Precision Medicine: Expanding Opportunities

OptumHealth Education – Essentials of Oncology, Solid Organ and Blood/Marrow Transplant Management

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Disclosure Information

1. PierianDx Knowledgebase Expert Panel
2. Bristol Myers Squibb Liquid Biopsy Advisory Board
3. Illumina provides reagents to my group to support circulating, cell-free nucleic acid studies
4. United States Patent No. 9,068,224: Measurement and Monitoring of Cell Clonality

My background and perspective

My clinical training is in Clinical Pathology, Molecular Genetic Pathology, and Clinical Cytogenetics.

My research training is classical genetics and cancer genomics.

1/3 of my effort is primarily clinical:

1. Sign out of molecular tests and assay development
2. Co-chair molecular tumor board
3. UNC Medical Center molecular oncology activities

2/3 of my effort is focused on translational research:

1. Design, oversight, and interpretation of correlative genomic testing for cancer clinical trials
2. Development and application of new technologies for clinical and translational application in cancer patients

Case – 50 year-old female w/ GBM

Glioblastoma (WHO grade IV), *IDH* wild-type

- S/P resection, radiation therapy with concurrent temozolomide
- Follow-up MRI revealed progression
- Patient experienced significant drop in performance status

Case – 50 year-old female w/ GBM

Clinical Test Result

- Two loss-of-function mutations were identified in *NF1*
- No gene fusions detected

Clinical Trial Test Result

- *TRIM2::NTRK2* gene fusion identified

Clinical Questions

1. Why are there apparently discrepant *NTRK2* fusion results?
2. Which assay is correct?

Outline

I. Background on genetics and genomics
II. Cancer biomarkers
III. NGS-based solid tumor testing
IV. Liquid biopsy
V. Conclusions and future directions

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Somatic vs. germline mutations

Germline DNA variation

- Heritable genetic changes that are generally found in all cells in the body
- Example: *BRCA1/2* mutations in patients with heritable breast and ovarian cancer syndrome
- Most cancer cases are not associated with heritable cancer predisposition mutations

Somatic DNA mutation

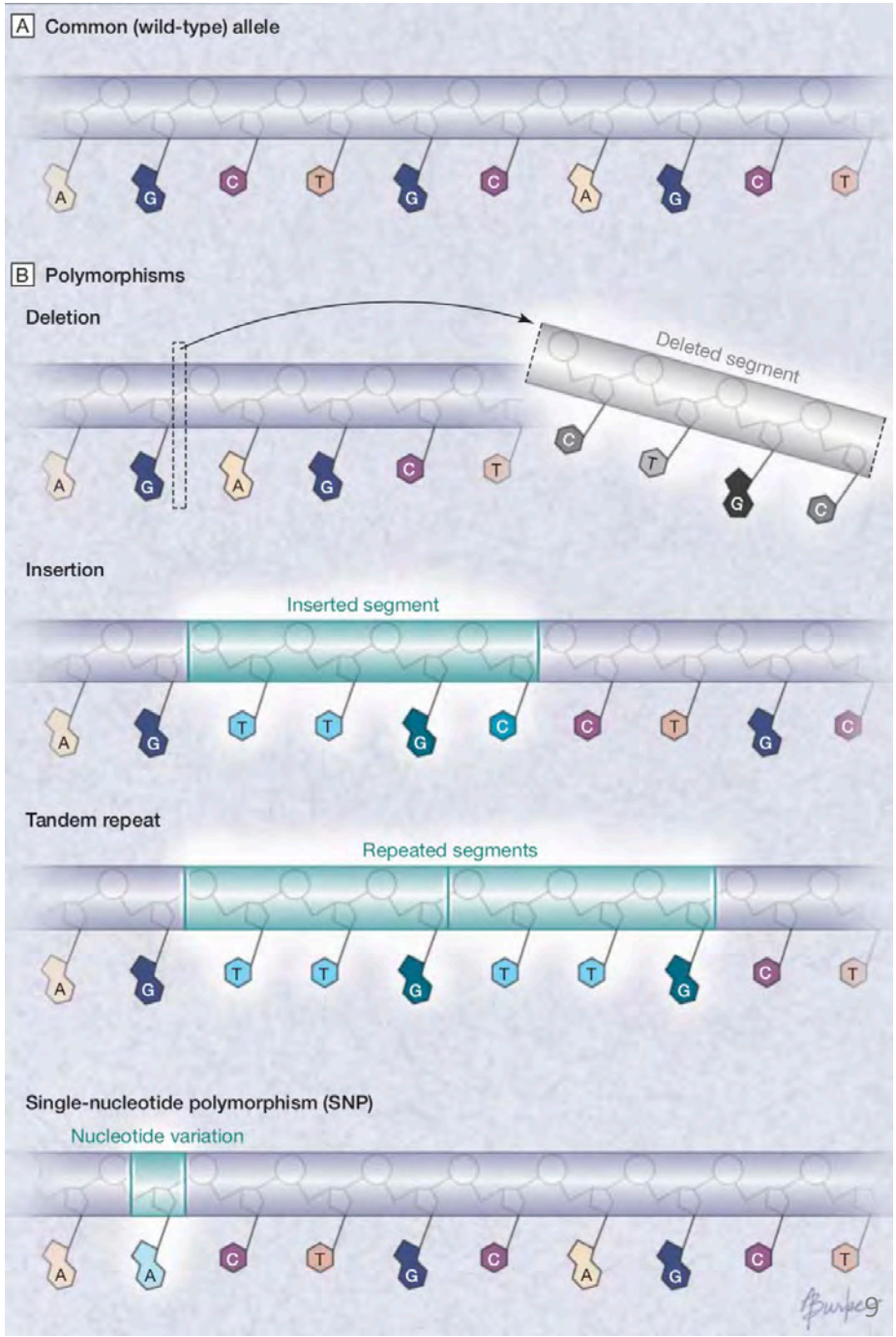
- Acquired genetic changes (e.g., found only in tumor cells)
- Cannot be inherited
- Example: *EGFR* mutation in lung cancer

Epigenetics

- Modify DNA or proteins that package DNA in the tumor (e.g., DNA methylation, histone modifications)
- Example: *MGMT* promoter methylation in glioblastoma

DNA variation

- Single-nucleotide variation
- Insertion
- Deletion
- Repeat expansion
- Copy number variation (insertions/deletions >=1,000 bp)
- Structural variation (e.g., gene fusions)



Attia J et al. JAMA. 2009;301:74

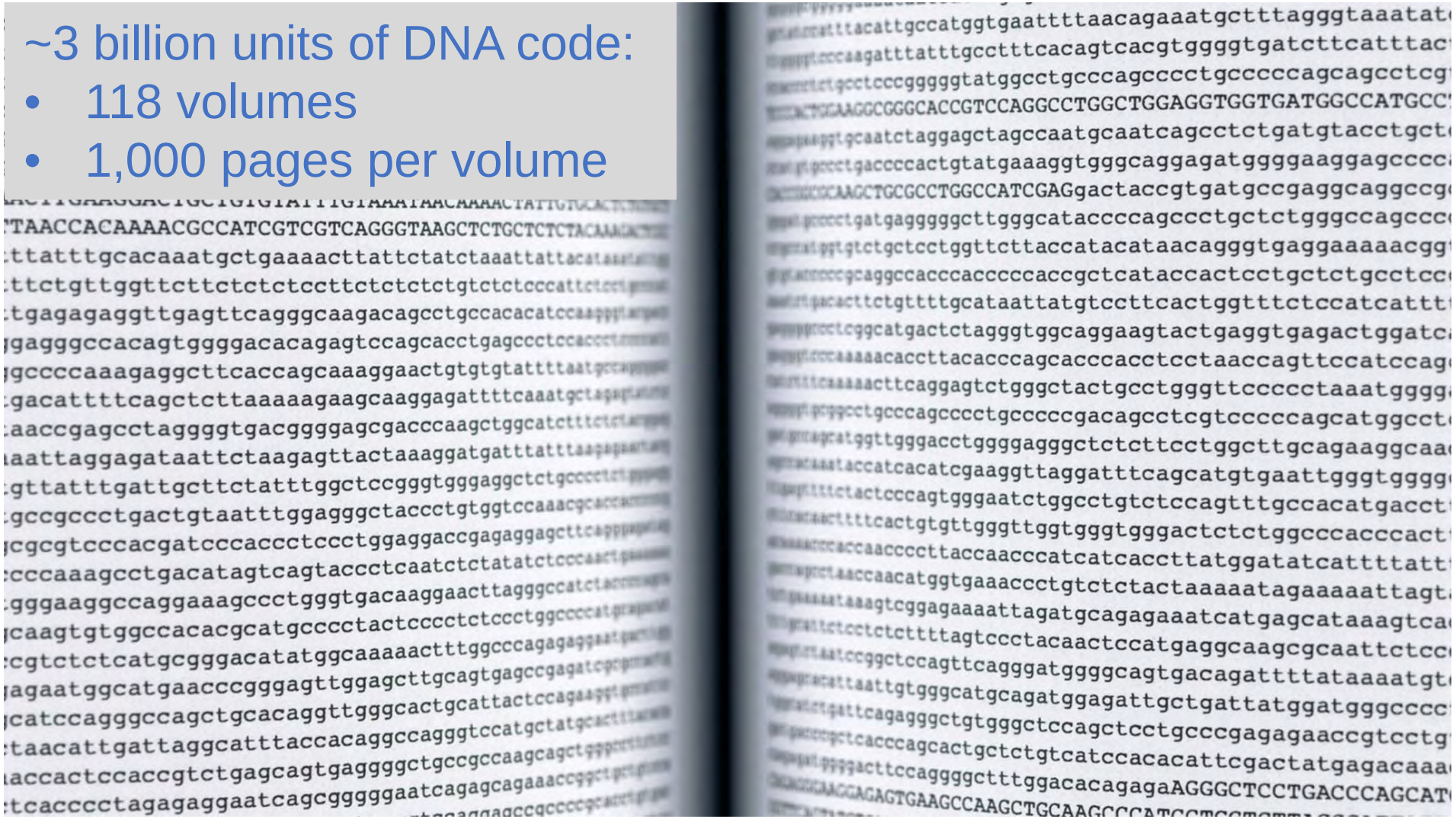
https://wellcomecollection.org/articles/WcvK4CsAANQR59Up; accessed 2/8/22
Photography by Ben Gilbert and Thomas Farnetti for Wellcome Collection

Printed Version of Human Genome

Wellcome Collection. Medicine Now.

~3 billion units of DNA code:

- 118 volumes
- 1,000 pages per volume

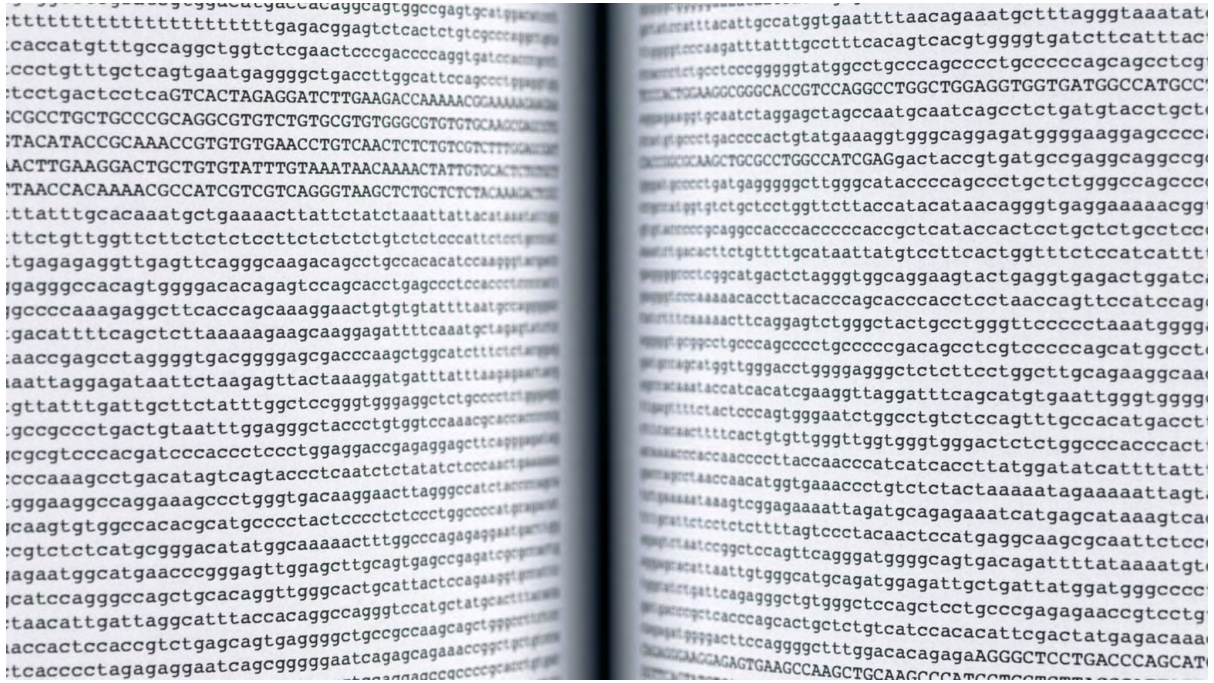


https://wellcomecollection.org/articles/WcvK4CsAANQR59Up; accessed 2/8/22
Photography by Ben Gilbert and Thomas Farnetti for Wellcome Collection

Size of targeted regions in example cancer assays

- Genome – 118 volumes
- Exome – 2 volumes
- 500 gene somatic CA panel – 100 pages
- 50 gene CA hotspot panel – 1 page
- Single gene – 1-2 lines

Amount of data generated for 500 gene cancer panel ≅ 1 bookcase



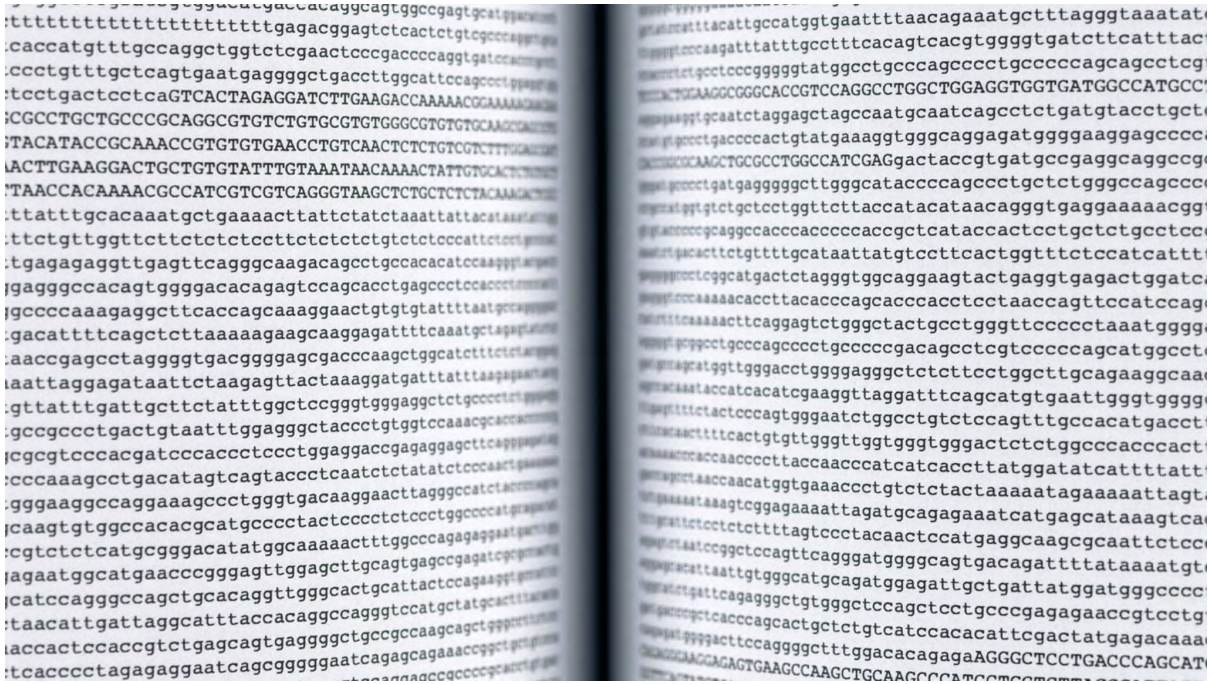
100 pages (target region)

X 1,000

(average coverage)

All files combined for tumor-normal panel ≅ 100 GB

Amount of data generated for 500 gene ctDNA panel ≅ 25 bookcases



100 pages (target region)

X 30,000

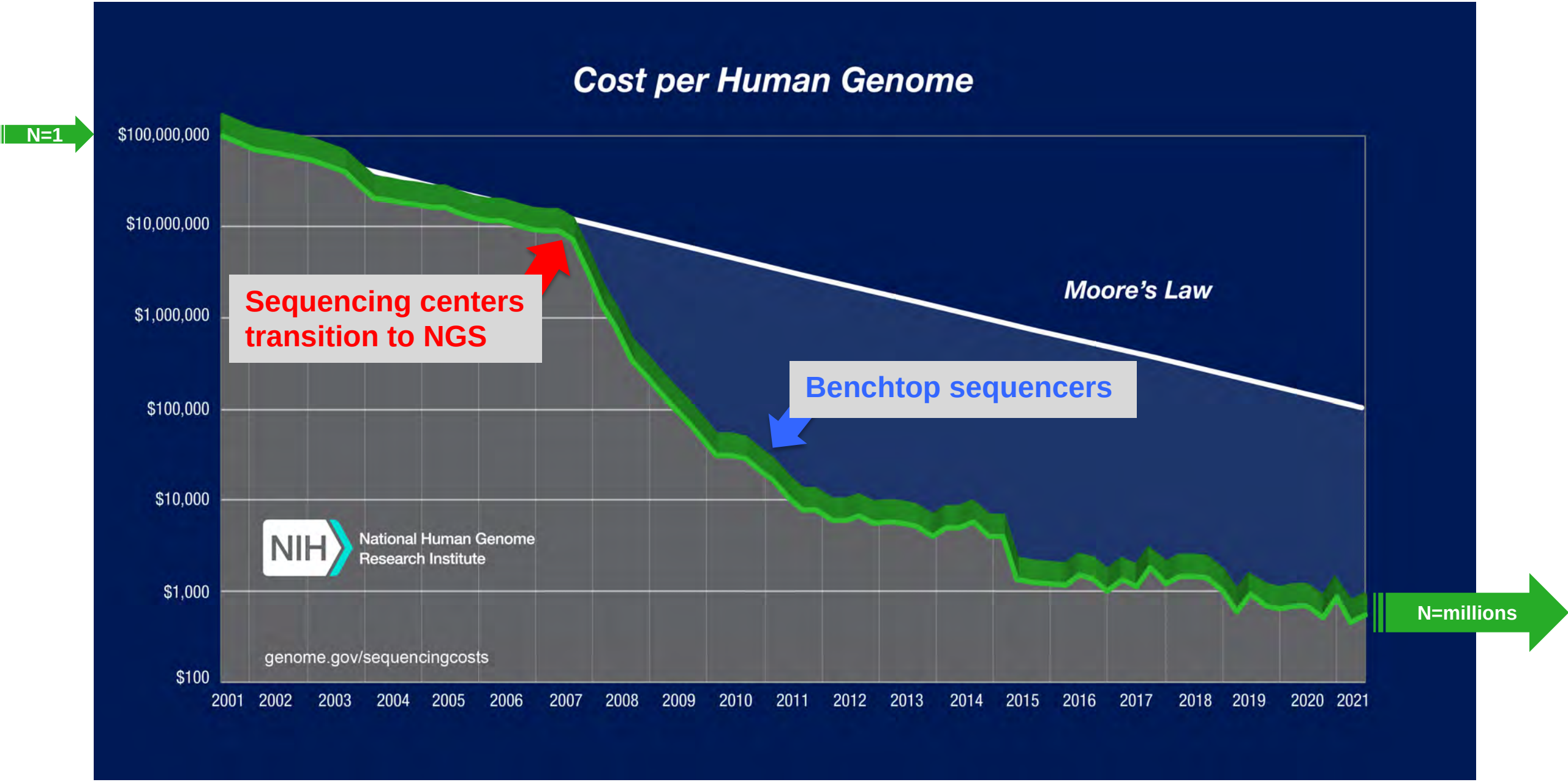
(average coverage)

=

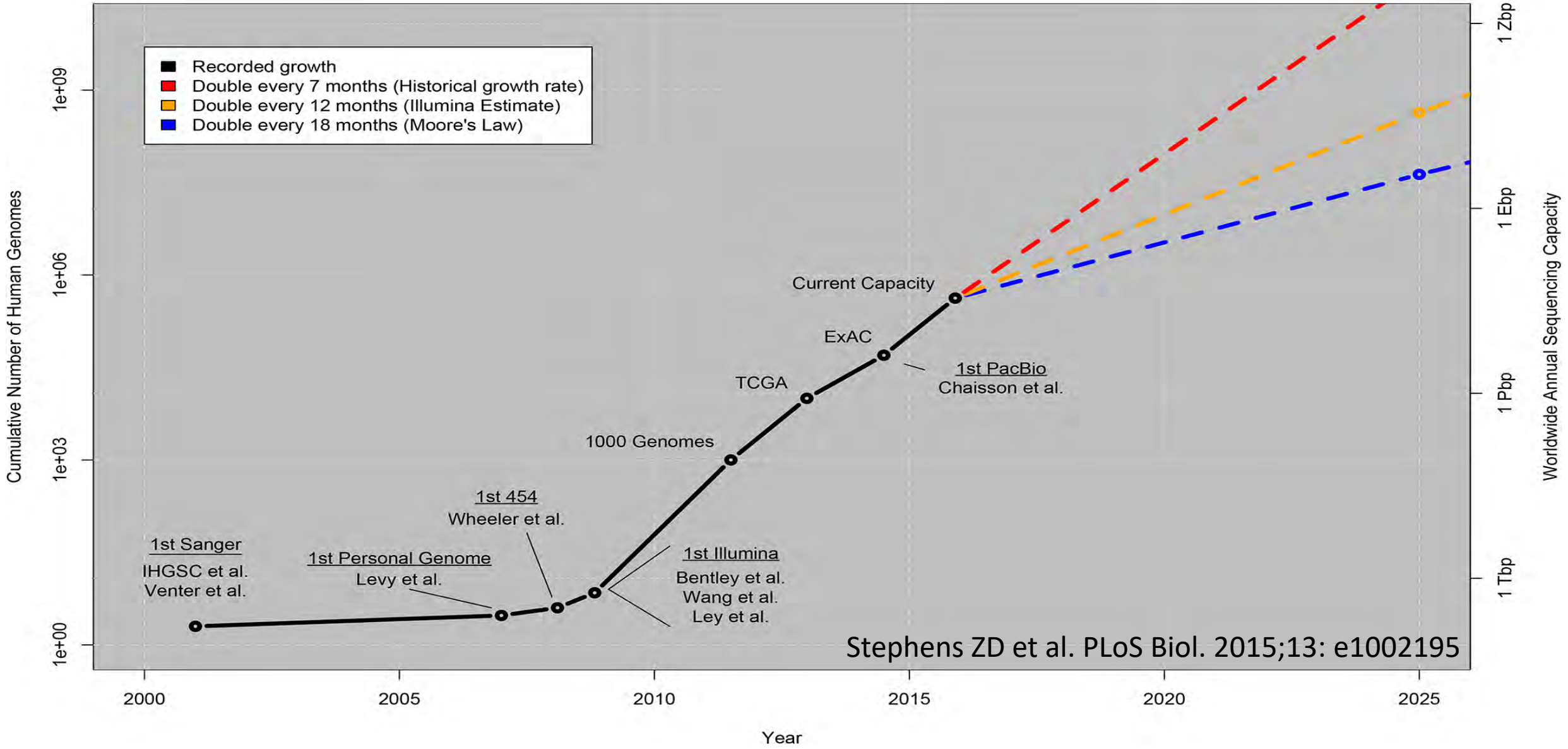
X 25

OR

3 TB (~1 TB compressed)


Cost per Human Genome
N=1
$100,000,000
$10,000,000
$1,000,000
$100,000
$10,000
$1,000
$100
Sequencing centers transition to NGS
Moore's Law
Benchtop sequencers
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021
N=millions

~1 billion human genomics sequences are estimated by 2025



Outline

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How are biomarkers used?

Diagnostic

- Assist with establishing diagnosis
- Example: *BCR/ABL1* gene fusion in chronic myeloid leukemia

Prognostic

- Assist with determining the likely aggressiveness or course of disease
- Example: *IDH1/2* mutations are associated with a relatively favorable prognosis in glioblastoma

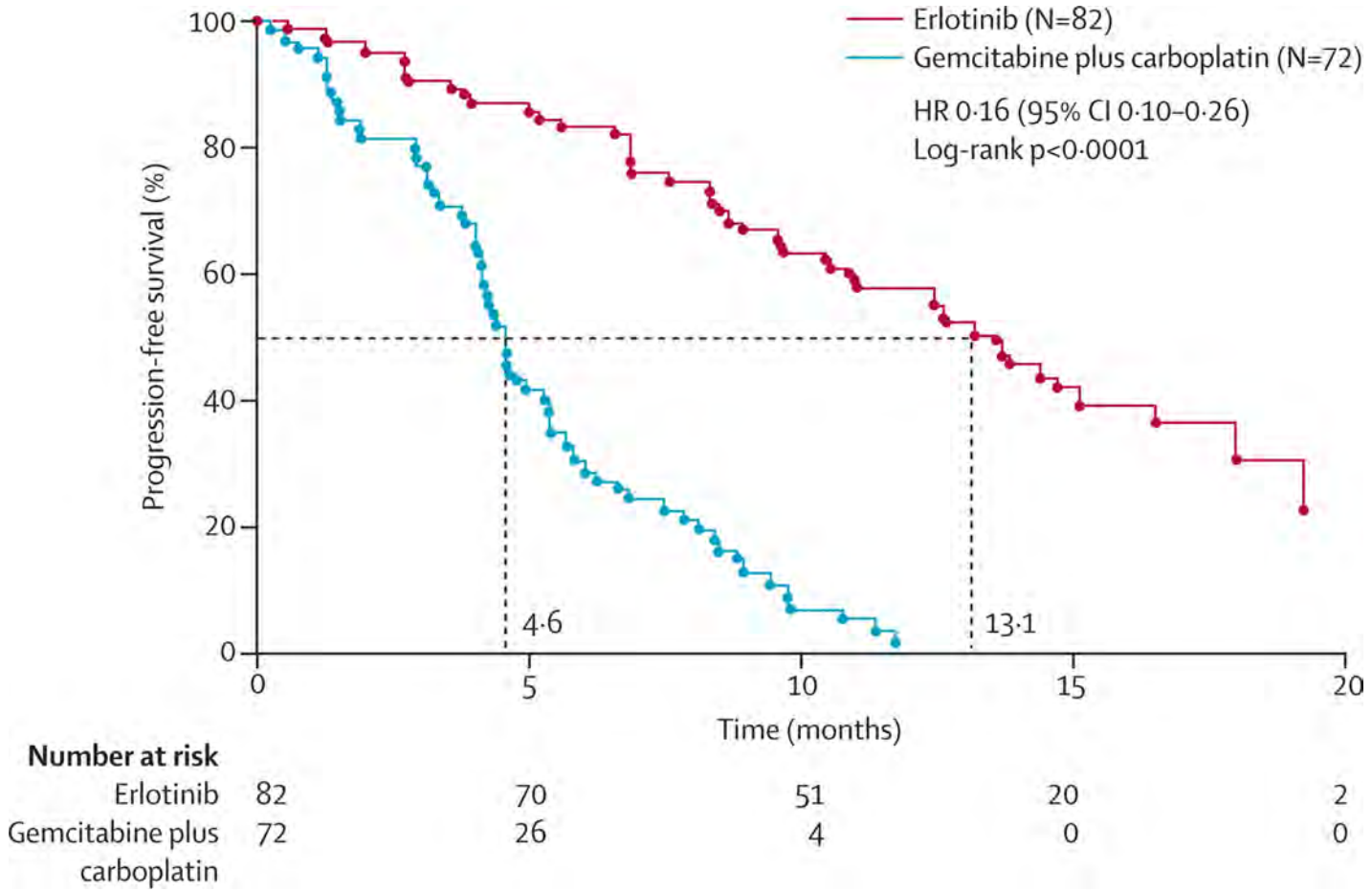
Therapeutic

- Assist with prediction of response or resistance to a given drug, biologic, or regimen
- Example: *EGFR* activating mutations are associated with response to EGFR tyrosine kinase inhibitor (TKI) therapy in Non-Small Cell Lung Cancer

Clinical Trial Eligibility

Example of a therapeutic biomarker – *EGFR*m in NSCLC

- In patients with advanced non-small cell lung cancer (NSCLC) and an activating *EGFR* mutation, treatment with erlotinib more than doubled median progression-free survival
- Erlotinib can be given orally and has a different side effect profile compared to cytotoxic chemotherapy

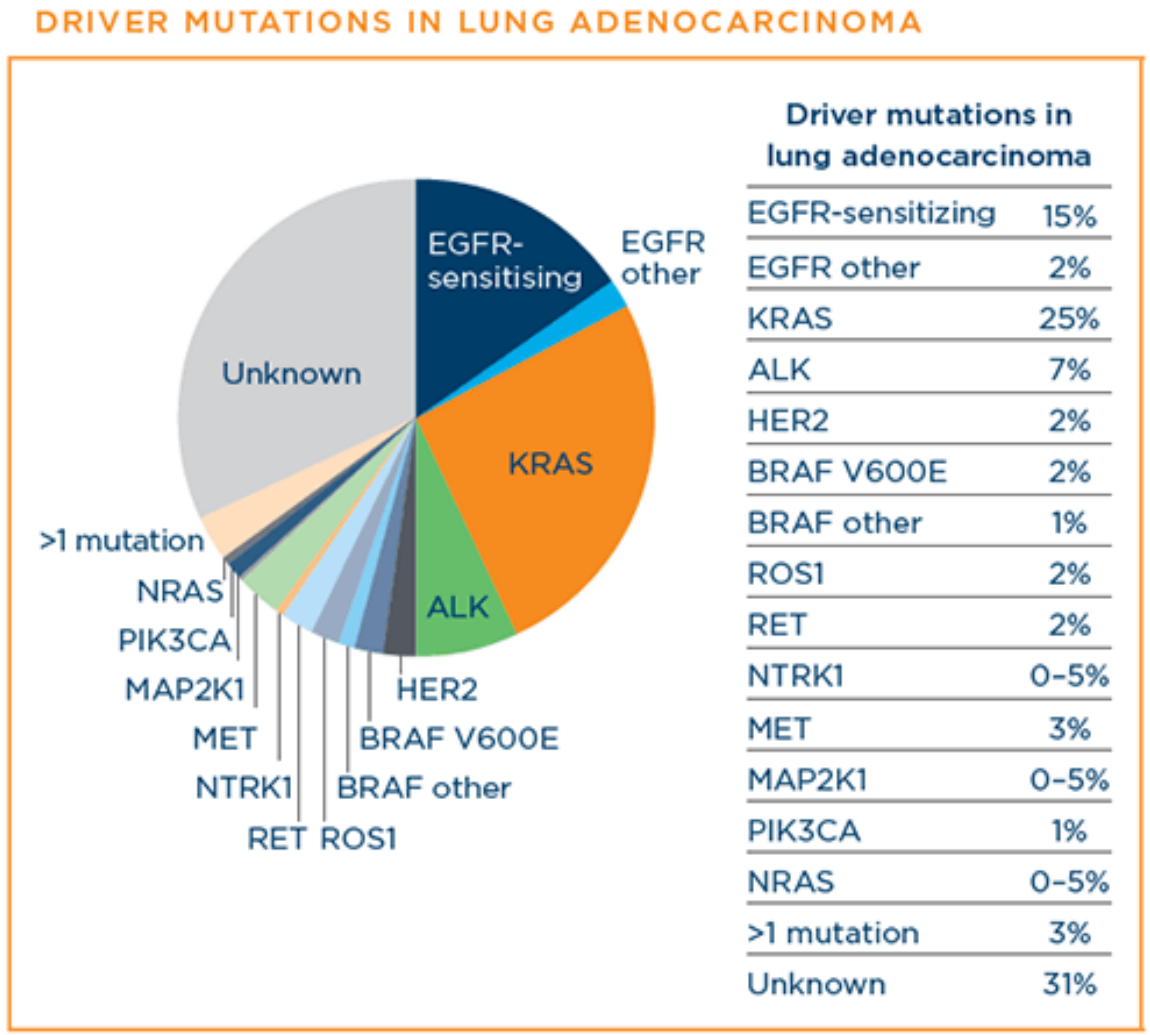


Zhou C et al. Lancet Oncol. 2011;12:735

Example therapeutic biomarkers in NSCLC

FDA-approved targeted therapies:

1. *EGFR* (15%)
2. *KRAS* G12C (10%)
3. *ALK* (7%)
4. *MET* exon 14 skipping (3%)
5. *BRAF* V600E (2%)
6. *RET* fusion (2%)
7. *ROS1* fusion (2%)
8. *NTRK* fusion (0.5%)



https://www.lungevity.org/for-patients-caregivers/navigating-your-diagnosis/biomarker-testing#10; accessed 2/9/22
Hirsch FR et al. Lancet. 2016;388:1012

FDA cleared or approved oncology biomarkers

>140 oncology-related companion diagnostic indications (diagnostic – therapeutic – tumor type)

- i.e., therapeutic label stipulates use of companion diagnostic device for use of a therapeutic in a specific tumor type

INTENDED USE

The **cobas**® EGFR Mutation Test is a real-time PCR test for the qualitative detection of exon 19 deletions and exon 21 (L858R) substitution mutations of the epidermal growth factor receptor (EGFR) gene in DNA derived from formalin-fixed paraffin-embedded (FFPET) human non-small cell lung cancer (NSCLC) tumor tissue. The test is intended to be used as an aid in selecting patients with NSCLC for whom (erlotinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated.

https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools; updated 11/30/21, accessed 2/9/22
https://www.accessdata.fda.gov/cdrh_docs/pdf12/P120019C.pdf; accessed 2/9/22

How do we test for biomarkers?

Common techniques:

1. Immunohistochemistry (IHC)
2. Chromosome analysis
3. Fluorescence in situ hybridization (FISH)
4. Amplification-based methods
5. Sanger sequencing
6. Next-generation (or massively parallel) sequencing

Considerations for biomarker test selection

Specimen

- Adequate size
- Sufficient tumor content

Assay

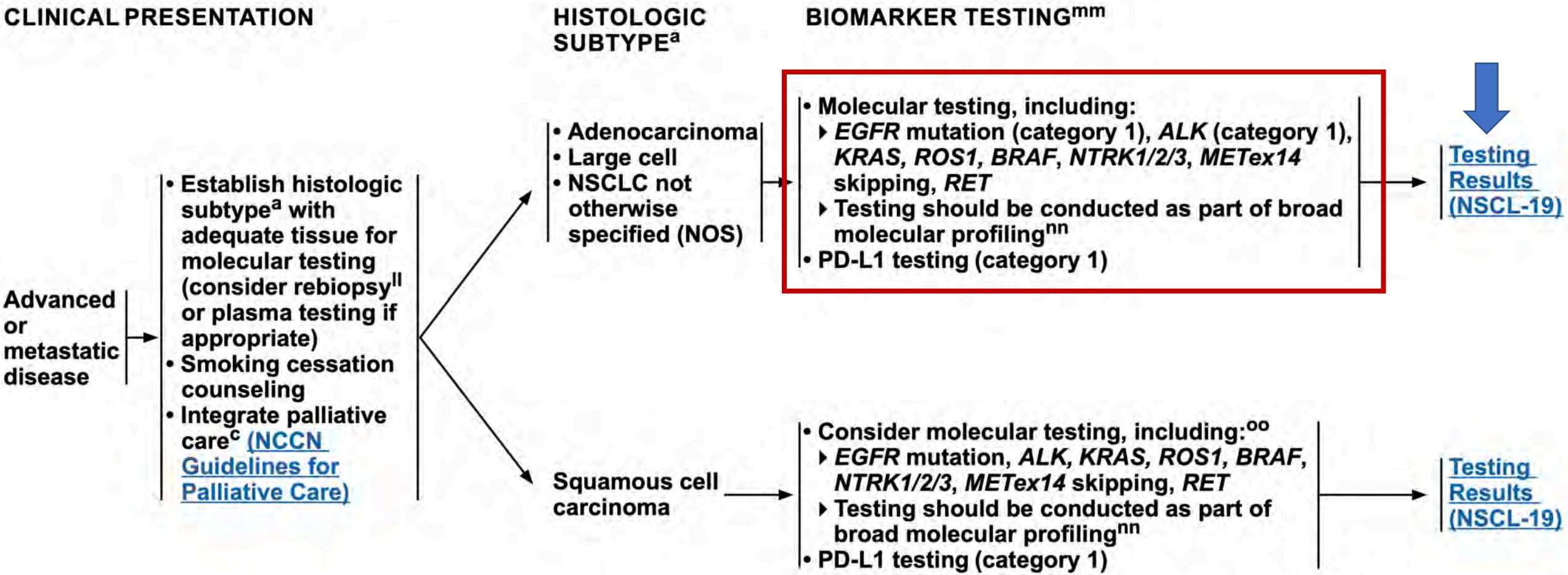
- Method associated with clinical indication
- Designed to somatic (acquired) and/or germline (heritable) variants
- Includes necessary genes
- Detects desired variant type (some panels cannot detect amplifications/losses or gene fusions)

Logistics

- Turnaround time
- Cost to patient
- Cost to institution (e.g., bundled payments)

Resources to help biomarker and test selection

NCCN Guidelines



NCCN Guidelines – Non-Small Cell Lung Cancer Version 1.2022

Resources to help with biomarker interpretation

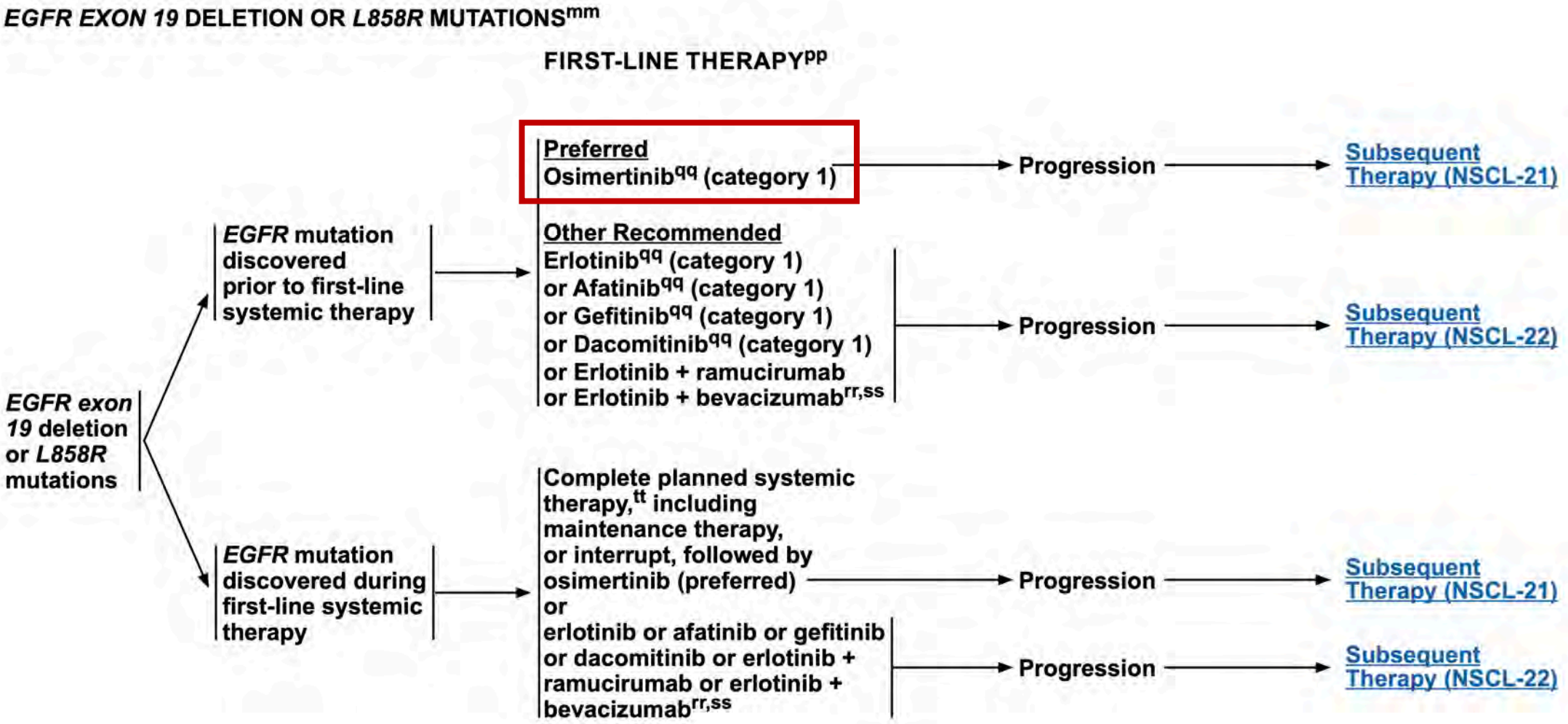
NCCN Guidelines

TESTING RESULTS[ll,mm]	
EGFR exon 19 **deletion or** ***L858R*** **mutation positive**	NSCL-20
EGFR S768I, L861Q, **and/or** ***G719X*** **mutation positive**	NSCL-23
EGFR exon 20 **insertion mutation positive**	NSCL-24
KRAS G12C **mutation positive**	NSCL-25
ALK **rearrangement positive**	NSCL-26
ROS1 **rearrangement positive**	NSCL-29
BRAF **V600E mutation positive**	NSCL-31
NTRK1/2/3 **gene fusion positive**	NSCL-32
METex14 **skipping mutation positive**	NSCL-33
RET **rearrangement positive**	NSCL-34
PD-L1 ≥50% and negative for actionable molecular biomarkers above	NSCL-35
PD-L1 ≥1%–49% and negative for actionable molecular biomarkers above	NSCL-36
PD-L1 <1% and negative for actionable molecular biomarkers above	NSCL-37

NCCN Guidelines – Non-Small Cell Lung Cancer Version 1.2022

Resources to help with biomarker interpretation

NCCN Guidelines



NCCN Guidelines – Non-Small Cell Lung Cancer Version 1.2022

Resources to help biomarker and test selection

NCCN Biomarkers Compendium

Guideline - Disease	Molecular Abnormality	Gene Symbol	NCCN Category	NCCN Recommendation
Non-Small Cell Lung Cancer	EGFR kinase domain mutations	EGFR	1	Stage IVA, M1a: pleural or pericardial effusion; M1b Advanced or metastatic disease: Adenocarcinoma, Large Cell, NSCLC not otherwise specified (NOS). Molecular testing, including: • *EGFR* mutation (category 1), *ALK* (category 1), *KRAS, ROS1, BRAF, NTRK1/2/3*, *MET* ex14 skipping, *RET* • Testing should be conducted as part of broad molecular profiling
Non-Small Cell Lung Cancer	ALK gene rearrangement	ALK	1	
Non-Small Cell Lung Cancer	EGFR kinase domain mutations, EGFR T790M mutation	EGFR	1	
Non-Small Cell Lung Cancer	CD274 (PD-L1) expression	CD274	1	

NCCN Biomarkers Compendium – Filters: Non-Small Cell Lung Cancer v.1.2022

Other resources

Molecular Tumor Board


William Kim, MD
Medical Oncology


Jason Merker, MD, PhD
Molecular Pathology


Amber Cipriani, PharmD
Pharmacy


Douglas Kirk, BA
LCCC Coordinator


Ashlynn Messmore, MS, CGC
Cancer Genetics


Shetal Patel, MD, PhD
Medical Oncology


Lori Ramkissoon, PhD
Molecular & Cytogenetics


Jaime Richardson, BA, RN, BSN
MTB Coordinator

Functions

- Assist HCPs with challenging case interpretation via e-consult (turnaround time based on clinical urgency)
- Run monthly molecular tumor boards and other educational activities
- Assist HCPs with test selection
- Guide molecular oncology test menu
- Facilitate use of molecular oncology testing across the healthcare system (e.g., EHR, CDS, clinical pathways)

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NGS-based solid tumor panels

- Most assays designed to be applied across multiple tumor types cover ~300-600 genes
- Turnaround time of ~2 weeks from receipt of tissue
- Most assays only test tumor tissue
- Report potential clinical trials based on molecular findings

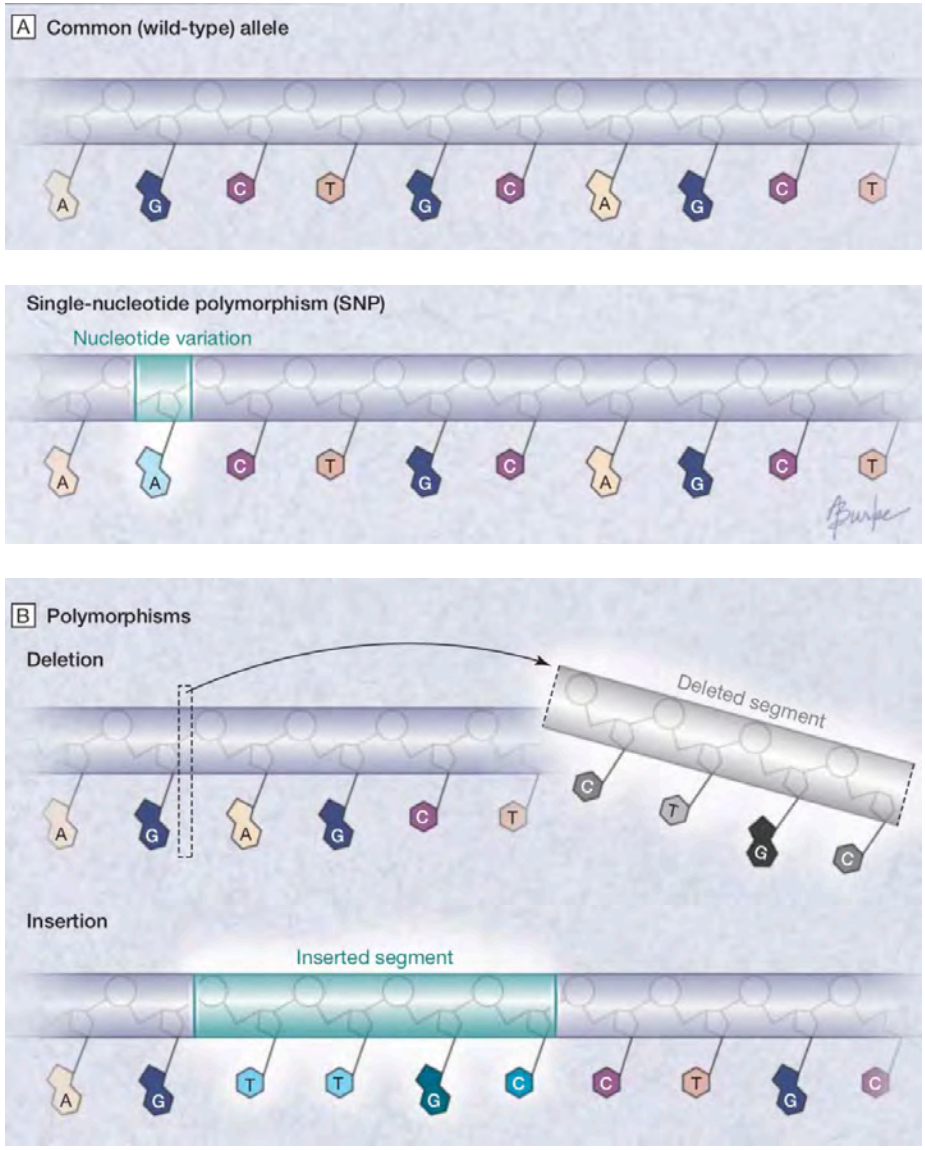
Variant types types detected by NGS panels

Most assays detect:

- Single-nucleotide variants (SNVs)
- Small insertions and deletions (<50 bp)

Many assays detect:

- Amplifications or losses
- Gene rearrangements/fusions



Wild-type (normal)

SNV

Deletion

Insertion

Attia J et al. JAMA. 2009;301:74

Genomic features commonly detected by NGS panels

Microsatellite Instability (MSI)

- Pattern of hypermutation involving changes in the length of short, repeated sequences

Tumor Mutation Burden (TMB)

- Number of mutations per Mb

Genomic Loss of Heterozygosity (LOH)

- Measure of genomic instability which suggests defective homologous recombination repair

National Human Genome Research Institute

Example clinical NGS-based solid tumor panels

	Assay 1	Assay 2	Assay 3
Number of genes	500	324	648
Germline DNA sequenced	N	N	**Y**
RNA sequencing	**Targeted**	N	**Comprehensive**
Maturity of EHR integration	+	++	**+++**
FDA cleared/approved	N	**Y**	N

Example of an expanded next-generation sequencing-based assay

Substitutions, insertions-deletions, copy-number changes

ABL1	ACVR1B	AKT1	AKT2	AKT3	ALK	ALOX12B	AMER1 (FAM123B)	APC
AR	ARAF	ARFRP1	ARID1A	ASXL1	ATM	ATR	ATRX	AURKA
AURKB	AXIN1	AXL	BAP1	BARD1	BCL2	BCL2L1	BCL2L2	BCL6
BCOR	BCORL1	BRAF	BRCA1	BRCA2	BRD4	BRIP1	BTG1	BTG2
BTK	C11orf30 (EMSY)	CALR	CARD11	CASP8	CBFB	CBL	CCND1	CCND2
CCND3	CCNE1	CD22	CD274 (PD-L1)	CD70	CD79A	CD79B	CDC73	CDH1
CDK12	CDK4	CDK6	CDK8	CDKN1A	CDKN1B	CDKN2A	CDKN2B	CDKN2C
CEBPA	CHEK1	CHEK2	CIC	CREBBP	CRKL	CSF1R	CSF3R	CTCF
CTNNA1	CTNNB1	CUL3	CUL4A	CXCR4	CYP17A1	DAXX	DDR1	DDR2
DIS3	DNMT3A	DOT1L	EED	EGFR	EP300	EPHA3	EPHB1	EPHB4
ERBB2	ERBB3	ERBB4	ERCC4	ERG	ERRFI1	ESR1	EZH2	FAM46C
FANCA	FANCC	FANCG	FANCL	FAS	FBXW7	FGF10	FGF12	FGF14
FGF19	FGF23	FGF3	FGF4	FGF6	FGFR1	FGFR2	FGFR3	FGFR4
FH	FLCN	FLT1	FLT3	FOXL2	FUBP1	GABRA6	GATA3	GATA4
GATA6	GID4 (C17orf39)	GNA11	GNA13	GNAQ	GNAS	GRM3	GSK3B	H3F3A
HDAC1	HGF	HNF1A	HRAS	HSD3B1	ID3	IDH1	IDH2	IGF1R
IKBKE	IKZF1	INPP4B	IRF2	IRF4	IRS2	JAK1	JAK2	JAK3
JUN	KDM5A	KDM5C	KDM6A	KDR	KEAP1	KEL	KIT	KLHL6
KMT2A (MLL)	KMT2D (MLL2)	KRAS	LTK	LYN	MAF	MAP2K1 (MEK1)	MAP2K2 (MEK2)	MAP2K4
MAP3K1	MAP3K13	MAPK1	MCL1	MDM2	MDM4	MED12	MEF2B	MEN1
MERTK	MET	MITF	MKNK1	MLH1	MPL	MRE11A	MSH2	MSH3
MSH6	MST1R	MTAP	MTOR	MUTYH	MYC	MYCL (MYCL1)	MYCN	MYD88
NBN	NF1	NF2	NFE2L2	NFKBIA	NKX2-1	NOTCH1	NOTCH2	NOTCH3
NPM1	NRAS	NT5C2	NTRK1	NTRK2	NTRK3	P2RY8	PALB2	PARK2
PARP1	PARP2	PARP3	PAX5	PBRM1	PDCD1 (PD-1)	PDCD1LG2 (PD-L2)		PDGFRA
PDGFRB	PDK1	PIK3C2B	PIK3C2G	PIK3CA	PIK3CB	PIK3R1	PIM1	PMS2
POLD1	POLE	PPARG	PPP2R1A	PPP2R2A	PRDM1	PRKAR1A	PRKCI	PTCH1
PTEN	PTPN11	PTPRO	QKI	RAC1	RAD21	RAD51	RAD51B	RAD51C
RAD51D	RAD52	RAD54L	RAF1	RARA	RB1	RBM10	REL	RET
RICTOR	RNF43	ROS1	RPTOR	SDHA	SDHB	SDHC	SDHD	SETD2
SF3B1	SGK1	SMAD2	SMAD4	SMARCA4	SMARCB1	SMO	SNCAIP	SOCS1
SOX2	SOX9	SPEN	SPOP	SRC	STAG2	STAT3	STK11	SUFU
SYK	TBX3	TEK	TET2	TGFBR2	TIPARP	TNFAIP3	TNFRSF14	TP53
TSC1	TSC2	TYRO3	U2AF1	VEGFA	VHL	WHSC1 (MMSET)	WHSC1L1	WT1
XPO1	XRCC2	ZNF217	ZNF703					

Select gene rearrangements

ALK	BCL2	BCR	BRAF	BRCA1	BRCA2	CD74	EGFR	ETV4
ETV5	ETV6	EWSR1	EZR	FGFR1	FGFR2	FGFR3	KIT	KMT2A (MLL)
MSH2	MYB	MYC	NOTCH2	NTRK1	NTRK2	NUTM1	PDGFRA	RAF1
RARA	RET	ROS1	RSPO2	SDC4	SLC34A2	TERC*	TERT (promoter only)**	
TMPRSS2								

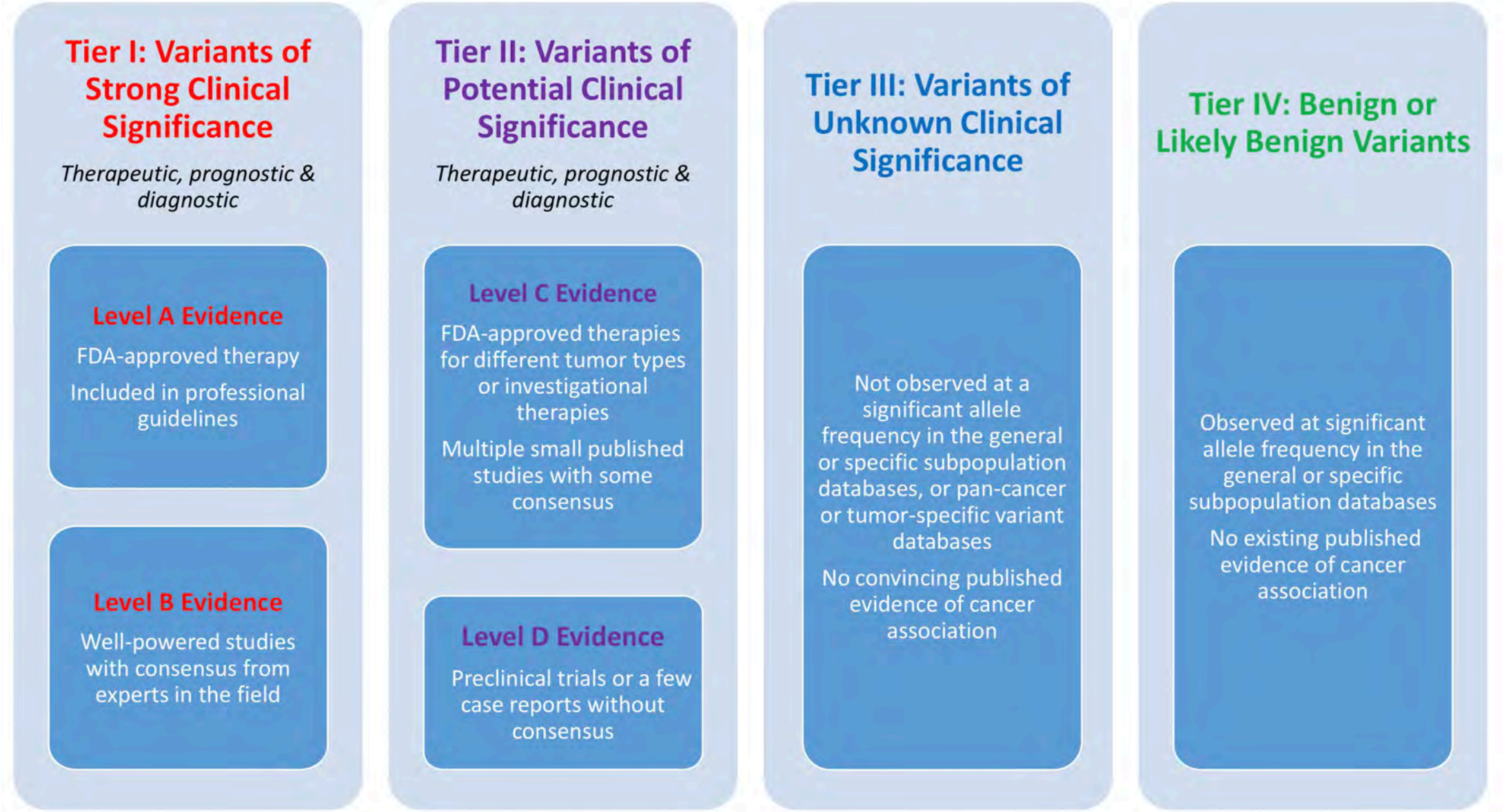
Tumor mutational burden (TMB)

Microsatellite instability (MSI)

Genomic loss of heterozygosity (LOH) – *some tumors*

https://assets.ctfassets.net/w98cd481qyp0/YqqKHaqQmFeqc5ueQk48w/c35460768c3a76ef738dcf88f8219524/F1CDx_Tech_Specs_072021.pdf accessed: 02/14/21

Somatic variant classification



AMP, ASCO, CAP Recommendations: Li MM et al. J Mol Diagn 2017;19:4

Case – 50 year-old female w/ GBM

Glioblastoma (WHO grade IV), *IDH* wild-type

- S/P resection, radiation therapy with concurrent temozolomide
- Follow-up MRI revealed progression
- Patient experienced significant drop in performance status

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- *TRIM2::NTRK2* gene fusion identified

Clinical Questions

1. Why are there apparently discrepant *NTRK2* fusion results?
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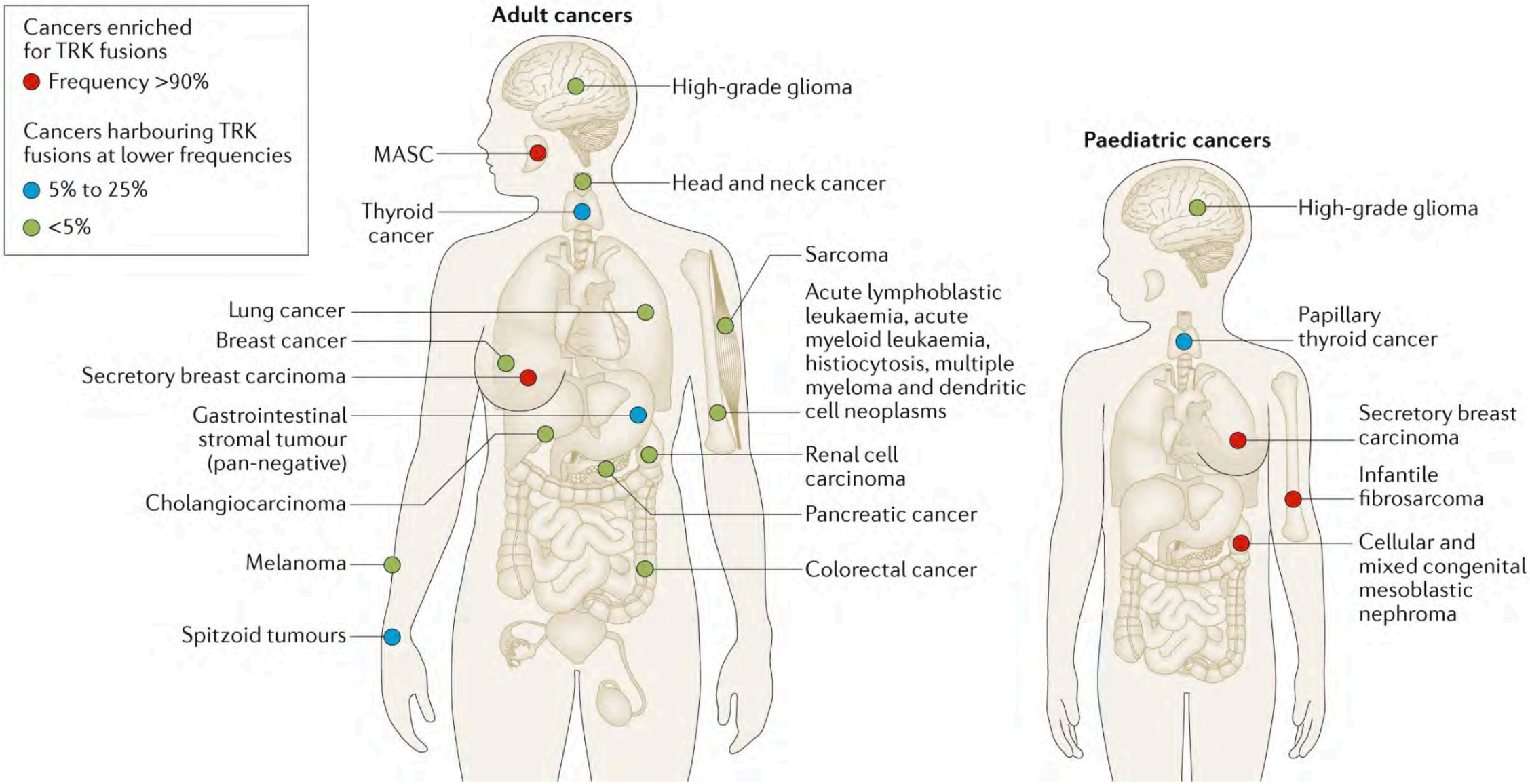
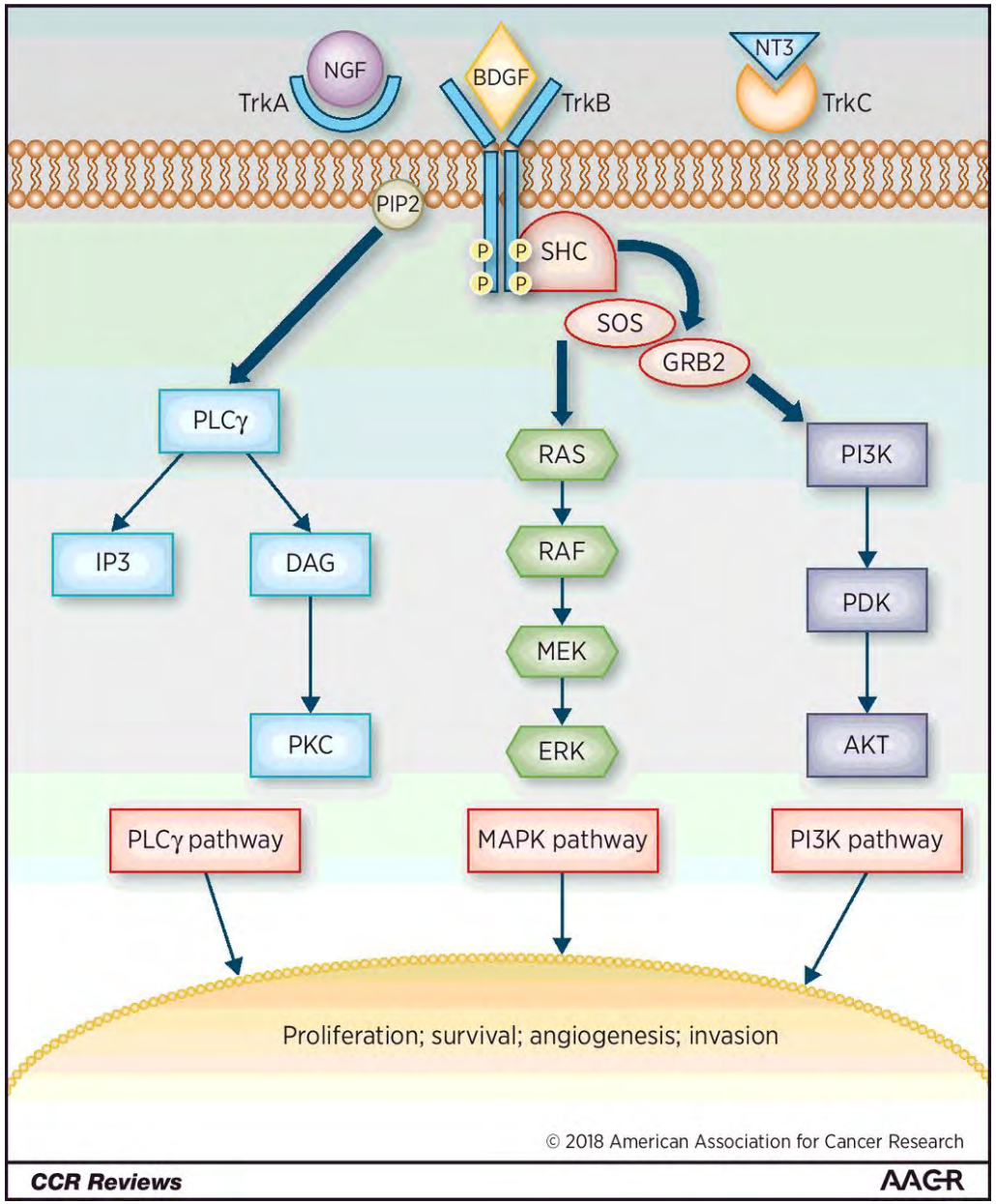
Frequency of *NTRK* fusions in adult and pediatric tumors



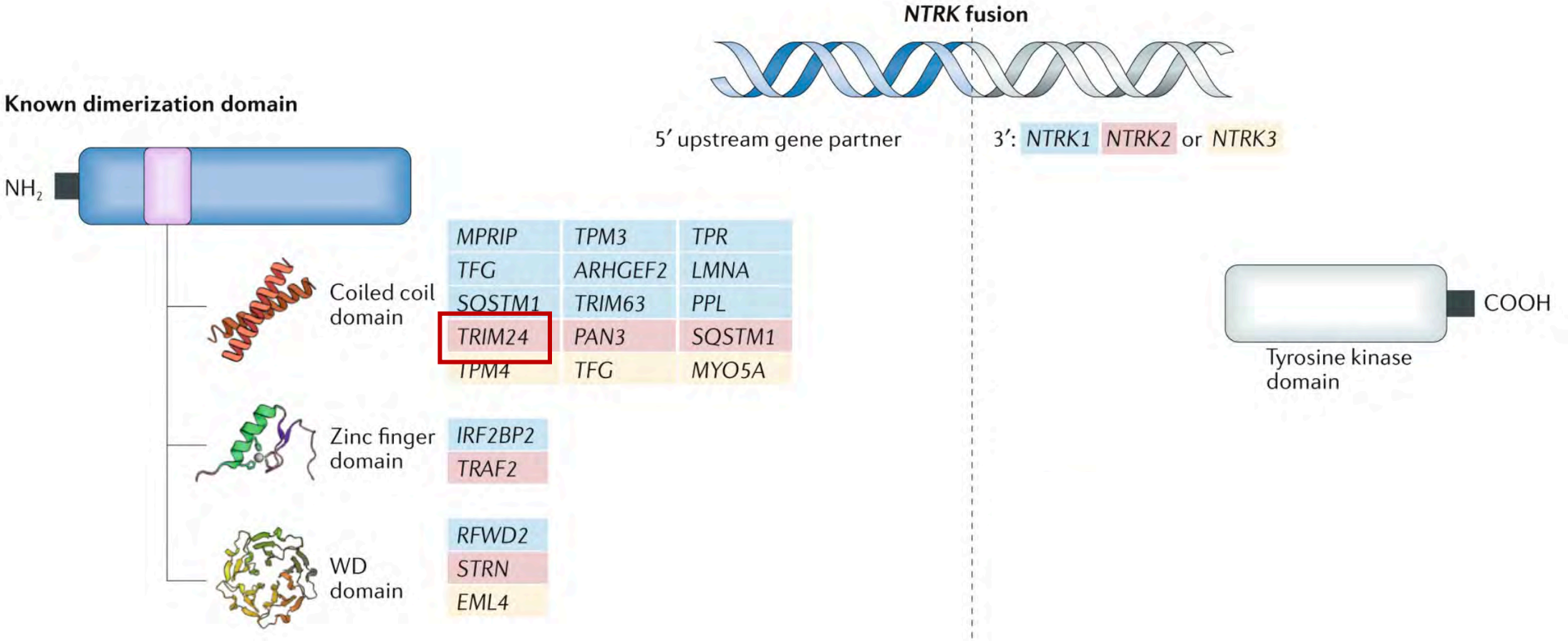
Cocco E et al. Nat Rev Clin Oncol. 2018;15:731

TRK biology and signaling



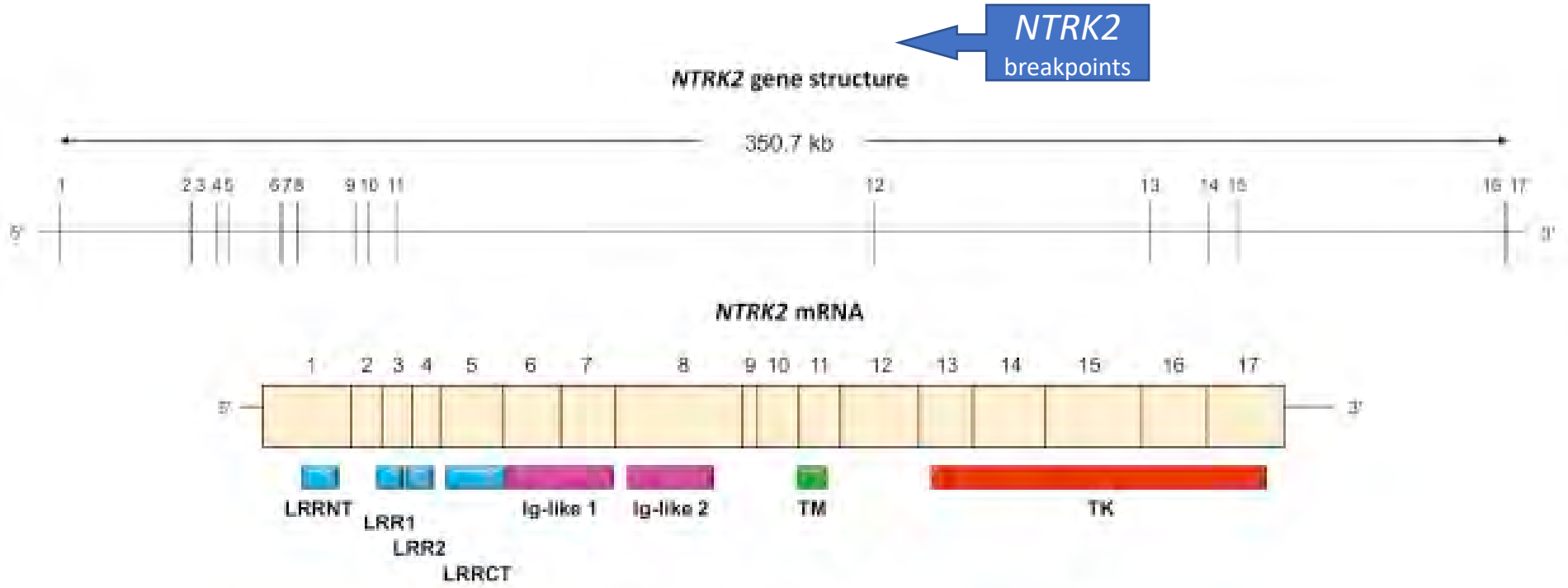
Kheder ES and Hong DS. Clin Cancer Res. 2018;24:5807

Activating mechanisms of *NTRK* fusions



Cocco E et al. Nat Rev Clin Oncol. 2018;15:731. PMID: 30333516
Stransky N et al. Nat Commun. 2014 10;5:4846. PMID: 25204415

The assays use different methods for fusion detection



DNA
350,700 bp

RNA
2,517 bp

Assay 1 (*NTRK* fusion not detected) uses DNA sequencing for fusion detection.

Assay 2 (*NTRK* fusion detected) uses RNA sequencing for fusion detection.

Detection of some gene fusions (e.g., *NTRK*) is challenging for DNA sequencing approaches.

Hsiao SJ et al. J Mol Diagn. 2019;21:553

DNA-based NGS assays miss a significant fraction of *NTRK* fusions

Table 42. Concordance between the CDx and RNA NGS LCTA methods for detection of NTRK gene fusions based on LCTA results and excluding invalid results

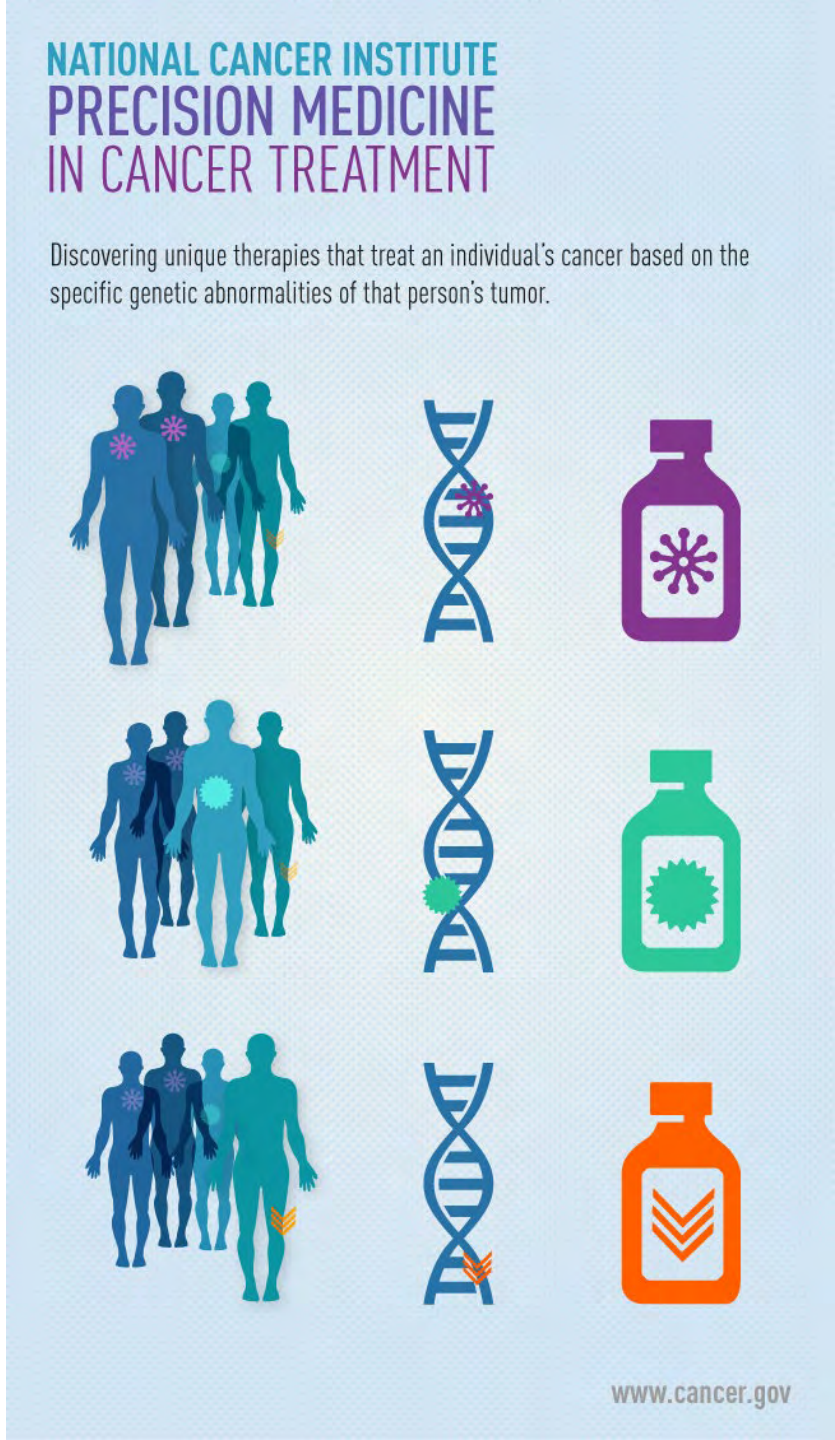
Measure of Agreement	% Agreement (N)	95% CI [(a)]
PPA	70.0% (14/20)	45.7%, 88.1%
NPA	100.0% (4/4)	39.8%, 100.0%
OPA	75.0% (18/24)	53.3%, 90.2%

[a] The 95% CI was calculated based on Clopper-Pearson exact method.

PMA P170019/S017: FDA Summary of Safety and Effectiveness Data

Case – 50 year-old female w/ GBM

- Patient started treatment with larotrectinib (Trk inhibitor) on the NCI-MATCH clinical trial
- Significant response to therapy with stable disease
- Resumption of normal activities



Case – 50 year-old female w/ GBM

- Progression after 18 months of therapy
- Stereotactic brain biopsy for molecular testing:

QUANTITY / QUALITY NOT SUFFICIENT

The requested assay could not be completed due to insufficient quantity and/or quality of nucleic acid.

Case – 50 year-old female w/ GBM

Clinical Test Result (from diagnosis)

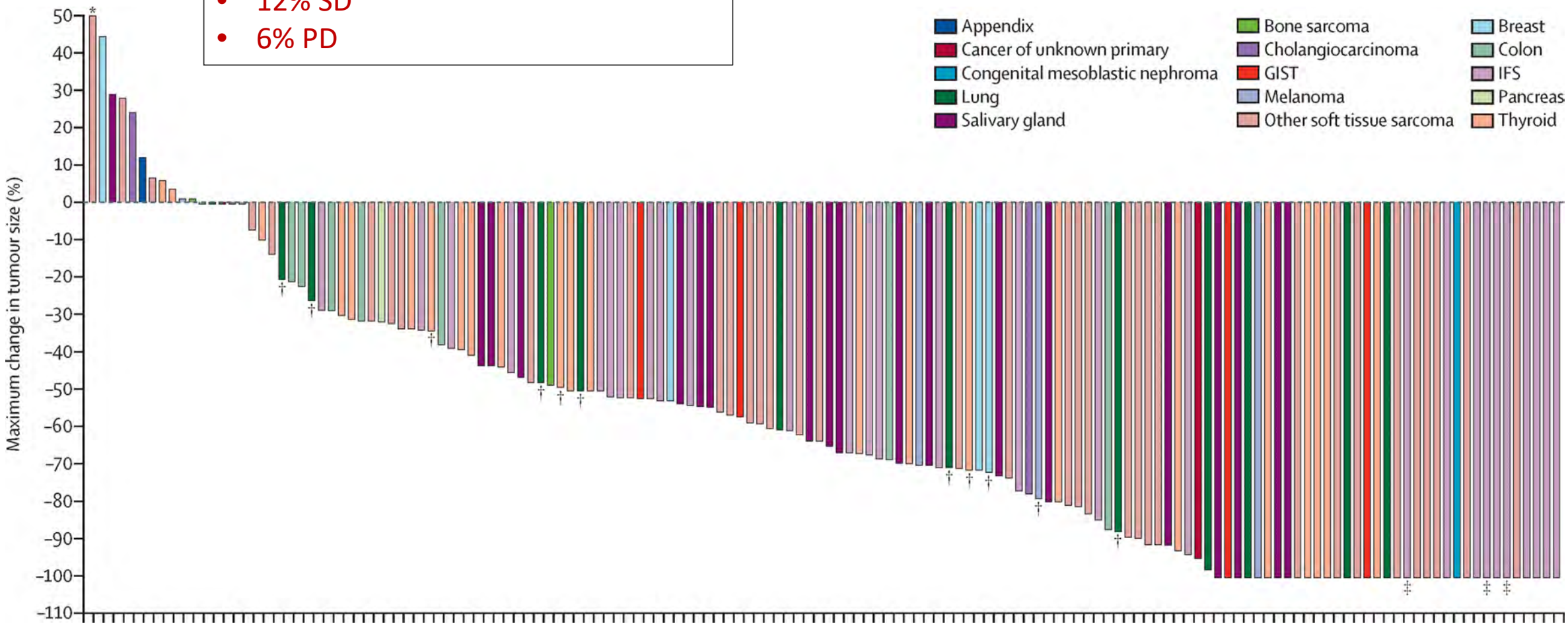
- Two loss-of-function mutations were identified in *NF1*
- Preclinical data and case reports suggest that NF1 inactivation may predict sensitivity to MEK inhibitors

Treatment following progression

- Patient started on trametinib (MEK inhibitor) + bevacizumab (VEGF inhibitor)
- Approaching 3 years from original diagnosis with stable disease

Larotrectinib in patients with TRK fusion-positive solid tumors

79% (121/153) objective response rate
- 16% CR
- 63% PR
- 12% SD
- 6% PD



Hong DS et al. Lancet Oncol. 2020;21:531

Outline

I. Background on genetics and genomics
II. Cancer biomarkers
III. NGS-based solid tumor testing
IV. Liquid biopsy
V. Conclusions and future directions

Liquid biopsy definition

- Pantel and Alix-Panabieres used the term *liquid biopsy* in a 2010 review of circulating tumor cells in cancer patients.
- Refers to a broad category of minimally invasive test done on blood or body fluids in an attempt to provide similar **genetic** information to that provided by a tissue biopsy.


Review

Cell PRESS

Circulating tumour cells in cancer patients: challenges and perspectives

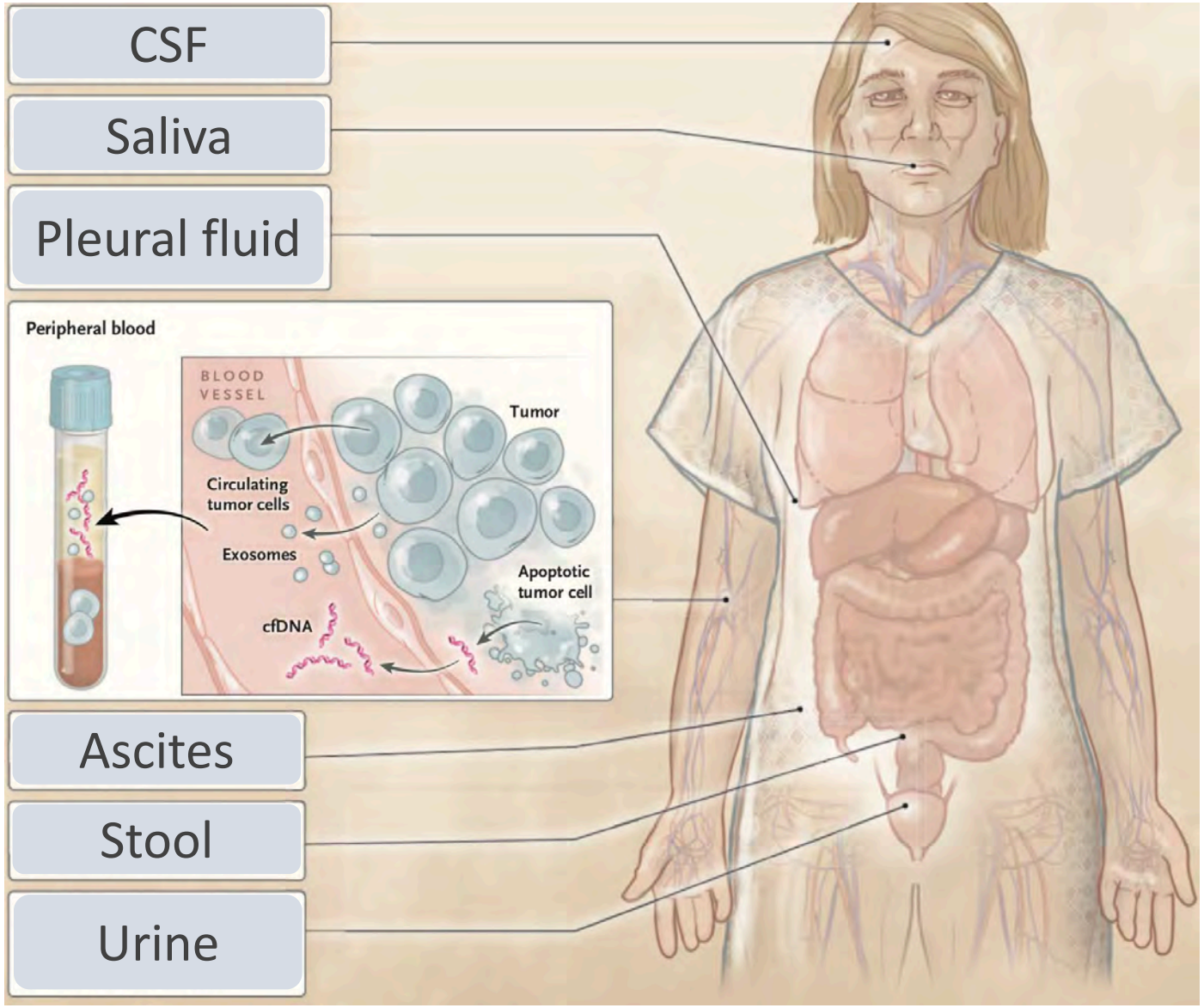
Klaus Pantel[1] and Catherine Alix-Panabières[2,3]

Trends Mol Med. 2010;16:398

Liquid biopsy sources

Liquid biopsy can include the measurement of:

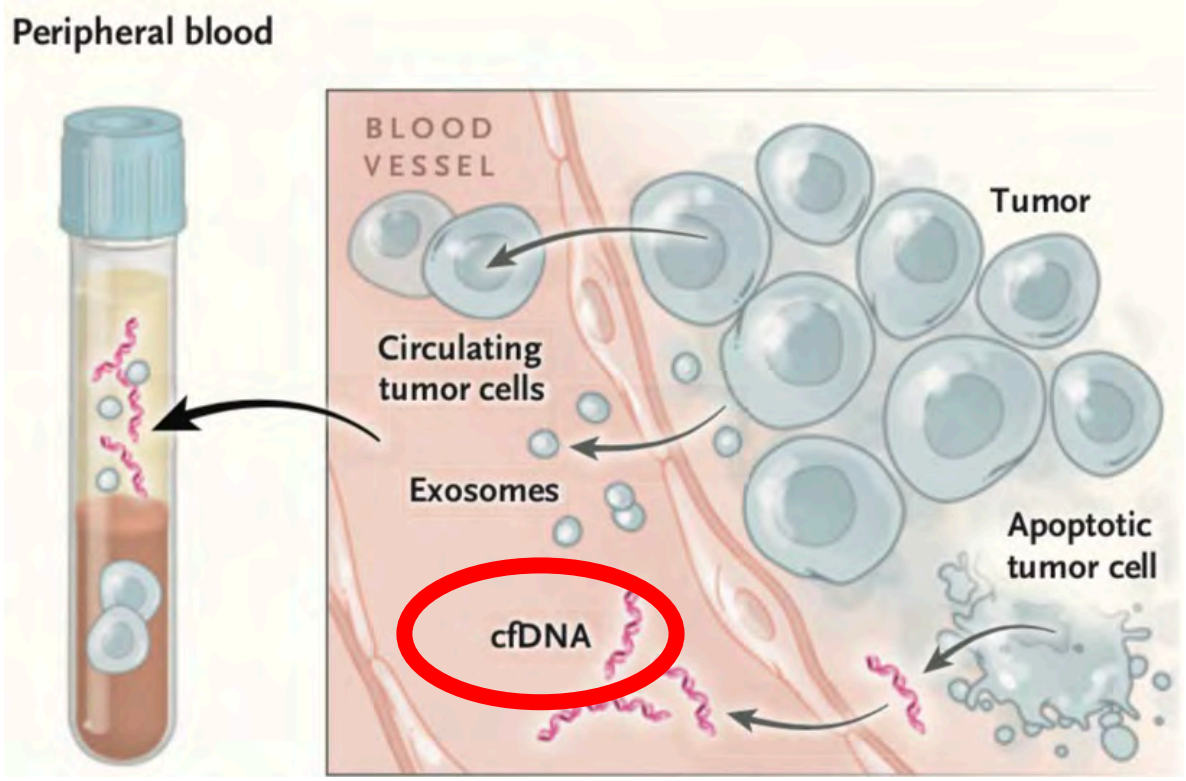
- Cell-free nucleic acids (DNA/RNA)
- Tumor cells
- Exosomes
- Proteins
- Other tumor markers



Adapted from Corcoran RB and Chabner BA. N Engl J Med. 2018;379:1754

This section will focus on analysis of cell-free DNA (cfDNA) in blood

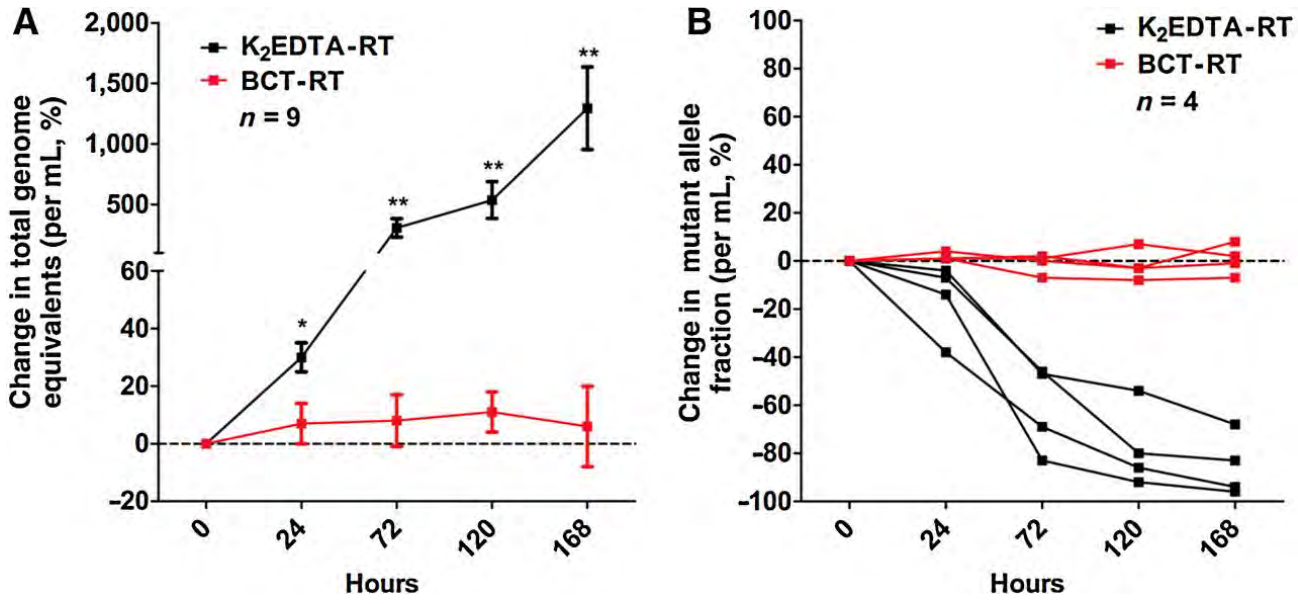
- cfDNA refers to DNA fragments in the plasma or serum, which can be derived from multiple sources, including tumor cells.
- Cell-free, circulating tumor DNA (ctDNA) is the subset of cfDNA that comes from the tumor cells



Corcoran RB and Chabner BA. N Engl J Med. 2018;379:1754

Plasma samples for cell-free DNA (cfDNA) studies should be collected in stabilizing tubes

- Plasma is preferred over serum
- EDTA tubes need to be centrifuged within 4-6 hours of collection
- For this reason, most groups use cells stabilization tubes, which can wait 48 hours or more before processing



Parpart-Li S et al. Clin Cancer Res. 2017;23:2471

Circulating cell-free DNA (cfDNA) described by Mandel and Metais in 1948

Les acides núcléiques du plasma sanguin chez l'Homme.

par P. MANDEL et P. MÉTAIS.

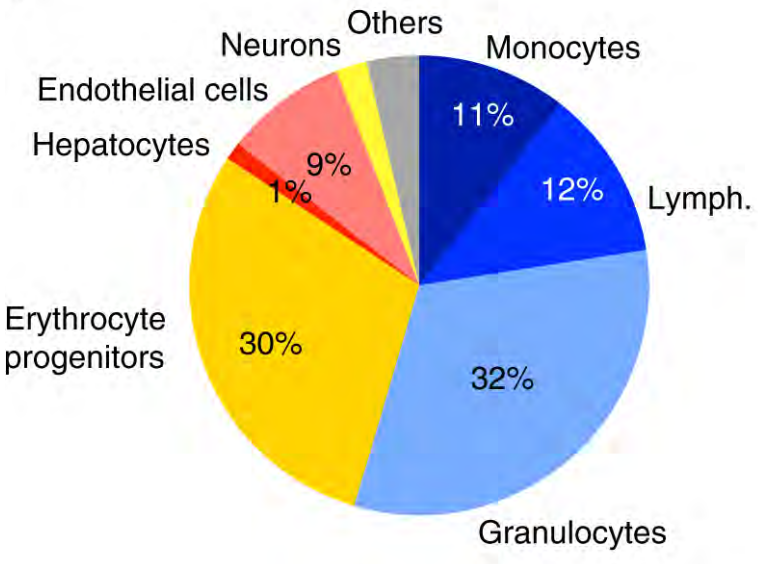
(1) *Bull. Soc. chim. biol.*, 1931, t. *13*, p. 685.
(2) *C. R. de la Soc. de biol.*, 1931, t. *107*, p. 1087.

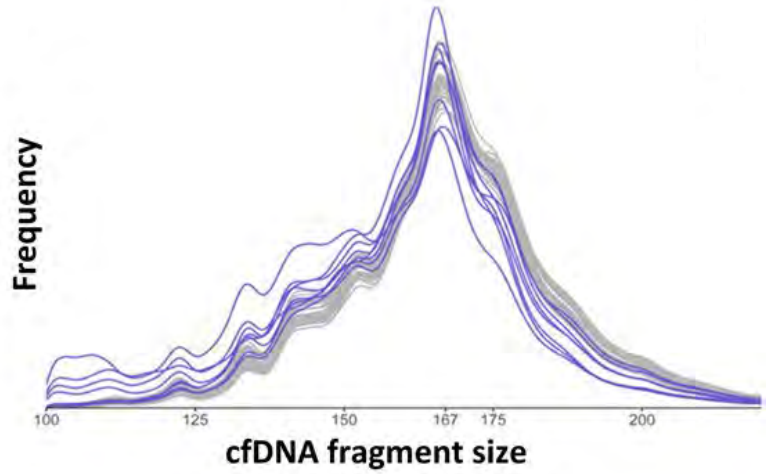
BIOLOGIE. COMPTES RENDUS. — N° 3-4, 1948. T. CXLII.

1948

Cell-free DNA (cfDNA) in healthy individuals

- Found at low levels in healthy individuals (~10 ng/mL).
- Mostly derived from blood cells.
- Primarily found as short fragments of double-stranded DNA (~160 – 180 bp).
- Short half-life (< 1 hour) due to rapid clearance. Total clearance within a couple of days.

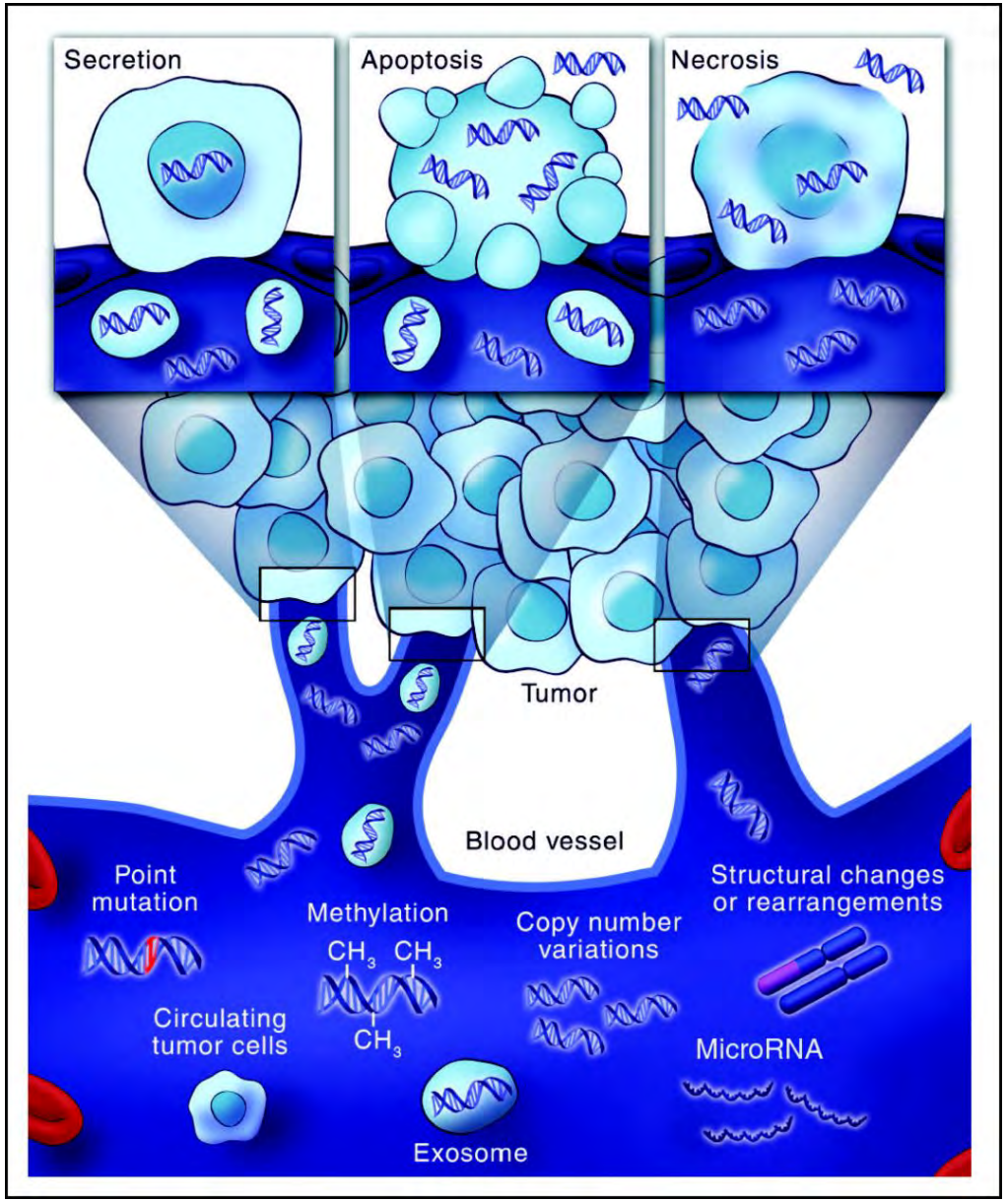




Moss J et al. Nat Commun. 2018;9:5068
Cristiano et al. Nature. 2019;570:385

Circulating cell-free nucleic acids

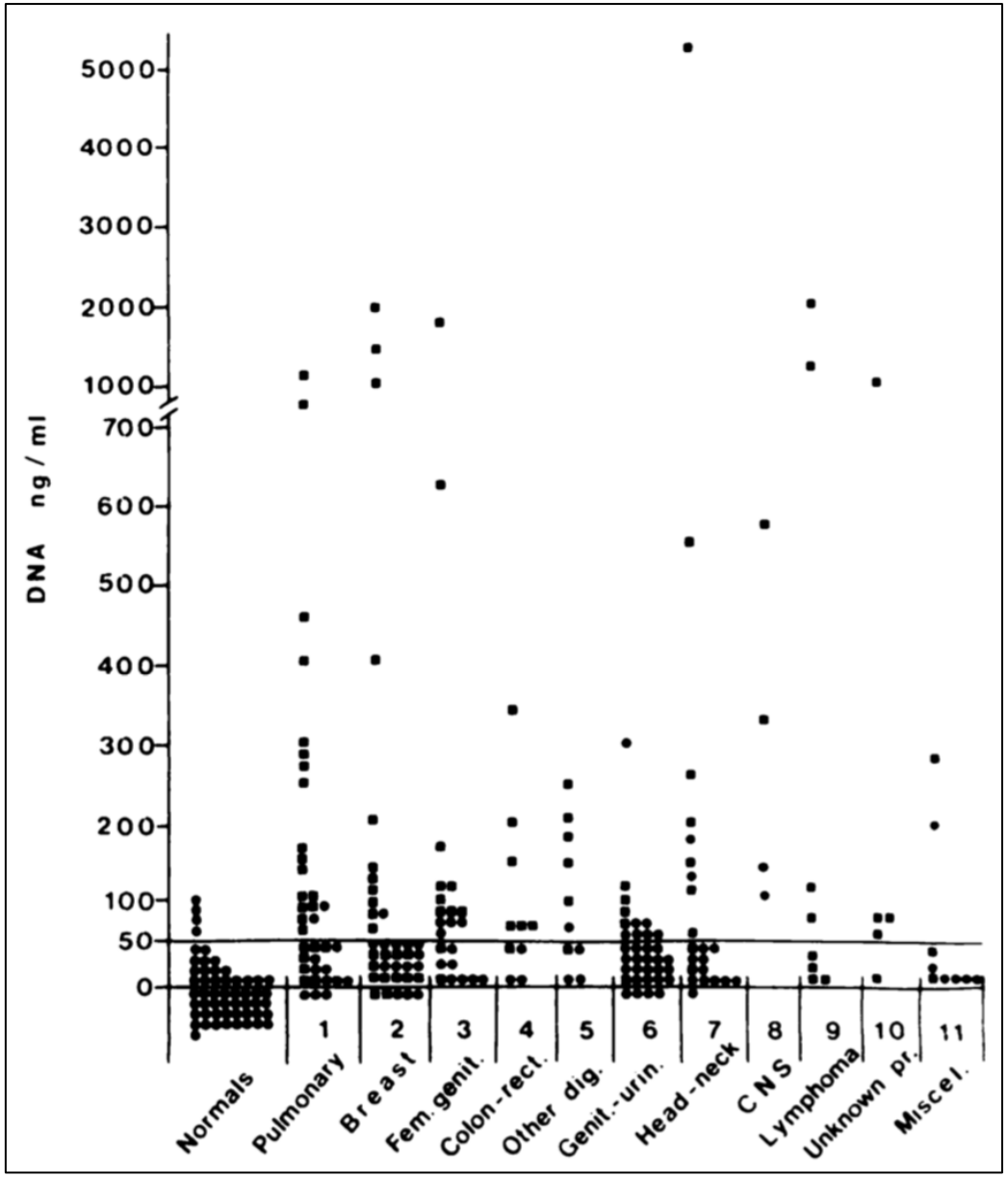
- Tumor cells release cell-free DNA (cfDNA) into the circulation by multiple mechanisms
- Circulating tumor cells, exosomes, and RNAs also released at low levels



Diaz LA and Bardelli. J Clin Oncol. 2014;32:579

Circulating tumor DNA (ctDNA) in cancer patients

- On average, patients with cancer have higher levels of cell-free DNA than those without cancer due, at least in part, to the contribution of circulating tumor DNA (ctDNA).
- ctDNA is the fraction of cell-free DNA that originates from tumor cells.
- This fraction can vary significantly from less than 0.01% to greater than 90%.

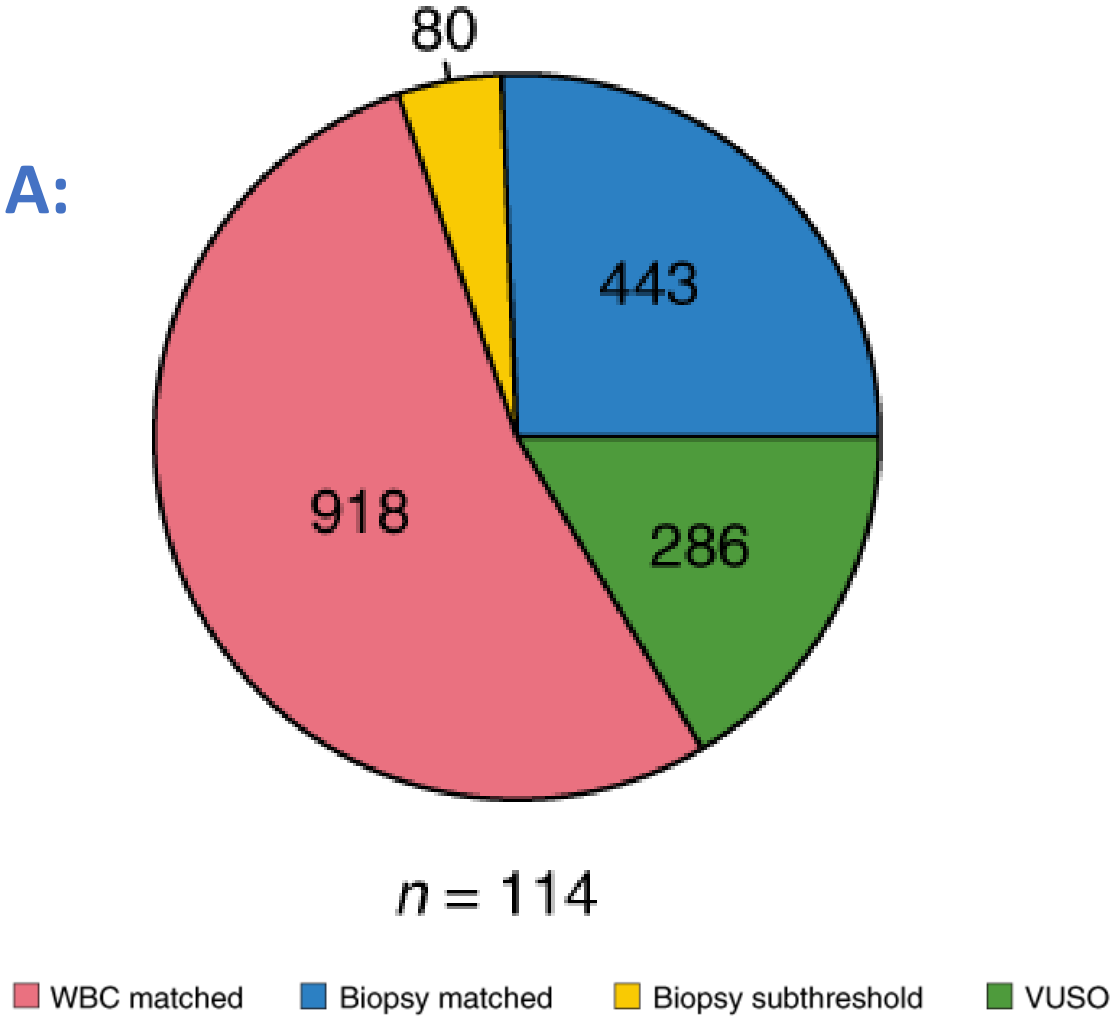


Leon SA et al. Cancer Res. 1977;37:646

Most cfDNA from advanced cancer patients is from white blood cells

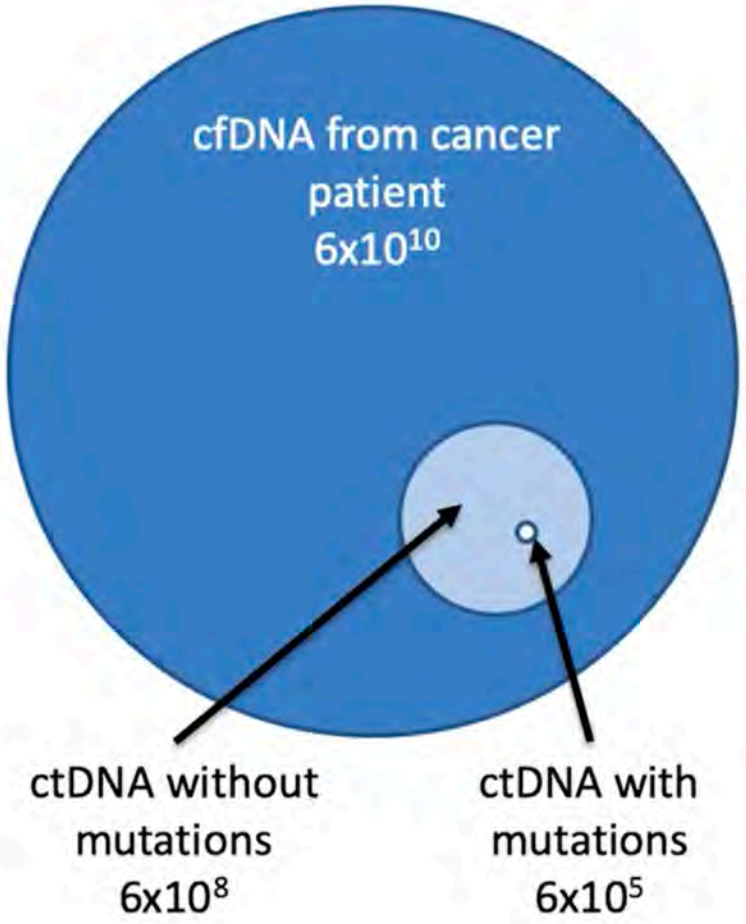
Source of somatic mutations in cfDNA:

- 53% WBCs
- 30% tumor
- 17% unknown



Razavi P et al. Nat Med. 2019;25:1928

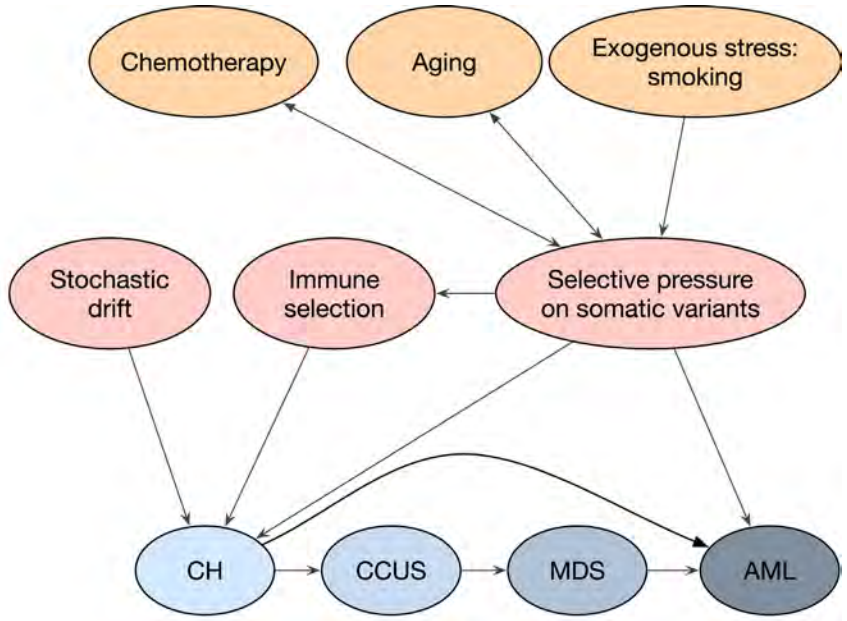
Circulating tumor DNA (ctDNA) is usually present at low levels and ctDNA fragments with mutations at very low levels

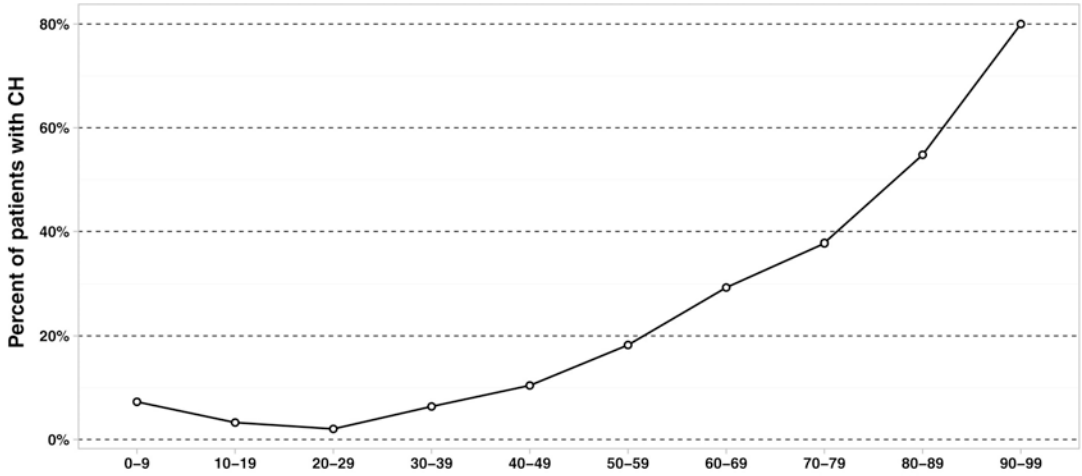


Cristiano et al. Nature. 2019;570:385

Clonal hematopoiesis (CH or CHIP) is major interpretive challenge in ctDNA testing

- Clonal hematopoiesis broadly describes the clonal expansion of blood cells with one or more mutations.
- Found in both healthy individuals and cancer patients.
- Associated with increasing age, therapy, and smoking.
- Confers increased risk of hematologic cancers, but also adversely impacts survival from solid tumors

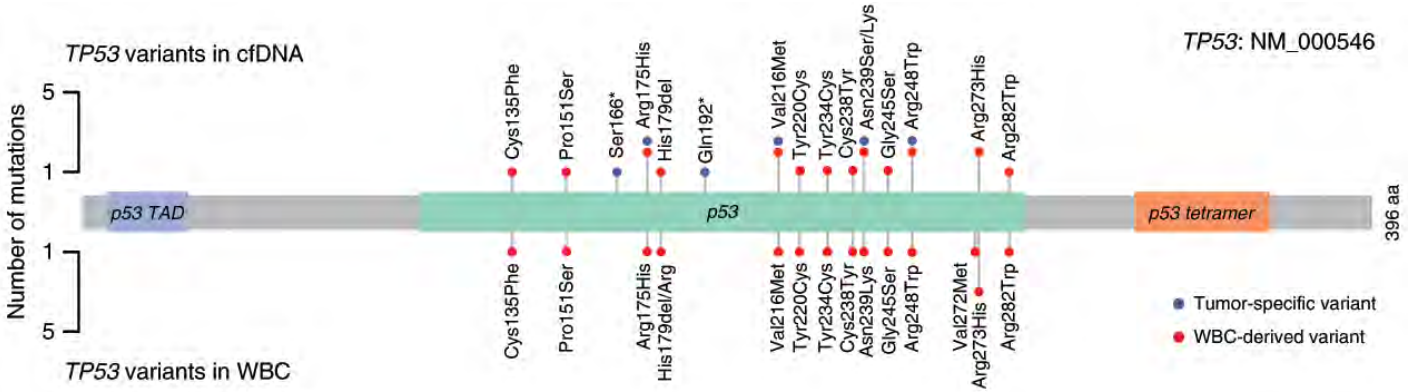




Bowman RL et al. Cell Stem Cell. 2018;22:157
Coombs CC et al. Cell Stem Cell. 2017;21:374

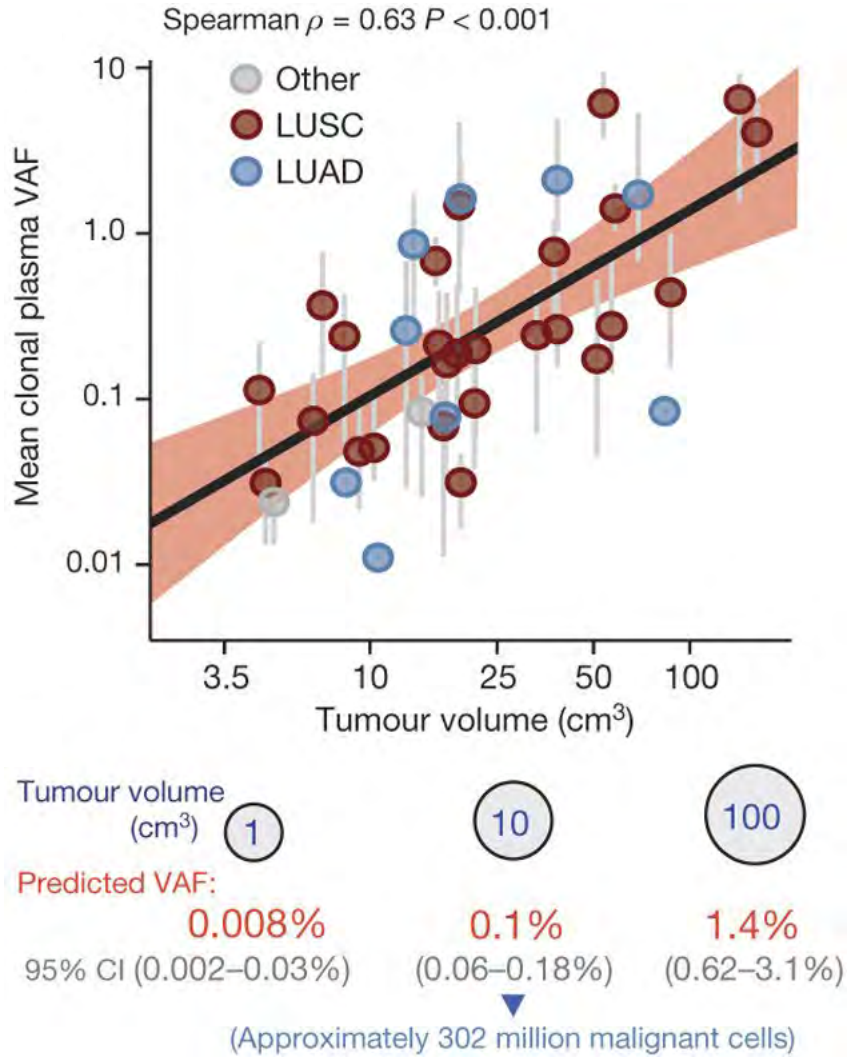
Clonal hematopoiesis (CH or CHIP) is major interpretive challenge in ctDNA testing

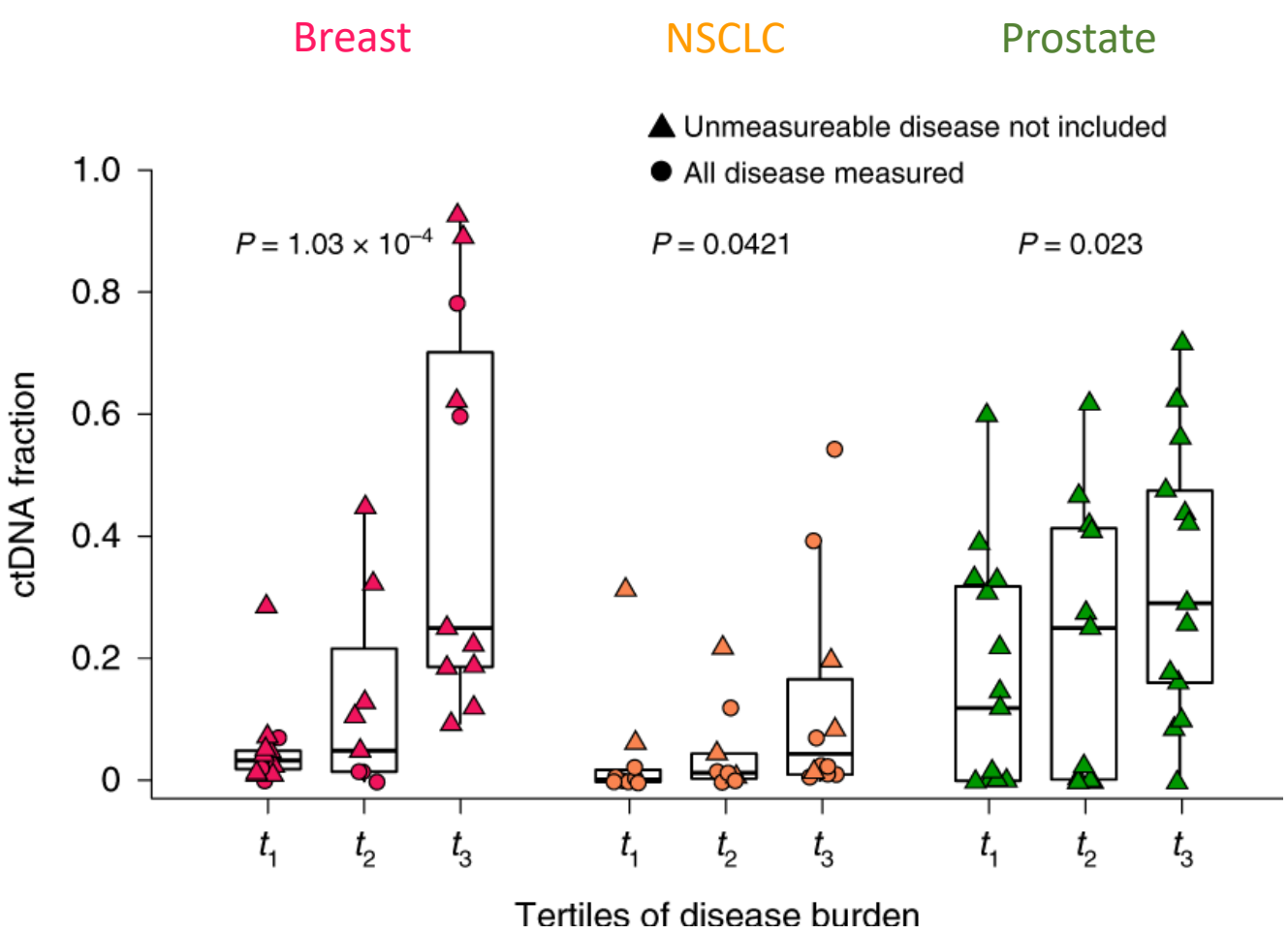
- Most commonly associated with recurrent somatic mutations in hematologic cancer genes.
- Significant challenge to interpret and report mutations in these genes since WBCs are not routinely sequenced.
- Somatic mutations are identified in WBCs at lower frequency in genes not implicated in hematologic cancers.



Coombs CC et al. Cell Stem Cell. 2017;21:374
Leal A et al. Nat Commun. 2020;11:525

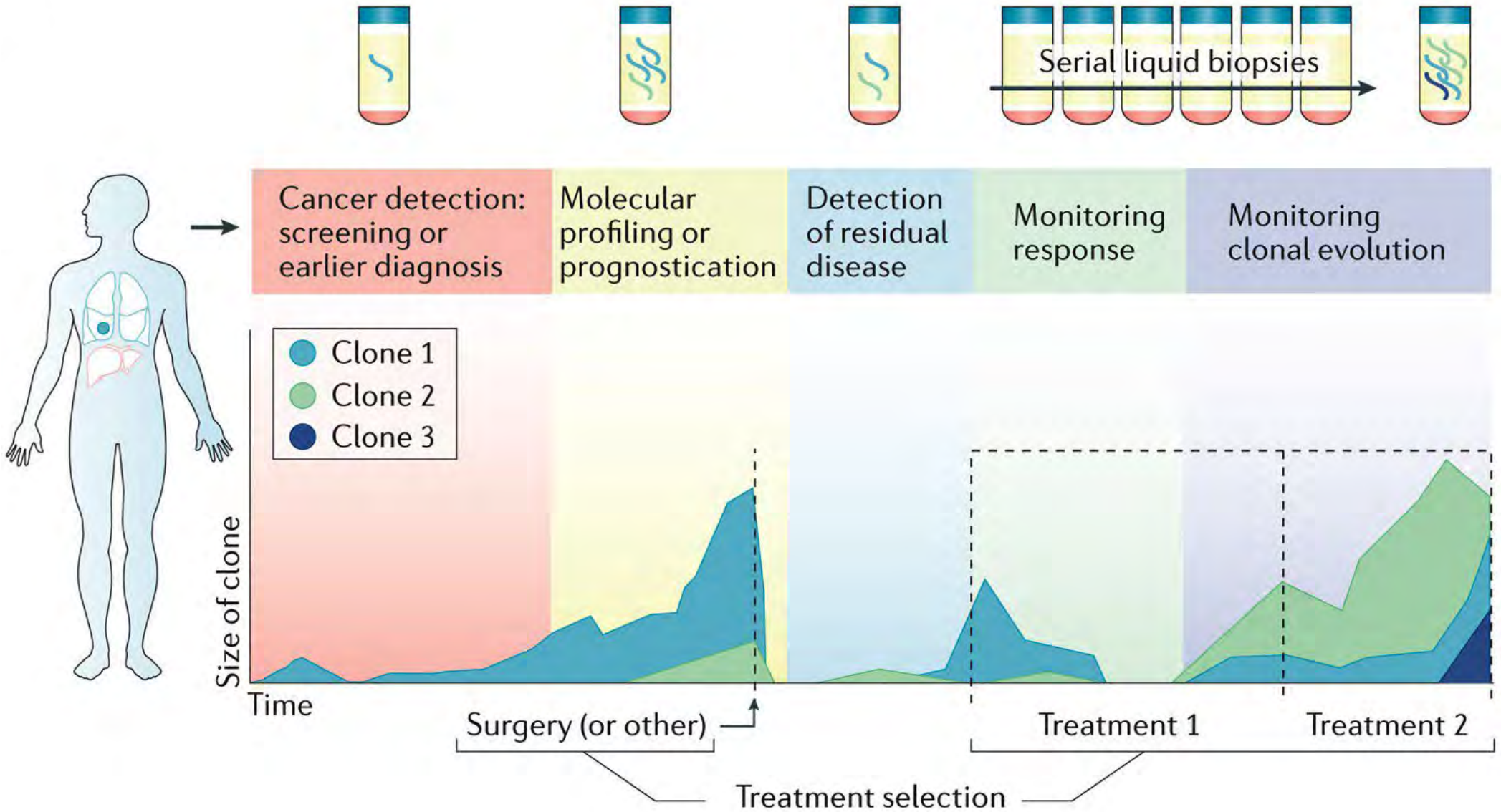
ctDNA demonstrates moderate correlation with tumor volume





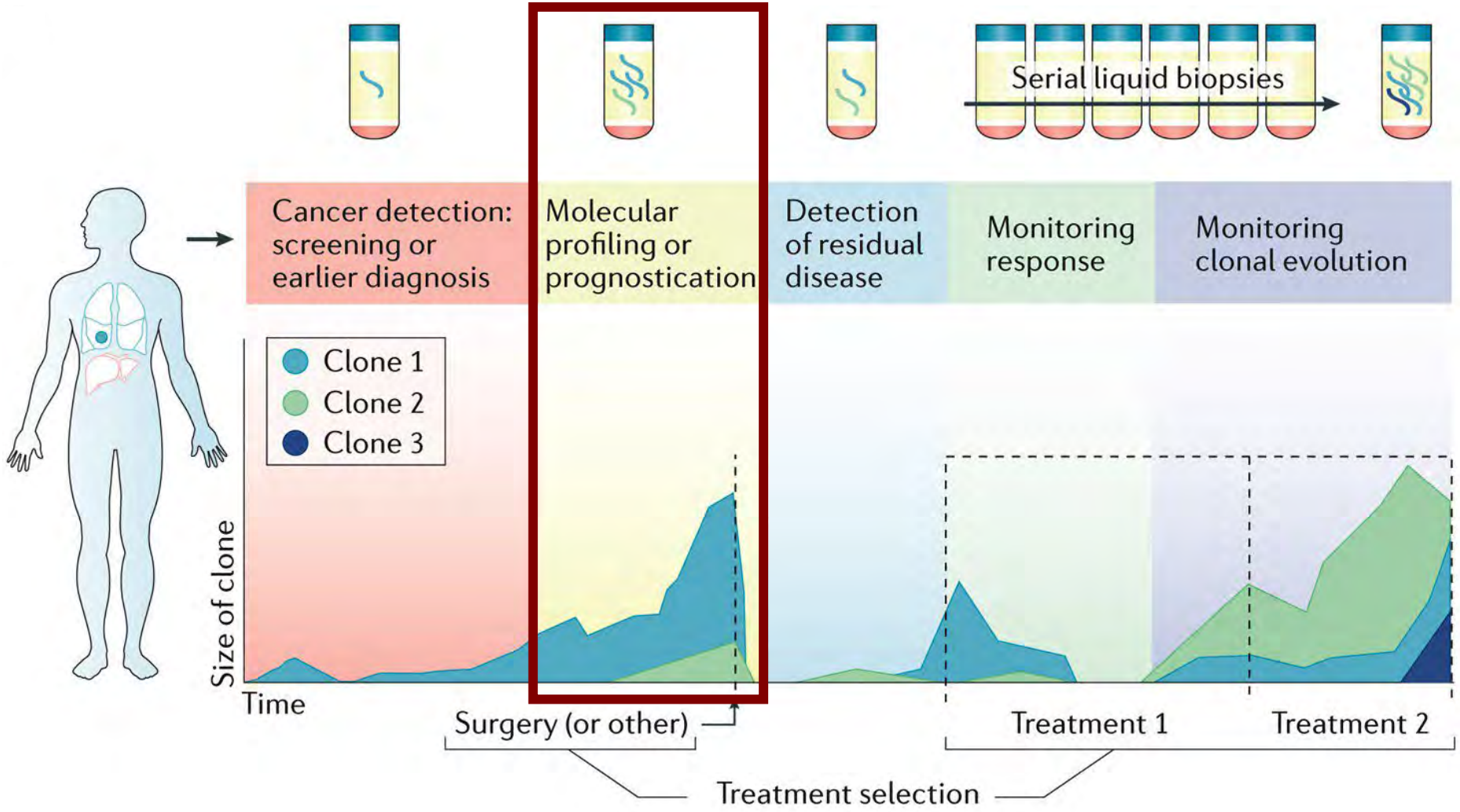
Abbosh C et al. Nature. 2017;545:446
Razavi P et al. Nat Med. 2019;25:1928

Potential applications of ctDNA analysis



Wan JCM et al. Nat Rev Cancer. 2017;17:223

Molecular profiling of advanced cancer is most frequent current clinical application of ctDNA testing



Wan JCM et al. Nat Rev Cancer. 2017;17:223

Comparison of ctDNA versus tumor tissue testing

Consideration	ctDNA Assay	Tissue Assay
Logistics	- Minimally invasive and low risk - Easier serial testing - More rapid turnaround time	- Often invasive with variable biopsy risks - Serial testing more difficult
Tumor Heterogeneity	- Theoretically may provide more complete representation of tumor heterogeneity	- Variable depending on sampling approach
Diagnosis	- Does not allow for pathologic diagnosis and staging	- Standard for pathologic diagnosis and staging
Clinical utility	- Evidence for utility for treatment selection in advanced cancer	- Substantial evidence for utility for treatment selection in multiple malignancies for early and advanced cancers

Example of ctDNA assay currently in clinical use – *PCR-based detection of EGFR mutations in NSCLC*

Assay: PCR test for qualitative detection of specific mutations in *EGFR*

Gene	Mutations	Drug
EGFR	Exon 19 deletions and L858R	erlotinib

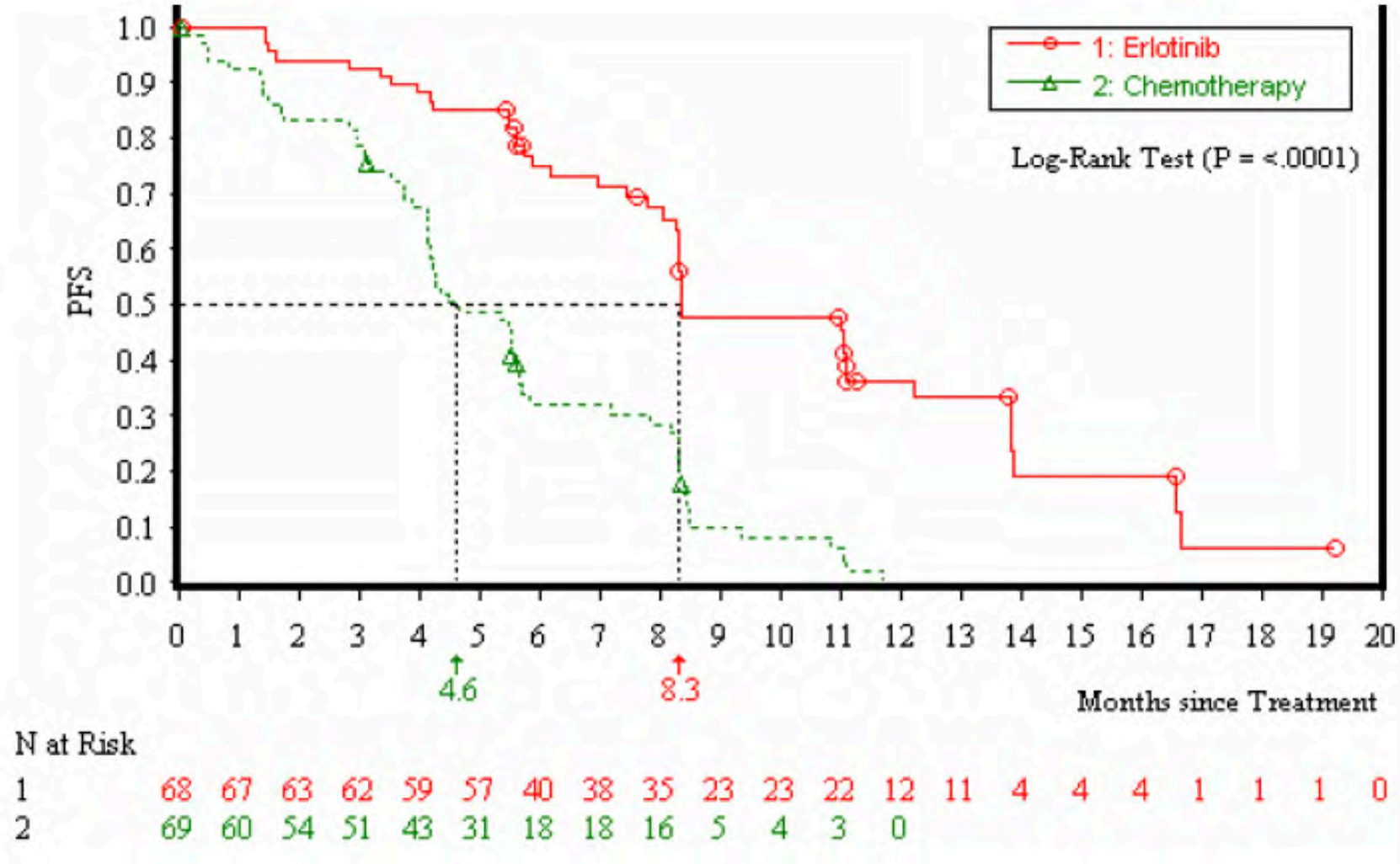
Population: Advanced non-small cell lung cancer (NSCLC) patients

Potential benefit: "Insofar as the test provides positive results, it may benefit patients who may be too ill or are otherwise unable to provide a tumor specimen for *EGFR* testing."

US FDA: Medical Devices: cobas EGFR Mutation Test v2 - P150047
https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P150047; accessed 2/14/22

PCR-based detection of *EGFR* mutations in NSCLC

Clinical utility – Improved PFS demonstrated



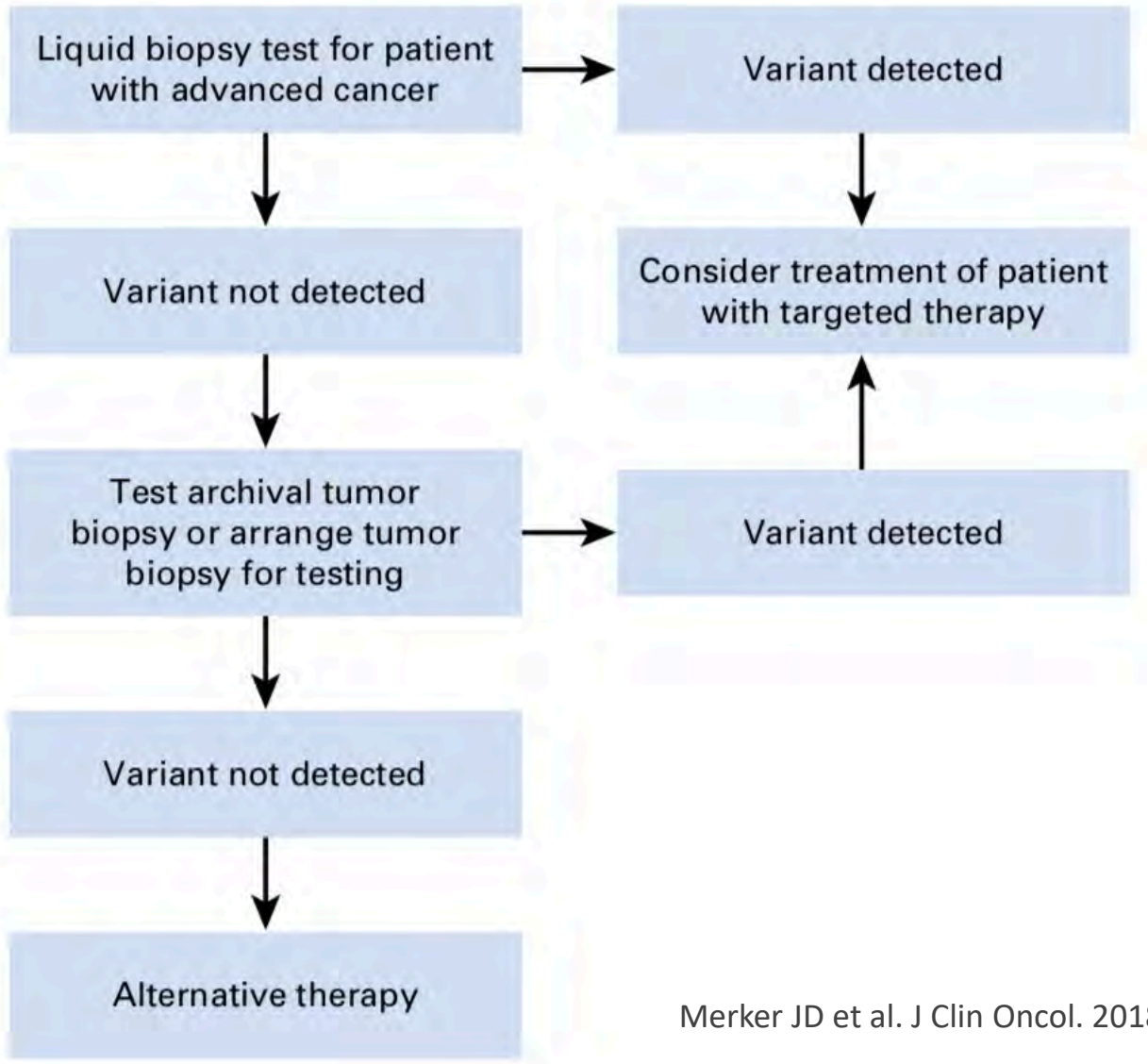
Improved progression-free survival (PFS) is observed in patients with an activating *EGFR* mutation detected by ctDNA analysis

US FDA: Medical Devices: cobas EGFR Mutation Test v2 - P150047
https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P150047; accessed 2/14/22

PCR-based detection of *EGFR* mutations in NSCLC

Patients with negative ctDNA results are reflexed to tissue testing

~30% of advanced lung cancer patients with an *EGFR* activating mutation in the tumor will have a negative ctDNA test result



Merker JD et al. J Clin Oncol. 2018;36:1631

Case – 60 year-old male w/ NSCLC

Metastatic lung adenocarcinoma

- 60 year-old male with 50+ pack year smoking history presented to the ED with worsening, atraumatic back pain
- X-rays concerning for malignancy
- MRI confirmed multiple metastases involving ribs and spine
- Bone biopsy confirmed metastatic carcinoma, most consistent with lung adenocarcinoma

Case – 60 year-old male w/ NSCLC

Molecular testing at diagnosis

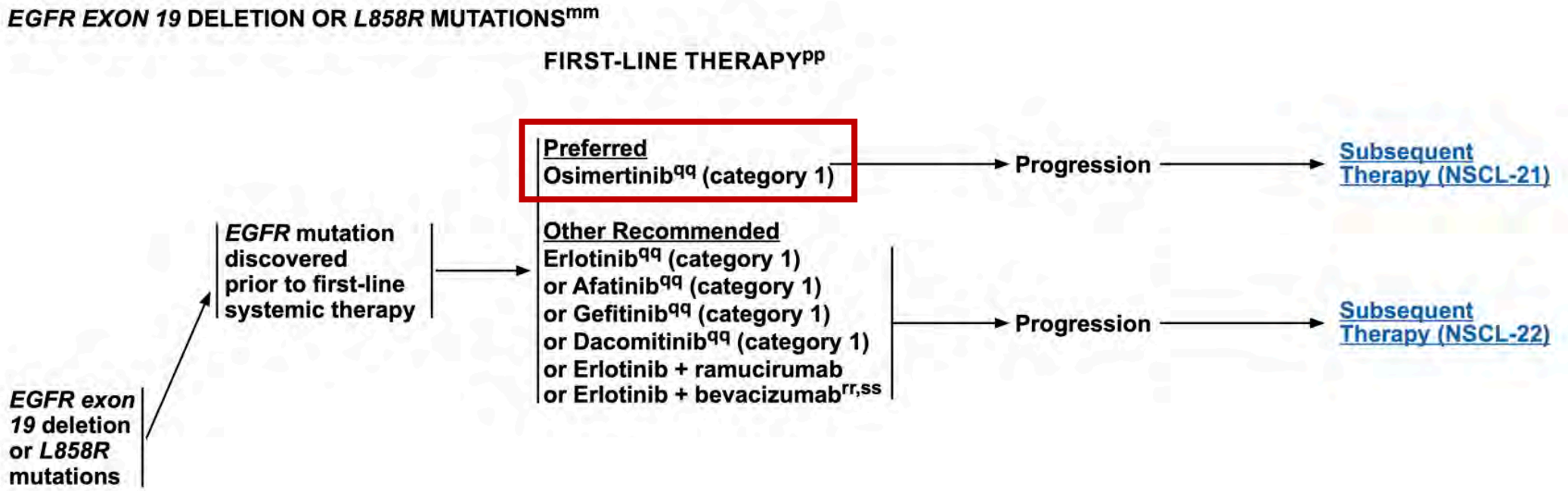
- PD-L1 – high expression (TPS > 50%, intensity 1+)
- NGS-based testing on bone biopsy unsuccessful:

QUANTITY / QUALITY NOT SUFFICIENT

The requested assay could not be completed due to insufficient quantity and/or quality of nucleic acid.

- NGS-based testing on plasma demonstrated one targetable alteration:
  - *EGFR* p.E746_A750del (canonical exon 19 deletion)
  - Oncogenic, activating EGFR mutation

Case – 60 year-old male w/ NSCLC



- Consistent with NCCN guidelines, patient started on Osimertinib
- Stable disease for ~20 months
- Progressive disease with new skull and potential cerebral metastasis

NCCN Guidelines – Non-Small Cell Lung Cancer Version 1.2022

Case – 60 year-old male w/ NSCLC

Molecular testing at disease progression

- NGS-based testing on plasma demonstrated one targetable alteration:
  - *EGFR* p.E746_A750del (canonical exon 19 deletion) – observed at diagnosis
  - *EGFR* p.C797S – associated with resistance to Osimertinib
- MTB review found that preclinical studies and case reports suggest that cases with the p.C797S resistance mutation may respond to reversible EGFR inhibitors, such as erlotinib
- Patient started on erlotinib with initial response

Outline

I. Background on genetics and genomics
II. Cancer biomarkers
III. NGS-based solid tumor testing
IV. Liquid biopsy
V. Conclusions and future directions

Conclusions

1. Molecular oncology biomarkers are being increasingly used to assist with diagnosis, prognostication and therapy selection.
2. Resources to assist with biomarker and test selection for patients with cancer include the NCCN Biomarkers Compendium, NCCN Guidelines other evidence-based guidelines.
3. Expanded NGS-based assay for testing solid tumors can detect genetic variants in several hundred genes along with genomic features.
4. Liquid biopsy refers to minimally invasive tests done on blood or other body fluids that attempt to provide similar genetic information to tissue-based testing.

Future Directions – 1

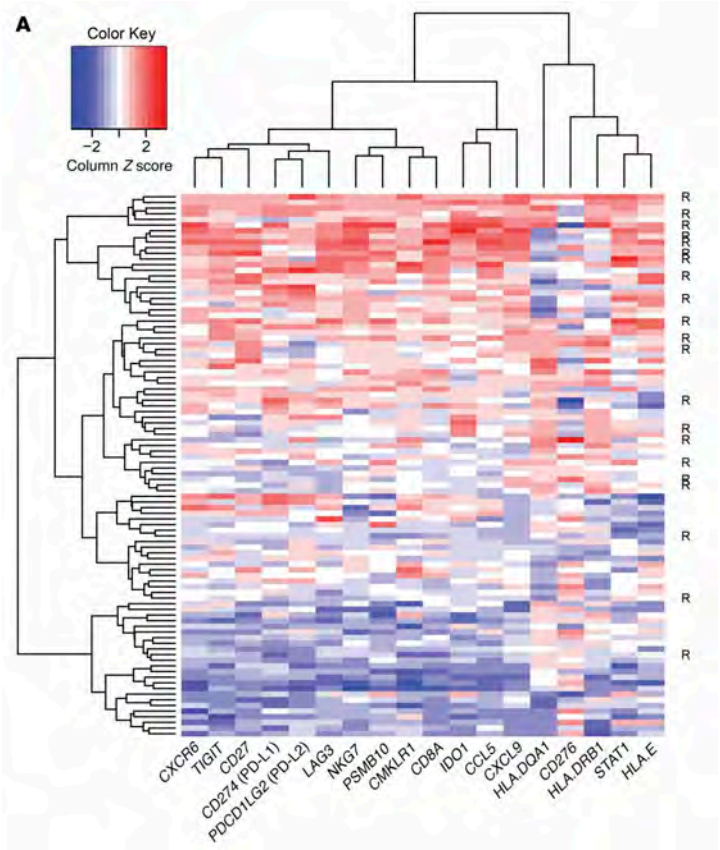
Increased use of paired tumor/normal testing to assist with detection of germline (heritable) cancer predisposition mutations

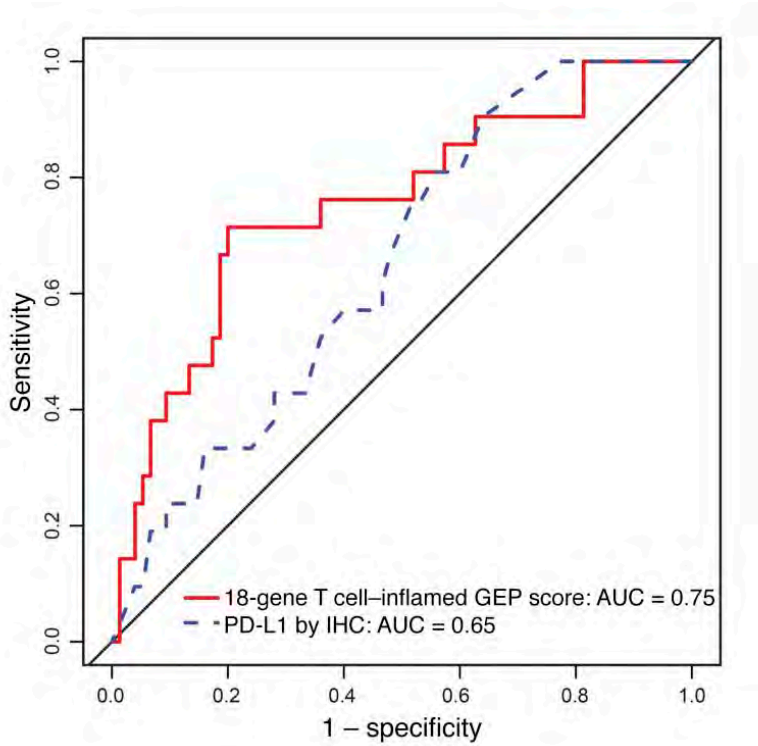
Cancer type	Prevalence of actionable pathogenic variants
Ovarian	15%
Breast	8%
Prostate (advanced)	8%
Pediatric	6%
Adults	4%

Kurian AW et al. J Clin Oncol. 2019 May 20;37(15):1305
Seifert BA et al. Clin Cancer Res. 2016;22:4087
Zhang J et al. N Engl J Med 2015;373:2336

Future Directions – 2

Expanded use genomics and related data to guide therapy selection (e.g., gene expression signatures to predict response to checkpoint inhibitors)

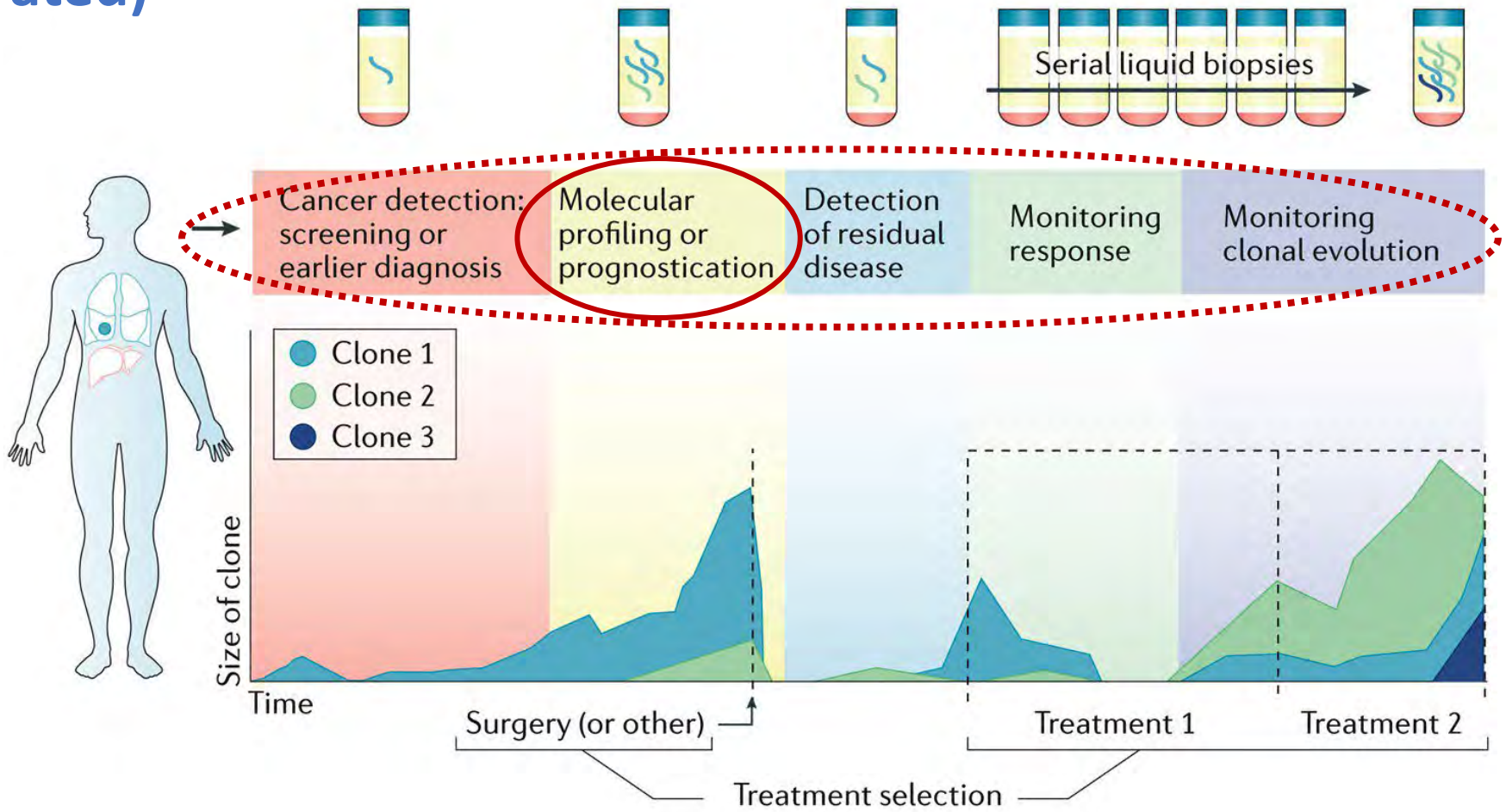




Ayers M et al. J Clin Invest. 2017;127:2930

Future Directions – 3

Expanded application of liquid biopsy technologies (as clinical utility is demonstrated)



Wan JCM et al. Nat Rev Cancer. 2017;17:223

Future Directions – Key need

Electronic health record and related tools to facilitate use of complex genetic and related data

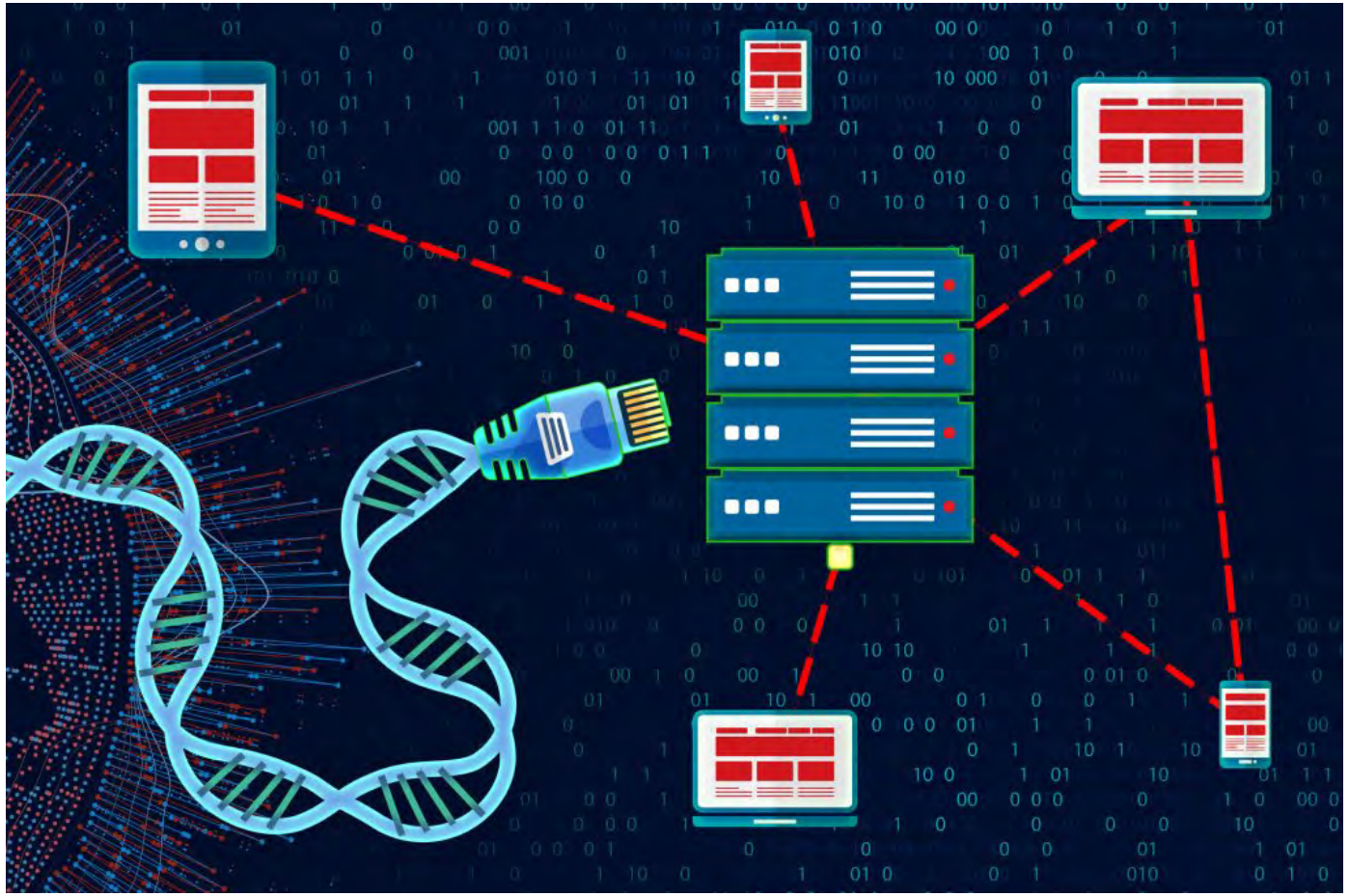


Image courtesy of National Human Genome Research Institute

UNC LINEBERGER COMPREHENSIVE CANCER CENTER

UNC CANCER CARE

Thank you!

UNC SCHOOL OF MEDICINE Pathology and Lab Medicine

UNC SCHOOL OF MEDICINE Genetics