Methylation, Proteomics and Other Efforts For Early Lung Cancer Detection

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Florida International University

Memorial Cancer Institute

CANCER CENTER of EXCELLENCE Florida HEALTH

Multiple Blood-Based MCED Tests in Development

- Standardized criteria not established (e.g., validity, benefit-risk, clinical utility)
- Study comparisons challenging (e.g., differences in eligibility, cancers targeted for detection, methodology, and performance metrics)

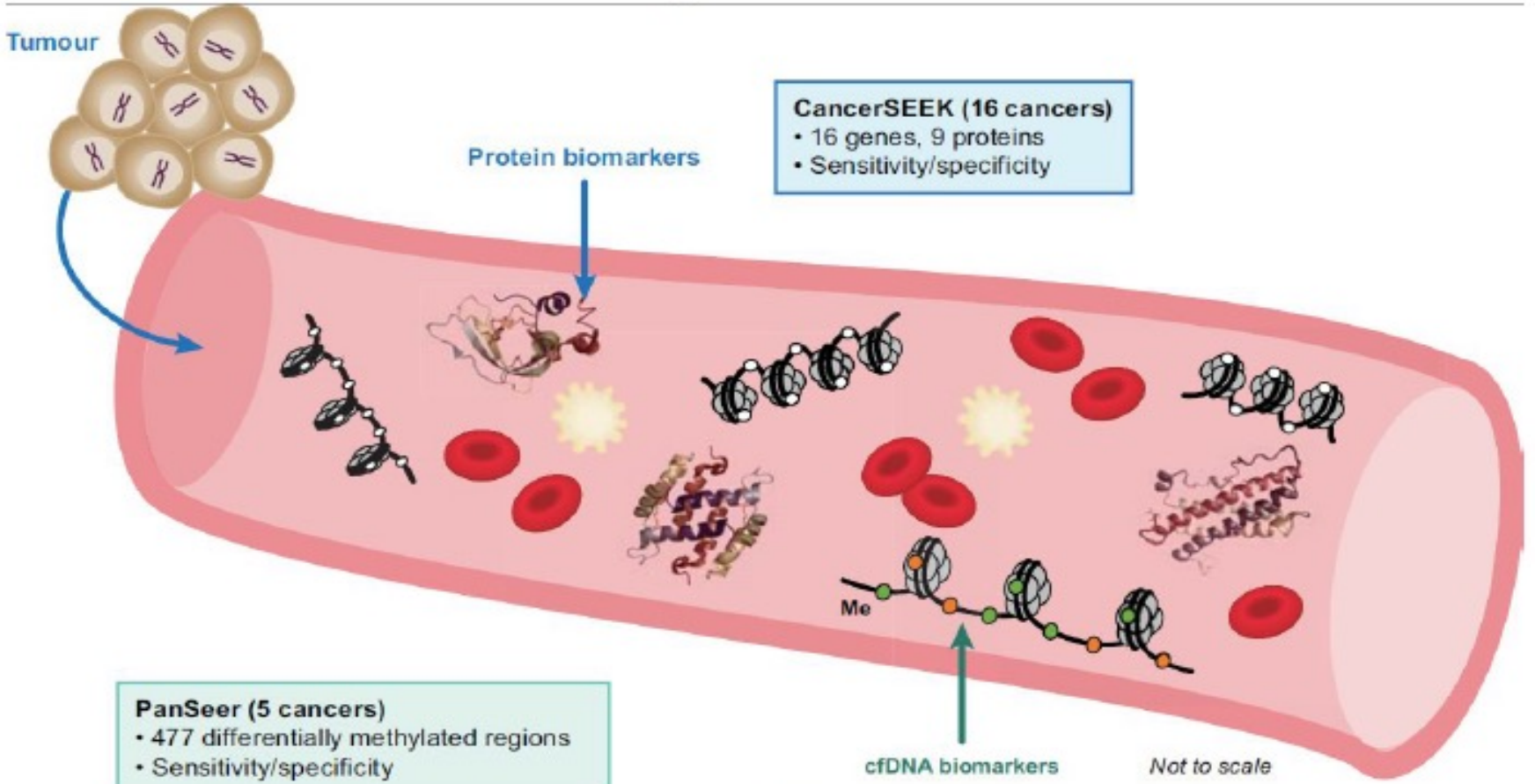
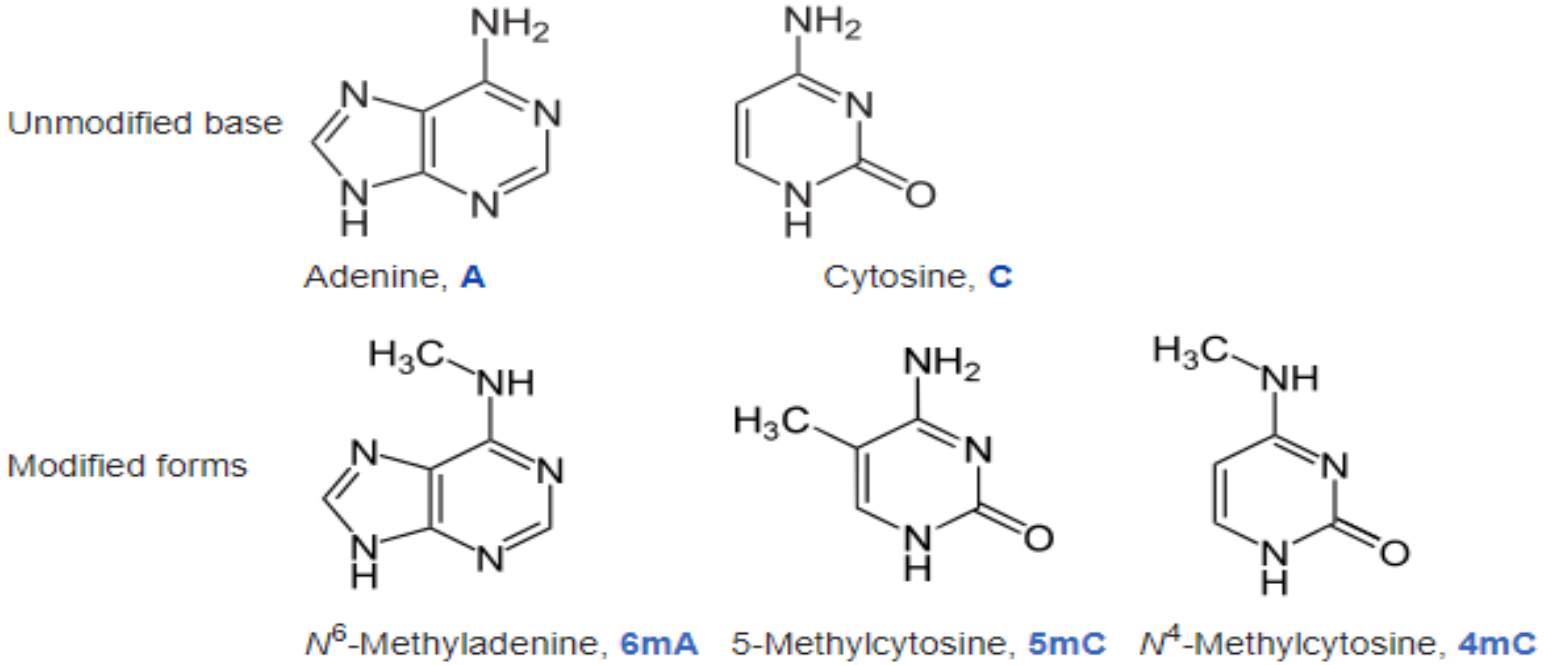


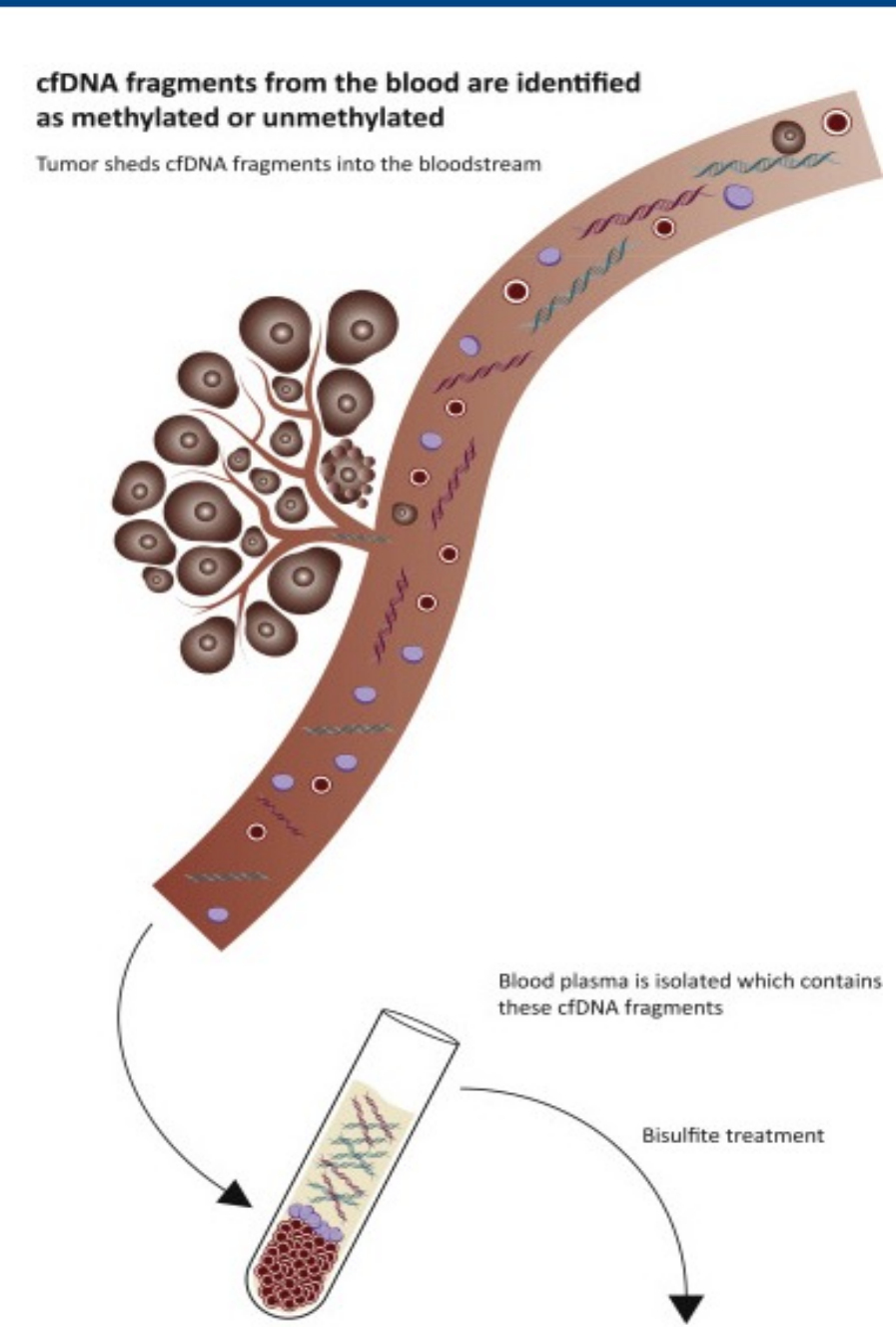
Figure adapted from Liu MC. *Br J Cancer*. 2021 Feb 9. DOI: 10.1038/s41416-020-01223-7.

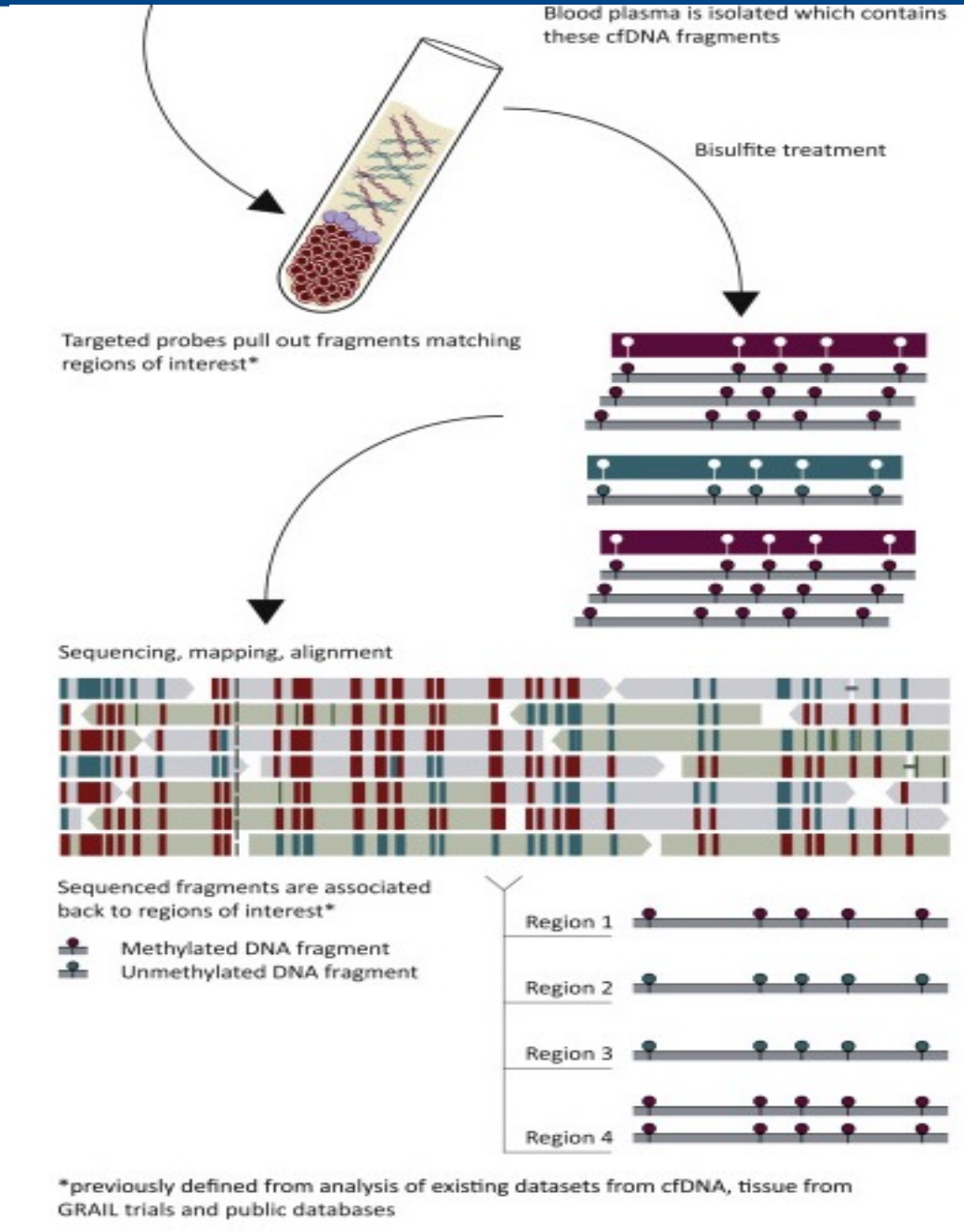
MCED, multi-cancer early detection; CSO, cancer signal origin.
Liu MC. *Br J Cancer*. 2021;124(9):1475-1477. doi: 10.1038/s41416-020-01223-7.

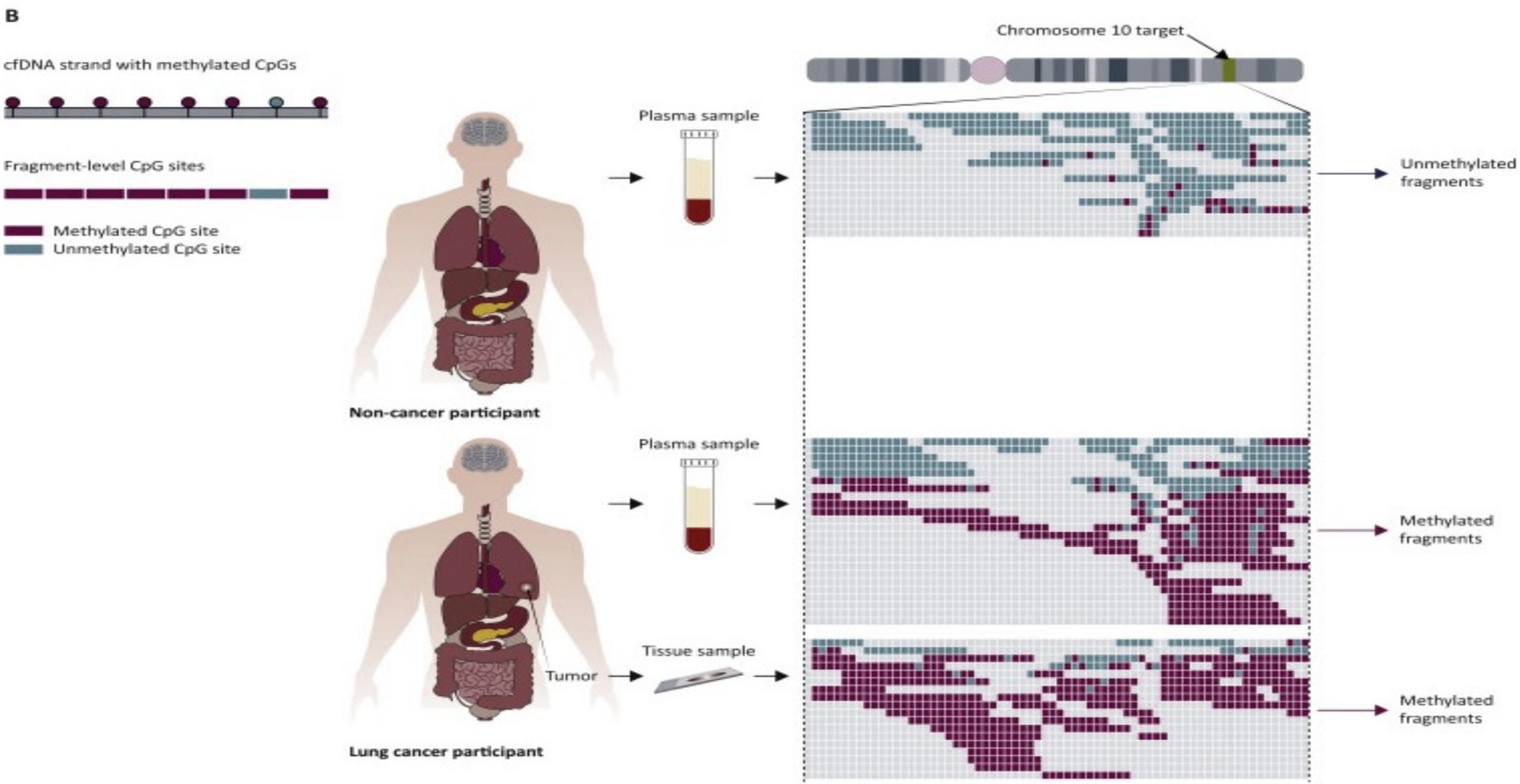
DNA Methylation

DNA methylation is an epigenetic mechanism that occurs by the addition of a methyl (CH_3) group to DNA, thereby often modifying the function of the genes and affecting gene expression. The most widely characterized DNA methylation process is the covalent addition of the methyl group at the 5-carbon of the cytosine ring resulting in 5-methylcytosine (5-mC), also informally known as the "fifth base" of DNA. These methyl groups project into the major groove of DNA and inhibit transcription




cfDNA fragments from the blood are identified as methylated or unmethylated
Tumor sheds cfDNA fragments into the bloodstream
Blood plasma is isolated which contains these cfDNA fragments
Bisulfite treatment


Blood plasma is isolated which contains these cfDNA fragments
Bisulfite treatment
Targeted probes pull out fragments matching regions of interest*
Sequencing, mapping, alignment
Sequenced fragments are associated back to regions of interest*
Methylated DNA fragment
Unmethylated DNA fragment
Region 1
Region 2
Region 3
Region 4
*previously defined from analysis of existing datasets from cfDNA, tissue from GRAIL trials and public databases


B
cfDNA strand with methylated CpGs
Fragment-level CpG sites
Methylated CpG site
Unmethylated CpG site
Chromosome 10 target
Plasma sample
Unmethylated fragments
Non-cancer participant
Plasma sample
Methylated fragments
Tissue sample
Tumor
Methylated fragments
Lung cancer participant

Galleri Test (Grail)

- Specificity for cancer signal detection was 99.5%
- Overall sensitivity for cancer signal detection was 51.5%; sensitivity increased with stage
  - Stage I: 16.8%, stage II: 40.4%, stage III: 77.0%, stage IV: 90.1%.
  - Stage I-III sensitivity was 67.6% in 12 pre-specified cancers that account for approximately two-thirds of annual USA cancer deaths
- Cancer signals were detected across >50 cancer types.
- Overall accuracy of CSO prediction in true positives was 88.7%

Klein JTO September 2021

The Circulating Cell-free Genome Atlas Study, sub-study 3: clinical validation

CCGA3: Cancer Signal Detection: Specificity and Overall Sensitivity

	Cancer (n=2823)	Non-cancer (n=1254)	Total (n=4077)
Test Positive	1453	6	1459
Test Negative	1370	1248	2618

Specificity:
99.5%
(95% CI: 99.0–99.8%)

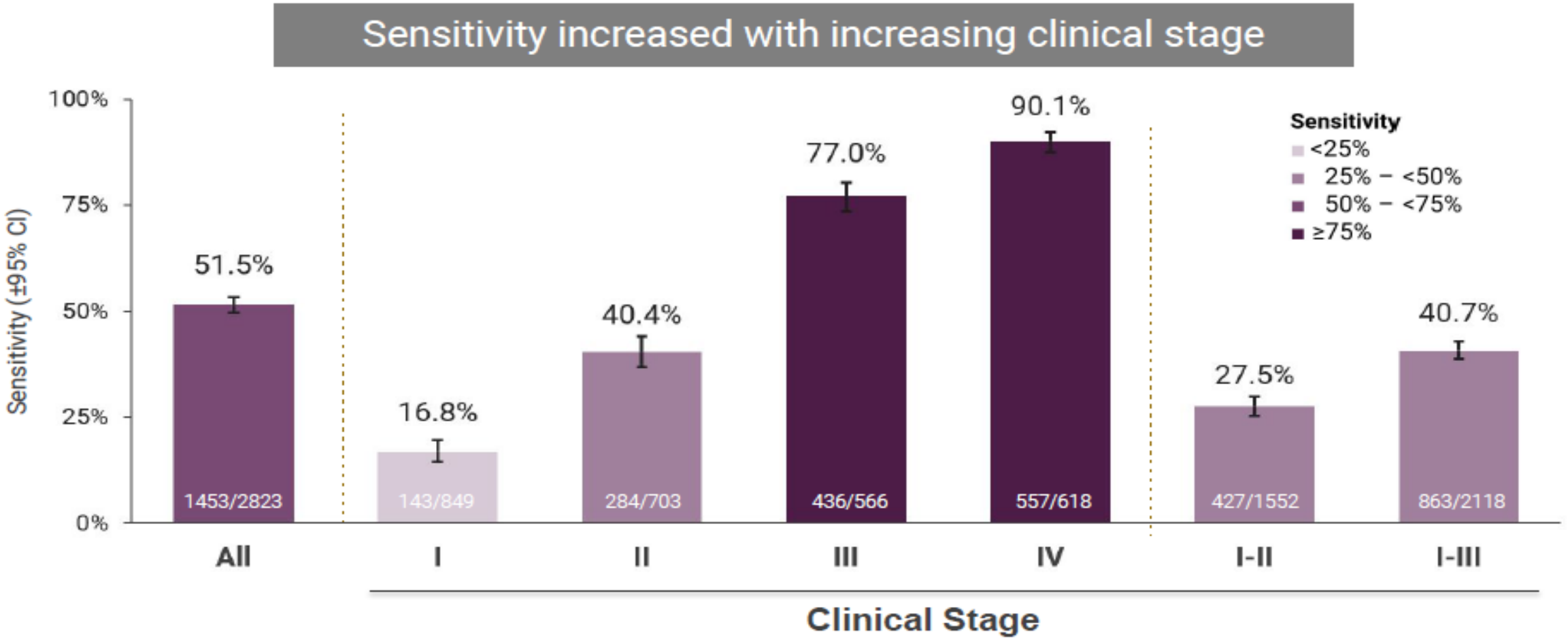
↓

0.5%
false-positive rate

Sensitivity:
51.5%
(95% CI: 49.6–53.3%)

Specificity indicates true negative rate. Sensitivity indicates true positive rate.
CI, confidence interval.
Klein E, et al. *Ann Oncol*. 2021;32(9):1167-1177. DOI: 10.1016/j.annonc.2021.05.806.

CCGA3: Sensitivity of Cancer Signal Detection by Clinical Stage

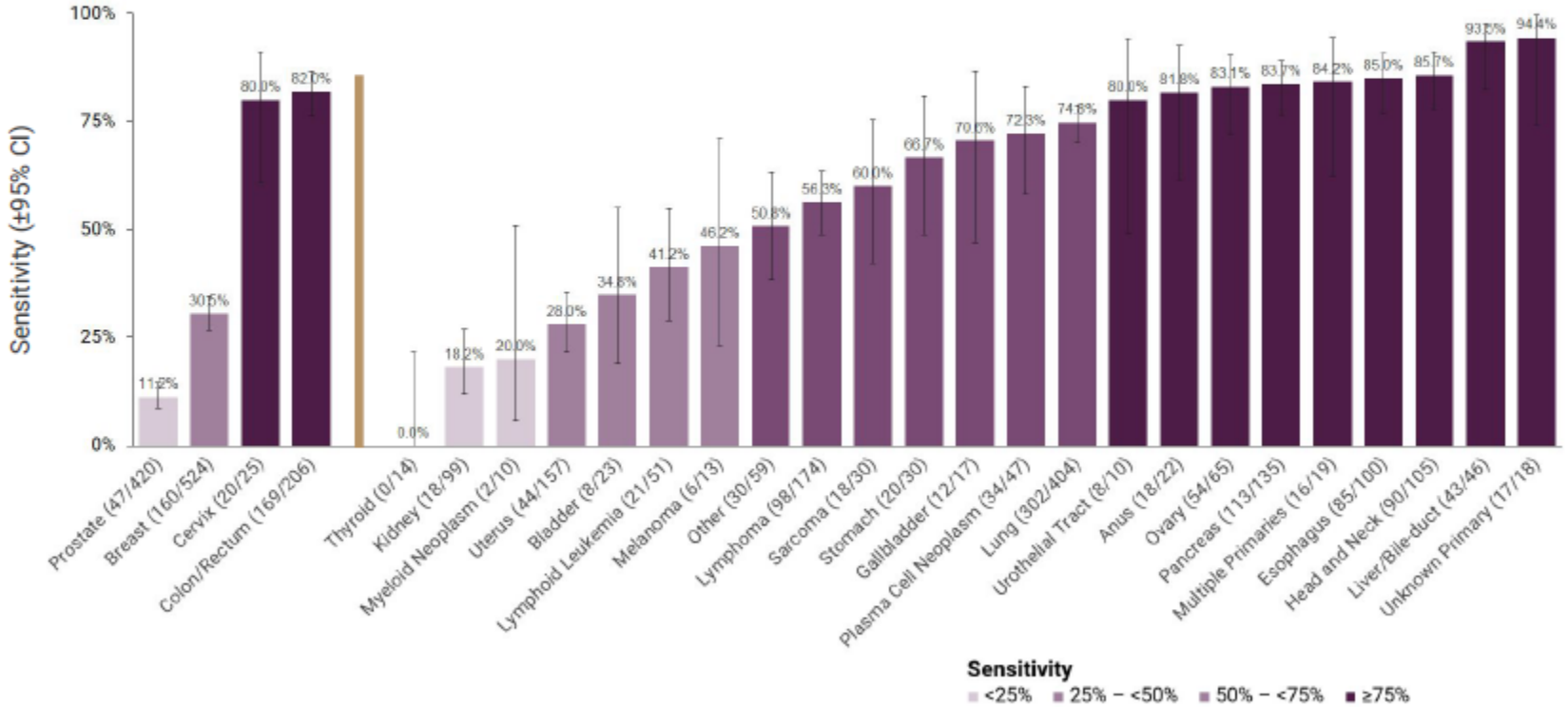


"All" comprises all cancer stages, including missing stage and cancer classes that do not have staging per American Joint Committee on Cancer (AJCC) staging manual.
CI, confidence interval.
Klein E, et al. *Ann Oncol*. 2021;32(9):1167-1177. DOI: 10.1016/j.annonc.2021.05.806.

CCGA3: Sensitivity of Cancer Signal Detection in Cancers With and Without Common Screening

With Common Screening Options:
33.7% (95% CI: 31.1–36.5%)

Without Common Screening Options:
63.8% (95% CI: 61.4–66.1%)



For multiple primaries, highest clinical stage was selected.
CI, confidence interval.
Klein E, et al. *Ann Oncol*. 2021;32(9):1167-1177. DOI: 10.1016/j.annonc.2021.05.806.

TABLE 4. Galleri Test Trials Summary[26-29]

Trial name	Status	Estimated completion	Trial design	Purpose	Participants
CCGA	Active, not recruiting	March 2024	Prospective, observational, longitudinal	Characterize the cfDNA in the blood of patients with cancer and without cancer	15,254 participants ≥20 years across 141 sites in the United States and Canada
STRIVE	Active, not recruiting	May 2025	Prospective, observational, longitudinal, cohort	Validate the test for early detection of cancer	99,481 women ≥18 years at time of mammogram screening across 35 sites in the United States
SUMMIT	Enrolling by invitation	August 2030	Prospective, observational, longitudinal, cohort	Validate the test by measuring cancer incidence	Estimated 50,000 participants 50-77 years without a cancer diagnosis, but with variable risks for cancer (specifically lung) at enrollment from London, United Kingdom
PATHFINDER	Recruiting	January 2022	Prospective clinical trial cohort	Evaluate implementation of test in clinical practice	Estimated 6200 participants ≥50 years, split into elevated risk group and nonelevated risk group

26. The Circulating Cell-free Genome Atlas Study (CCGA). ClinicalTrials.gov. Updated August 31, 2020. Accessed October 19, 2020. clinicaltrials.gov/ct2/show/NCT02889978?term=NCT02889978&draw=2&rank=1
27. The STRIVE Study: Development of a Blood Test for Early Detection of Multiple Cancer Types. ClinicalTrials.gov. Updated July 31, 2020. Accessed October 19, 2020. clinicaltrials.gov/ct2/show/NCT03085888?term=NCT03085888&draw=2&rank=1
28. The SUMMIT Study: A Cancer Screening Study. ClinicalTrials.gov. Updated May 2, 2019. Accessed October 19, 2020. clinicaltrials.gov/ct2/show/NCT03934866?term=NCT03934866&draw=2&rank=1clinicaltrials.gov/ct2/results?cond=&term=NCT03934866&cntry=&state=&city=&dist=
29. Assessment of the Implementation of an Investigational Multi-Cancer Early Detection Test Into Clinical Practice. ClinicalTrials.gov. Updated August 5, 2020. Accessed October 19, 2020. clinicaltrials.gov/ct2/show/NCT04241796?term=NCT04241796&draw=2&rank=1

REFLECTION

A prospective, observational study of patients administered the MCED test as part of their medical care in a real-world setting

Study Objectives

Purpose of the Study

- To understand MCED test performance in clinical settings
- To understand MCED test impact on patients and healthcare providers

Primary Objective

- To assess the performance of MCED in a real-world setting

Study Design

~35,000 adult participants[a] recruited over 24 months → **Blood drawn and processed[b]** → **Initial MCED test results to providers** → **Providers share test results with patients** → **Clinical follow up** performed at provider's discretion

Active data capture[c] (up to 5 years from enrollment): MCED test result(s), diagnostic/treatment data, patient-reported outcomes

Passive data capture (after active phase)[d]: linkage to cancer registries and vital statistics; participant screening continued per standard of care

First patient first visit was August 23, 2021.

[a]Participants will be recruited to an intervention arm; there will also be an external control arm. [b]Participants may receive subsequent MCED test(s) post baseline. [c]Patients actively followed through medical record data collection and self-reported questionnaires administered pre-test, post-test, 6- and 12-months post consent, and annually for 5 years. [d]Patients passively followed through linkages to cancer registries and other administrative health databases up to time of death, loss to follow-up, withdrawal of informed consent, or per institutional guidelines on duration of data collection, whichever occurs sooner.

MCED, multi-cancer early detection.

1,000 volunteers to be enrolled at Memorial Healthcare System!

Memorial Cancer Institute

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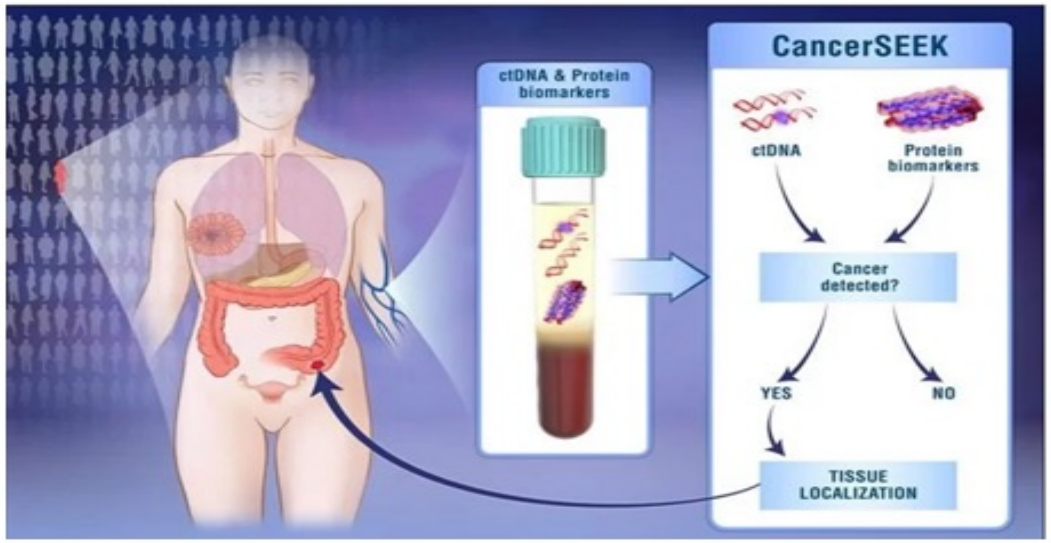
(Cancerseek, Thrive-Exact Sciences)

Science

Detection and localization of surgically resectable cancers with a multi-analyte blood test



Cohen et al. Science 2018

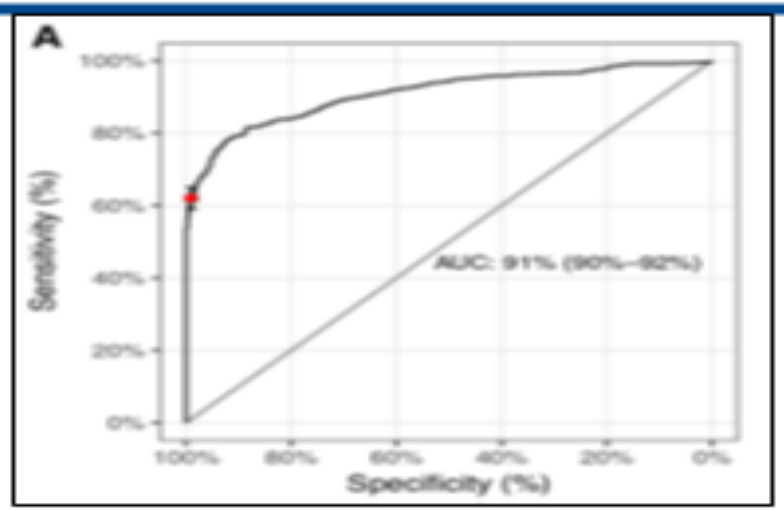
Multiplex PCR analysis of circulating cell-free tumour DNA (ctDNA) enables the detection of mutations at 2,001 genomic positions across 16 genes, whereas levels of the protein biomarkers are assessed using immunoassays.

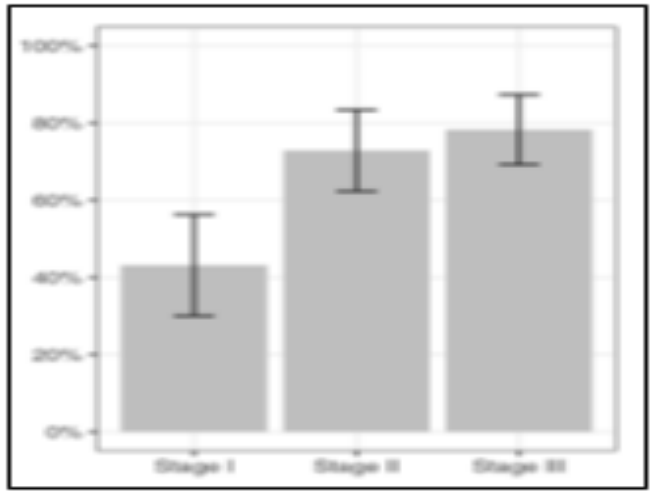
The eight proteins are:

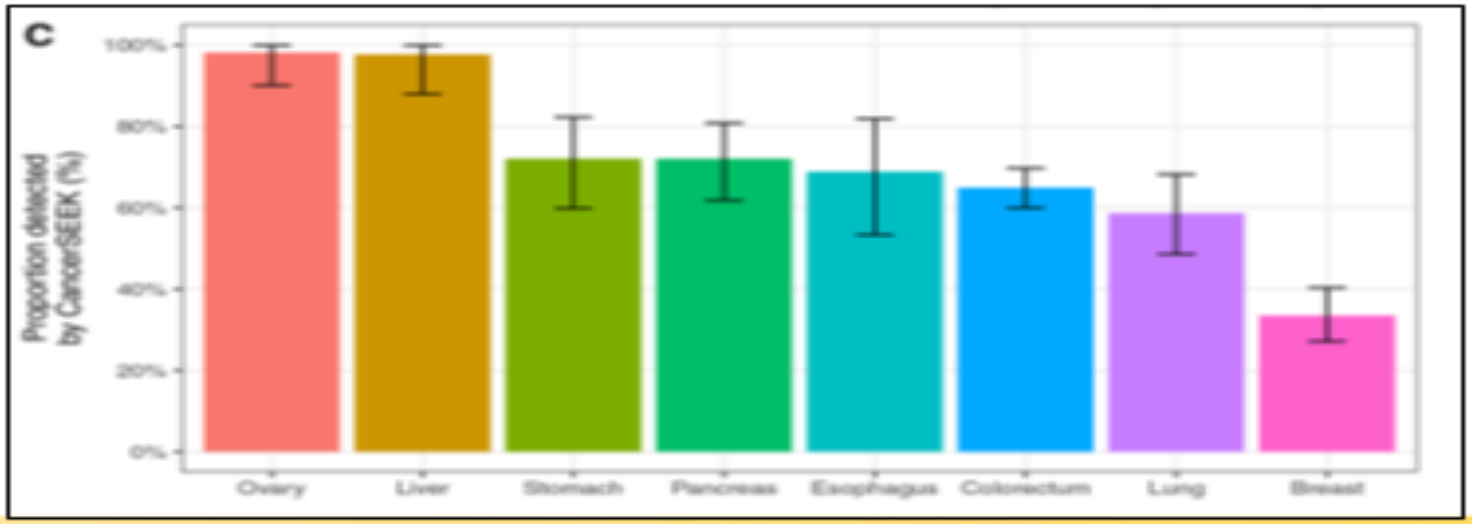
cancer antigen 125, carcinoembryonic antigen, cancer antigen 19-9, prolactin, hepatocyte growth factor, osteopontin, myeloperoxidase, and tissue inhibitor of metalloproteinases 1.

Science

Detection and localization of surgically resectable cancers with a multi-analyte blood test







- N=1005 patients with eight different types of clinically detected cancer (early stage)
- N=812 healthy controls
- Platform: cfDNA (DNA in regions of interest from 16 genes) and proteins (39)
- Median Sensitivity: 70%
- Median Specificity: 99%

Cohen et al. Science 2018

Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention (Cancerseek, Thrive-Exact Sciences)

- blood test that detects ctDNA mutations in 16 genes and 8 protein biomarkers of cancer in a prospective, interventional study of 10,006 women who were 65 to 75 years old and who had no prior history of cancer. Positive blood tests were followed by PET-CT.
- Detection: Of 96 cancers incident during the study period, 26 were first detected by blood testing and 24 additional cancers by conventional screening. Fifteen of the 26 patients in whom cancer was first detected by blood testing underwent PET-CT imaging, and 11 patients developed signs or symptoms of cancer after the blood test that led to imaging procedures other than PET-CT.
- Specificity and PPV of blood testing alone were 98.9% and 19.4%, respectively, and combined with PET-CT, specificity and PPV increased to 99.6% and 28.3%.

- The blood test first detected 14 of 45 cancers (31%) in seven organs for which no standard-of-care screening test is available.
- Of the 26 cancers first detected by blood testing, 17 (65%) had localized or regional disease. Of the 15 participants with positive blood tests as well as positive PET-CT scans, 9 (60%) underwent surgery with curative intent.
- Blood testing could be combined with conventional screening, leading to detection of more than half of the total incident cancers observed during the study period. Blood testing did not deter participants from undergoing mammography, and surveys revealed that 99% of participants would join a similar, subsequent study if offered.
- Only 0.22% underwent an unnecessary invasive diagnostic procedure as a result of a false-positive blood test.

Lennon, AM. Vol 369, issue 6499, April 2020

TABLE 3. CancerSEEK Trials Summary[23,24]

Trial name	Status	Estimated completion	Trial design	Purpose	Participants
DETECT-A[a]	Complete	--	Prospective, interventional	Identify multiple cancer types using test	10,006 women 65-75 years old with no history of cancer
ASCEND	Recruiting	June 2020	Prospective, observational, cohort	Validate test	Estimated 3000 participants ≥50 years; 1000 with a cancer diagnosis and 2000 with no prior history of cancer in the United States

[a]Trial is not registered on clinicaltrials.gov; publication results used for summary.

Lennon AM, Buchanan AH, Kinde I, et al. Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention. Science. 2020;369(6499):eabb9601. doi: 10.1126/science.abb9601

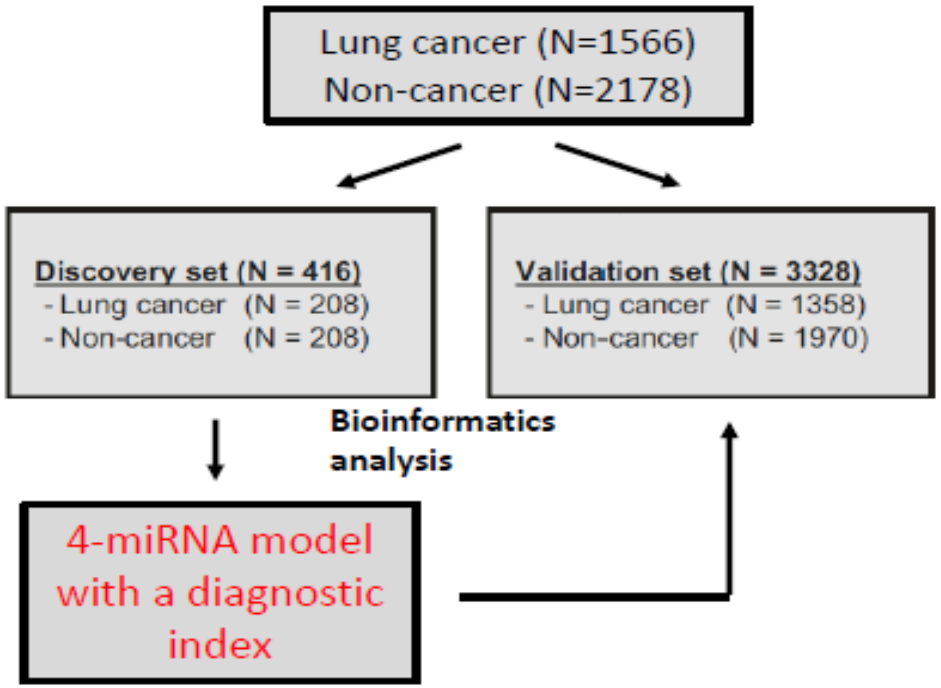
Detecting Cancers Earlier Through Elective Plasma-based CancerSEEK Testing (ASCEND). ClinicalTrials.gov. Updated January 14, 2020. Accessed October 19, 2020. clinicaltrials.gov/ct2/show/NCT04213326?term=NCT04213326&draw=2&rank=1

A Novel Blood-Based microRNA Diagnostic Test With Very High Accuracy For Early Lung Cancer Screening And Monitoring

Andrew Zhang[1] and Hai Hu[2]

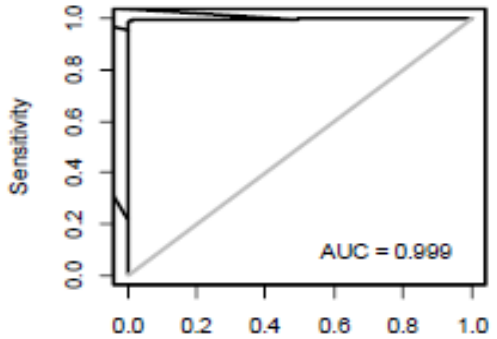
[1]Del Norte High School, San Diego, CA;
[2]Chan Soon-Shiong Institute of Molecular Medicine at Windber, Windber, PA USA

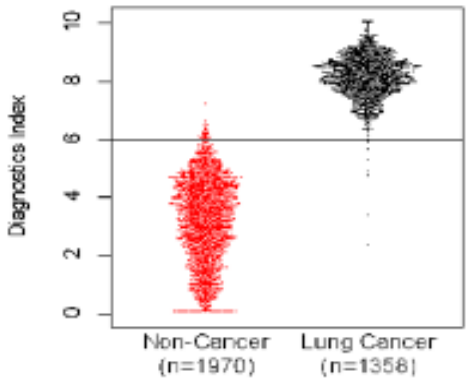
The New 4-miRNA Diagnostic Model: Comparison with the Published 2-miRNA Model



- The new 4-miRNA diagnostic model had superior performance (ROC, sensitivity & specificity) than the original published model, reaching nearly perfect accuracy in detecting lung cancer

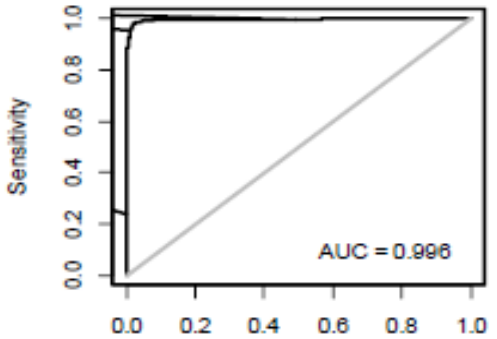
NEW 4-miRNA Model

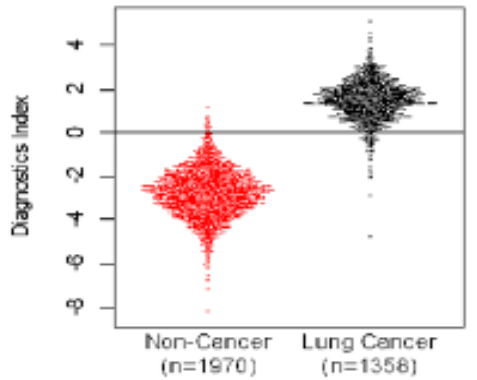




Sensitivity: 99%
Specificity: 99%

Published 2-miRNA Model





Sensitivity: 95%
Specificity: 99%

TAKE HOME MESSAGE

- A novel **blood based** diagnostic model based on expression of 4 miRNAs has been developed that showed **extraordinary accuracy** (99% sensitivity and specificity), and especially demonstrated >99% sensitivity in detecting early stage lung cancer such as stage 1 cancer
- The performance of the new diagnostic model is superior to that of the original published model in all clinically relevant patient subgroups. The magnitude of improvement is clinically important to minimize false negatives if the model will be developed into a test to target large population size.
- The 4 miRNAs are likely tumor derived, and therefore the novel diagnostic test may also have the potential to be a tumor recurrence monitoring test.

- **Acknowledgement:** I want to thank the original study authors for making the data publicly available, and would like to thank all the patients and participants who donated blood to this important study.

IASLC
INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER
IASLC 19th World Conference on Lung Cancer
September 23–26, 2018 Toronto, Canada
WCLC2018.IASLC.ORG
#WCLC2018

Blood Based Biomarkers: RNA, KRAS and PD-L1 Strongly Matching with Tissue and Showing Correlation with Clinical Responses In NSCLC Patient's

Luis E. Raez[1], Joshua Usher[2], Cheryl Habaue[1], Kathleen Danenberg[2], Brian Hunis[1], Yolanda Jaimes[2], Shahrooz Rabizadeh[2], Aurelio Castrellon[1], Peter Danenberg[3];

[1]Memorial Cancer Institute/Florida International University, FL/USA
[2]Liquid Genomics/Nanth Health, Torrance, CA/USA
[3]University of Southern California/USA

760 samples from patients with metastatic disease were enrolled.

44 gastric cancers (GC), 212 colorectal cancers (CRC), 320 non-small cell lung cancers (NSCLC), 24 breast cancers (BC), and 88 prostate cancers (PC).

Patient samples were from USC Norris Comprehensive Cancer Center, Memorial Cancer Institute or University Hospital Essen, West German Cancer Center.

Ten milliliters of blood were collected in each of two tubes containing a proprietary nucleic acid preservation cocktail and transferred to Liquid Genomics, Inc.

Table 1. Detection frequencies and relative values of PD-L1 gene expression in plasma from patients with various cancer types and in cancer free individuals

Plasma from individuals with	Detection frequency (%)	Detection frequency by IHC (ref.)	P values for cancer-healthy difference
No cancer	0.0 (0/19)	-	-
Gastric Ca.	31.8 (14/44)	29.6 (24)	0.006
CRC	44.8 (96/212)	25.8 (25)	<0.001
NSCLC	63.8 (204/320)	25.0(26)	<0.001
Breast Ca.	25.0 (24/96)	56.6 (27)	0.012
Prostate Ca.	23.9 (21/88)	52.2 (28)	0.022
All cancer	47.2 (359/760)	-	<0.001

T. Ishiba, (LE Raez), et al. Frequencies and expression levels of programmed death ligand 1(PD-L1) in circulating tumor RNA (ctRNA) in various cancer types. Biochem Biophys Res Commun. 2018 Jun 7;500(3):621-625.

Raez *et al. Cancer Drug Resist* 2021;4:1060-70
DOI: 10.20517/cdr.2021.78

Cancer
Drug Resistance

Original Article

Open Access

Using cfRNA as a tool to evaluate clinical treatment outcomes in patients with metastatic lung cancers and other tumors

Luis E. Raez[1], Kathleen Danenberg[2], Daniel Sumarriva[1], Joshua Usher[2], Jacob Sands[3], Aurelio Castrellon[1], Pablo Ferraro[1], Adriana Milillo[1], Eric Huang[4], Patrick Soon-Shiong[2], Sandeep Reddy[2], Peter Danenberg[5]

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[2]Nanth Health, Culver City, 2040 E Mariposa Ave, El Segundo, CA 90245, USA.
[3]Thoracic Oncology Program, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02215, USA.
[4]Burning Rock, Beijing 100022, China.
[5]Department of Biochemistry and Molecular Medicine, University of Southern California, Los Angeles, CA 90089, USA.

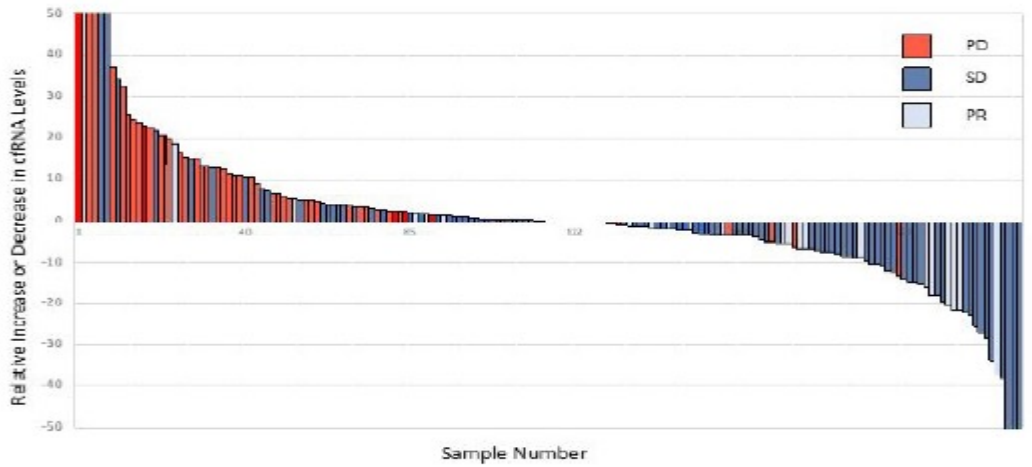


Figure 5. Changes in total cfRNA *vs.* outcome in response to various therapies across different tumor types. The bars represent analyses of 154 BC, 84 CRC, 135 NSCLC patient samples. BC: Breast cancer; CRC: colorectal cancer; NSCLC: non-small cell lung cancer.

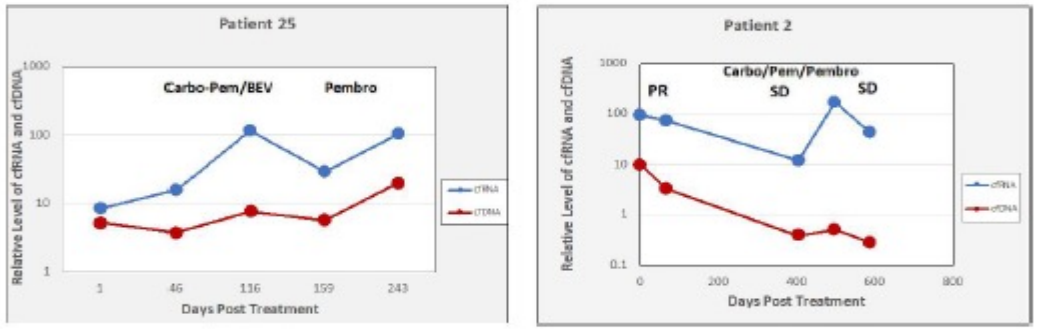


Figure 3. Changes in levels of cfRNA and cfDNA during therapy. cfRNA and cfDNA were measured at initiation of therapy and at various times during the chemotherapy using PCR amplification of B-actin. Treatment efficacy was determined by CT scans. PCR: Polymerase chain reaction.

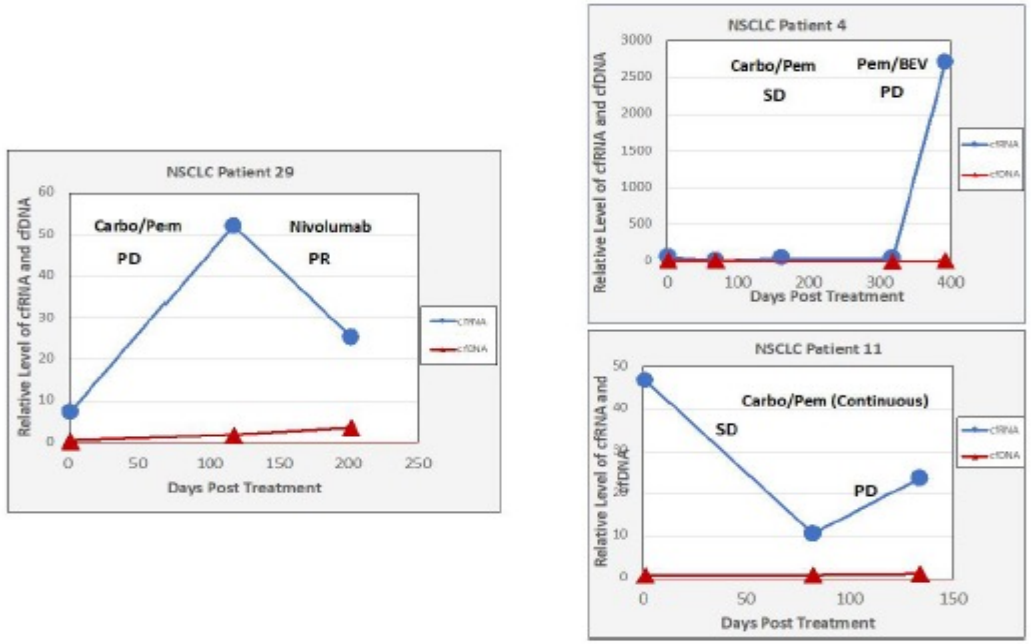
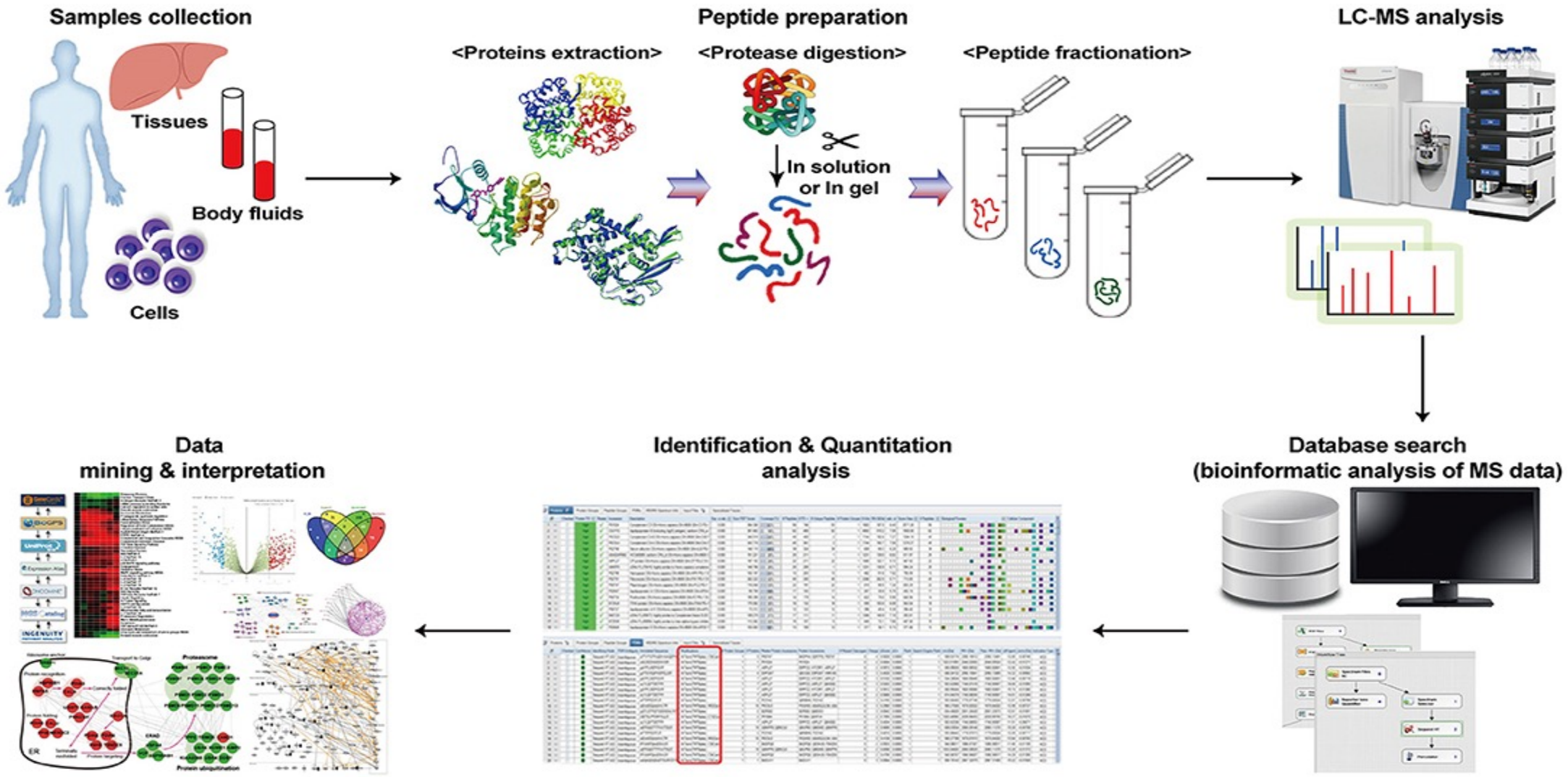


Figure 4. Changes in cfRNA levels during therapy when cfDNA is unmeasurable. cfRNA and cfDNA were measured at initiation of treatment and at various times during chemotherapy using PCR amplification of B-actin. Treatment efficacy of chemotherapy regimens was determined by CT scans. PCR: Polymerase chain reaction.

Proteomics

- Proteomics is the study of the entire set of proteins expressed in a cell, tissue, or individual.
- With the advent of mass spectrometry (MS)-based protein analysis technology, large-scale protein analysis has now become widely used.
- Proteomics involves a wide range of processes such as protein expression profiling, protein modifications, protein-protein interactions, protein structure, and protein function. The results obtained from such tasks can be used to decipher disease processes, provide diagnosis and prognosis of diseases, aid in drug development, and deliver the basis for biological discovery.
- With the development of proteomics technology and its application to various diseases, especially cancer, significant progress has been made in identifying clinically applicable biomarkers and new therapeutic targets.

Proteomics


Samples collection
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Body fluids
Cells
Peptide preparation
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or In gel
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LC-MS analysis
Database search
(bioinformatic analysis of MS data)
Identification & Quantitation
analysis
Data
mining & interpretation

Advances in Respiratory Medicine 2021, vol. 89, no. 4, pages 419–426

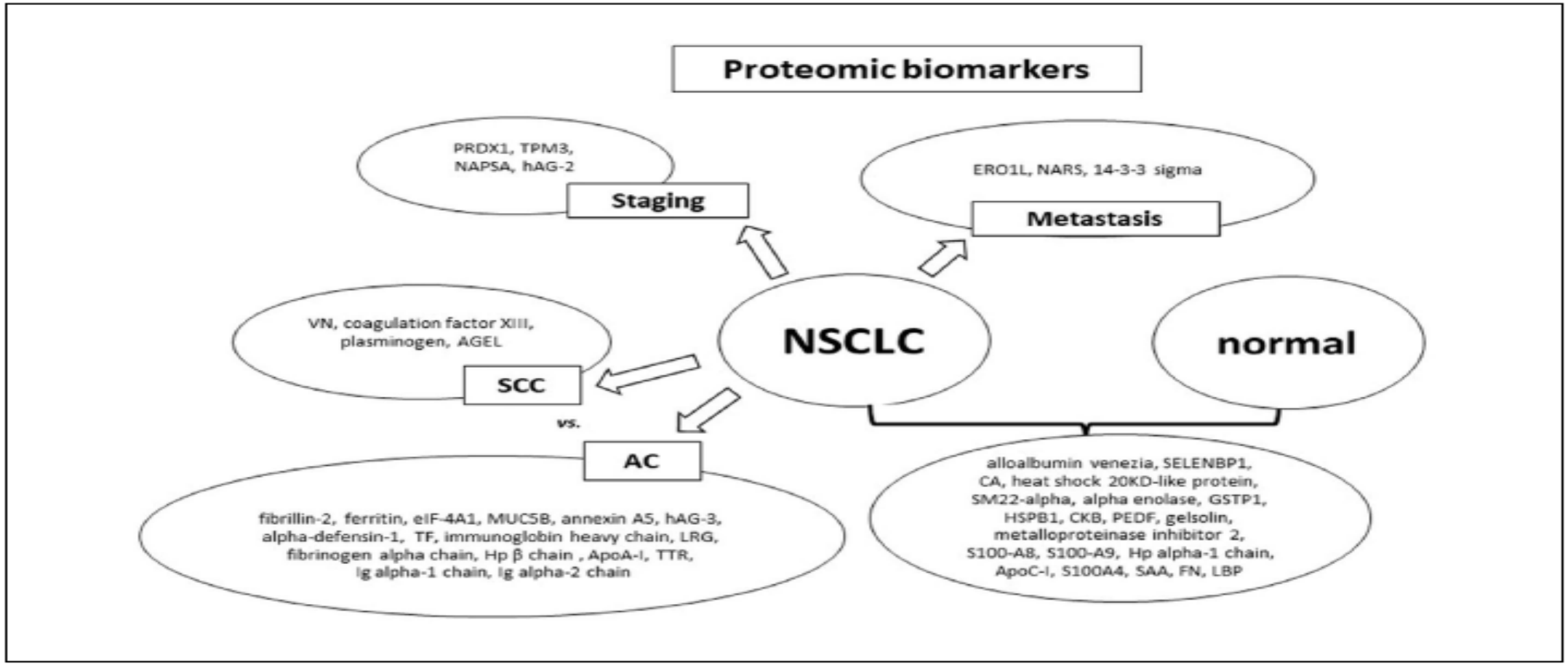

Figure 1. Proteomic biomarkers of NSCLC patients

AC — adenocarcinoma; AGEL — gelsolin; CA — carbonic anhydrase; CKB — creatine kinase brain-type; eIF-4A1 — eukaryotic translation initiation factor 4A1; ERO1L — ERO1-like protein alpha; FN — fibronectin; GSTP1 — glutathione S-transferase P1; hAG-2 — anterior gradient protein 2 homolog; hAG-3 — anterior gradient protein 3; Hp — haptoglobin; HSPB1 — heat shock protein beta-1; LBP — lipopolysaccharide binding protein; LRG — leucine-rich alpha-2-glycoprotein; MUC5B — mucin-5B; NAPSA — napsin-A; NARS — asparagine-tRNA ligase; NSCLC — non-small cell lung cancer; PEDF — pigment epithelium-derived factor; PRDX1 — peroxiredoxin 1; SAA — serum amyloid A; SCC — squamous cell carcinoma; SELENBP1 — selenium-binding protein 1; TF — transferrin; TPM3 — tropomyosin alpha-3 chain; TTR — transthyretin; VN — vitronectin


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