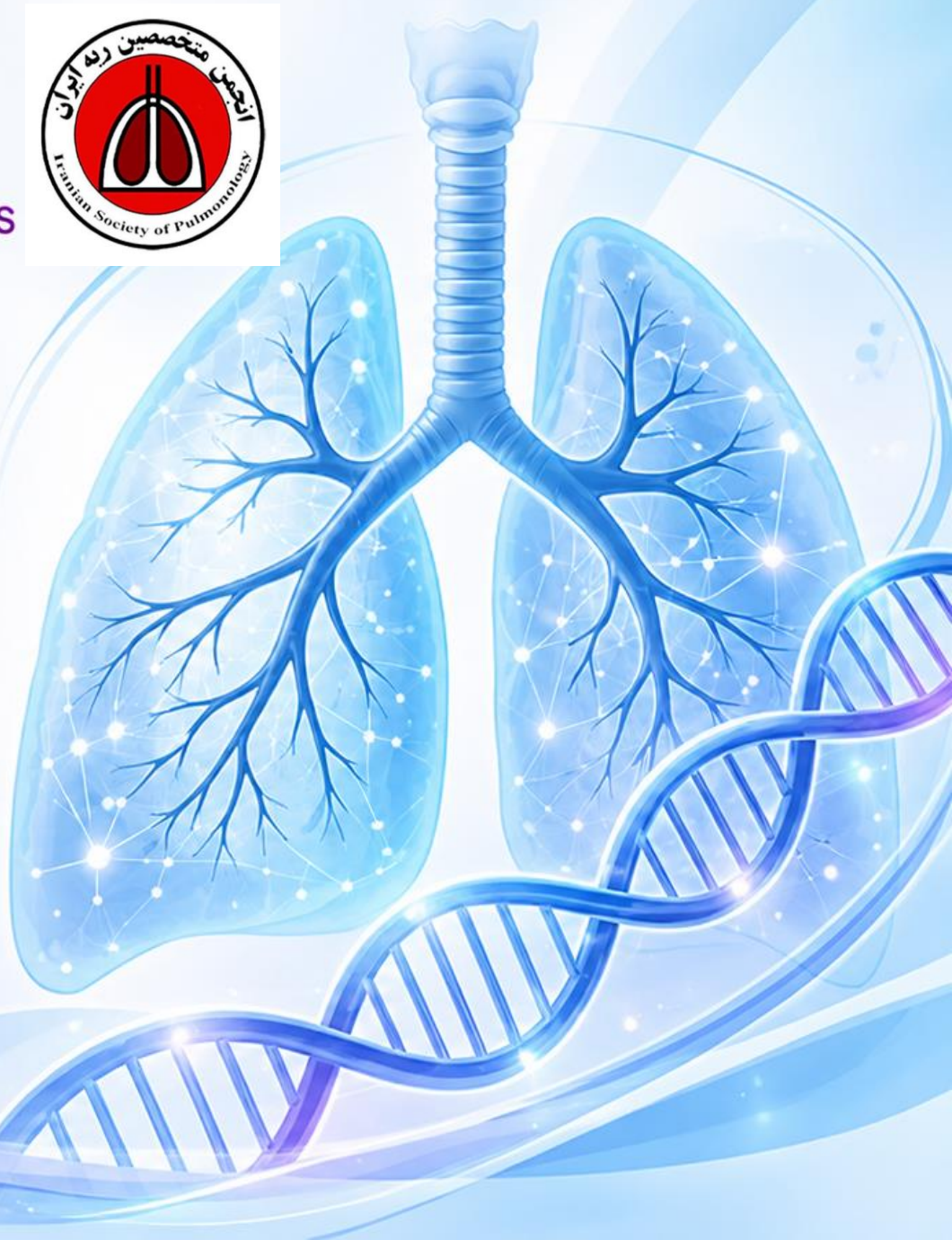
The NAME of GOD in our every BREATH!



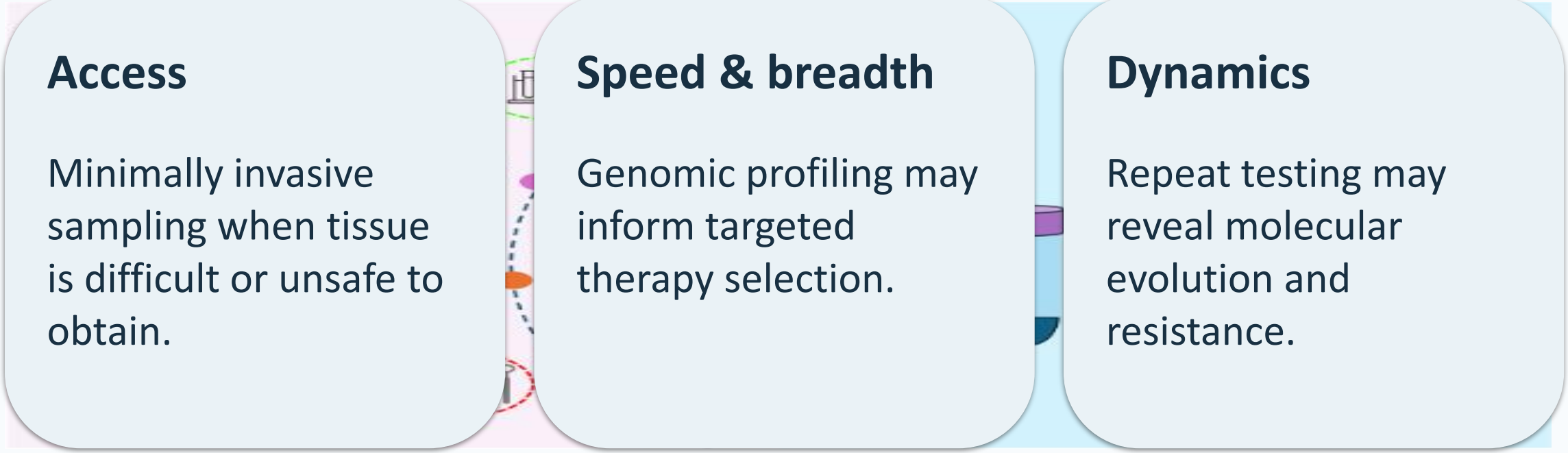
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Liquid Biopsy in Lung Cancer From Sampling to Precision Oncology



Why liquid biopsy matters



Access

Minimally invasive sampling when tissue is difficult or unsafe to obtain.

Speed & breadth

Genomic profiling may inform targeted therapy selection.

Dynamics

Repeat testing may reveal molecular evolution and resistance.

What is measured?

ctDNA / cfDNA

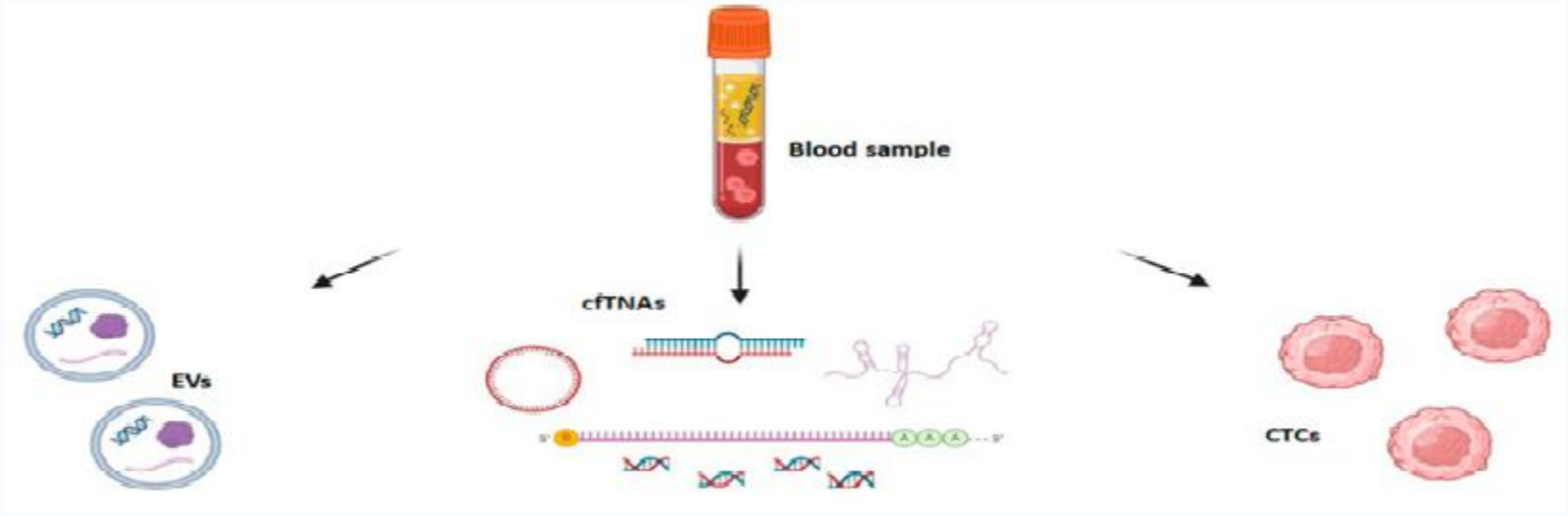
Somatic variants, copy-number signals and methylation.

CTCs

Intact cells; phenotypic and cellular information.

Other signals

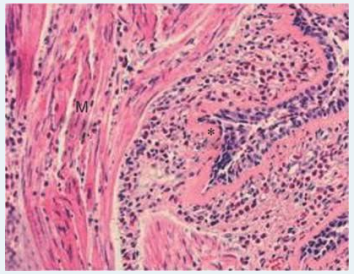
cfRNA, exosomes, proteins and emerging multimodal assays.



Which Body Fluids Can Be Used?

Tissue

Histology, architecture, IHC and PD-L1; comprehensive assessment.



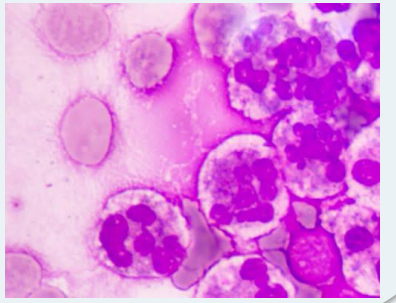
Plasma

Repeatable systemic ctDNA profiling; sensitivity depends on shedding.



Pleural fluid

Cytology/cell block plus cell-free supernatant for molecular analysis.



A negative plasma assay is not equivalent to a negative tumor genotype.

From sample to molecular result

SAMPLE
Plasma / pleural fluid

→

PROCESS
Centrifugation

→

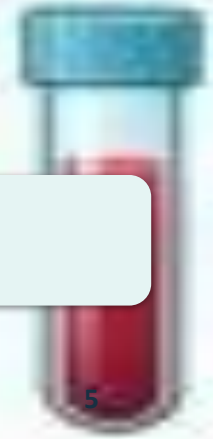
FRACTIONATE
Cell pellet + supernatant

↓

CELL PELLET
Cytology • Cell block • IHC

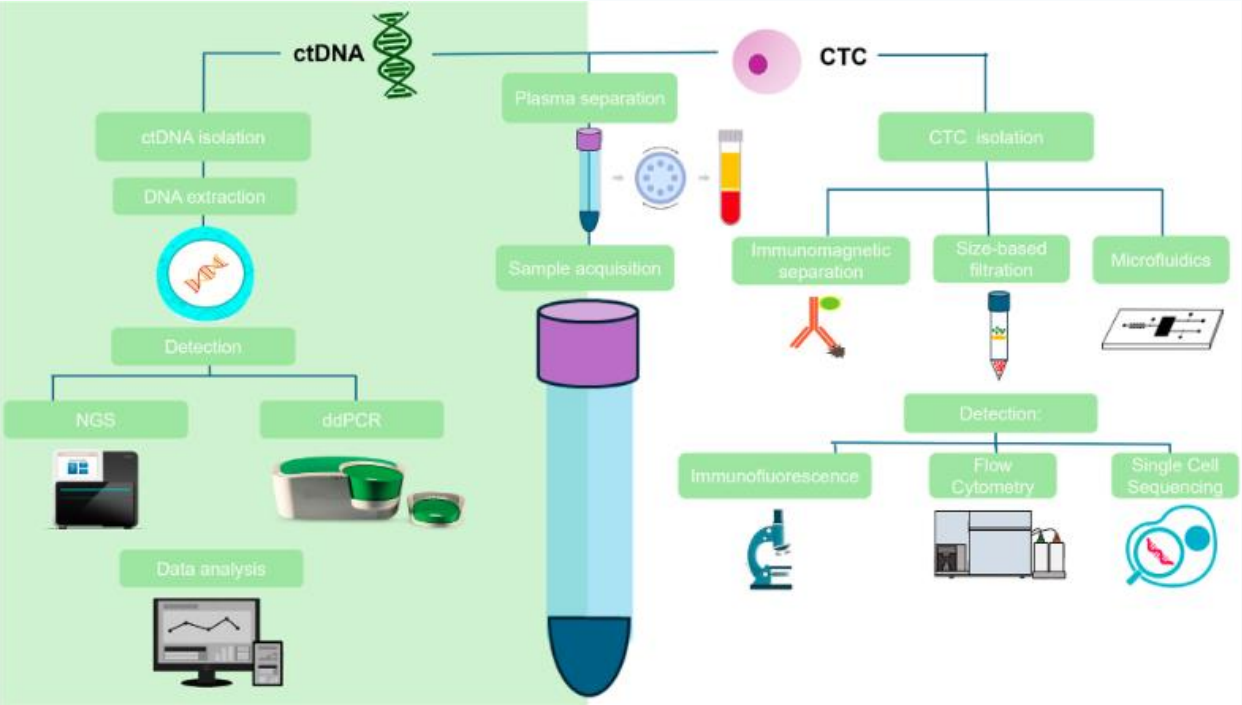
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SUPERNATANT
cfDNA → ctDNA → NGS / ddPCR



Collection tubes, processing delay and assay design affect interpretability.

What Are We Looking For?



Different analysis provide different biological information.

CTCs

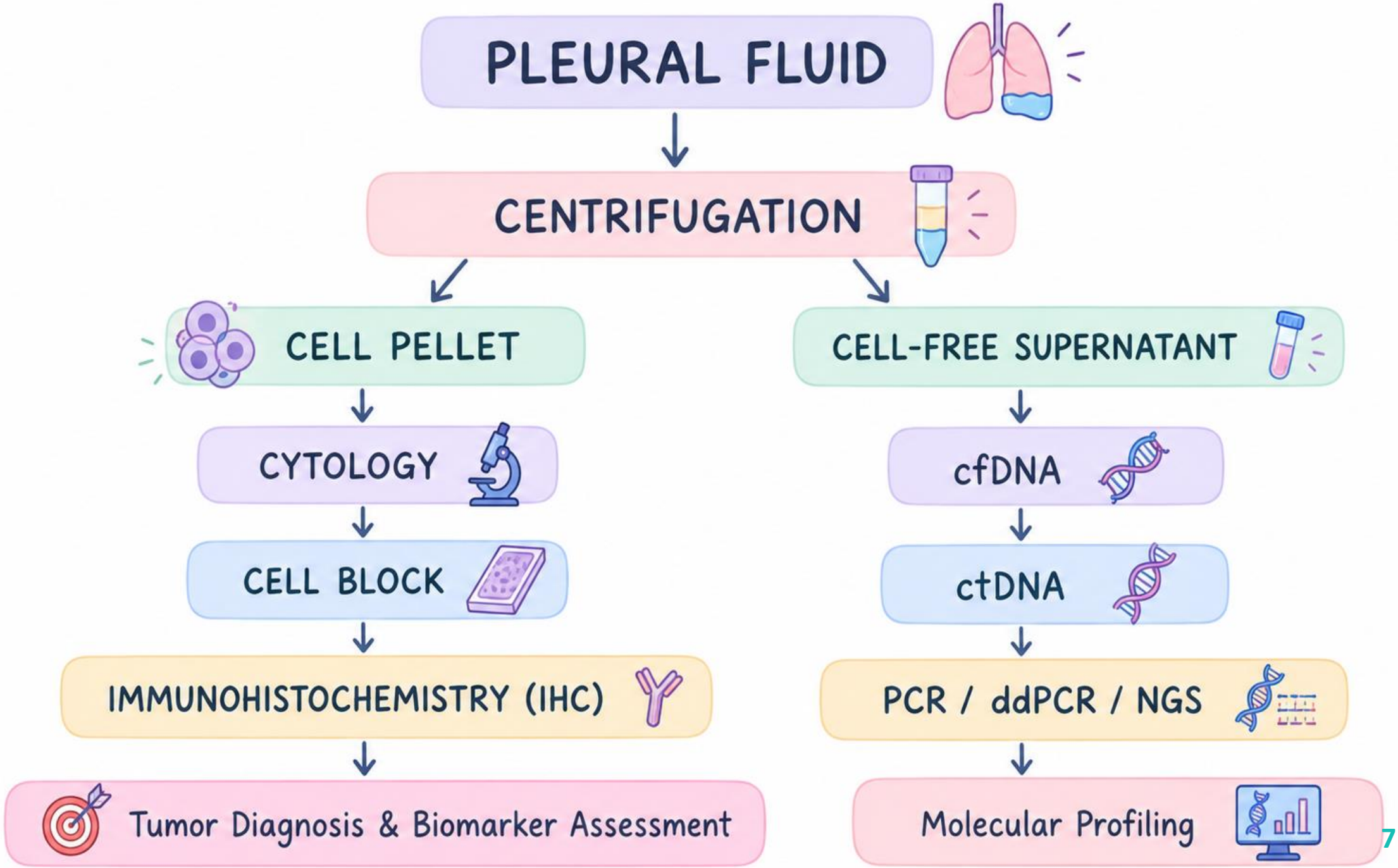
cfDNA / ctDNA

cfRNA / miRNA

Extracellular vesicles / exosomes

Methylation biomarkers

Protein biomarkers


PLEURAL FLUID
CENTRIFUGATION
CELL PELLET
CELL-FREE SUPERNATANT
CYTOLOGY
CELL BLOCK
IMMUNOHISTOCHEMISTRY (IHC)
cfDNA
ctDNA
PCR / ddPCR / NGS
Tumor Diagnosis & Biomarker Assessment
Molecular Profiling

Pleural fluid cytology from sample in lab



The high-value pulmonary scenario

78-year-old patient • lung mass • large pleural effusion

Dyspnea • multimorbidity • high risk for invasive biopsy

Clinical question: Can thoracentesis yield BOTH a diagnosis and actionable molecular data?



Pleural fluid: two diagnostic fractions

CELLULAR PELLET

Cytology → cell block → histology-supporting IHC and selected molecular tests.

Cells → Cell block → IHC

CELL-FREE SUPERNATANT

cfDNA / ctDNA → mutation and fusion profiling when validated locally.

Supernatant → ctDNA → NGS

Do not discard the supernatant before considering molecular testing.

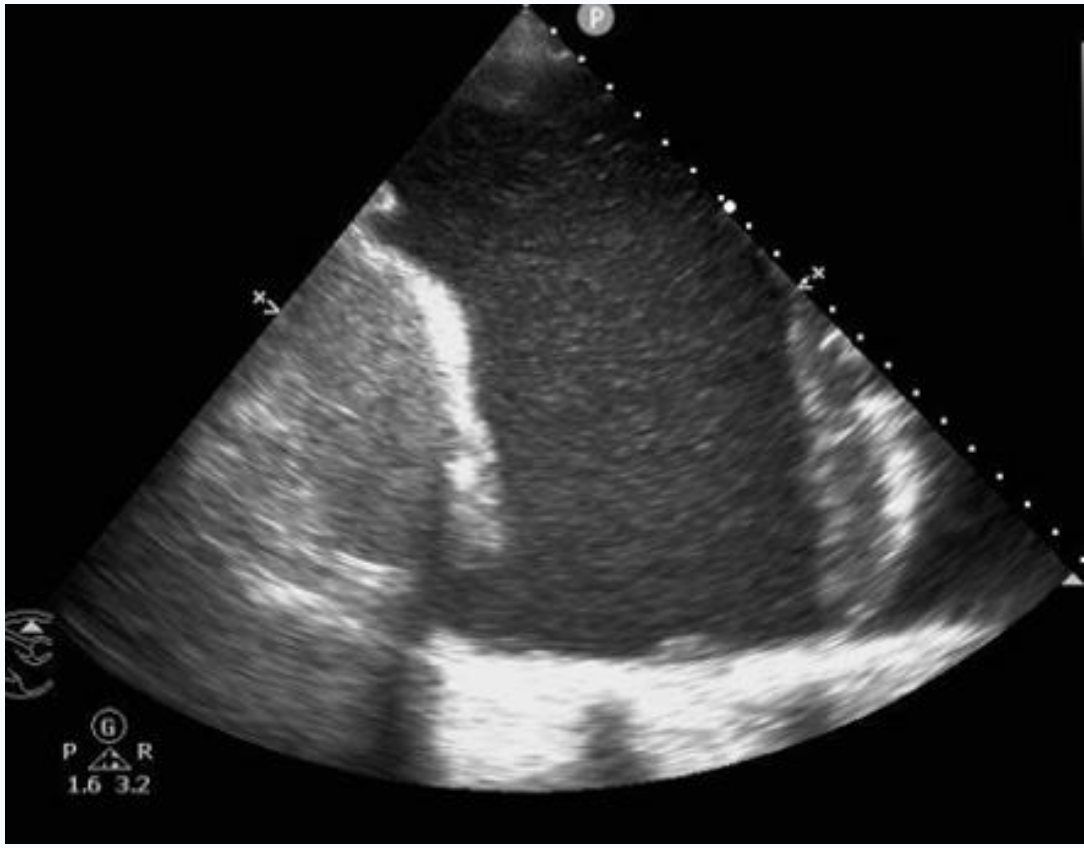
When is pleural fluid adequate?

Tumor cell content and tumor fraction

DNA/RNA yield, integrity and handling

Assay sensitivity, breadth and validation

Availability of cytology and cell-block material



“Adequate” is assay-specific—not a property of the specimen alone.

Pleural fluid versus plasma

Potential advantage

In malignant pleural effusion, tumor-proximal fluid may yield stronger tumor DNA signals.

Important caveat

Performance is heterogeneous; collection and tumor biology matter.

Practical implication

Use available pleural fluid strategically, alongside cytology and tissue assessment.

Tumor-proximal sampling can improve molecular yield in selected malignant effusions — not universally

Molecular profiling in NSCLC

DNA NGS: EGFR, KRAS, BRAF, MET, ERBB2 and other alterations

RNA NGS: particularly useful for selected fusions/rearrangements (ALK, ROS1, RET, NTRK)

Targeted PCR/ddPCR: rapid interrogation of known variants

NGS is a testing platform—not a liquid-biopsy specimen type.

What a positive result means

A validated, actionable alteration can guide treatment selection.

Confirm assay coverage, variant classification and clinical context.

Beware non-tumor sources of variants, including clonal hematopoiesis.

Why a negative result is different

Low tumor burden / low ctDNA shedding

Compartmentalized disease (including CNS-predominant disease)

Insufficient tumor fraction or limited assay coverage

Pre-analytical degradation / technical limits

NOT DETECTED ≠ NOT PRESENT → consider tissue or alternative specimen.

False positives: CHIP and artifacts

Clonal hematopoiesis can release variants into plasma unrelated to the lung tumor.

Also consider germline variants and sequencing artifacts.

Interpret molecular findings with pathology and the clinical phenotype.

LONGITUDINAL USE

At progression: re-profile biology



Identify acquired resistance • Integrate imaging and tissue when needed

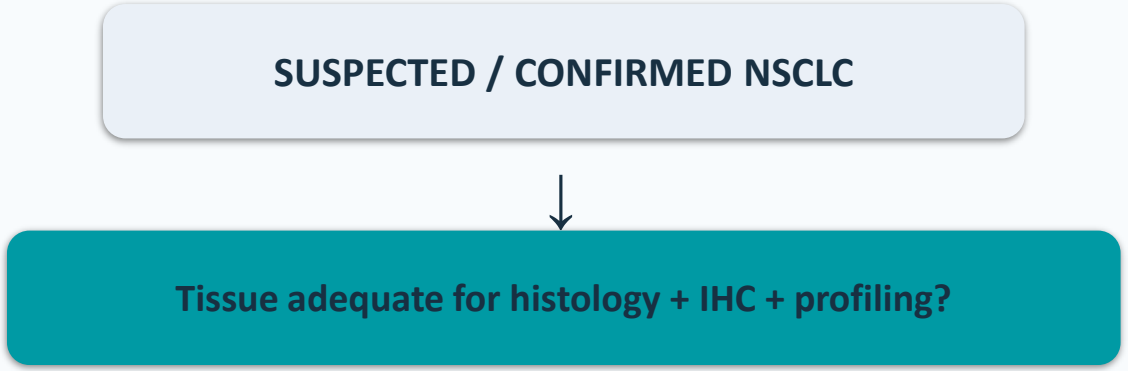
MRD and early detection

Post-treatment ctDNA detection can signal molecular residual disease and recurrence risk.

However, ctDNA-guided adjuvant changes and broad screening applications remain evolving—not interchangeable with established care.

DECISION PATHWAY

A practical pulmonary algorithm



YES → Tissue diagnosis + biomarker testing

NO / HIGH RISK → Plasma ctDNA ± pleural fluid

Integrate specimen availability, procedural risk and turnaround time.

Five take-home messages

1. Liquid biopsy is more than a blood test.
2. Pleural fluid can provide cellular AND molecular data.
3. Positive actionable findings can be treatment-informing.
4. Negative ctDNA does not exclude an alteration.
5. Multimodal sampling is the future of precision care.



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THANK YOU!

FOR YOUR ATTENTION

