UNC | LINEBERGER COMPREHENSIVE CANCER CENTER

UNC CANCER CARE

Optimizing value in molecular testing

North Carolina Oncology Association Annual Conference

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September 10, 2022

Disclosure of Conflicts of Interest

Jason D. Merker, MD, PhD has the following financial relationships to disclose:

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

Off-Label Use of Drugs

During this presentation I discuss the use of trametinib in combination with bevacizumab for the treatment of glioblastoma.

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.

1/2 of my effort is primarily clinical:

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

1/2 of my effort is focused on translational cancer research:

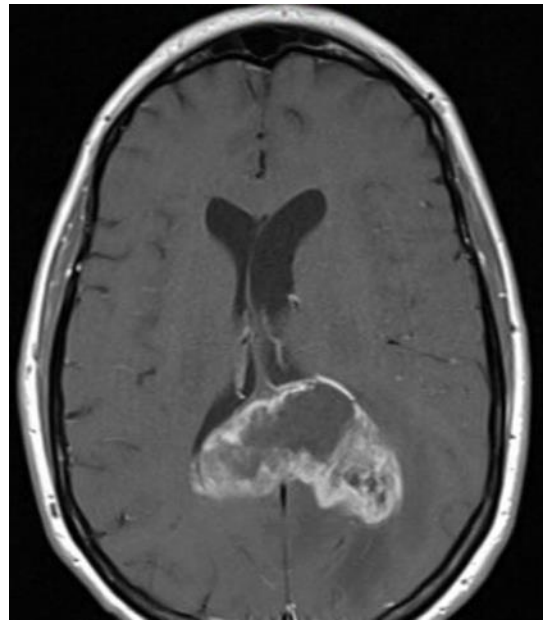
1. Correlative genomic testing for cancer clinical trials
2. New technology development

Case – 60 year-old male w/ GBM

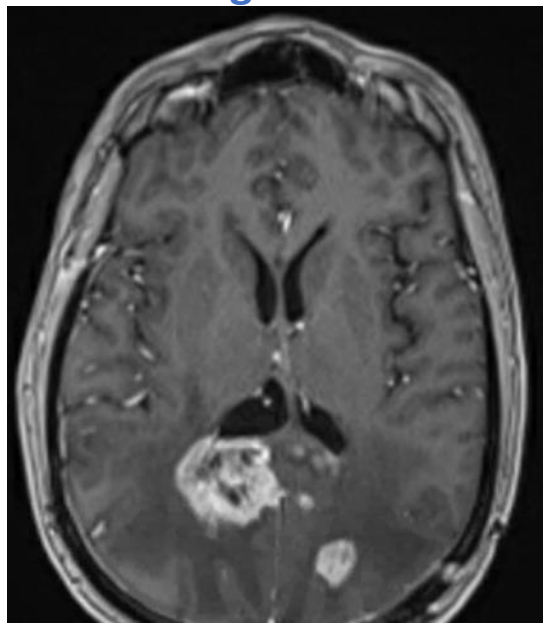
Glioblastoma (WHO grade IV), *IDH* wild-type

- S/P resection, radiation therapy with concurrent temozolomide
- Follow-up MRI after C1 of maintenance temozolomide revealed progression
- Patient experienced significant drop in performance status

Diagnosis



Progression



Genetic Testing – 60 year-old male w/ GBM

	Targeted testing	Clinical sequencing	Clinical trial sequencing
SQSTM1-NTRK2	**Not tested**	**(-)**	**(+)**
***NF1* (2 inactivating mutations)**	**Not tested**	**(+)**	**Not tested**
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***TERT* p.C228T**	**(+)**	**(+)**	**(+)**
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Clinical Questions

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

Outline

I. Genetic considerations for molecular oncology testing
II. Expanded NGS-based tissue assays
III. Liquid biopsy assays
IV. Molecular oncology assay selection

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Somatic vs. germline mutations

Germline DNA variation

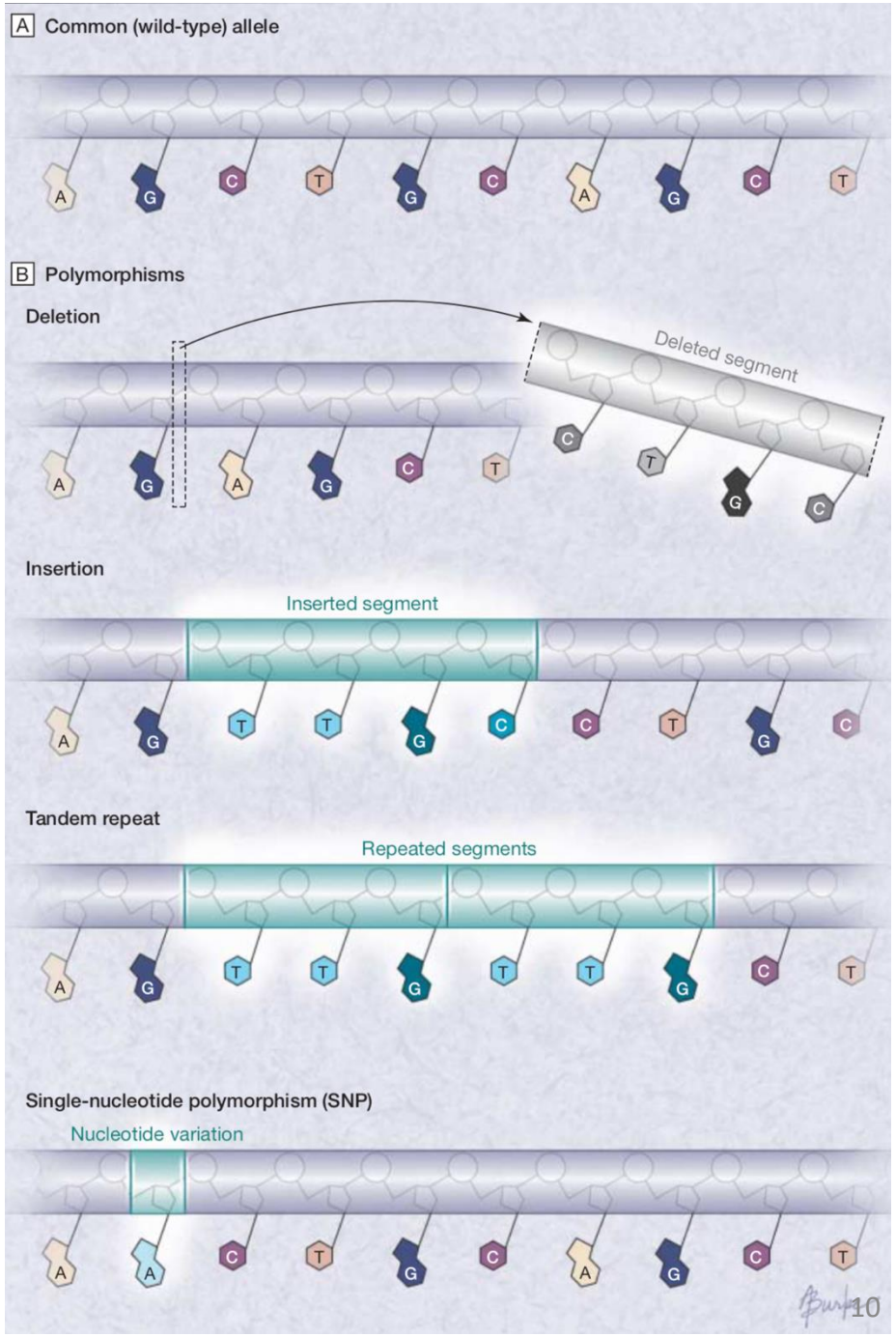
- Heritable genetic changes that are generally found in all cells in the body
- Example: *BRCA1/2* mutations in patients with heritable breast and ovarian cancer syndrome
- Most cancer cases are not associated with heritable cancer predisposition mutations

Somatic DNA mutation

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

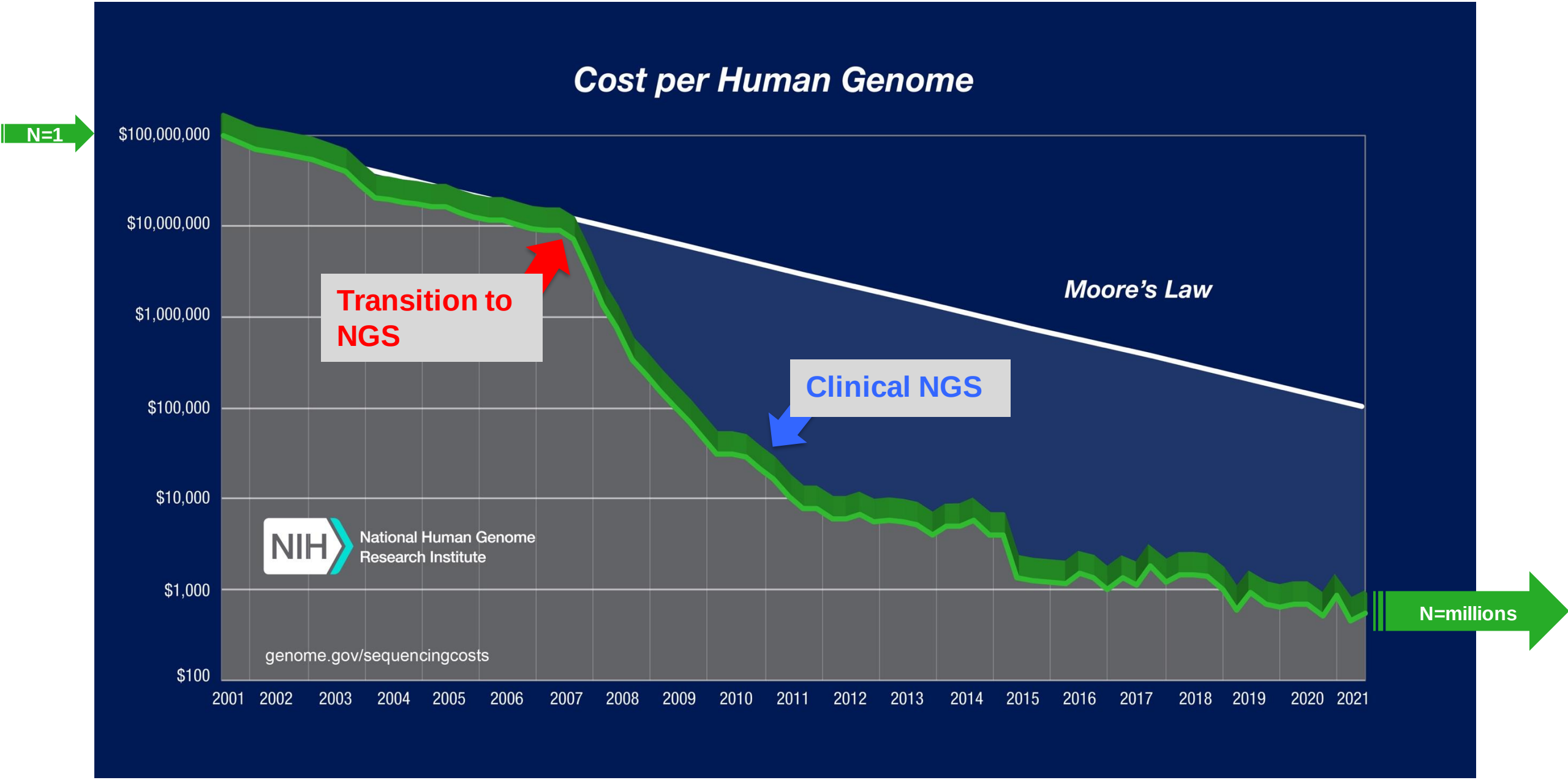
DNA variation

- Single-nucleotide variation
- Insertion
- Deletion
- Amplifications or losses
- Gene fusions or rearrangements
- Repeat expansion
- Genomic features (MSI, TMB, LOH)

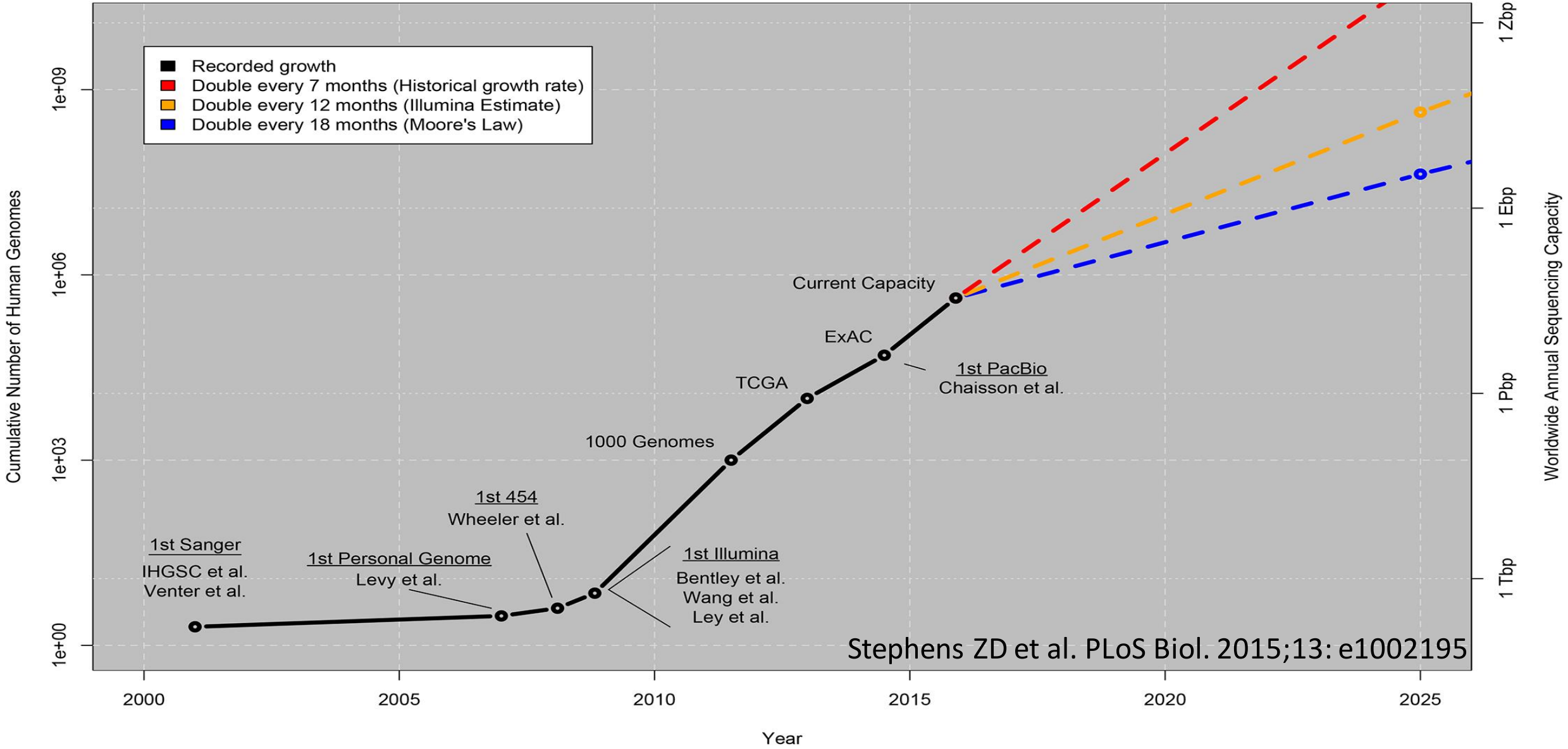


Attia J et al. JAMA. 2009;301:74

https://wellcomecollection.org/articles/WcvK4CsAANQR59Up; accessed 8/11/22
Photography by Ben Gilbert and Thomas Farnetti for Wellcome Collection


Cost per Human Genome
N=1
$100,000,000
$10,000,000
$1,000,000
$100,000
$10,000
$1,000
$100
Transition to NGS
Moore's Law
Clinical NGS
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



There are a lot of clinical somatic cancer tests available

In U.S., there are approximately:

- 1,765 clinical somatic tests
- 131 clinical laboratories

https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=target_somatic%5BPROP%5D; accessed 8/11/22

Key trends in clinical molecular oncology testing

1. Move to broader assays that can detect multiple variant types and genomic features
2. Increased use of smaller FFPE specimens
3. Increased use of circulating biomarkers requiring more sensitive assays
4. Need for more rapid turnaround time

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NGS tissue-based solid tumor panels

- Most assays designed to be applied across multiple tumor types cover ~300-600 genes
- Genes selected to include FDA-approved therapies and professional guidelines (NCCN, ASCO, ESMO)
- Turnaround time of ~2 weeks from receipt of tissue
- Most assays only test tumor tissue

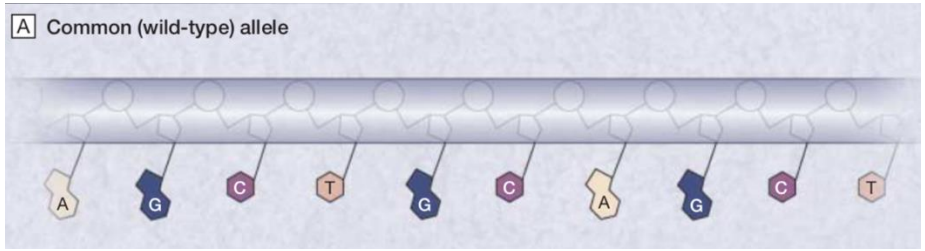
Variant types detected by tissue NGS panels

Vast majority of assays detect:

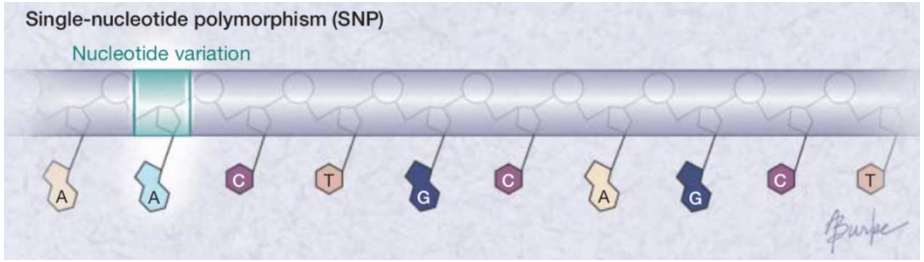
- Single-nucleotide variants (SNVs)
- Small insertions and deletions (<50 bp)

Many assays detect (but often only in a subset of genes and with variable performance):

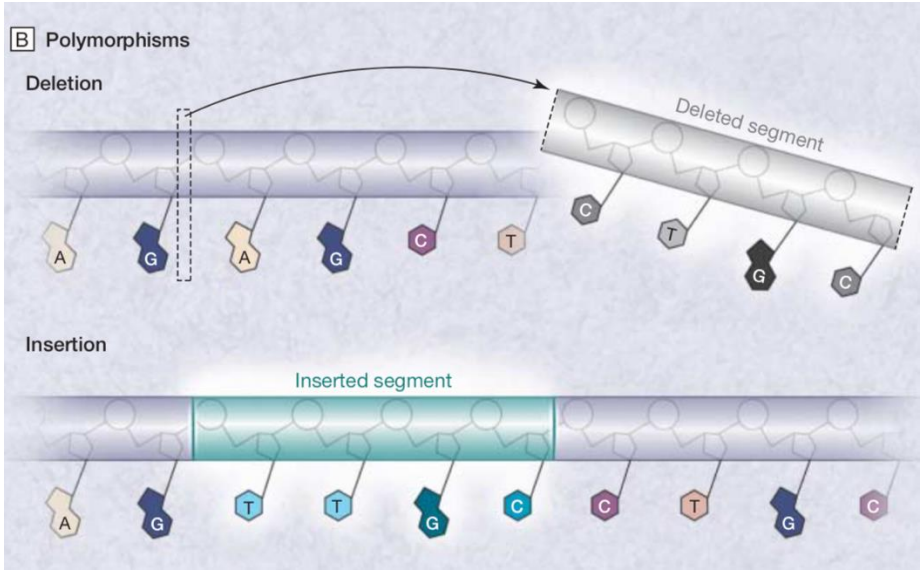
- Amplifications (>6-8 copies) or losses (biallelic)
- Gene rearrangements/fusions



Wild-type (normal)



SNV



Deletion

Insertion

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

Genomic features often detected by tissue 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 of an expanded NGS-based tissue 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								

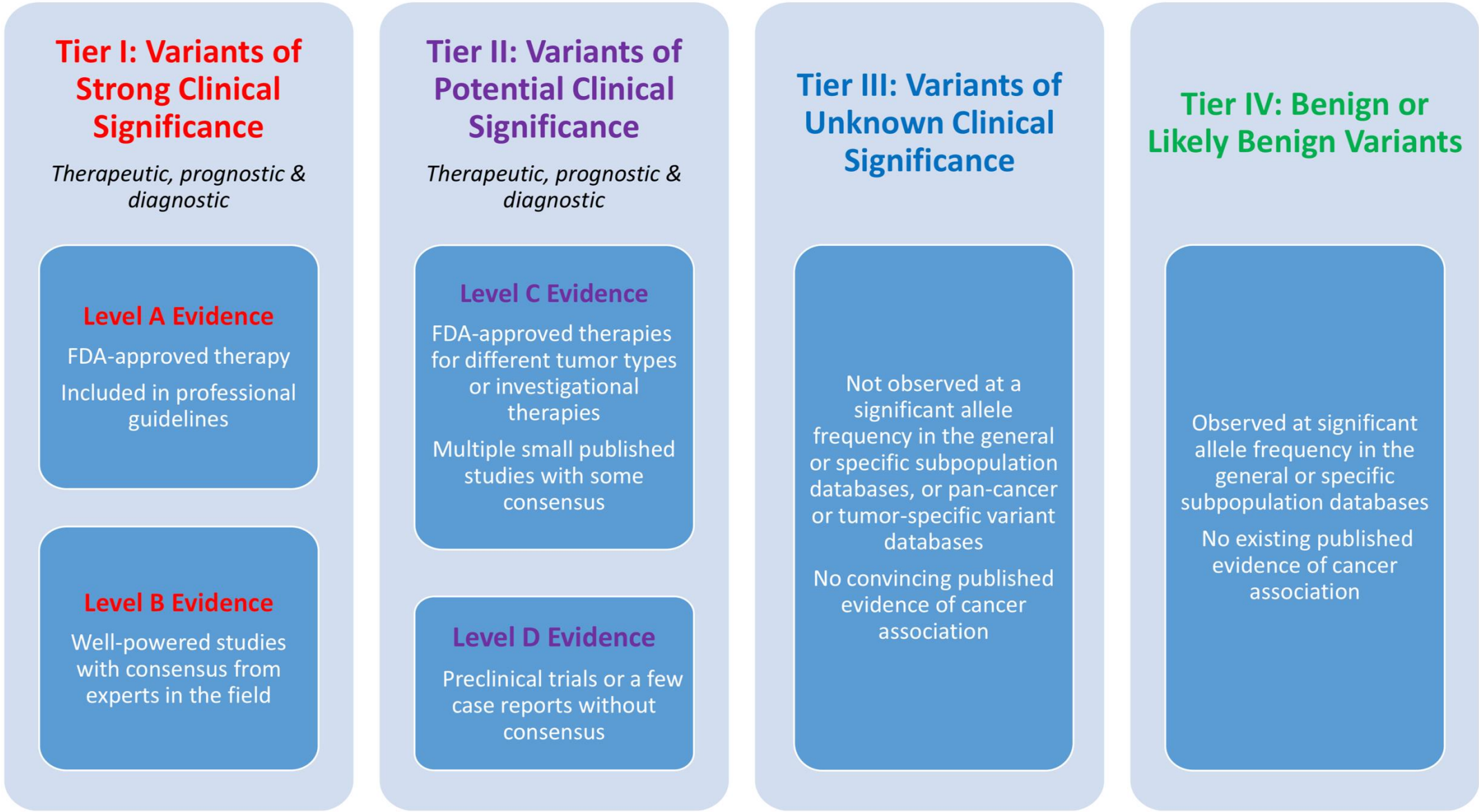
Tumor mutational burden (TMB)

Microsatellite instability (MSI)

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

https://assets.ctfassets.net/w98cd481qyp0/YqqKHaqQmFeqc5ueQk48w/c35460768c3a76ef738dcf88f8219524/F1CDx_Tech_Specs_072021.pdf accessed: 8/11/22

Somatic variant classification



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

Case – 60 year-old male w/ GBM

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

- S/P resection, radiation therapy with concurrent temozolomide
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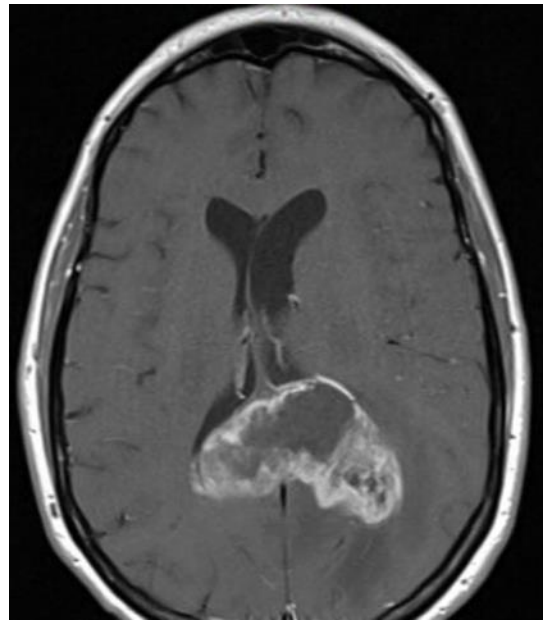
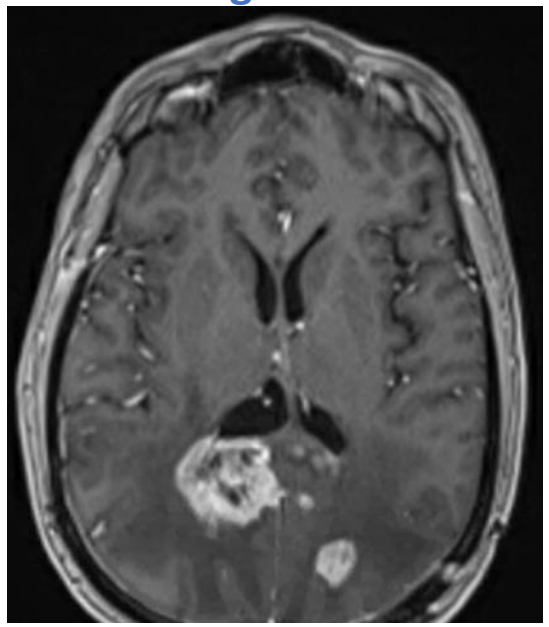


Progression



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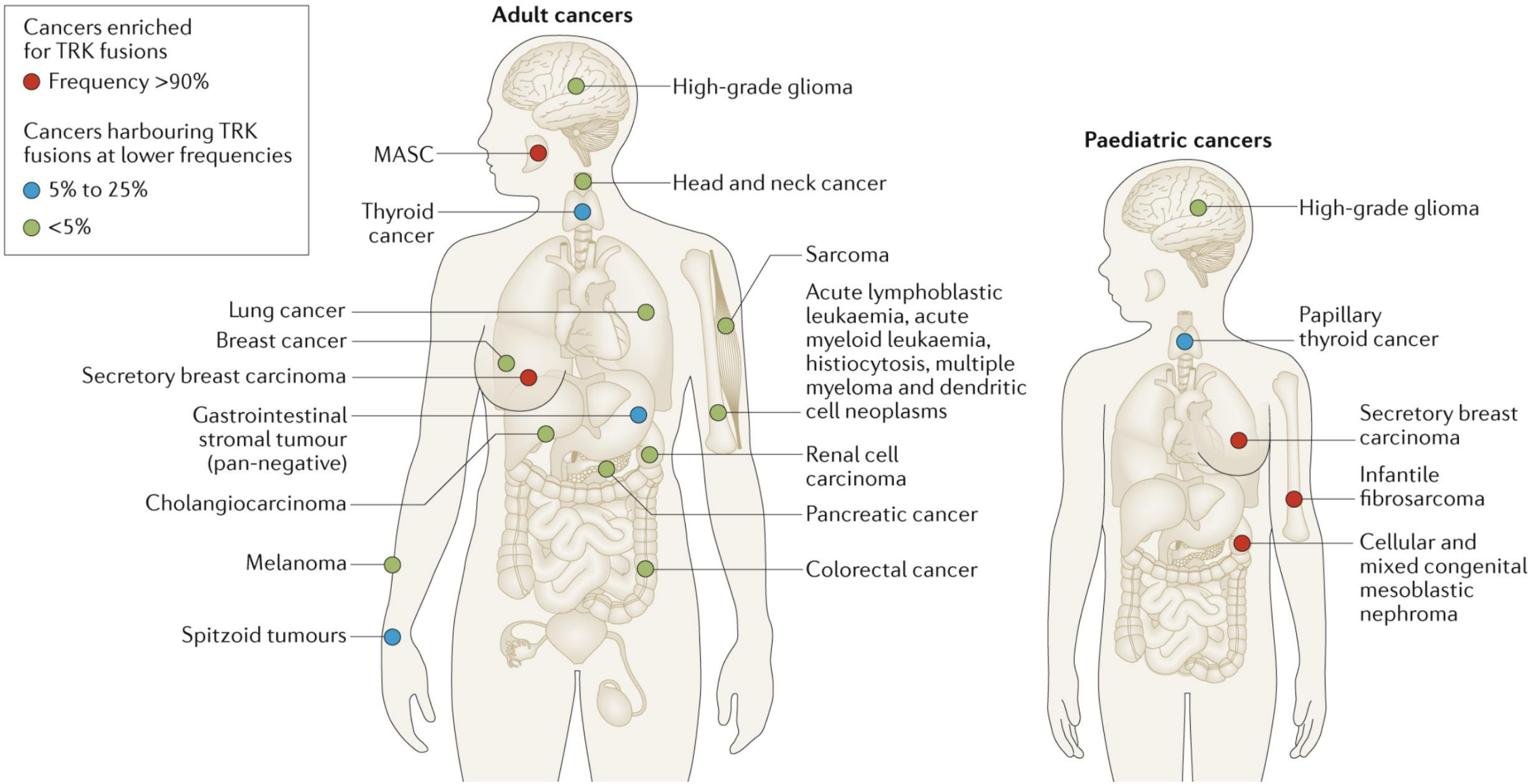
Clinical Questions

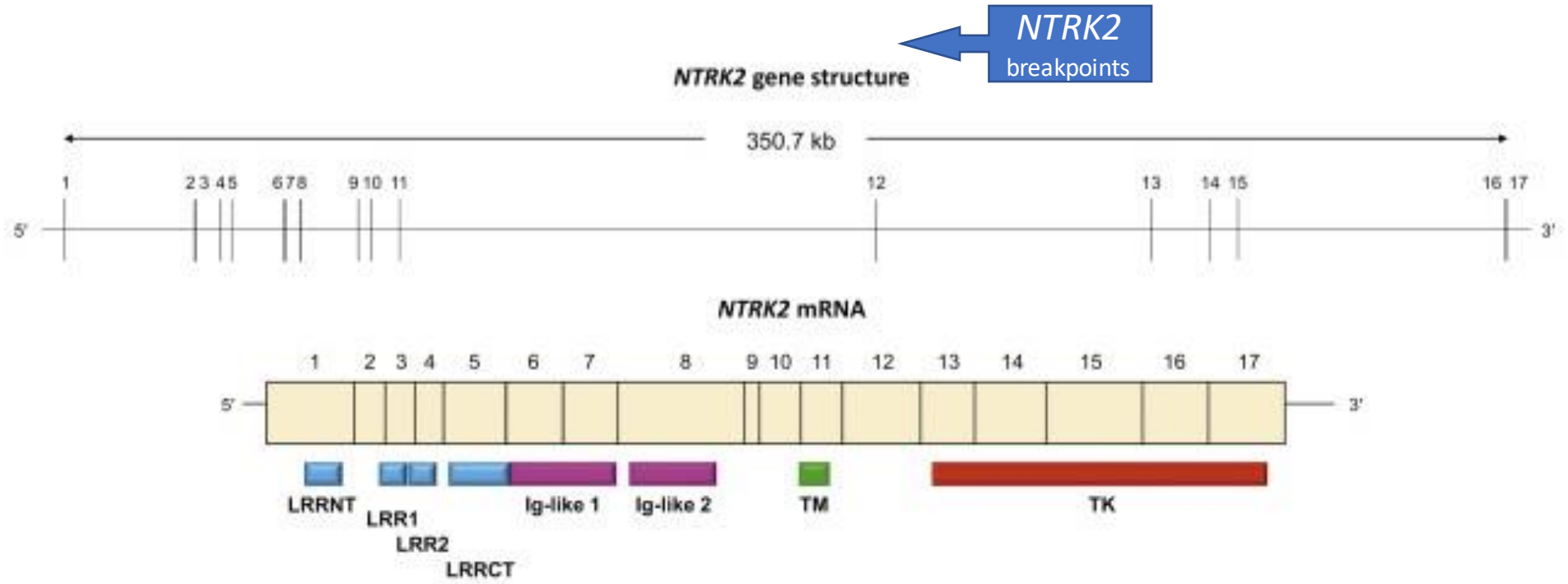
1. Why are there apparently discrepant *NTRK2* fusion results?
2. Which assay is correct?

Frequency of *NTRK* fusions in adult and pediatric tumors



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

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

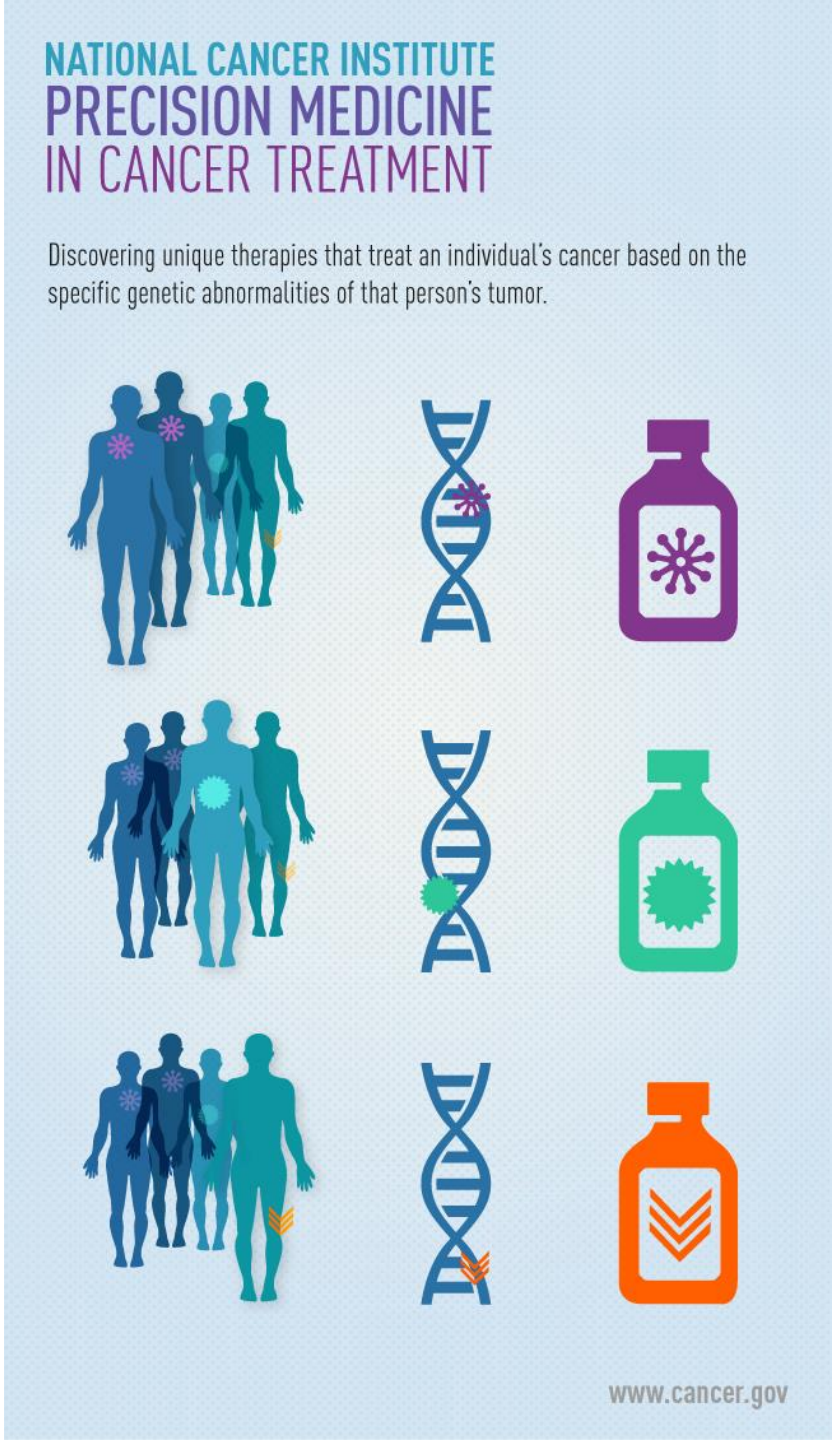
Measure of Agreement	% Agreement (N)	95% CI [(a)]
PPA	70.0% (14/20)	45.7%, 88.1%
NPA	100.0% (4/4)	39.8%, 100.0%
OPA	75.0% (18/24)	53.3%, 90.2%

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

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

Case – 60 year-old male w/ GBM

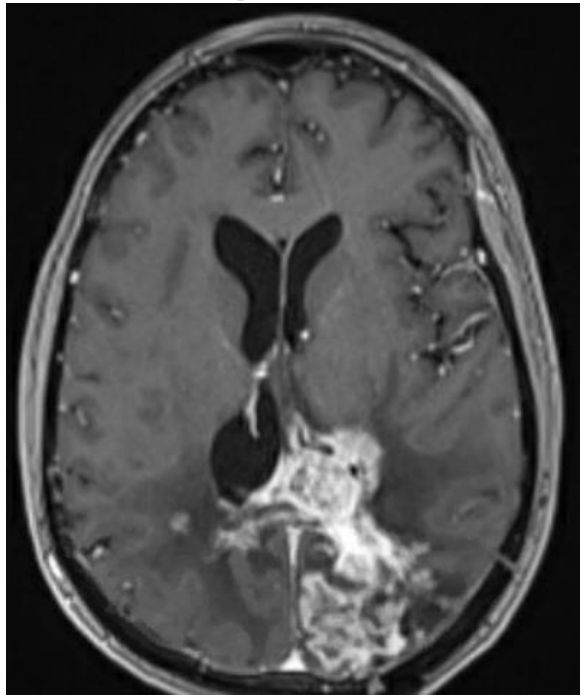
- Patient started treatment with larotrectinib (Trk inhibitor) on the NCI-MATCH clinical trial
- Significant response to therapy with stable disease
- Resumption of normal activities



Case – 60 year-old male w/ GBM

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

Progression



QUANTITY / QUALITY NOT SUFFICIENT

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

Molecular Tumor Board – 60 year-old male 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

William Kim, MD
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Pharmacy

Douglas Kirk, BA
LCCC Coordinator

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Cancer Genetics

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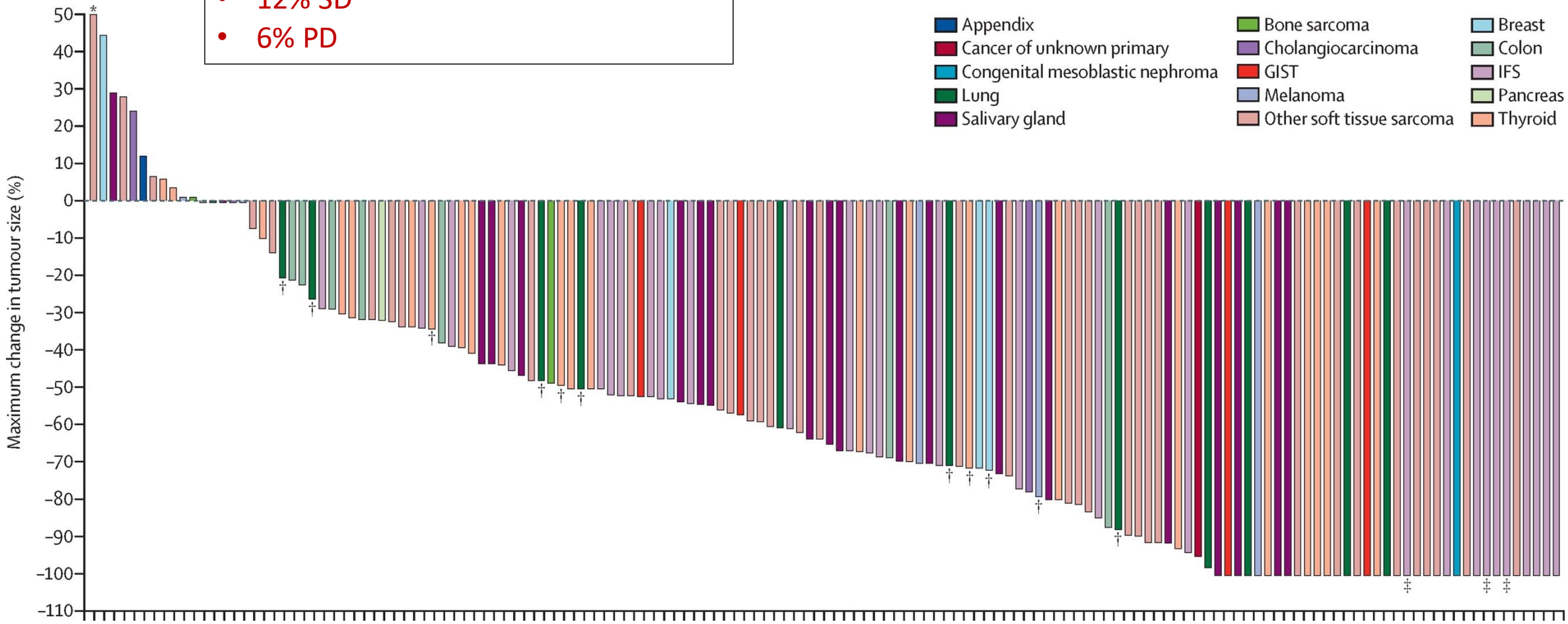
Lori Ramkissoon, PhD
Molecular & Cytogenetics

Jaime Richardson, BA, RN, BSN
MTB Coordinator

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

Key challenges with expanded NGS-based tissue assays

1. Variable performance in detection of amplification, losses, and gene fusions.
2. Genomic features may not be comparable across different panels (although harmonization efforts are underway).
3. Assay failure rate may be higher in smaller formalin specimens.
4. Turnaround time may not be optimal

Outline

I. Genetic considerations for molecular oncology testing

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Liquid biopsy definition

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

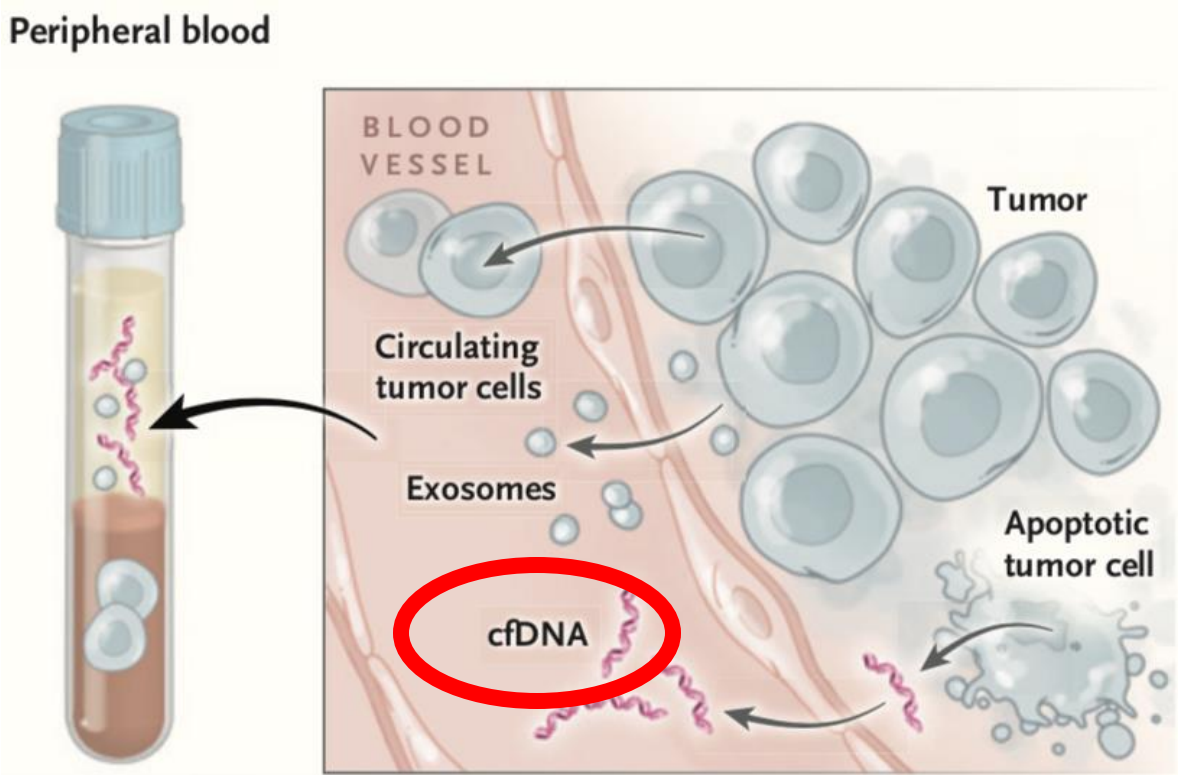
Circulating tumour cells in cancer patients: challenges and perspectives

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

Trends Mol Med. 2010;16:398

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

- cfDNA refers to DNA fragments in the plasma, 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

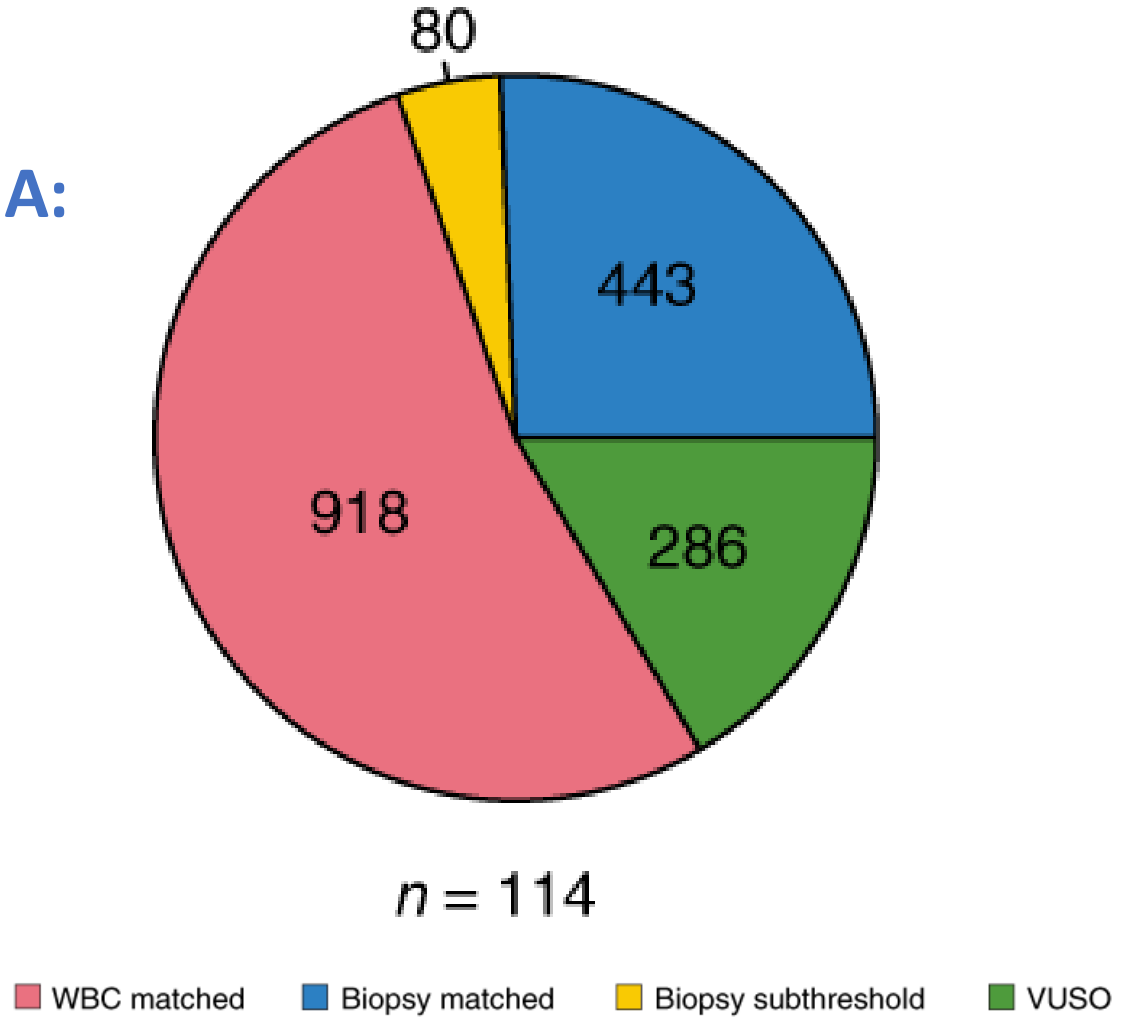


Corcoran RB and Chabner BA. N Engl J Med. 2018;379:1754

Most cfDNA from advanced cancer patients is from white blood cells

Source of somatic mutations in cfDNA:

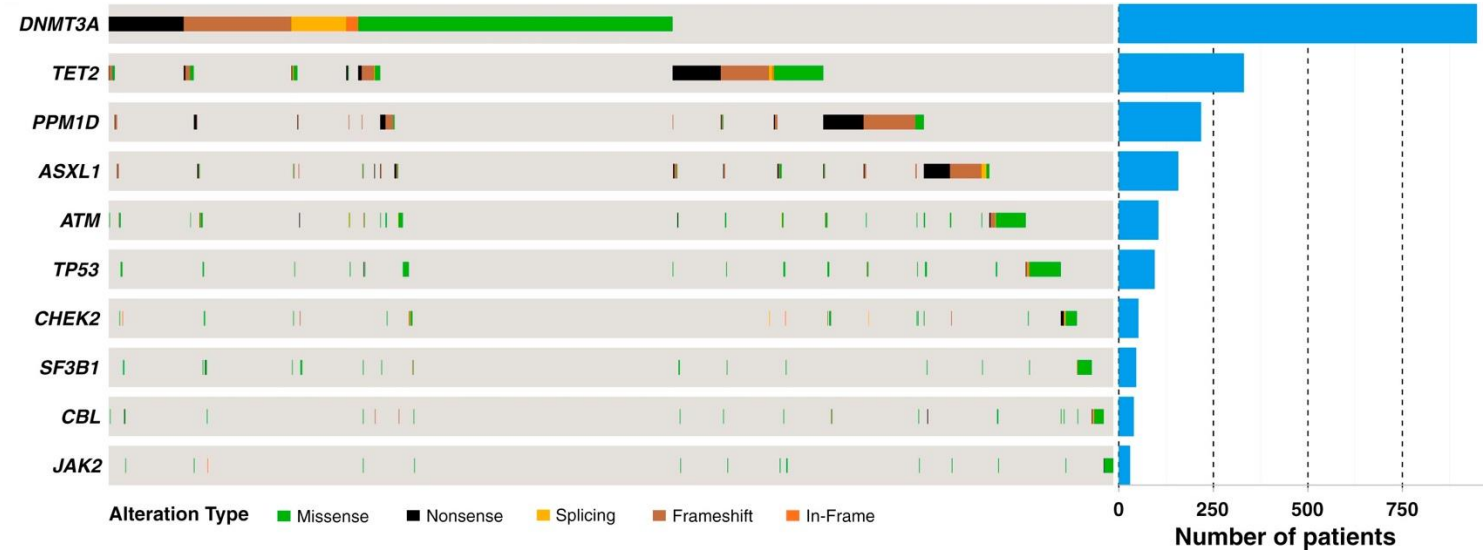
- 53% WBCs
- 30% tumor
- 17% unknown

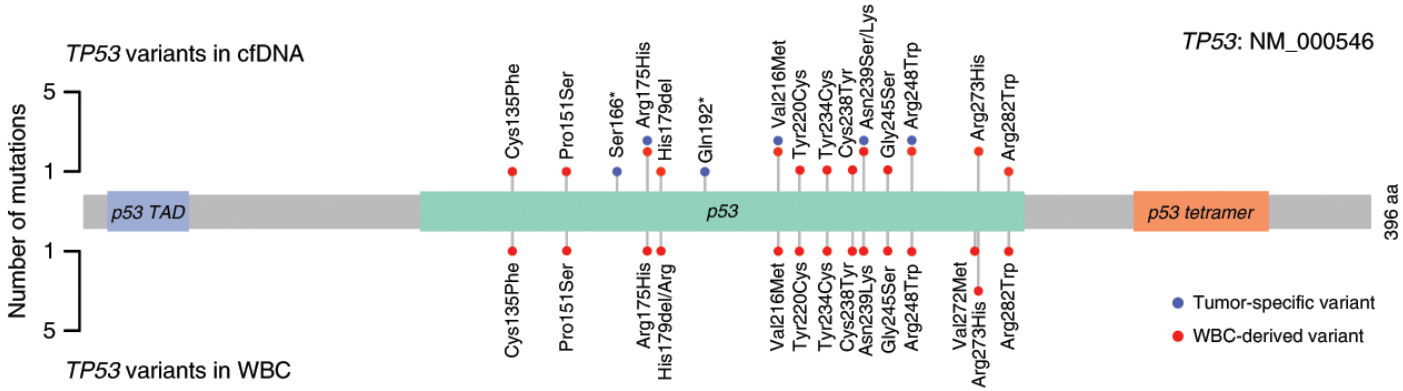


Razavi P et al. Nat Med. 2019;25:1928

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

- Clonal hematopoiesis broadly describes the expansion of blood cells with one or more mutations in genes associated with hematologic cancers.
- Significant challenge to interpret and report mutations in these genes since WBCs are not routinely sequenced.



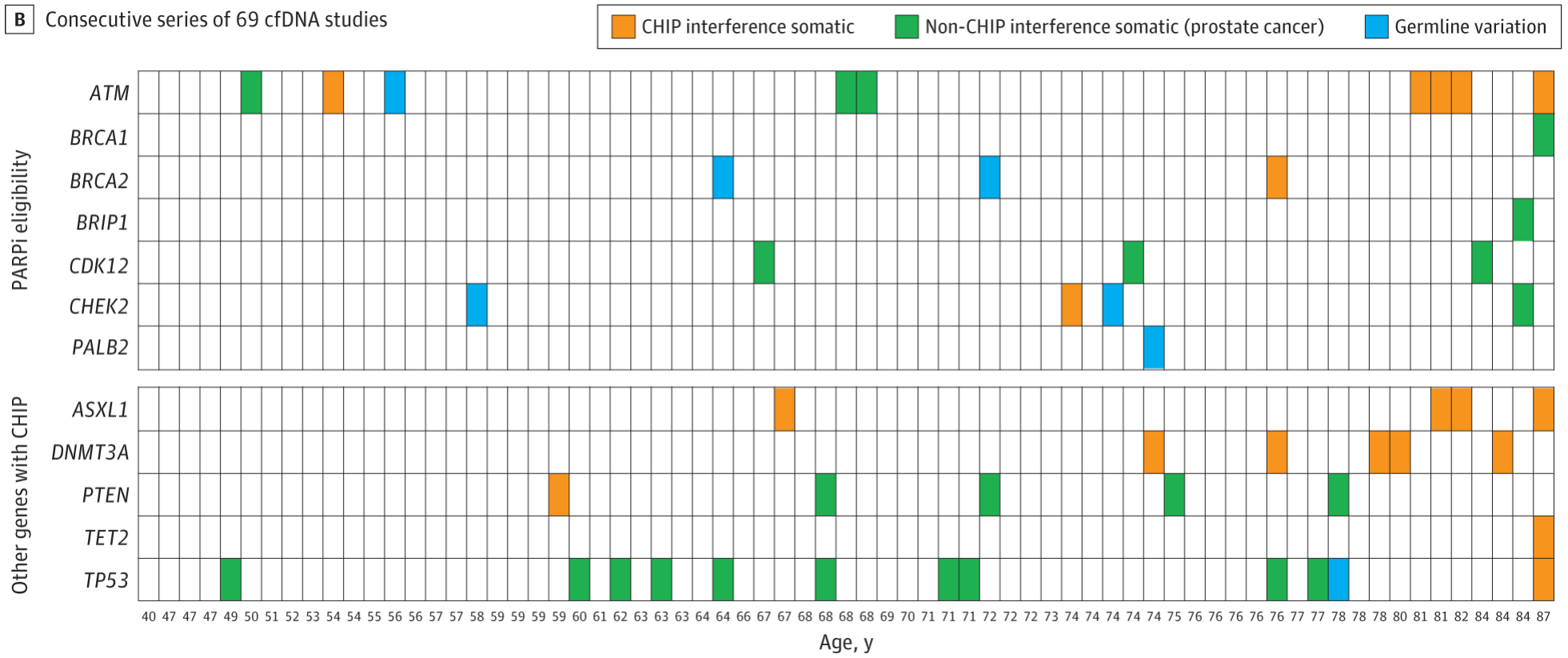


Coombs CC et al. Cell Stem Cell. 2017;21:374
Leal A et al. Nat Commun. 2020;11:525

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

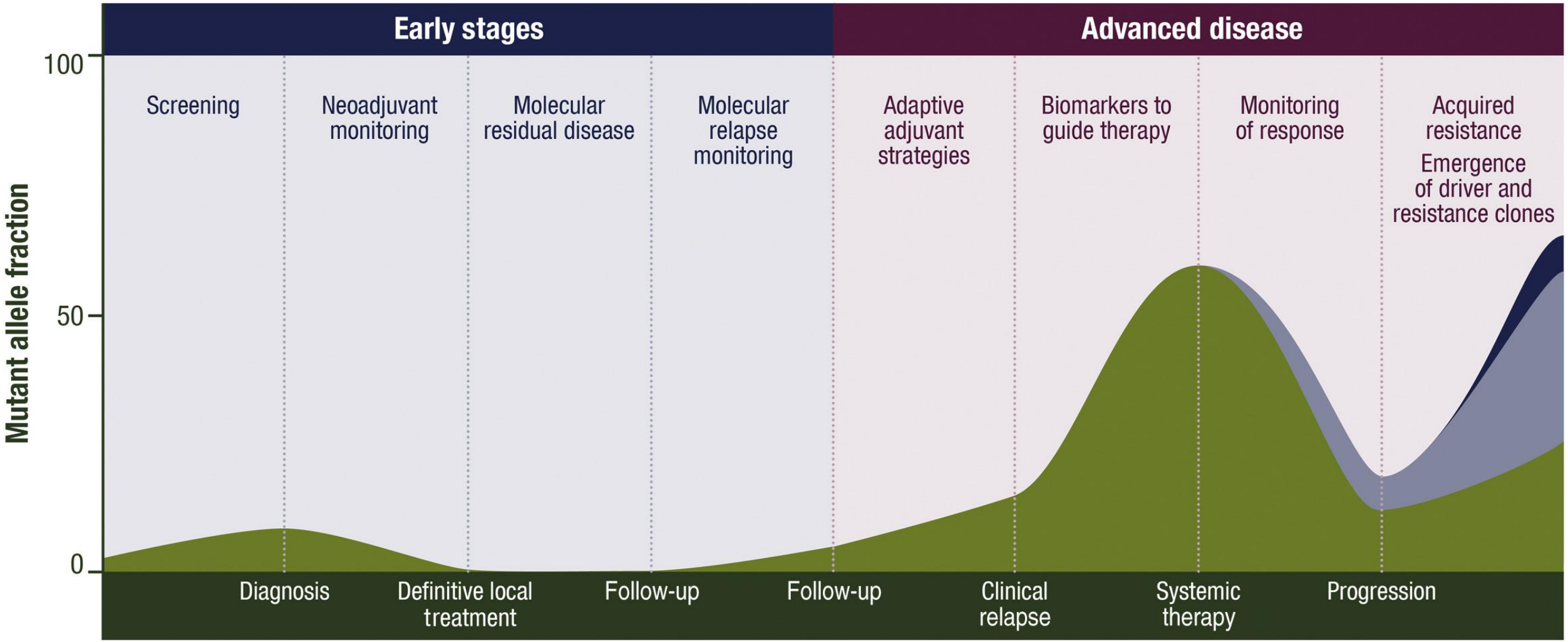
Advance prostate cancer:

- 19% (13/69) cases demonstrated CHIP
- 10% (7/69) cases demonstrated CHIP in DNA repair genes used for PARPi selection



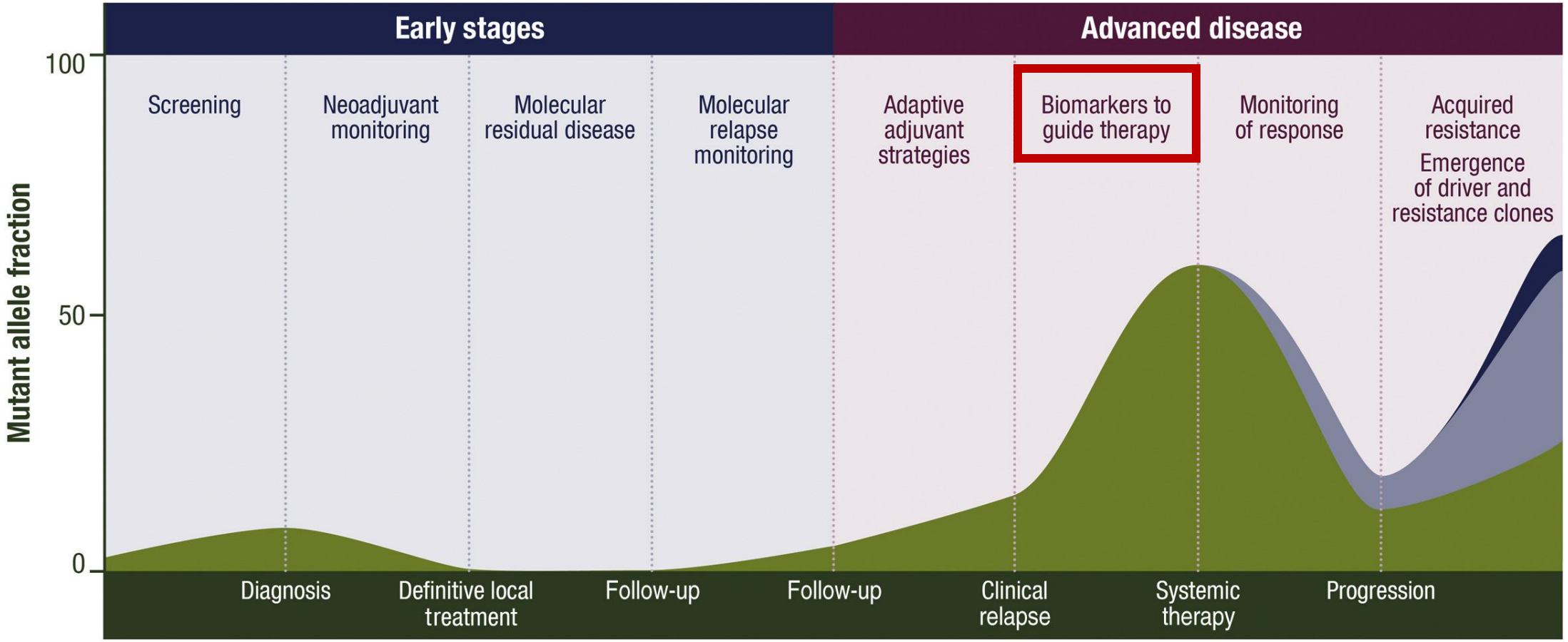
Jensen K et al. JAMA Oncol. 2021;7:107

Potential applications of ctDNA analysis



Pascual J et al. Ann Oncol. 2022;33:750

Potential applications of ctDNA analysis

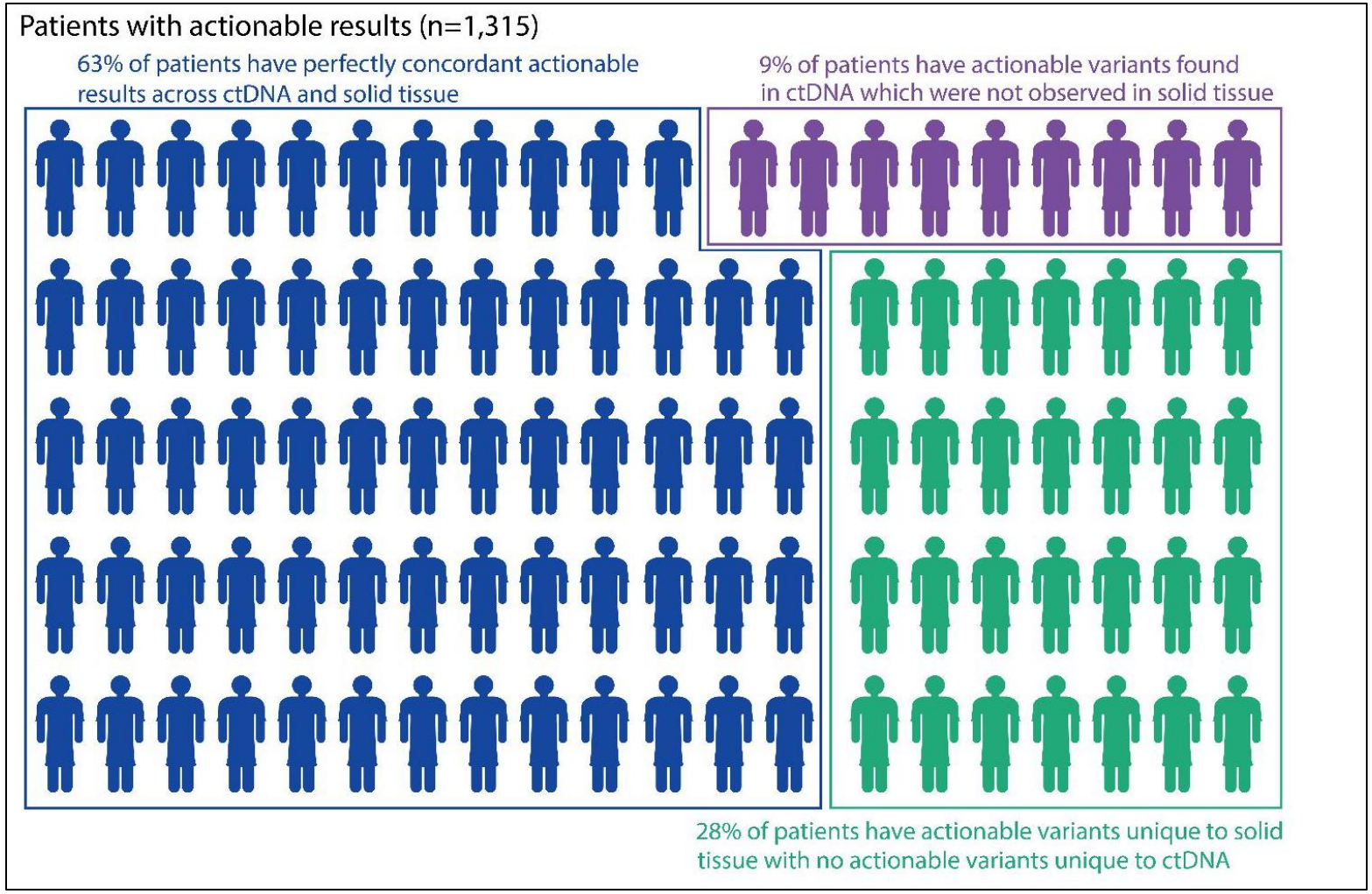


ESMO recommendations on the use of ctDNA assays for patients with cancer:

The level of evidence for the clinical validity of ctDNA assays is such that validated and adequately sensitive ctDNA testing (for SNVs and small indels) can be used in routine practice for advanced disease genotyping, provided that limitations are understood and taken into account.

Pascual J et al. Ann Oncol. 2022;33:750

Plasma-based testing will miss ~30% of actionable variants



1,315 patients with actionable results:

- 63% found in plasma and tissue
- 28% found only in tissue
- 9% found only in plasma

Stage 4 patients:

- NSCLC
- Colorectal
- Breast
- Prostate

MacKay M. et al. 2022 ASCO abstract #3017

Key challenges with plasma-based ctDNA assays

1. Plasma-based ctDNA testing has an appreciable false-negative rate.
2. Detection of amplifications/losses, gene fusions, and genomic features (TMB, MSI, and HRD) is an emerging area.
3. Clonal hematopoiesis (CHIP) may be major interpretive challenge when the tumor biomarker overlaps with CHIP genes (e.g., prostate cancer)
4. Applications other than therapy selection in advanced cancer are specific to cancer type and other clinical information

Outline

I. Genetic considerations for molecular oncology testing

II. Expanded NGS-based tissue assays

III. Liquid biopsy assays

IV. Molecular oncology assay selection

Practical approach to molecular oncology assay selection

1. Assay content
2. Analytical validity
3. Turnaround time
4. Ordering/billing logistics
5. Other practice-related considerations

Assay content – NGS expanded tissue panel

1) Does the assay cover genes, variant types, and genomic features covered by guidelines in my tumor type (e.g., FDA, NCCN 2A and above)?

Molecular Abnormality	FDA approval or NCCN Category
NTRK gene fusions	FDA
MSI-H	FDA
TMB-H	FDA
ATRX mutation	2A
BRAF fusion and/or mutation	2A
IDH1/2 mutation	2A
TERT promoter mutation	2A
H3F3A mutation	2A
HIST1H3B mutation	2A

Other molecular assays	FDA approval or NCCN Category
MGMT promoter methylation	2A
1p19q co-deletion by FISH	2A

NCCN Biomarkers Compendium. CNS Cancers v.1.2022, accessed 8/16/22

Assay content – NGS expanded tissue panel

Substitutions, insertions-deletions, copy-number changes

ABCB1	BLM	CHEK2**	EPHB1	FGFR2	HLA-C	JAK1	MIB1	PAK1	PTCH1	SH2B3	TFEC
ABCC3	BMPR1A**	CIC	EPHB2	FGFR3	HLA-DMA	JAK2	MITF	PALB2**	PTCH2	SHH	TGFBR1
ABL1	BRAF	CIITA	EPOR	FGFR4	HLA-DMB	JAK3	MKI67	PALLD	PTEN**	SLC26A3	TGFBR2
ABL2	BRCA1*	CKS1B	ERBB2 (HER2)	FH**	HLA-DOA	JUN	MLH1**	PAX3	PTPN11	SLC47A2	TIGIT
ABRAXAS1	BRCA2**	CREBBP	ERBB3	FHIT	HLA-DOB	KAT6A	MLH3	PAX5	PTPN13	SLC9A3R1	TMEM127
ACTA2	BRD4	CRKL	ERBB4	FLCN**	HLA-DPA1	KDM5A	MLLT3	PAX7	PTPN22	SLIT2	TMEM173
ACVR1 (ALK2)	BRIP1**	CRLF2	ERCC1	FLT1	HLA-DPB1	KDM5C	MN1	PAX8	PTPRD	SLX4	TMPRSS2
ACVR1B	BTG1	CSF1R	ERCC2	FLT3	HLA-DPB2	KDM5D	MPL	PBRM1	PTPRT	SMAD2	TNF
AGO1	BTK	CSF3R	ERCC3	FLT4	HLA-DQA1	KDM6A	MRE11	PCBP1	QKI	SMAD3	TNFAIP3
AJUBA	BUB1B	CTC1	ERCC4	FNTB	HLA-DQA2	KDR	MS4A1	PDCD1	RAC1	SMAD4**	TNFRSF14
AKT1	C11orf65	CTCF	ERCC5	FOXA1	HLA-DQB1	KEAP1	MSH2**	PDCD1LG2	RAD21	SMARCA1	TNFRSF17
AKT2	C3orf70	CTLA4	ERCC6	FOXL2	HLA-DQB2	KEL	MSH3**	PDGFRA	RAD50	SMARCA4	TNFRSF9
AKT3	C8orf34	CTNNA1	ERG	FOXO1	HLA-DRA	KIF1B	MSH6**	PDGFRB	RAD51	SMARCB1	TOP1
ALK	CALR	CTNNB1	ERRFI1	FOXO3	HLA-DRB1	KIT	MTAP	PDK1	RAD51B	SMARCE1	TOP2A
AMER1	CARD11	CTRC	ESR1	FOXP1	HLA-DRB5	KLF4	MTHFD2	PHF6	RAD51C**	SMC1A	TP53**
APC**	CARM1	CUL1	ETS1	FOXQ1	HLA-DRB6	KLHL6	MTHFR	PHGDH	RAD51D**	SMC3	TP63
APLNR	CASP8	CUL3	ETS2	FRS2	HLA-E	KLLN	MTOR	PHLPP1	RAD54L	SMO	TPM1
APOB	CASR	CUL4A	ETV1	FUBP1	HLA-F	KMT2A	MTRR	PHLPP2	RAF1	SOCS1	TPMT
AR	CBFB	CUL4B	ETV4	FUS	HLA-G	KMT2B	MUTYH**	PHOX2B	RANBP2	SOD2	TRAF3
ARAF	CBL	CUX1	ETV5	G6PD	HNF1A	KMT2C	MYB	PIAS4	RARA	SOX10	TRAF7
ARHGAP26	CBLB	CXCR4	ETV6**	GABRA6	HNF1B	KMT2D	MYC	PIK3C2B	RASA1	SOX2	TSC1**
ARHGAP35	CBLC	CYLD	EWSR1	GALNT12	HOXA11	KRAS	MYCL	PIK3CA	RB1**	SOX9	TSC2**
ARID1A	CBR3	CYP1B1	EZH2	GATA1	HOXB13	L2HGDH	MYCN	PIK3CB	RBM10	SPEN	TSHR
ARID1B	CCDC6	CYP2D6	FAM46C	GATA2**	HRAS	LAG3	MYD88	PIK3CD	RECQL4	SPINK1	TUSC3
ARID2	CCND1	CYP3A5	FANCA	GATA3	HSD11B2	LATS1	MYH11	PIK3CG	RET**	SPOP	TYMS
ARID5B	CCND2	CYSLTR2	FANCB	GATA4	HSD3B1	LCK	NBN**	PIK3R1	RHEB	SPRED1	U2AF1
ASNS	CCND3	DAXX	FANCC	GATA6	HSD3B2	LDLR	NCOR1	PIK3R2	RHOA	SRC	UBE2T
ASPSCR1	CCNE1	DDB2	FANCD2	GEN1	HSP90AA1	LEF1	NCOR2	PIM1	RICTOR	SRSF2	UGT1A1
ASXL1	CD19	DDR2	FANCE	GLI1	HSPH1	LMNA	NF1	PLCG1	RINT1	STAG2	UGT1A9
ATIC	CD22	DDX3X	FANCF	GLI2	IDH1	LMO1	NF2**	PLCG2	RIT1	STAT3	UMPS
ATM**	CD274 (PD-L1)	DICER1	FANCG	GNA11	IDH2	LRP1B	NFE2L2	PML	RNF139	STAT4	VEGFA
ATP7B	CD40	DIRC2	FANCI	GNA13	IDO1	LYN	NFKBIA	PMS1	RNF43	STAT5A	VEGFB
ATR	CD70	DIS3	FANCL	GNAQ	IFIT1	LZTR1	NHP2	PMS2**	ROS1	STAT5B	VHL**
ATRX	CD79A	DIS3L2	FANCM	GNAS	IFIT2	MAD2L2	NKX2-1	POLD1**	RPL5	STAT6	VSIR
AURKA	CD79B	DKC1	FAS	GPC3	IFIT3	MAF	NOP10	POLE**	RPS15	STK11**	WEE1
AURKB	CDC73	DNM2	FAT1	GPS2	IFNAR1	MAFB	NOTCH1	POLH	RPS6KB1	SUFU	WNK1
AXIN1	CDH1**	DNMT3A	FBXO11	GREM1	IFNAR2	MAGI2	NOTCH2	POLQ	RPTOR	SUZ12	WNK2
AXIN2**	CDK12	DOT1L	FBXW7	GRIN2A	IFNGR1	MALT1	NOTCH3	POT1	RRM1	SYK	WRN
AXL	CDK4	DPYD	FCGR2A	GRM3	IFNGR2	MAP2K1	NOTCH4	POU2F2	RSF1	SYNE1	WT1**
B2M	CDK6	DYNC2H1	FCGR3A	GSTP1	IFNL3	MAP2K2	NPM1	PPARA	RUNX1**	TAF1	XPA
BAP1	CDK8	EBF1	FDPS	H2BC5	IKBKE	MAP2K4	NQO1	PPARD	RUNX1T1	TANC1	XPC
BARD1	CDKN1A	ECT2L	FGF1	H3F3A	IKZF1	MAP3K1	NRAS	PPARG	RXRA	TAP1	XPO1
BCL10	CDKN1B	EGF	FGF10	HAUS3	IL10RA	MAP3K7	NRG1	PPM1D	SCG5	TAP2	XRCC1
BCL11B	CDKN1C	EGFR**	FGF14	HAVCR2	IL15	MAPK1	NSD1	PPP1R15A	SDHA	TARBP2	XRCC2
BCL2	CDKN2A **	EGLN1	FGF2	HDAC1	IL2RA	MAX	NSD2	PPP2R1A	SDHAF2**	TBC1D12	XRCC3
BCL2L1	CDKN2B	EIF1AX	FGF23	HDAC2	IL6R	MC1R	NT5C2	PPP2R2A	SDHB **	TBL1XR1	YEATS4
BCL2L11	CDKN2C	ELF3	FGF3	HDAC4	IL7R	MCL1	NTHL1	PPP6C	SDHC**	TBX3	ZFHX3
BCL6	CEBPA**	ELOC (TCEB1)	FGF4	HGF	ING1	MDM2	NTRK1	PRCC	SDHD**	TCF3	ZMYM3
BCL7A	CEP57	EMSY	FGF5	HIF1A	INPP4B	MDM4	NTRK2	PRDM1	SEC23B	TCF7L2	ZNF217
BCLAF1	CFTR	ENG	FGF6	HIST1H1E	IRF1	MED12	NTRK3	PREX2	SEMA3C	TCL1A	ZNF471
BCOR	CHD2	EP300	FGF7	HIST1H3B	IRF2	MEF2B	NUDT15	PRKAR1A	SETBP1	TERT*	ZNF620
BCORL1	CHD4	EPCAM**	FGF8	HIST1H4E	IRF4	MEN1**	NUP98	PRKDC	SETD2	TET2	ZNF750
BCR	CHD7	EPHA2	FGF9	HLA-A	IRS2	MET	OLIG2	PRKN	SF3B1	TFE3	ZNRF3
BIRC3	CHEK1	EPHA7	FGFR1	HLA-B	ITPKB	MGMT	P2RY8	PRSS1	SGK1	TFEB	ZRSR2

Select gene rearrangements – DNA sequencing

ABL1	EGFR**	FGFR3	NTRK2	PML	TFE3
ALK	ETV6**	MYB	NTRK3	RARA	TMPRSS2
BCR	EWSR1	NRG1	PAX8	RET	
BRAF	FGFR2	NTRK1	PDGFRA	ROS1	

Gene fusions – RNA exome

Tumor mutational burden (TMB)

Microsatellite instability (MSI)

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

https://www.tempus.com/oncology/genomic-profiling/ accessed: 8/16/22

Analytical validity – NGS expanded tissue panel

2) Has the assay been reviewed by an independent group for analytical validity?

CLIA Validation Requirements:

- **P**recision
- **A**ccuracy
- **R**eportable Range
- **R**eference Range
- **A**nalytical **S**ensitivity (LOD)
- **A**nalytical **S**pecificity
- Calibration/control procedures
- Other performance characteristics

Review prior to assay implementation
FDA-approved or cleared assays
New York State DOH

Laboratory accreditation with NGS-specific requirements
College of American Pathologists
ISO (International Organization for Standardization)

Turnaround time – NGS expanded tissue panel

3) What is the assay turnaround time?

Considerations

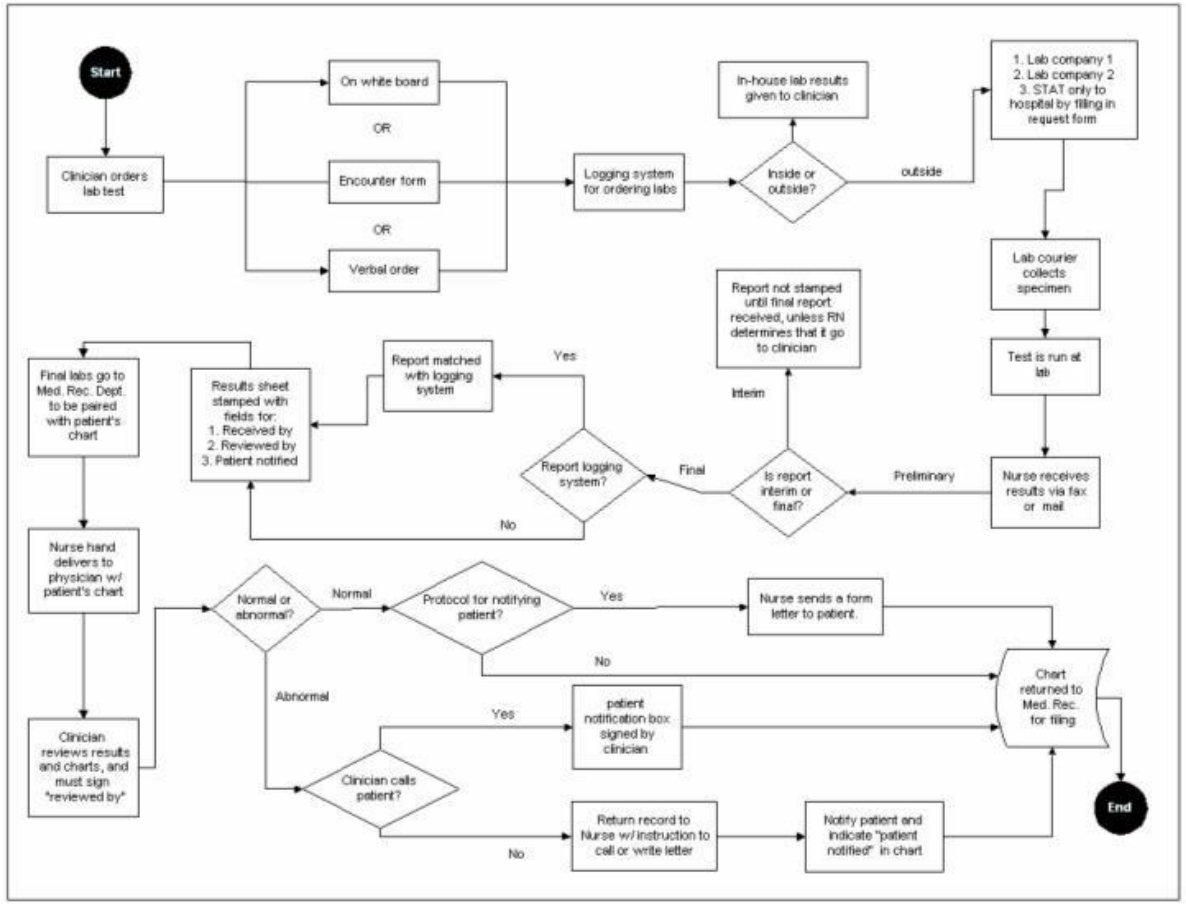
- Usually does not include time for tissue procurement
- Local (courier) vs. national (shipping)
- Calendar vs. business days

Ordering/billing logistics – NGS expanded tissue panel

4) What support is provided to facilitate ordering and billing?

Considerations

- Ease of ordering
- Support for tissue or other specimen procurement (request and monitoring)
- Support for pre-authorization and billing
- Cost to patient and institution



Advances in Patient Safety: From Research to Implementation (Volume 3: Implementation Issues). Henriksen K et al., editors. Rockville (MD): AHRQ (US); 2005 Feb.

Other practice-related considerations

5) What differentiates your laboratory?

Considerations

- Handling of small specimens (success rate and liquid reflex options)
- Support for other assay needs
- Report format and options for accessing
- Support for challenging case consultation (e.g., molecular tumor board)

Questions to approach to molecular oncology assay selection

1. Does the assay cover genes, variant types, and genomic features covered by guidelines in relevant tumor types?
2. Has the assay been reviewed by an independent group for analytical validity?
3. What is the assay turnaround time?
4. What support is provided to facilitate ordering and billing?
5. What differentiates your laboratory?

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Thank you!

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