



Precision Medicine: Expanding Opportunities

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

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March 15, 2022

SCHOOL OF MEDICINE Pathology and Lab Medicine



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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)





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

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

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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 \cong 1 bookcase



All files combined for tumornormal panel ≅ 100 GB

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100 pages (target region)

OR 3 TB (~1 TB compressed)

100 pages (target region)

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X 30,000 = (average coverage)



Amount of data generated for 500 gene ctDNA panel \cong 25 bookcases



N=1

~1 billion human genomics sequences are estimated by 2025



Cumulative Number of Human Genomes

Outline

- I. Background on genetics and genomics
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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 nonsmall 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



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%)



20

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

DRIVER MUTATIONS IN LUNG ADENOCARCINOMA

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 21 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*



Resources to help with biomarker interpretation *NCCN Guidelines*

TESTING RESULTS^{II,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*

EGFR EXON 19 DELETION OR L858R MUTATIONSmm

FIRST-LINE THERAPYPP



Resources to help biomarker and test selection NCCN Biomarkers Compendium

Guideline 1 - 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: <i>EGFR</i> mutation (category 1), <i>ALK</i> (category 1), <i>KRAS, ROS1, BRAF, NTRK1/2/3, MET</i> ex14 skipping, <i>RET</i> 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	

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 econsult (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



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	Ν	Ν	Y
RNA sequencing	Targeted	Ν	Comprehensive
Maturity of EHR integration	+	++	+++
FDA cleared/approved	Ν	Y	Ν

Example of an expanded next-generation sequencingbased assay

Substitutions, insertions-deletions, copy-number changes								
ABLI	ACVRIB	AKTI	AKT2	AKT3	ALK	ALOX12B	AMERI (FAM123B)	APC
AR	ARAF	ARFRPI	ARIDIA	ASXLI	ATM	ATR	ATRX	AURKA
AURKB	AXINI	AXL	BAPI	BARD1	BCL2	BCL2L1	BCL2L2	BCL6
BCOR	BCORL1	BRAF	BRCAI	BRCA2	BRD4	BRIPI	BTG1	BTG2
BTK	C11orf30 (EMSY)	CALR	CARDII	CASP8	CBFB	CBL	CCND1	CCND2
CCND3	CCNE1	CD22	CD274 (PD-L1)	CD70	CD79A	CD79B	CDC73	CDHI
CDK12	CDK4	CDK6	CDK8	CDKNIA	CDKN1B	CDKN2A	CDKN2B	CDKN2C
CEBPA	CHEKT	CHEK2	C/C	CREBBP	CRKL	CSFIR	CSF3R	CTCF
CTNNA1	CTNNB1	CUL3	CUL4A	CXCR4	CYP17A1	DAXX	DDRI	DDR2
DIS3	DNMT3A	DOTIL	EED	EGFR	EP300	EPHA3	EPHB1	EPHB4
ERBB2	ERBB3	ERBB4	ERCC4	ERG	ERRF11	ESR1	EZH2	FAM46C
FANCA	FANCC	FANCG	FANCL	FAS	FBXW7	FGF10	FGF12	FGF14
FGF19	FGF23	FGF3	FGF4	FGF6	FGFR1	FGFR2	FGFR3	FGFR4
FH	FLCN	FLTI	FLT3	FOXL2	FUBPI	GABRA6	GATA3	GATA4
GATA6	GID4 (C17or139)	GNATT	GNA13	GNAQ	GNAS	GRM3	GSK3B	H3F3A
HDAC1	HGF	HNF1A	HRAS	HSD3B1	ID3	IDH1	IDH2	IGFIR
IKBKE	IKZF1	INPP4B	IRF2	IRF4	IRS2	JAKI	JAK2	JAK3
JUN	KDM5A	KDM5C	KDM6A	KDR	KEAPI	KEL	KIT	KLHL6
KMT2A (MLL)	KMT2D (MLL2)	KRAS	LTK	LYN	MAF	MAP2KI (MEKI)	MAP2K2 (MEK2)	MAP2K4
MAP3KI	MAP3K13	MAPKI	MCLI	MDM2	MDM4	MED12	MEF2B	MENT
MERTK	MET	MITE	MKNKI	MLHI	MPL	MREIIA	MSH2	MSH3
MSH6	MSTIR	MTAP	MTOR	MUTYH	MYC	MYCL (MYCLI)	MYCN	MYD88
NBN	NFI	NF2	NFE2L2	NFKBIA	NKX2-1	NOTCHI	NOTCH2	NOTCH3
NPM1	NRAS	NT5C2	NTRKI	NTRK2	NTRK3	P2RY8	PALB2	PARK2
PARPI	PARP2	PARP3	PAX5	PBRMI	PDCD1 (PD-D)	PDCDILG2 (PD-L	2)	PDGFRA
PDGFRB	PDKI	PIK3C2B	PIK3C2G	PIK3CA	PIK3CB	PIK3R1	PIMI	PMS2
POLD1	POLE	PPARG	PPP2R1A	PPP2R2A	PRDMI	PRKARIA	PRKCI	PTCHI
PTEN	PTPNII	PTPRO	QKI	RAC1	RAD21	RAD51	RAD51B	RAD51C
RADSID	RAD52	RAD54L	RAFI	RARA	RB1	RBM10	REL	RET
RICTOR	RNF43	ROS1	RPTOR	SDHA	SDHB	SDHC	SDHD	SETD2
SF3B1	SGKI	SMAD2	SMAD4	SMARCA4	SMARCB1	SMO	SNCAIP	SOCSI
SOX2	SOX9	SPEN	SPOP	SRC	STAG2	STAT3	STKII	SUFU
SYK	TBX3	TEK	TET2	TGFBR2	TIPARP	TNFAIP3	TNFRSF14	TP53
TSC1	TSC2	TYRO3	U2AFT	VEGFA	VHL	WHSCI (MMSET)	WHSCIL1	WTT
XPO1	XRCC2	ZNF217	ZNF703					

Select gene rearrangements

	0010	202		22211	20012	0074		
ALK	BCL2	BCR	BRAF	BRCAL	BRCAZ	CD/4	EGFR	EIV4
ETV5	ETV6	EWSRI	EZR	FGFRI	FGFR2	FGFR3	KIT	KMT2A (MLL)
MSH2	MYB	MYC	NOTCH2	NTRKI	NTRK2	NUTMI	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

Tier I: Variants of Strong Clinical Significance

Therapeutic, prognostic & diagnostic

Level A Evidence

FDA-approved therapy Included in professional guidelines

Level B Evidence

Well-powered studies with consensus from experts in the field

Tier II: Variants of Potential Clinical Significance

Therapeutic, prognostic & diagnostic

Level C Evidence

FDA-approved therapies for different tumor types or investigational therapies Multiple small published studies with some consensus

Level D Evidence

Preclinical trials or a few case reports without consensus

Tier III: Variants of Unknown Clinical Significance

Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases

No convincing published evidence of cancer association

Tier IV: Benign or Likely Benign Variants

Observed at significant allele frequency in the general or specific subpopulation databases

No existing published evidence of cancer association

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
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?

Frequency of NTRK fusions in adult and pediatric tumors



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

TRK biology and signaling



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



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

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

NATIONAL CANCER INSTITUTE PRECISION MEDICINE IN CANCER TREATMENT

Discovering unique therapies that treat an individual's cancer based on the specific genetic abnormalities of that person's tumor.



- 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.

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



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.



Liquid biopsy sources

Liquid biopsy can include the measurement of:

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



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



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



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. — Nº 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



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%.



Most cfDNA from advanced cancer patients is from white blood cells

Source of somatic mutations in cfDNA:

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



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



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 <u>https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P150047</u>; accessed 2/14/22

PCR-based detection of EGFR mutations in NSCLC Clinical utility – Improved PFS demonstrated



Improved progressionfree 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 <u>https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P150047</u>; 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



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

EGFR EXON 19 DELETION OR L858R MUTATIONSmm

FIRST-LINE THERAPYPP



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

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

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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





