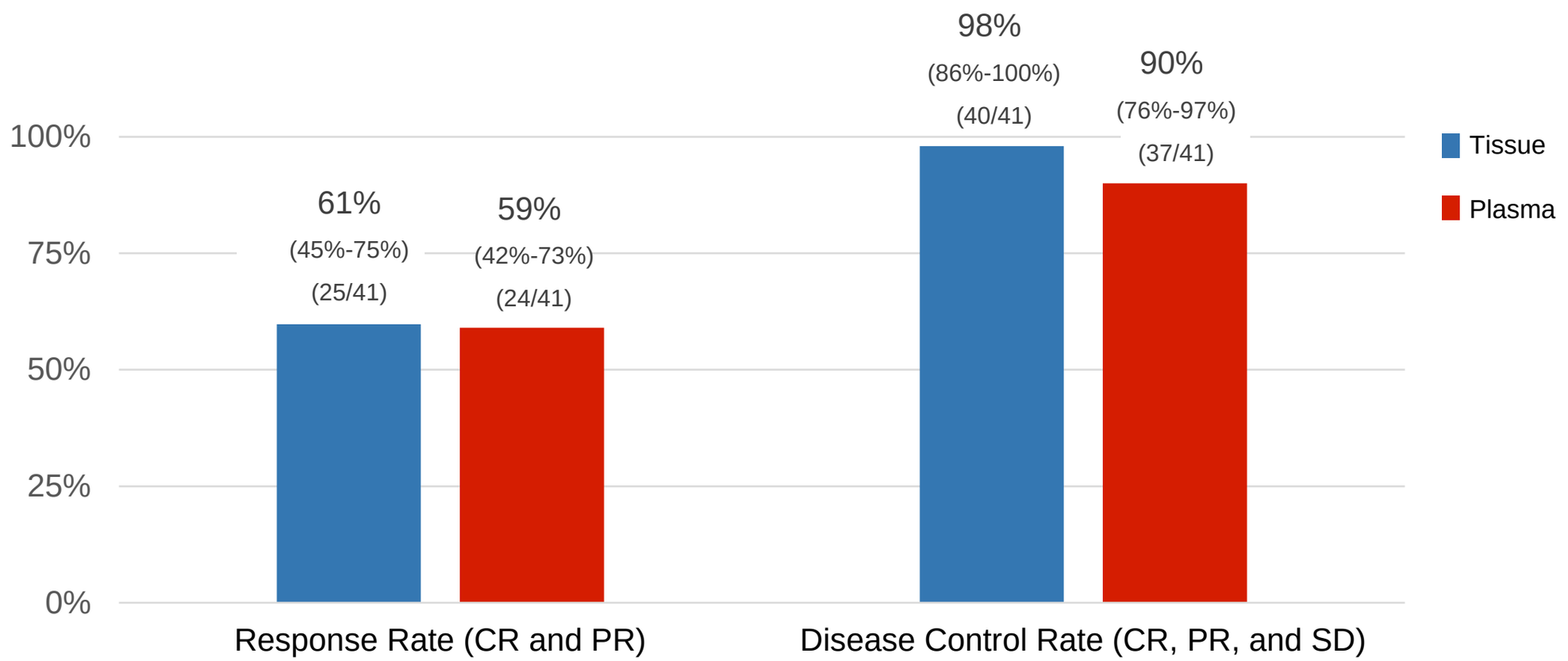
B - *EGFR* L858R or exon 19 Del Measured in Plasma (N = 49)
Median PFS 995% CI) by qPCR or TaqMan:
Erlotinib arm 12.3 mos (8.4 – 14.7)
Chemotx arm 5.5 mos (4.5 – 6.7)

Whether measured in tissue or blood, *EGFR* L858R and ex19 deletions responded to erlotinib. This is intuitive since the mutations in the blood come from the tissue.

Karachaliou et al. 2015 EURTAC trial *JAMA Oncology*

Plasma *EGFR* T790M Responds to 3rd Generation TKI Even When Tissue Negative

Plasma (BEAMing dPCR) vs. Tissue "Non-Reference Standard" (N=38)	
Sensitivity	81%
Specificity	58%



Thress et al. 2015 *Lung Cancer*

Case Two: Low-MAF ALK Fusion
NSCLC but Tissue QNS for Genotyping

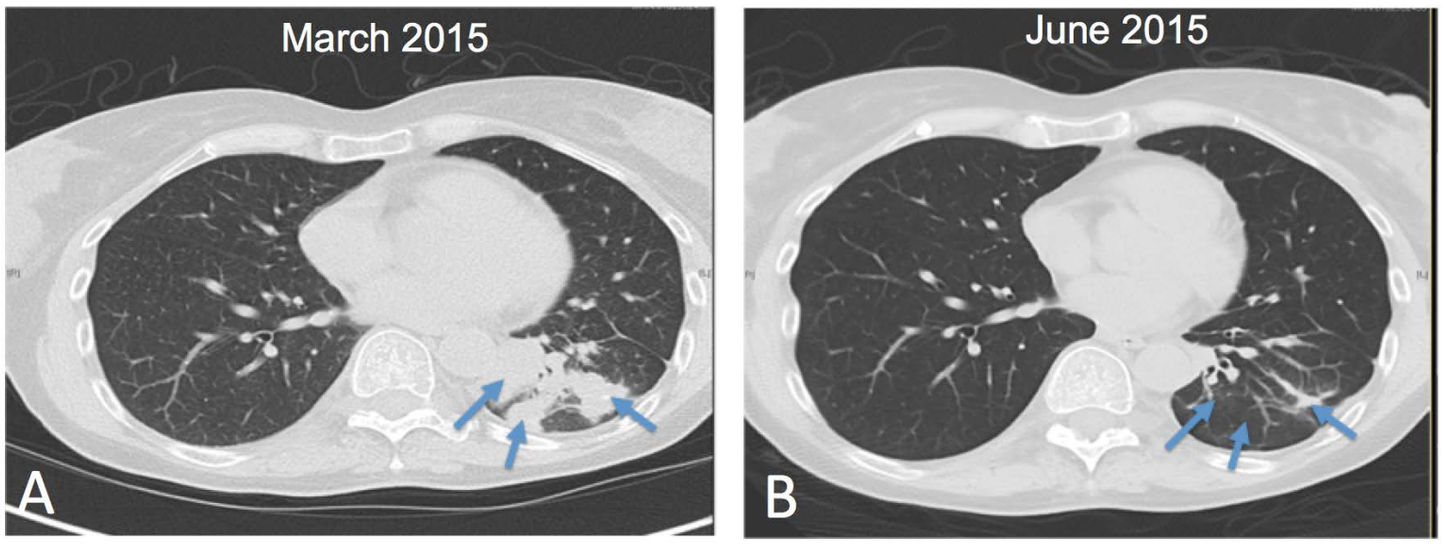
Clinical Case:

— 58-year-old female non-smoker presents with metastatic NSCLC

— Progression on empirical chemotherapy but tissue is QNS for genotyping despite three biopsy attempts

— Guardant360 demonstrated **TWO MOLECULES of *EML4-ALK* fusion at 0.1% VAF.**

— Major durable response to crizotinib



— From no options to response with a simple blood test

Courtesy Nisha Mohindra, Northwestern & Jyoti Patel, Univ. Chicago (2015 WCLC/IASLC Abstract)

MET Exon 14 Skipping Indel at 0.1% MAF in Undergenotyped Patient

72-year-old male never-smoker with metastatic lung adenocarcinoma.
Had 3 prior lines: carbo/taxol/avastin, then nivolumab, then carbo/pem. Could no longer walk on the beach.

Alteration		% cfDNA	
TP53	*S241F*	0.5	"First hit", tumor suppressor gene inactivating mutation
MAP2K2	*Y134C*	0.1	*MEK2* Variant of Uncertain Significance (VUS)?
MET	*Exon 14 Skipping Indel*	0.1	*MET* oncogene activation targetable with crizotinib or cabozantanib

Guardant360 reveals *MET* mutation: MET inhibitor produced PR (30%) with major clinical response, ECOG performance now 0.

Courtesy Sandip Patel MD, UCSD

Guardant360 Publications (1-6)

Author/Journal (Year)	Key Findings	AV, CV, CU
Zill et al. 2015 Cancer Discovery (pancreatic cancer and cholangiocarcinoma)	• 35% of tissue biopsies insufficient in 100% "rescued" with Guardant360, i.e. ctDNA positive for driver mutation • 92% sensitivity for 5 major genes	CV
Ko et al. 2015 Clinical Cancer Research (pancreatic cancer)	• 100% concordance for *KRAS*	CV
Kim et al. 2015 Oncotarget (colorectal ca/melanoma)	• 90%+ sensitivity for *KRAS* and *BRAF* • Mean turnaround 10 days (N=75 patients)	CV
Lanman et al. 2015 PLOS One (multiple stage III/IV solid tumor cancers)	• Analytic sensitivity to 1-2 molecules (0.1% variant allele fraction), analytic specificity 99.9999% • Clinical sensitivity 85%, clinical specificity 99.6%, accuracy 99.3%	AV, CV
Lokhandwala et al. 2016 Clin Lung Cancer (lung cancer)	• Lung biopsy average cost ($14,600) driven by complication rate (19.3%) in Medicare population	Health Econ
Raghav et al. 2016 Oncotarget (advanced colorectal carcinoma)	• 22% of anti-EGFR inhibitor-resistant metastatic colorectal cancer patients *MET* amplified, but not treatment-naive or *RAS* mutant	CV

Note: Most Clinical Utility Studies are actual clinical practice (outside of an investigational setting)

Guardant360 Publications (7-11)

Author/Journal (Year)	Key Findings	AV, CV, CU
Liang et al. 2015 **Breast Cancer Res Treat** (metastatic breast cancer)	• 100% specificity for *ERBB2* (HER2) amplification • 86% clinical response rate	CV, CU
Schwaederlé et al. 2015 **Oncotarget** (advanced pan-cancer)	• 54-gene/3-gene amp test version, 69% were actionable w/ FDA-approved drug • Mean turnaround 13 days (95%CI 12-13), N=171)	CV, CU
Piotrowska et al. 2016 **J Thor Oncol** (non-small cell lung cancer (NSCLC))	• Case report of *EGFR* T790M Guardant360 “rescue” and response when biopsy tissue was insufficient for genotyping	CV, CU
Schwaederlé et al. 2016 **Clin Canc Res** (advanced pan-cancer)	• CtDNA-targeted patients with response or stable disease in 5 of 12 evaluable patients (42%) superior to 2 of 28 unmatched patients (7.1%), p=0.02	CV, CU
Villaflor et al. 2016 **Oncotarget** (advanced NSCLC)	• 1st clinic-based NSCLC cohort, 83% had ctDNA detected • Tissue not genotyped in 54% • Six (11%) of all ctDNA positive patients had *EGFR* mutations (two were T790M), one initial EGFR driver was found in plasma but not tissue • 8 responded (75%) & 2 stable – for 100% disease control rate • Median PFS of 11.5 months in the expected range based on published studies using tissue genotyping	CV, CU

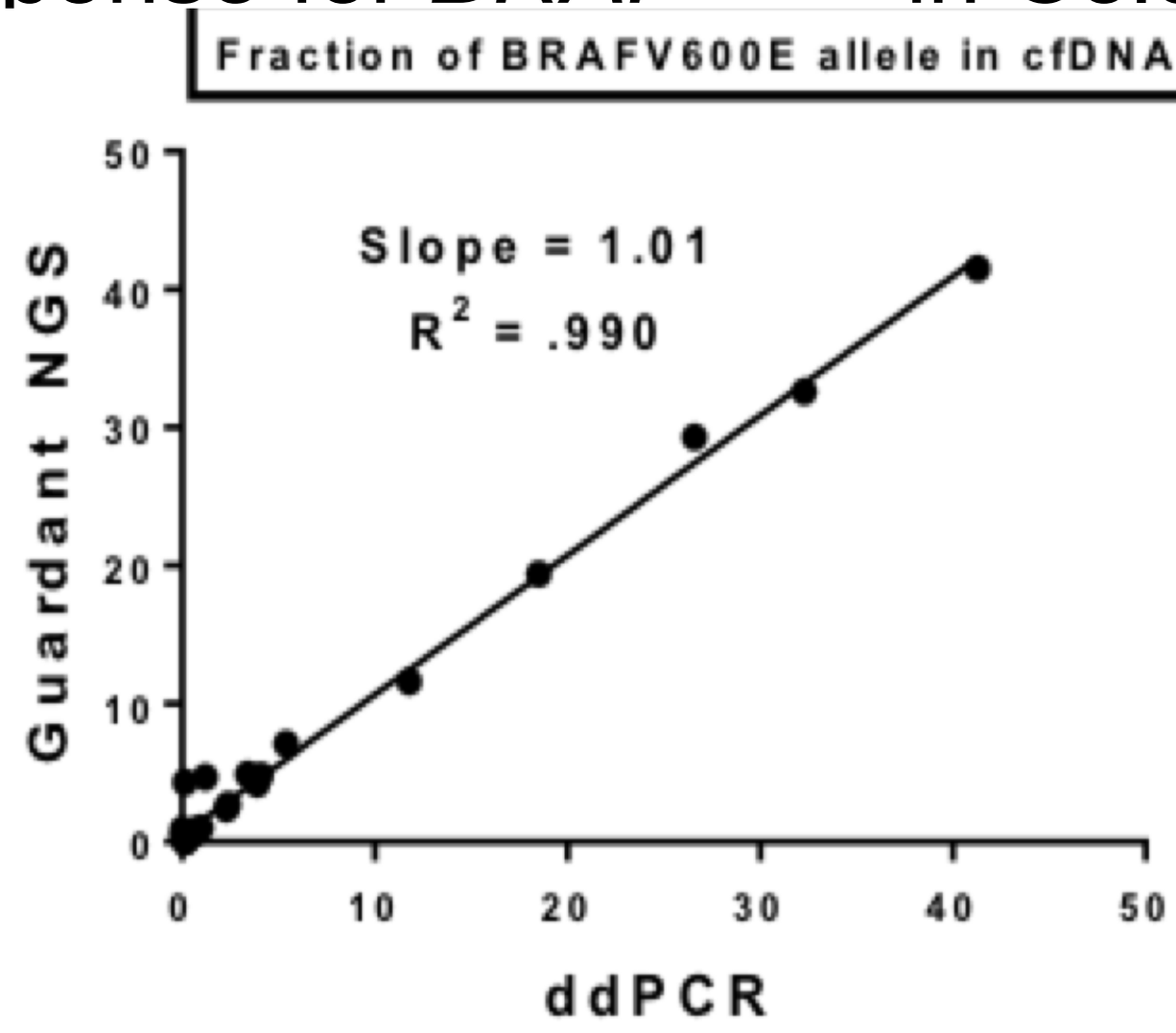
Note: Most Clinical Utility Studies are actual clinical practice (outside of an investigational setting)

Guardant360 Publications (12-14)

Author/Journal (Year)	Key Findings	AV, CV, CU
Thompson et al. 2016 Clinical Cancer Research (advanced NSCLC)	• Prospective clinic-based NSCLC cohort (N=102), 53 1st line, 47 at progression (≥ 2nd line) • Tissue not genotyped in 51% – 7 not biopsy-able and 45 QNS • CtDNA positive in 84.3%, including 8 with *EGFR* T790M where tissue failed • 31% had on-label Rx, 55% off-label and 70% were trial eligible	CV, CU
Hong et al. 2016 Cancer Discovery *In Press* (advanced colorectal cancer (mCRC))	• Prospective combination therapy study of 17 mCRC patients with *BRAF* V600E: 35% response rate (RECIST) and 88% disease control rate - median PFS 7.7 months vs. 2.5 months • Guardant BRAF V600E correlated to plasma ddPCR: R^2 = 0.99 • *BRAF* V600E cfDNA correlated with radiographic response	CV, CU
Rozenblum et al. 2016 Journal of Thoracic Oncology *In Press* (advanced NSCLC)	• 19 tissue insufficient for tissue NGS, treatment decision was changed in 32% (6 of 19) who had NCCN genomic targets *EGFR* (2), *RET* (2), *MET* (1), *ERBB2* (HER2) (1) • Matched therapy resulted in three PR and two SD out of five treated (60% objective response, 100% disease control rate by RECIST) • All but one of the six with genomic targets (84%) were missed with local *EGFR* qPCR and *ALK* FISH only testing	CV, CU

Note: Most Clinical Utility Studies are actual clinical practice (outside of an investigational setting)

“Blood to Blood” Validation of ctDNA NGS to ddPCR and Response for *BRAF*^{V600E} in Colorectal Cancer

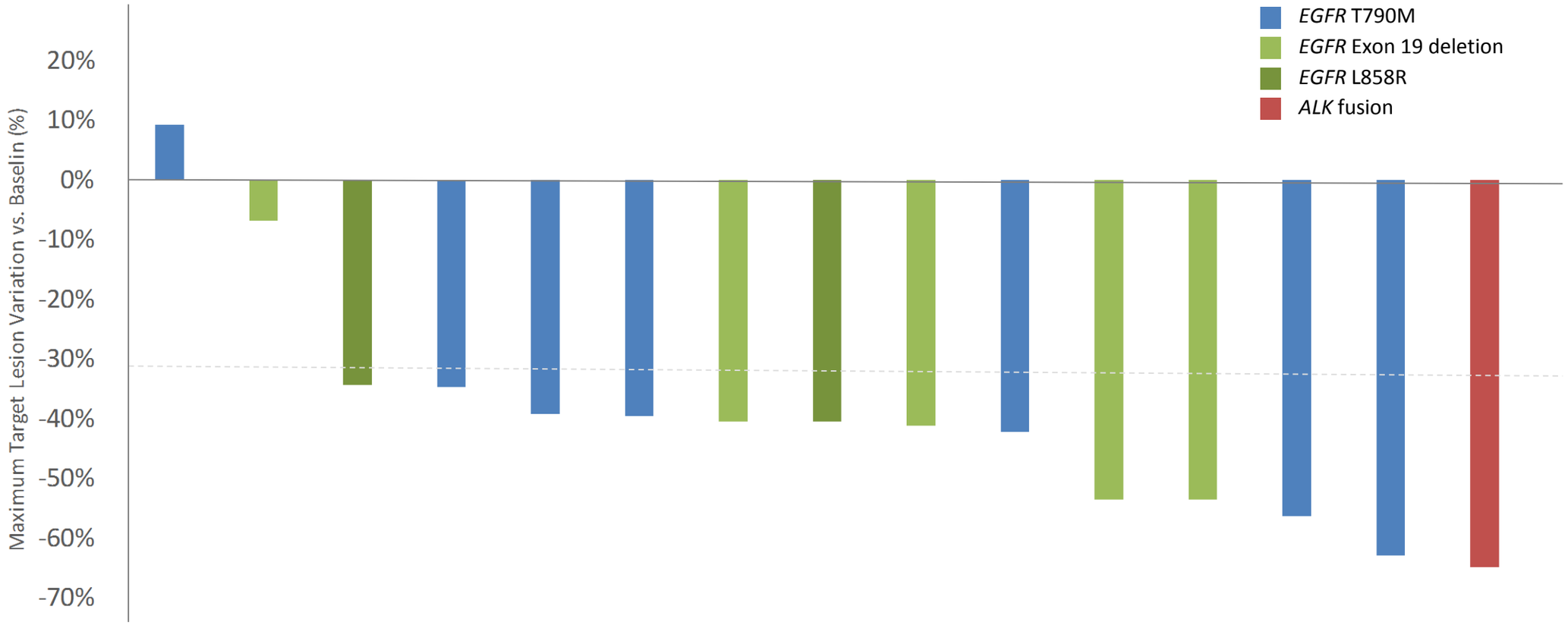


1. Six of 17 evaluable patients (35%) achieved a radiographic response by RECIST 1.1 criteria with vemurafenib, cetuximab & Irinotecan. Median progression-free survival was 7.7 months.
2. *BRAF*^{V600E} cfDNA trends correlated with radiographic changes (response and progression)
3. Acquired mutations from cfDNA in genes critical to MAPK signaling were observed at progression before their first restaging scan.

Hong et al. 2016 *Cancer Discovery In Press*

Prospective cfDNA-based Matching Trial, an Interim Analysis: NEXT-2 NSCLC

87% Response Rate and 100% Disease Control Rate



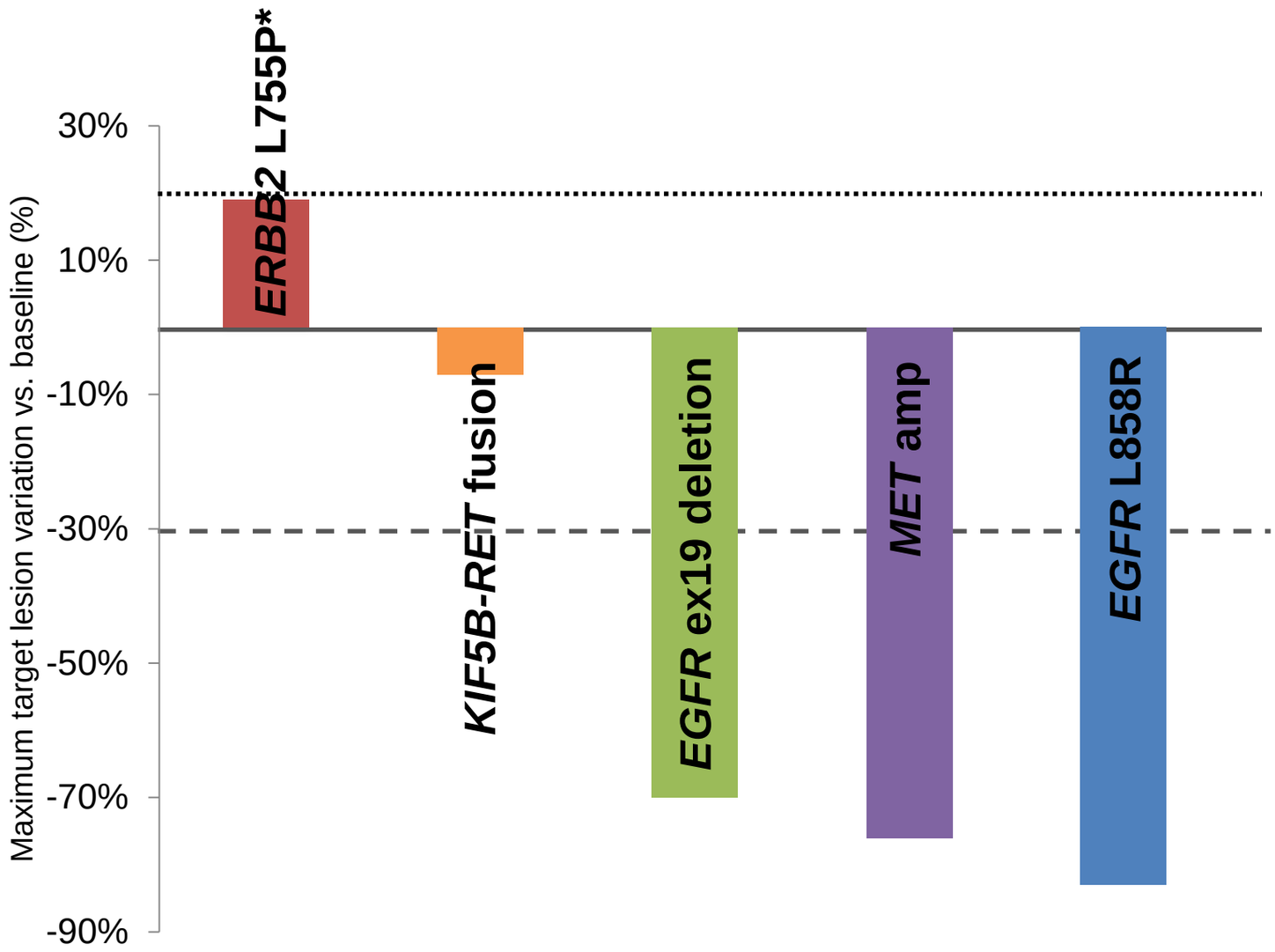
Matched Therapy	afatinib + IGF ligand MAb	rociletinib	gefitinib	olmutinib	osimertinib	olmutinib	afatinib	erlotinib	afatinib	olmutinib	afatinib	afatinib	olmutinib	osimertinib	crizotinib
Targeted Alteration % cfDNA	**6.3%**	**0.5%**	**0.2%**	**3.2%**	**0.6%**	**4.8%**	**0.8%**	**3.8%**	**0.1%**	**12.7%**	**0.1%**	**0.2%**	**40.6%**	**1.5%**	**0.1%**
ECOG PS	1	1	1	1	1	1	1	1	1	2	1	1	1	1	2
Line of Therapy	2	1	1	2	2	2	1	3	1	9	1	1	3	2	2
Best Response (RECIST 1.1)	9%	-7%	-35%	-35%	-39%	-40%	-41%	-41%	-41%	-42%	-54%	-54%	-56%	-63%	-65%

Abbreviations: IGF, insulin-like growth factor; MAb, monoclonal antibody; cfDNA, cell-free DNA

Kim et al. 2016 ASCO Abstract *J Clinical Oncology* 34;15_suppl

Treatment Decision Changed in 32% (6 of 19) of NSCLC with Biopsy Insufficient for Tissue NGS

Local EGFR & ALK Testing Missed 83% (5 of 6) ctDNA-Detected Alterations



Matched therapy	Trastuzumab	Cabozantinib	Afatinib	Crizotinib	Afatinib
Alteration AF or Copy Number*	4.1%	0.1%	0.4%	35^	1.0%
Line of Therapy	2	3	1	3	2

Rozenblum et al. 2016 Journal of Thoracic Oncology Note: 6th patient with CCDC6-RET fusion not yet evaluated
* Clinically stable disease; ^copy number in plasma

GUARDANT HEALTH

Prospective cfDNA-based Matching Trial, an Interim Analysis: NEXT-2 Gastric Carcinoma

67% Response Rate and 100% Disease Control Rate

Matched Therapy	lapatinib	taxol + AKT1i	FGFR2b MAb	capecitabine + oxaliplatin + lapatinib	taxol + AKT1i	capecitabine + oxaliplatin + lapatinib	IND	capecitabine + oxaliplatin + lapatinib	capecitabine + oxaliplatin + lapatinib
ctDNA amp	**6.81**	**N/A**	**3.92**	**5.08**	**N/A**	**10.43**	**10.28**	**2.55**	**12.15**
ECOG PS	1	1	1	1	1	1	1	1	1
Line of Therapy	3	2	6	1	2	1	1	1	1

Abbreviations: AKT1i, AKT1 inhibitor; MAb, monoclonal antibody; ctDNA, circulating tumor DNA; IND, investigational new drug

Kim et al. 2016 ASCO Abstract *J Clinical Oncology* 34;15_suppl

86% Response Rate to ctDNA-Detected *ERBB2* (HER2) Amplification in Metastatic Breast Cancer

Patient *ERBB2* Amplified in plasma	Tissue HER2+ Confirmed by IHC and/or FISH	Treatment	Response
23.6	✓	paclitaxel/trastuzumab/pertuzumab	✓
3.9	✓	paclitaxel/trastuzumab/pertuzumab	✓
7.1	✓	paclitaxel/trastuzumab/pertuzumab	✓
2.3	✓	trastuzumab/pertuzumab	✓
2.7	✓	trastuzumab/emtansine/lapatinib	✓
8.6	✓	trastuzumab/emtansine/lapatinib	✓
2.7	✓	trastuzumab emtansine monotherapy	✗
Total	**100% Concordant**		**86% Response Rate**

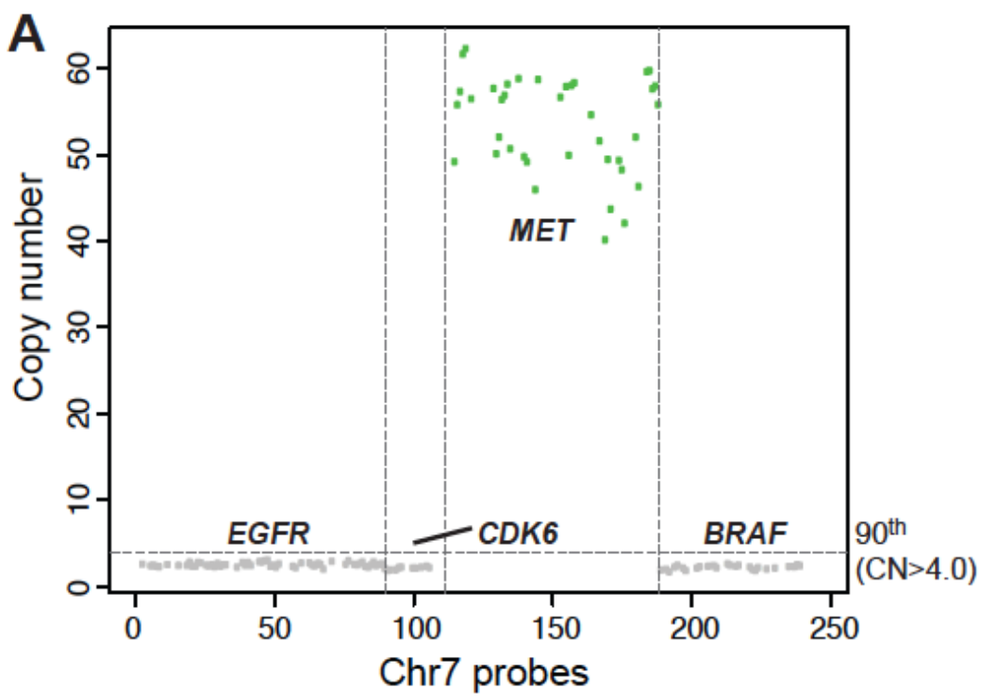
Liang et al. 2015 *Breast Cancer Research & Treatment*

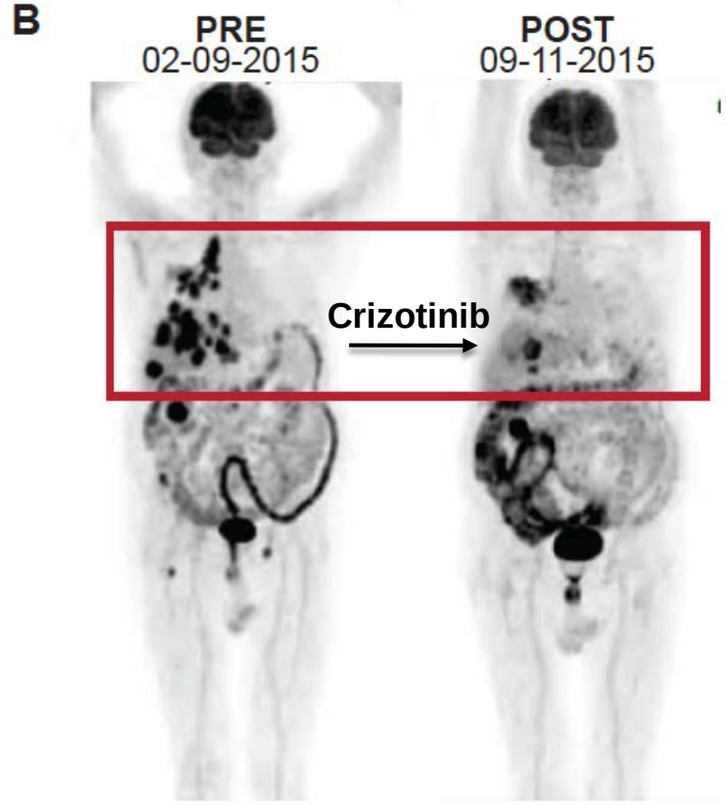
Case Three: *MET* Gene Amplification in NSCLC

Summary of Alterations & Associated Treatment Options

The percentage, or allele frequency, of altered circulating cell-free DNA (% cfDNA) in blood is related to the unique tumor biology of this patient. Factors that may affect the amount/ percentages of detected genomic alterations in circulating cell-free DNA in blood include tumor growth, turn-over, size, heterogeneity, vascularization, disease progression, or treatment.

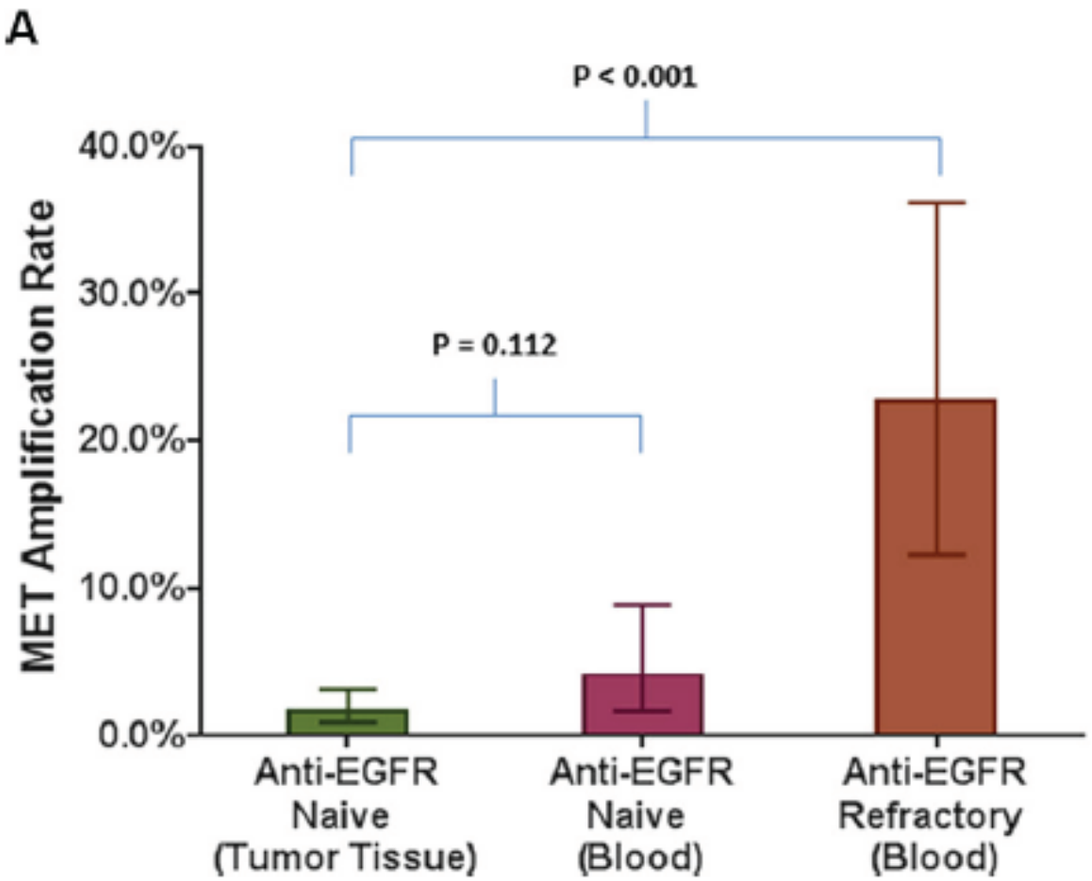
Alteration		% cfDNA	cfDNA Amplification	FDA Approved in Indication	Available for Use in Other Indications	Clinical Drug Trials
MET	*AMP*		+++	None	Cabozantinib, Crizotinib	Trials Available





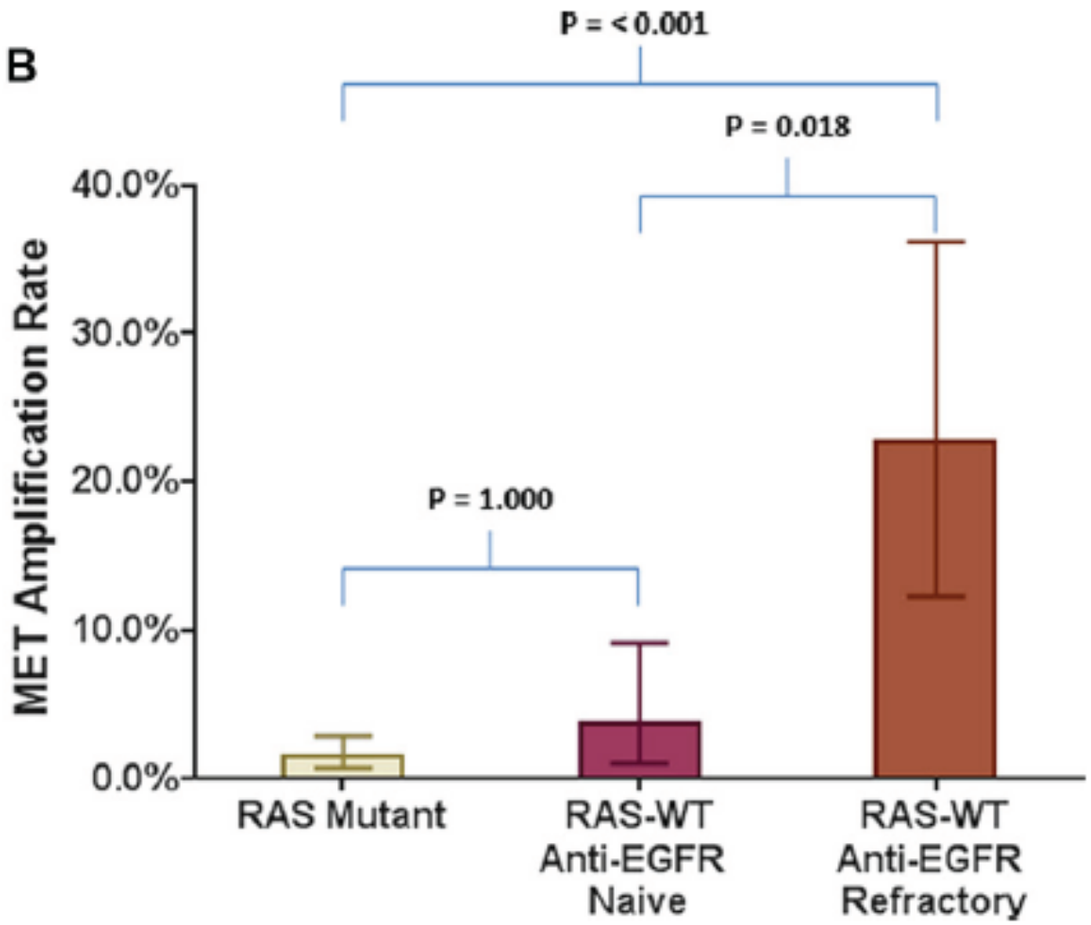
Courtesy Nir Peled MD, Sheba Medical Center, Tel Aviv University

MET Amplification: a Function of anti-EGFR Therapy in Metastatic Colorectal Cancer



Raghav (Kopetz) et al. 2016 *Oncotarget*

MET Amplification: An Actionable Resistance Target in Metastatic Colorectal Cancer (ctDNA)



Raghav (Kopetz) et al. 2016 *Oncotarget*

“Comprehensive” Means Fusions & Amps Too!

“plasma NGS is now moving into the clinical space and can make accurate and precise calls”

Table. Clinically Available Assays for Genotyping of Plasma Cell-Free DNA (cfDNA)

	PCR Assays		NGS Assays	
Characteristic	Allele-Specific PCR	Emulsion PCR	Amplicon-Based Targeted NGS	Capture-Based Targeted NGS
Variants potentially detected	Known recurring mutations	Known recurring mutations	Any exonic mutations, copy number gains	Exonic mutations, intronic gene fusions, copy number gains
Quantitation	Semiquantitative (against standard curve)	Absolute or relative quantitation, wide dynamic range	Quantitation of relative AF, but vulnerable to PCR amplification bias	Quantitation of relative AF
Speed and complexity	Rapid, relatively easy to interpret	Rapid, relatively easy to interpret	Potentially rapid, less complex bioinformatics	Potentially slower, more complex bioinformatics
Examples	Cobas (Roche); therascreen (Qiagen)	Droplet digital PCR (Biorad); BEAMing (Sysmex Inostics)	Tam-seq (Inivata)	Guardant360 (Guardant); cancerselect (Personal Genome Diagnostics)

Oxnard et al. 2016 *J Clinical Oncology*

Should the Hurdle for Clinical Utility for a Diagnostic be Higher Than for a Matched Therapy Drug?

"*High ORR (e.g. statistically exceeding an ORR of 30%) is an appropriate end point for single-arm trials aiming to demonstrate breakthrough activity of a single-agent anticancer therapy*"

Table. A Range of Statistical End Points for Single-Arm Trials and the Estimated Test Characteristics for Their Ability to Predict for Regulatory Approval

Percentage (95% CI)				
ORR	Sensitivity	Specificity	PPV	NPV
Single-Agent Therapies				
10	70 (49-87)	86 (74-94)	68 (46-85)	88 (76-95)
15	67 (45-84)	91 (81-97)	76 (53-92)	87 (75-94)
20	50 (29-71)	91 (81-97)	71 (44-90)	81 (70-90)
25	42 (22-63)	95 (85-99)	77 (46-95)	79 (68-88)
30	33 (16-55)	98 (91-100)	89 (52-100)	78 (66-87)
35	33 (16-55)	98 (91-100)	89 (52-100)	78 (66-87)
40	29 (13-51)	98 (91-100)	88 (47-100)	77 (65-86)
45	25 (10-47)	100 (94-100)	100 (54-100)	76 (65-85)
50	13 (3-32)	100 (94-100)	100 (29-100)	73 (62-82)
55	13 (3-32)	100 (94-100)	100 (29-100)	73 (62-82)
60	13 (3-32)	100 (94-100)	100 (29-100)	73 (62-82)

Oxnard et al. 2016 *JAMA Oncology*

GUARDANT HEALTH

31% of Lung Adenocarcinoma is Targetable

NCCN Genomic Targets: EGFR, BRAF, MET, ERBB2 (HER2), ALK, ROS1, RET