Plasma Genotyping in NSCLC: Layering in the liquid and future applications

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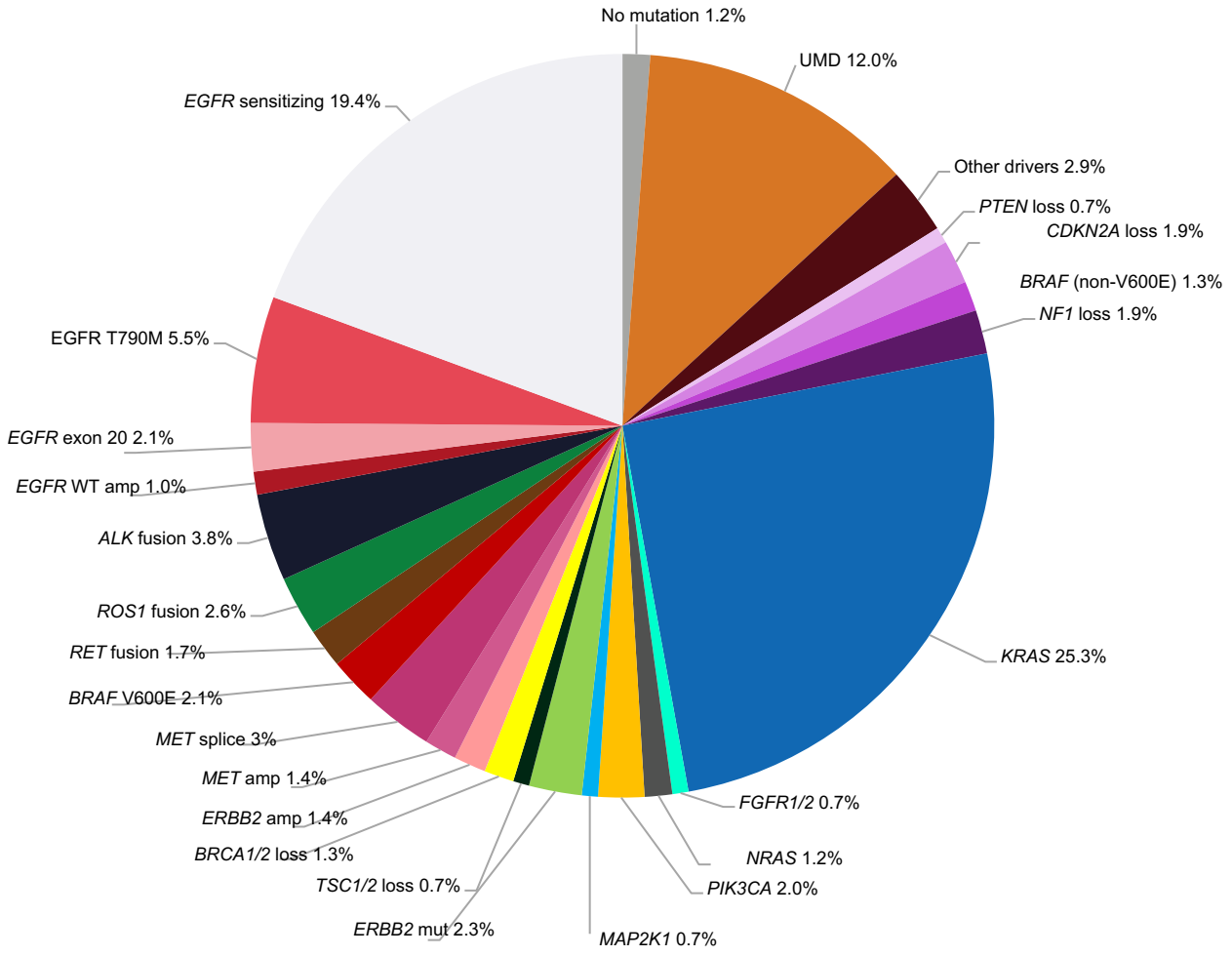
Biomarker Testing Demands and Targeted Therapy Options for Lung Adenocarcinoma Continue to Expand[1]



Target	Approved Drugs
EGFR (common mutations)	Gefitinib, erlotinib, afatinib, dacomitinib, osimertinib, erlotinib/ramucirumab
EGFR (exon 20)	Amivantamab, mobocertinib
ALK	Crizotinib, ceritinib, alectinib, brigatinib, lorlatinib
ROS1	Crizotinib, entrectinib
RET	Selpercatinib, pralsetinib
***NTRK*1/2/3**	Larotrectinib, entrectinib
***BRAF* V600E**	Dabrafenib + trametinib
***MET* exon 14**	Capmatinib, tepotinib
***KRAS* G12C**	Sotorasib

1. Jordan et al. *Cancer Discov*. 2017;7:596-609.

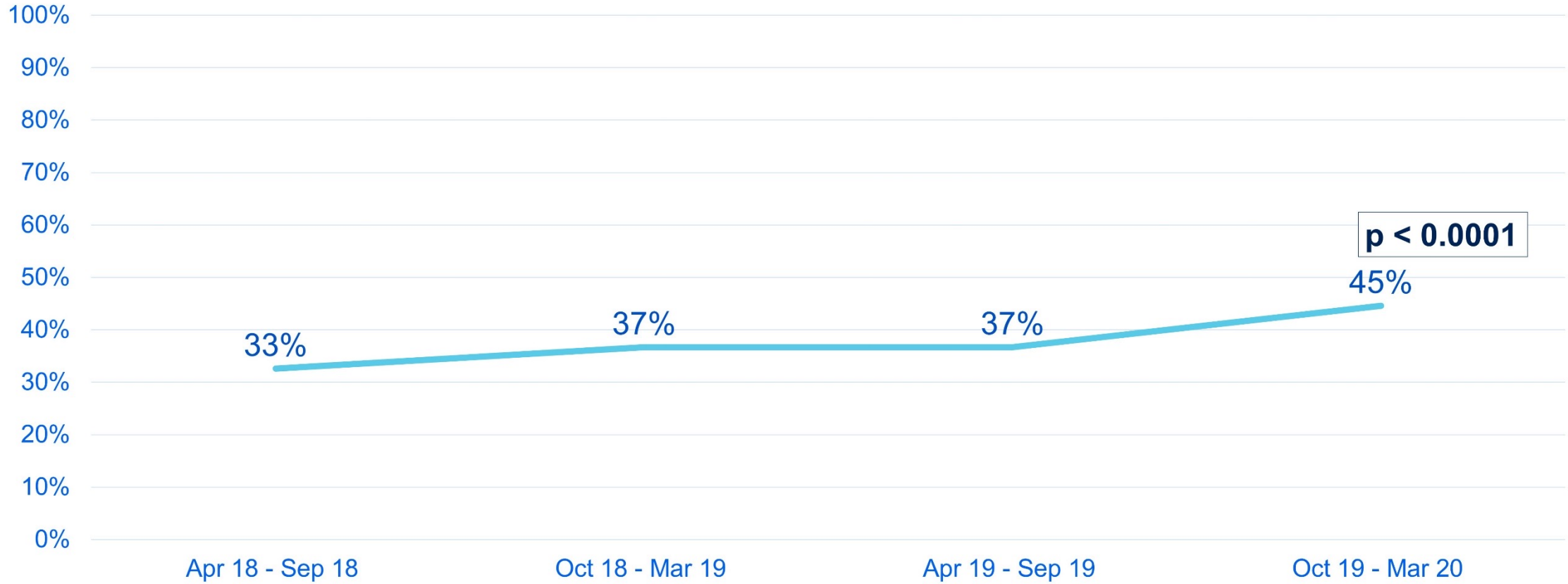
Methods

- Retrospective, observational chart review
- Patients with mNSCLC initiating first-line (1L) systemic therapy between April 1, 2018 and March 31, 2020
- Data from practices within The US Oncology Network of community oncology practices that utilize a similar electronic health record

Patient Characteristics

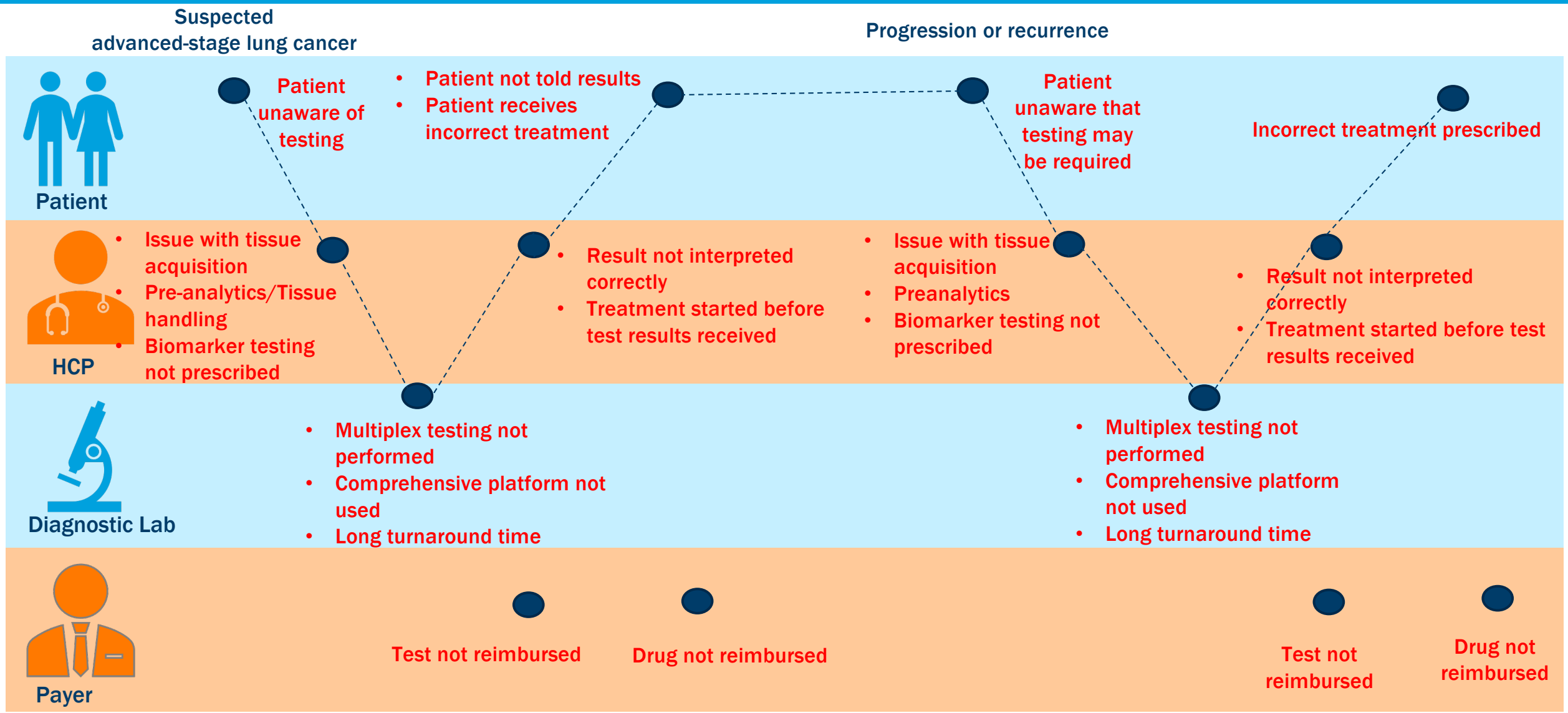
	Overall N=3474	Nonsquamous N=2820
Age at mNSCLC, years		
Median(Min, Max)	69 (23,90+)	69 (24,90+)
Gender, %		
Female	51.1	53.9
Male	48.9	46.1
Race, %		
White	65.3	64.4
Black Or African American	8.3	8.3
Other	5.8	6.0
Not documented	20.7	21.2
Practice region, %		
South	46.4	45.5
West	35.3	36.2
Midwest	11.6	12.1
Northeast	6.6	6.3

NGS testing rates over time for the overall population

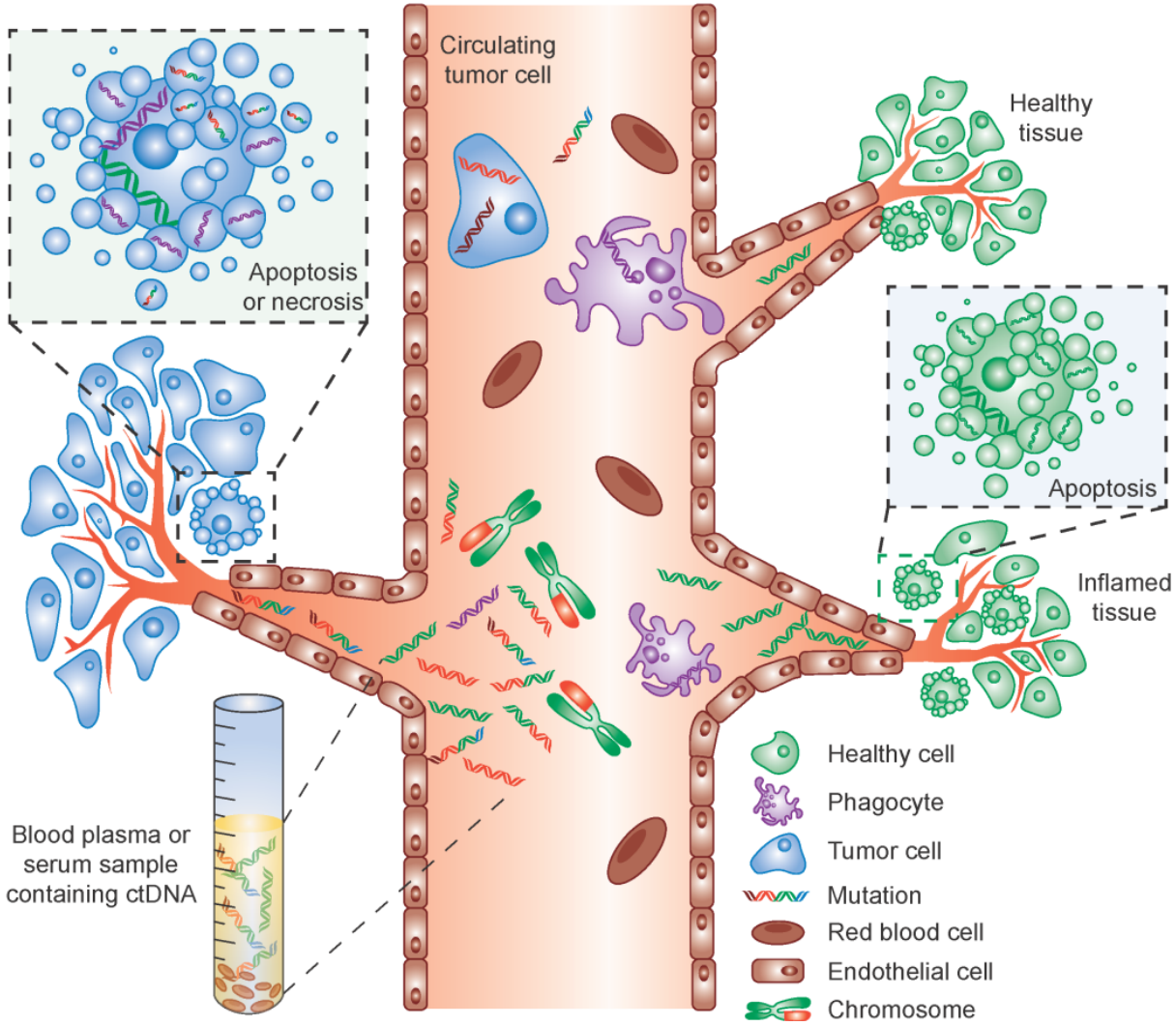


p value: Cochrane Armitage test for trend analysis

PAIN POINTS IN THE JOURNEY (ADVANCED-STAGE NONSQUAMOUS NCSLC)



Plasma ctDNA "Liquid Biopsies": Rationale and Methods[1]



- Capture of ctDNA via a simple noninvasive blood test
- Shedding of ctDNA is product of apoptosis and necrosis, two relevant processes in cancer
- New, more sensitive diagnostic platforms have the capability to genetically interrogate isolated DNA from the blood
- Circumvent the need for tissue biopsies

Technique	Sensitivity, %	Optimal Application
Sanger sequencing	>10	Tumor tissue
Pyrosequencing	10	Tumor tissue
NGS	2	Tumor tissue
Quantitative PCR	1	Tumor tissue
ARMS	0.10	Tumor tissue
BEAMing, PAP, digital PCR, TAm-Seq	≤0.01	ctDNA, rare variants in tumor tissue

1. Diaz LA Jr, Bardelli A. *J Clin Oncol*. 2014;32:579-586.

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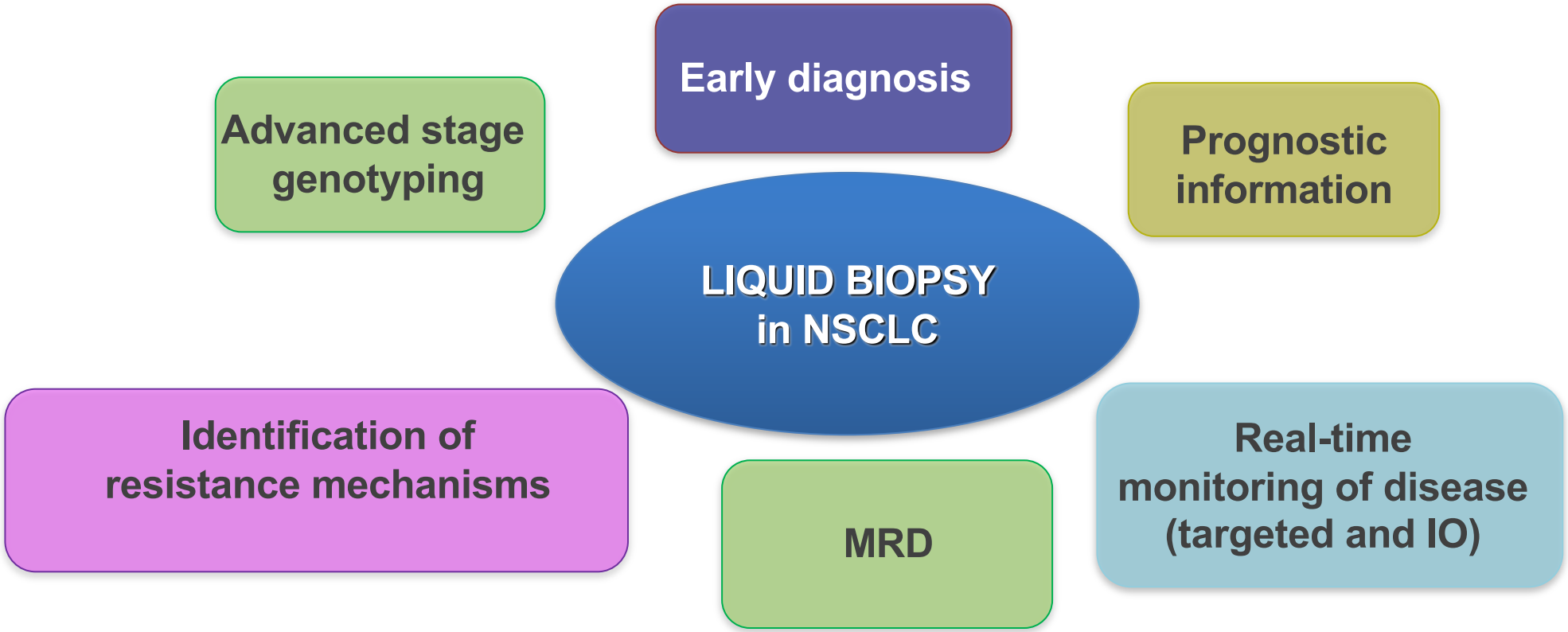
Technologies for Detection of ct-DNA

Principles of Detection	Method Ex.	Notes
Quantitative PCR	Real-time PCR ARMS/Scorpion PCR Mutant allele-specific PCR	Lowest cost, ease of use
Digital PCR	BEAMing Droplet digital PCR (ddPCR) Microfluidic digital PCR	Highest sensitivity, limited genomic loci
Next-Generation Sequencing	Hybrid capture based NGS CAPP-Seq TAm-Seq	High sensitivity, broad range of genomic coverage

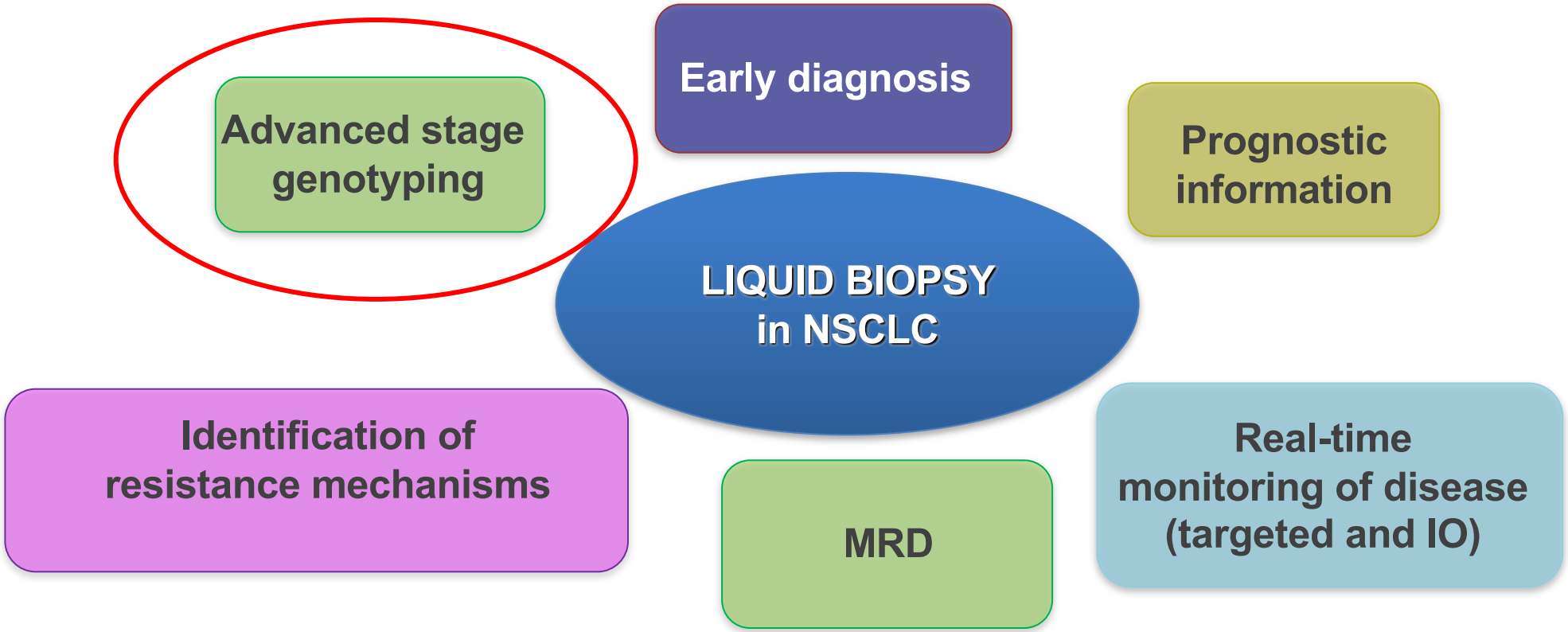
Adapted, Qin et al., Chinese Journal of Cancer 2016

Liquid Biopsy: Clinical Application



Castiglia M et al. *Biochim Biophys Acta*. 2014;1846(2):539-46.

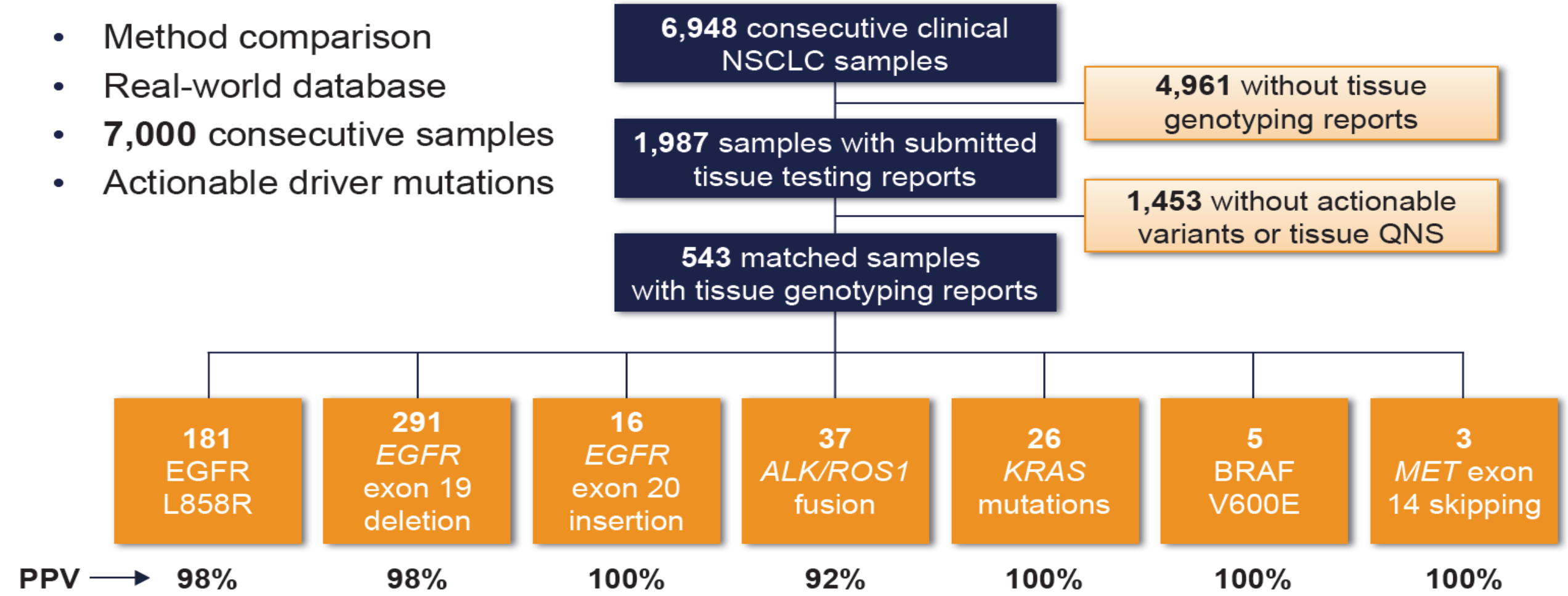
Liquid Biopsy: Clinical Application



Castiglia M et al. *Biochim Biophys Acta*. 2014;1846(2):539-46.

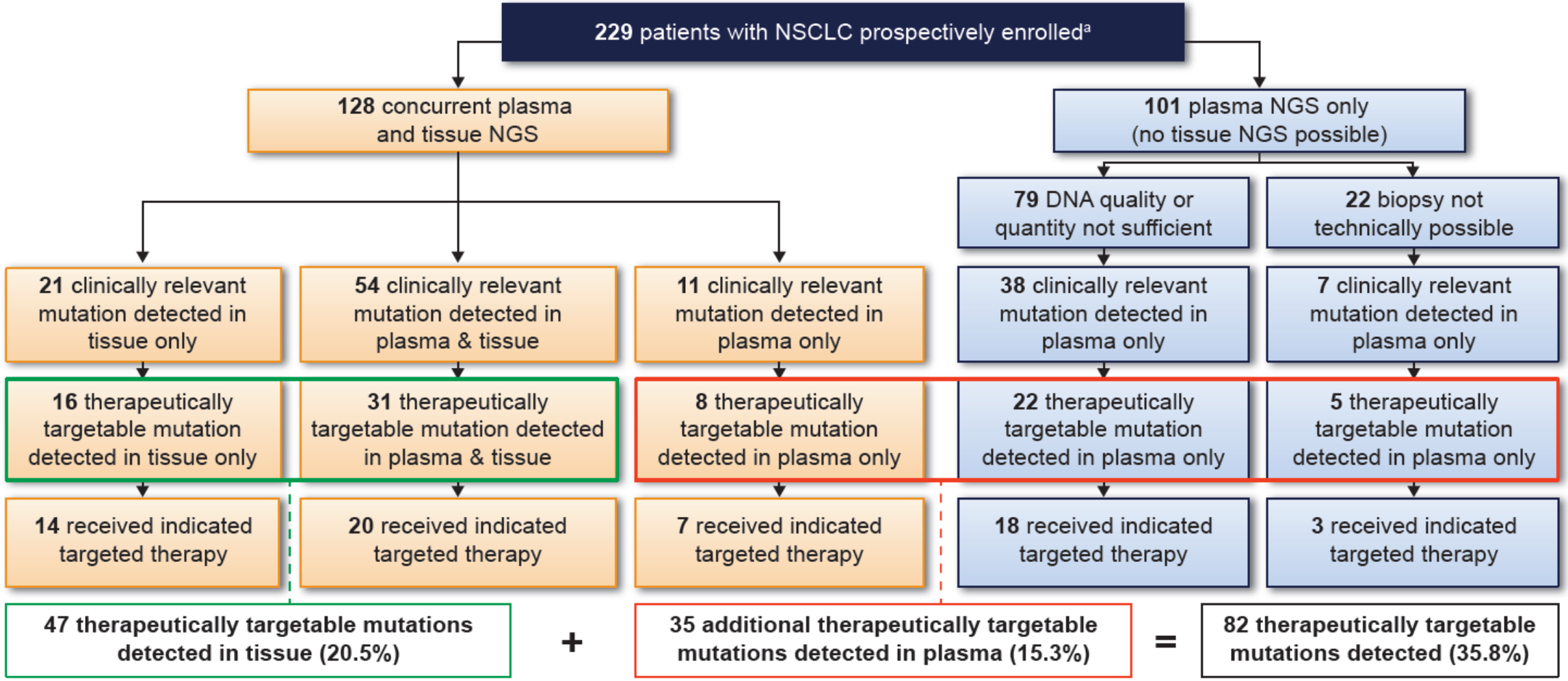
High PPV for NGS Panel[1]

- Method comparison
- Real-world database
- **7,000** consecutive samples
- Actionable driver mutations



1. Zill OA et al. *J Clin Oncol*. 2016;34(suppl 18). Abstract LBA11501.

Clinical Implications of Plasma ctDNA Testing in Metastatic NSCLC[1]

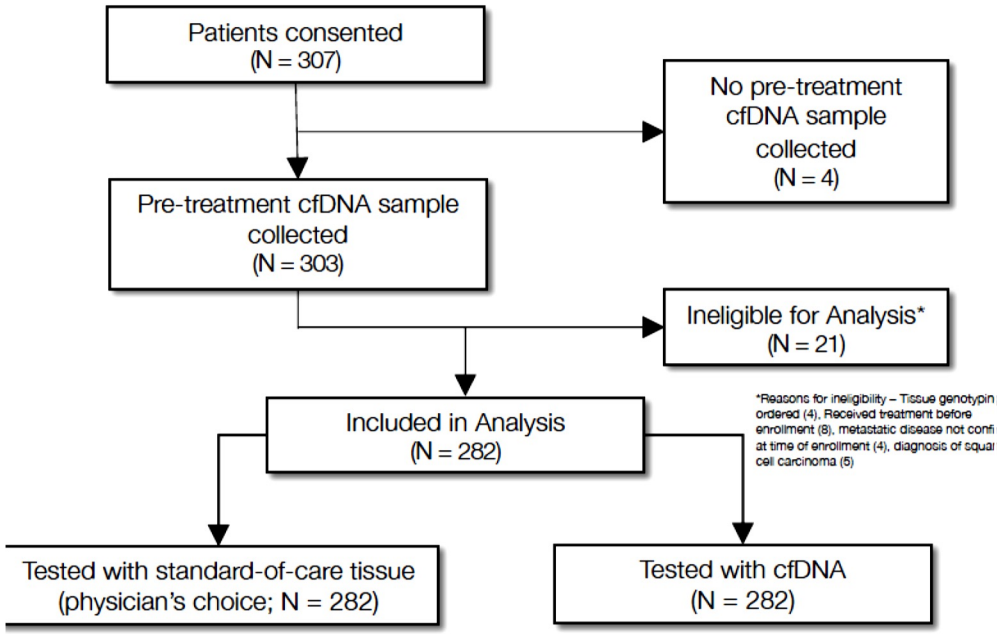


[a] Patients were either enrolled at time of initial diagnosis or at disease progression.

1. Aggarwal C et al. *JAMA Oncol*. 2019;5:173-180.

NILE Study

Study Cohort



Primary Objective

- Detection of guideline recommended biomarkers

Clinical follow-up at one year or at disease progression

		Number	Percentage (%)
Gender	Female	153	54.3
	Male	129	45.7
Median Age at diagnosis (range) in years		69 (26 – 100)	
Race	Asian	17	6.0
	Black or African American	18	6.4
	Native Hawaiian or other Pacific Islander	1	0.4
	White	231	81.9
	Other	8	2.8
	Unknown	7	2.5
Ethnicity	Hispanic	23	8.2
	Non-Hispanic	259	91.8
ECOG status at enrollment	0	71	25.2
	1	151	53.5
	2	36	12.8
	3	12	4.3
	Unknown/missing	12	4.3
History of prior chemotherapy for early stage NSCLC	Yes	45	16.0
	No	237	84.0
Stage of NSCLC at enrollment	IIIb	7	2.5
	IV	275	97.5
Type of NSCLC at enrollment	Adenocarcinoma	271	96.1
	Large cell carcinoma	5	1.8
	Other*	6	2.2
Smoking History	Non-smoker	61	21.4
	Previous Smoker	153	54.4
	Current Smoker	61	21.7
	Unknown	7	2.5

Leighl N et al. AACR 2019. Abstract 4460.

NILE Study

Results – cfDNA Biomarker Detection Rate

- Primary endpoint of cfDNA non-inferiority was met, with physician discretion SOC tissue genotyping identifying 60 patients (21.3%) with a guideline recommended biomarker and cfDNA identifying 77 patients (27.3%) ($p<0.0001$ for non-inferiority)

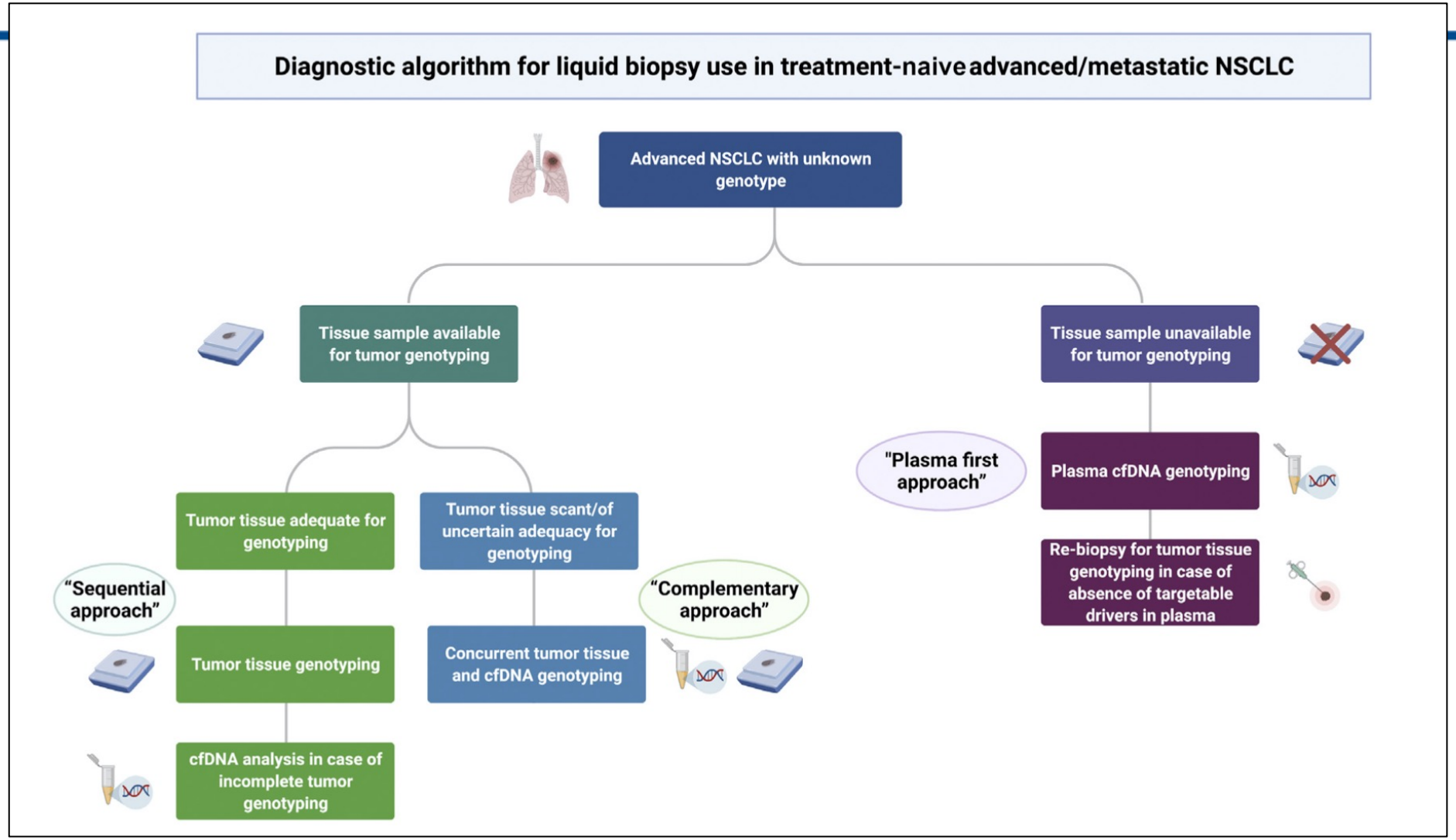
Guideline-recommended biomarker positivity by sample type		Tissue		
		Positive	Negative	Total
cfDNA	Positive	48	29	77
	Negative	12	193	205
	Total	60	222	282

- Biomarker positive patients increased from 60 using tissue alone to 89 using tissue + cfDNA
  - cfDNA found biomarkers in patients with negative (N = 7), not assessed (N = 16), or insufficient tissue results (QNS; N = 6)
- When restricted to the 64 patients with guideline complete tissue genotyping attempted (N = 13) or completed (N = 51), tissue and cfDNA each identified 22 patients with a guideline recommended biomarker (19 concordant)
- cfDNA results were returned significantly faster than tissue results (median 9 vs 15 days; $p<0.0001$)

Leighl (Papadimitrakopoulou). 2019. American Association for Cancer Research Annual Meeting, Abstract #4460.

Leighl N et al. AACR 2019. Abstract 4460.

Patients with Advanced Treatment-naive NSCLC

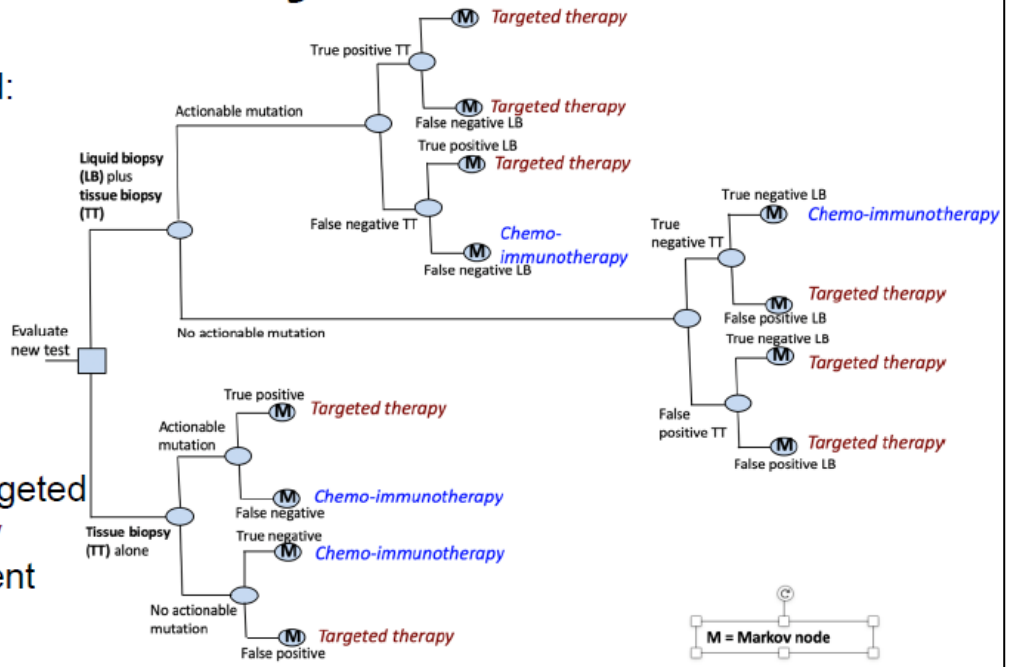


Rolfo et al, JTO 2021 Oct;16(10):1647-1662

Is this cost effective?

VALUE Economic Analysis

- Decision analytic Markov model compared:
  1. ***Tissue biopsy alone versus***
  2. ***Liquid biopsy in addition to tissue biopsy***
- Perspective: Canadian public health care system.
- Time horizon: lifetime (10 years).
- Genomic alterations were considered:
  - **Actionable** if approved or off-label targeted treatment available→Targeted therapy
  - **Non-actionable** if no targeted treatment available → Chemo-immunotherapy



Is this cost effective?

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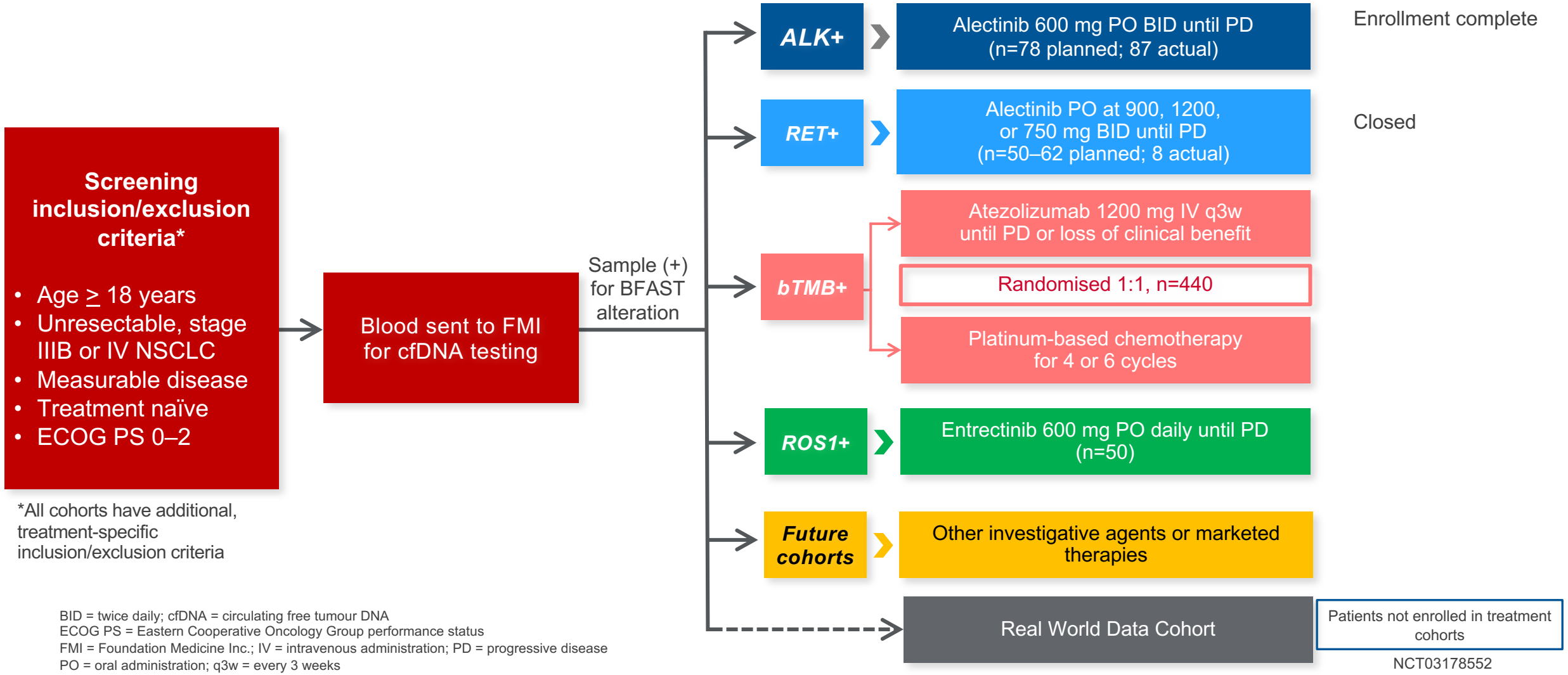
Results

IASLC

Stage IV NSCLC	Targeted therapy (n=82)	Non-targeted therapy (n=48)
Median PFS, months (95%CI)	11.4 (8.3 - not reached)	9.8 (4.4 – 19.5)
Median OS, months (95% CI)	Not reached	19.5 (10.2 – 19.5)

Testing strategy	Cost (CAD$)	QALY	Incremental cost (CAD$)
Liquid biopsy + Tumour tissue biopsy	1,305,524	7.17	Reference
Tumour tissue biopsy alone	1,342,740	7.10	37,216

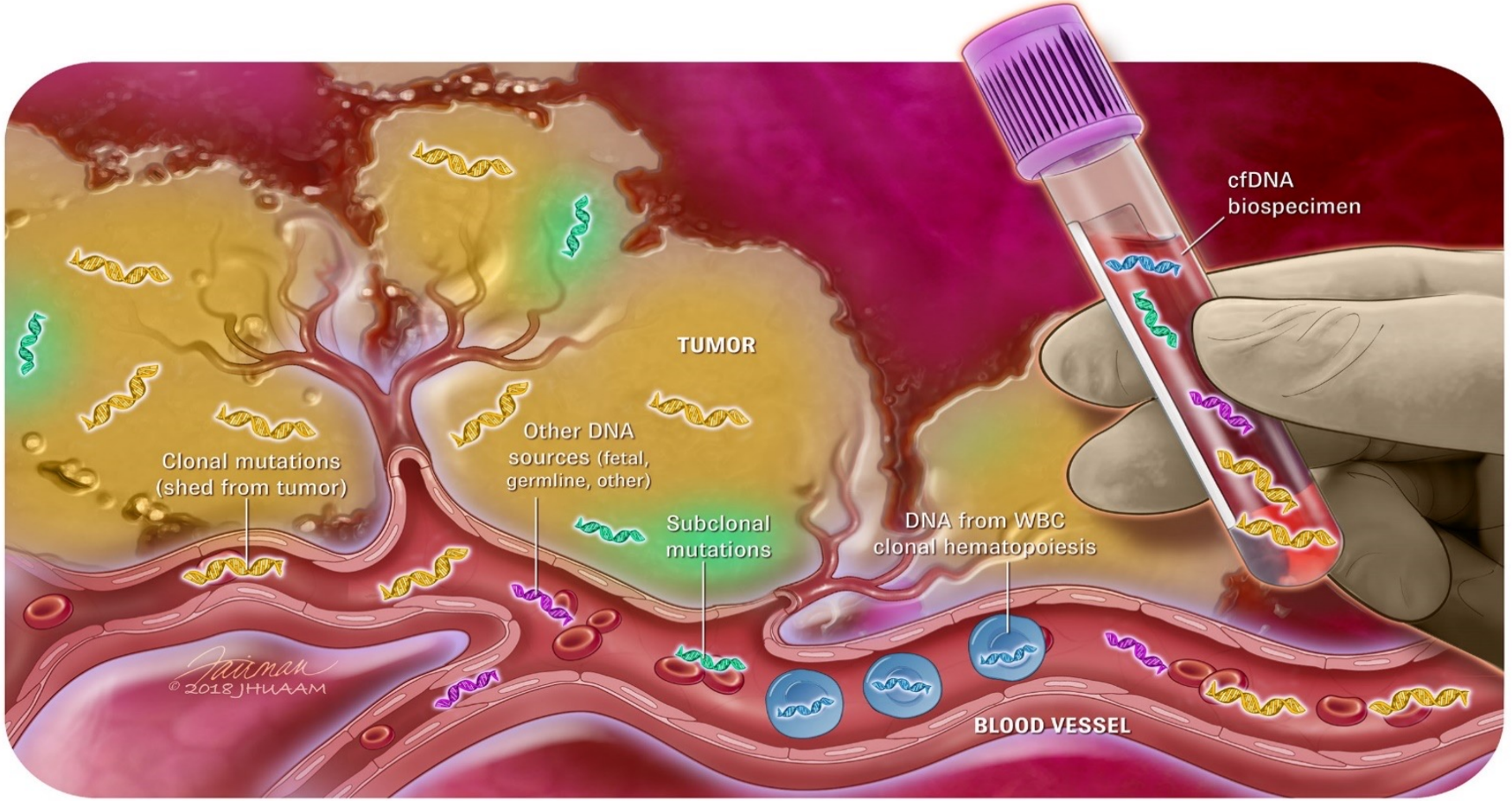
Study Design: BFAST



BID = twice daily; cfDNA = circulating free tumour DNA
ECOG PS = Eastern Cooperative Oncology Group performance status
FMI = Foundation Medicine Inc.; IV = intravenous administration; PD = progressive disease
PO = oral administration; q3w = every 3 weeks

Special considerations

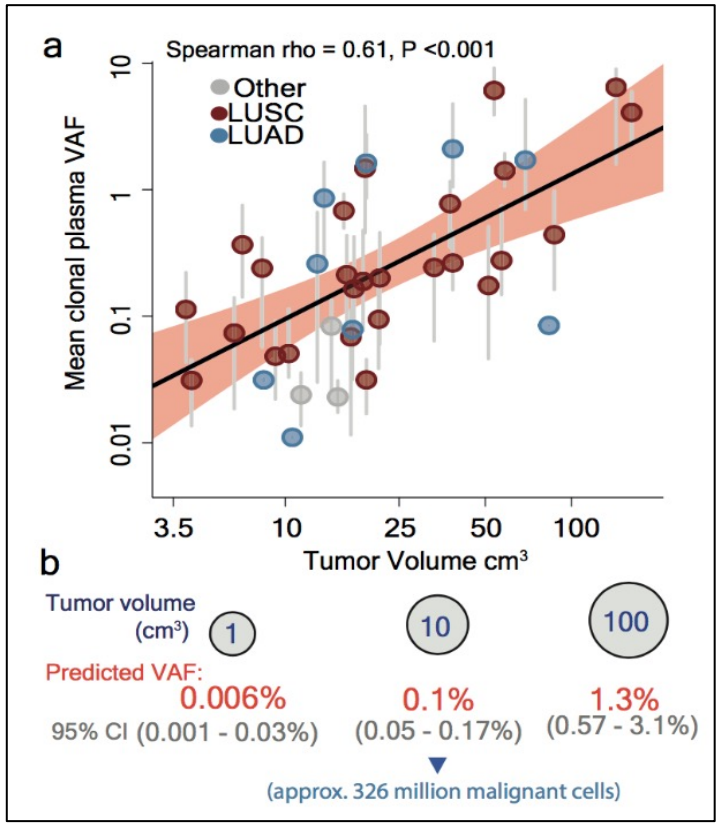
cfDNA: A Complex Biospecimen



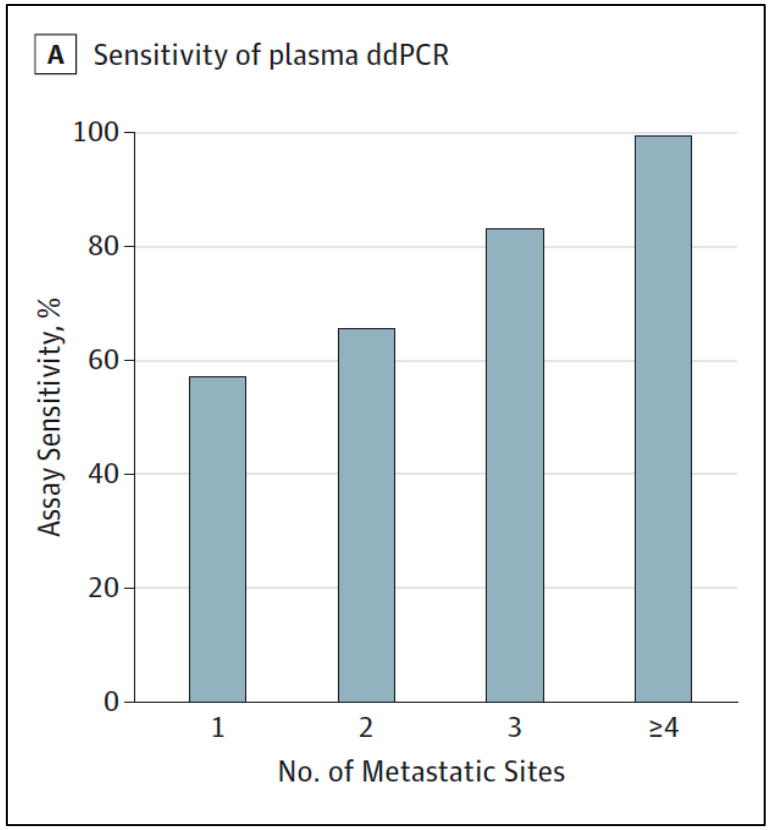
Bauml J et al. *Clin Cancer Res.* 2018;24(18):4352-4354.

Special considerations
Sensitivities and Improving Pretest Probability

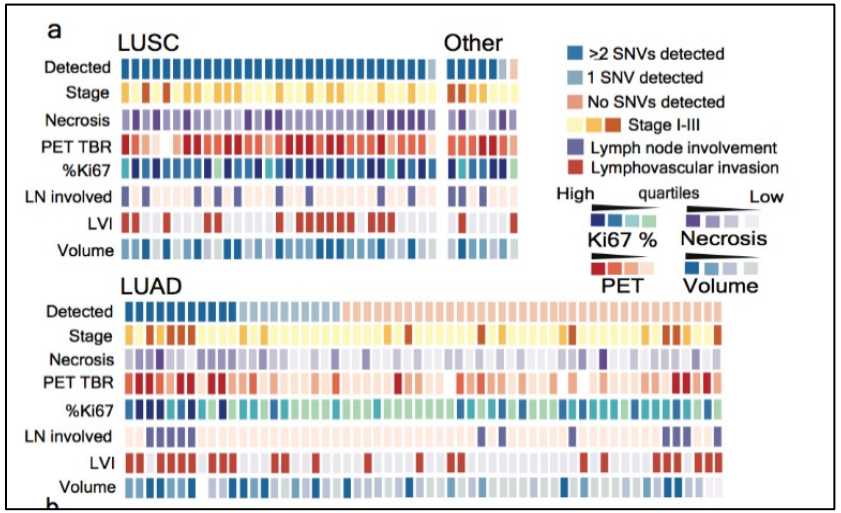
Tumor size



Metastatic burden



Tumor features



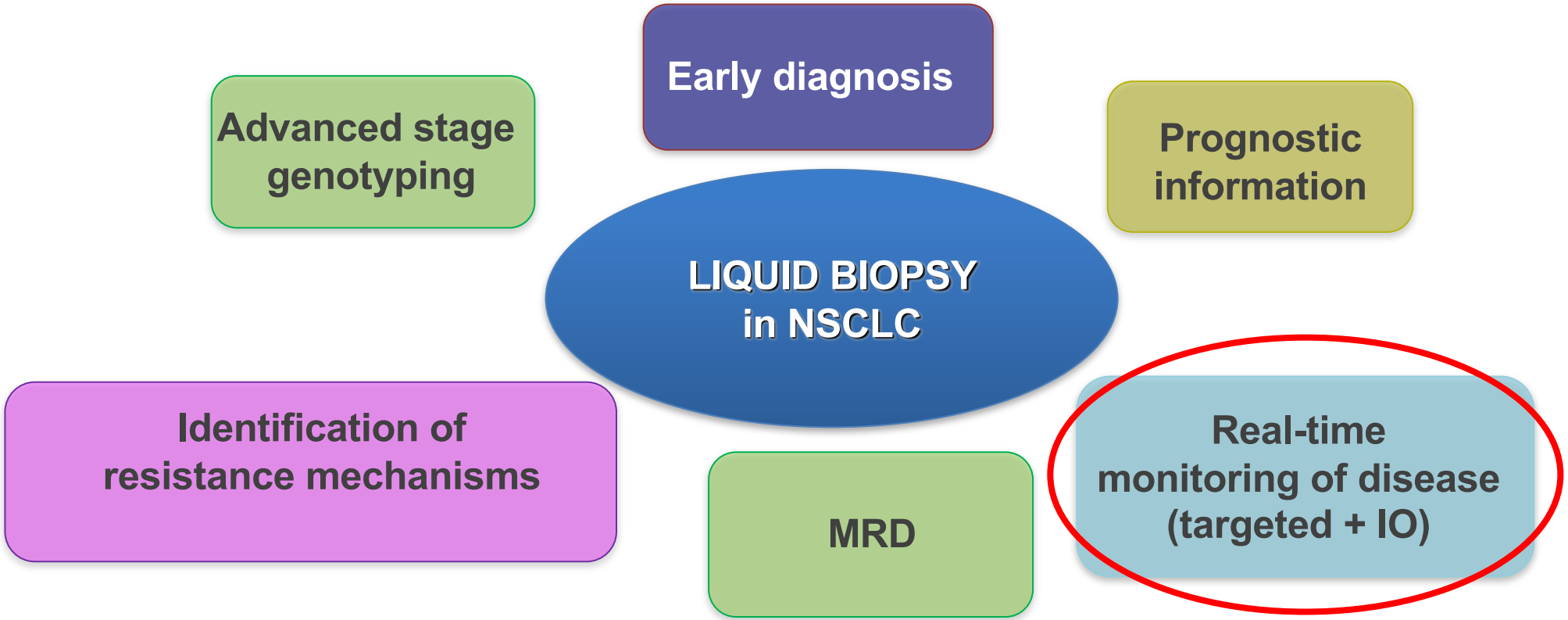
GGO 10x lower DNA concentration

Abbosh C et al. *Nature*. 2017;545:446-451; Sacher AG et al. *JAMA Oncol.* 2016;1;2(8):1014-22; Chen CL et al. *Sci Rep.* 2016;6:21471.

Special Considerations: Germline

RELEVANT FOR THERAPY SELECTION	%CFDNA OR AMPLIFICATION	FDA APPROVED IN INDICATION	AVAILABLE FOR USE IN OTHER INDICATIONS	CLINICAL DRUG TRIALS
BRCA2 E1308*	32.3%	None	Olaparib	Trials Available Other Therapies
TP53 Y220H	4.9%	None	None	Trials Available Other Therapies
EGFR Exon 19Deletion	3.3%	Erlotinib Afatinib Gefitinib	None	Trials Available Other Therapies
MYC Amplification	+	None	None	Trials Available Other Therapies
PIK3CA Amplification	+	None	Everolimus Temsirolimus	Trials Available Other Therapies

Liquid Biopsy: Clinical Application



Castiglia M et al. *Biochim Biophys Acta*. 2014;1846(2):539-46

Early Prediction of Response to Tyrosine Kinase Inhibitors by Quantification of *EGFR* Mutations in Plasma of NSCLC Patients

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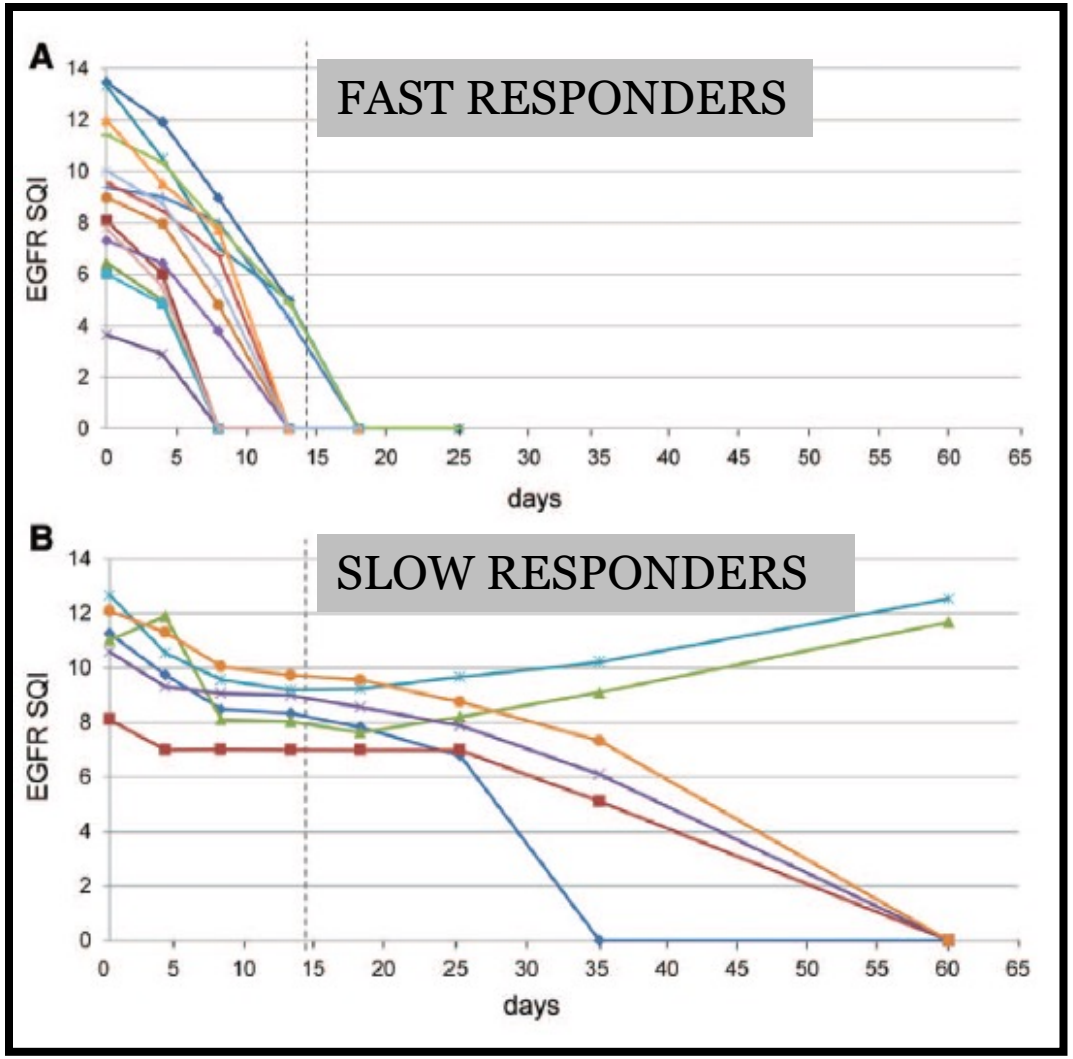

Marchetti A et al. *J Thorac Oncol* . 2015;10(10):1437-43.

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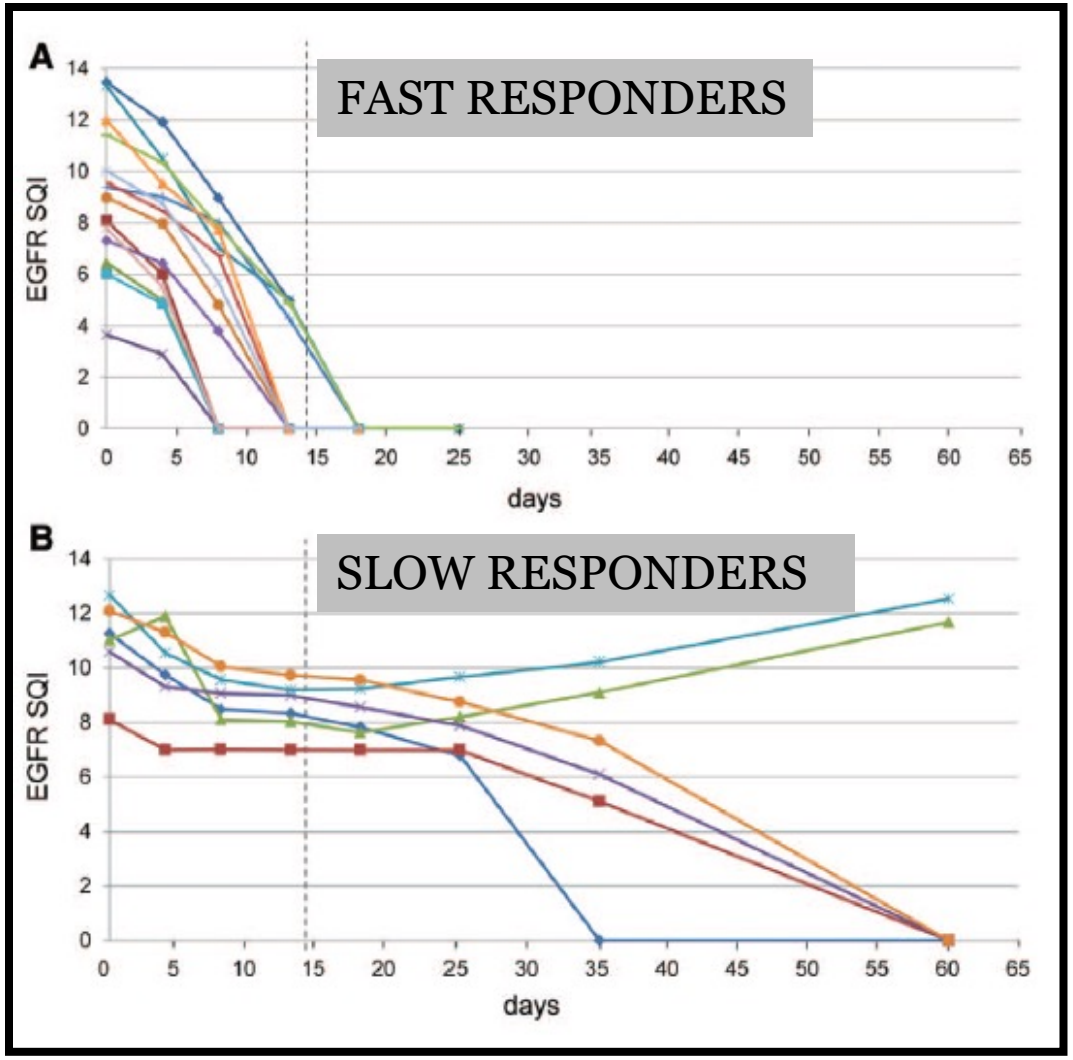
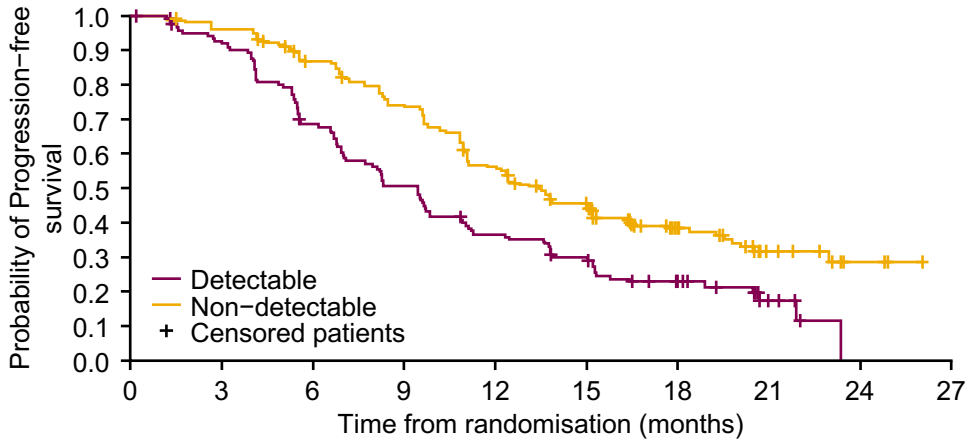


Mean % of Tumor Shrinkage: **60%**

Mean % of Tumor Shrinkage: **18%**

Marchetti A et al. *J Thorac Oncol* . 2015;10(10):1437-43.

Abstract 9019: FLAURA plasma samples: [Platform: ddPCR; clearance]

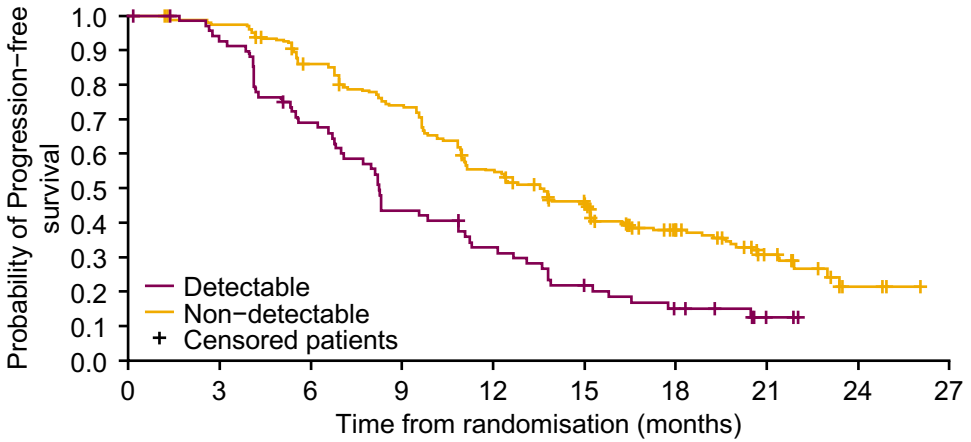


Number of patients at risk

Non−detectable	208	198	174	147	111	86	41	13	3	0
Detectable	126	114	84	62	44	34	20	6	0	0

a) Clearance of plasma EGFRm at week 3

	Detectable EGFRm (n=126)	Non-detectable EGFRm (n=208)
Events,n (maturity,%)	99 (79)	128 (62)
mPFS, months (95% CI)	9.5 (7.0, 10.9)	13.5 (11.1, 15.2)
HR (95% CI); p value	0.57 (0.4, 0.7) p<0.0001	
ORR, % (95% CI)	78 (69.5, 84.7)	87 (81.7, 91.3)



Number of patients at risk

Non−detectable	258	249	216	184	137	109	54	18	3	0
Detectable	70	63	46	29	21	13	8	2	0	0

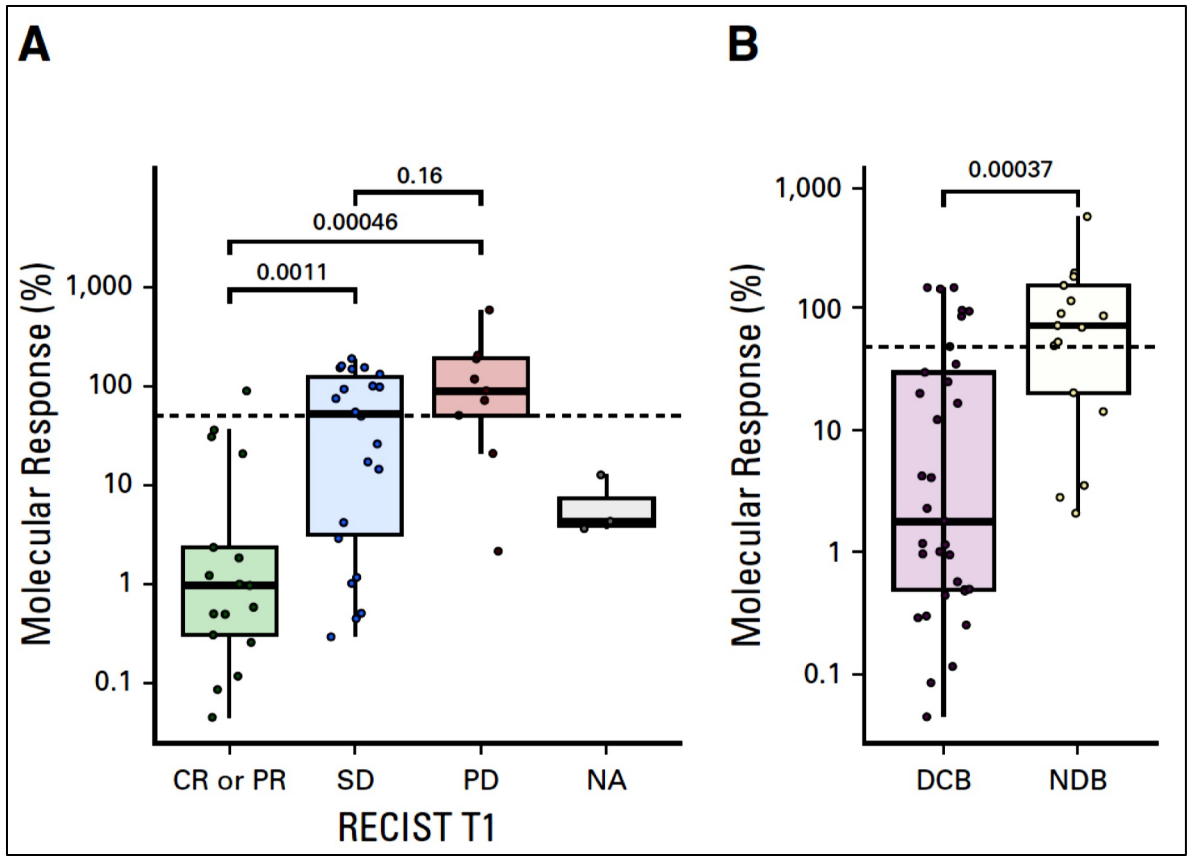
b) Clearance of plasma EGFRm at week 6

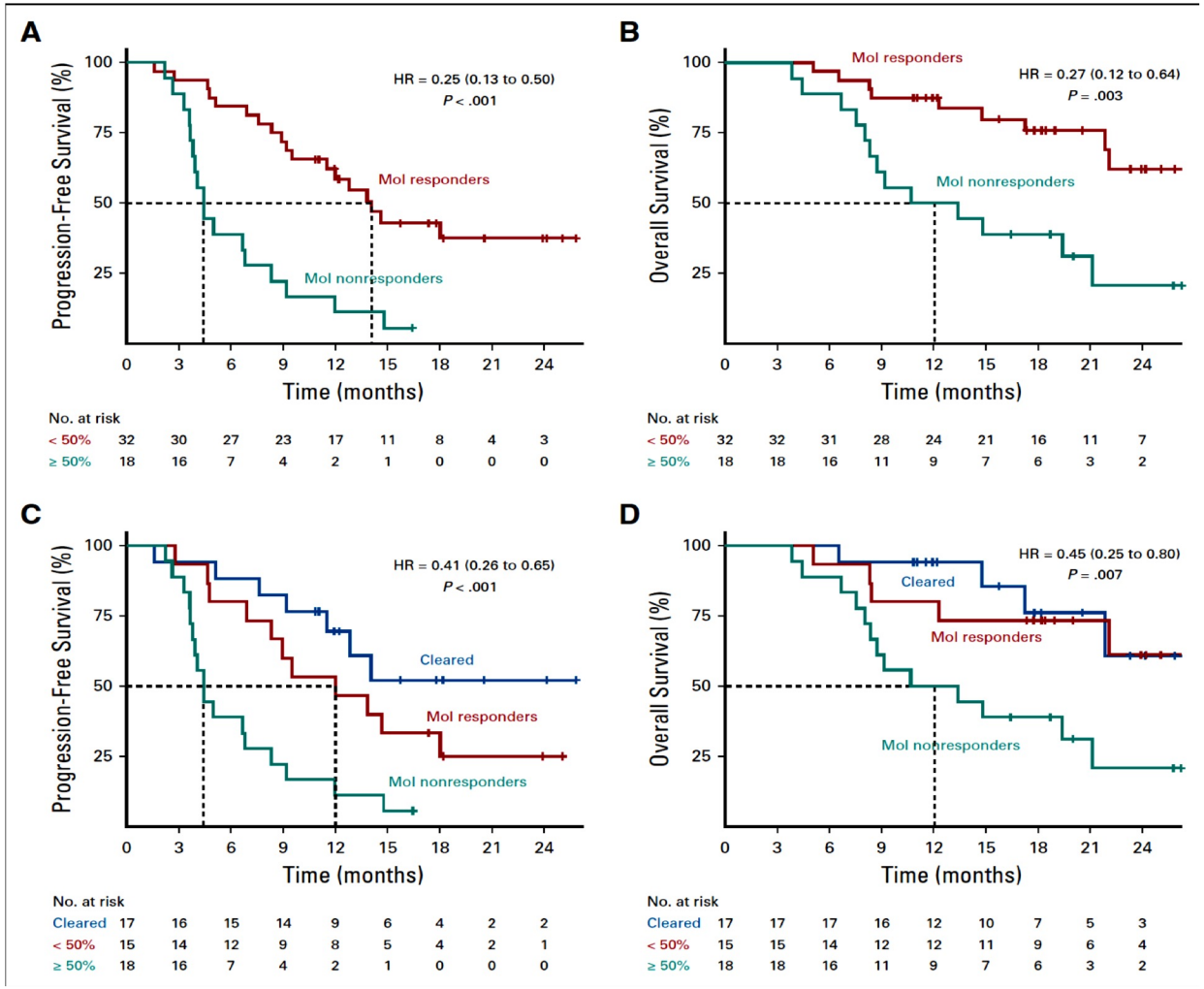
	Detectable EGFRm (n=70)	Non-detectable EGFRm (n=258)
Events,n (maturity,%)	57 (81)	165 (64)
mPFS, months (95% CI)	8.2 (6.8, 10.9)	13.5 (11.1, 15.2)
HR (95% CI); p value	0.51 (0.4, 0.7) p<0.0001	
ORR, % (95% CI)	73 (60.9, 82.8)	88 (83.4, 91.7)

*Clearance refers to undetectable plasma EGFR mutations, where they were detectable at baseline, using ddPCR

CI, confidence interval; EGFR, epidermal growth factor receptor; EGFRm, EGFR-TKI sensitizing mutations (ex19del or L858R); EGFR-TKI; EGFR-tyrosine kinase inhibitor; HR, hazard ratio; mPFS, median progression-free survival

Serial Monitoring of ctDNA as a Biomarker of Response to Pembrolizumab-based Treatment





Thompson JC et al. *JCO Precis Oncol.* 2021;5:510-524..

ctDNA and TCR Dynamics-Sustained Response: Second-line nivo

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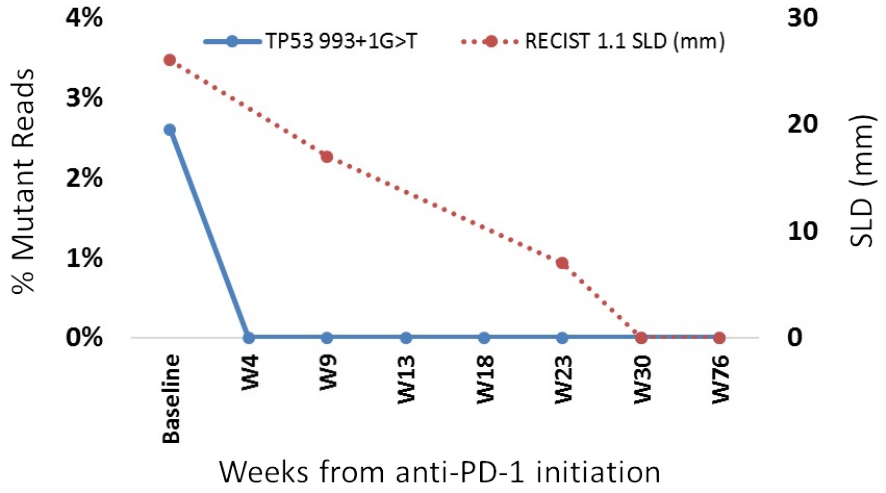
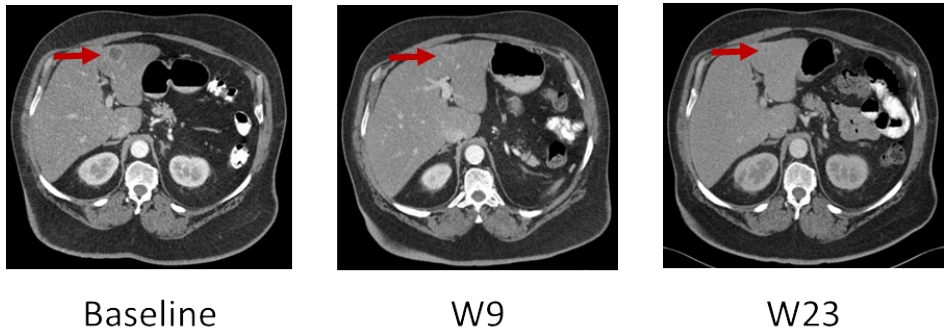
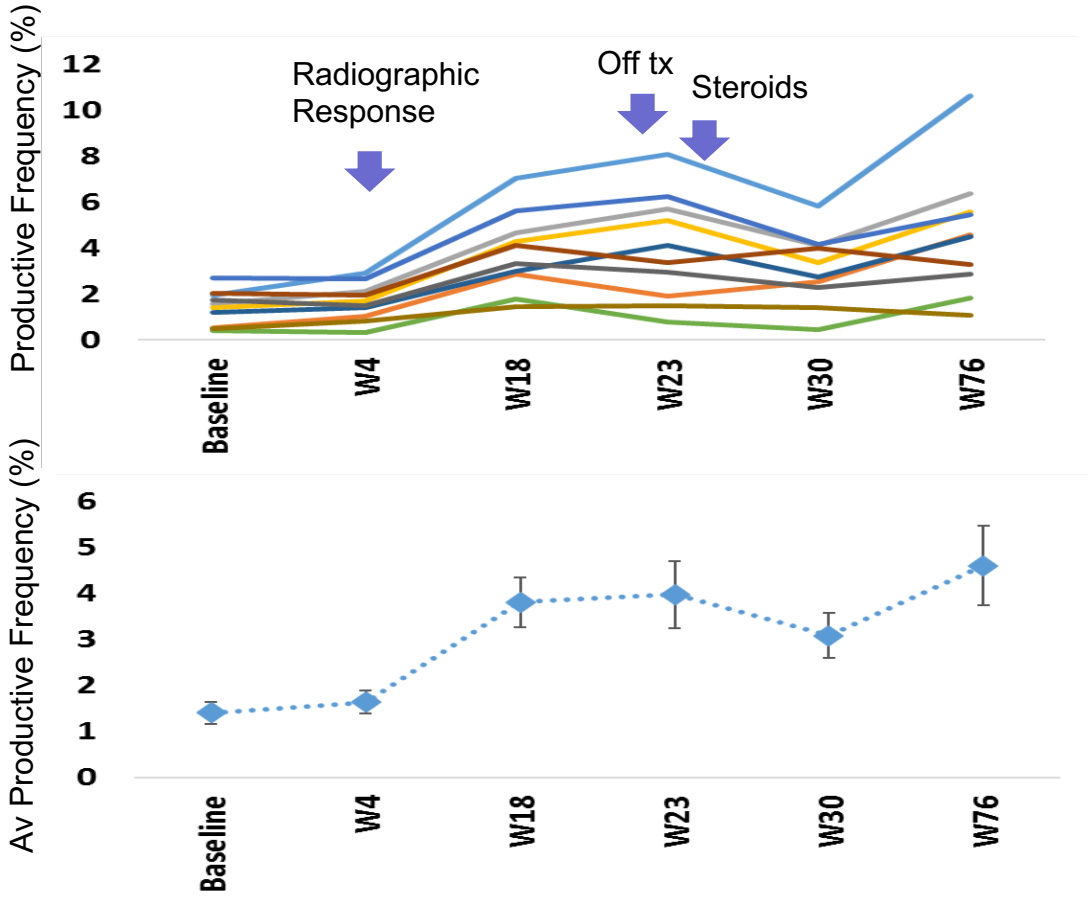
ctDNA trends of intratumoral variants



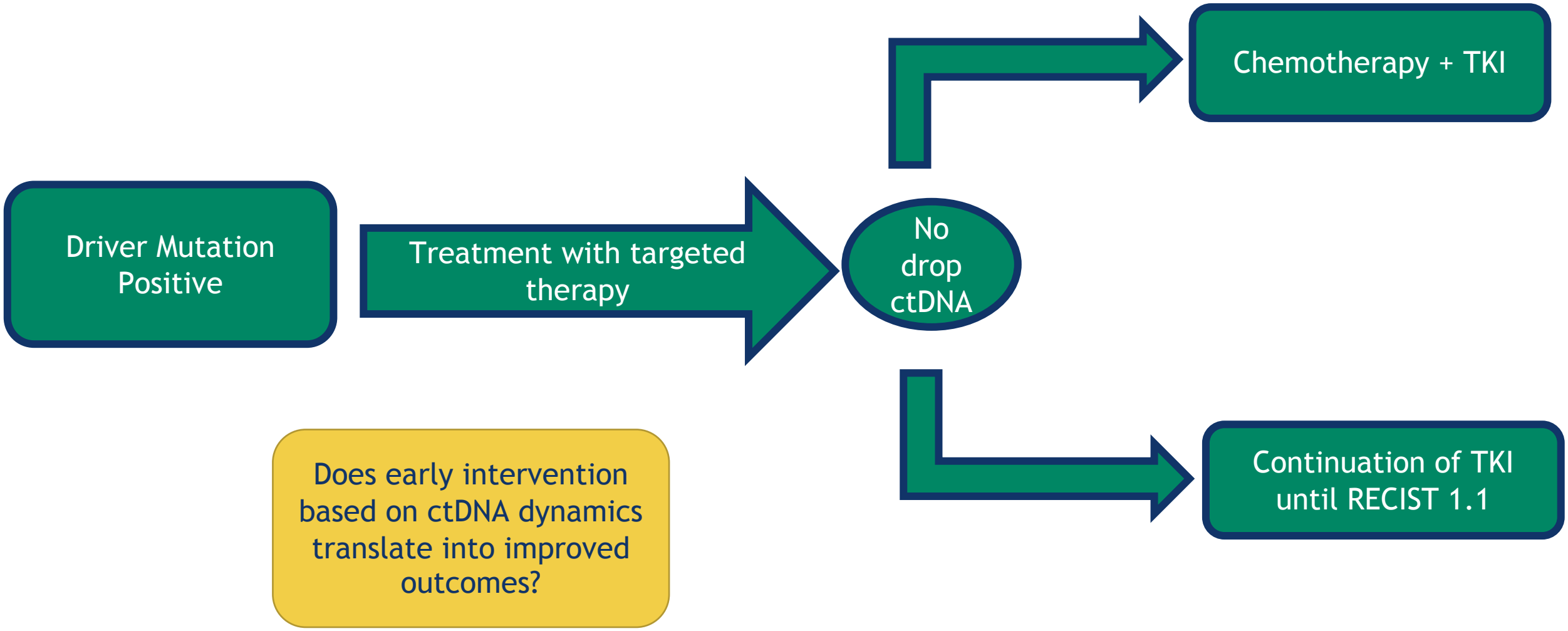


Differentially abundant TCR clones at response



Anagnostou V et al. *Cancer Res*. 2019;79(6):1214-1225..

Moving Forward: Early Assessment



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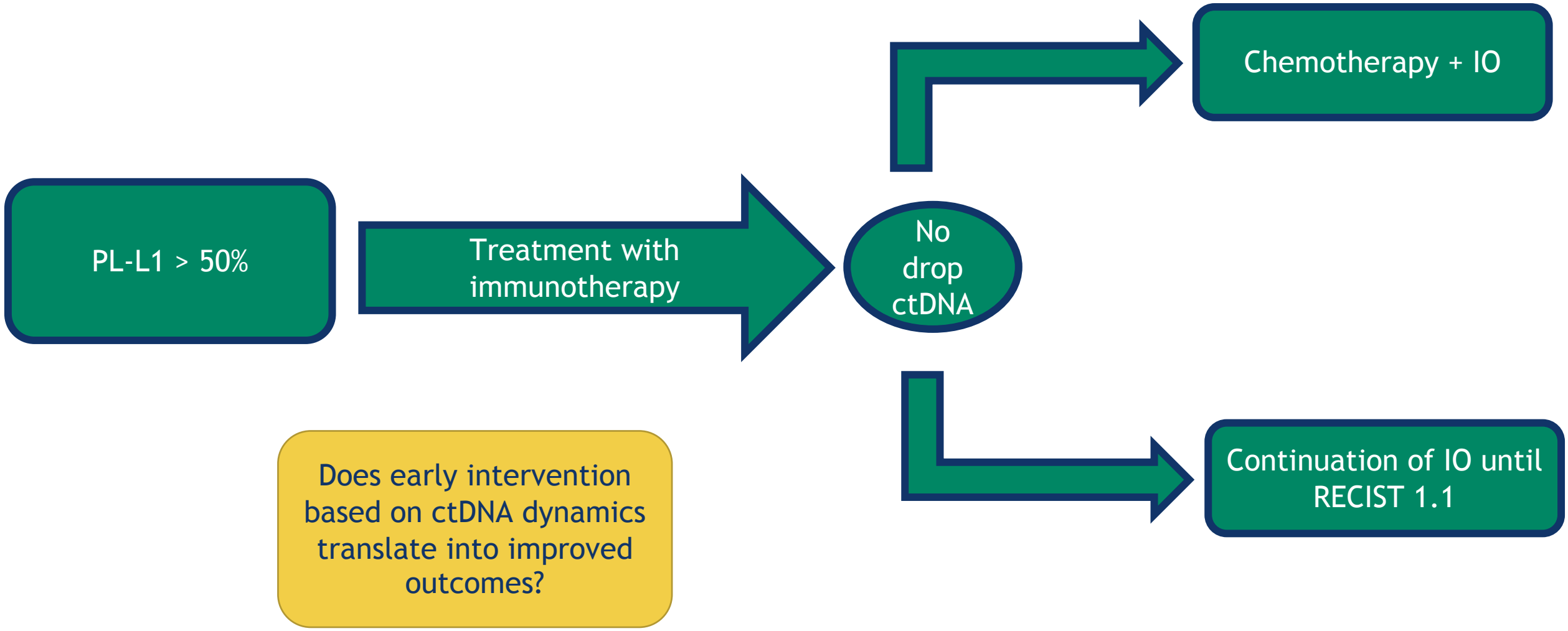
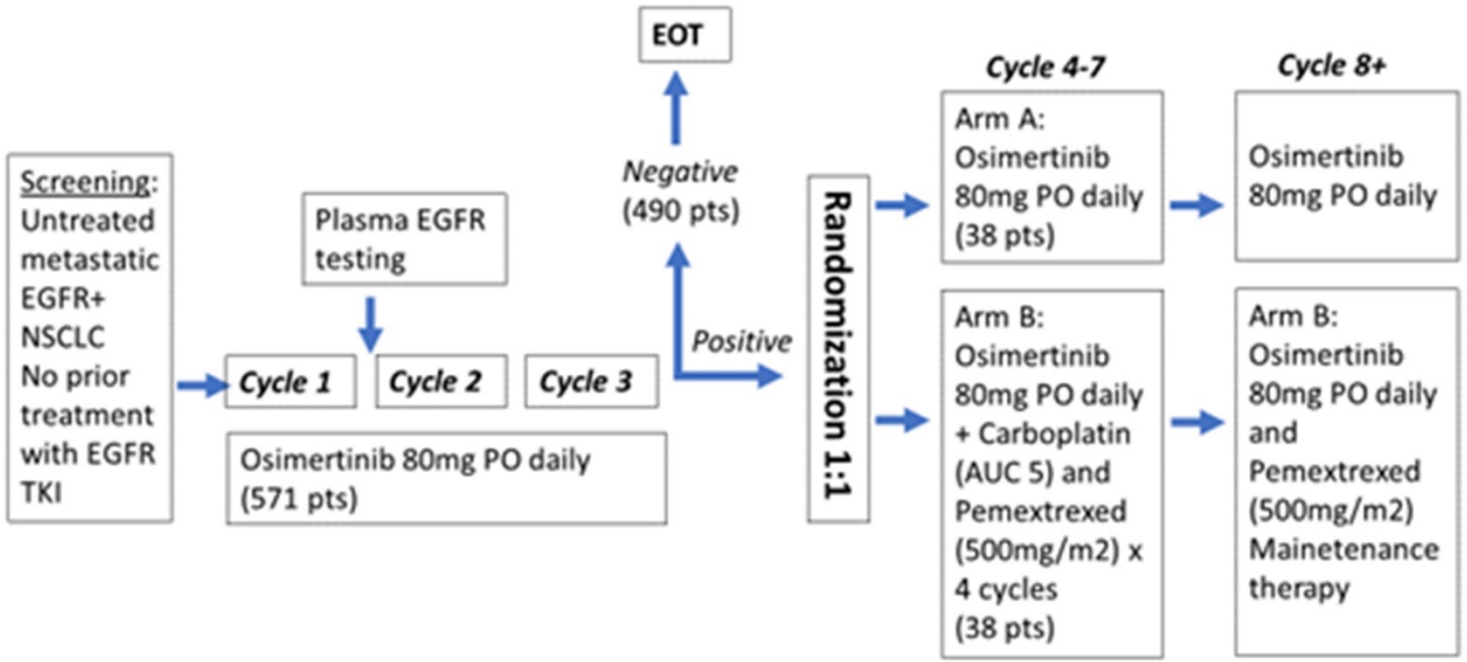


Moving ctDNA Toward Clinical Action



Primary Endpoint:
PFS

Osimertinib Alone or With Chemotherapy for EGFR-Mutant Lung Cancers
Dr. Helena Yu, NCT04410796

Outstanding Question

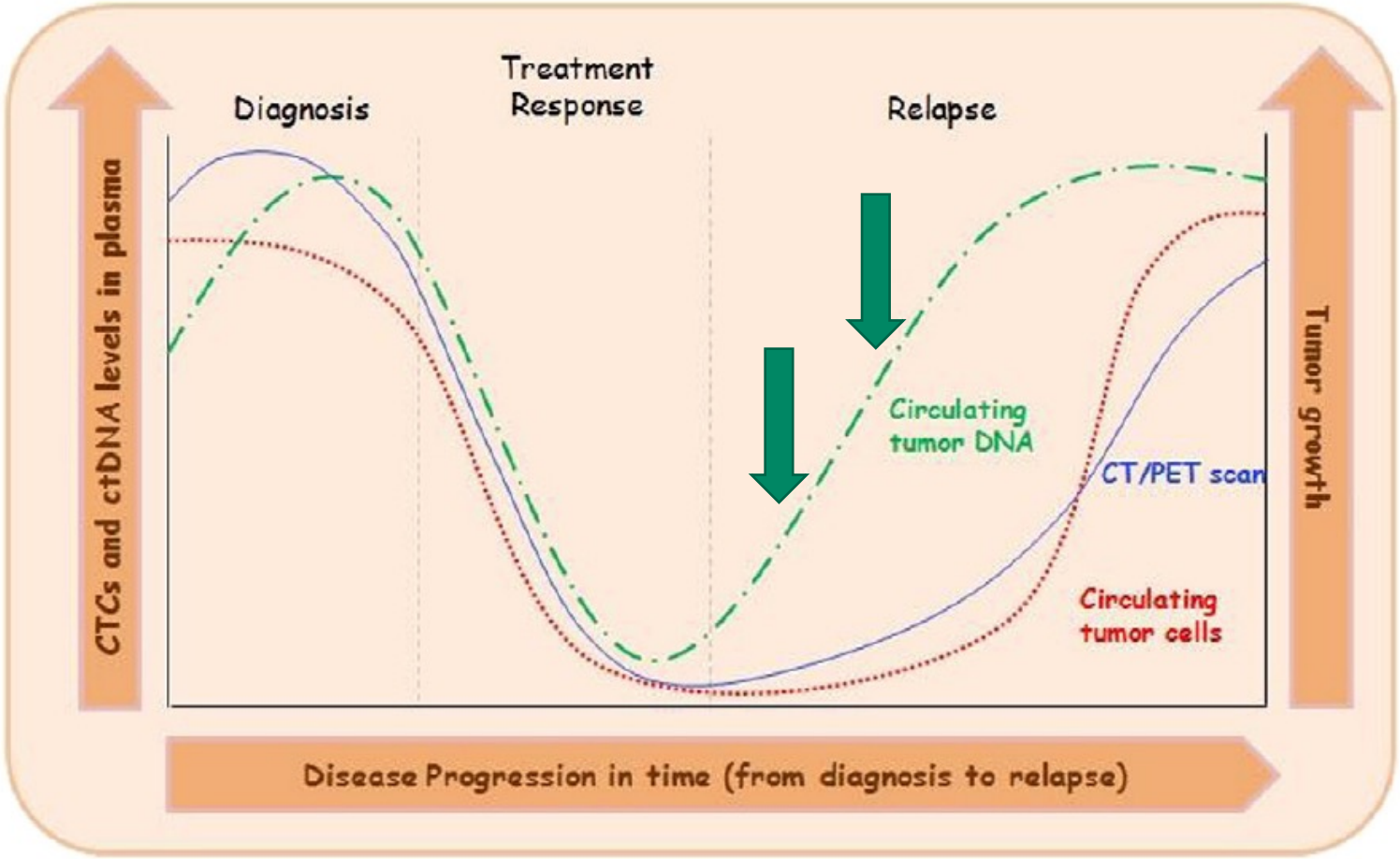
We will need to define what change in ctDNA is meaningful

Clearance?

Percentage Drop?

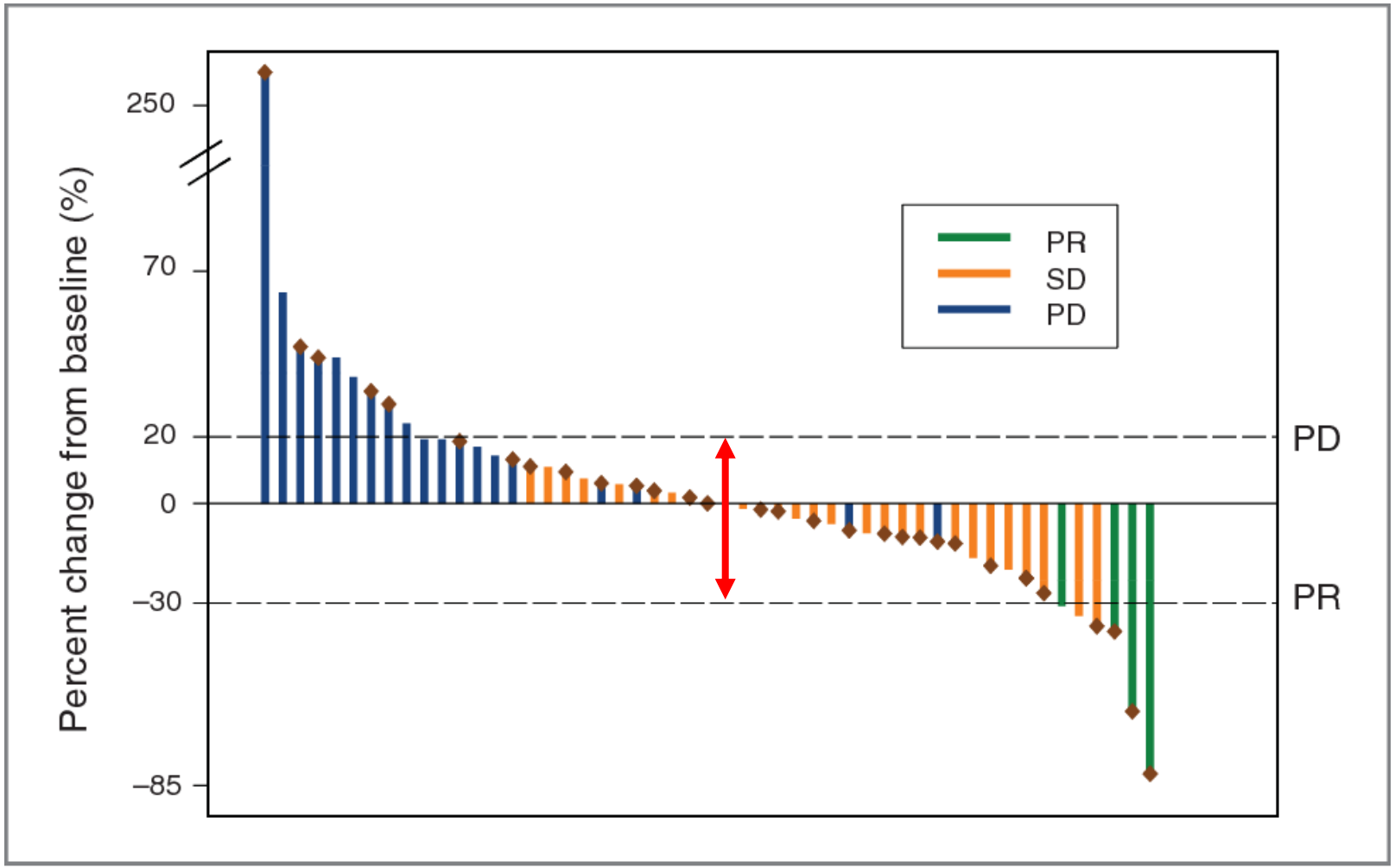
What time Point?

Moving Forward: Identifying early resistance

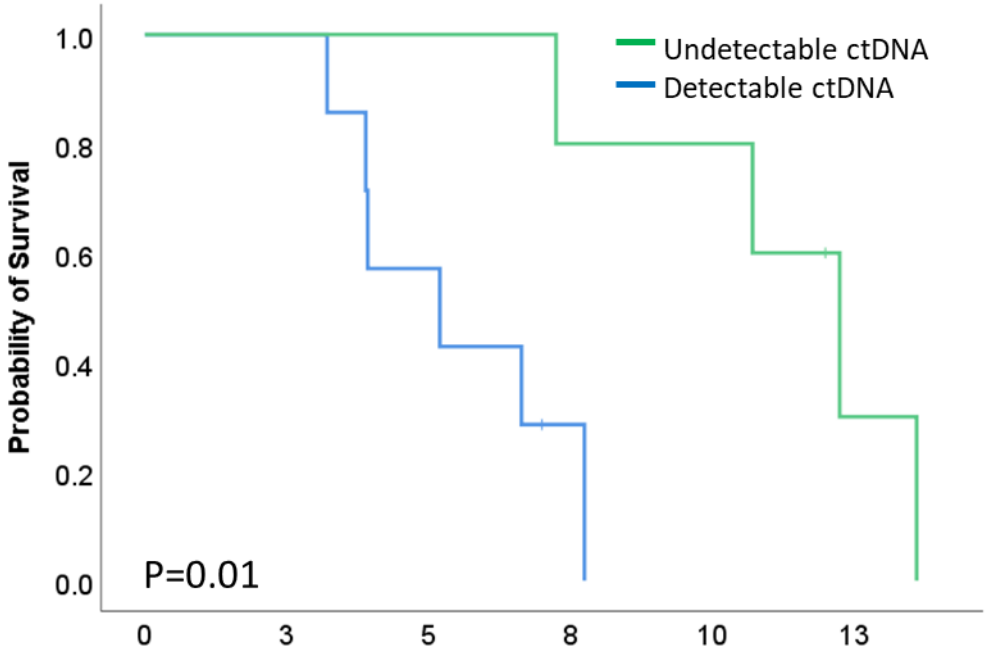


Does a therapeutic switch based on early detection of resistance or rise in sensitizing mutation prior to scans improve outcome?

Stable Disease: A wide spread



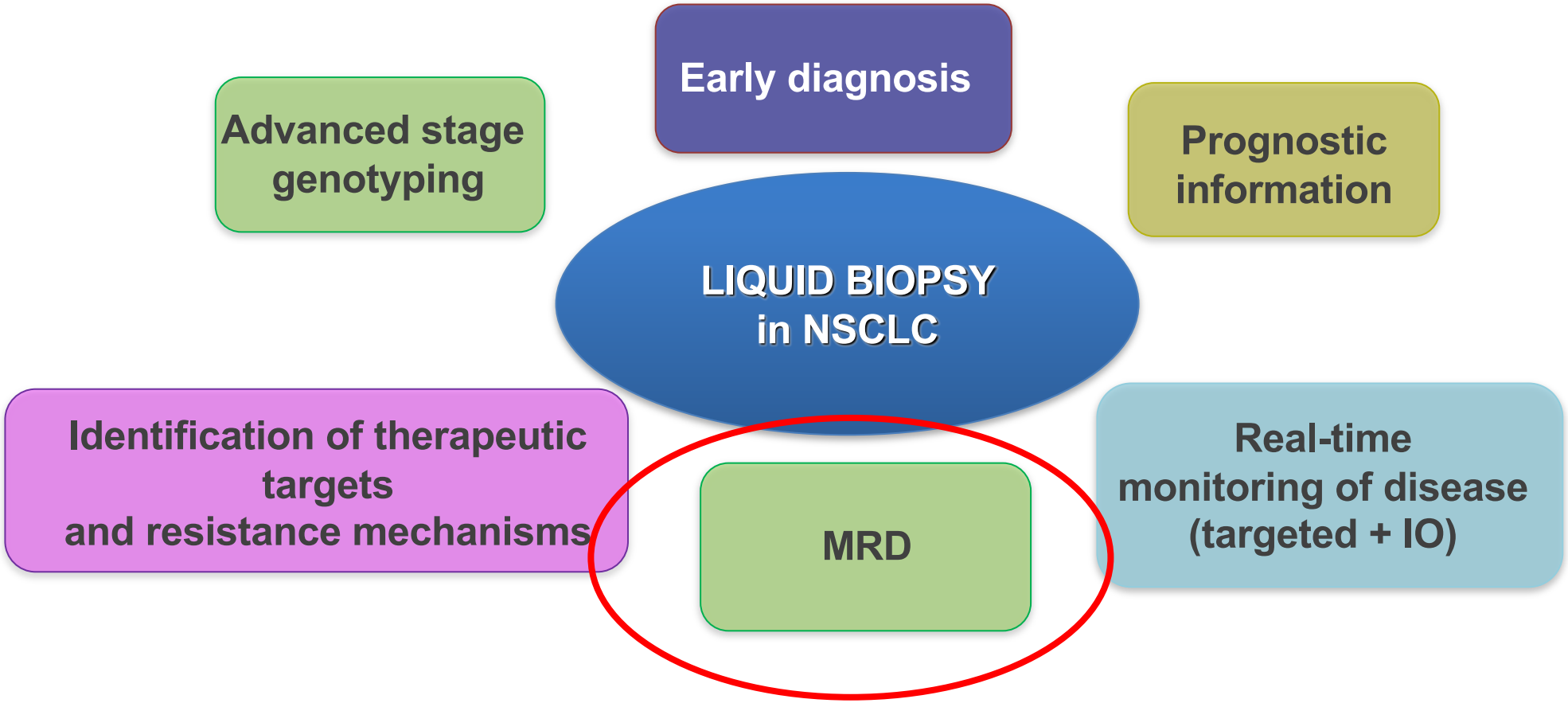
Molecular-Radiologic Response Concordance



- Patients with radiographically stable disease (n=12) had differential responses to immune checkpoint blockade that were consistent with their molecular response pattern.

Anagnostou et al., *Cancer Research*, 2019

Liquid Biopsy: Clinical Application

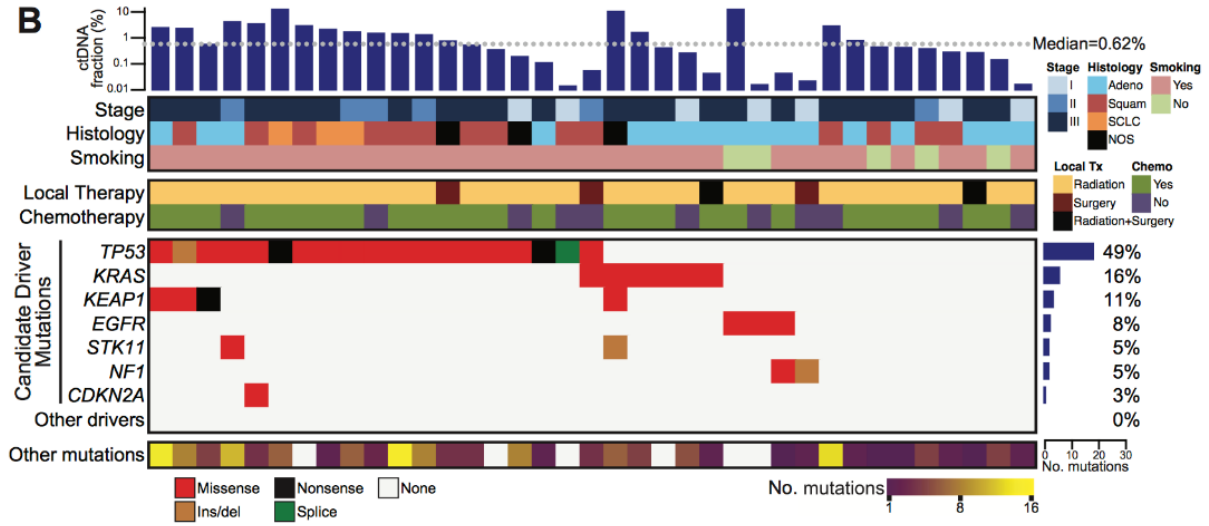


Castiglia M et al. *Biochim Biophys Acta*. 2014;1846(2):539-46

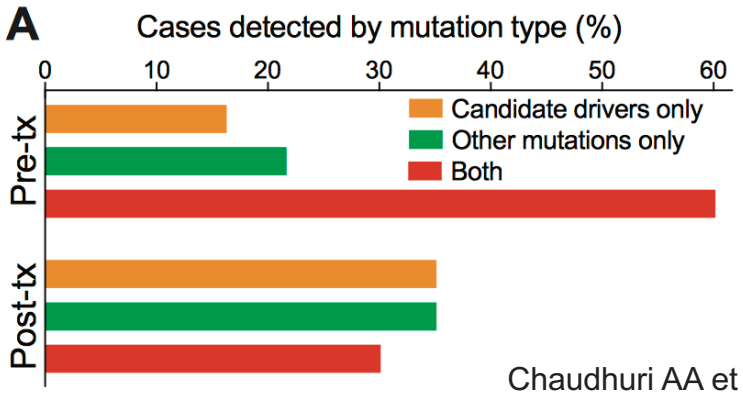
High Sensitivity cfDNA to Detect Recurrence

- **Cancer Personalized Profiling by deep sequencing (CAPP-seq)**
  - 128 recurrently mutated genes
  - 188 kb total
  - Lower limit of detection 0.002%

- 40 patients undergoing curative intent therapy
  - 37 NSCLC, 3 SCLC
  - Stage IB (n=7)
  - Stage II/ III (n=33)

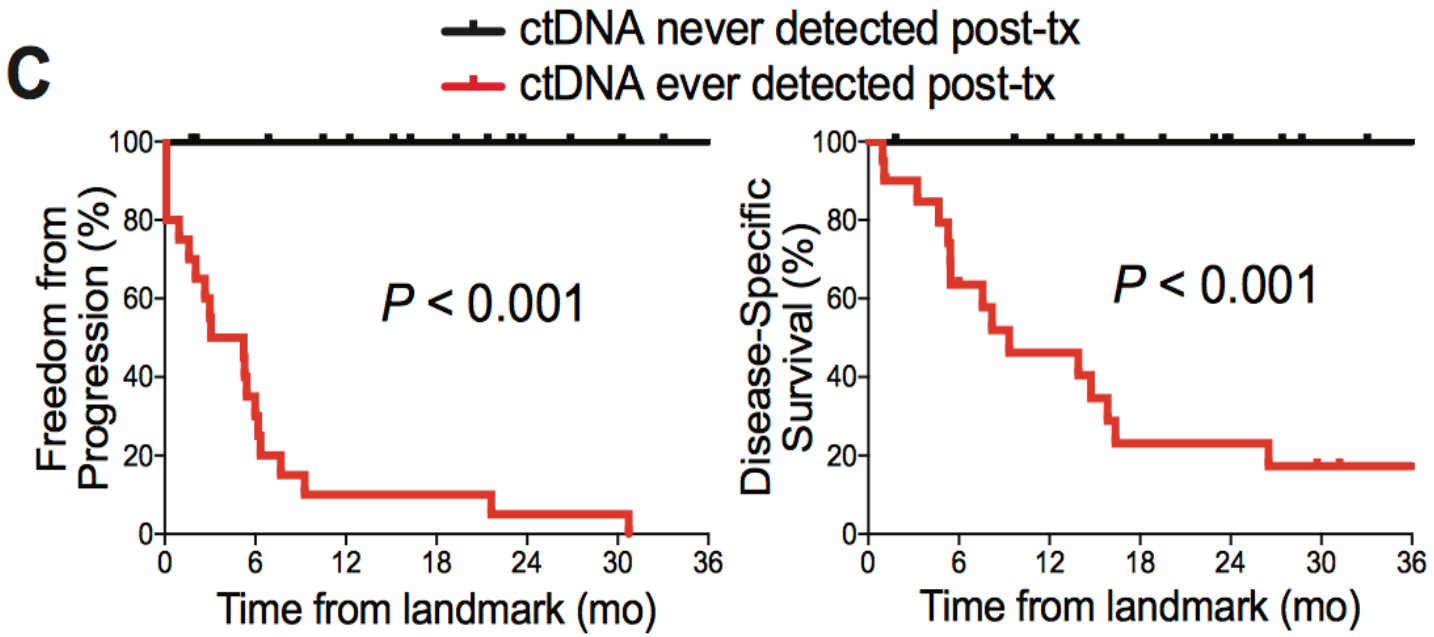


18% mutations deemed drivers

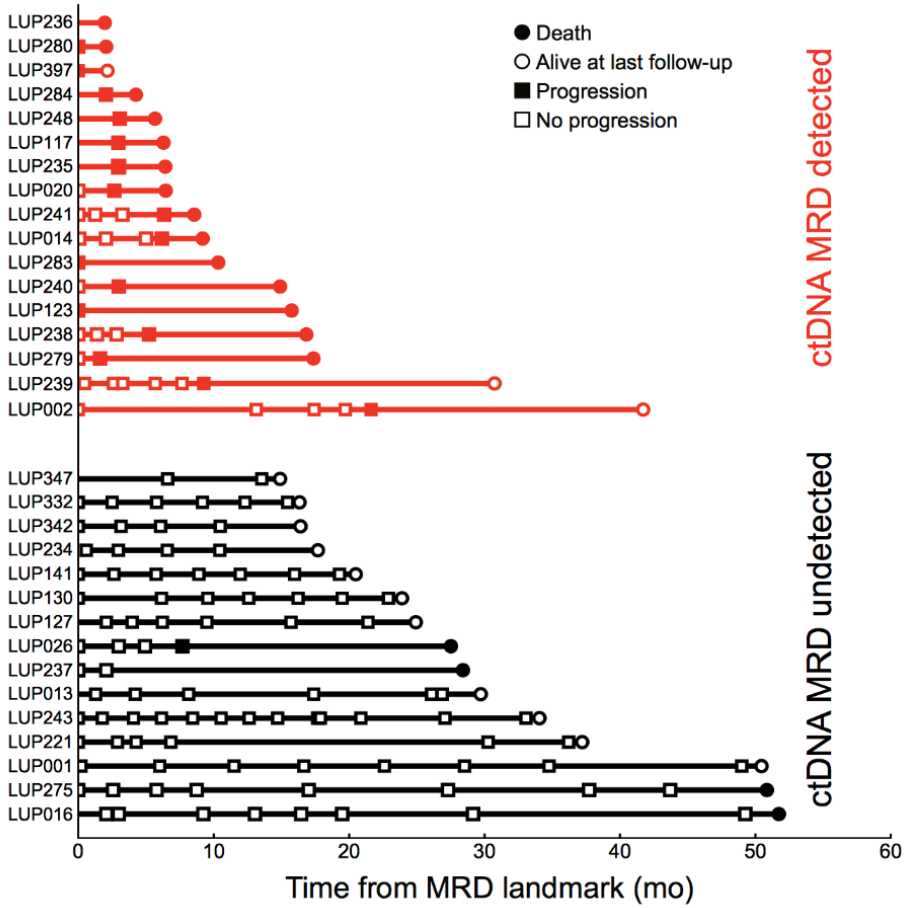


Chaudhuri AA et al. *Cancer Discov*. 2017;7(12):1394-1403.

Detecting Minimal Residual Disease



Detection of ctDNA preceded radiographic progression in **72% of patients** by median of **5.2 months.**

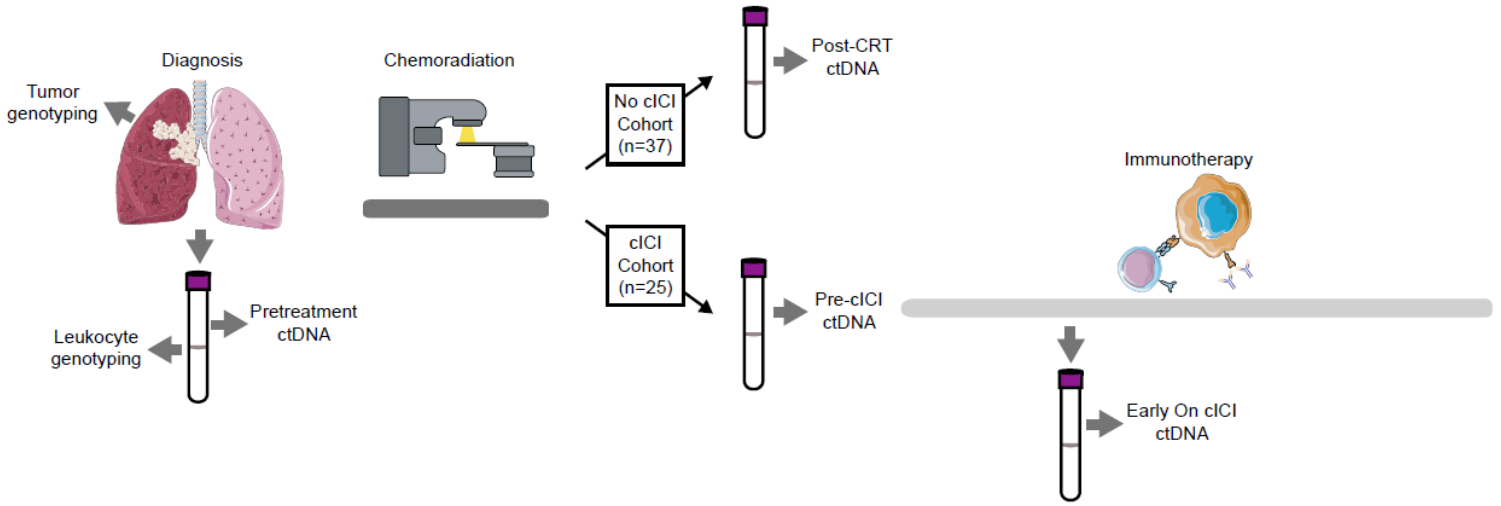


Chaudhuri AA et al. *Cancer Discov*. 2017;7(12):1394-1403.

Detecting Minimal Residual Disease: Stage III

Clinical Trial to Test Effect of Consolidation Immunotherapy in ctDNA MRD+ NSCLC

- Retrospective study of 62 patients with Stage III NSCLC
- *In silico* model of ctDNA-guided trial
- No differences in baseline characteristics between cohorts

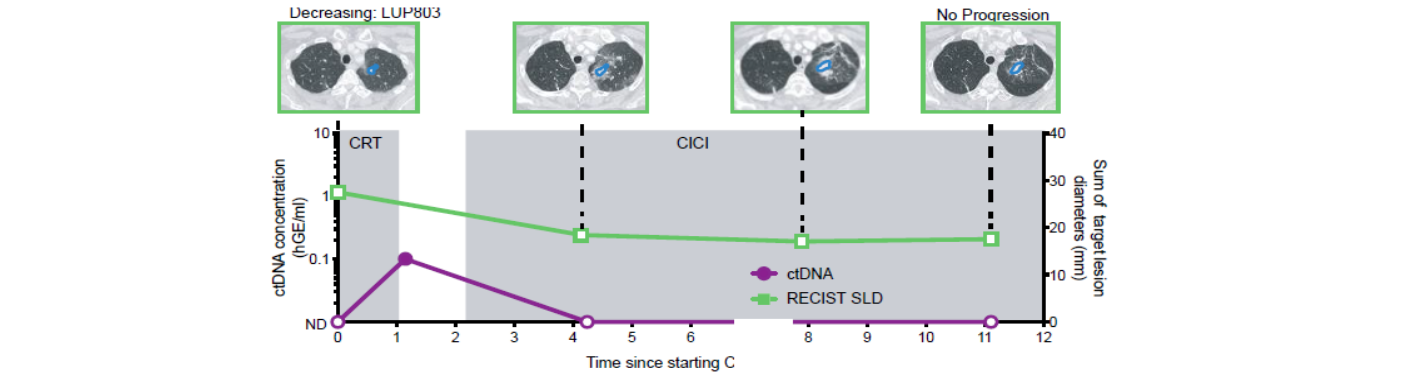


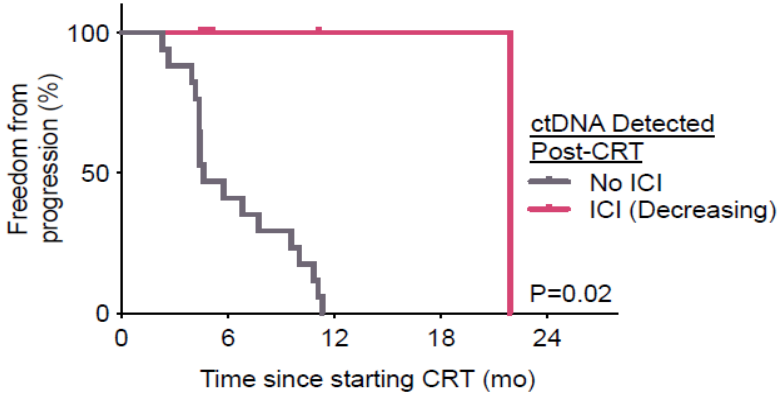
E. Moding et al. Nature Cancer 2020

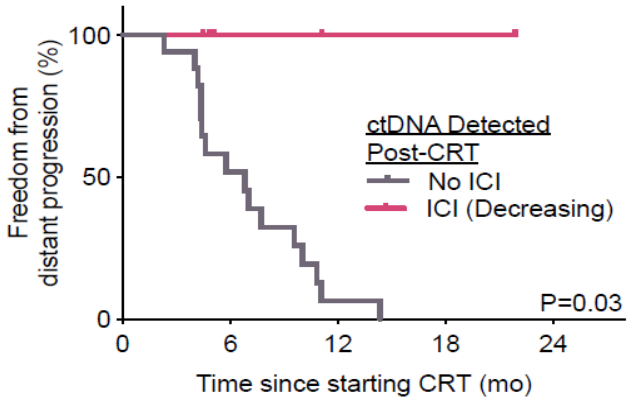
Detecting Minimal Residual Disease: Stage III

JOHNS HOPKINS MEDICINE

ctDNA Clearance During Consolidation ICI is Associated With Improved Outcomes



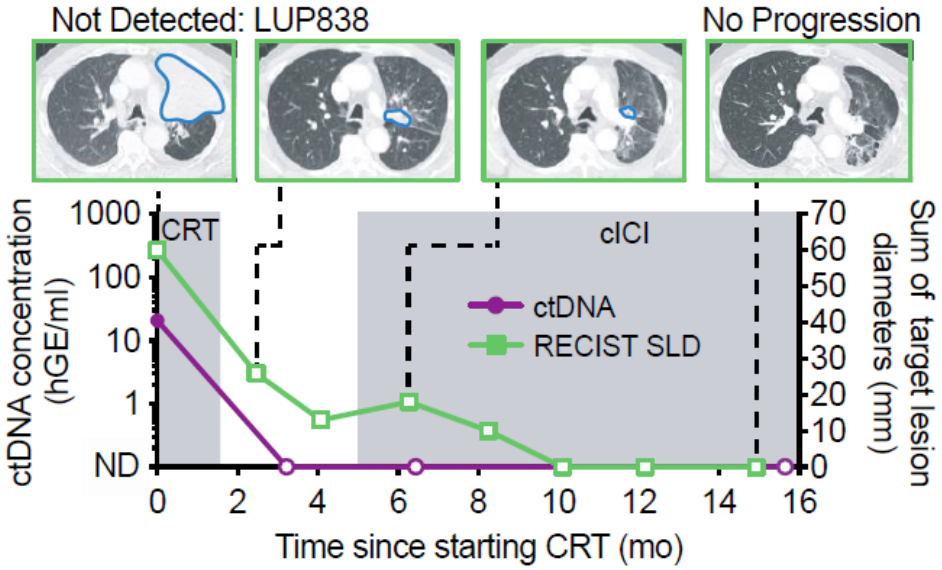


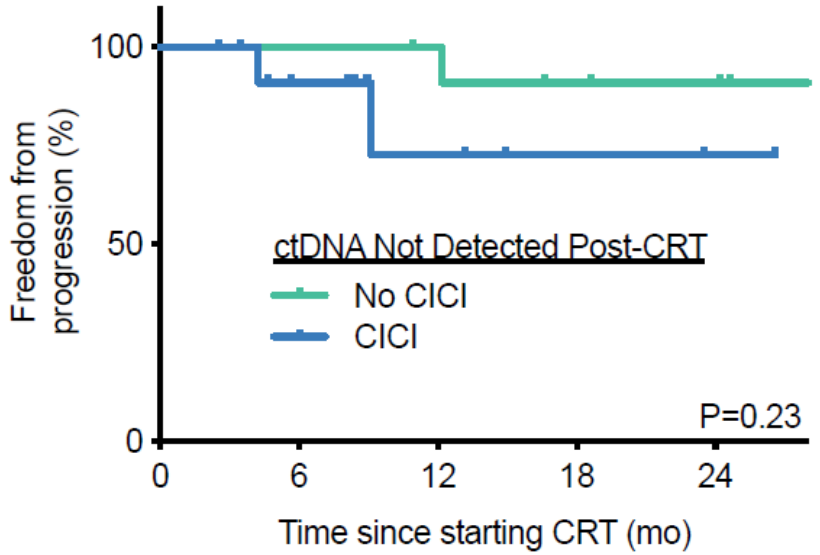


E. Moding et al. Nature Cancer 2020

Detecting Minimal Residual Disease: Stage III

Outcomes in Patients with Undetectable ctDNA After CRT





E. Moding et al. Nature Cancer 2020

Phase III IMpower010 adjuvant study in resected NSCLC

Study design

Completely resected stage IB-IIIA NSCLC per UICC/AJCC v7

- Stage IB tumours ≥4 cm
- ECOG PS 0-1
- Lobectomy/pneumonectomy
- Tumour tissue for PD-L1 analysis

1-4 cycles cisplatin + pemetrexed, gemcitabine, docetaxel or vinorelbine

N=1280

R 1:1

No crossover permitted

Atezolizumab *1200 mg IV q21d 16 cycles*

N=1005

BSC

Survival follow-up

Randomisation stratification factors: sex, stage (IB vs II vs IIIA), histology, PD-L1 tumour expression status per VENTANA SP142 assay (TC2/3 and any IC vs TC0/1 and IC2/3 vs TC0/1 and IC0/1)

Study endpoints

Primary endpoint

- Investigator-assessed DFS (hierarchically tested)[a]

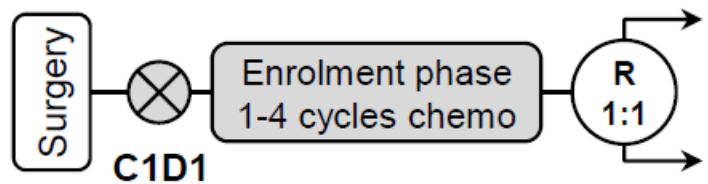
Key secondary endpoints

- OS in ITT population[a,b]
- DFS in PD-L1 TC ≥50% stage II-IIIA population[c]

Exploratory endpoints

- DFS in additional subgroups defined by PD-L1
- DFS defined by baseline ctDNA status

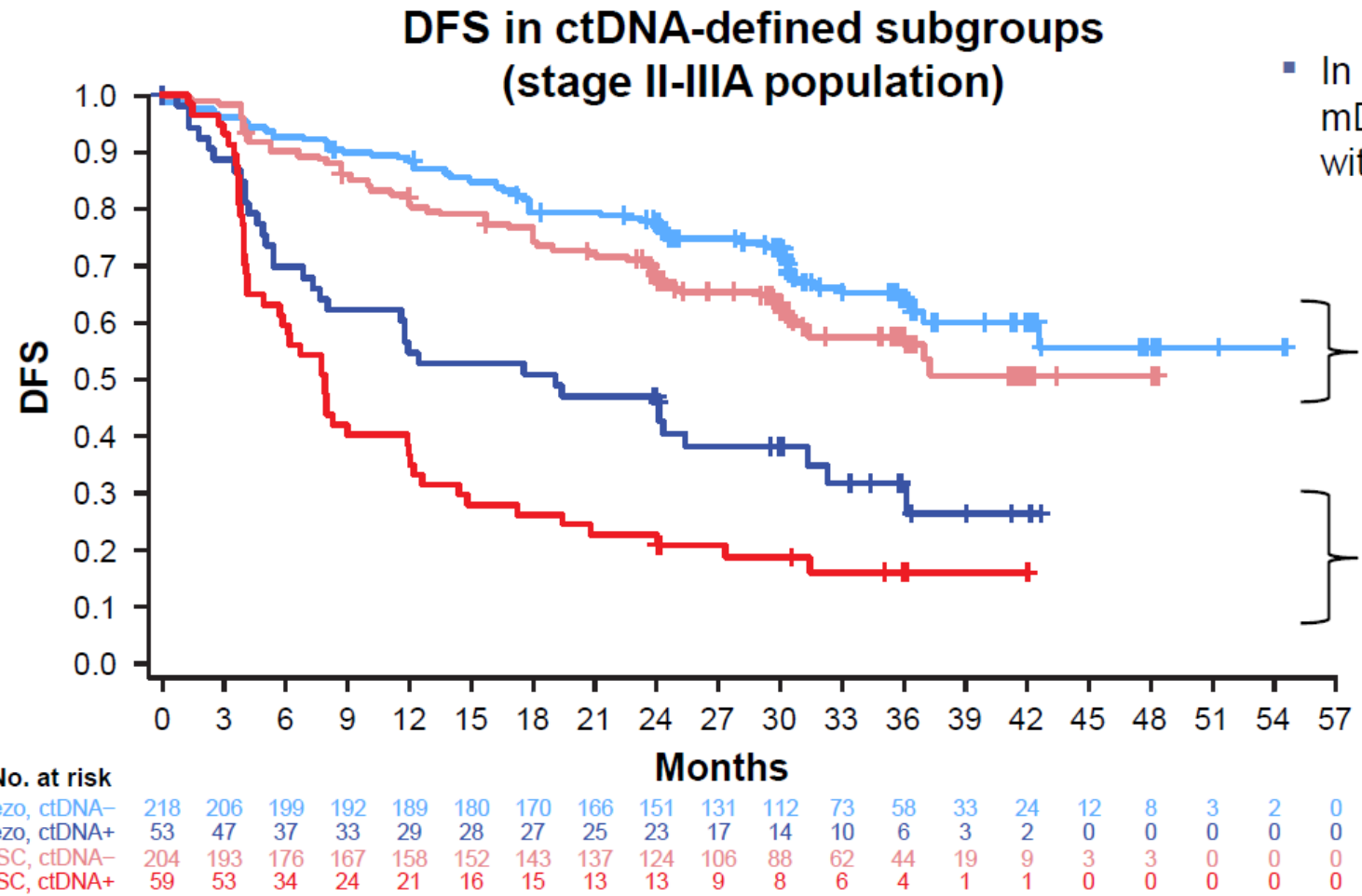
Plasma collection for ctDNA analysis



ctDNA samples were collected on C1D1 of the enrolment phase (after surgery, prior to chemo) and retrospectively tested using the Natera Signatera assay

C1D1, cycle 1, day 1; IC, tumour-infiltrating immune cells; [a] Hierarchical statistical design: DFS tested in (1) PD-L1 TC ≥1%[c] stage II-IIIA patients; then if positive, (2) in all randomised stage II-IIIA patients; then if positive, (3) in the ITT population[b]; then if positive, (4) OS tested in the ITT population. [b] The ITT population includes the all-randomised stage IB-IIIA population. [c] Per SP263 assay.

ctDNA positivity was strongly prognostic, with DFS favouring atezo in both ctDNA+ and ctDNA– patients



- In all ctDNA-evaluable stage II-IIIA patients, mDFS was NR (atezo) vs 31.4 months (BSC), with an HR of 0.69 (95% CI: 0.53, 0.89)

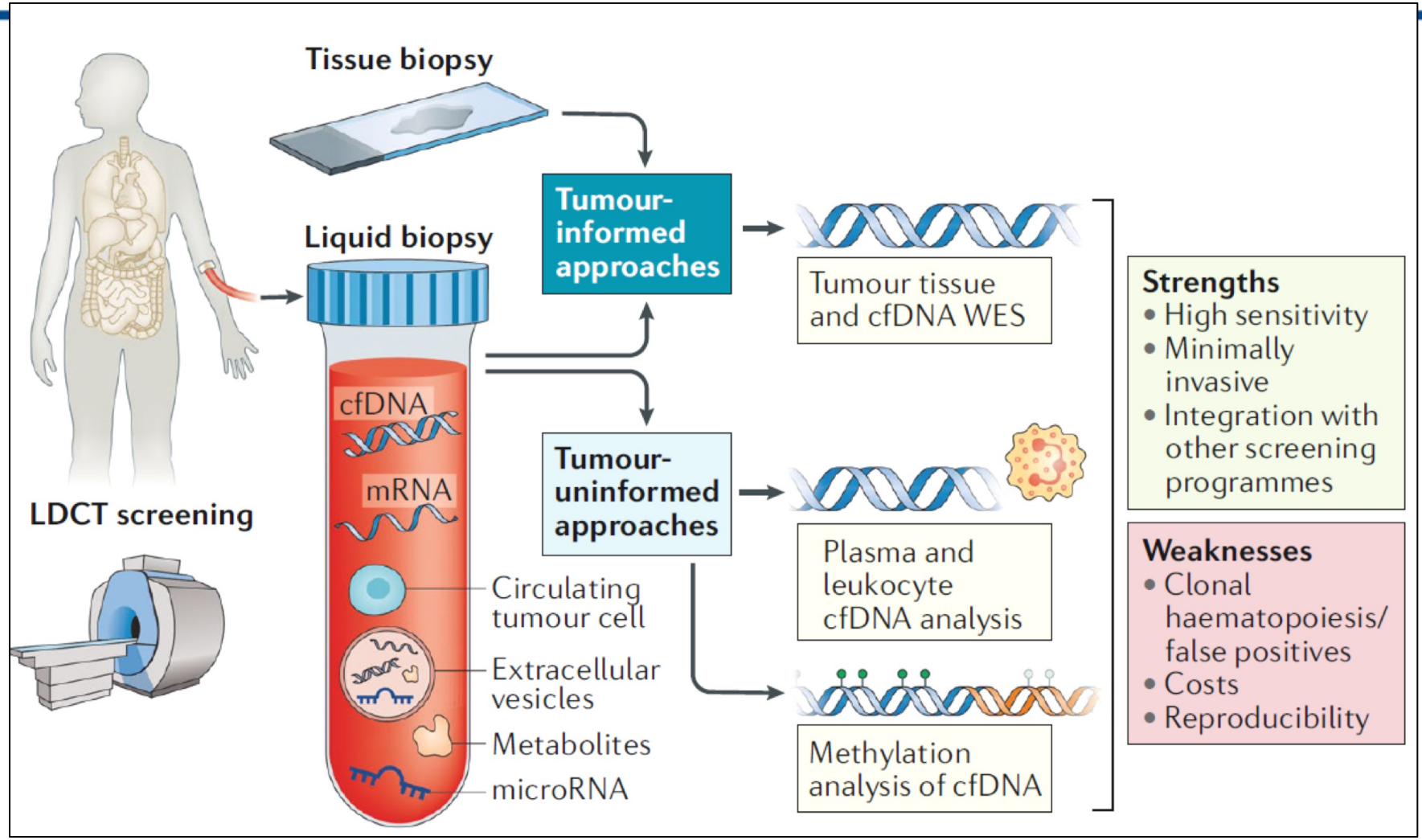
ctDNA–	Atezo (n=218)	BSC (n=204)
mDFS, mo	NR	NR
HR (95% CI)	0.72 (0.52, 1.00)	

ctDNA+	Atezo (n=53)	BSC (n=59)
mDFS, mo	19.1	7.9
HR (95% CI)	0.61 (0.39, 0.94)	

Clinical cutoff: 21 January 2021. Unstratified HRs are shown.

Zhou et al. IMpower010 biomarkers. https://bit.ly/3F2KriO

Minimal Residual Disease Platforms



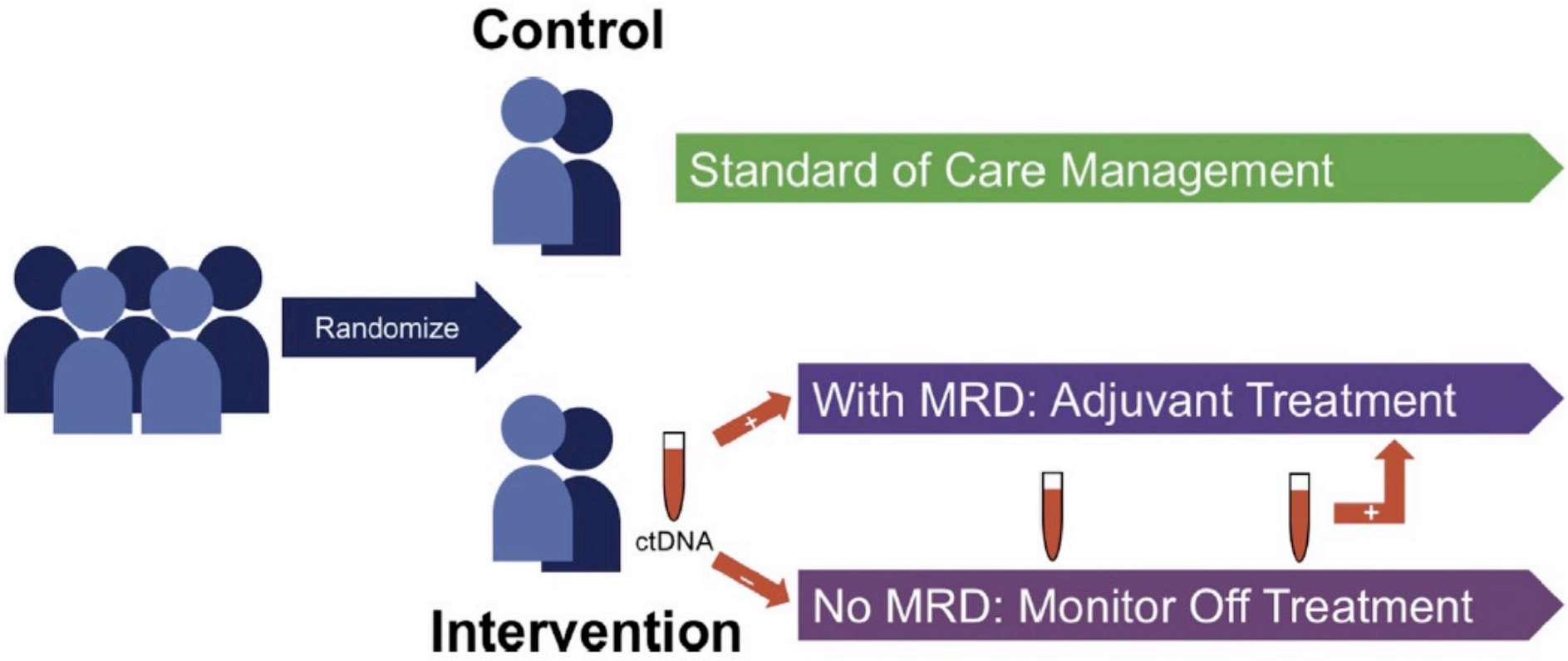
Rolfo et al. Nature Reviews clinical oncology May 2020

Minimal Residual Disease Platforms

	"Tumor naïve"	"Tumor informed"
Gene coverage	Large panel of commonly altered genes	Limited panel of genes personalized to the patient's tumor
Tissue sequencing required?	No	Yes
Key applications	• MRD • Assess heterogeneity • Detect actionable alterations • Identify drivers of resistance • Serial monitoring	• Detect MRD • Assess treatment response • Serial recurrence monitoring
Screens out germline, CHIP alterations?	No*	Yes
Turnaround time	1-2 wks	First test: 2-3 wks (includes tissue WES profiling) Subsequent tests: 1 wk

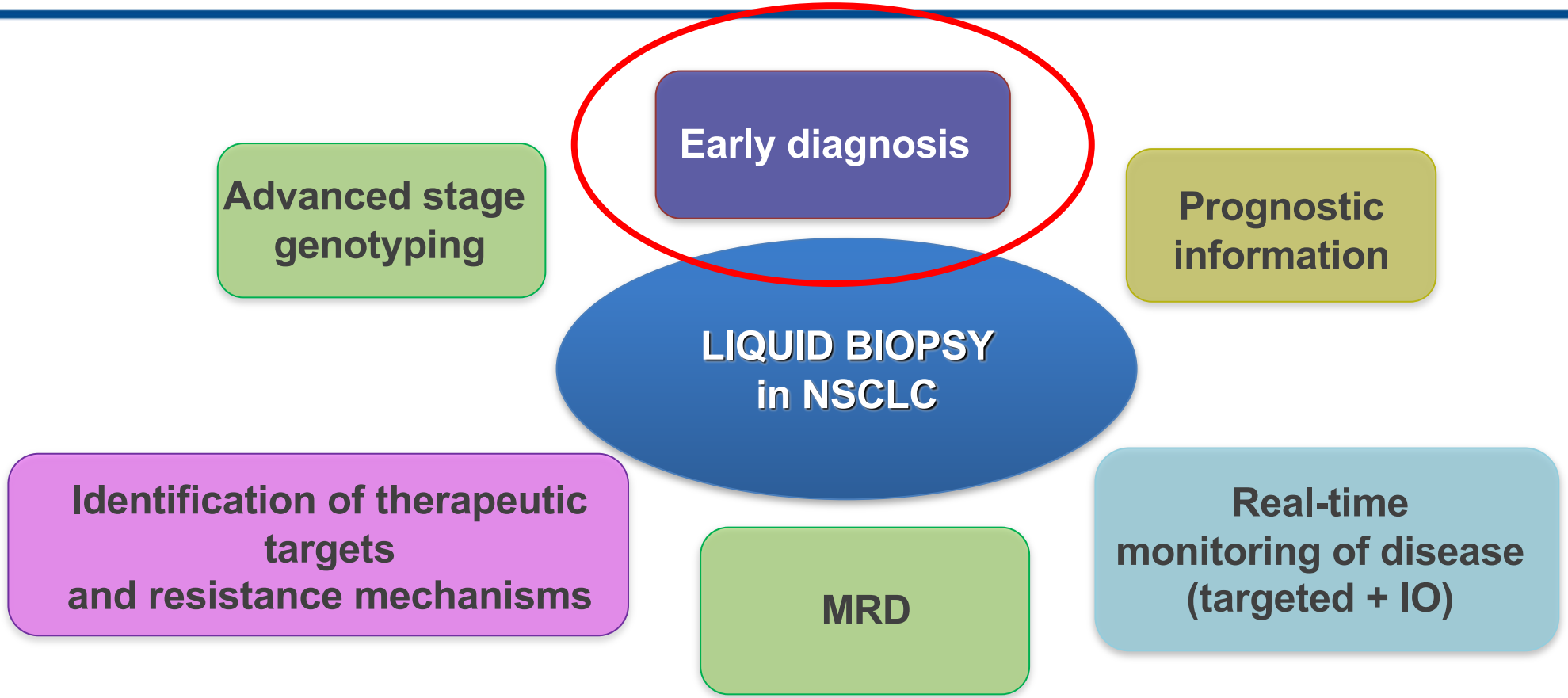
Slide courtesy of Aparna Parkikh

What's the optimal trial design?



Chae et al. JCO 2018

Liquid Biopsy: Clinical Application

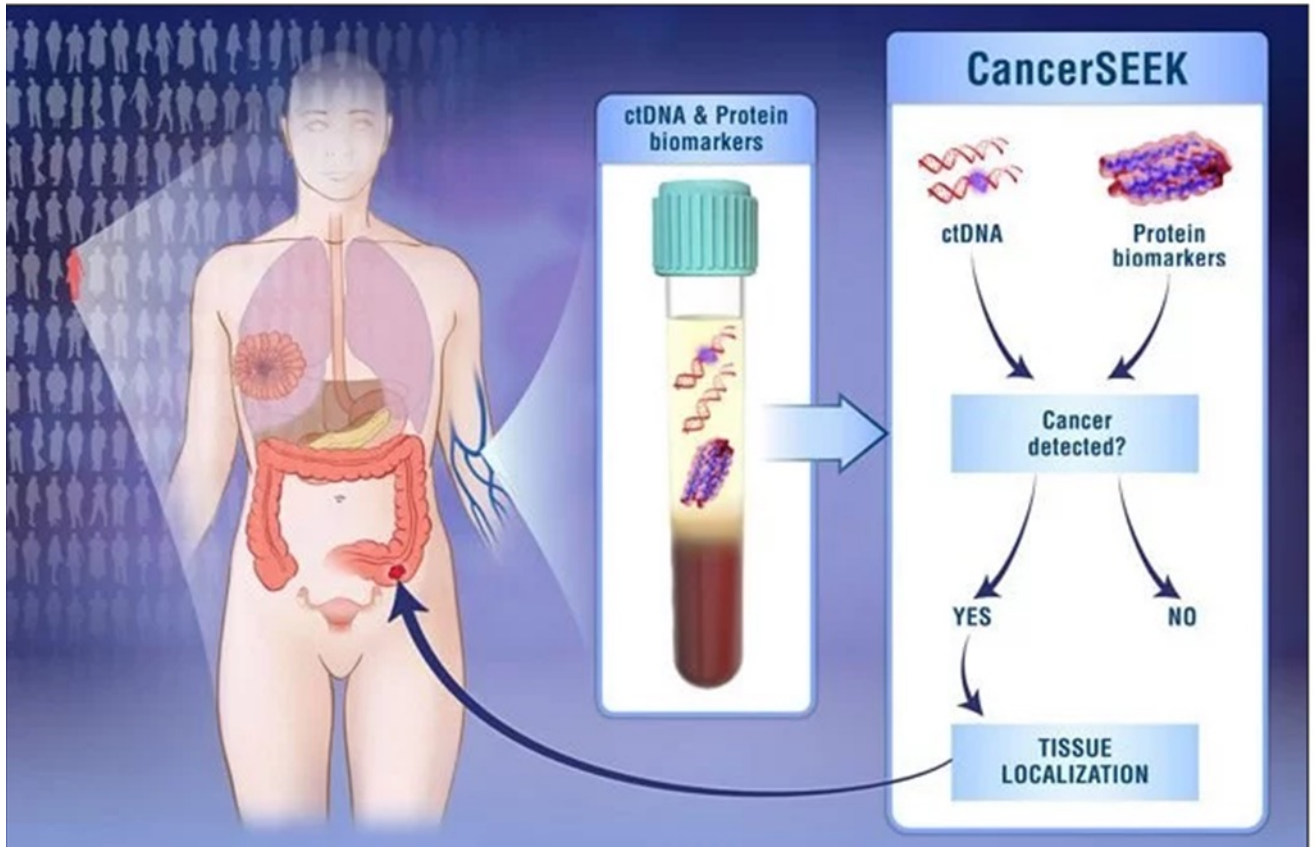


Castiglia M et al. *Biochim Biophys Acta*. 2014;1846(2):539-46

Science

JOHNS HOPKINS MEDICINE

Detection and localization of surgically resectable cancers with a multi-analyte blood test

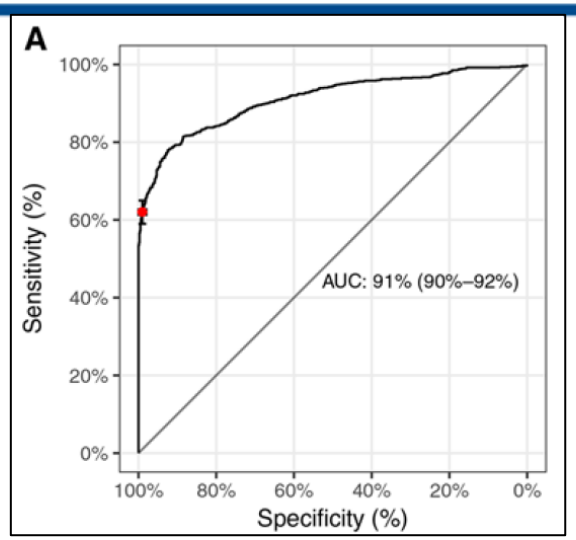


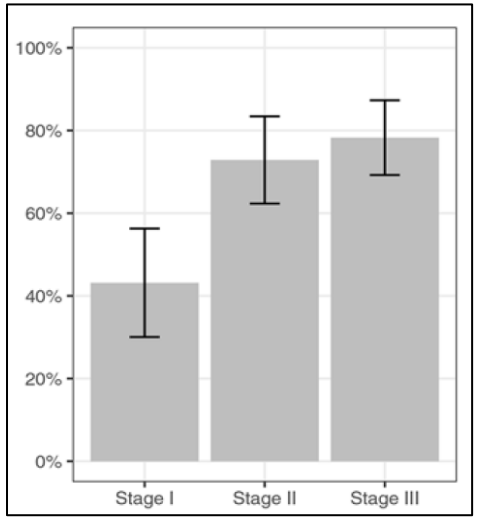
Cohen et al. Science 2018

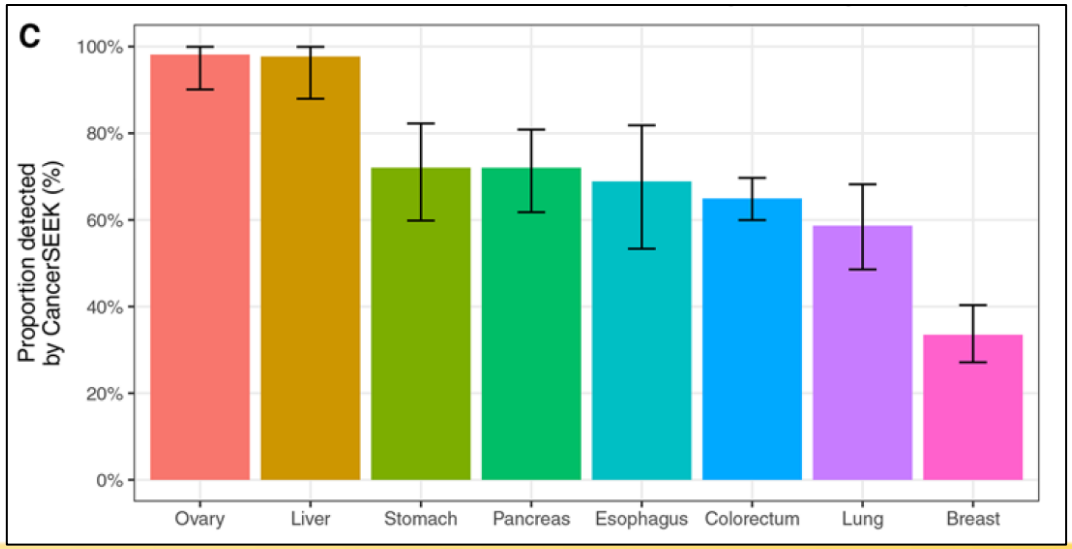
Science

Detection and localization of surgically resectable cancers with a multi-analyte blood test

JOHNS HOPKINS MEDICINE







- N=1005 patients with eight different types of clinically detected cancer (early stage)
- N=812 healthy controls
- Platform: cfDNA (DNA in regions of interest from 16 genes) and proteins (39)
- Median Sensitivity: 70%
- Median Specificity: 99%

Cohen et al. Science 2018

The CCGA study

15 254 participants at 142 sites
56% with cancer; 44% without cancer
(anticipated enrollment period, ~24 months)

Blood (all) and tissue (cancer only) samples collected

Samples divided among three pre-specified CCGA substudies

CCGA substudy 1

Discovery
Training, *n* = 1785 Validation, *n* = 1015

Three independent methods evaluated
1. Targeted sequencing
2. Whole genome sequencing (copy number variants)
3. Whole genome bisulfite sequencing (whole genome methylation)

Whole genome methylation
- Identified as method to be used for further development

CCGA substudy 2

Development of assay and classifier and initial validation
Training, *n* = 3133 Validation, *n* = 1354

Plasma cfDNA underwent bisulfite sequencing targeting a panel of >100 000 informative methylation regions. A classifier was developed/validated for cancer detection and CSO

Targeted methylation
- Identify key methylation regions
- Training and validation of the selected and updated targeted methylation assay and classifier

Further refinement of assay and classifier informed by training set

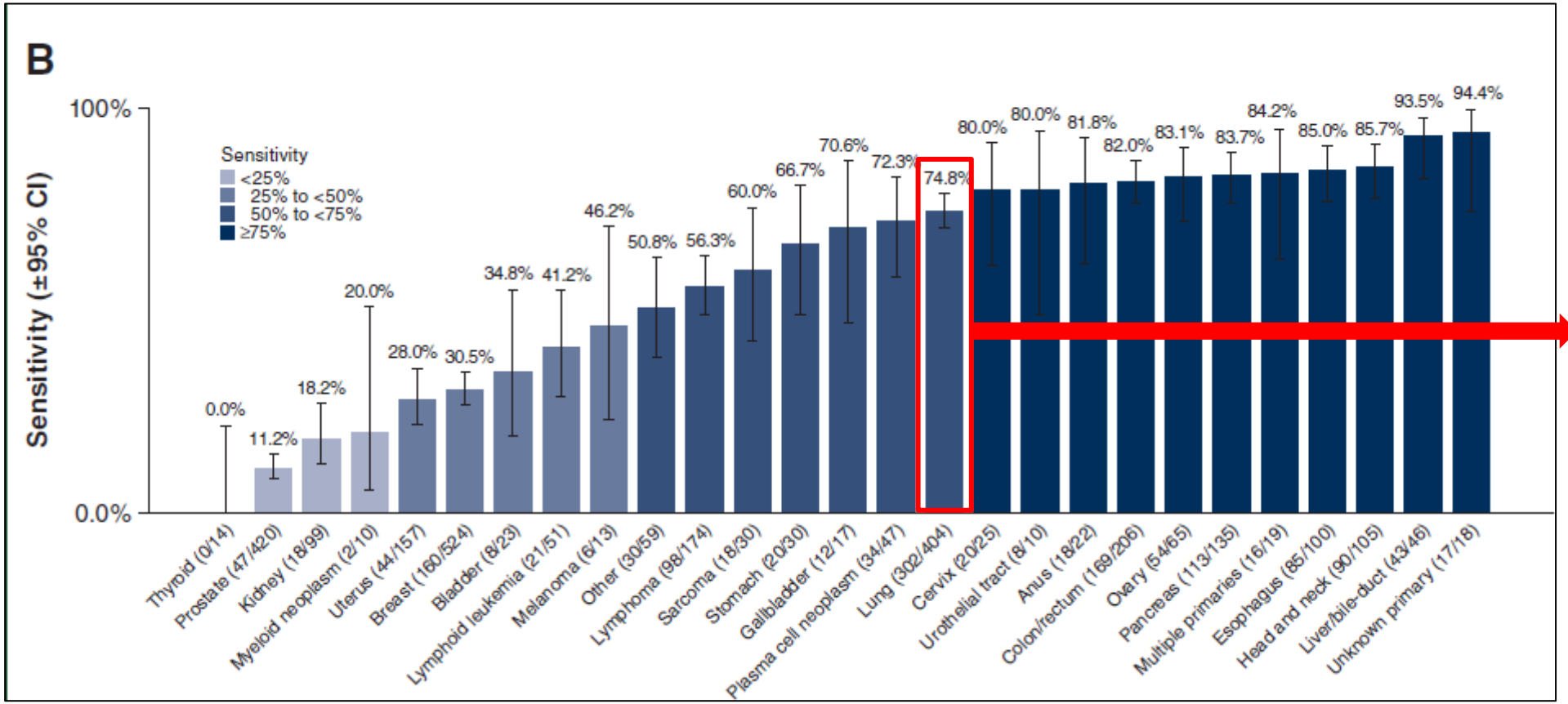
CCGA substudy 3

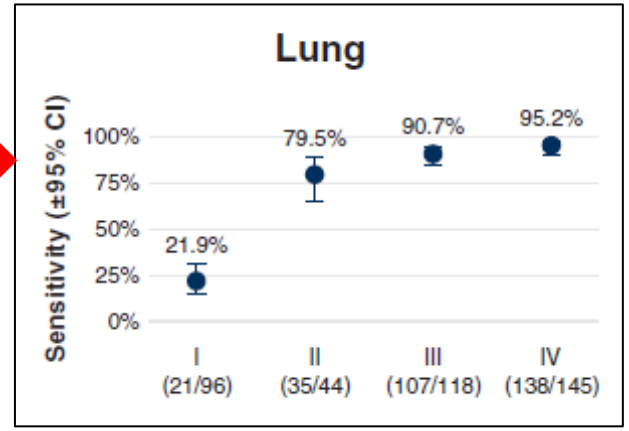
Large-scale clinical validation
n = 5309 participants (cancer = 3237; non-cancer = 2069)
n = 4077 confirmed status set (cancer = 2823; non-cancer = 1254)

Locked assay and classifier for screening (Galleri™) validated in independent validation set

Follow-up for 5 years
(vitals & cancer status)

Test Performance for cancer signal detection





Conclusions

- Genetic interrogation in paramount in optimizing front-line decision making and helping to select genotype-driven therapies in the resistant setting
- cfDNA has demonstrated promise in its ability to:
  - Serve as a molecular proxy in identifying genetic alterations in treatment naïve patients
  - Assess real-time monitoring as a predictor of response
- Studies evaluating cfDNA platforms in monitoring residual disease post curative intent therapy as well identification of early stage disease are promising