Clinical applications of cell-free DNA (cfDNA) genotyping for cancer care

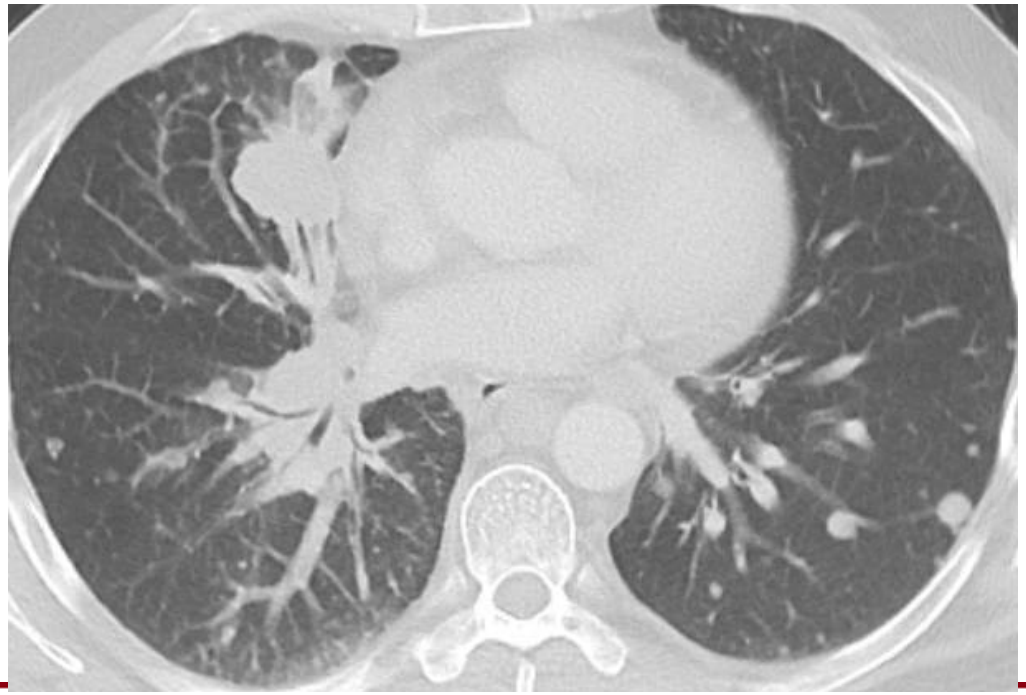
Geoffrey R. Oxnard, MD
Damon Runyon-Gordon Family Clinical Investigator
Assistant Professor of Medicine
Dana-Farber Cancer Institute

Disclosures

- Consulting fees or honoraria from AstraZeneca, Ariad, Boehringer-Ingelheim, Clovis, Chugai, Genentech, Inivata, Sysmex
- Ongoing research collaborations with multiple pharma and biotech partners in this space

Case

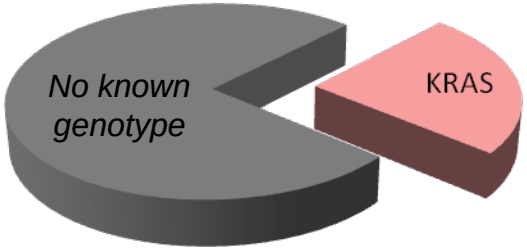
- 49 yo M never-smoker p/w several weeks of cough, headache
  - Chest CT shows adenopathy, pulm nodules



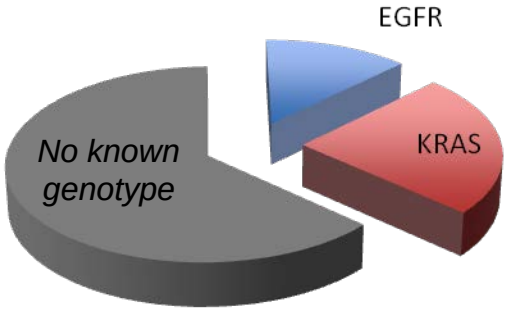
Case

- 49 yo M never-smoker p/w several weeks of cough, headache
  - Chest CT shows adenopathy, pulm nodules
  - Brain MRI with 8mm cerebellar lesion, cannot rule out lepto
  - Supraclav biopsy shows NSCLC
- Presents to oncology 4 days post-biopsy
  - Path not yet finalized, genomics not started

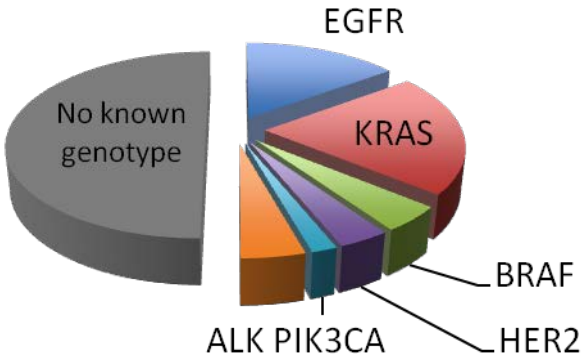
Lung cancer genotyping



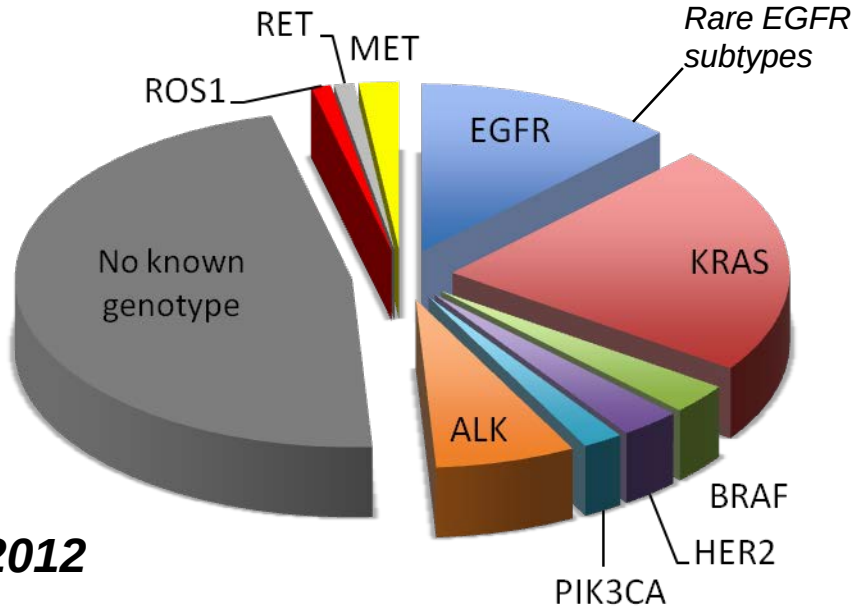
1984 - 2003



2004



2009



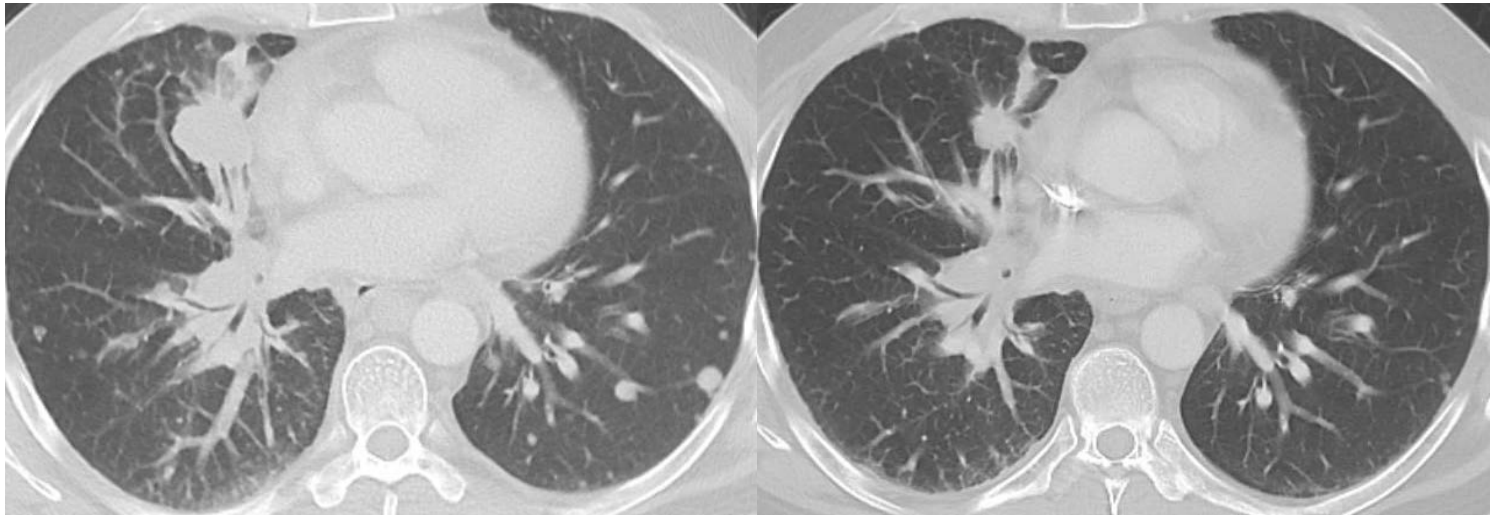
2012

Case

- 49 yo M never-smoker p/w stage IV NSCLC metastatic to brain
  - Tumor genotyping pending
- Plasma genotyping of EGFR ordered
  - Seen on a Monday, blood drawn that day
  - Results reported on Wednesday
  - EGFR L858R detected at 34% AF

Case

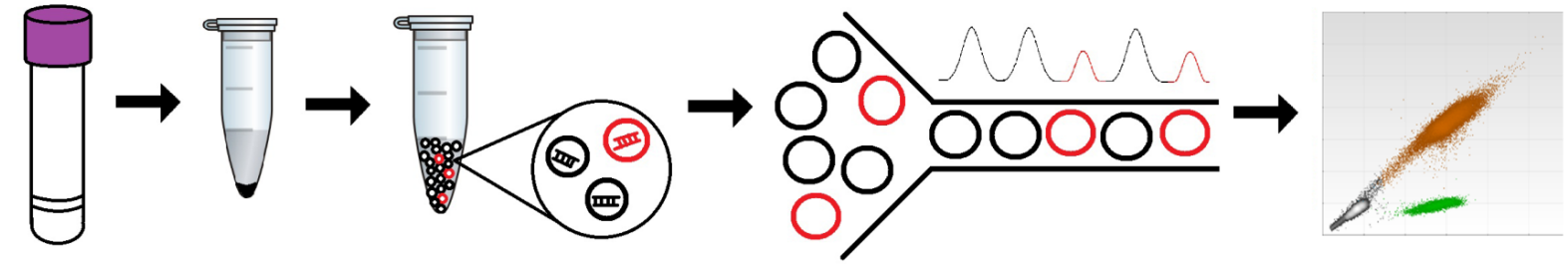
- 49 yo M never-smoker p/w stage IV NSCLC metastatic to brain
- Erlotinib initiated, patient symptoms rapidly improve



Baseline *2 months*

Plasma ddPCR

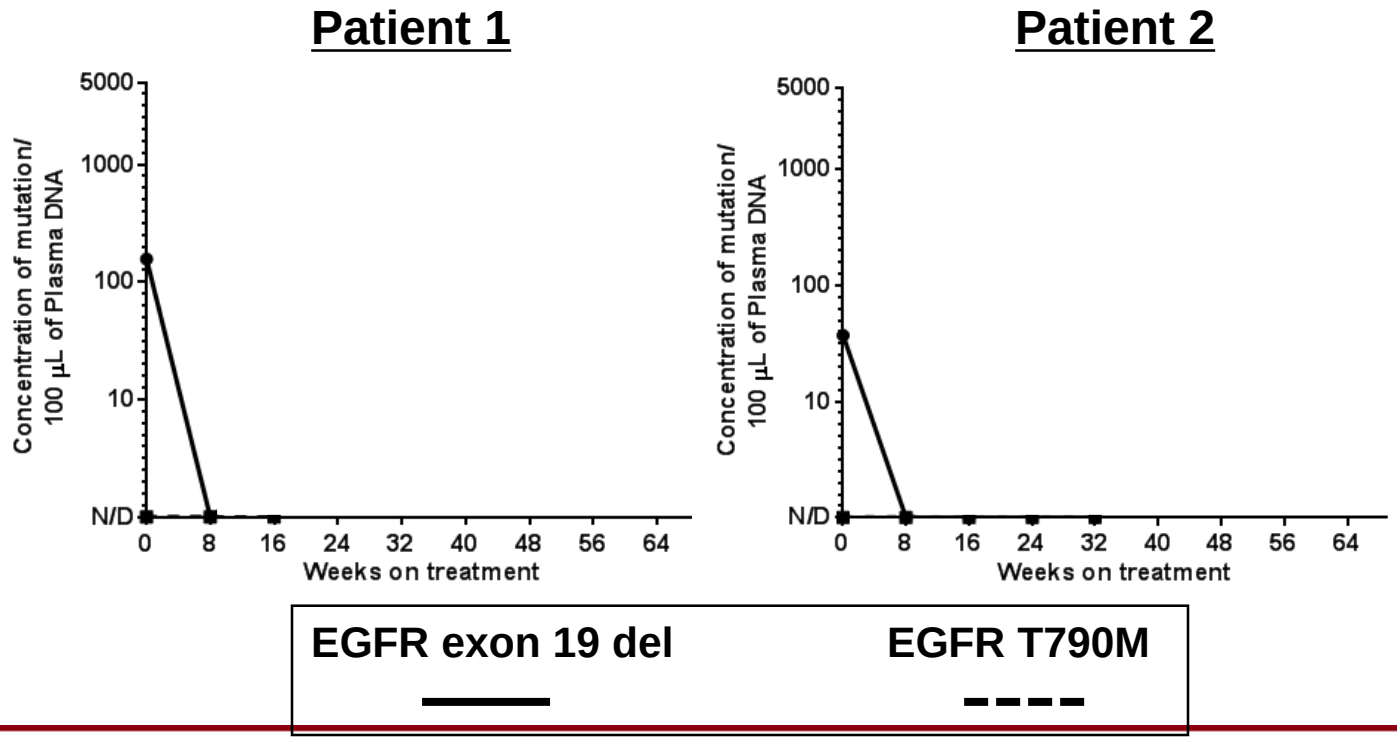
- Droplet digital PCR is a highly sensitive, quantitative assay for detection of hotspot mutations (e.g. EGFR, KRAS, BRAF, PIK3CA)
  - 20,000 droplets generated each carrying mutant or wildtype DNA



Oxnard & Paweletz et al, CCR, 2014

Plasma ddPCR

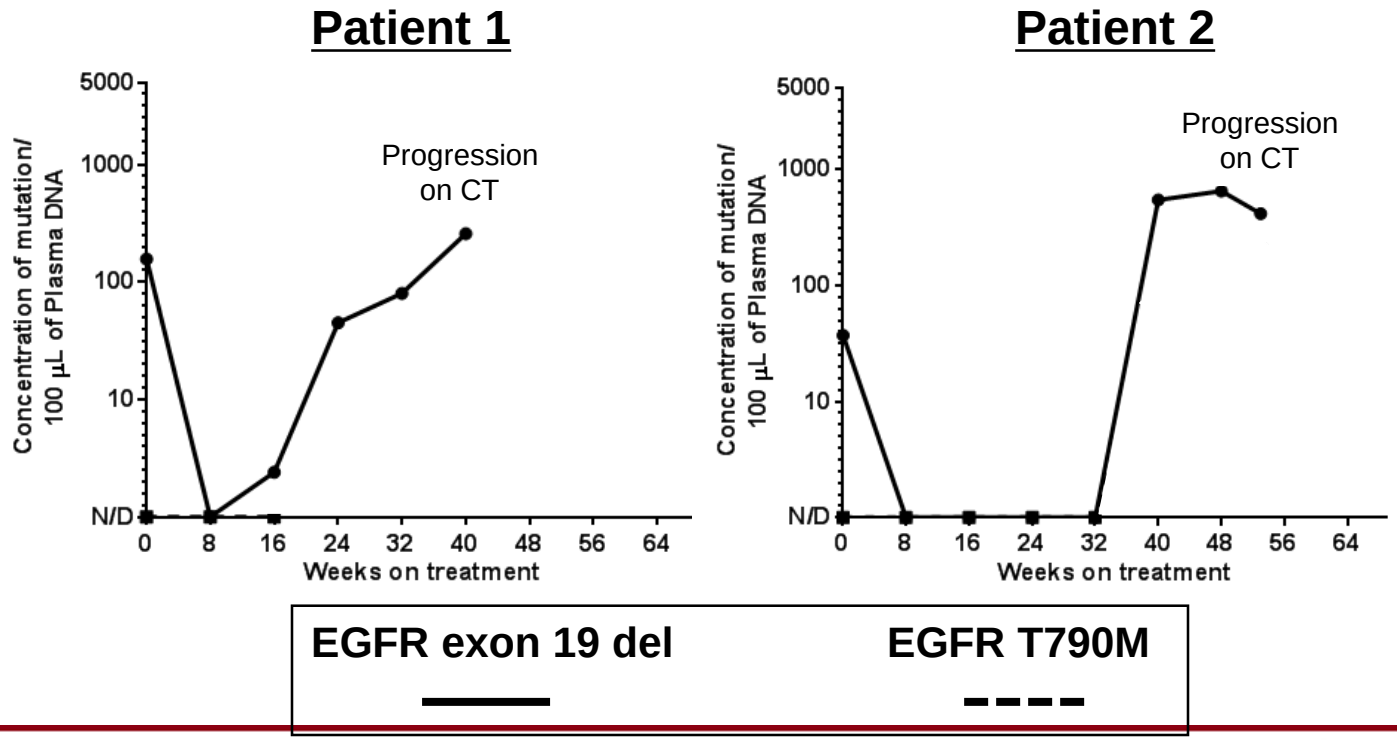
- In EGFR-mutant NSCLC, ddPCR can detect response and resistance



Oxnard & Paweletz et al, CCR, 2014

Plasma ddPCR

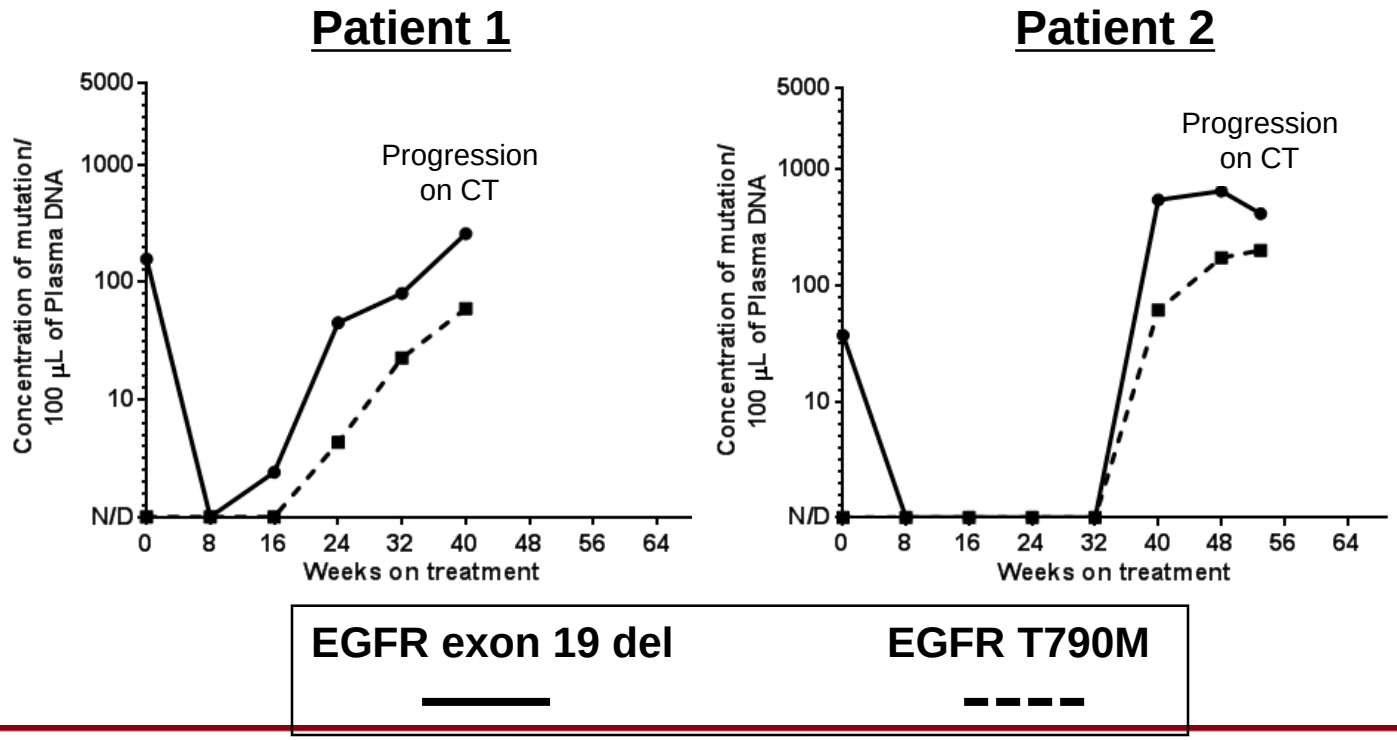
- In EGFR-mutant NSCLC, ddPCR can detect response and resistance



Oxnard & Paweletz et al, CCR, 2014

Plasma ddPCR

- In EGFR-mutant NSCLC, ddPCR can detect response and resistance



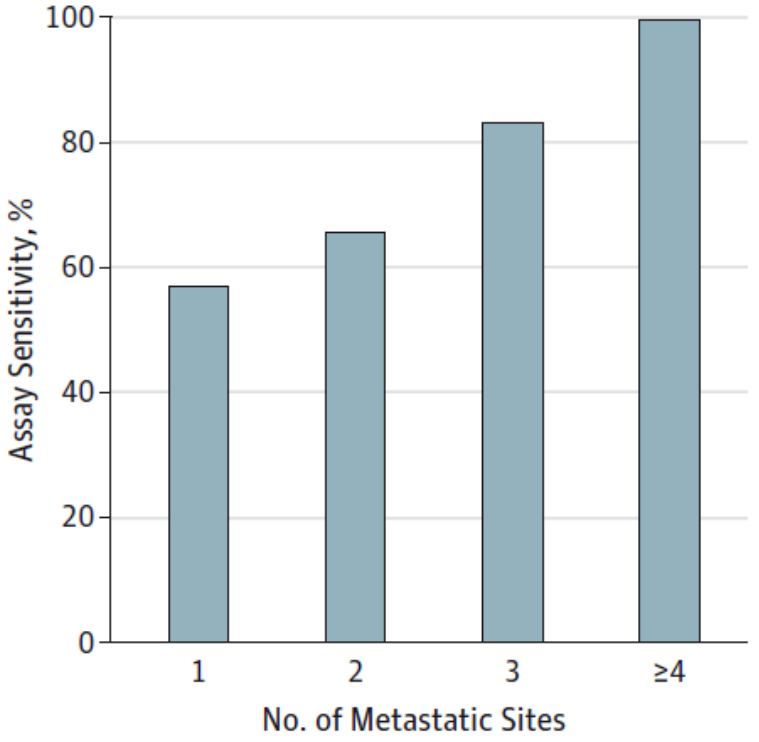
Oxnard & Paweletz et al, CCR, 2014

Plasma genotyping

- Several clinical applications to consider:
  1. Cancer genotyping at initial therapy
  2. Cancer genotyping at resistance
  3. Assessment of response / PD effect
  4. Cancer screening / diagnosis

Lung cancer genotyping

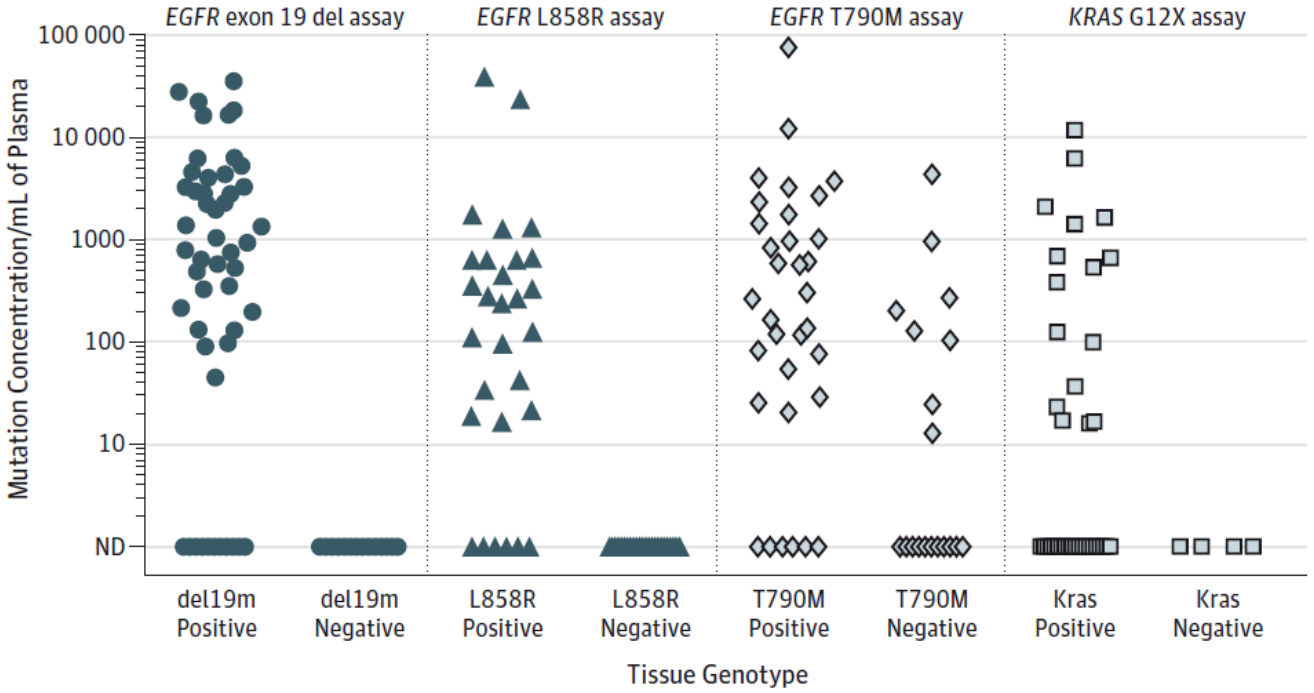
- We recently completed a prospective validation of plasma ddPCR in 180 patients with NSCLC
  - Overall sensitivity of 64-82% for detection of known tumor genotype
  - Rate of detection increases with increased tumor burden
  - 3-day TAT



Sacher et al, JAMA Onc, 2016

Lung cancer genotyping

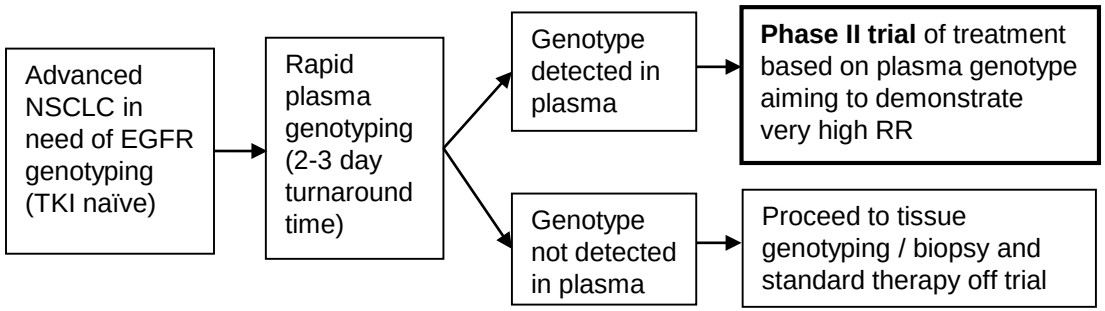
- We recently completed a prospective validation of plasma ddPCR in 180 patients with NSCLC
  - 100% specificity (0% FPR) for driver mutations
  - 63% specificity for T790M resistance mutation



Sacher et al, JAMA Onc, 2016

Lung cancer genotyping

- Now using our validated plasma ddPCR assay as a CLIA test at BWH
- DFCI has launched a clinical trial of plasma EGFR genotyping for rapid initiation of erlotinib (NCT02770014)



Lung cancer genotyping

- Now using our validated plasma ddPCR assay as a CLIA test at BWH
- DFCI has launched a clinical trial of plasma EGFR genotyping for rapid initiation of erlotinib (NCT02770014)
- This is the setting in which the FDA approved the Cobas plasma assay as a screening test, with reflex to tumor analysis if negative

NGS of plasma cfDNA

Strengths of ddPCR genotyping

- Rapid
- Quantitative
- Inexpensive

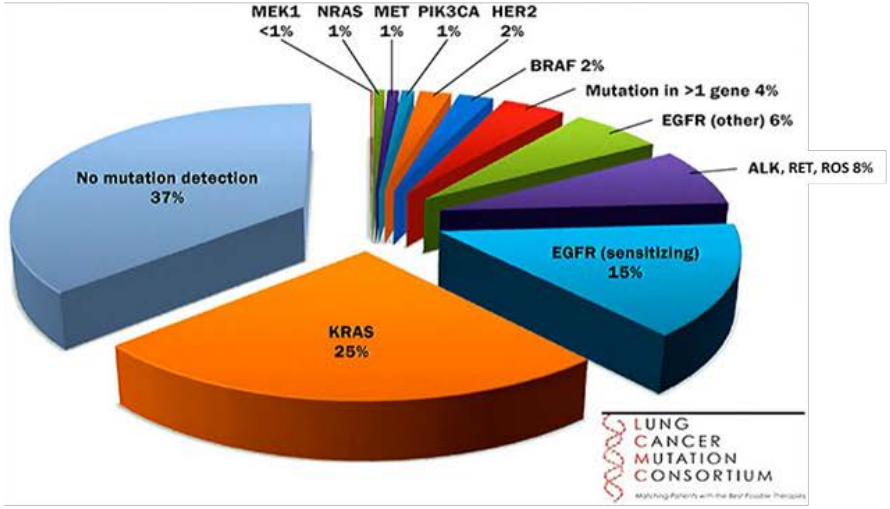
Limitations of ddPCR genotyping

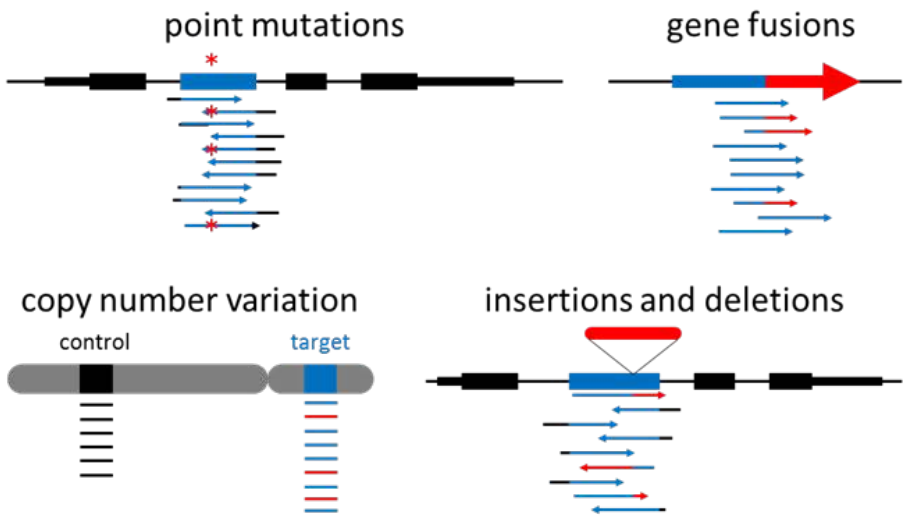
- Only tests for known genotypes
- Difficult to multiplex
- Cannot detect rearrangements

Paweletz et al, CCR, 2015

NGS of plasma cfDNA

- Using NGS of plasma, one may detect all targetable alterations in lung cancer rapidly and noninvasively



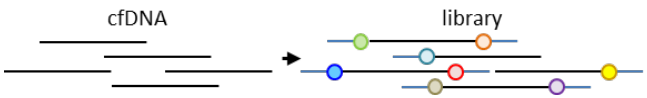


Challenges of advanced genomics in plasma

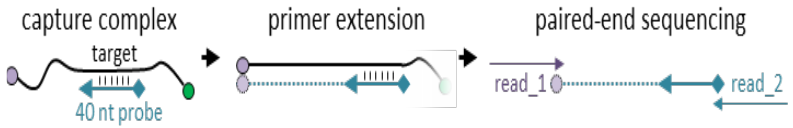
- **Small quantities**
- **Mostly germline**
- **Fragmented**

NGS of plasma cfDNA

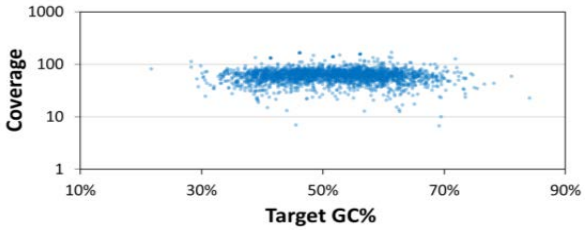
- Ultra-high-efficiency cloning of cfDNA with tagged adaptors



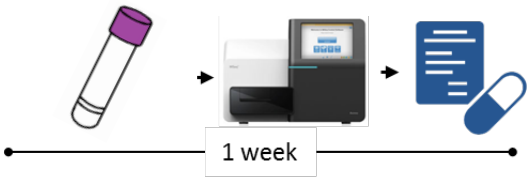
- Methods that yield on-target rates >90% and generate “primer-indexed” reads



- Proprietary nucleic acids chemistry that neutralizes GC bias



- Synergies between chemistry and molecular biology reduce sequencing demands and turnaround times



Paweletz et al, CCR, 2015

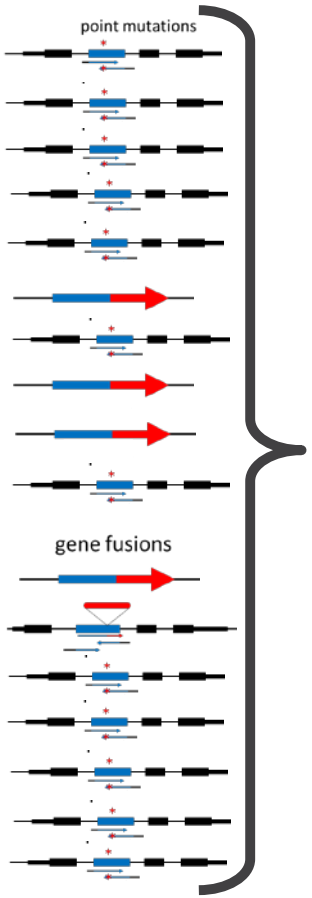
NGS of plasma cfDNA

Actionable genes

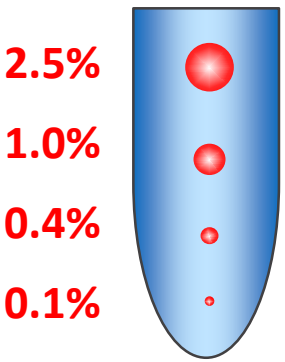
ALK
BRAF
EGFR
ERBB2
KRAS
MAP2K1
cMET
NRAS
PIK3CA
RET
ROS

Cell line validation

Cell line	Gene	Mutant allele
A549	KRAS	G12S
H1666	BRAF	G466V
H1781	ERBB2	G776VC
H1975	EGFR	T790M
H1975	EGFR	L858R
H2228	ALK	EML4
H2347	NRAS	Q61R
H3122	ALK	EML4
HCC78	ROS1	SLC34A2
HCT116	KRAS	G13D
HCT116	PIK3CA	H1047R
LC2	RET	CCDC6
PC9	EGFR	KELREA745K
SKMEL28	BRAF	V600E
SKMEL28	EGFR	P753S
SW48	EGFR	G719S
SW48	MAP2K1	Q56P
SW480	KRAS	G12V



Admix measurement



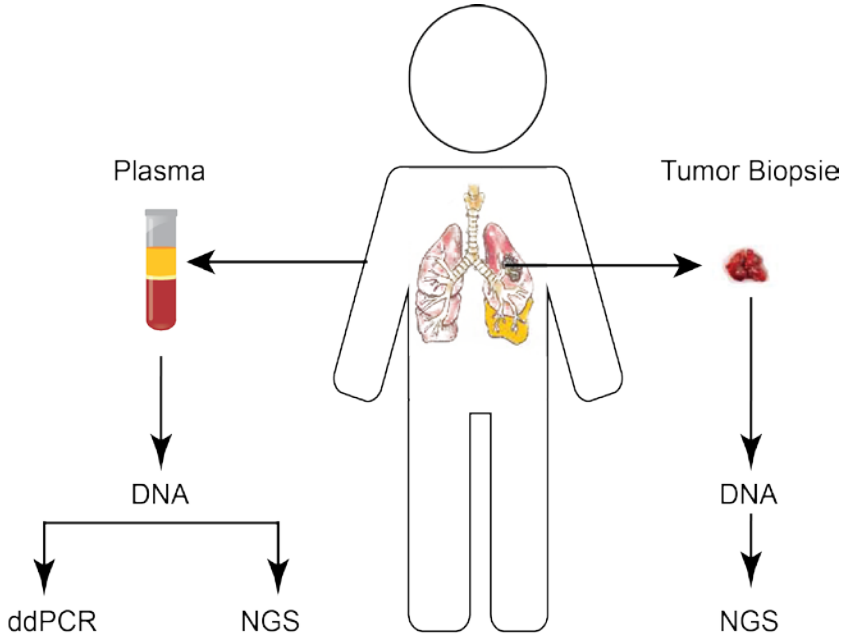
Sensitivity & Specificity

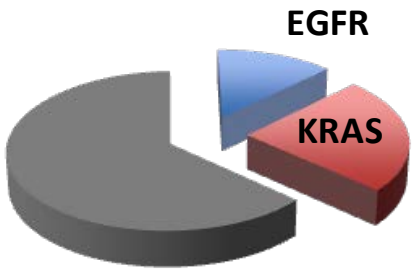
100%/100% at ≥ 1.0%

90%/100% at ≥ 0.1%

Paweletz et al, CCR, 2015

NGS of plasma cfDNA

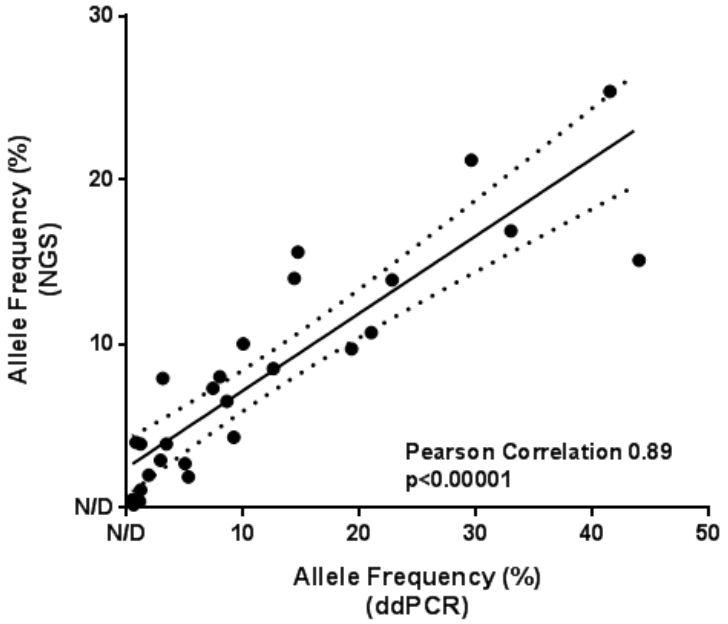




Sample	Tissue Genotype	ddPCR	NGS	GE In NGS library
105	*EGFR* del19	●	●	15733
510	*EGFR* del19	●	●	4680
053	*EGFR* del19	●	●	3619
091	*EGFR* del19	●	●	3021
081	*KRAS* G12C	●	●	2071
004	*EGFR* del19	●	●	1672
044	*EGFR* del19	●	●	1510
522	*EGFR* del19	●	●	1510
001	*KRAS* G12C	●	●	1256
011	*EGFR* del19	●	●	1087
017	*EGFR* del19	●	●	1035
039	*EGFR* del19	●	●	1001
095	*EGFR* del19	●	●	746
048	*EGFR* L858R	●	●	605
061	*KRAS* G12C	●	●	463
045	*EGFR* del19	●	●	382
028	*EGFR* L858R	●	●	382
070	*KRAS* G12C	●	●	300
008	*KRAS* G12C	●	●	289
074	*EGFR* del19	●	●	100
094	*KRAS* G12V	●	●	89
109	*EGFR* del19	●	●	17

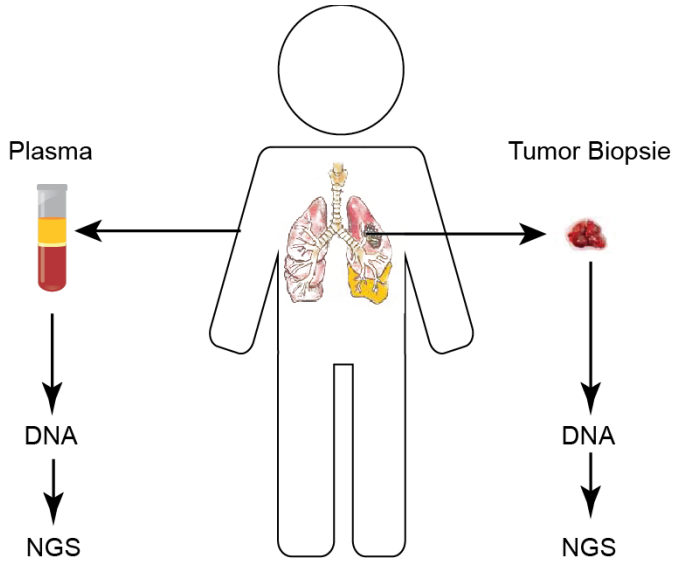
NGS of plasma cfDNA

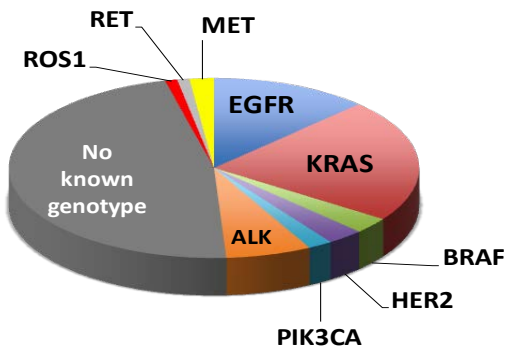
Sample	Tissue Genotype	ddPCR	NGS	GE In NGS library
105	*EGFR* del19	●	●	15733
510	*EGFR* del19	●	●	4680
053	*EGFR* del19	●	●	3619
091	*EGFR* del19	●	●	3021
081	*KRAS* G12C	●	●	2071
004	*EGFR* del19	●	●	1672
044	*EGFR* del19	●	●	1510
522	*EGFR* del19	●	●	1510
001	*KRAS* G12C	●	●	1256
011	*EGFR* del19	●	●	1087
017	*EGFR* del19	●	●	1035
039	*EGFR* del19	●	●	1001
095	*EGFR* del19	●	●	746
048	*EGFR* L858R	●	●	605
061	*KRAS* G12C	●	●	463
045	*EGFR* del19	●	●	382
028	*EGFR* L858R	●	●	382
070	*KRAS* G12C	●	●	300
008	*KRAS* G12C	●	●	289
074	*EGFR* del19	●	●	100
094	*KRAS* G12V	●	●	89
109	*EGFR* del19	●	●	17



Paweletz et al, CCR, 2015

NGS of plasma cfDNA





Sample	Tissue Genotype	NGS	GE in NGS library
127	*ROS1 fusion*	0.5	4562
018	*ALK-EML4 fusion*	4.0	4429
015	*ROS1-CD74 fusion*	0.4	3990
081	*PIK3CA* E545K	3.9	2071
036	*EGFR* G719A	6.0	1253
022	*EGFR* G719A	2.1	858
903	*Her2-neu 2311-2322 dup*	14	696
137	*RET fusion*	4.0	475
115	*KRAS* Q61L		420
089	*KRAS* G13D		312
108	*BRAF* V600E		265
904	*Her2-neu 2332-2340 dup*	8.0	181
202	*ALK-EML4 fusion*	0.5	180

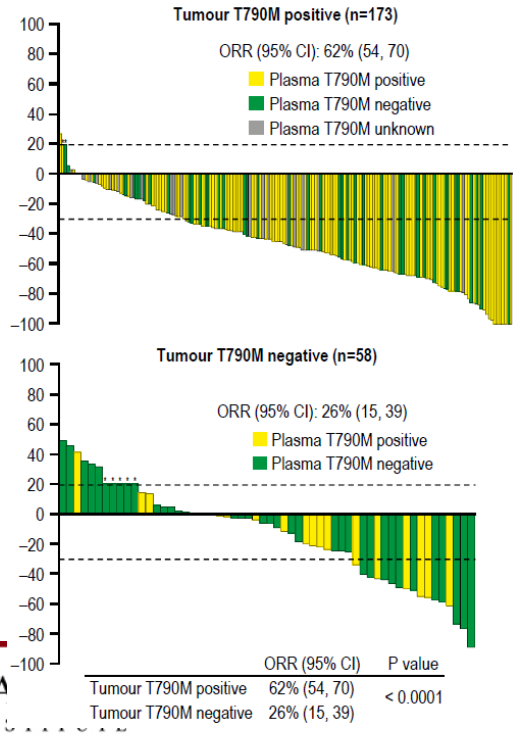
Paweletz et al, CCR, 2015

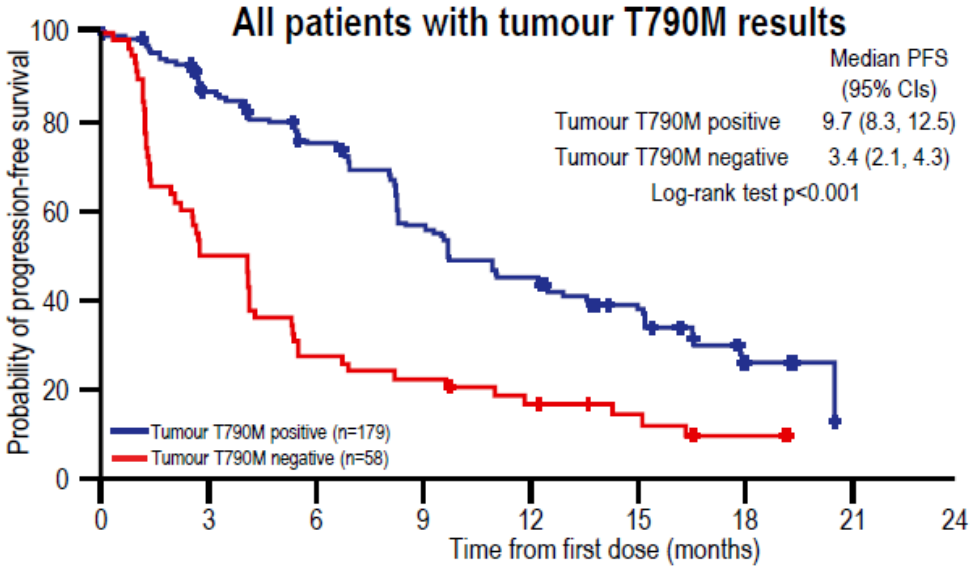
Resistance genotyping

- Osimertinib is approved in multiple countries for EGFR-mutant NSCLC with T790M+ resistance

T790M+ in tumor: 62% RR

T790M- in tumor 26% RR

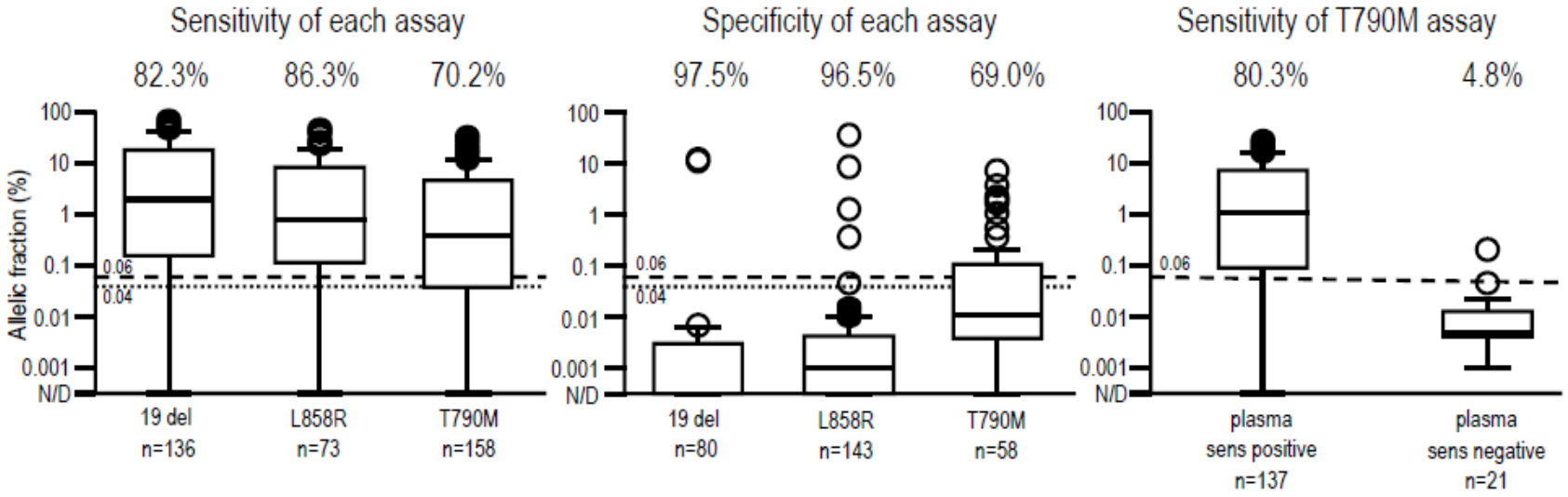




Oxnard et al, JCO, 2016

Resistance genotyping

- Plasma from phase I trial sent for BEAMing
  - Similarly found that sensitivity was 70%-86%
  - Similarly found a high specificity (>95%) for driver EGFR mutations but only 69% specificity for T790M



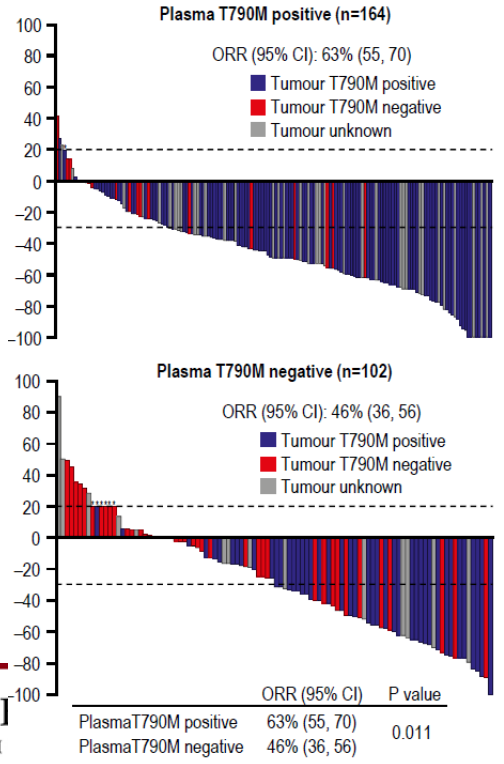
Oxnard et al, JCO, 2016

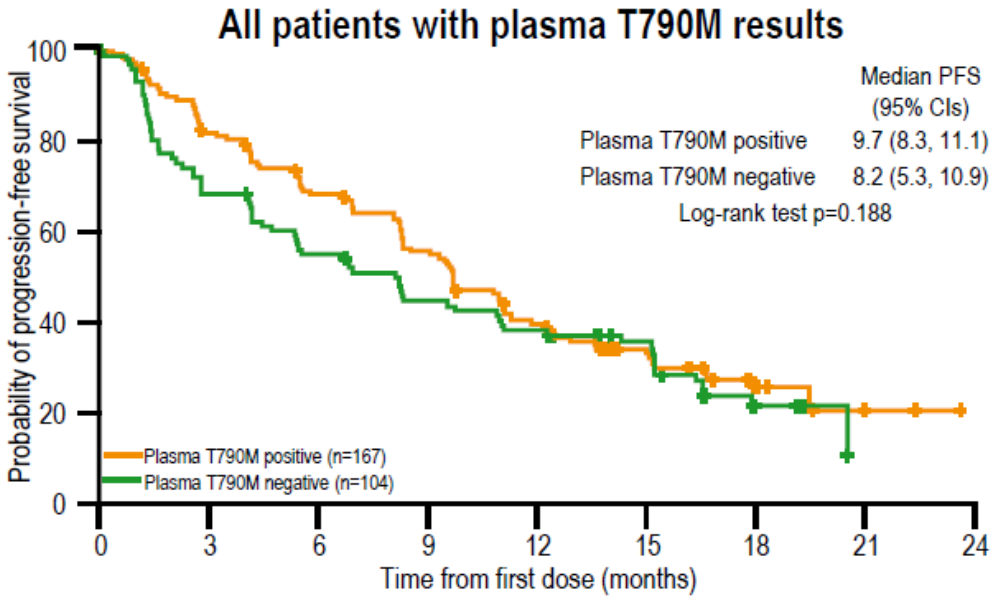
Resistance genotyping

- Plasma from phase I trial sent for BEAMing
  - Despite the false positives, plasma T790M+ cases do well, like tumor T790M+
  - But plasma T790M- cases do better than expected

T790M+ in plasma: 63% RR

T790M- in plasma 46% RR

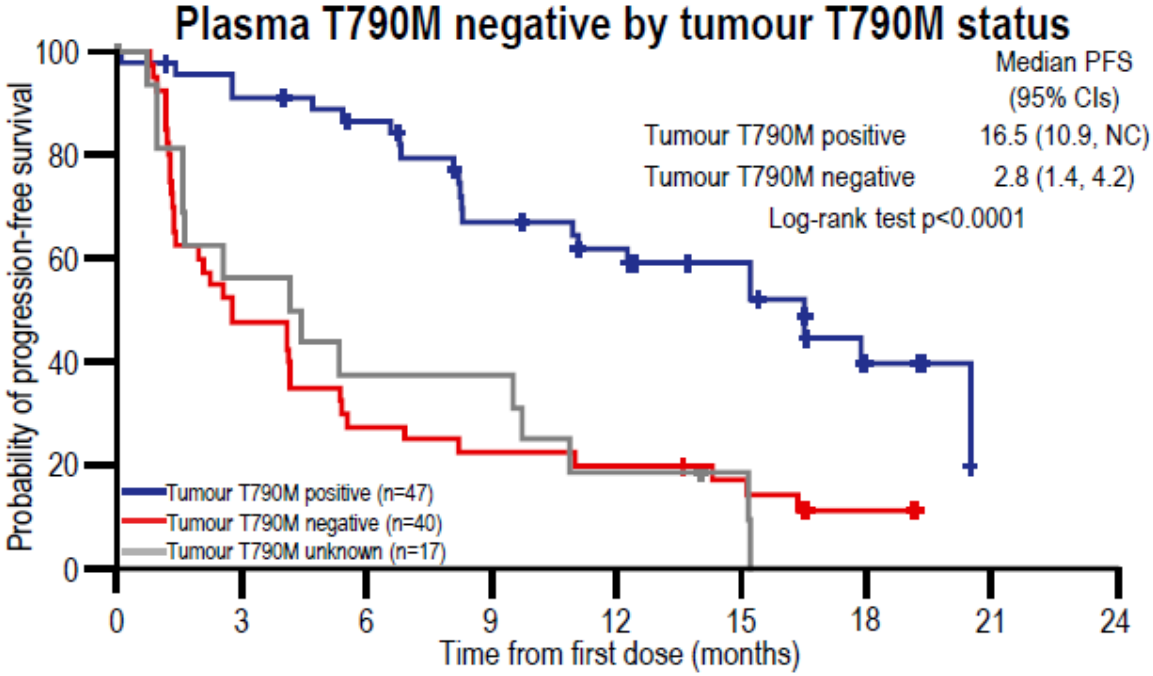




Oxnard et al, JCO, 2016

Resistance genotyping

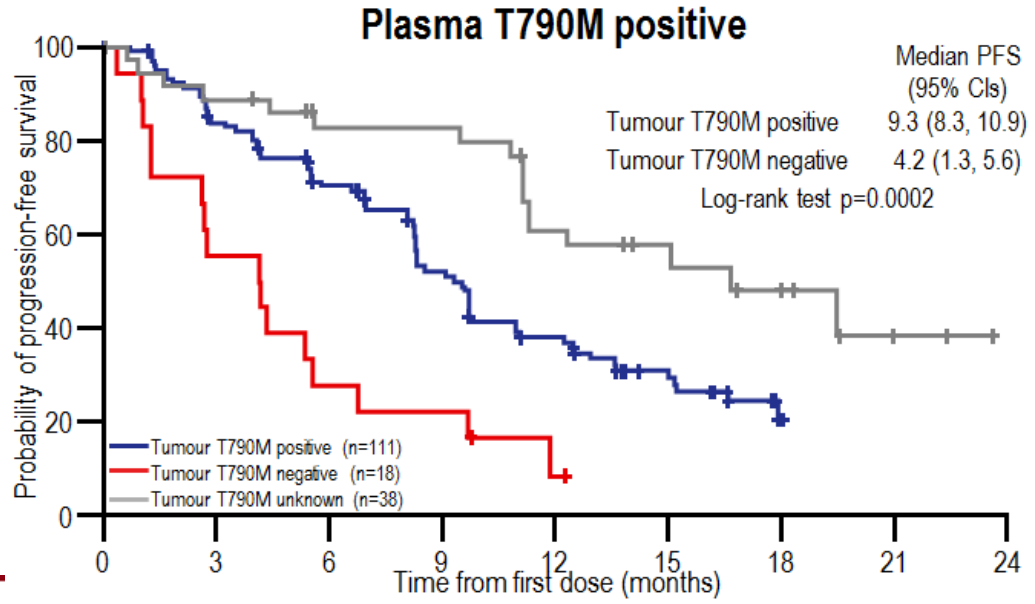
- Which is better, tumor vs plasma?
  - Tumor genotyping can clarify which plasma T790M- patients do better or worse on osimertinib



Oxnard et al, JCO, 2016

Resistance genotyping

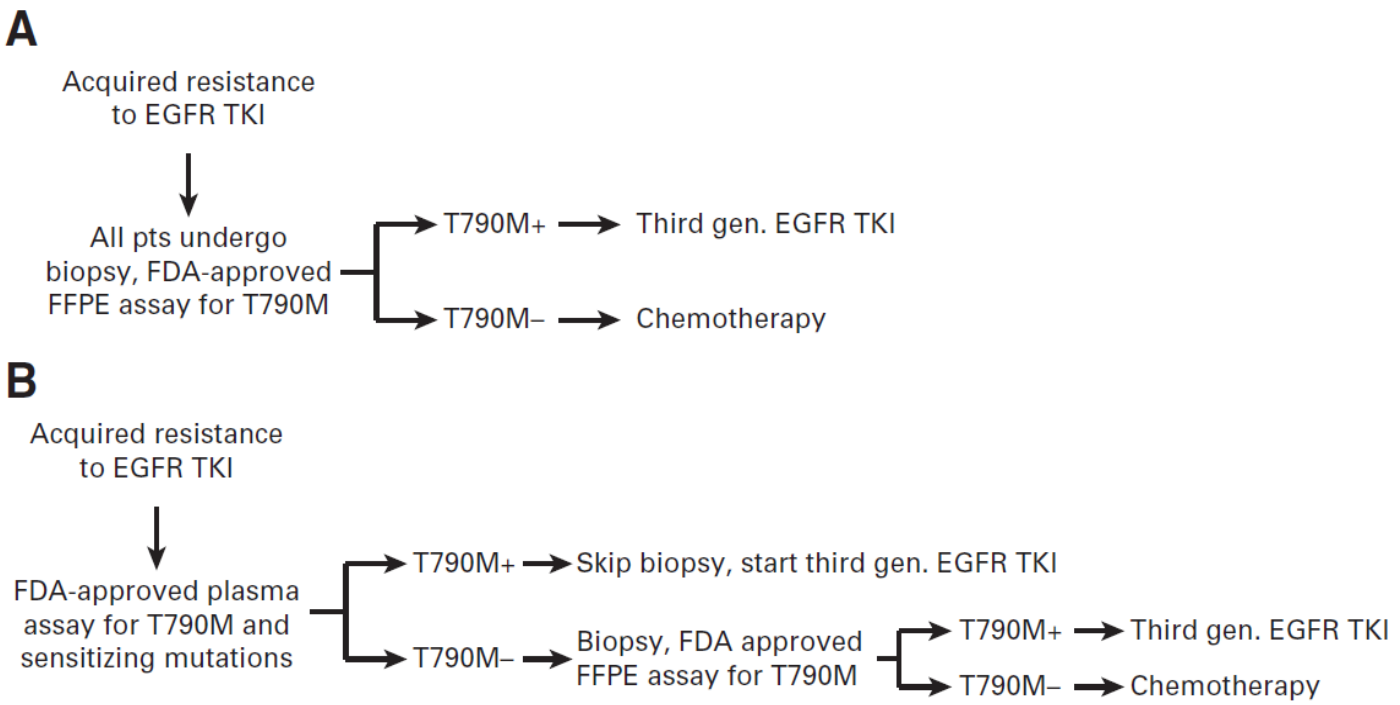
- Which is better, tumor vs plasma?
  - Tumor genotyping can clarify which plasma T790M- patients do better or worse on osimertinib
  - Tumor genotyping also clarifies which plasma T790M+ patients do better or worse on osimertinib



Oxnard et al, JCO, 2016

Resistance genotyping

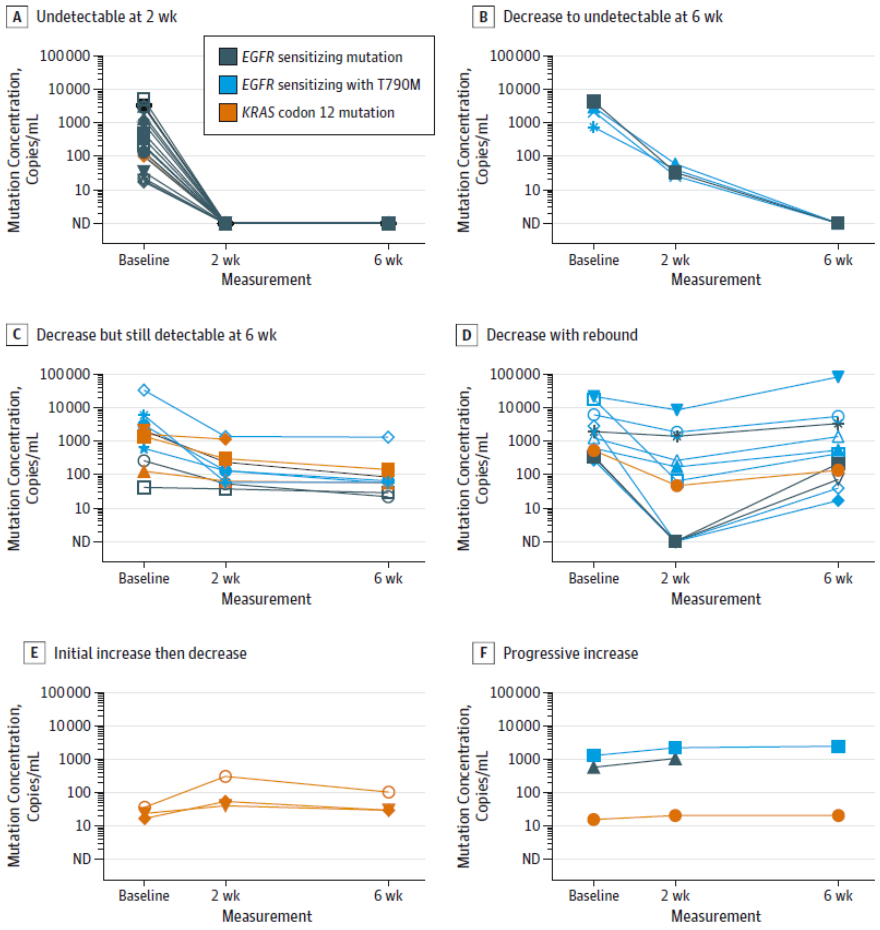
- Plasma T790M is a compelling resistance biomarker but heterogeneity is a challenge
- Would be clinically valuable as a screening assay:



Oxnard et al, JCO, 2016

Plasma response

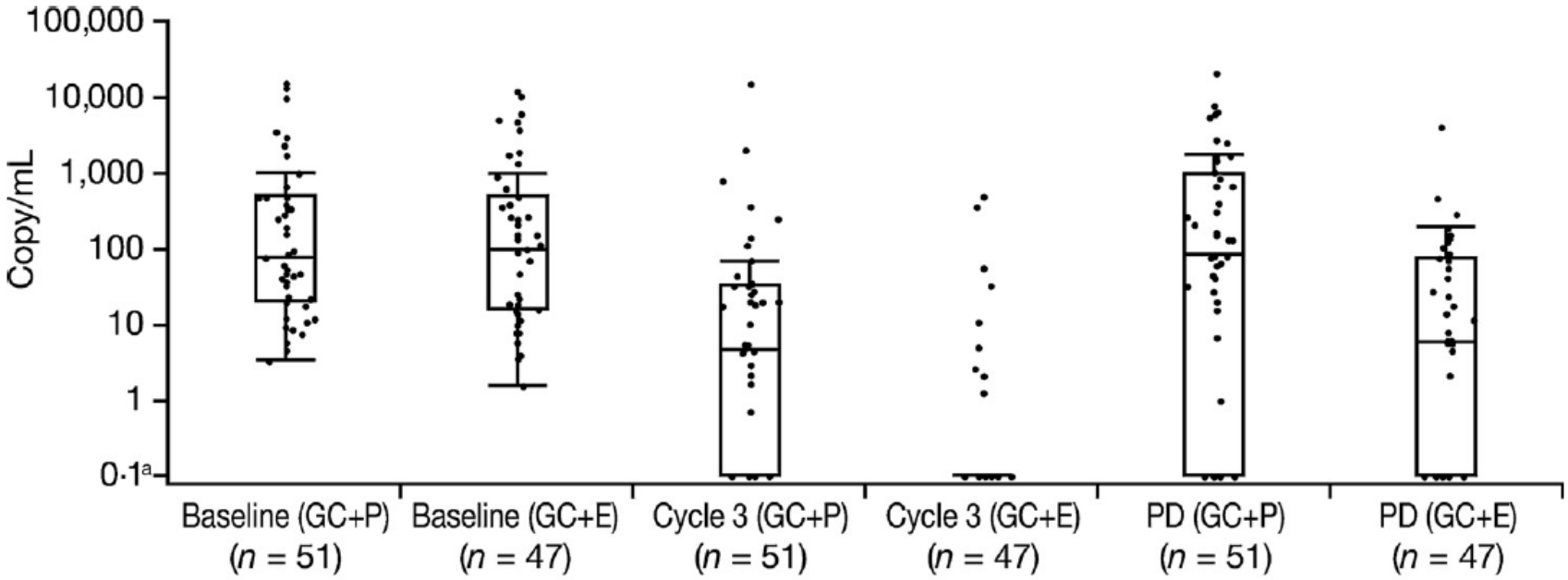
- Levels of circulating mutations appear to track with disease status
- Various patterns of plasma response kinetics seen using ddPCR



Sacher et al, JAMA Onc, 2016

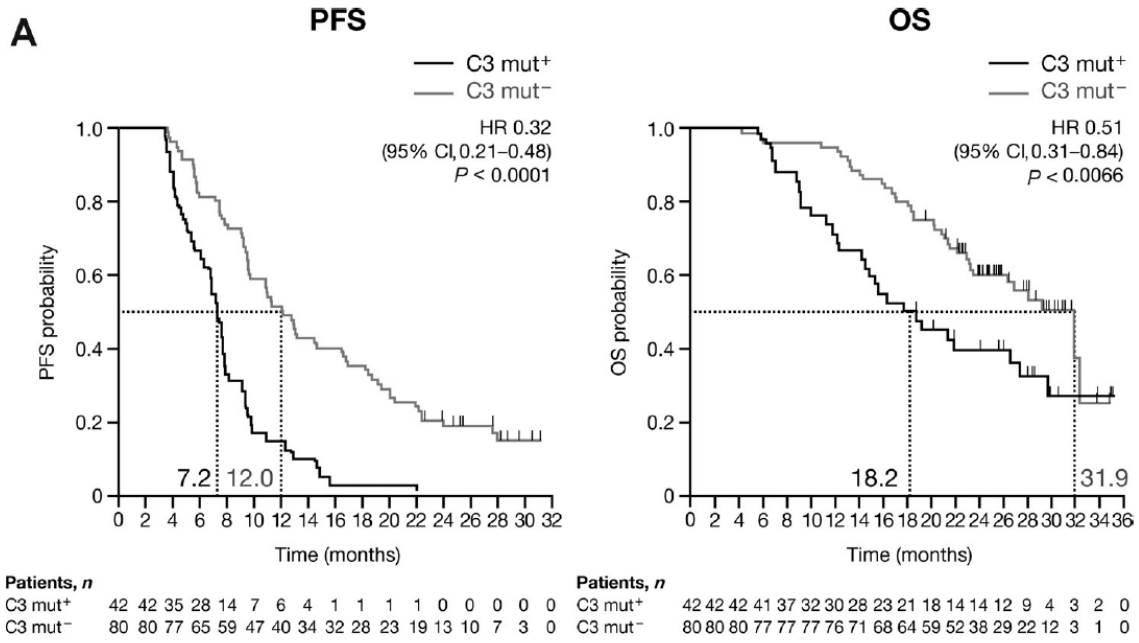
Plasma response

- Mok et al, CCR, 2015
  - Studied advanced EGFR-mutant NSCLC
  - Drop in plasma EGFR levels on therapy



Plasma response

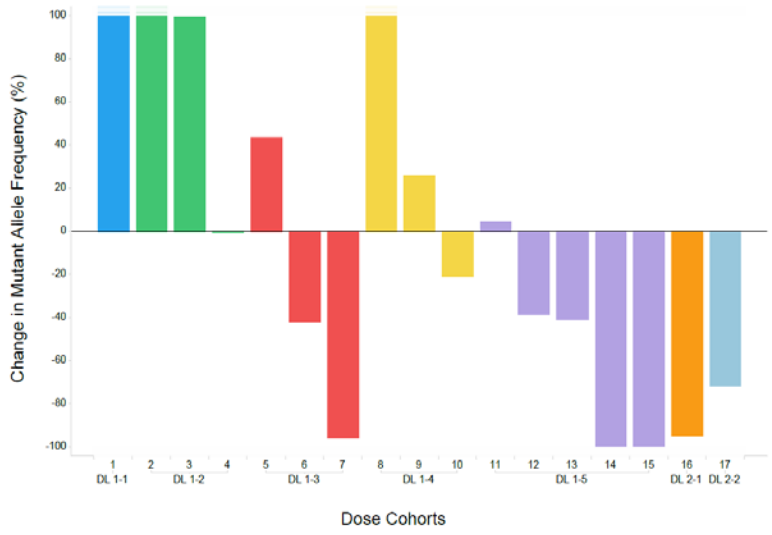
- Mok et al, CCR, 2015
  - Studied advanced EGFR-mutant NSCLC
  - Drop in plasma EGFR levels on therapy
  - Worse PFS in those without plasma “CR”



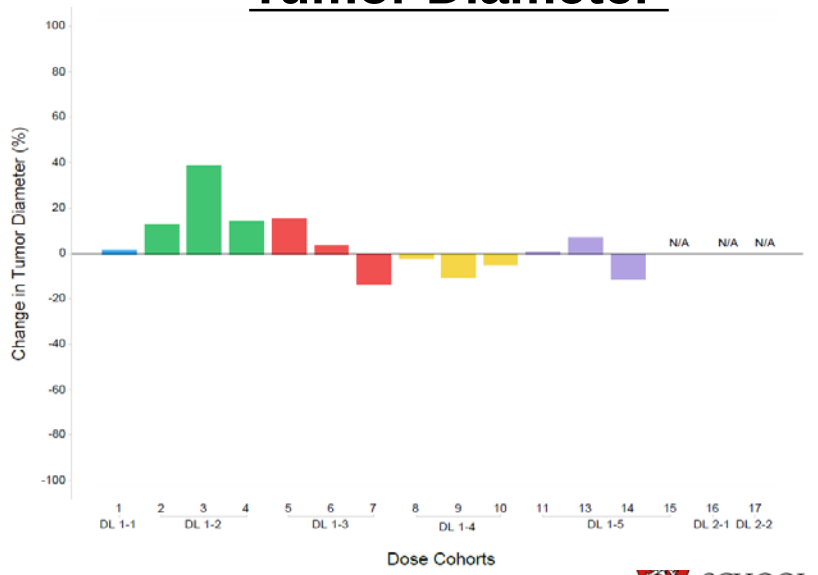
Plasma response

- Have studied plasma ddPCR to complement dose finding in phase I trials:
  - Combination of CDK4/6 and MEK inhibition in KRAS-mutant cancers
  - Compared plasma and tumor response

Mutant cfDNA



Tumor Diameter



Paweletz CP, AACR, 2016

Plasma response

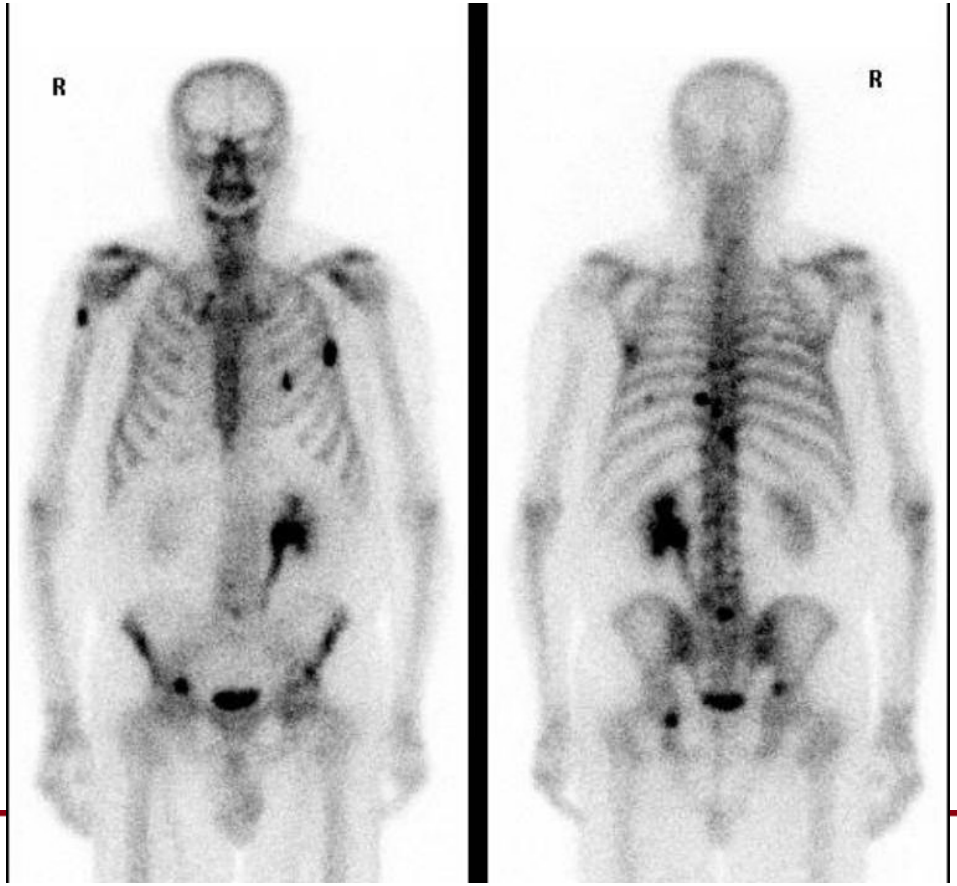
- Available data supports the idea that levels of tumor DNA in plasma track with disease status
- Unclear if it will be practical / clinically valuable / cost effective to routinely monitor cfDNA in patients with advanced cancer
- There could be a role for monitoring assays which are *very rapid* and *very inexpensive*

Case

- 74 yo M never-smoker with a prior history of resected NSCLC p/w bone lesions
  - Stage II adenocarcinoma resected 3 years prior, followed by adjuvant chemo
  - Surveillance CT shows new sclerotic lesions in bilateral ribs
  - Bone scan confirms abnormal uptake in rubs, spine, pelvis suspicious for a metastatic process

Case

- 74 yo M never-smoker with a prior history of resected NSCLC p/w bone lesions



Case

- 74 yo M never-smoker with h/o resected NSCLC p/w suspected recurrence
- Plasma genotyping for EGFR & KRAS
  - Positive for EGFR L858R, 3.5% AF
- Does this confirm recurrence of his NSCLC?

Case

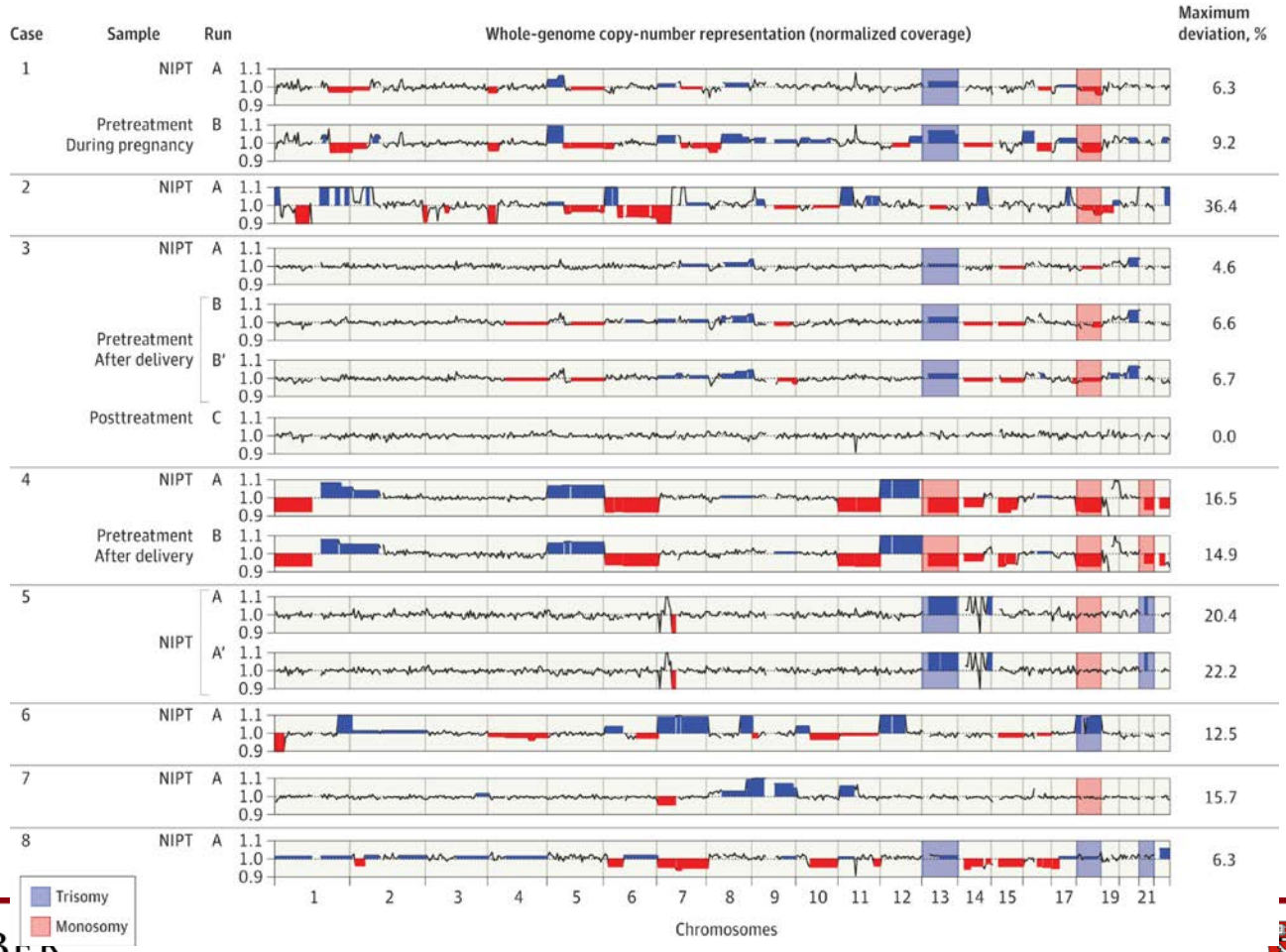
- 74 yo M never-smoker with h/o resected NSCLC p/w suspected recurrence
- Plasma genotyping for EGFR & KRAS
  - Positive for EGFR L858R, 3.5% AF
- Does this confirm recurrence of his NSCLC?
  - We confirmed the diagnosis with a bone biopsy before starting erlotinib

Cancer screening

- Bianchi et al, JAMA, 2015
  - Through a collaboration with Illumina, investigators queried NIPT results from 125,426 asymptomatic pregnant women
  - Aneuploidy identified in 3757 (3%)
  - In 8 cases, clinician voluntarily informed the lab that cancer was subsequently diagnosed, and the patient was consented for further study
  - All had abnormal NIPT; 7 had fetal karyotype performed and all were normal

Cancer screening

- Bianchi et al, JAMA, 2015



Cancer screening

- Bianchi et al, JAMA, 2015
  - Cancers detected in cfDNA were largely advanced or hematologic malignancies:
    - 4 cases of lymphoma
    - 1 case of leukemia
    - Stage IIIC colorectal cancer
    - Stage IIIB anal cancer
    - Stage IV neuro-endocrine carcinoma

Cancer screening

- Bianchi et al, JAMA, 2015
  - Cancers detected in cfDNA were largely advanced or hematologic malignancies:
  - Can plasma NGS be used to identify early-stage, curable cancers pre-diagnosis?
  - What will the false positive rate of such a screening approach be?
  - What if plasma NGS is abnormal but extensive imaging does not identify a cancer?

Conclusions

- cfDNA genotyping is a powerful tool for noninvasive genotyping
  - Can be rapid and convenient
  - Offers insight into the heterogeneity of resistance
  - Can allow noninvasive monitoring
  - However, not all tumor shed tumor DNA
- PCR and NGS assays likely have complementary roles going forward for clinical application and research

Acknowledgements

- Lowe Center for Thoracic Oncology
  - Pasi Jänne, Ryan Alden, Adrian Sacher, Emmy Hu
- Trans. Research Lab / Belfer Center
  - Cloud Paweletz, Yanan Kuang, Nora Feeney
- Department of pathology, BWH
  - Lynette Sholl, Neal Lindeman
- Industry Collaborators
  - AstraZeneca, Resolution Bioscience, Guardant, Illumina, Astellas
- Funding
  - Damon Runyon Cancer Research Foundation, DOD, Anna Fuller Fund, Stading-Younger Cancer Research Fund