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An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage

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- **Performance assessment**
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stages in NSCLC

- occult (hidden) stage
- stage 0 (carcinoma in situ)
- stage I
- stage II
- stage III
  - stage IIIa/stage IIIb
- stage IV

- occult (hiden) stage:
  - cancer cannot be seen by imaging/ bronchoscopy
  - cancer cells are found in sputum/or bronchial washing

- stage 0 (carcinoma in situ):
  - abnormal cells are found in the airways
  - cells may become cancer

    →spread into nearby normal tissue

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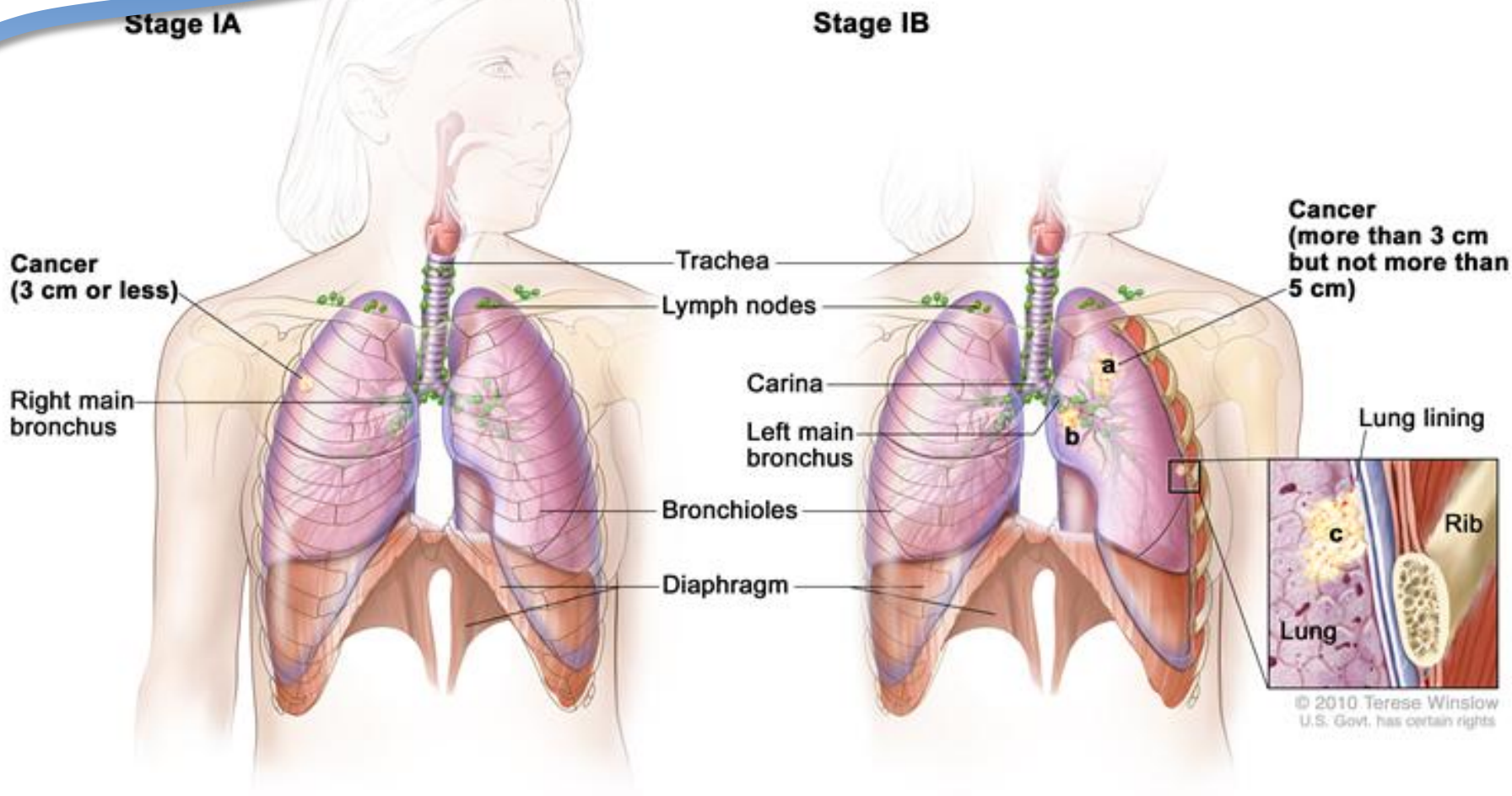
stage I



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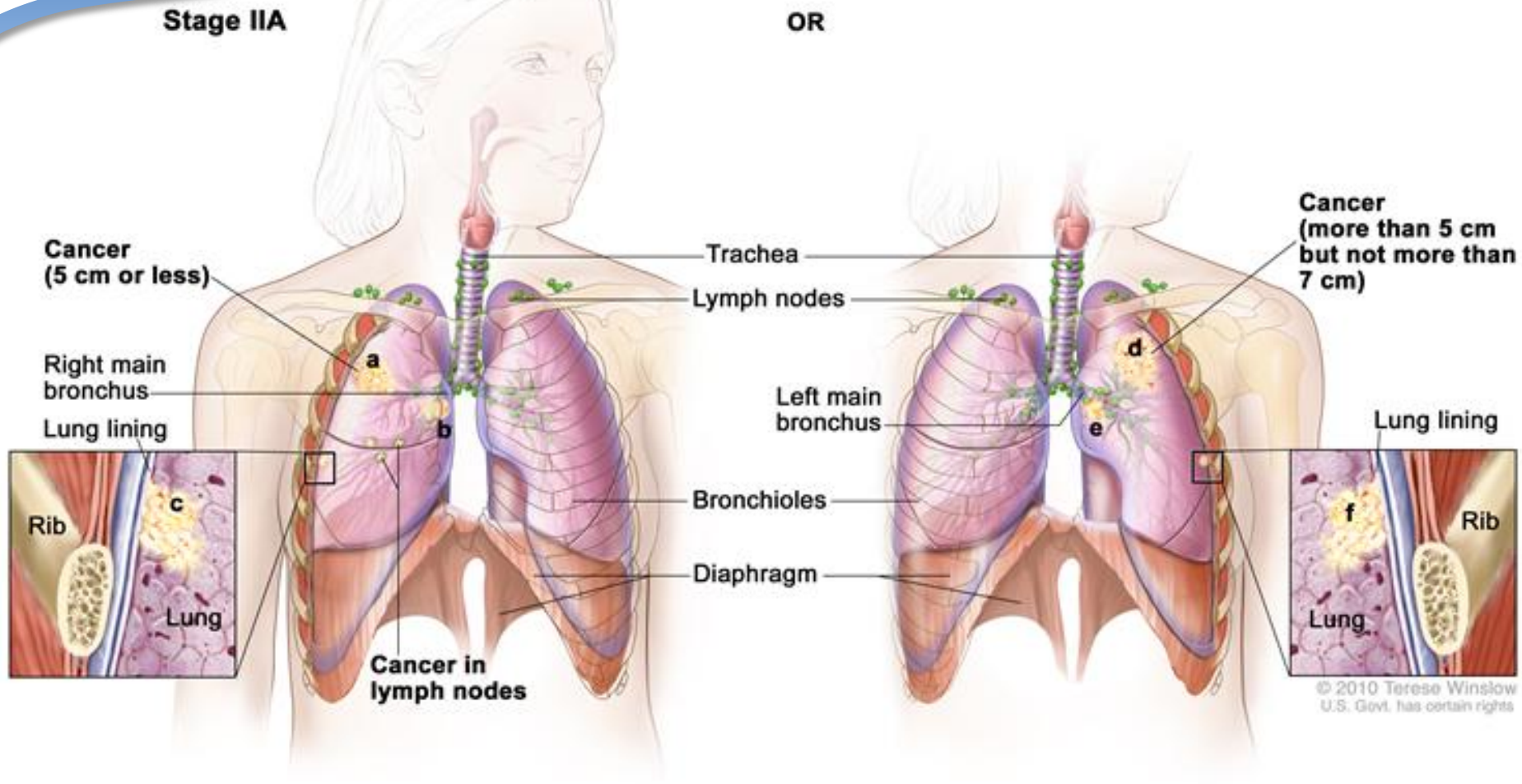
stage IIa



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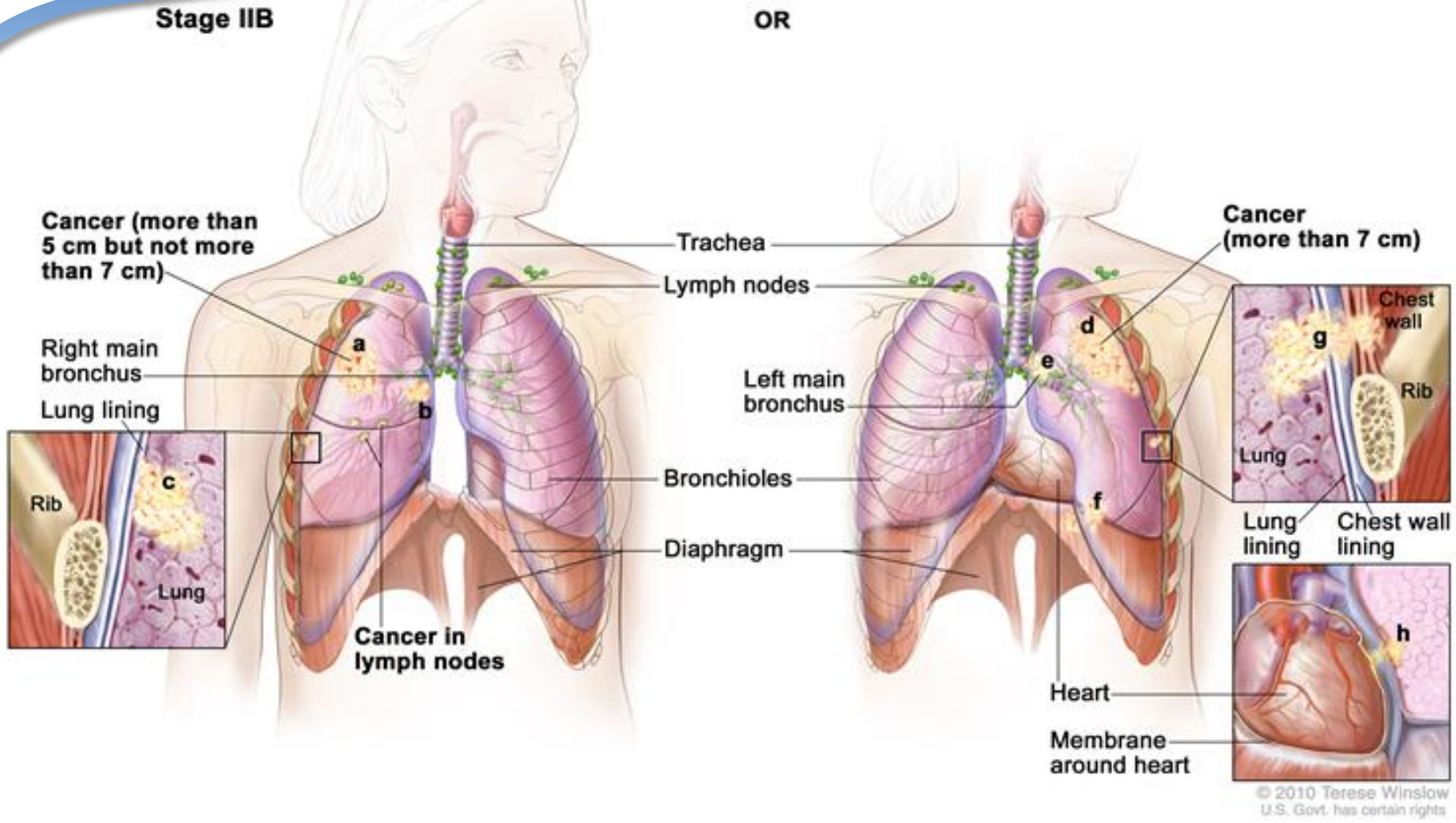
stage IIb



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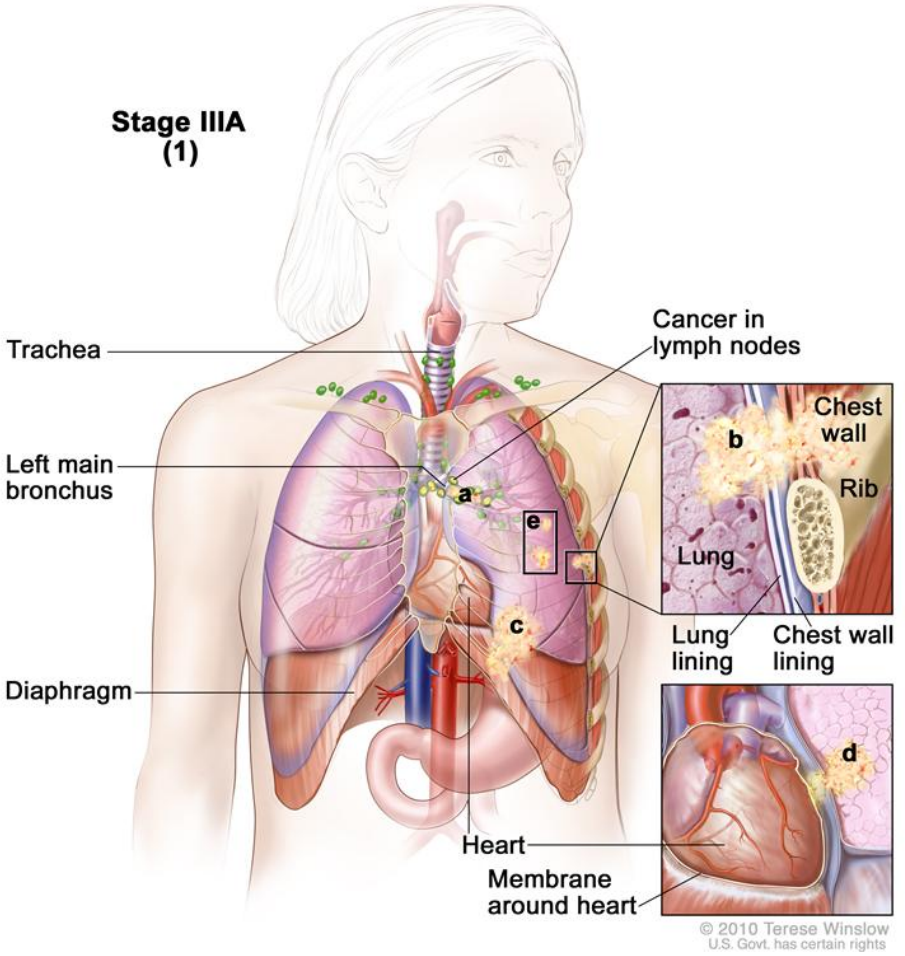
stage IIIA

divided into three sections depending on

- the size of the tumor
- where the tumor is found
- which lymph nodes have cancer



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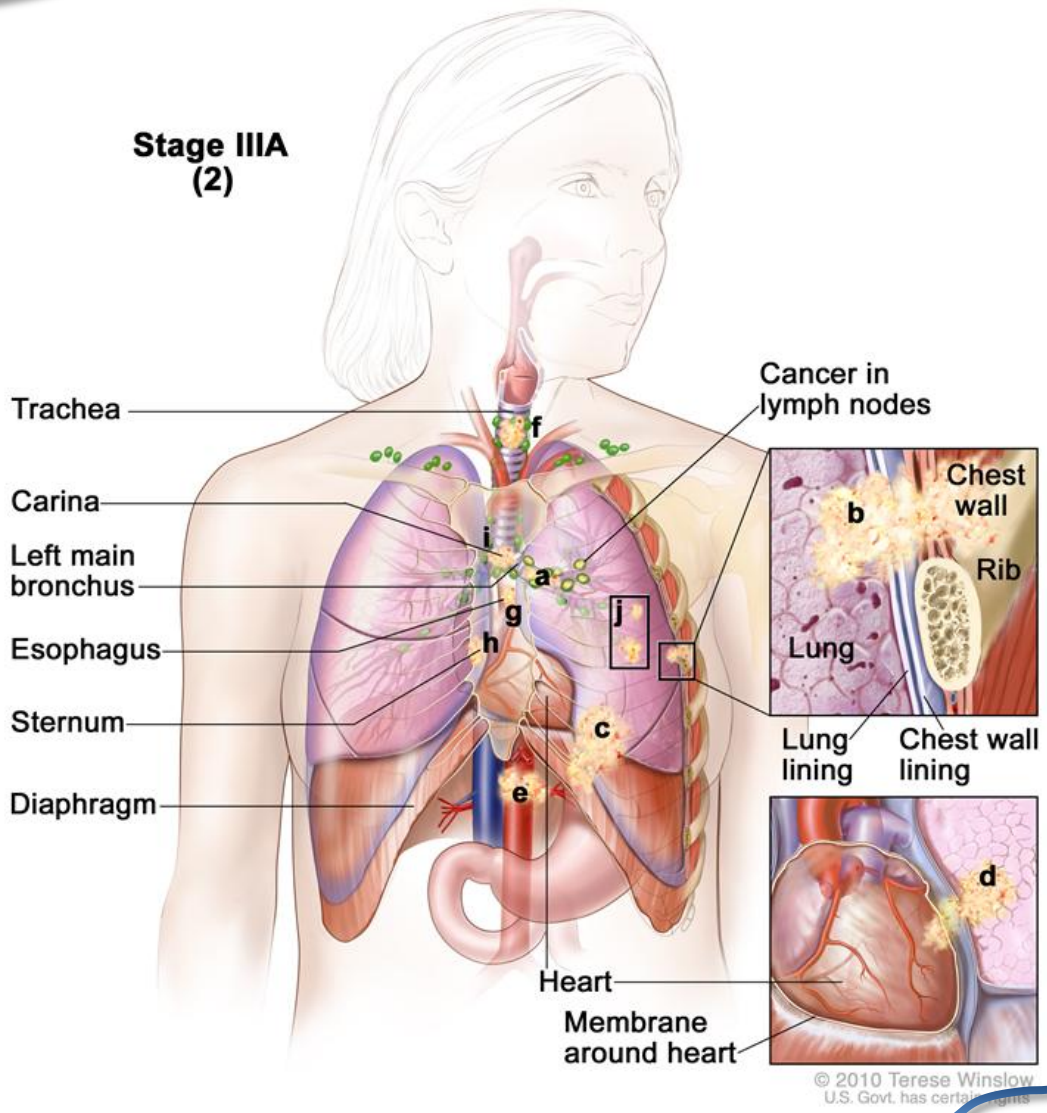
stage IIIA



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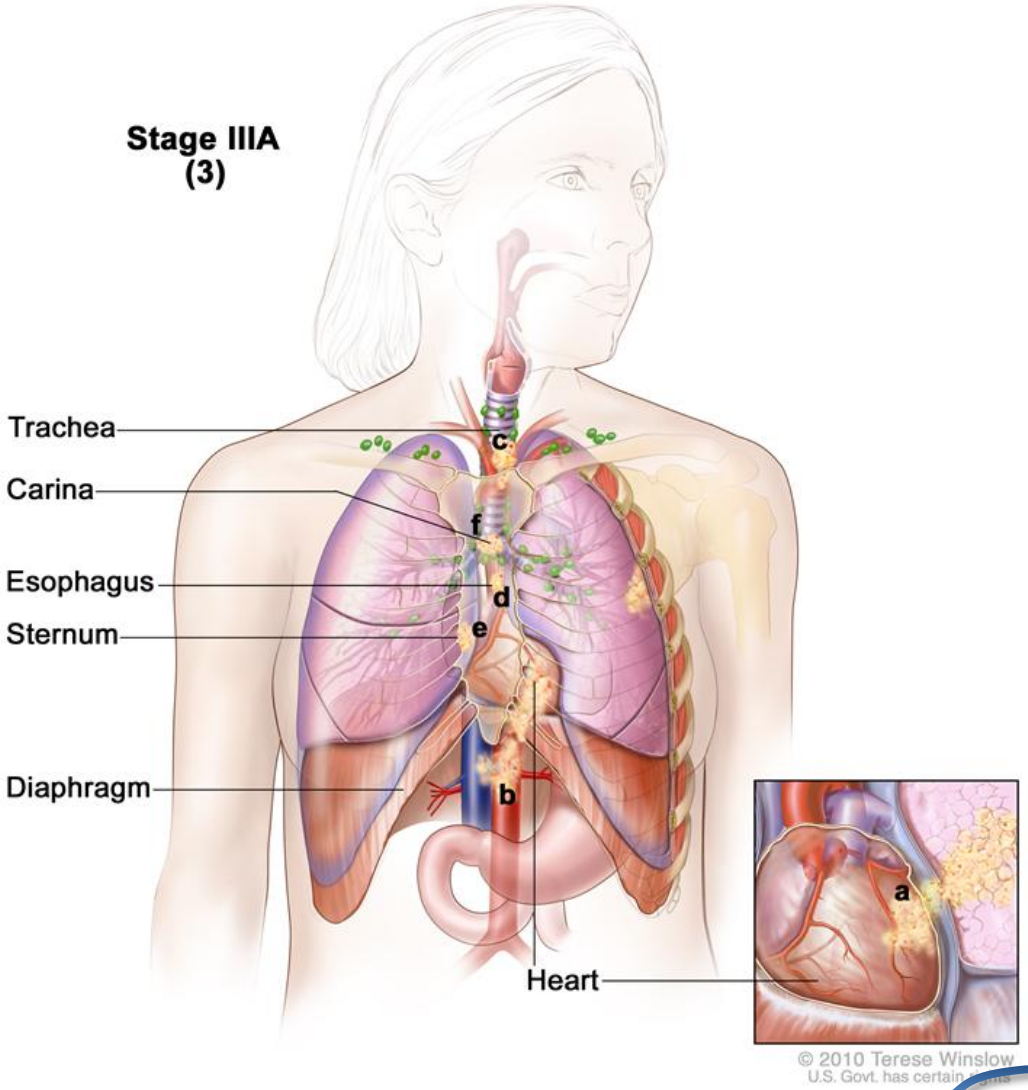
stage IIIA



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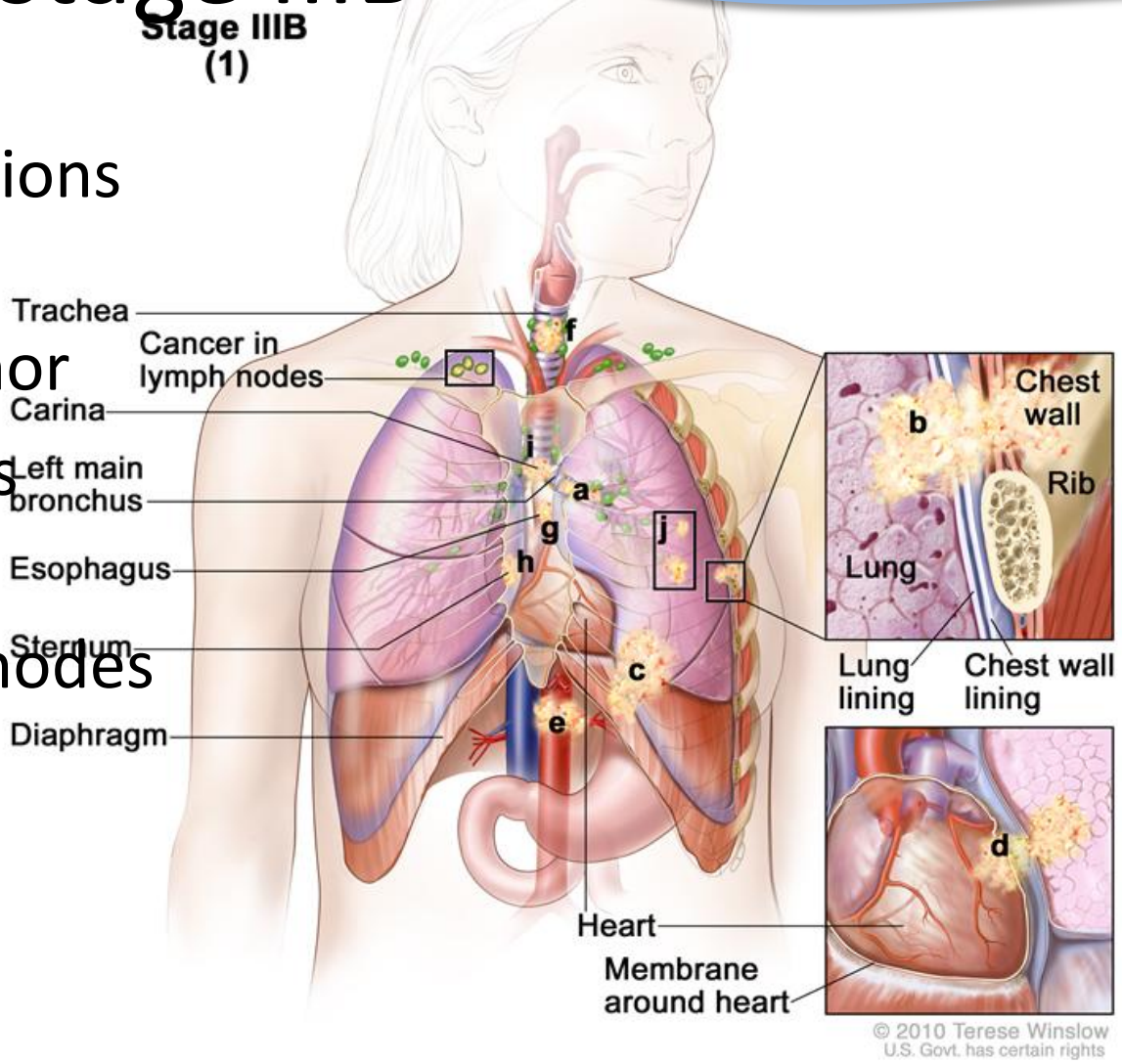
stage IIIB

divided into two sections depending on

- the size of the tumor
- where the tumor is found
- and which lymph nodes have cancer



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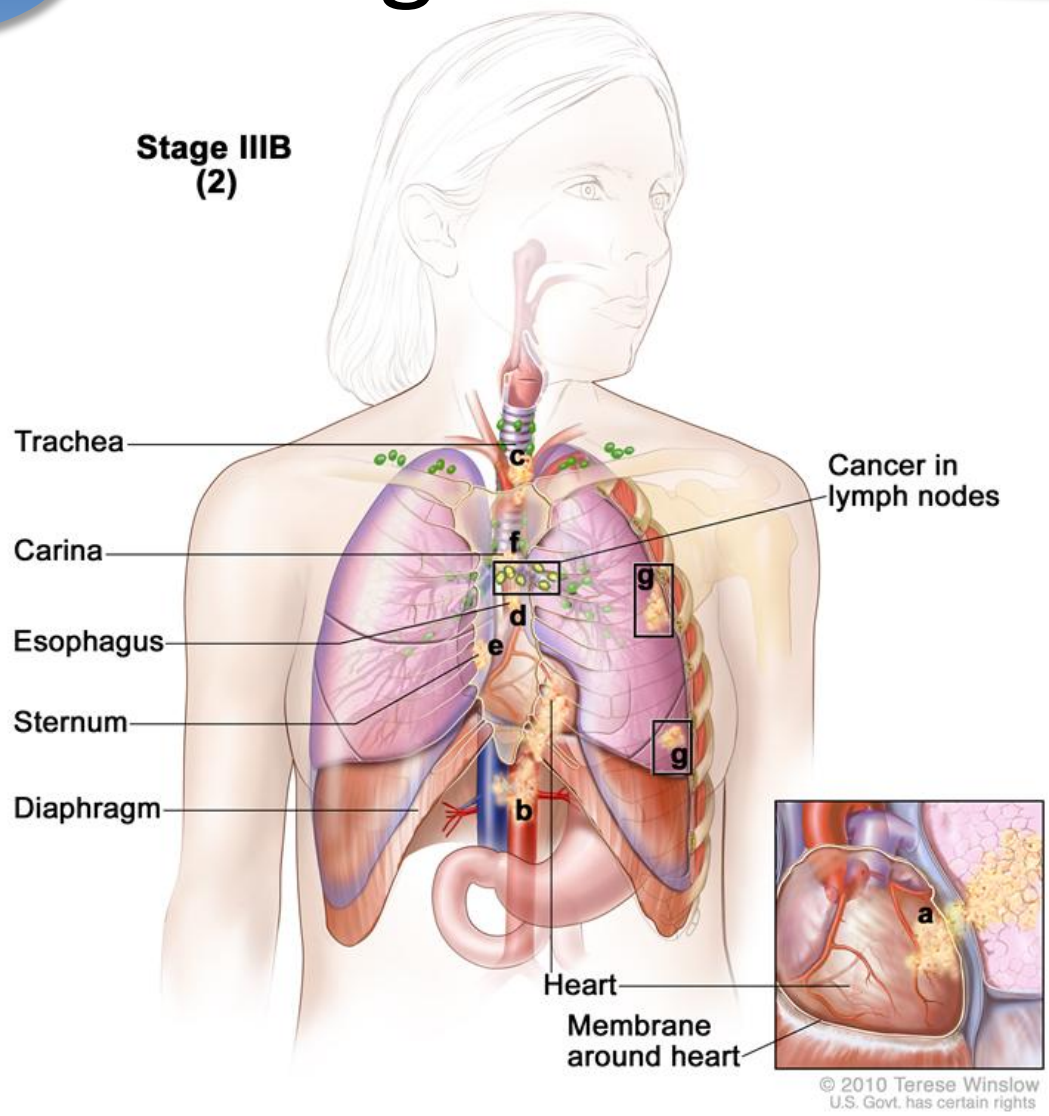
stage IIIB



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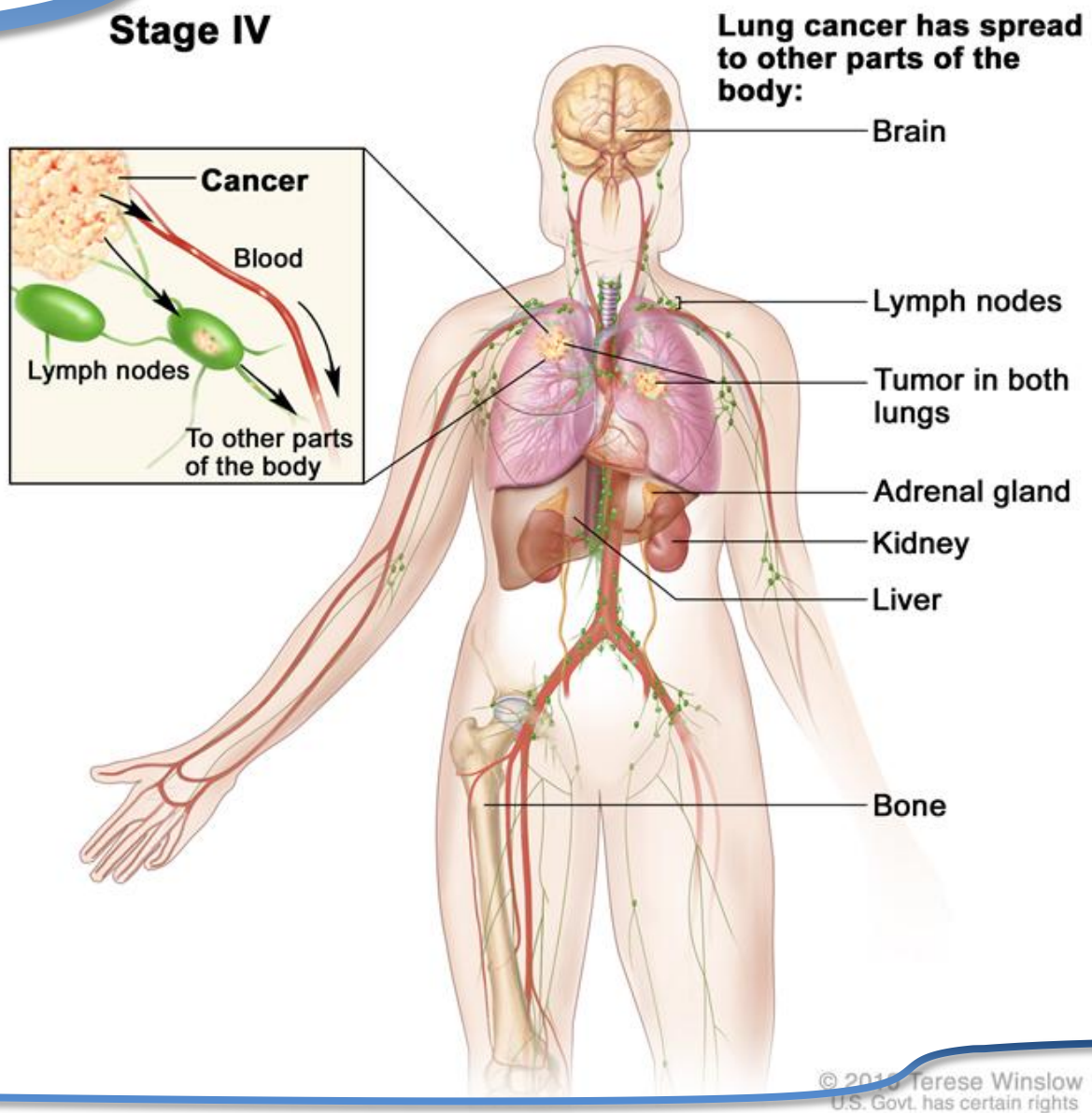
stage IV



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Introduction

Circulating tumor DNA (ctDNA) = promising biomarker for noninvasive assessment of cancer burden

existing ctDNA detection methods have insufficient sensitivity:

--*PCR-based assays* (majority of patients lack mutations in recurrent point mutations as KRAS/EGFR)

--*parallel sequencing* (modest sensitivity)

they developed a cancer personalized profilying by deep sequencing

CAPP-Seq selector= biotinylated DNA oligonucleotides

→target mutated regions in cancer

CAPP-Seq overcomes this limitations

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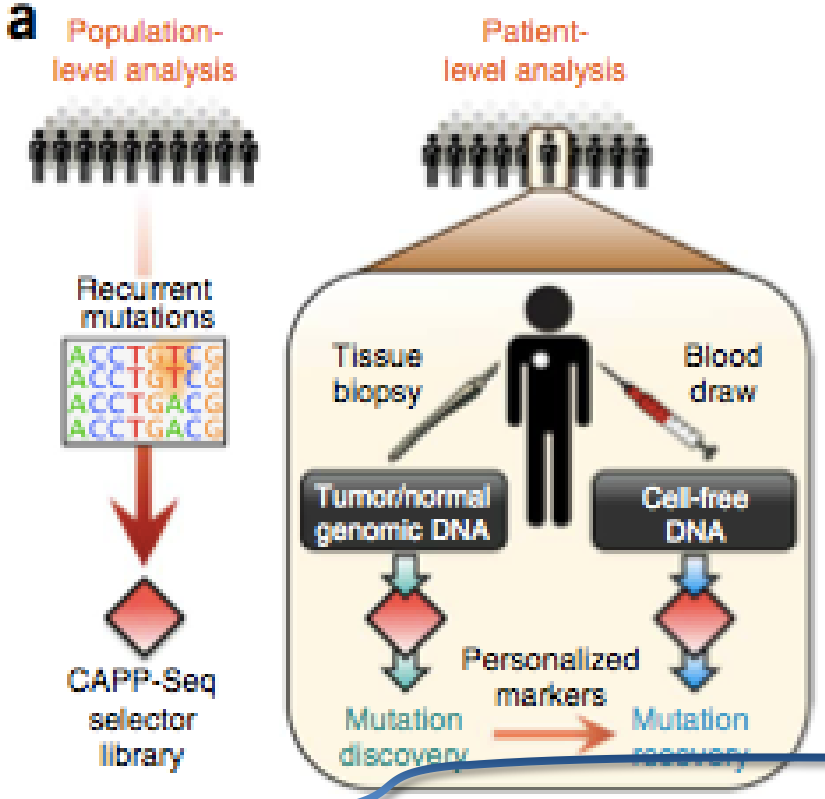
Introduction

1.)CAPP selector applied to tumor DNA

→ to identify a patients cancer-specific genetic aberations

2.) directly applied to circulating DNA quantify it



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Design for a CAPP-Seq Selector for NSCLC

1.) including Exons covering recurrent mutations in potentional driver genes

2.) addition of Exons containing recurrent SNVs

(using WES data from TCGA (lung cancer/squamos cell carcinom)

3.)addition of exons/introns harbouring breakpoints in rearrangements

involving ALK, ROS1, RET =tyrosine kinase genes

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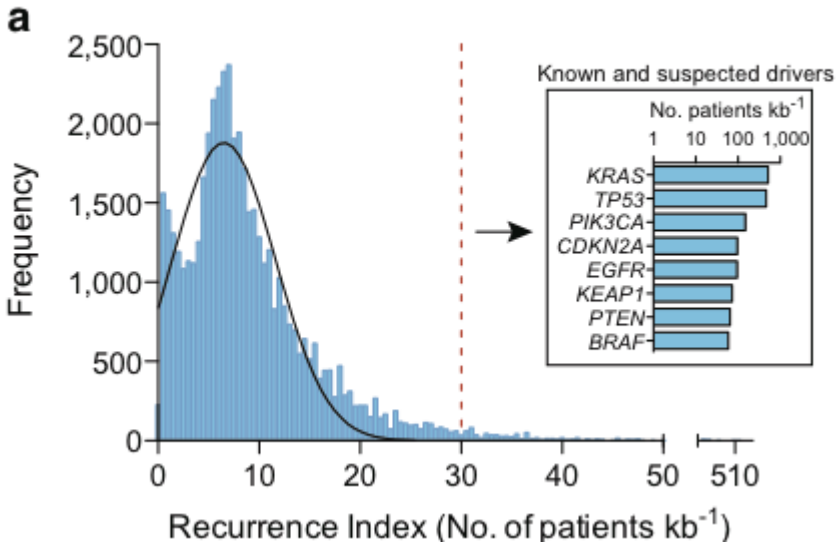
Statistical enrichment of recurrently mutated NSCLC exons



- RI=number of patients with somatic mutations per kilobase of an exon
- Known and suspected NSLC drivers are highl enriched at RI 30

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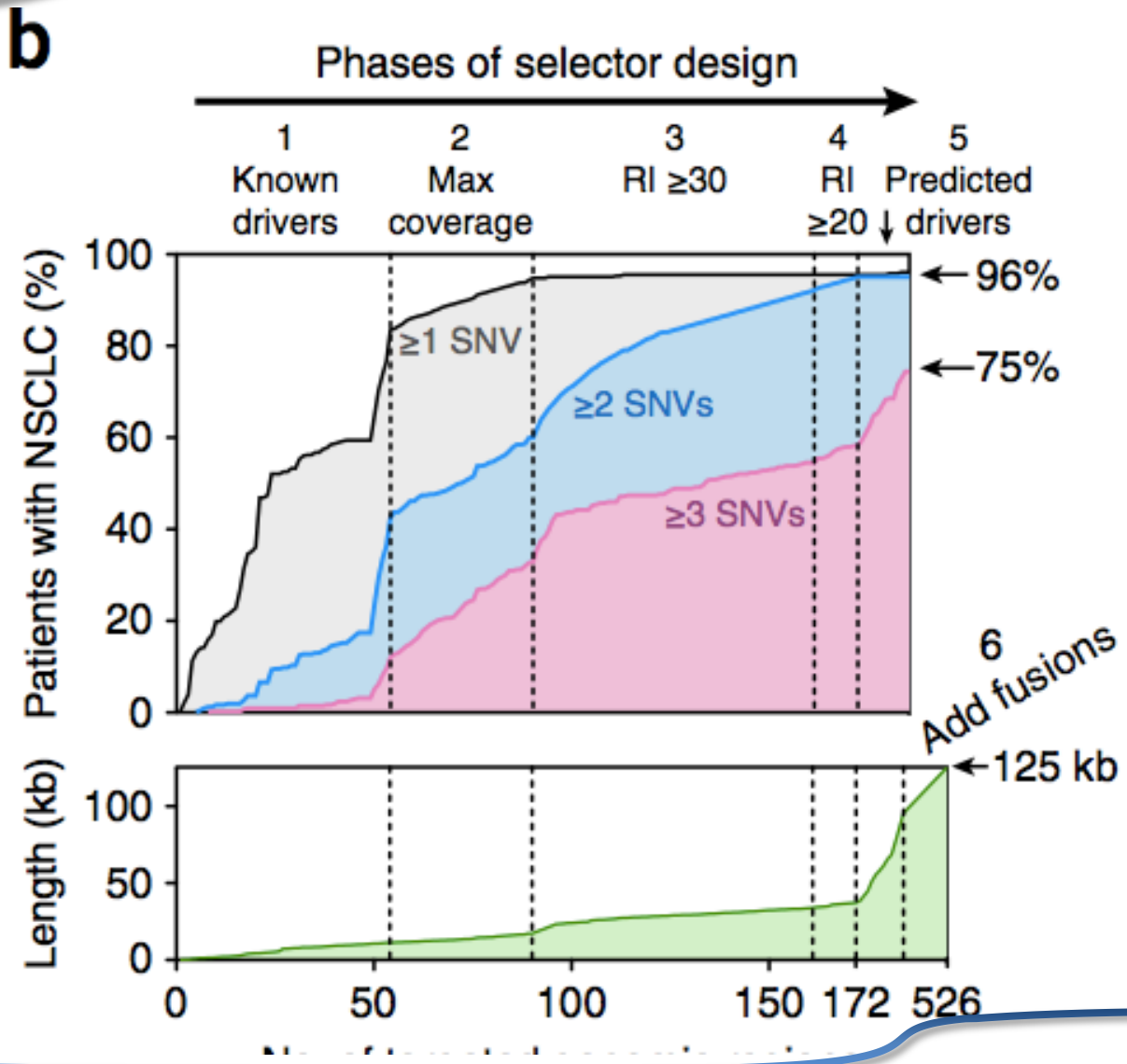
b
Phases of selector design
1
Known drivers
2
Max coverage
3
RI ≥30
4
RI ≥20
5
Predicted drivers
Patients with NSCLC (%)
100
80
60
40
20
0
≥1 SNV
≥2 SNVs
≥3 SNVs
96%
75%
6
Add fusions
Length (kb)
100
50
0
125 kb
0
50
100
150
172
526

- Selector targets: 521 Exons/13 introns from139 recurrently mutated genes in total kovering 125 kb

- Small target(0.004% of human genom)
  - Selector identifies a median of 4 SNV
  - Covers 96% of patients with lung adenocarcinom/squamous cell carcinoma

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Independent cohort

- To validate the number of mutations covered per tumor:

->examined the selector region in WES data: independent cohort of 183 patients with lung adenocarcinoma

- Selector covered 88% of patients with a median of four SNVs per Patient.

Better than random sampling from the exome

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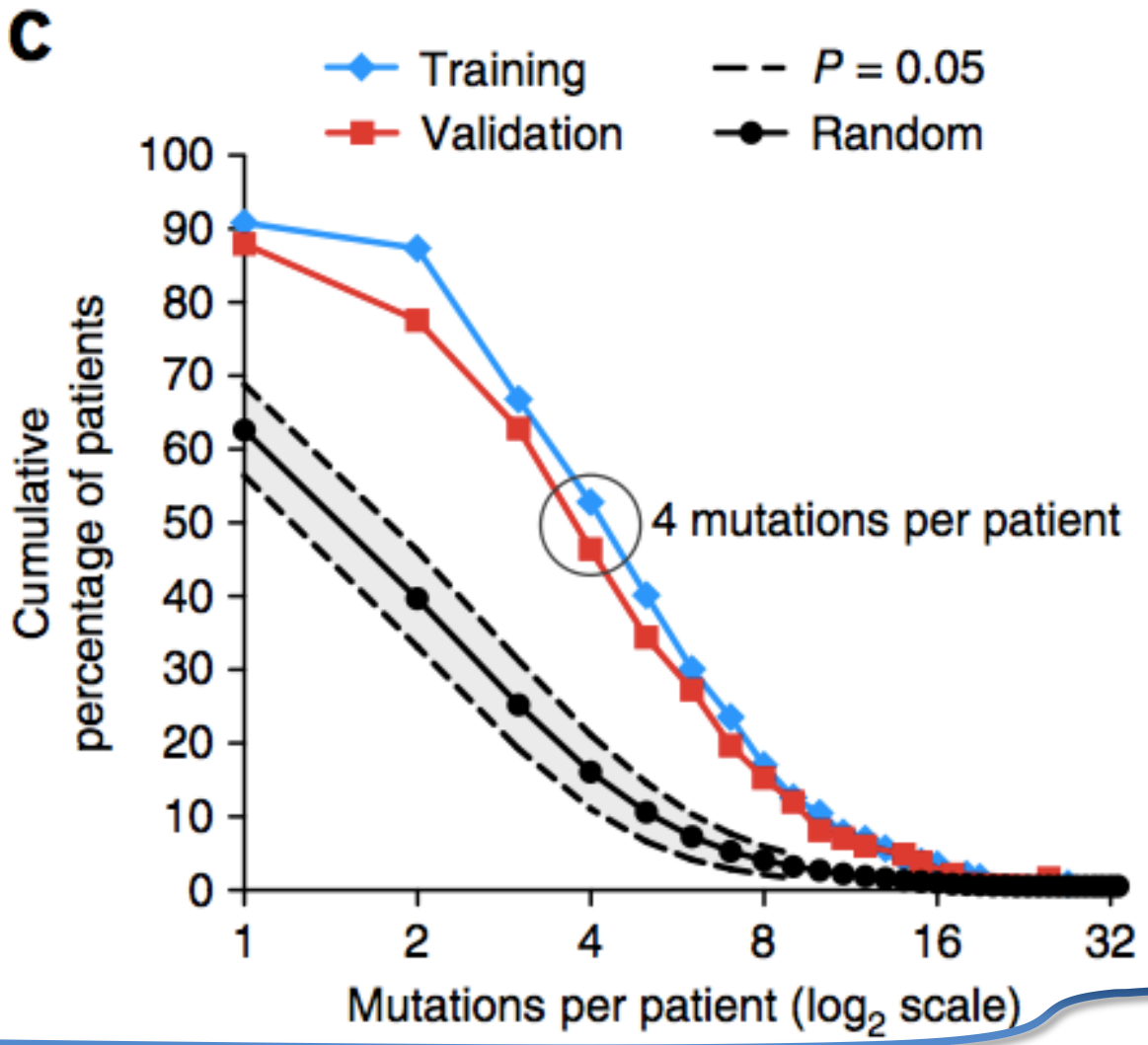
Independent cohort



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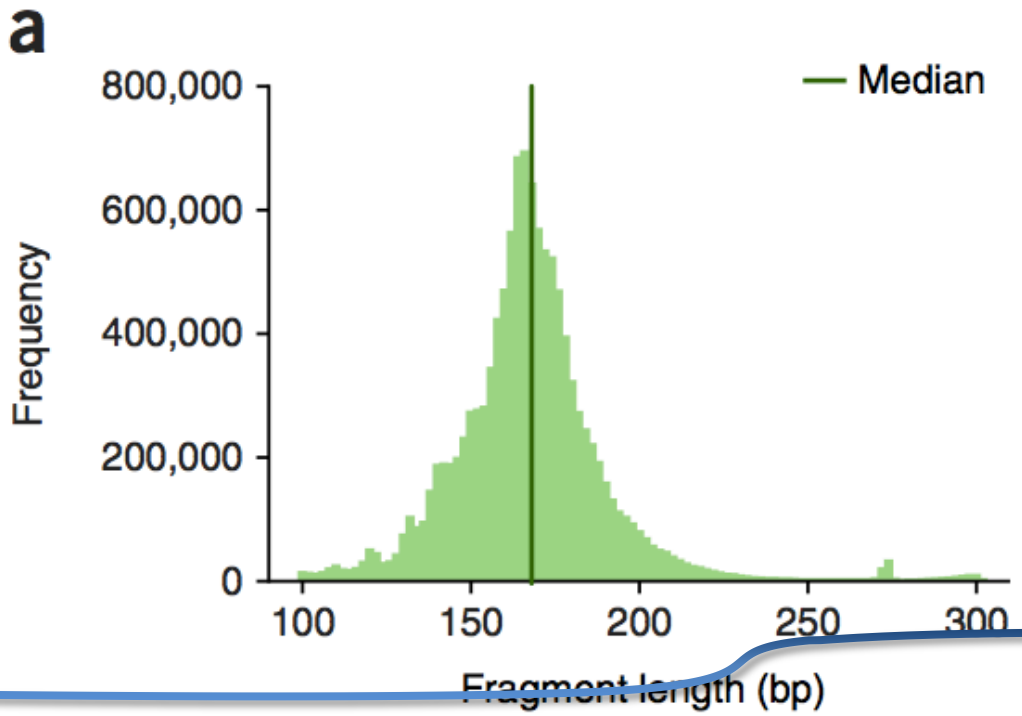
Performance assessment

- Performed deep sequencing with NSCLC selector to achieve 10 000 * coverage

profiled a total of 90 samples:

- two NSCLC cell lines
- 17 primary tumor samples with matched peripheral blood leukocytes (PBLs)
- 40 plasma samples from 18 human subjects,
  (5 healthy adults and 13 patients with NSCLC)

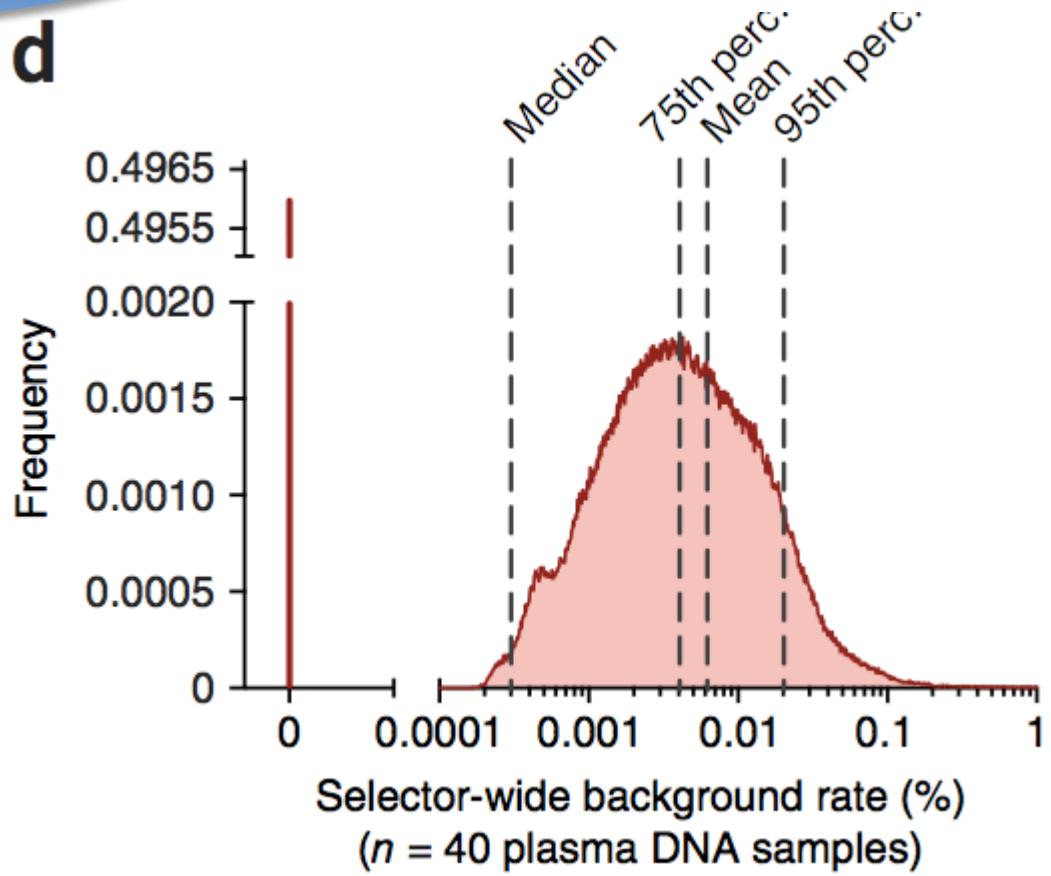
- They applied selector to circulating DNA purified from healthy control plasma
  - Observed capture of genomic DNA
- Sequenced plasma DNA fragments:
  - median length of 170bp



Nonreference Alleles

- Distribution of nonreference alleles across the selector for 40 plasma samples
  - Mean background rate:0.006%
  - Median background rate:0.003%

Nonreferece alleles



→Lower than existing NGS-based methods for ctDNA analysis

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Somatic mutation detection

- Applied CAPP-Seq to discovery of somatic mutations in tumor samples
  - 17 patients with NSCLC
    - Formalin-fixed surgical resections
    - Needle biospy
- mean sequencing depth of ~5,000× in tumor samples
  - Detected 100% of previously identified SNVs and fusions
  - Discoverd many additional somatic variants
  - Characterized breakpoints at base-pair resolution
  - Identified partner genes for each of eight known fusions (incl. ALK1/ROS)

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Table 1 Patient characteristics and pretreatment CAPP-Seq monitoring results

									Fusion		Pretreatment		
Case	Age	Sex	Histology	Stage	TNM	Smoking history	No. of SNVs (nonsilent)	Indels	*ALK* or *ROS1*	Partner	ctDNA (%)	ctDNA (pg ml^{-1})	Tumor (ml)
P12	86	F	SCC	IA	T1bN0M0	Heavy	6 (3)	1			ND	ND	5.5
P1	66	M	Adeno	IB	T2aN0M0	Heavy	12 (3)	4			0.025	1.9	23.1
P16	82	F	Adeno	IB	T2aN0M0	Heavy	26 (5)	2			0.019	2.5	22.5
P17	85	F	Adeno	IB	T2aN0M0	Heavy	2 (2)	0			ND	ND	10.2
P13	90	F	SCC	IIB	T3N0M0	Heavy	5 (4)	0			1.78	269.8	339.3
P2	61	M	Large cell	IIIA	T3N1M0	Heavy	12 (3)	1			0.896	64.7	23.1
P3	67	F	Adeno	IIIB	T1bN3M0	Light	1 (1)	0			0.095	16.2	7.9
P14	55	M	Adeno	IIIB	T1aN3M0	Heavy	8 (5)	0			0.05	10.2	5.2
P15	41	M	Adeno	IIIB	T3N3M0	Light	25 (10)	1			0.58	108.1	121.8
P4	47	F	Adeno	IV	T2aN2M1b	Heavy	3 (2)	0			0.039	2.1	12.4
P5	49	F	Adeno	IV	T1bN0M1a	None	4 (3)	0			3.2	143.8	82.1
P6	54	M	Adeno	IV	T3N2M1b	None	3 (2)	0	*ALK*	*KIF5B*	1.0	350.2	NA
P9	49	M	Adeno	IV	T4N3M1a	None	0	0	*ALK*	*EML4*	0.04	3.8	66.2
									ROS1	*MKX, FYN*			
P10	35	F	Adeno	IIIA	T4N0M0	None	0	0	*ROS1*	*SLC34A2*	–	–	–
P11	38	F	Adeno	IIIA	T3N2M0	None	2 (1)	0	*ROS1*	*CD74*	–	–	–
P7	50	M	Adeno	IV	T1aN2M1b	Light	0	0	*ALK*	*EML4*	–	–	–
P8	48	F	Adeno	IV	T4N0M1b	None	1 (0)	0	*ALK*	*EML4*	–	–	–

ND, mutant DNA was not detected above background (Online Methods); NA, tumor volume could not be reliably assessed. Dashes indicate a plasma sample was not available. Smoking history, ≥20 pack years (Heavy), >0 and <20 pack years (Light). SCC, small cell cancer; Adeno, adenocarcinoma; TNM, tumor, node and metastasis classification system. Additional details are provided in **Supplementary Tables 3** and **4**.

- fusions: never smokers/ few SNVs
- no fusions: median of 6 SNVs per patient

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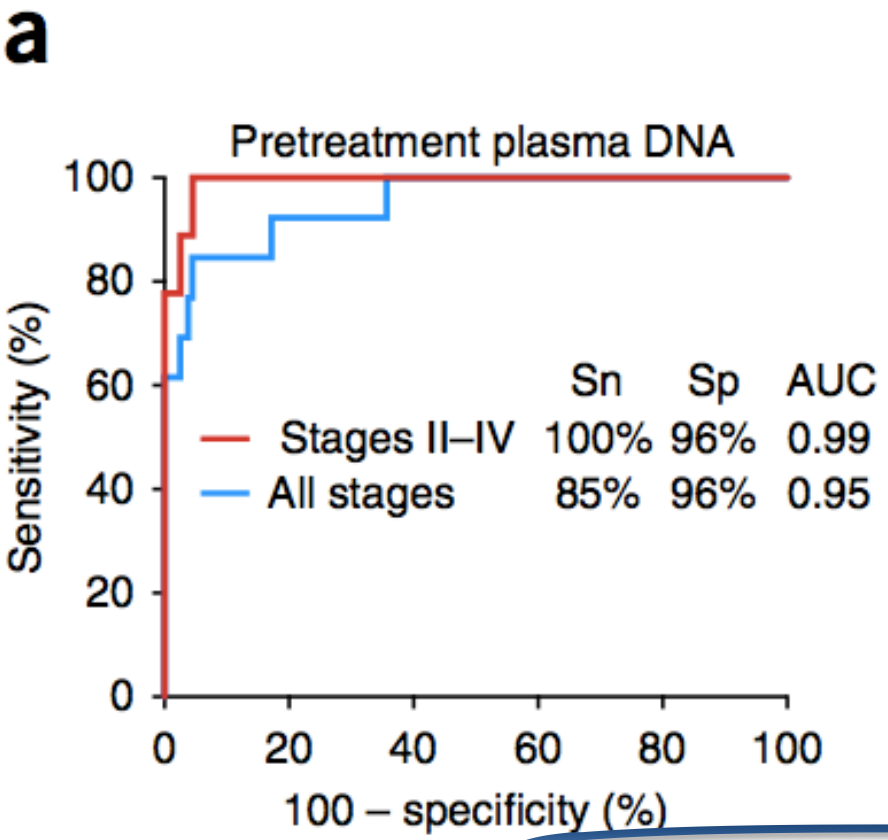
Sensitivity/Specifity of CAPP-Seq for disease monitoring

- plasma samples from 5 healthy controls and 35 samples collected from 13 patients with NSCLC (Table 1)

- ROC analysis:
- CAPP-Seq: AUC-0.95
  - Sensitivity: 85%
  - Specifity:96%



Monitoring of NSCLC tumor burden in plasma samples

whether levels of ctDNA correlate with radiographically measured tumor volumes

Fractions of ctDNA ranged from ~0.02% to 3.2%;
-median of ~0.1% in pretreatment samples.

- levels of ctDNA in pretreatment plasma significantly correlated with tumor volume as measured by CT and PET imaging:

(R2 = 0.89, P = 0.0002)

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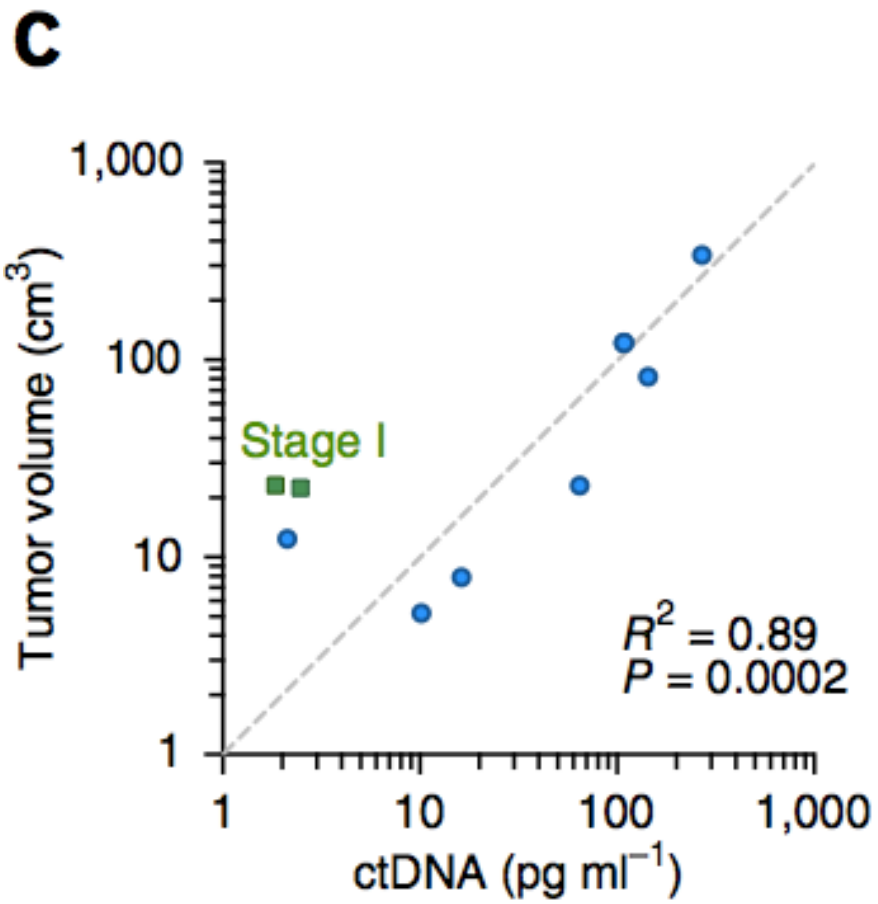
ctDNA level correlates with tumor volume



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ctDNA concentrations reflect disease burden

- analyzed plasma DNA from three patients with advanced NSCLC undergoing distinct therapies

ctDNA levels highly correlated with tumor volumes during therapy

- R2 = 0.95 (P15)
- R2 = 0.85 for (P9)

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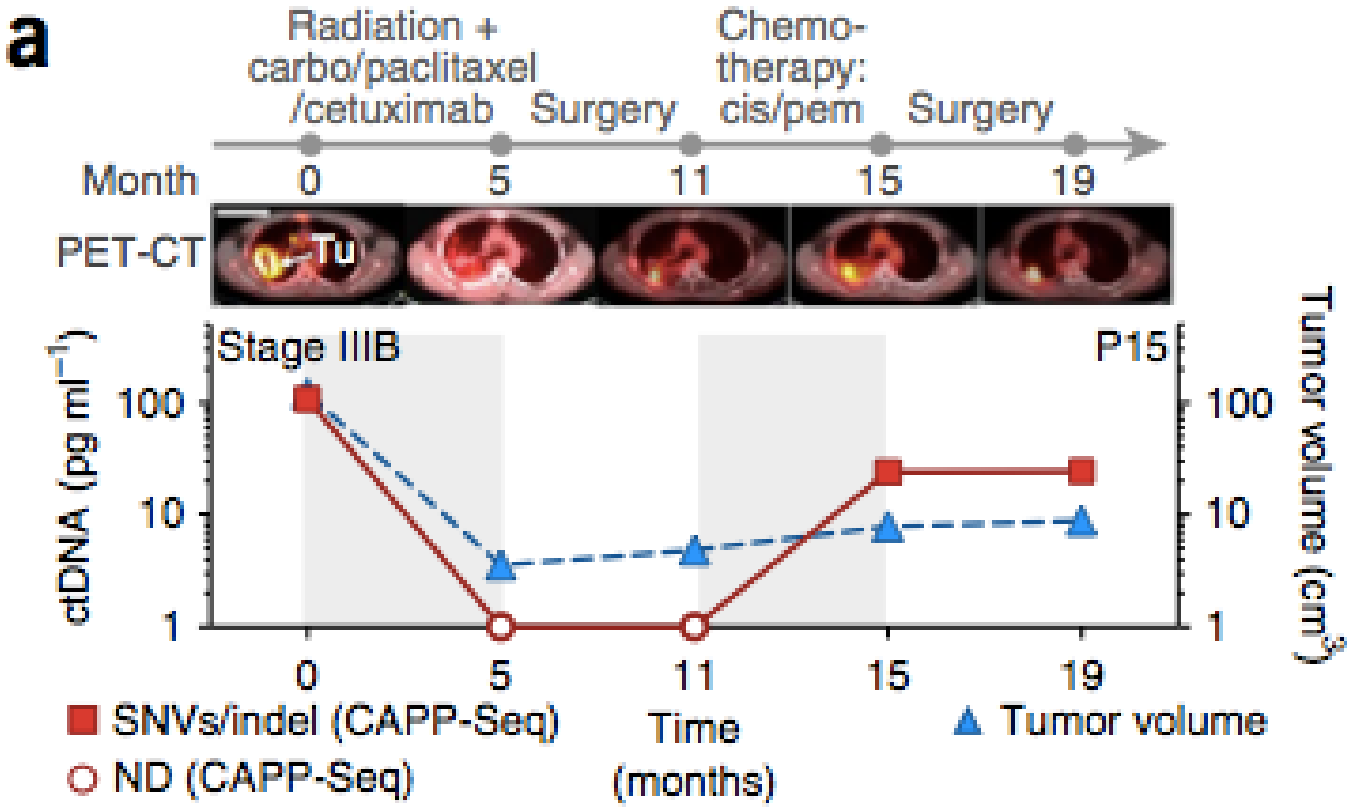
Patient 15



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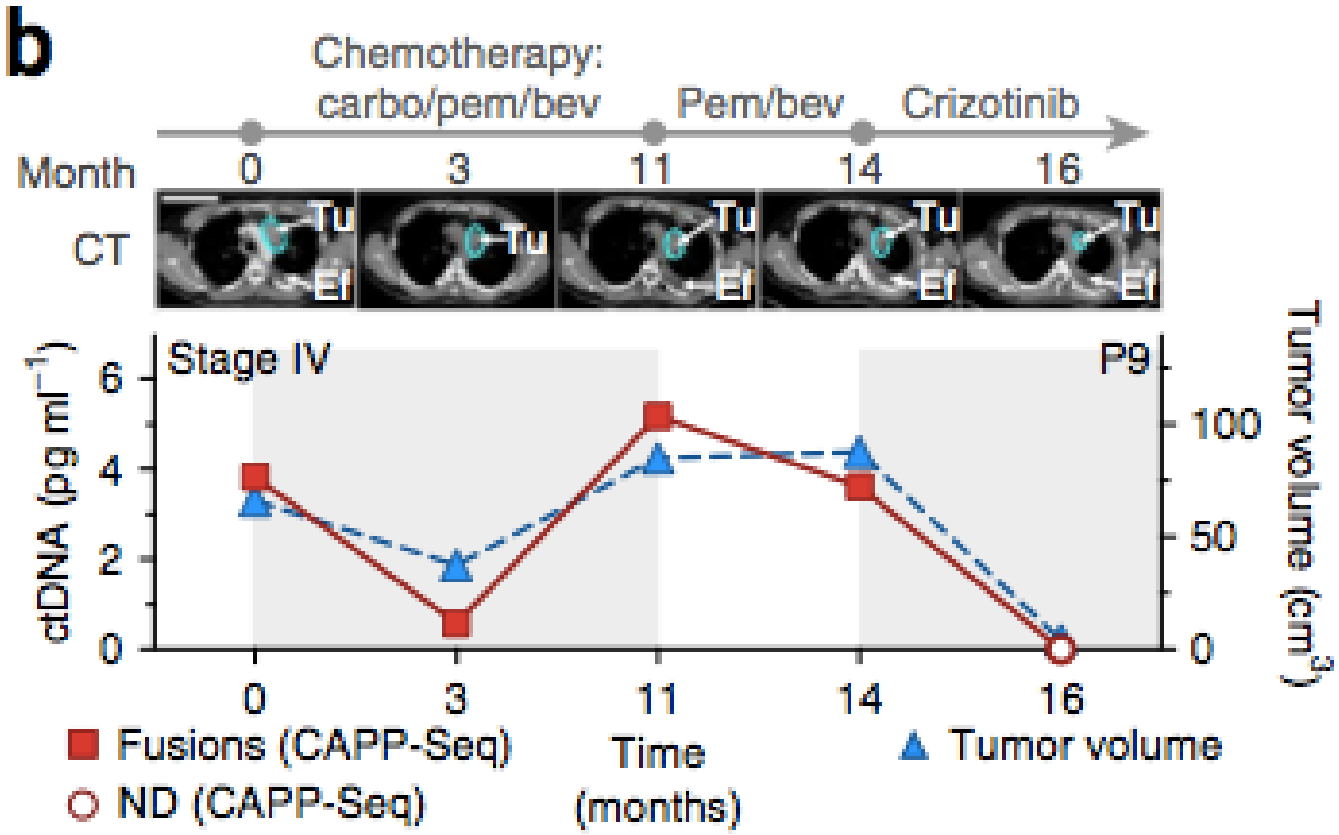
Patient 9



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Patient 13

- surveillance CT or PET-CT scans difficult to interpret
  →radiation-induced inflammatory and fibrotic changes in the lung and surrounding tissues

P13, who was treated with radiotherapy for stage IIB NSCLC,
follow-up imaging: large mass
-> interpreted to represent residual disease

-ctDNA at the same time point was undetectable
--patient remained disease free for 22 months

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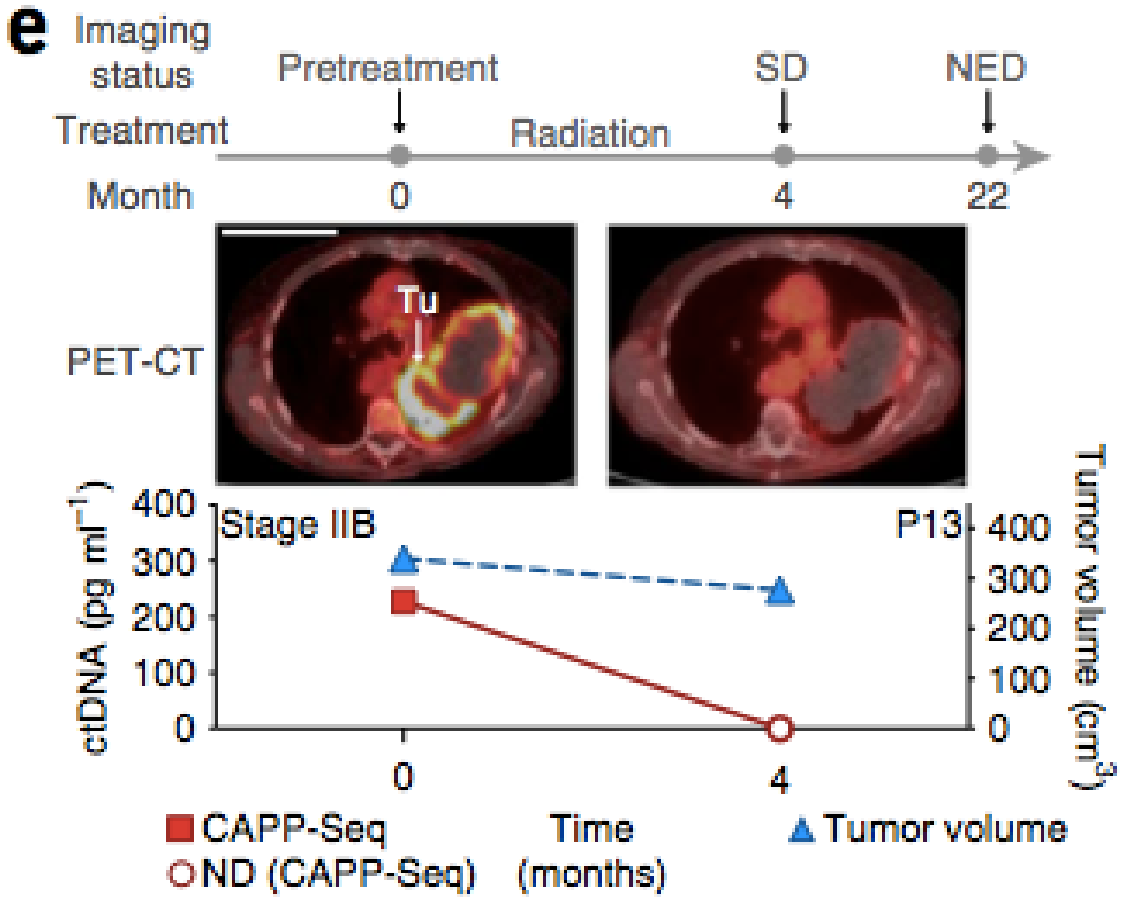
Patient 13



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Patient 14

- (P14): treated with chemoradiotherapy for stage IIIB NSCLC
  - follow-up imaging revealed a near-complete response
- ctDNA concentration slightly increased following therapy
  - suggesting progression of occult microscopic disease

clinical progression was detected 7 months later
ultimately succumbed to NSCLC

ctDNA analysis: identifying residual disease after therapy

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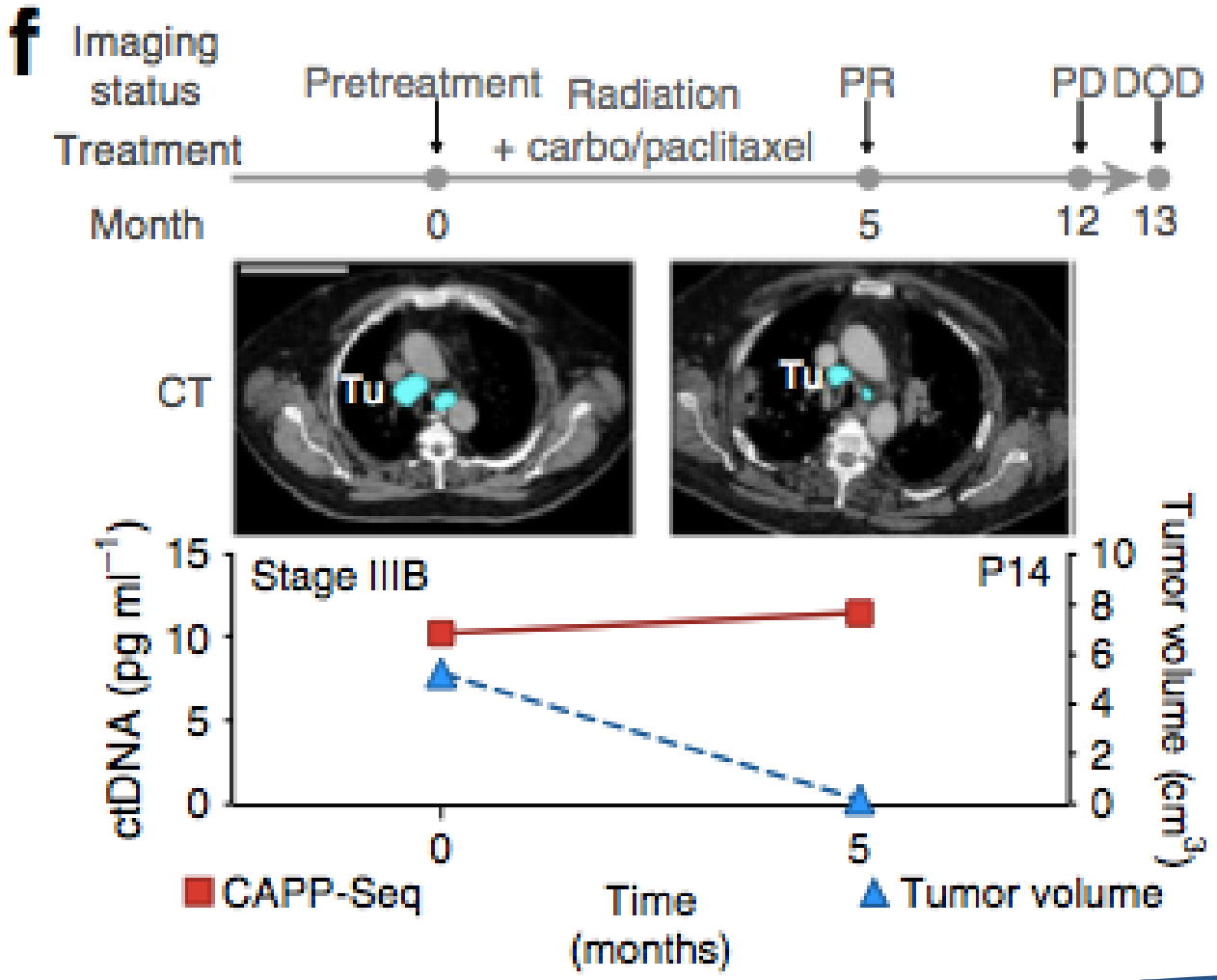
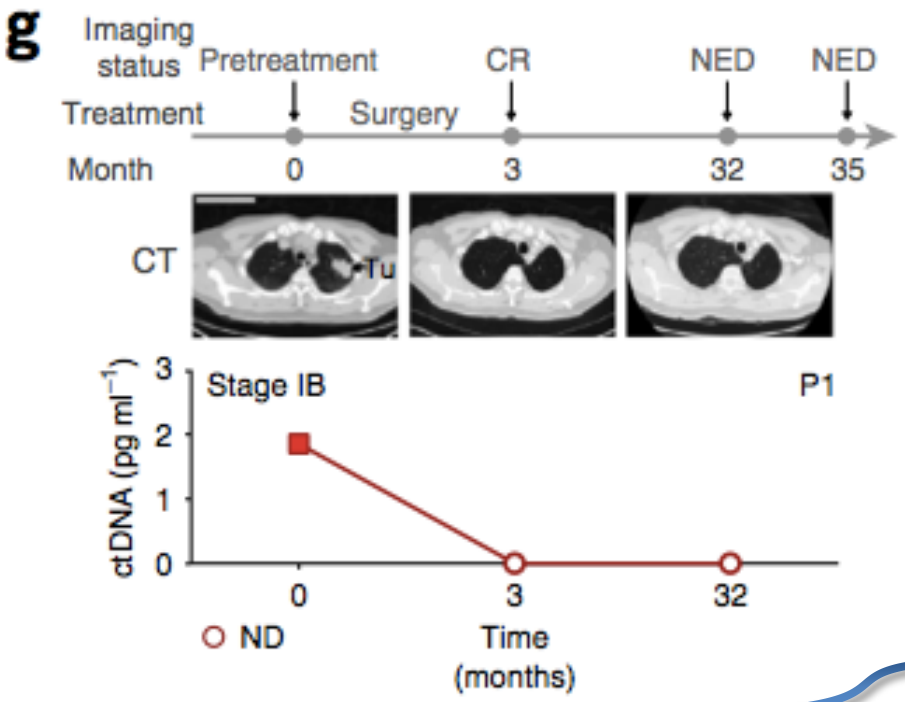
Patient 14



early-stage NSCLC

CAPP-Seq allows monitoring in early stage NSCLC.

- Patients P1/P16 : surgery and stereotactic ablative radiotherapy for stage IB NSCLC.
- P1: ctDNA in pretreatment plasma but not at 3 or 32 months following surgery, which suggests that this patient was free of disease and probably cured

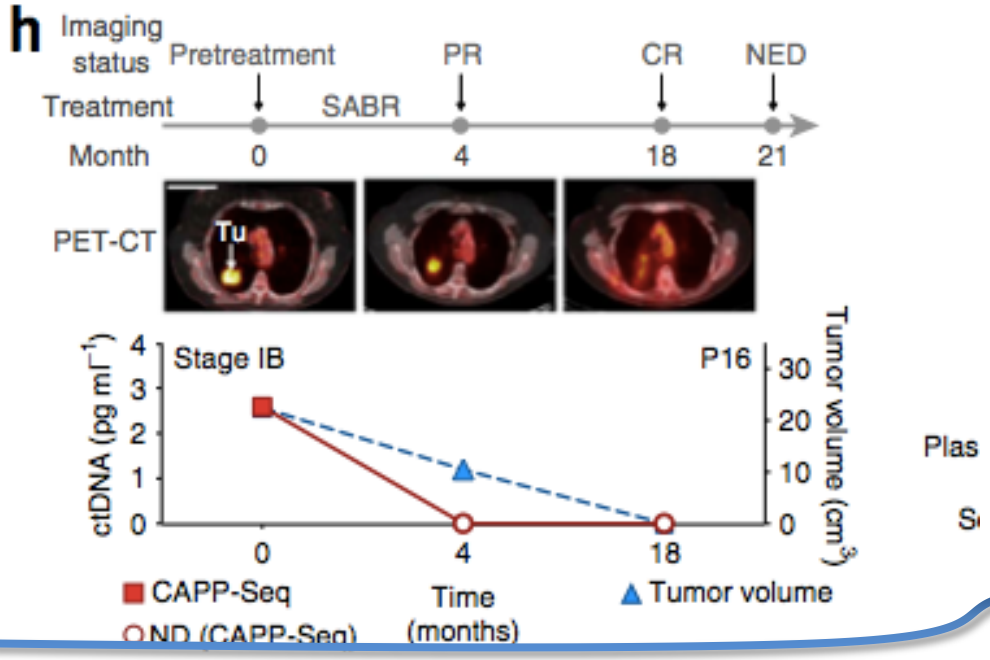


early-stage NSCLC

- P16, PET-CT showed a residual mass
  - represent residual tumor
  - or postradiotherapy inflammation

- ctDNA levels stayed low

  →patient remained free of disease at last follow-up 21 months after therapy.



Biopsy-free cancer screening

- they blinded themselves to the mutations present in each patient's tumor
  - tested for the presence of cancer DNA in each plasma sample

- correctly classified 100% of patient plasma samples with ctDNA above fractional abundances of 0.4%

false-positive rate of 0%

→CAPP-Seq : improve the low positive predictive value of low-dose CT screening

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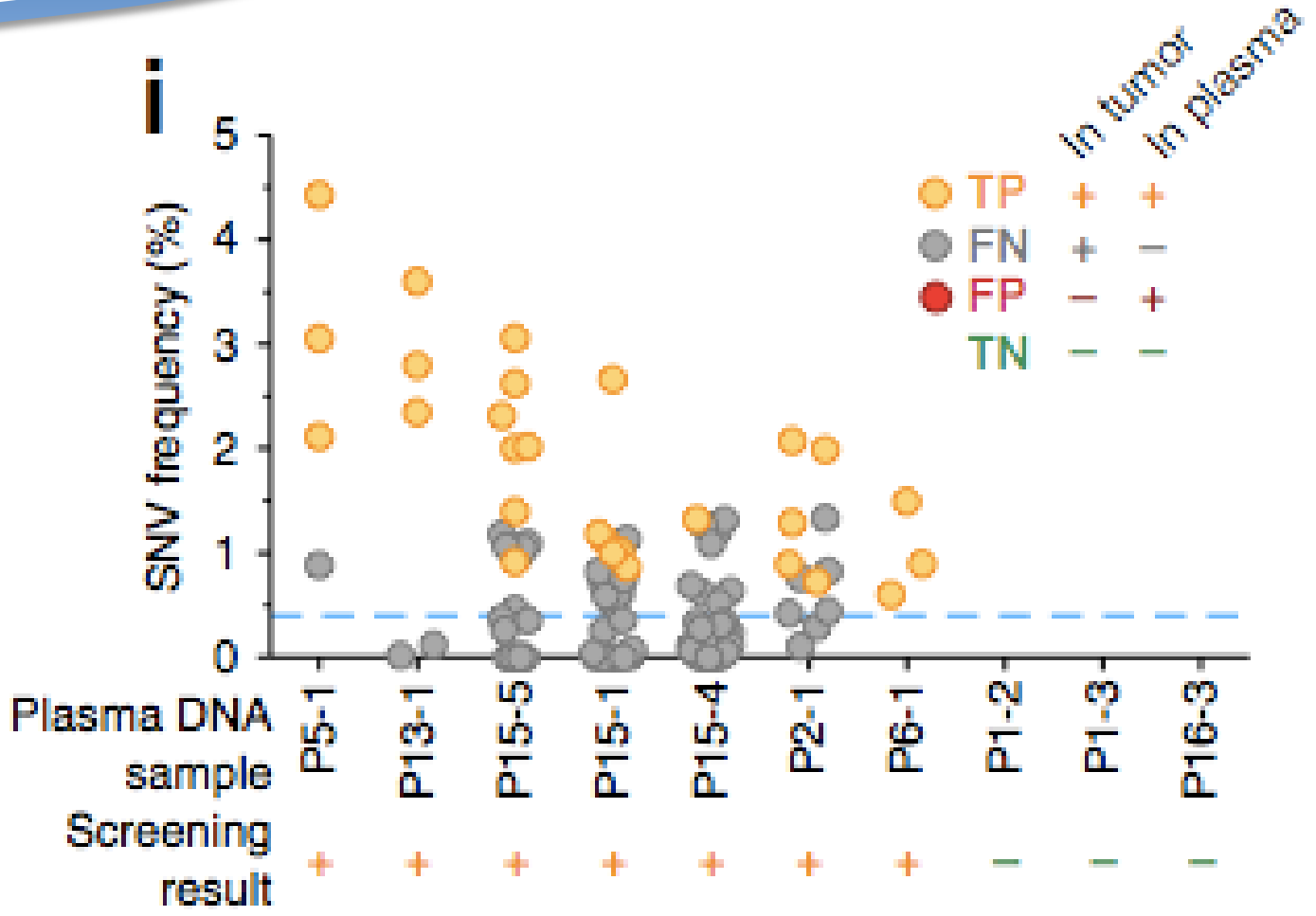

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Conclusion

- CAPP-Seq: a new method for ctDNA quantitation
  - high sensitivity and specificity
  - lack of a need for patient-specific optimization
  - coverage of nearly all patients with NSCLC

- First NGS-based method for ctDNA analysis
  - ultralow detection limit and broad patient coverage at a reasonable cost

ctDNA were highly correlated with tumor volume/ distinguished between residual disease and treatment-related imaging changes

Paper focused on NSCLC; method could be applied to any malignancy for which recurrent mutation data are available

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Conclusion

- levels of ctDNA are considerably lower than the detection thresholds of previously described sequencing-based methods
- pretreatment ctDNA concentration is <0.5% in the majority of patients with lung and colorectal carcinomas.
- Following therapy, ctDNA concentrations drop
  → thus requiring even lower detection thresholds

whole-exome whole-genome sequencing would not be sensitive enough to detect ctDNA in most patients with NSCLC

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Conclusion

CAPP-Seq selctor

-routinely applied clinically and

-accelerating the personalized detection, therapy , monitoring of cancer

CAPP-Seq will prove

- valuable in a variety of clinical settings,
  - assessment of cancer DNA in alternative biological fluids and specimens with low cancer cell content

additional gains in the detection threshold are desirable

- increasing the amount of plasma used for ctDNA: more than~1.5 ml
- the potential for inefficient capture of fusions
  → underestimates of tumor burden (P9)

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- Thank you for your attention!