UNDERSTANDING THE ROLE OF BIOMARKERS IN PERSONALIZED HEALTH CARE FOR CANCER

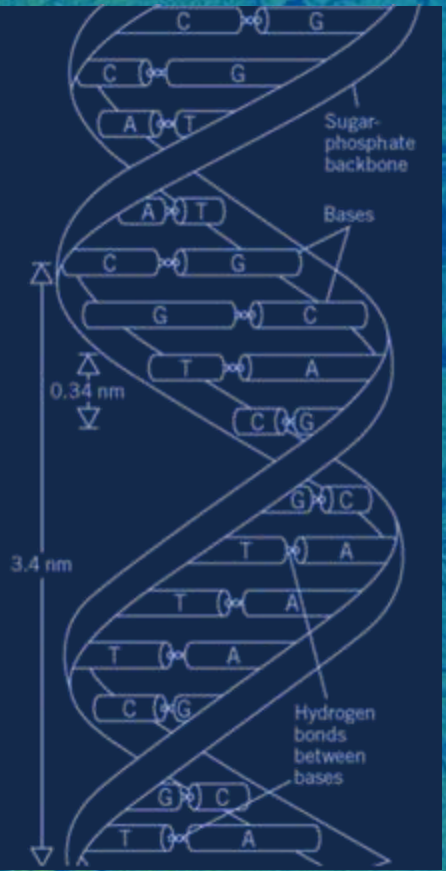

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TABLE OF CONTENTS

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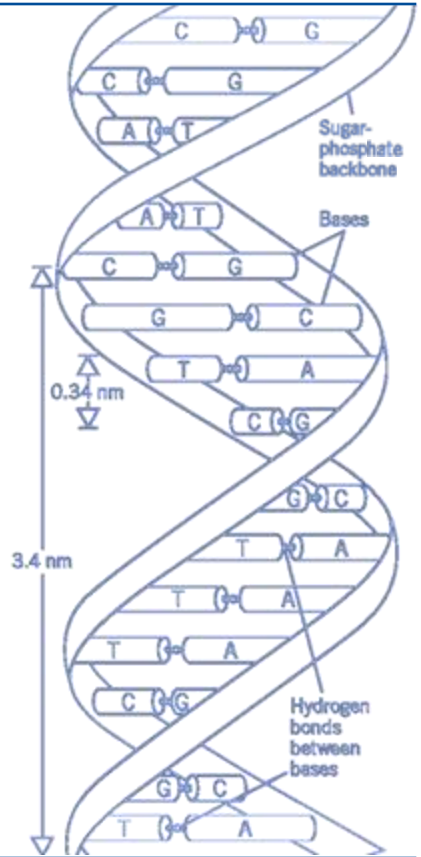
SECTION 1

Oncology Biomarkers Overview



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WHAT IS A BIOMARKER?

A measurable indicator of biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[1]

Biomarkers can include:

Physiological Measurements

Blood Tests

Molecular Analyses of Biopsies

Genetic or Metabolic Data

Imaging Measurements

Biomarkers are critical in diagnosing diseases, predicting disease progression, and monitoring responses to treatment.[2]

1. Wagner JA, Atkinson AJ Jr. Clin Pharmacol Ther. 2015;98(1):2-5. 2. Ahmad A, et al. Pharmaceutics. 2023;15(6):1630. Published 2023 May 31.

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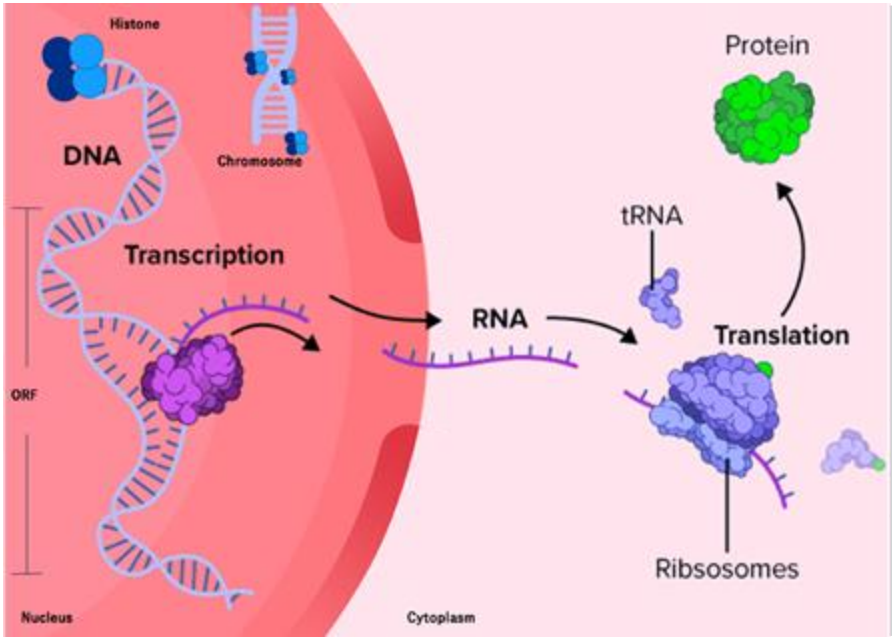
BIOMARKERS ACROSS THE CENTRAL DOGMA: STRUCTURE, FUNCTION, AND MEASUREMENT



Central Dogma

Component	Method	Readout
Chromosome - Structure composed of DNA and proteins carrying genetic material.	Karyotyping, FISH, array CGH, WGS, WES, RNAseq	Structural alterations (e.g., translocations, aneuploidy)
Histones - Proteins that package and order DNA into structural units called nucleosomes.	ChIP-seq, mass spectrometry, western blot	Post-translational modifications (e.g., methylation, acetylation)
DNA - Hereditary molecule encoding gene sequences and regulatory elements.	DNA NGS, PCR, digital droplet PCR (ddPCR)	Mutations, copy number variations, insertions/deletions
RNA - Transcribed from DNA, RNA carries genetic messages for protein synthesis.	RNA-seq, qRT-PCR, microarrays	Gene expression levels, splice variants
Ribosome - Molecular machine that translates RNA into protein.	Electron microscopy, ribosome profiling	Translation efficiency, ribosome occupancy
Protein - Final functional product of gene expression with cellular or extracellular roles.	IHC, ELISA, western blot, mass spectrometry	Protein abundance, isoforms, post-translational modifications

CGH, comparative genomic hybridization; ChIP-seq, chromatin immunoprecipitation sequencing; ddPCR, digital droplet polymerase chain reaction; DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; ORF, open reading frame; PCR, polymerase chain reaction; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RNA, ribonucleic acid; RNA-seq, RNA sequencing; tRNA, transfer RNA; WES, whole exome sequencing; WGS, whole genome sequencing.

CK-12 Foundation. What is the central dogma? CK-12 Life Science. https://www.ck12.org/flexi/life-science/protein-synthesis/what-is-the-central-dogma/. Published 2024. Accessed April 17, 2025.

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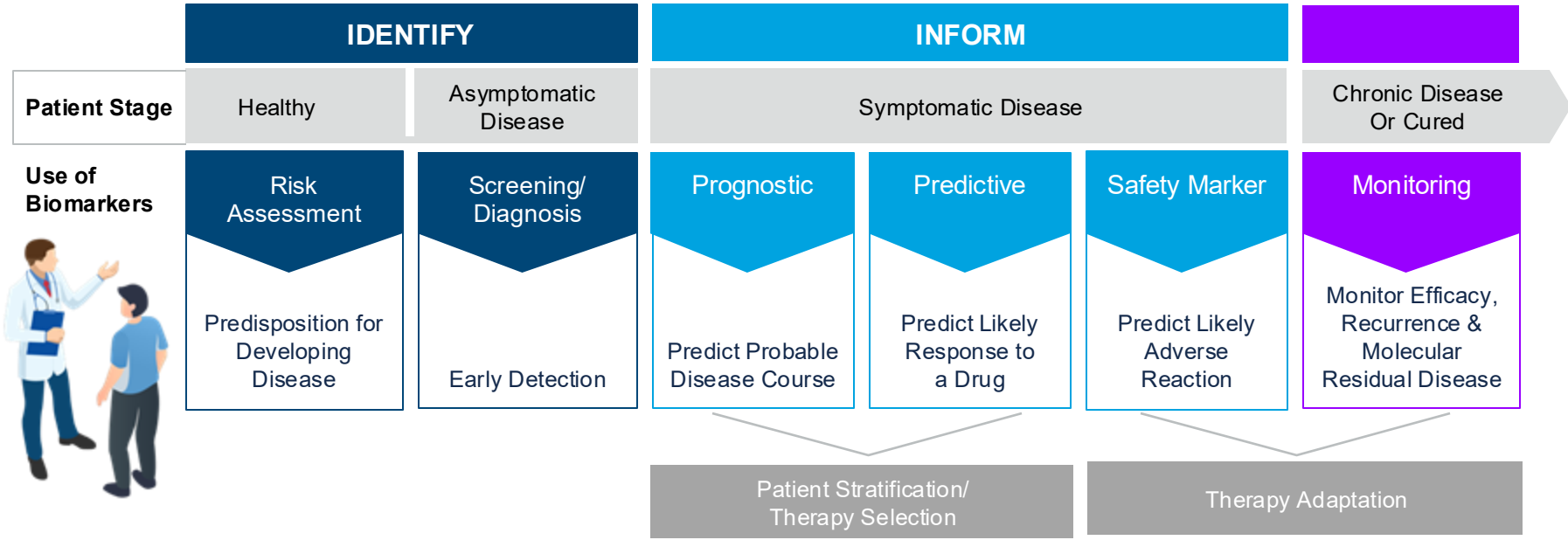
BIOMARKER INTEGRATION ACROSS THE PATIENT TREATMENT JOURNEY[1,2]

Biomarker testing at each stage is used to identify disease and prognosis, inform treatment decisions, and monitor for recurrence.



1. FDA: About Biomarkers, https://www.fda.gov/drugs/biomarker-qualification-program/about-biomarkers-and-qualification, 2021. Accessed 06/13/2025. 2. Nalejska E, et al. Mol Diagn Ther. 2014;18:273-284.

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HOW BIOMARKERS ARE USED TO INFORM TREATMENT

Biomarkers are **critical** for identifying novel targeted **therapies** that **improve cancer patients' survival outcomes**. Therefore, **awareness and testing** are essential in clinical practice.

PROGNOSTIC

Provides information about the patient's overall cancer outcome, regardless of therapy

E.g. Biomarkers

- ctDNA MRD
- Ki-67
- *TP53*

VS

PREDICTIVE

Predicts response to a particular therapy

E.g. Biomarkers

- *ALK*
- PD-L1
- TMB
- *KRAS*
- *ESR1*
- *PIK3CA*
- MSI-H/ dMMR
- *HER2*

ALK, anaplastic lymphoma kinase; ctDNA, circulating tumor deoxyribonucleic acid; dMMR, defective mismatch repair; ESR1, estrogen receptor 1; HER2, human epidermal growth factor receptor 2; HRD, homologous recombination deficiency; Ki-67, antigen KI-67; KRAS, Kirsten rat sarcoma virus; MRD, minimal residual disease; MSI-H, microsatellite instability high; PD-L1, programmed death-ligand 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; TMB, tumor mutation burden.

Oldenhuis CNAM, et al. Eur J Cancer. 2008;44:946-953

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MULTIPLE TYPES OF **BIOMARKER DIAGNOSTIC ASSAYS CAN INFORM PATIENT THERAPY**

Companion Diagnostic (CDx)[1]

- An *in vitro* diagnostic (IVD) device that provides essential information for the safe and effective use of a corresponding therapeutic product.*
- An FDA-approved test for use with a specific therapeutic as stipulated in labels of both the diagnostic device and the corresponding therapeutic product.

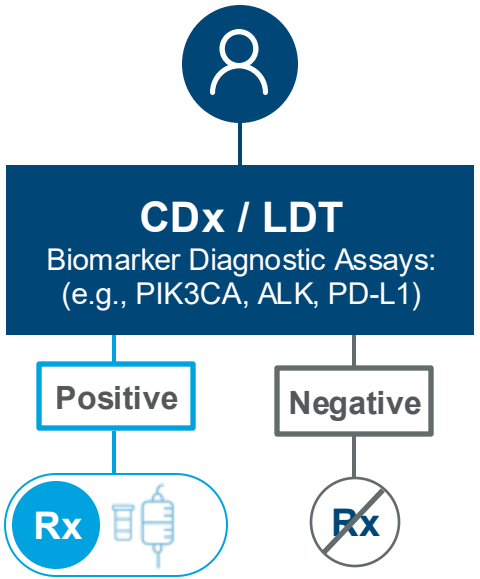
FDA status: **FDA-approved**

OR

Laboratory Developed Tests (LDTs)[2,3]

- *In vitro* diagnostics designed, manufactured, and used within a single laboratory.
- Certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA).

FDA status: **Not FDA-approved**



*The companion diagnostic assays are linked to a specific drug and region requirement for administration of that drug.

ALK, anaplastic lymphoma kinase; FDA, Food and Drug Administration; PD-L1, programmed death-ligand 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; Rx, prescription.

1. Jørgensen JT. Trends Cancer. 2016;2:706-712. 2. FDA: Laboratory Developed Tests, 02/04/2025. Accessed 02/20/2025. 3. New York State Department of Health, Wadsworth Center: Search Approved Laboratory Developed Tests. Accessed 02/20/2025.

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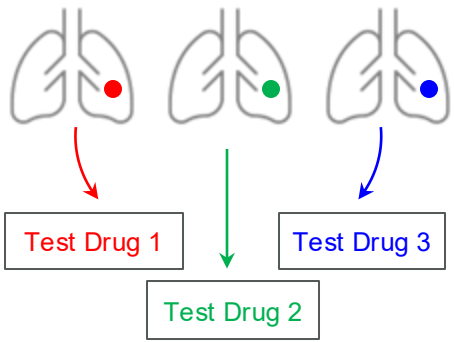
BIOMARKER-DRIVEN CLINICAL TRIALS: UMBRELLA VS. BASKET

Biomarker-Driven Oncology Trial Designs

UMBRELLA TRIAL

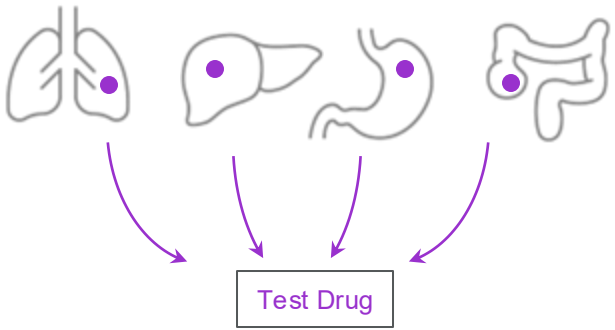
1 type of cancer
Different actionable biomarkers (● ● ●)



An **Umbrella Trial** assesses how well new drugs or other interventions work in patients who have the same type of tumor, but different gene alterations or other biomarkers.

BASKET TRIAL

Multiple types of cancer
1 common biomarker (●)



In contrast, a **Basket Trial** assesses how well new drugs or other interventions work in patients who have different types of cancer that all have the same genomic alteration or biomarker.

These are both biomarker-selected trials.
NCI Dictionary of Cancer Terms. Accessed 05/01/2025.

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BIOMARKER TESTING IS INCLUDED IN NATIONAL GUIDELINES ACROSS MANY TUMOR TYPES

	Non-Small Cell Lung Cancer[1]	**NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)**
	Breast Cancer[2]	
	Colon Cancer[3]	
	Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology[4]	College of American Pathologists IASCLC AMP
	HER2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update[5] Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update[6]	College of American Pathologists ASCO®
	Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology[6]	College of American Pathologists ASCO® AMP ASCP

AMP, Association for Molecular Pathology; ASCO, American Society of Clinical Oncology; ASCP, American Society for Clinical Pathology; IASCLC; International Association for the Study of Lung Cancer; NCCN, National Comprehensive Cancer Network.

1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.4.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed May 30, 2025. To view the most recent and complete version of the guideline, go online to NCCN.org. 2. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer V.4.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed April 30, 2025. To view the most recent and complete version of the guideline, go online to NCCN.org. 3. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Colon Cancer V.3.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed April 30, 2025. To view the most recent and complete version of the guideline, go online to NCCN.org. 4. Lindeman NI, et al. Arch Pathol Lab Med. 2018;142:321-346. 5. Wolff AC, et al. J Clin Oncol 2018 Jul 10;36(20):2105-2122. 6. Allison KH, et al. Arch Pathol Lab Med (2020) 144 (5): 545–563. 7. Sepulveda AR, et al. J Clin Oncol 2017 May 1;35(13):1453-1486.

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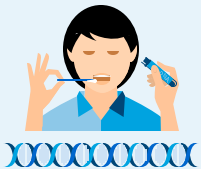
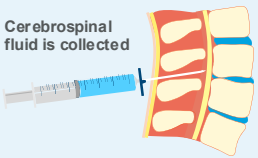
BIOMARKER SAMPLES CAN BE COLLECTED FROM A WIDE VARIETY OF BIOLOGICAL TISSUES AND FLUIDS

Saliva: gDNA

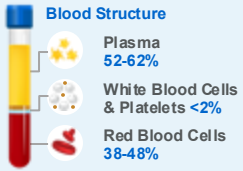


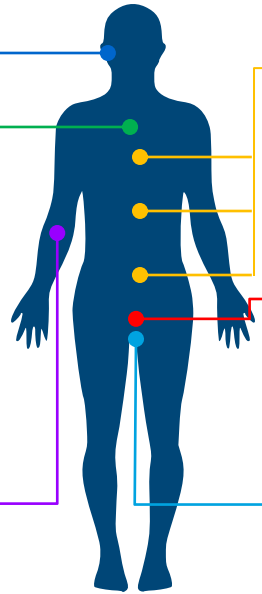
Cerebrospinal fluid (CSF): miRNA, cytokines, ctDNA



Blood: ctDNA, cytokines, DNA

- Plasma, serum, PBMC, WBC, buffy coat





Tissue: (PD-L1, HER2, dMMR/MSI-H, RNA subtyping)

- Fine needle aspirate (FNA), core needle biopsy, biopsy, resection, excision, EBUS (lung)
- FFPE, fresh frozen

Urine: (utDNA, cytokines)

- Urine, cell pellets

Stool: DNA, microbiome

ctDNA, circulating tumor DNA; dMMR, Mismatch Repair deficient; EBUS, endobronchial ultrasound; FFPE, formalin fixed paraffin embedded; gDNA, genomic deoxyribonucleic acid; HER2, human epidermal growth factor receptor 2; miRNA, micro ribonucleic acid; MSI-H, Microsatellite Instability high; PBMC, peripheral mononuclear cells; PD-L1, programmed death receptor ligand 1; utDNA, urine tumor DNA; WBC, white blood cells.

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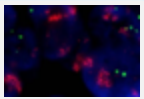
COMPREHENSIVE BIOMARKER TESTING INCORPORATES SEVERAL WELL-DEVELOPED TECHNIQUES TO ASSESS BIOMARKERS IN CLINICAL PRACTICE

Comprehensive biomarker testing can provide the fullest information about possible biomarkers for a patient's tumor.

Innovation (time)

Biomarkers/test:	Testing Method	Advantages	Type of biomarker	Samples
≥ 10 targets up to full genome	Next-Generation Sequencing (NGS)[1-4] 	Highly quantitative/ Highly multiplexed	Gene RNA expression, fusions & DNA alterations, copy number, TMB, MSI, etc	Tumor tissue and blood
< 100 targets	Quantitative Polymerase Chain Reaction (qPCR)[1-3] 	Highly sensitive, moderately multiplexed	RNA gene expression or DNA alterations	Tumor tissue and blood
1 – 2 targets	In-situ Hybridization (ISH)[1-3] 	Spatial localization info	RNA gene copies or DNA fusions	Tumor tissue
1 – 2 targets	Immunohistochemistry (IHC)[2-5] 	Spatial localization info	Protein over-expression	Tumor tissue

DNA, deoxyribonucleic acid; MSI, microsatellite instability; RNA, ribonucleic acid; TMB, tumor mutational burden.

1. Han HS, et al. Clin Breast Cancer. 2016;16:166-79. 2. Song JL, et al. Cancer Med. 2016;5:3475-3488. 3. Raja R, et al. Pharmaceut Med. 2017;31:217-233. 4. Cummings CA, et al. Clin Transl Sci 2016;9:283-292. 5. Yuan J, et al. J Immunother Cancer. 2016;4:3.

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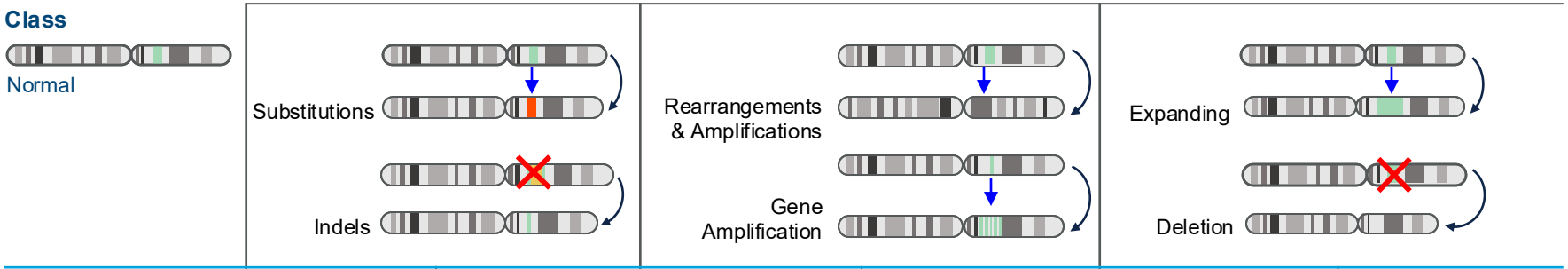
THERE ARE THREE MAIN CLASSES OF DNA ALTERATIONS

Large NGS panels (tissue or blood-based) can detect all 3 classes of DNA alterations from 100s of genes in a single assay.



Type	Substitution	Insertion/ Deletion (Indels)	Rearrangement/ Translocation	Gene Amplification	Expanding trinucleotide repeat	Gene Deletion
Description	1 base replaces the original	1+ nucleotides inserted or deleted	A part of the chromosome is incorrectly fused to another	Number of tandem copies of a locus is increased	The normal number of 3 bases is expanded	Single (heterozygous) or both copies of a gene are lost (homozygous)
Examples in Cancer	*EGFR, BRAF, KRAS, PIK3CA, ESR1*	*EGFR*	*ALK, BCR-ABL,* 11q23	*HER2, PIK3CA*	Microsatellite Instability (MSI)	*PTEN*

ALK, anaplastic lymphoma kinase; BCR-ABL, breakpoint cluster region–Abelson murine leukemia viral oncogene homolog fusion gene; DNA, deoxyribonucleic acid; EGFR, epidermal growth factor receptor; ESR1, estrogen receptor 1; HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; NGS, next-generation sequencing; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN, phosphatase and tensin homolog.

Adapted from: Clancy, S. (2008) Genetic mutation. Nature Education 1(1):187

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EXAMPLE NGS REPORT & RESOURCES TO LINK OUT

Component	Sample Report	Description	Purpose
Patient Information	Patient name / Report Date / Diagnosis Date of birth / Client / Specimen received Gender / Physician / Specimen site Lab case # / Addtl recipient / Collection method Medical record # / Labl client # / Specimen date Block ID / Pathologist / Specimen type	Includes demographics, medical identifiers, ordering physician, and specimen collection details.	Ensures accurate patient-sample matching and traceability for clinical decision-making.
Results	Actionable Test Results: NGS genomic alterations detected; NGS genomic signatures detected; Other bundled test results (IHC, etc); eg: *KRAS*, *PIK3CA*, *ALK*, MSI-H, PD-L1 All Test Results, including VUS: All genomic alterations detected; All genomic signatures identified; Additional disease-relevant genes with no reportable alterations detected	Summarizes the tumor type and lists genomic alterations identified by NGS.	Provides the molecular profile necessary to inform treatment and trial considerations.
Therapeutic Indications	Therapeutic Implications Genomic Alterations Detected / FDA Approved Therapies (In patient's tumor type) / FDA Approved Therapies (in another tumor type) / Potential Clinical Trials *PTEN* *KRAS* *APC* *BRAF*	Lists genomic alterations with matched FDA-approved therapies and available clinical trials.	Translates molecular findings into clinically actionable options for therapy or enrollment.

ALK, anaplastic lymphoma kinase , *APC*, adenomatous polyposis coli; *BRAF*, B-Raf proto-oncogene; FDA, Food and Drug Administration; *KRAS G12C*, IHC, immunohistochemistry; Kirsten rat sarcoma viral oncogene homolog with glycine-to-aspartic acid substitution at codon 12; MSI-H; microsatellite instability high; NGS, next-generation sequencing; PD-L1, programmed death-ligand 1; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog VUS, variants of unknown significance.

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EQUAL HEALTH IMPACT FOR ALL CANCER PATIENTS

Healthcare disparities include differences closely linked to social, economic, or environmental disadvantage [1,2]

The affect people who have systematically experienced obstacles to achieving health [1,2]

- Race and ethnicity
- Sexual orientation/ gender identity
- Age
- Gender
- Mental/ physical disability

"Even when studies have a reasonable 'relative' representation of racial and ethnic minorities, the overall 'absolute' number of minorities examined may not be enough to detect small differences in the cancer's genome"[3]

"We need to know what mutations are present in patients of different races. Otherwise, we may be unintentionally widening disparities."[3]

Daniel Spratt, MD,
University of Michigan Medical School

1. Jooma S, et al. Ethn Dis. 2019; 29:173-178. 2. Ignoffo RJ, et al. J Oncol Pract. 2020;27(1):5-13. 3. Science Daily, August 18, 2016. https://www.sciencedaily.com/releases/2016/08/160818131531.htm. Accessed June 7, 2019.

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UNEQUAL ACCESS TO COMPREHENSIVE BIOMARKER TESTING REMAINS A BARRIER FOR SOME PATIENTS

Comprehensive biomarker testing, including NGS and IHC, can help improve patient outcomes and reduce cost burden to patients and favorably impact the healthcare system [1]

Key Barriers to Equal Access to Genomic Medicine[1,2]

3rd party payer rejection and high out-of-pocket costs

Inadequate preparation, communication, and awareness

Variable health literacy

Different expectations

Genomic medicine language

NGS: next generation sequencing, IHC: immunohistochemistry.

1. National Academies of Sciences, Engineering, and Medicine 2018. Understanding Disparities in Access to Genomic Medicine: Proceedings of a Workshop. Washington, DC: The National Academies Press. https://doi.org/10.17226/25277. Accessed June 7, 2019. 2. Amendola LM, et al. Am J Hum Genet. 2018 Sep 6;103(3):319-327.

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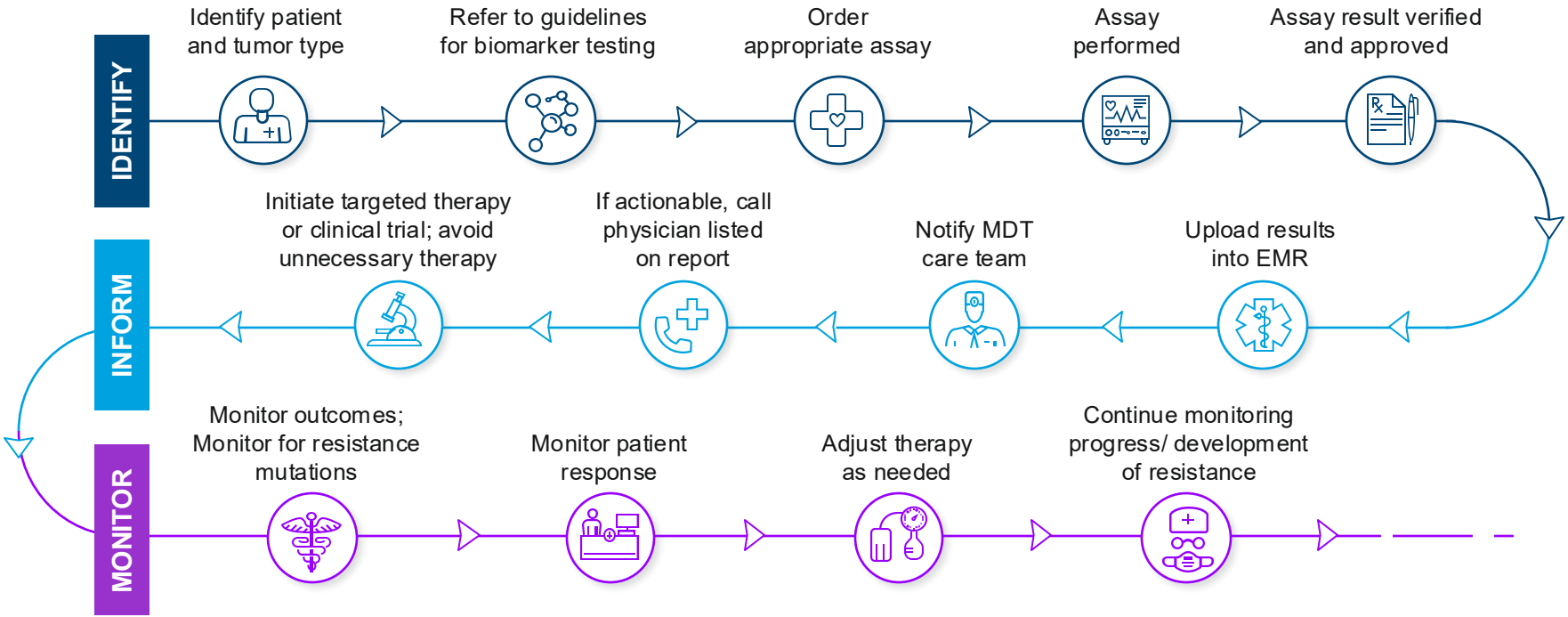
A SIMPLIFIED COMPREHENSIVE BIOMARKER TESTING WORKFLOW[1,2]



MDT, multidisciplinary team; EMR, electronic medical records.

1. Gagan J, et al. Genome Med. 2015;7:80. 2. Cutting EM, et al. AMIA Annu Symp Proc. 2015:466-474. https://pure.johnshopkins.edu/en/publications/using-workflow-modeling-to-identify-areas-to-improve-genetic-test, accessed 06/13/25.

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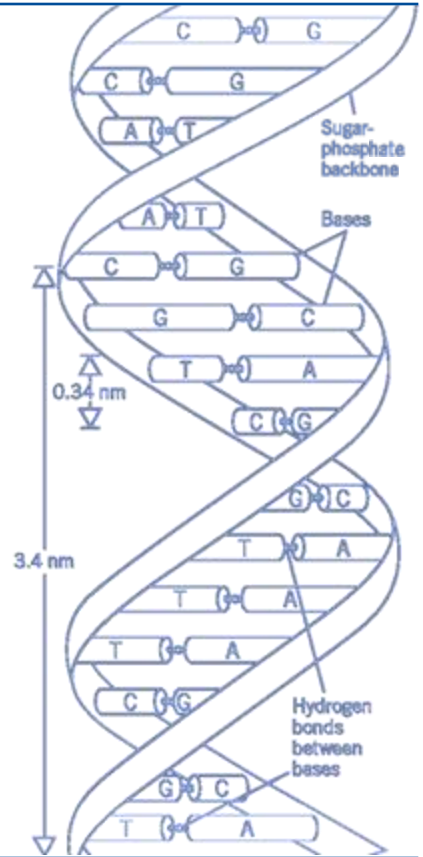
SECTION 2

Biomarkers For Solid Tumors



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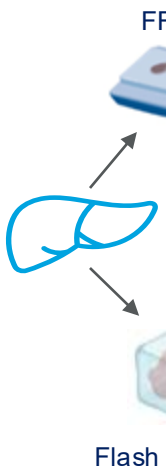
PREANALYTICAL FACTORS AFFECTING MOLECULAR (AND HISTOLOGICAL) TESTING FOR TISSUE SPECIMENS[1]

6 Key Preanalytical Factors Affecting Molecular (and Histological) Testing for Tissue Specimens[1]

1. **Time to start stabilization**
   - Warm ischemia
     - ASAP, dependent upon procedure
   - Cold ischemia
     - ≤ RT, < 60min
2. **Method of stabilization**
   - i.e., fixative, total time in fixative, acid decalcification
3. **Method of processing**
4. **Tissue processor variables**
5. **Storage conditions**
6. **Documentation data**



Pathologist using a microtome in a lab to process an FFPE sample for testing.[2]

Pathologists flash freezing tissue for cryosectioning of samples.[3]

Potential Assays

- IHC
- RNAseq
- WES/WGS

Potential Assays

- scRNAseq
- ROSE – at bedside

ASAP, as soon as possible; FFPE, formalin fixed paraffin embedded; IHC, immunohistochemistry; NGS, next-generation sequencing; RNA, ribonucleic acid; ROSE, rapid on-site evaluation; RT, room temperature; scRNAseq, single-cell RNA sequencing.

1. Compton CC, et al. Arch Pathol Lab Med. 2019;143:1346-1363. 2. https://kids.britannica.com/students/article/microtome/329998, accessed August 22, 2023. 3. Shabihkhani M, et al. Clin Biochem. 2014;47(4-5):258-266.

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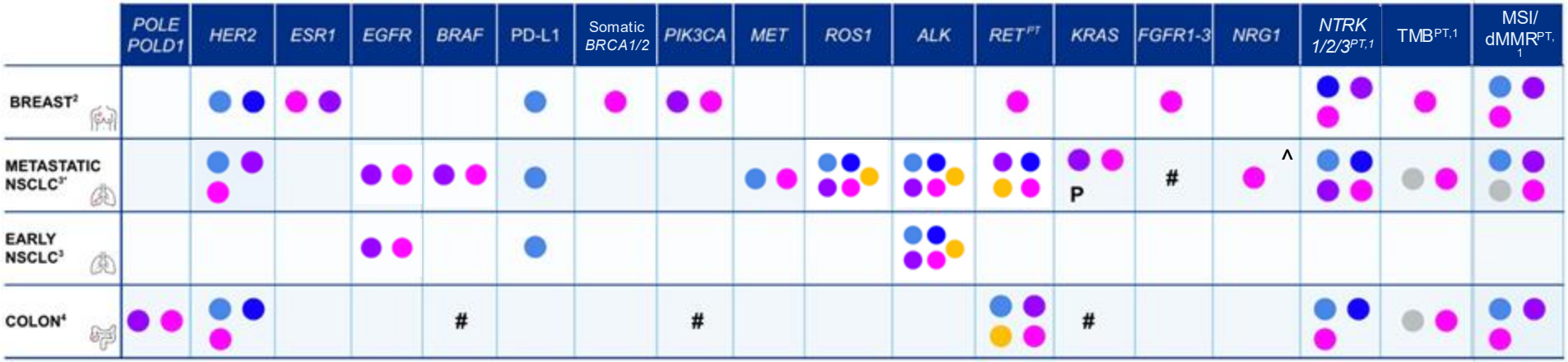
EXAMPLES OF ACTIONABLE SOLID TUMOR BIOMARKERS THAT IMPACT CLINICAL ONCOLOGY TREATMENT



Testing methodologies: IHC ISH qPCR NGS No NCCN Guidelines Listing Screening **#:** testing listed by NCCN with no methodology stated **P:** prognostic biomarker

ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; BRCA1/2, breast cancer gene 1 and 2; dMMR, deficient mismatch repair; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; KRAS, Kirsten rat sarcoma viral oncogene homolog; MET, mesenchymal-epithelial transition factor; MSI, microsatellite instability; NCCN, National Comprehensive Cancer Network; NGS, next generation sequencing; NSCLC, non-small cell lung cancer; NTRK 1/2/3, neurotrophic tyrosine receptor kinase genes 1, 2, and 3; PALB2, partner and localizer of BRCA2; PD-L1, programmed death-ligand 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; POLE, DNA polymerase epsilon catalytic subunit; POLD1, DNA polymerase epsilon catalytic subunit; PT, FDA pan-tumor drug approval; qPCR, quantitative polymerase chain reaction; RET, rearranged during transfection; ROS1, c-ros oncogene 1; TMB, tumor mutational burden; P, prognostic biomarker; #, testing listed by NCCN with no methodology stated; FDA, U.S. Food and Drug Administration.

1. FDA.gov. Accessed 04/30/25. 2. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer V.4.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed April 30, 2025. To view the most recent and complete version of the guideline, go online to NCCN.org. 3. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.4.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed May 30, 2025. To view the most recent and complete version of the guideline, go online to NCCN.org. 4. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Colon Cancer V.3.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed April 30, 2025. To view the most recent and complete version of the guideline, go online to NCCN.org. ^NRG1 testing is recommended as part of broad molecular profiling by the NCCN Guidelines for NSCLC, which is commonly NGS-based. *The NCCN Guidelines for NSCLC provide recommendations for certain individual biomarkers that should be tested in certain patients with metastatic NSCLC and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories.

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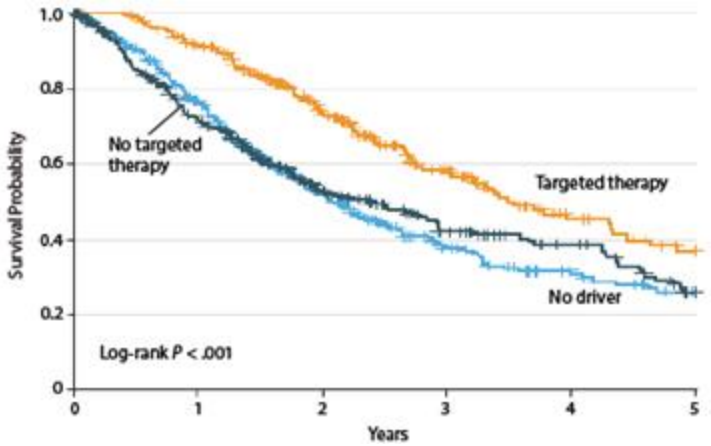
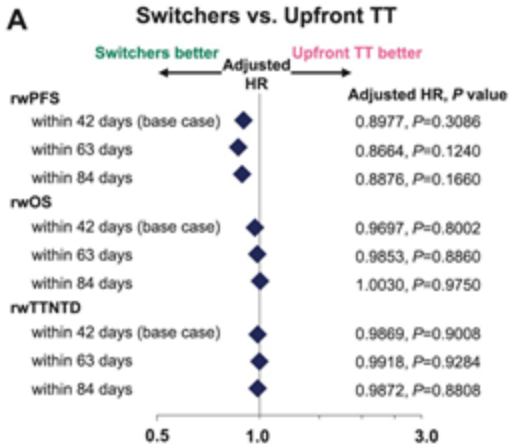
COMPREHENSIVE BIOMARKER TESTING (CBT) CAN FAVORABLY IMPACT PATIENT OUTCOMES

Timely targeted therapy had significantly better outcomes compared to those who didn't switch to TT in a NSCLC patients with driver mutations

Non-switchers had worse outcomes than Upfront TT regardless of the time period from CBT results



Comparable outcomes between Upfront TT and Switchers within 84 days **after CBT results**



NSCLC, non-small cell lung cancer; HRs, hazard ratios; rwOS, real world overall survival; rwPFS, real world progression free survival; rwTTNTD, real world time to next treatment or death; TT, targeted therapy;
Upfront TT, received 1L targeted treatment within 42 days of test result; Switchers, received non-targeted treatment then switched targeted treatment; Non-switchers,received non-targeted treatment and never switched to targeted. Stricker et al. The Oncologist, Volume 29, Issue 6, June 2024, Pages 534–542.

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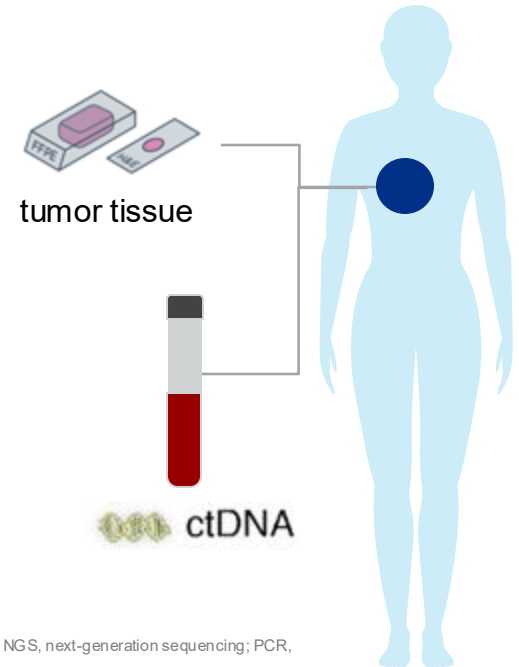
PIK3CA MUTATION TESTING IS CURRENTLY RECOMMENDED FOR INVASIVE HR+/HER2- BREAST CANCER[1]

NCCN Guidelines®: Breast Cancer

- Upon initial workup for recurrent/metastatic disease, comprehensive germline and somatic profiling are recommended to identify candidates for targeted therapies.
- For HR-positive/HER2-negative, recurrent unresectable or metastatic breast cancer, **assess for *PIK3CA* mutations on tumor tissue or ctDNA in peripheral blood (liquid biopsy)** to identify candidates for targeted treatment.
- Tissue-based assays have greater sensitivity, but ctDNA may reflect tumor heterogeneity more accurately. **If one specimen is negative for actionable biomarkers, testing on the alternative specimen can be considered.**



ctDNA, circulating tumor DNA; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; NCCN, National Comprehensive Cancer Network® (NCCN®); NGS, next-generation sequencing; PCR, polymerase chain reaction; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

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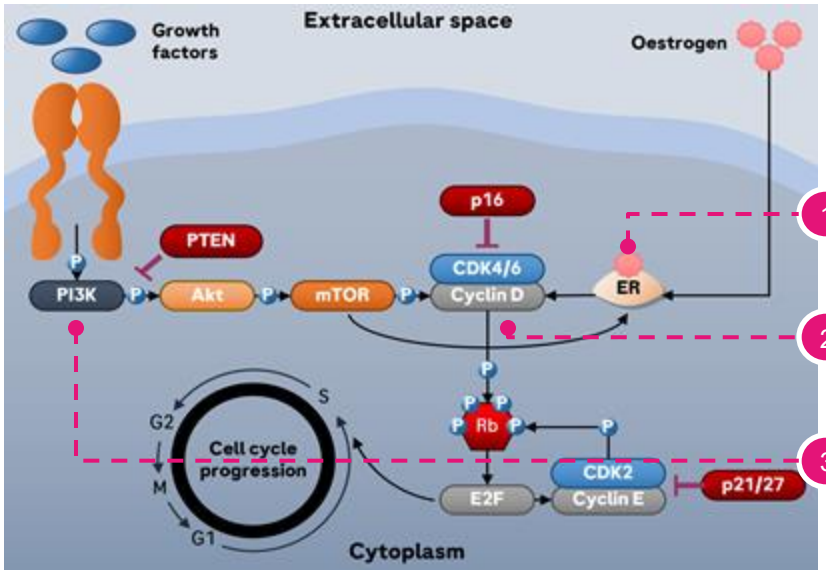
CROSSTALK BETWEEN THE PI3K/AKT/mTOR AND ER PATHWAYS PROVIDES A RATIONALE FOR COMBINATION THERAPIES

PI3K/AKT/mTOR and ER pathway crosstalk

- There is an important crosstalk between the ER and PI3K/AKT/mTOR pathways, highlighted by the high frequency of *PIK3CA* mutations (~40%) in patients with HR+ BC[1]
- Activation of the PI3K/AKT/mTOR pathway may promote resistance to endocrine therapy in ER+ BC[2,3]



ER and PI3K/AKT/mTOR cell signaling pathways[5-10]

AKT, protein kinase B; BC, breast cancer; CDK, cyclin-dependent kinase; ER, estrogen receptor; mTOR, mammalian target of rapamycin; P, phosphate; PI3K, phosphatidylinositol 3-kinase; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN, phosphatase and tensin homolog; Rb, retinoblastoma protein.

1. Vasan N, et al. Ann Oncol. 2019;30:x3–x11; 2. Burris III HA. Cancer Chemother Pharmacol. 2013;71:829–842. 3. Presti D, Quaquarini E. Cancers. 2019;11:1242. 5. Brufsky AM, Dickler MN. Oncologist. 2018;23:528. 6. Anderson EJ, et al. Int J Breast Cancer. 2020;2020:3759179 7. Miller TW, et al. J Clin Oncol. 2011;29:4452–4461. 8. LoRusso PM, et al. J Clin Oncol. 2016;34:3803–3815. 9. Martínez-Sáez O, et al. Breast Cancer Res. 2020;22:45. 10. Fillbrunn M, et al. BMC Cancer. 2022;22(1):1002.

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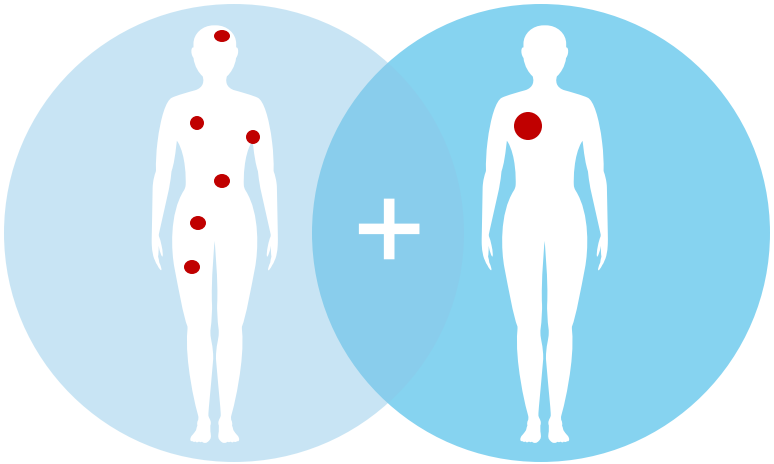
BIOMARKER TESTING IS CONDUCTED IN BOTH THE LATE-STAGE AND EARLY-STAGE SETTINGS

- Since 2013, biomarker testing has been used for HER2-directed therapeutics in the **neoadjuvant and adjuvant setting** for **breast cancer**, by an FDA approved test.[1,2]
- The National Comprehensive Cancer Network® (NCCN®) recommends testing for *ALK*, *EGFR* and PD-L1 in **early NSCLC**, using an **FDA-approved test.**[3]
- Further biomarker tests are **US guideline-recommended** for use in clinical decision-making for **risk stratification** of disease progression and aggressiveness, including MammaPrint®[,4] (breast).
- Others have **CMS pricing decisions**, such as DetermaRx™[,5] and Signatera™[,6].



ALK, anaplastic lymphoma kinase; CMS, Centers for Medicare & Medicaid Services; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; HER2, human epidermal growth factor receptor 2; NSCLC, non-small cell lung cancer; PD-L1, programmed death-ligand 1; US, United States.

1. Amiri-Kordestani, L, et al. Clin Canc Res. 2014 Nov 1;20(21):5359-64. 2. FDA.gov. Accessed 04/09/21. 3. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.4.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed May 30, 2025. To view the most recent and complete version of the guideline, go online to NCCN.org. 4. Following the 2016 Publication of MINDACT, the 2017 ASCO guidelines were updated to indicate favorable results in patients with 1-3 positive lymph nodes. MammaPrint's 510(k) FDA clearance includes breast cancer patients with Stage 1 or Stage II disease, with tumor size ≤ 5.0 cm and lymph node negative. (Agendia is committed to delivering results in less than 10 business days, and results are provided within 6 business days for the majority of cases). 5. Oncocyte.com. Accessed 08/08/23. 6. Natera.com. Accessed 05/02/25.

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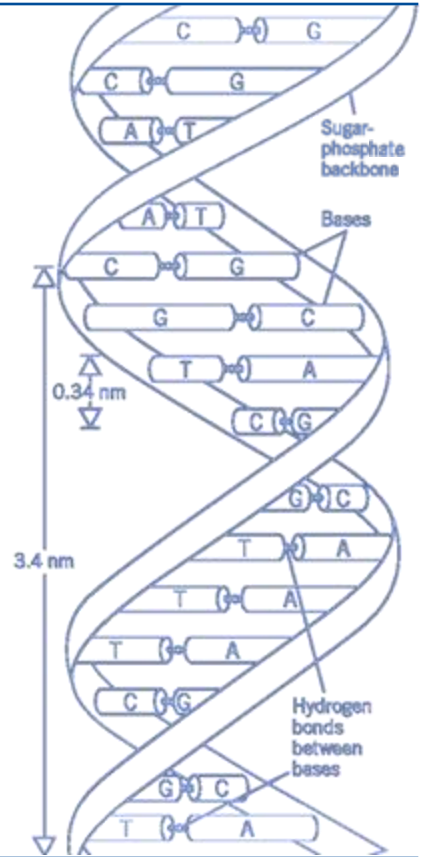
SECTION 3

ctDNA Liquid Biopsy



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CELL-FREE DNA (cfDNA) AND CIRCULATING TUMOR DNA (ctDNA)

Liquid Biopsy

A non-invasive diagnostic technique that involves the analysis of circulating tumor components in body fluids such as blood, urine, cerebrospinal fluid, and saliva.

cfDNA

- Cells shed DNA and it can appear as short fragments in plasma from **normal tissue**
- Methylation and fragmentation patterns can provide additional insights into the tissue of origin and tumor-specific epigenetic changes.

ctDNA

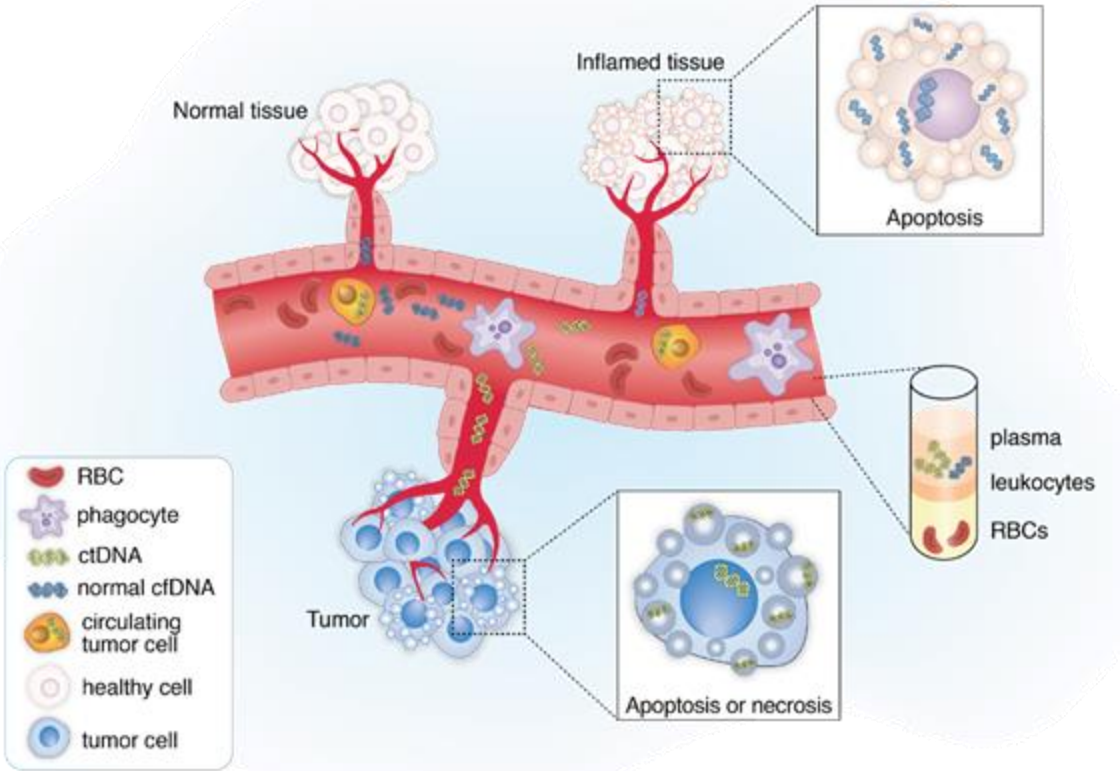
- Tumor cells can shed DNA as ctDNA, which is primarily found in plasma.

ctDNA reflects

- Genomic alterations from the tumor.

ctDNA prevalence in the blood

- Fluctuates with treatment during cancer therapy.
- May correlate with disease response and relapse across wide range of cancers (solid and heme) and treatments.



RBC, red blood cell.

Nikanjam M, et al. J Hematol Oncol. 2022;15(1):131. Published 2022 Sep 12.

Adapted from Crowley, E. et al. Nat. Rev. Clin. Oncol. 10, 472–484 (2013).

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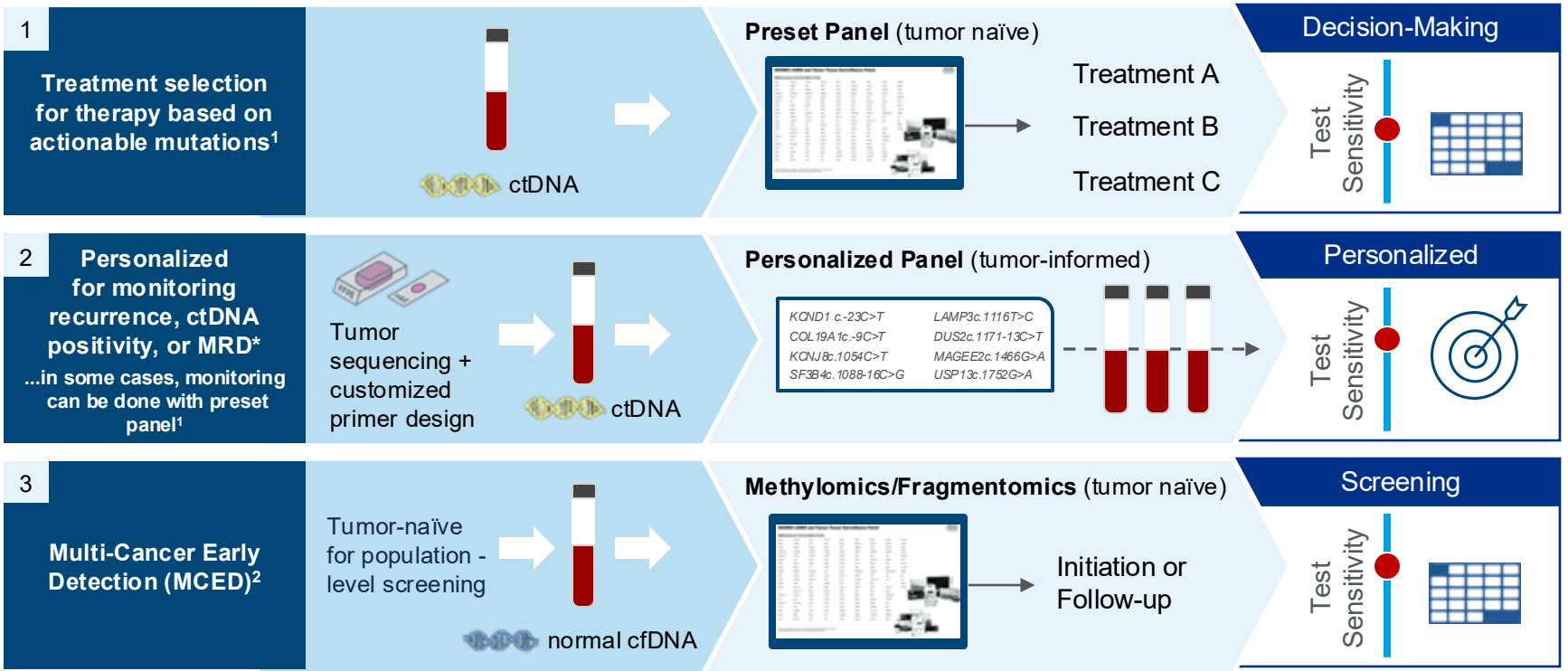
MAIN PRINCIPLES GUIDE ctDNA and cfDNA DETECTION APPROACH[1,2]



cfDNA, normal cell-free DNA; ctDNA, circulating tumor DNA; MCED, Multi-Cancer Early Detection; MRD, molecular residual disease.
*minimal residual disease.

1. Duffy MJ and Crown J. J Pers Med. 2022;12(1):99. 2. Basharat S and Horton J. CADTH Horizon Scan. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; April 2022.

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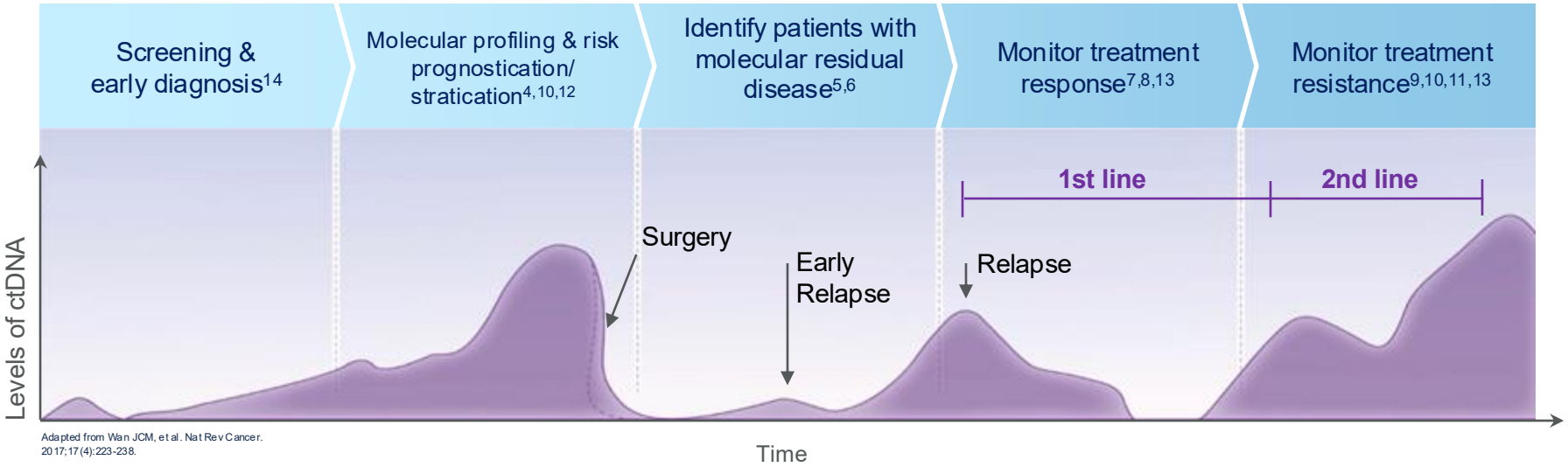
POTENTIAL USE OF ctDNA DURING CLINICAL PRACTICE[1,2]

Timeline of Potential Events and Corresponding ctDNA Levels During Clinical Practice, with examples spanning lung, breast, bladder and prostate cancers[3]



ctDNA, circulating tumor DNA; MRD, molecular residual disease; R, relapse; 1st line, first line of treatment; 2nd line, second line of treatment; ΔctDNA, change in circulating tumor DNA.

1. Sumbal S, et al. Exp Hematol. 2018;65:17–28. 2. Zhou C, et al. J Hematol Oncol. 2018;11(1):129. 3. Wan JCM, et al. Nat Rev Cancer. 2017;17(4):223–238, https://core.ac.uk/reader/83939186?utm_source=linkout, accessed 06/13/25. 4. Christensen E, et al. J Clin Oncol. 2019;37(18):1547–1557. 5. Powles T, et al. Nature. 2021;595(7867):432–437. 6. ClinicalTrials.gov. Updated January 10, 2025. Accessed January 13, 2025. https://clinicaltrials.gov/study/NCT04660344. 7. Powles T, et al. Nat Med. 2024;30(9):2508–2516. 8. Tolmeijer SH, et al. Clin Cancer Res. 2023;29(15):2835–2844 9. Fonseca NM, et al. Nat Commun. 2024;15:1828. 10. Goldberg SB, et al. Clin Cancer Res. 2018 Apr 15;24(8):1872-1880. 11. Jacob et al. Clin Cancer Res. 2021;27(5):1361-1370. 12. Turner NC, et al. N Engl J Med 2024;391:1584-96. 13. Chiang AC, et al. JTO Clinical and Research Reports 2025. https://doi.org/10.1136/jitc-2024-011363, accessed 06/13/25. 14. Wan JCM, et al. Nat Rev Clin Oncol (2025).

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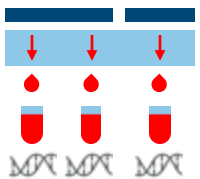
POTENTIAL USE OF ctDNA DURING CLINICAL TRIALS[1,2]

Integrating ctDNA Into Standard and Adaptive Phase Treatment Clinical Trials[3]

Standard phase	Adaptive phase
Treatment 	Continue to monitor ctDNA as appropriate to address research question 

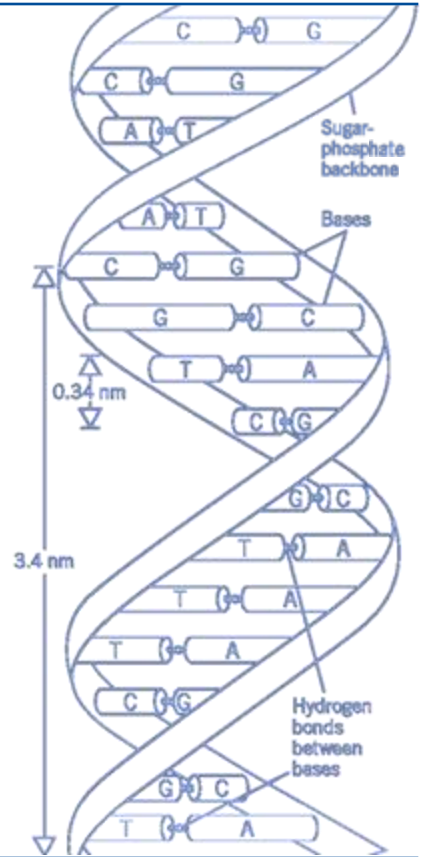
Escalation trial	△ctDNA meets threshold		Continue to progression 	**Potential methods of escalation** • Switch to more aggressive therapy • Increase the dose of a treatment • Add a second treatment in combination
	△ctDNA does not meet threshold		Escalate treatment 	
De-escalation trial	△ctDNA meets threshold		De-escalate treatment 	**Potential methods of de-escalation** • Switch to less toxic therapy • Reduce the dose of a treatment • Remove an agent from the combination
	△ctDNA does not meet threshold		Continue to progression 	

ctDNA, circulating tumor DNA; MRD, molecular residual disease; R, relapse; 1st line, first line of treatment; 2nd line, second line of treatment; ΔctDNA, change in circulating tumor DNA.

1. Sumbal S, et al. Exp Hematol. 2018;65:17–28. 2. Zhou C, et al. J Hematol Oncol. 2018;11(1):129. 3. Adapted from Sanz-Garcia, E. et al. Science Advances. Vol 8, Issue 4 (2022).

SECTION 4

Biomarkers for Hematological Cancers



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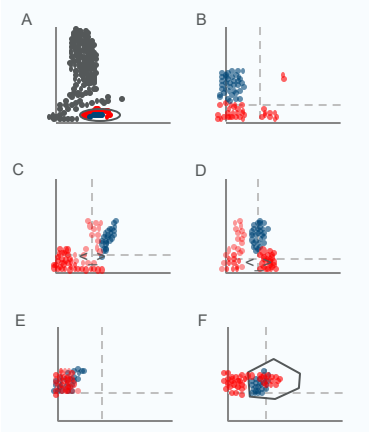
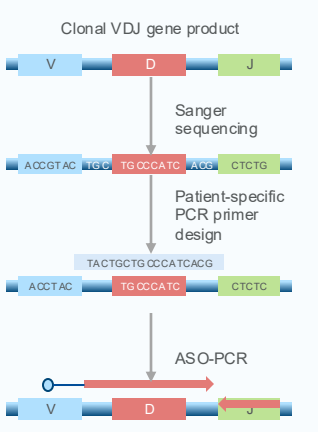
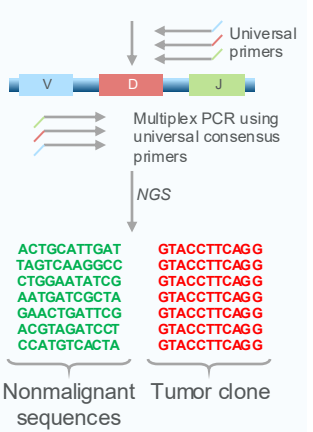
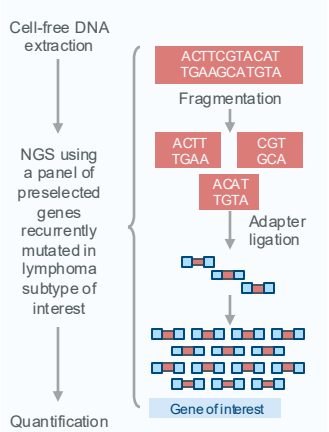
MRD TECHNOLOGIES FOR HEMATOLOGICAL MALIGNANCIES[1,2]

Different MRD measuring technologies are available, but not all are universally used in all hematological indications:

- AML (Flow and NGS)
- CLL (Flow, PCR and NGS)
- MM (Flow and NGS)
- DLBCL (ctDNA NGS)
- FL (PCR, ctDNA)

	Multicolor Flow Cytometry	RQ-PCR and ASO-PCR	Ig NGS	Panel Directed NGS
				
SENSITIVITY:	10^{-4} to 10^{-5}	10^{-5}	10^{-6}	10^{-6} + molecular profile
SAMPLE INPUT:	Cells from blood or bone marrow	DNA from blood or bone marrow	DNA from blood or bone marrow	cfDNA from plasma or DNA from PBMC/BMMCs
+ / -	+ Standard FC widely available - Requires presence of CTCs	+ Stable sample - Limited to research	+ Commercially available, uses universal reagents - Limited genomic information	+ Non-invasive sample - Limited to research

ALM, acute myeloid leukemia; ASO-PCR, allele-specific oligonucleotide polymerase chain reaction; BMMC, bone marrow mononuclear cells; cfDNA, circulating free DNA; CLL, chronic lymphocytic leukemia; CTC, circulating tumor cell; DLBCL, diffuse large B-cell lymphoma; FC, flow cytometry; FL, follicular lymphoma; Ig NGS, Immunoglobulin Gene Next Generation Sequencing; MM, multiple myeloma; MRD, minimal residual disease; NGS, next-generation sequencing; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; RQ-PCR, real-time quantitative polymerase chain reaction.

1. Herrera AF and Armand P. J Clin Oncol. 2017;35:3877-3887. 2. Fürstenau M, et al. Hemasphere. 2019;3:e287.

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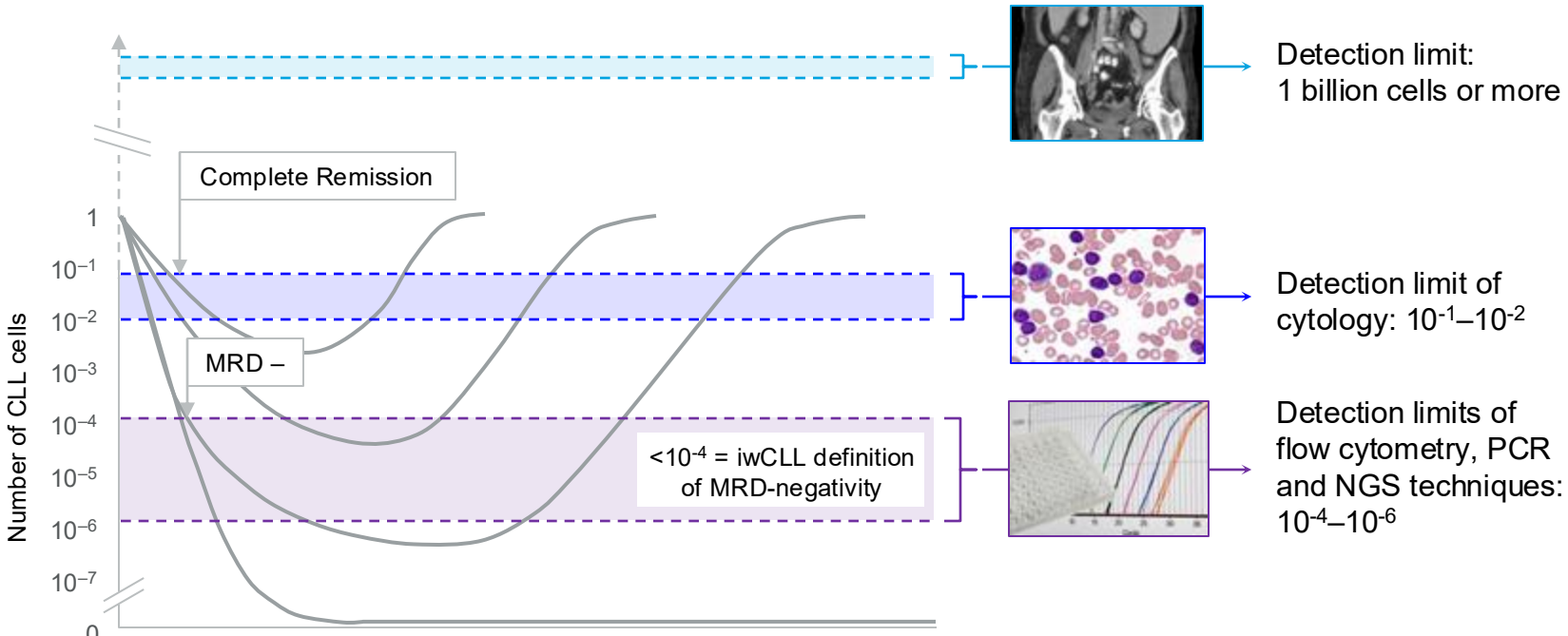
MRD MEASUREMENT IS A SENSITIVE TEST FOR PRESENCE OF DISEASE IN BLOOD AND BONE MARROW IN CLL[1-4]



CLL, chronic lymphocytic leukemia; iwCLL, International Workshop on CLL; MRD, minimal residual disease; NGS, next-generation sequencing; PCR, polymerase chain reaction.
Illustration is conceptual: references contain definitions and descriptions.

1. Moreno C, et al. *Best Pract Res Clin Haematol* 2010; 23:97-107. 2. Hallek M, et al. *Blood* 2018;131:2745-2760. 3. Del Monte U. *Cell Cycle*. 2009;8:505-506. 4. Adewoyin AS and Nwogoh B. *Ann Ib Postgrad Med*. 2014;12:71-79.

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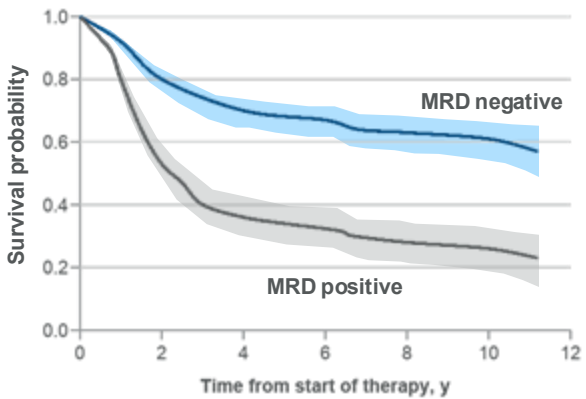
MRD IS A PROGNOSTIC MARKER IN ACUTE MYELOID LEUKEMIA (AML)

Association of Measurable Residual Disease with Survival Outcomes in Patients With AML

Systematic review and meta-analysis of 81 articles (11,151 patients)

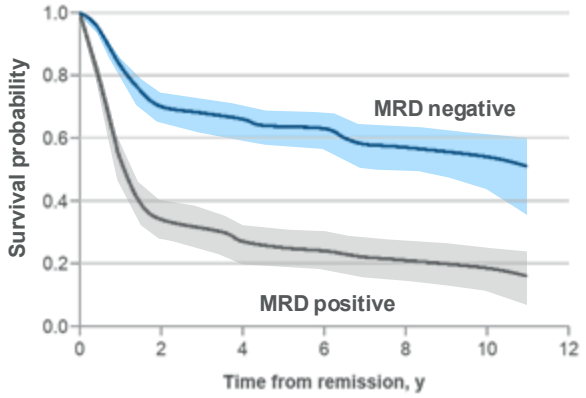
- 17 OS only (3118 patients)
- 20 DFS only (1783 patients)
- 44 Both (6250 patients)

A Overall survival



Overall survival (OS) (A): The curves show the posterior means of survival distribution in the bayesian hierarchical analysis. The shadings of each curve show the 95% bayesian credible intervals (CrIs) for the survival proportion at the corresponding point in time of follow-up.
The 5-year OS was 68% (95% CrI, 63%-73%) for the MRD-negative group and 34% (95% CrI, 28%-40%) for the MRD-positive group. The average hazard ratio for OS was 0.36 (95% CrI, 0.33-0.39), with a 5-year restricted mean survival time difference of 15.37 months (95% CrI, 13.58-17.19 months).

B Disease-free survival



Disease-free survival (DFS) (B): The 5-year DFS was 64% (95% CrI, 59%-70%) for the MRD-negative group and 25% (95% CrI, 20%-32%) for the MRD-positive group. The average hazard ratio for DFS was 0.37 (95% CrI, 0.34-0.40), with a 5-year restricted mean survival time difference of 19.61 months (95% CrI, 17.33-21.92 months).

AML, acute myeloid leukemia; CrI, credible interval; DFS, disease-free survival; MRD, measurable residual disease; OS, overall survival.

Short NJ, et al. JAMA Oncol. 2020;6:1890-1899.

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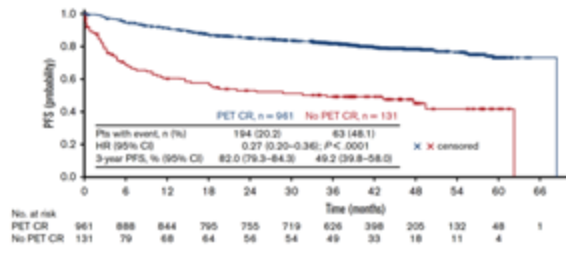
RECENT DEVELOPMENTS IN HEME MRD

2018: FDA allows using uMRD4 as an endpoint in CLL clinical trials.[1]

2024: The FDA's Oncologic Drugs Advisory Committee voted 12 to 0 that the totality of available data supports the use of minimal residual disease (MRD) as an end point for accelerated approval of new treatments for patients with Multiple Myeloma.[2]

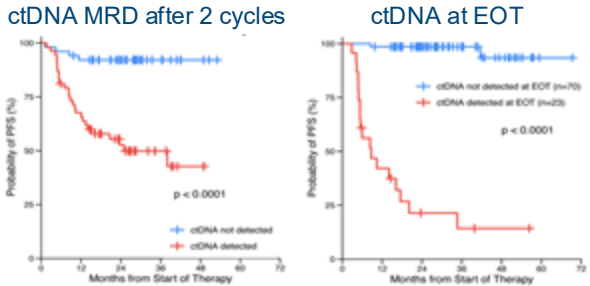
2025: The NCCN Guidelines recommend ctDNA testing as an alternative to biopsy for evaluating positive PET imaging results in patients with DLBCL at the end of first-line therapy, particularly those who have achieved a partial response or have progressive disease.[3,7]

PET Scans have a high false-positive rate, and at EOT are Prognostic but Not Specific for Lymphoma[4,5]



20% had a PFS event after PET CR

PhasED-Seq MRD is Prognostic After 2 Cycles and EOT[4,6]



CI, Confidence Interval; CLL, Chronic Lymphocytic Leukemia; CR, Complete Response; ctDNA, circulating tumor DNA; DLBCL, Diffuse Large B-Cell Lymphoma; EOT, end of therapy; FDA, Food and Drug Administration; HR, Hazard Ratio; MRD, minimal residual disease; NCCN, National Comprehensive Cancer Network; ODAC, Oncologic Drugs Advisory Committee; PET, positron emission tomography; PFS, Progression-Free Survival; uMRD4, undetectable Minimal Residual Disease at a sensitivity of 10^{-4}.

1. Ghia P and Rawstron A. Leukemia. 2018;32(6):1307-1316. 2. International Myeloma Foundation. International Myeloma Foundation. A historic turning point: ODAC unanimously votes in favor of MRD testing as an early endpoint in myeloma clinical trials to support accelerated approvals of new treatments. April 18, 2024. Accessed April 29, 2025. 3. Foresight Diagnostics, Inc. NCCN guidelines updated to include ctDNA-MRD testing recommendation for B-cell lymphoma. GlobeNewswire. Published January 10, 2025. Accessed April 29, 2025. 4. M, Lindenberg L, et al. Presented at: American Society of Hematology (ASH) Annual Meeting; December 9, 2023; San Diego, CA. Accessed April 29, 2025. 5. Kostakoglu et al. Blood Adv 2021;5(5):1283-1290. 6. Roschewski et al. Hematological Oncology 41(S2):177-179, ICML 2023. 7. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for B-Cell Lymphomas V.2.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed May 2, 2025. To view the most recent and complete version of the guideline, go online to NCCN.org.

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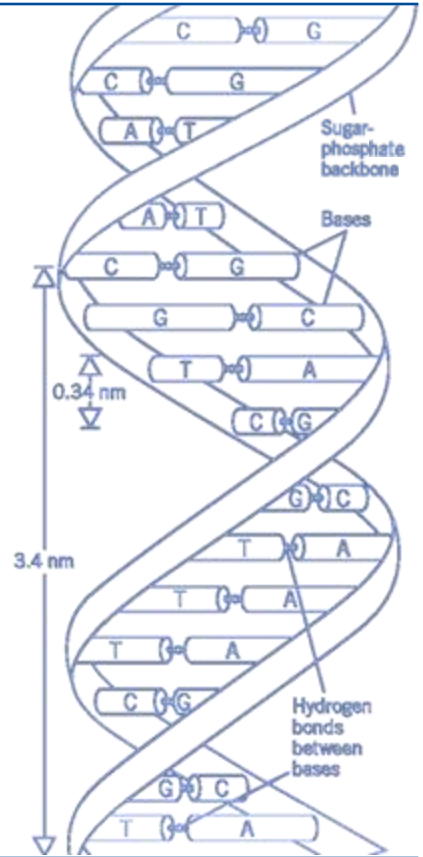
SECTION 5

Emerging Applications



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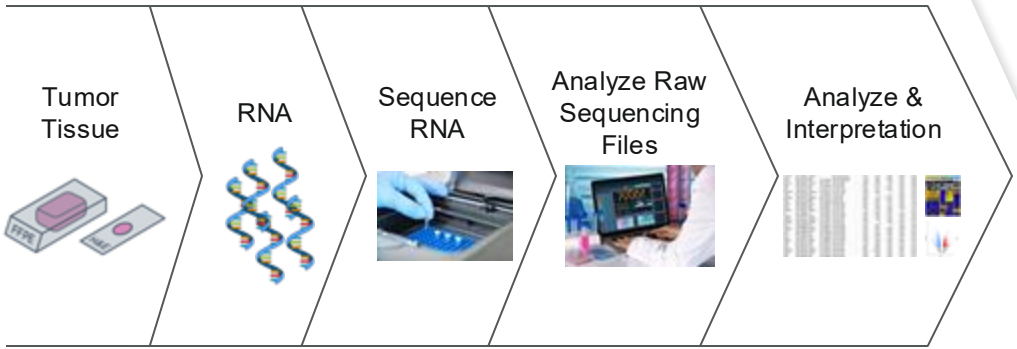
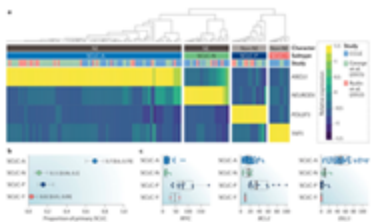
AI/ML IS RAPIDLY TRANSFORMING RESEARCH AND DRUG DEVELOPMENT AS AN INTEGRAL PART OF LAB-IN-A-LOOP





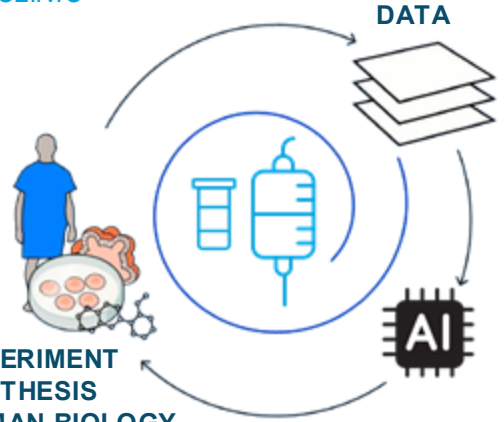
Key transcription regulators define molecular subtypes in many indications, including SCLC

Further investigation of the biology of these subtypes and prospective application in subtype-specific clinical investigations, could result in better, personalized treatments for SCLC patients.[1]

LAB*-In-A-LOOP

To transform drug discovery with data, RT, and AI

*OR CLINIC



AI, artificial intelligence; E2E, efficacy-to-effectiveness; ML, machine learning; RNA, ribonucleic acid; RT, reverse translation; SCLC, small cell lung cancer.

1. Roche's Virtual IR Digitalization Day, November 25, 2024. Accessed 03/04/25.

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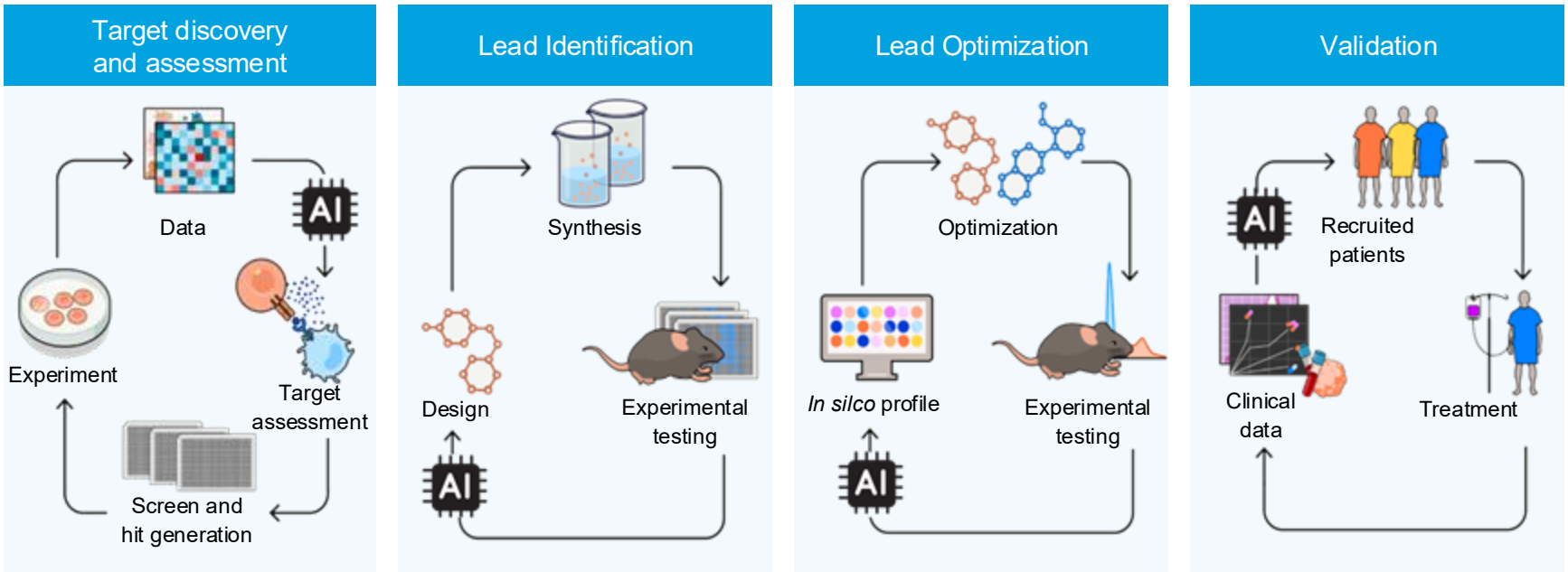
LAB-IN-A-LOOP IN DRUG R&D: EMBEDDED, END TO END, FROM TARGET DISCOVERY TO THE CLINIC



AI, artificial intelligence; R&D, research and development.

1. Roche's Virtual IR Digitalization Day (November 25, 2024), Accessed 03/04/25.

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USING AI/ML TO IDENTIFY FEATURES IN AN IMAGE (STAINED SECTION OR RADIOGRAPHIC) ASSOCIATED WITH PROGNOSIS OR RESPONSE TO THERAPY: DIGITAL PATHOLOGY (DP)[1-3]

Computer vision models are trained using pathologist annotations to label cells and segment tissue regions across multiple indications

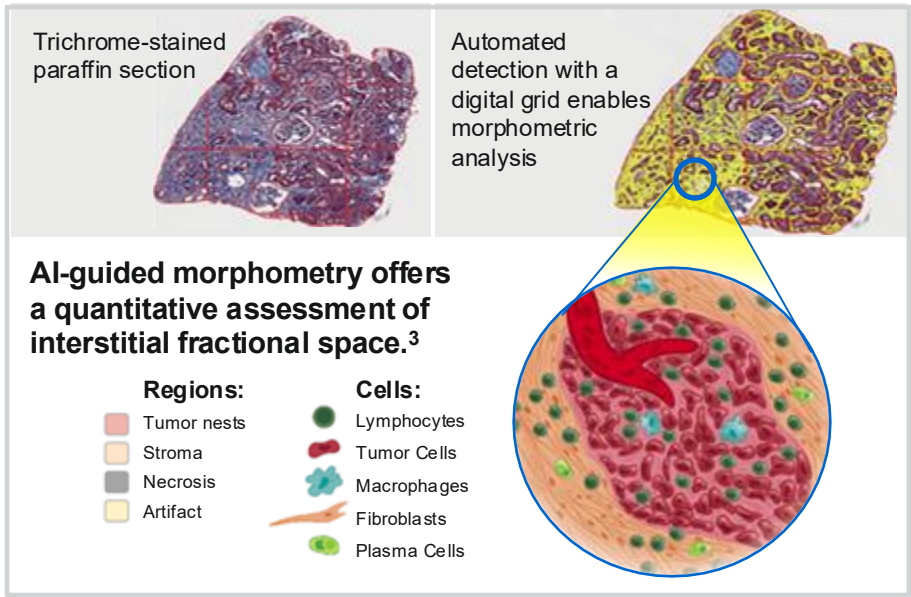
Human interpretable features (HIFS) are computed by combining these cell and tissue region predictions with derived spatial measures

HIFs extracted which exhaustively quantify & spatially characterize the tumor microenvironment

Higher Fidelity

Examples:

- Potentially faster and less resource-intensive identification of biomarker status for:
  - Clinical risk stratification
  - PD-L1 IHC and immunophenotype status
  - single gene driver alterations or RNAseq-based molecular subtype status



AI, artificial intelligence; DP, digital pathology; ML, machine learning; PD-L1, Programmed Cell Death Ligand 1; RNA, ribonucleic acid; RNAseq, RNA sequencing.
HIFS: Human Interpretable Features combinatorial and calculated feature outputs from image models trained with ground truth labeled by medical pathologist (MD or DVM).

1. Taylor-Weiner, Giltnane, et al. Machine learning based identification of predictive features of the tumor microenvironment and vasculature in NSCLC patients using the IMpower150 study (ASCO 2020, April 08, 2022).
2. Qamra, Amitai, et al. Digital pathology based prognostic & predictive biomarkers in metastatic non-small cell lung cancer (AACR 2023, April 04, 2023). 3. Barisoni L, et al. Nat Rev Nephrol. 2020;16(11):669-685.

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SUMMARY & KEY TAKEAWAYS

- Biomarkers are an integral part of clinical management across the entirety of a cancer patient's treatment journey.
  - Biomarkers are critical for the clinical care of patients with both solid or hematological cancers.
  - increasingly in both the early- and late-stage to identify disease status, inform decision-making, and monitor for recurrence.
  - Positive biomarkers (e.g., in NSCLC) enable targeted therapy with improved outcomes.
- Comprehensive Biomarker Testing (CBT) is essential across the oncology care continuum:
  - Enables diagnosis, prognosis (e.g., risk stratification), screening, and prediction of efficacy and safety outcomes.
  - Identifies actionable prognostic and predictive biomarkers for therapy selection.
  - Supports safer and more effective treatment decisions.
  - Liquid biopsy and tissue-based testing methods can both have key roles in CBT.
- ctDNA based testing is a powerful non-invasive testing platform:
  - ctDNA is used for both treatment selection and Molecular Residual Disease (MRD) monitoring for recurrence.
  - ctDNA and tumor-naïve panels also enable multi-cancer early detection (MCED) screening.
- AI/ML is rapidly transforming research and drug development as an integral part of lab-in-a-loop, including biomarker discovery and clinical testing and implementation.

cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; MCED, Multi-Cancer Early Detection; MRD, molecular residual disease; NSCLC, non-small cell lung cancer.

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Doing now what patients need next