SESSION V: ctDNA AND LIQUID BIOPSY
Thursday, 27 November 2025
17:30 - 17:45 hours

GECP
lung cancer
research

16th
CONGRESS
Lung ON
CANCER

BARCELONA
27 / 28
NOVEMBER 2025

NEW DEVELOPMENTS IN LIQUID BIOPSY

Dr. Valeria Denninghoff

DECLARATION OF COMPETING INTEREST

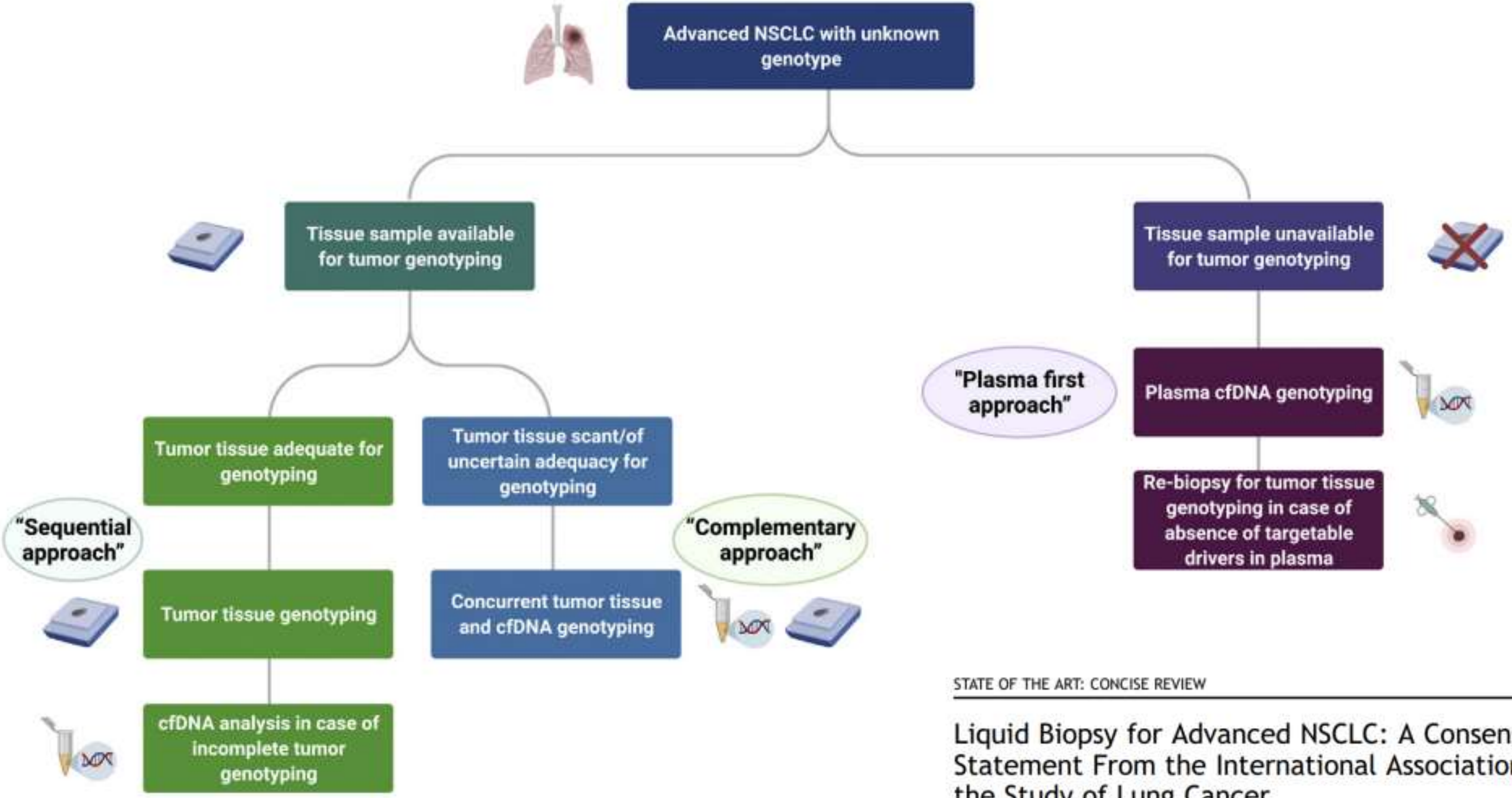
- Collaborative project with AstraZeneca and Miltenyi
- Research funding from Illumina and Tempus
- Educational activities supported by Pfizer
- Oncomine reviewer and research funding from Thermo Fisher Scientific

NEW DEVELOPMENTS IN LIQUID BIOPSY

☐ Clinical approach
☐ Technical approach
☐ Sample approach

Diagnostic algorithm for liquid biopsy use in treatment-naive advanced/metastatic NSCLC

GECP lung cancer research



Advanced NSCLC with unknown genotype

Tissue sample available for tumor genotyping

Tumor tissue adequate for genotyping

"Sequential approach"

Tumor tissue genotyping

cfDNA analysis in case of incomplete tumor genotyping

Tumor tissue scant/of uncertain adequacy for genotyping

"Complementary approach"

Concurrent tumor tissue and cfDNA genotyping

Tissue sample unavailable for tumor genotyping

"Plasma first approach"

Plasma cfDNA genotyping

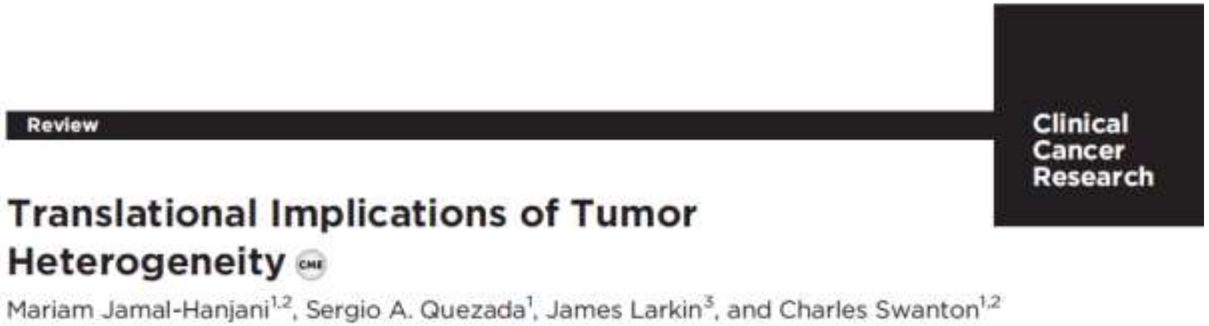
Re-biopsy for tumor tissue genotyping in case of absence of targetable drivers in plasma

IASLC

STATE OF THE ART: CONCISE REVIEW

Liquid Biopsy for Advanced NSCLC: A Consensus Statement From the International Association for the Study of Lung Cancer

Check for updates

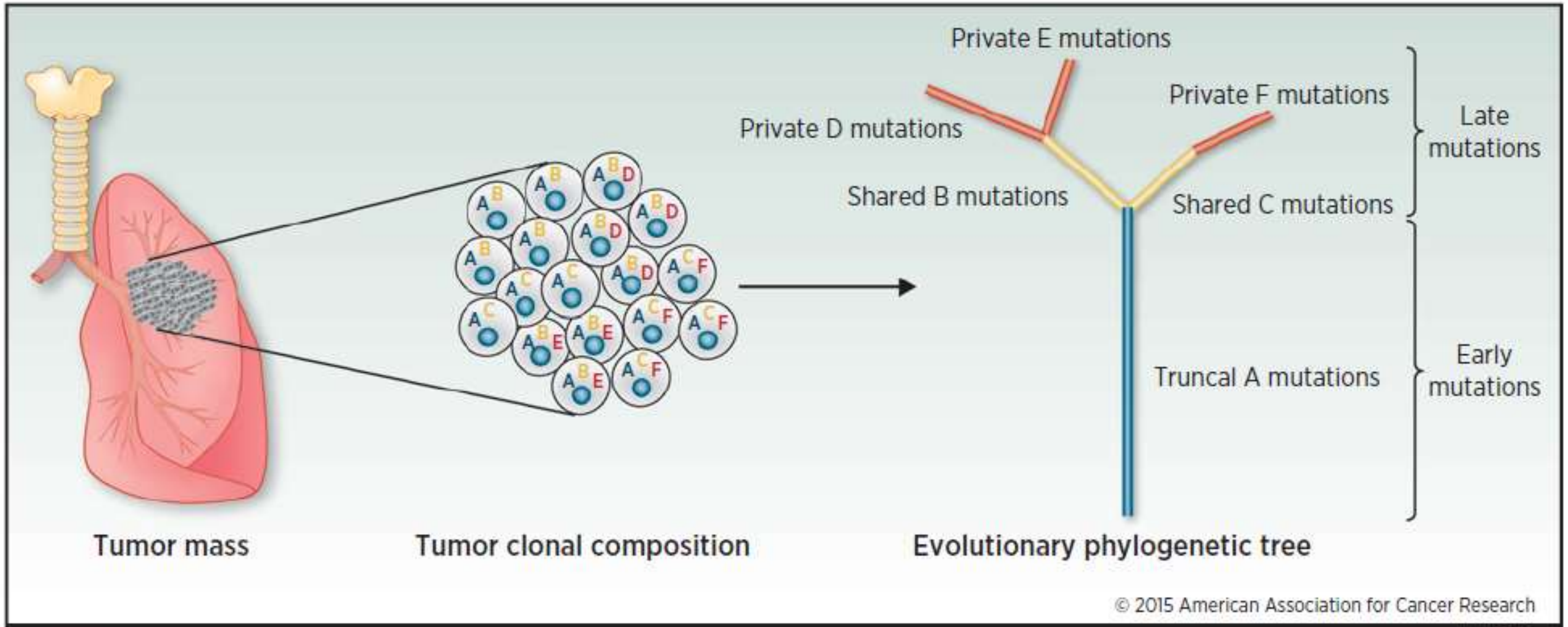


Review

Clinical Cancer Research

Translational Implications of Tumor Heterogeneity

Mariam Jamal-Hanjani[1,2], Sergio A. Quezada[1], James Larkin[3], and Charles Swanton[1,2]



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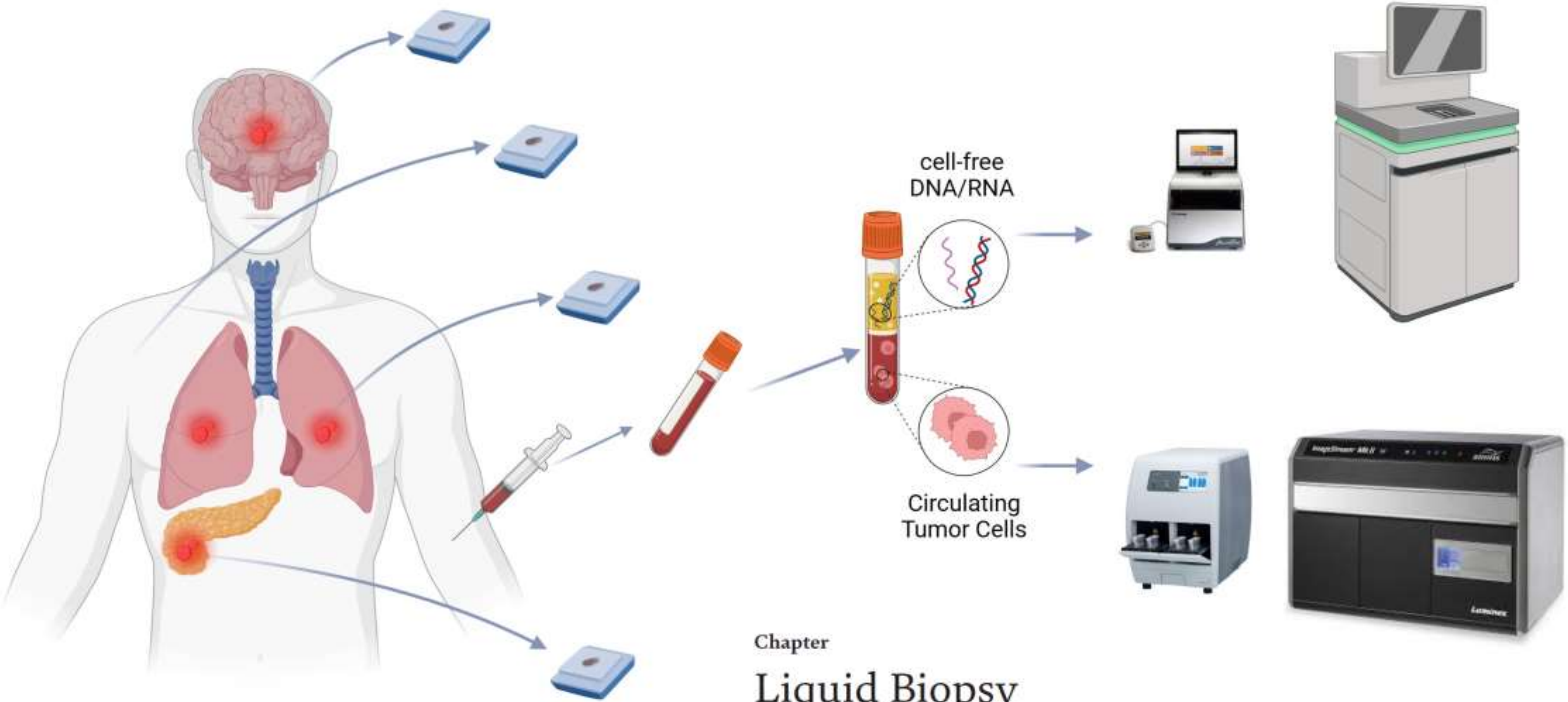
CCR Reviews

AACR



Chapter

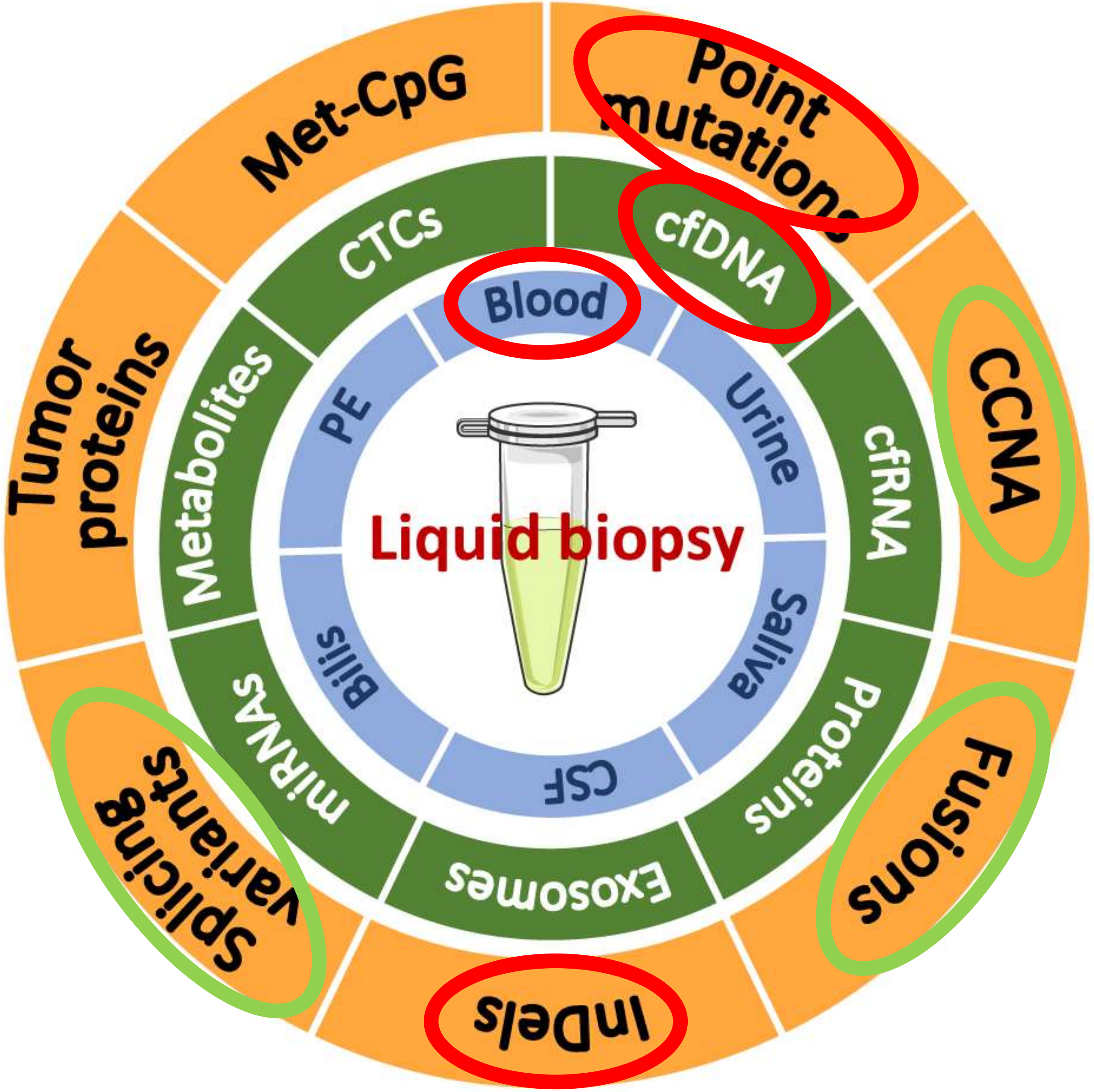
Liquid Biopsy

Valeria Denninghoff and Maria Jose Serrano

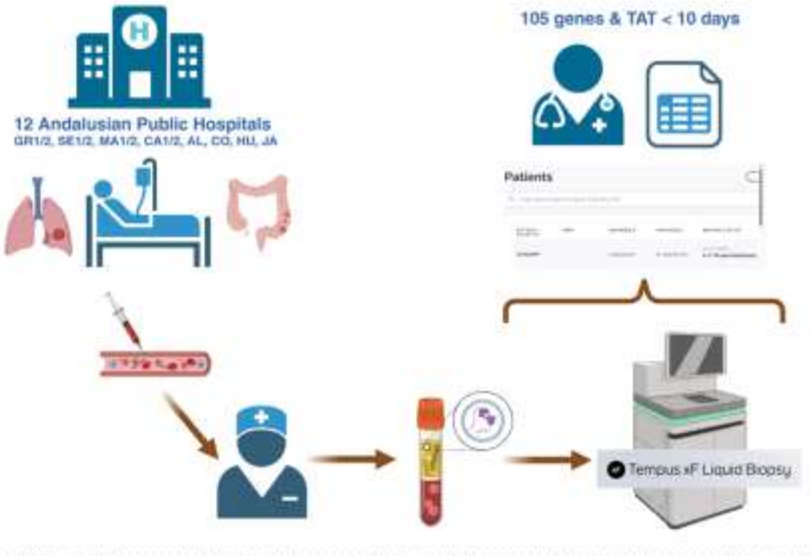
🟦 Biofluids

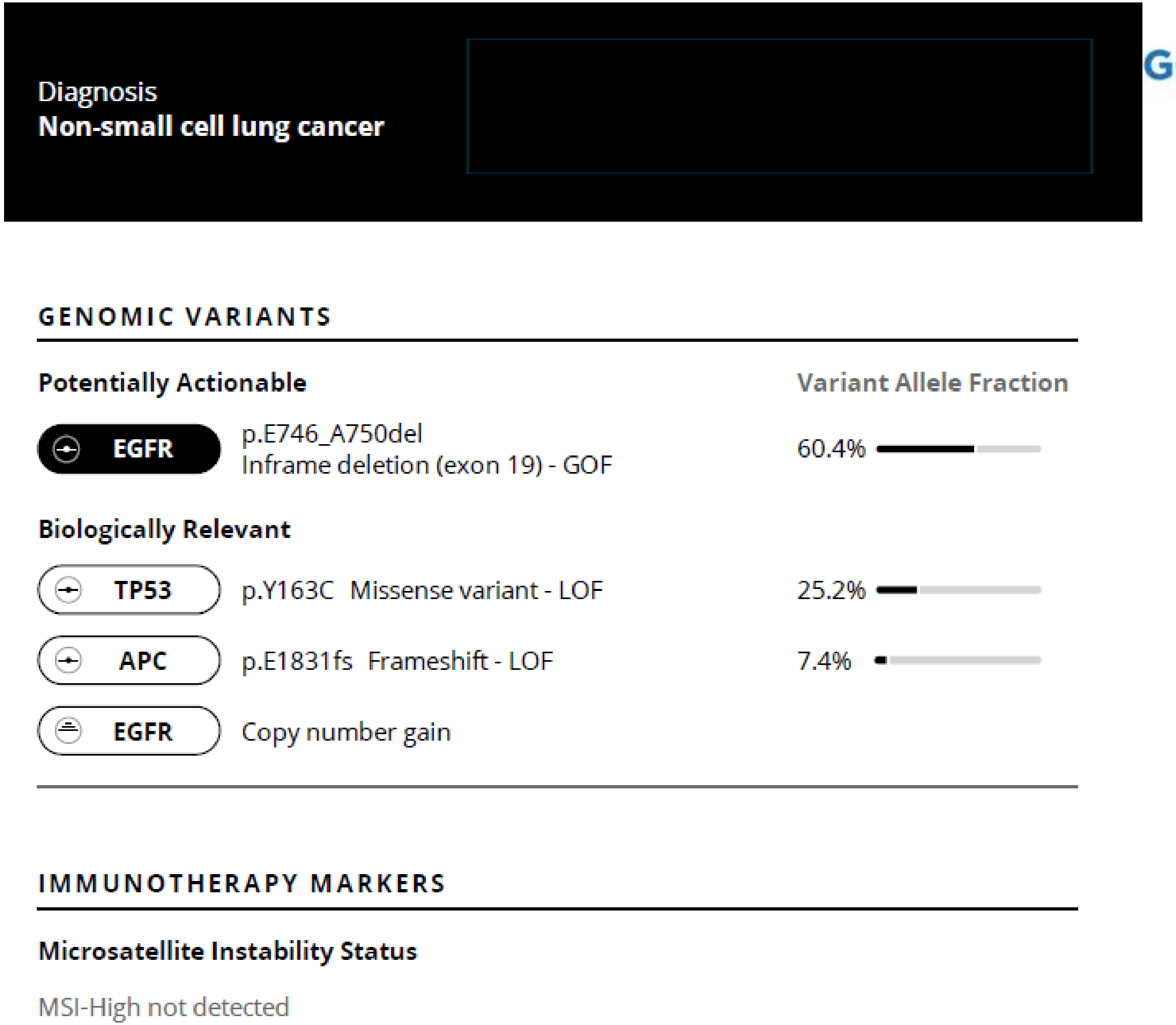
🟩 Analytes

🟧 Biomarkers



Genomics





Diagnosis
Non-small cell lung cancer

[redacted]

GENOMIC VARIANTS

Potentially Actionable		Variant Allele Fraction
EGFR	p.E746_A750del Inframe deletion (exon 19) - GOF	60.4%

Biologically Relevant		
TP53	p.Y163C Missense variant - LOF	25.2%
APC	p.E1831fs Frameshift - LOF	7.4%
EGFR	Copy number gain	

IMMUNOTHERAPY MARKERS

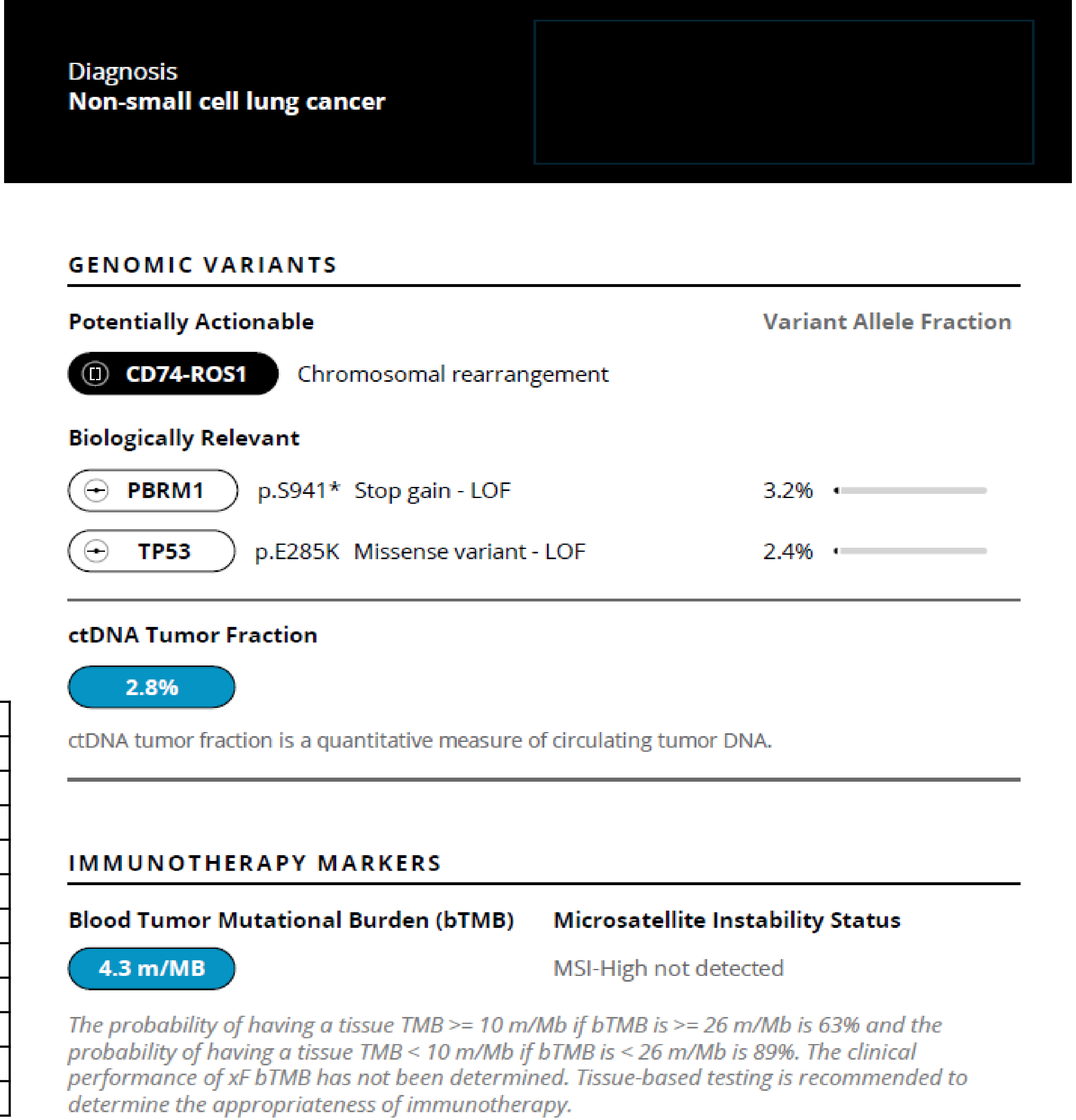
Microsatellite Instability Status

MSI-High not detected

Genomics

https://clinicaltrials.gov/ NCT06997458

Hospital	PI-Hospital	N
Hospital Universitario Virgen del Rocío, Sevilla	Amparo Sánchez-Gastaldo	39
Hospital Universitario Reina Sofía, Córdoba	Isidoro Barneto-Aranda	35
Hospital Universitario de Jaén, Jaén	Ana Laura Ortega-Granados	33
Hospital Universitario Puerta del Mar, Cádiz	Esperanza Arriola-Arellano	31
Hospital Regional Universitario de Málaga, Málaga	Manuel Cobo-Dols	28
Hospital Universitario Torrecárdenas, Almería	Victoria Castellón-Rubio	25
Hospital Universitario Virgen de las Nieves, Granada	Javier Valdivia-Bautista	20
Hospital Universitario Clínico San Cecilio, Granada	Silvia Sequero-Lòpez	16
Hospital Universitario Juan Ramon Jiménez, Huelva	Ángel Inoriza	12
Hospital Universitario Virgen de la Macarena, Sevilla	David Vicente-Baz	11
Hospital Universitario de Jerez de la Frontera, Cádiz	Amelia Muñoz-Lerma	6



Diagnosis
Non-small cell lung cancer

GENOMIC VARIANTS

Potentially Actionable			Variant Allele Fraction
CD74-ROS1	Chromosomal rearrangement		

Biologically Relevant			
PBRM1	p.S941*	Stop gain - LOF	3.2%
TP53	p.E285K	Missense variant - LOF	2.4%

ctDNA Tumor Fraction

2.8%

ctDNA tumor fraction is a quantitative measure of circulating tumor DNA.

IMMUNOTHERAPY MARKERS

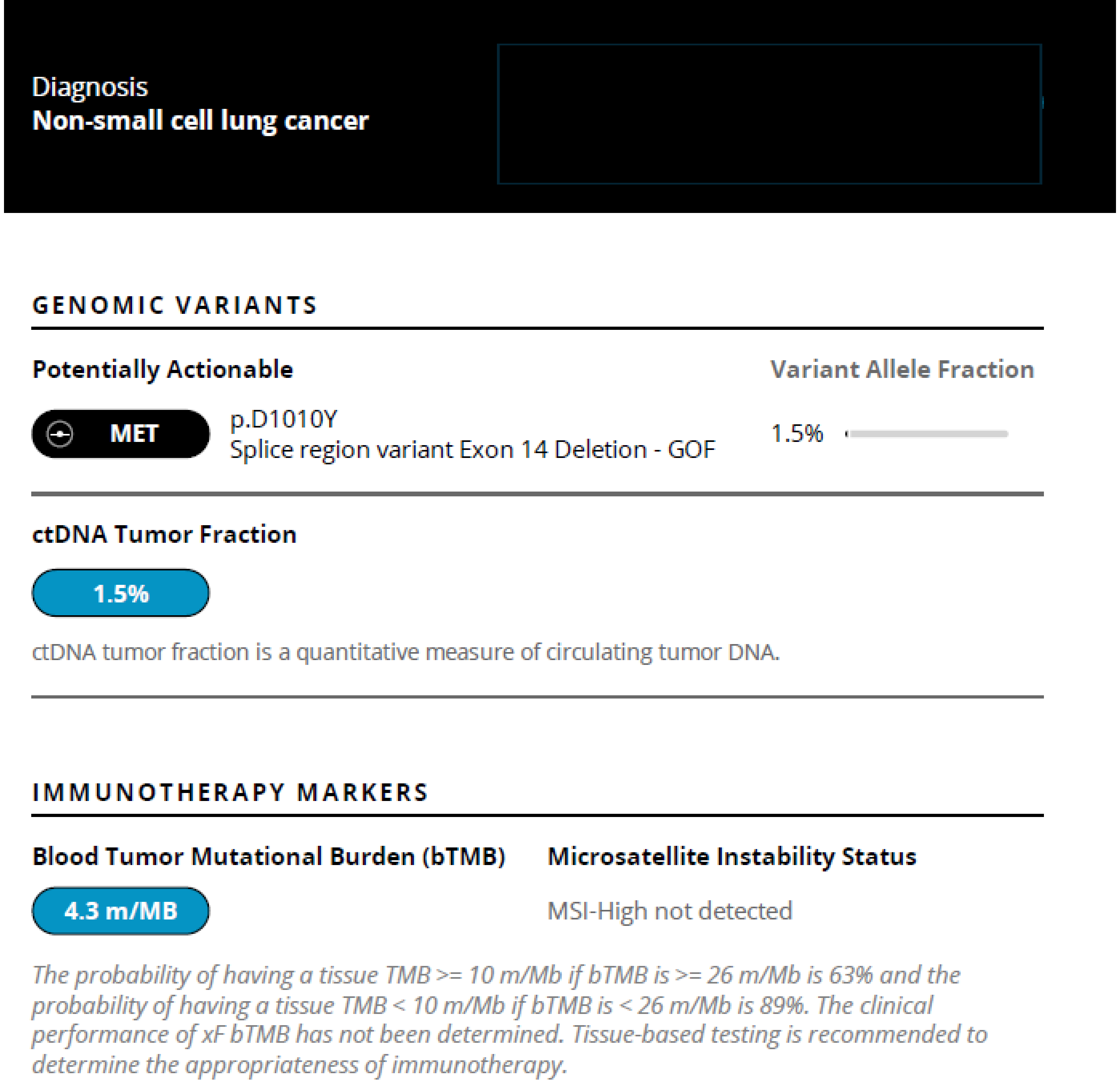
Blood Tumor Mutational Burden (bTMB)	Microsatellite Instability Status
4.3 m/MB	MSI-High not detected

The probability of having a tissue TMB >= 10 m/Mb if bTMB is >= 26 m/Mb is 63% and the probability of having a tissue TMB < 10 m/Mb if bTMB is < 26 m/Mb is 89%. The clinical performance of xF bTMB has not been determined. Tissue-based testing is recommended to determine the appropriateness of immunotherapy.

Genomics

Turnaround Time (TAT)
Median (Q1-Q3) = 10 (9-11)*
*first sample

Median depth = 4375



Diagnosis
Non-small cell lung cancer

[redacted]

GENOMIC VARIANTS

Potentially Actionable		**Variant Allele Fraction**
MET	p.D1010Y Splice region variant Exon 14 Deletion - GOF	1.5%

ctDNA Tumor Fraction

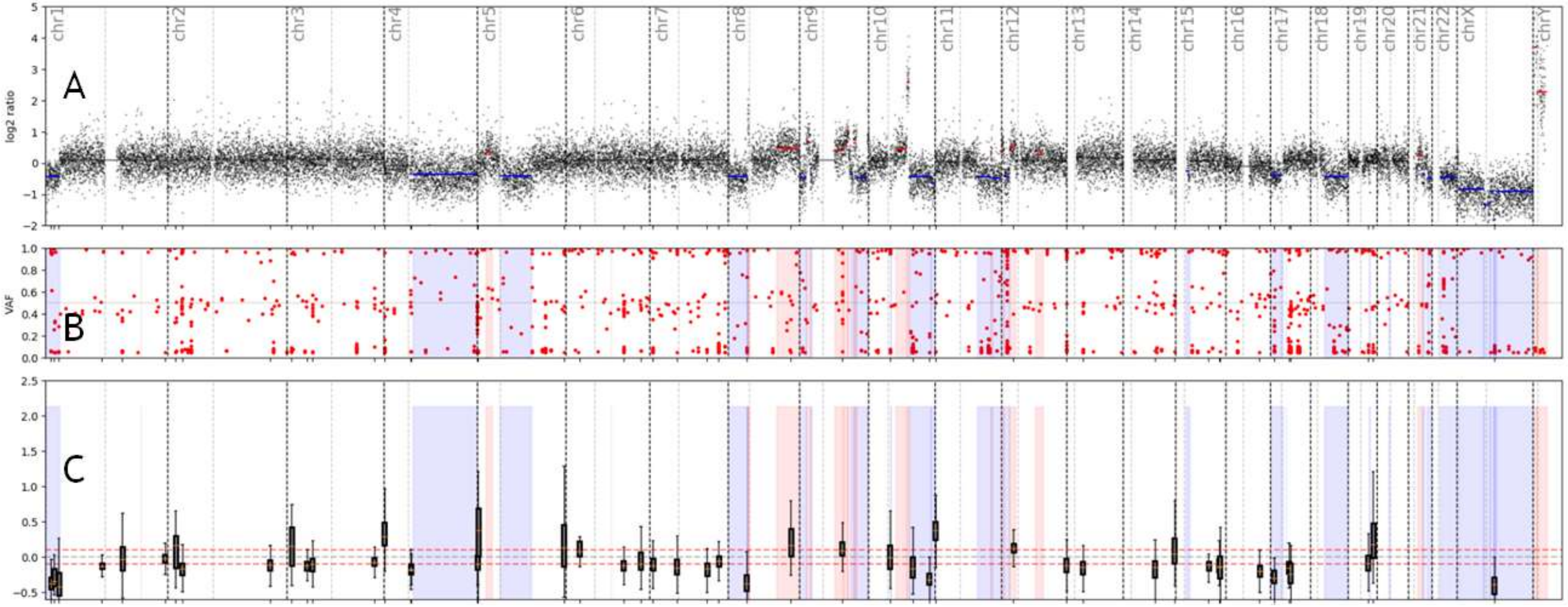
1.5%

ctDNA tumor fraction is a quantitative measure of circulating tumor DNA.

IMMUNOTHERAPY MARKERS

Blood Tumor Mutational Burden (bTMB)	**Microsatellite Instability Status**
4.3 m/MB	MSI-High not detected

The probability of having a tissue TMB >= 10 m/Mb if bTMB is >= 26 m/Mb is 63% and the probability of having a tissue TMB < 10 m/Mb if bTMB is < 26 m/Mb is 89%. The clinical performance of xF bTMB has not been determined. Tissue-based testing is recommended to determine the appropriateness of immunotherapy.



GECP lung cancer research

Kuilman *et al. Genome Biology* (2015) 16:49
DOI 10.1186/s13059-015-0617-1

Genome **Biology**

METHOD **Open Access**

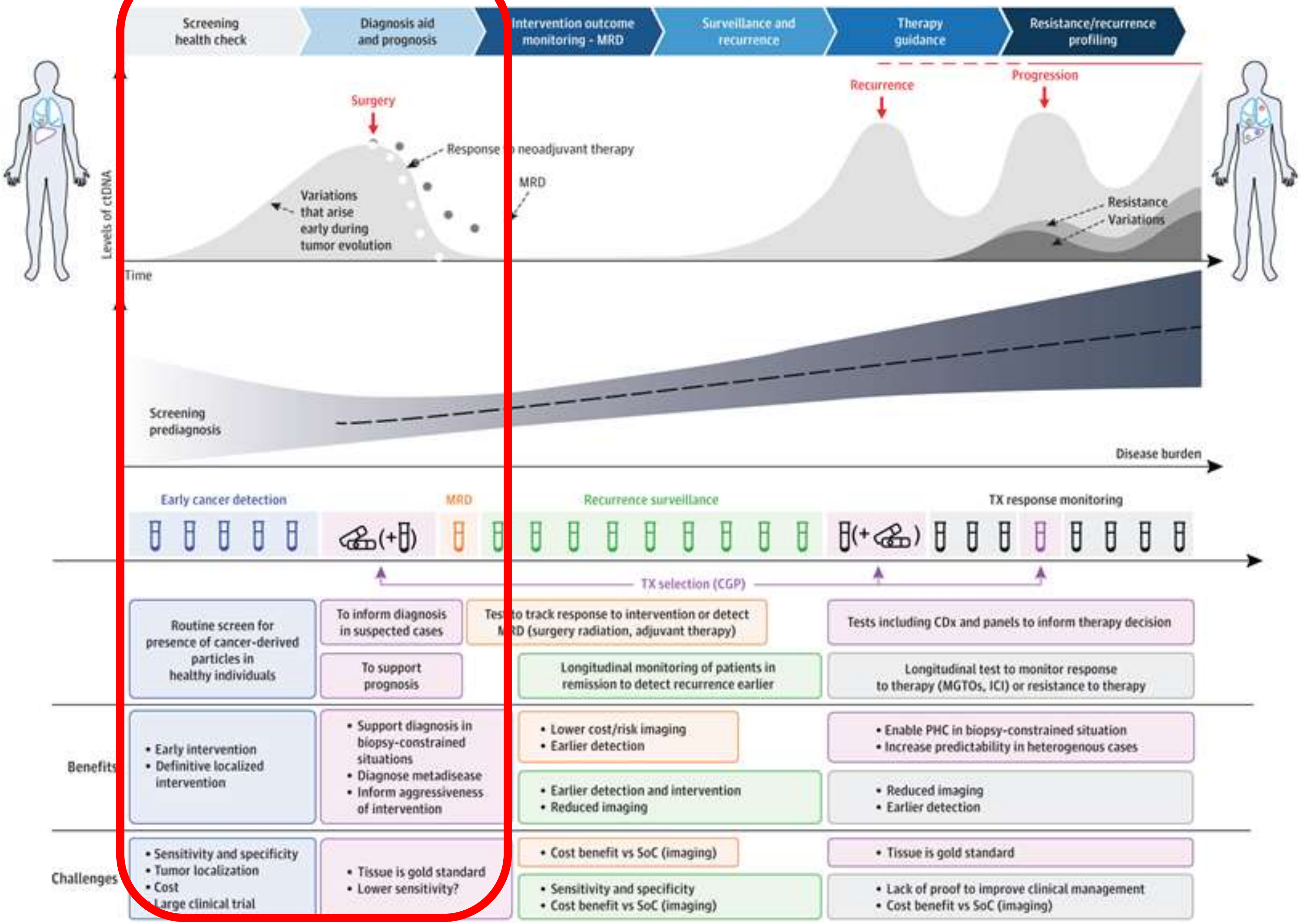
CopywriteR: DNA copy number detection from off-target sequence data

Thomas Kuilman[1], Arno Velds[2], Kristel Kemper[1], Marco Ranzani[3], Lorenzo Bombardelli[4], Marlous Hoogstraat[5], Ekaterina Nevedomskaya[5,6], Guotai Xu[1], Julian de Ruiter[5,6], Martijn P Lolkema[7], Bauke Ylstra[8], Jos Jonkers[6], Sven Rottenberg[1,9], Lodewyk F Wessels[5], David J Adams[3], Daniel S Peeper[1*] and Oscar Krijgsman[1]

Junta de Andalucía
Consejería de Salud y Consumo
FONDO EUROPEO DE DESARROLLO REGIONAL
"Una manera de hacer Europa"

BIOPSIA LIQUIDA

GeCP
lung cancer research



Routine screen for presence of cancer-derived particles in healthy individuals

To inform diagnosis in suspected cases

To support prognosis

Tes to track response to intervention or detect M RD (surgery radiation, adjuvant therapy)

Longitudinal monitoring of patients in remission to detect recurrence earlier

Tests including CDx and panels to inform therapy decision

Longitudinal test to monitor response to therapy (MGTOs, ICI) or resistance to therapy

Benefits

- Early intervention
- Definitive localized intervention

- Support diagnosis in biopsy-constrained situations
- Diagnose metadisease
- Inform aggressiveness of intervention

- Lower cost/risk imaging
- Earlier detection

- Earlier detection and intervention
- Reduced imaging

- Enable PHC in biopsy-constrained situation
- Increase predictability in heterogenous cases

- Reduced imaging
- Earlier detection

Challenges

- Sensitivity and specificity
- Tumor localization
- Cost
- Large clinical trial

- Tissue is gold standard
- Lower sensitivity?

- Cost benefit vs SoC (imaging)

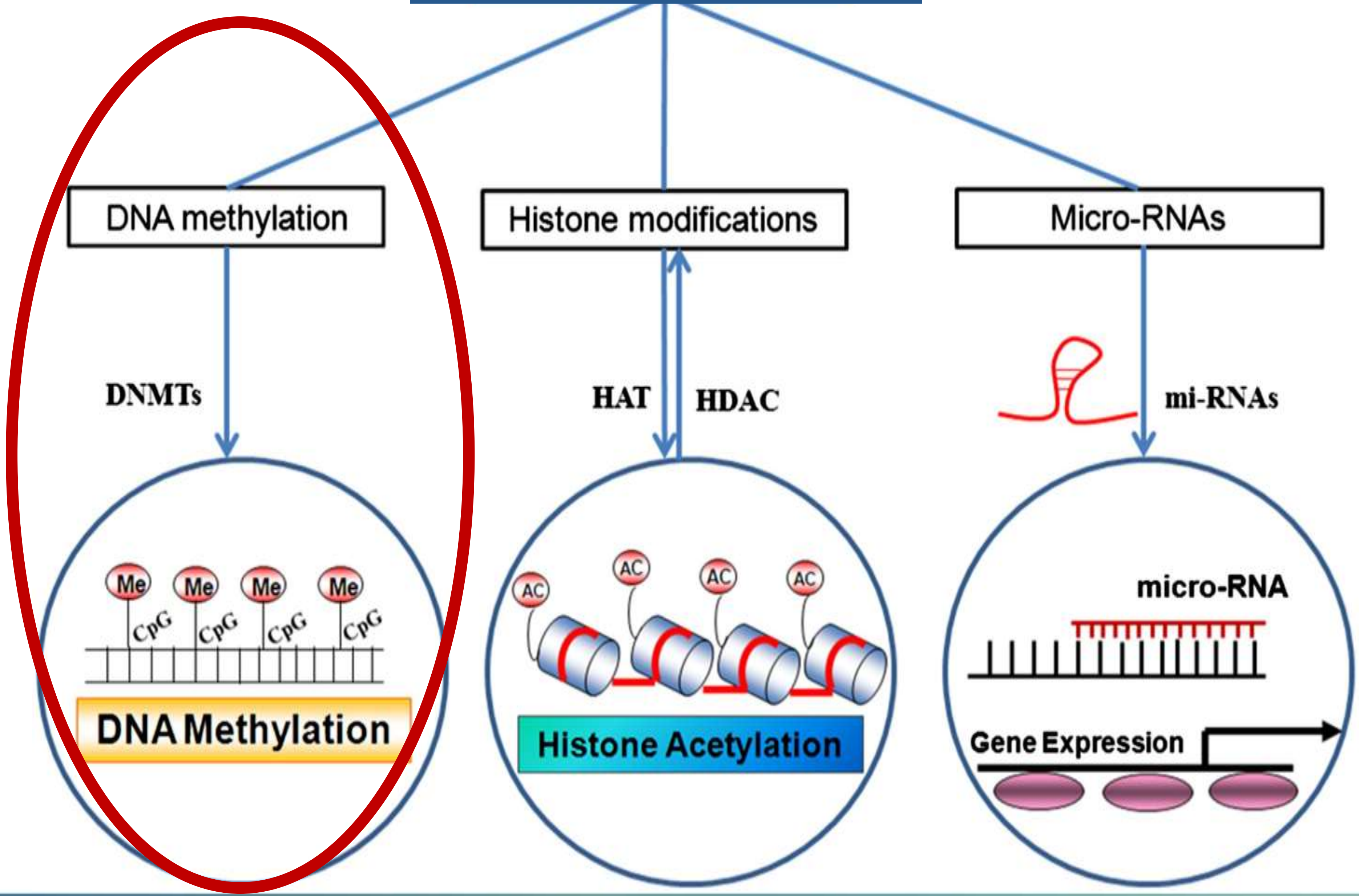
- Sensitivity and specificity
- Cost benefit vs SoC (imaging)

- Tissue is gold standard

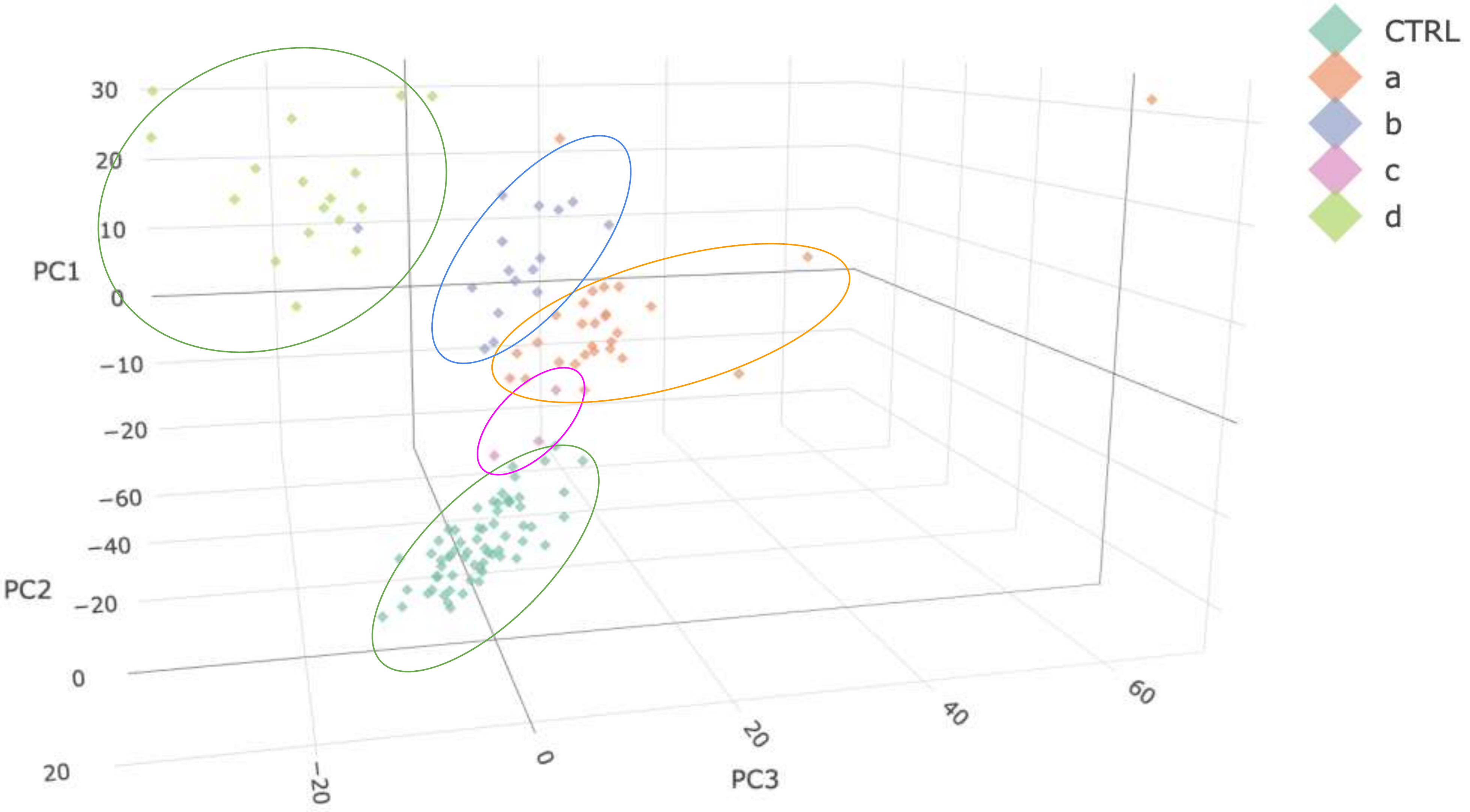
- Lack of proof to improve clinical management
- Cost benefit vs SoC (imaging)

Krebs 2022

Epigenomics

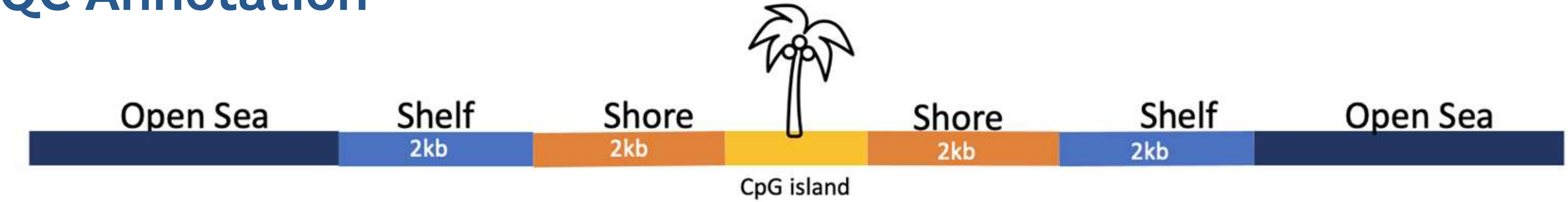


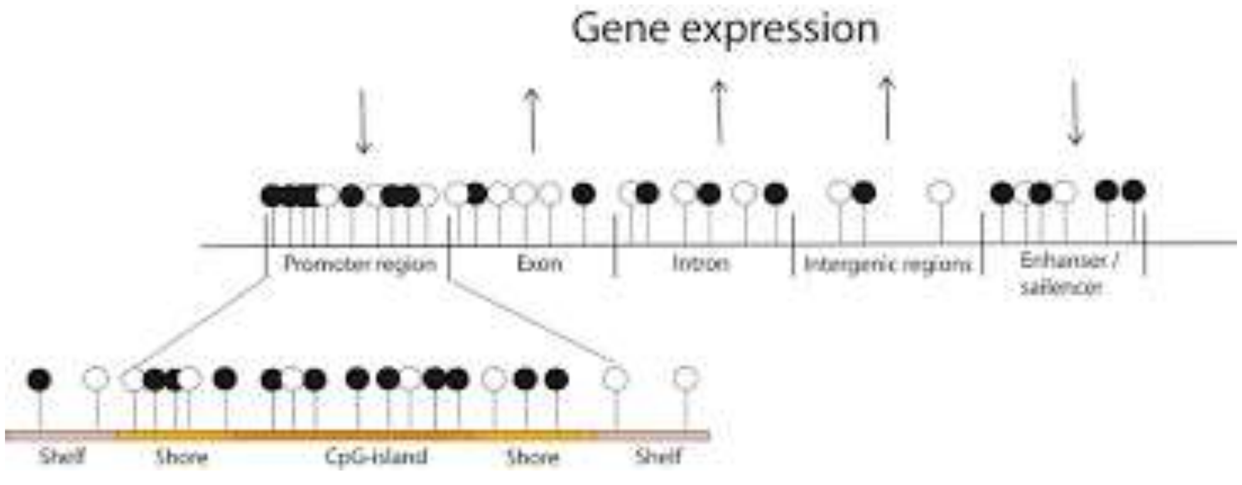
Principal component analysis (PCA)

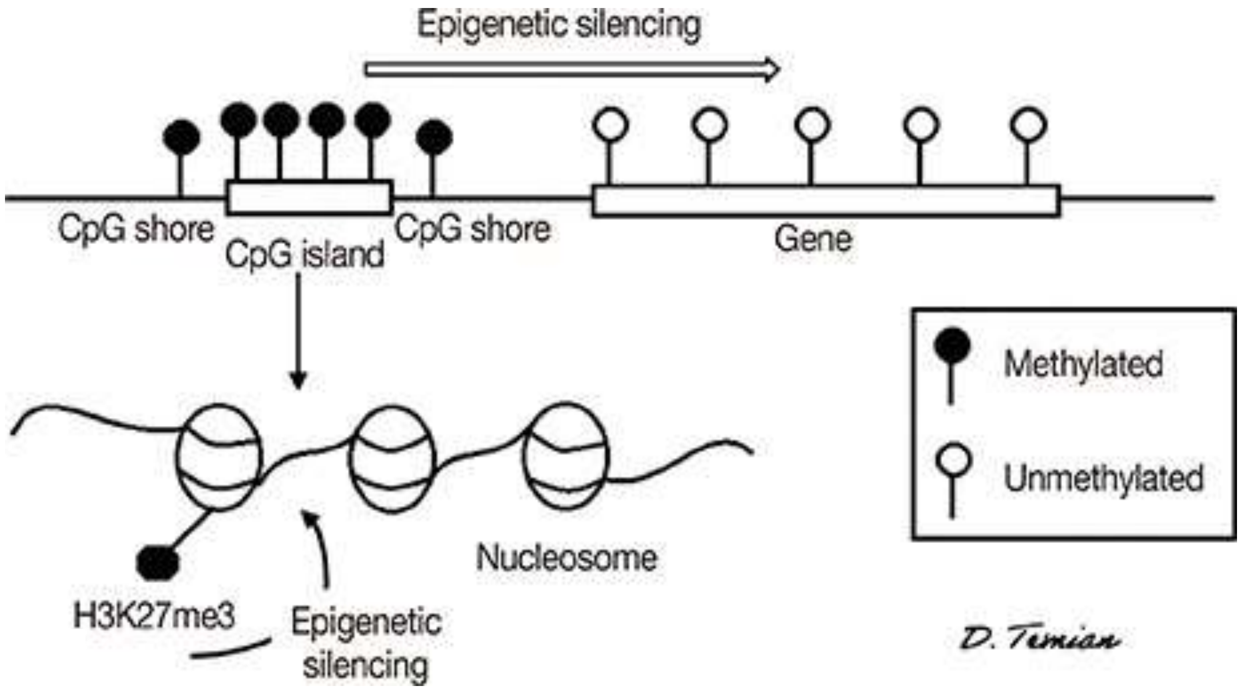


Data deposited in the NCBI Gene Expression Omnibus: GSE289742

QC Annotation

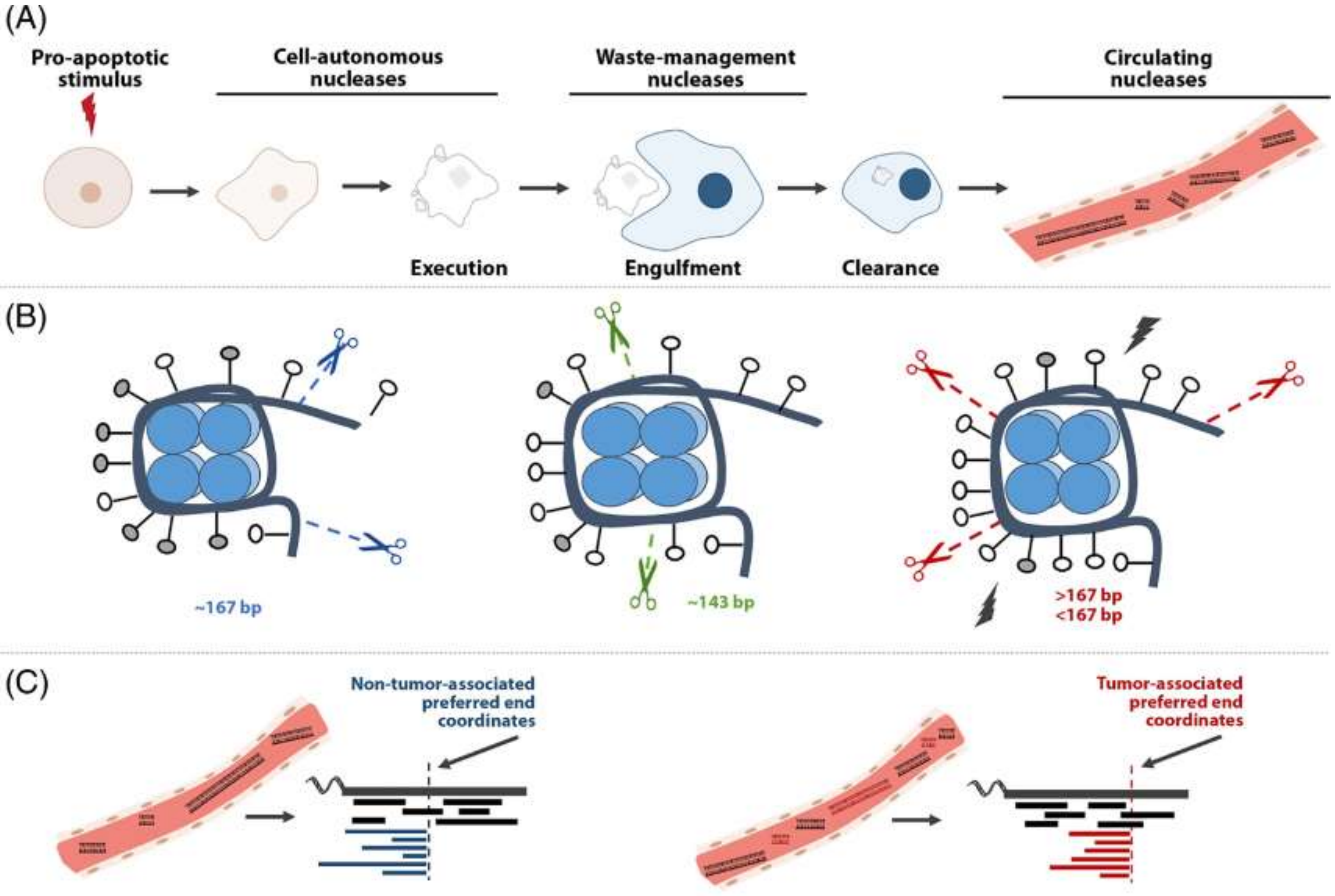






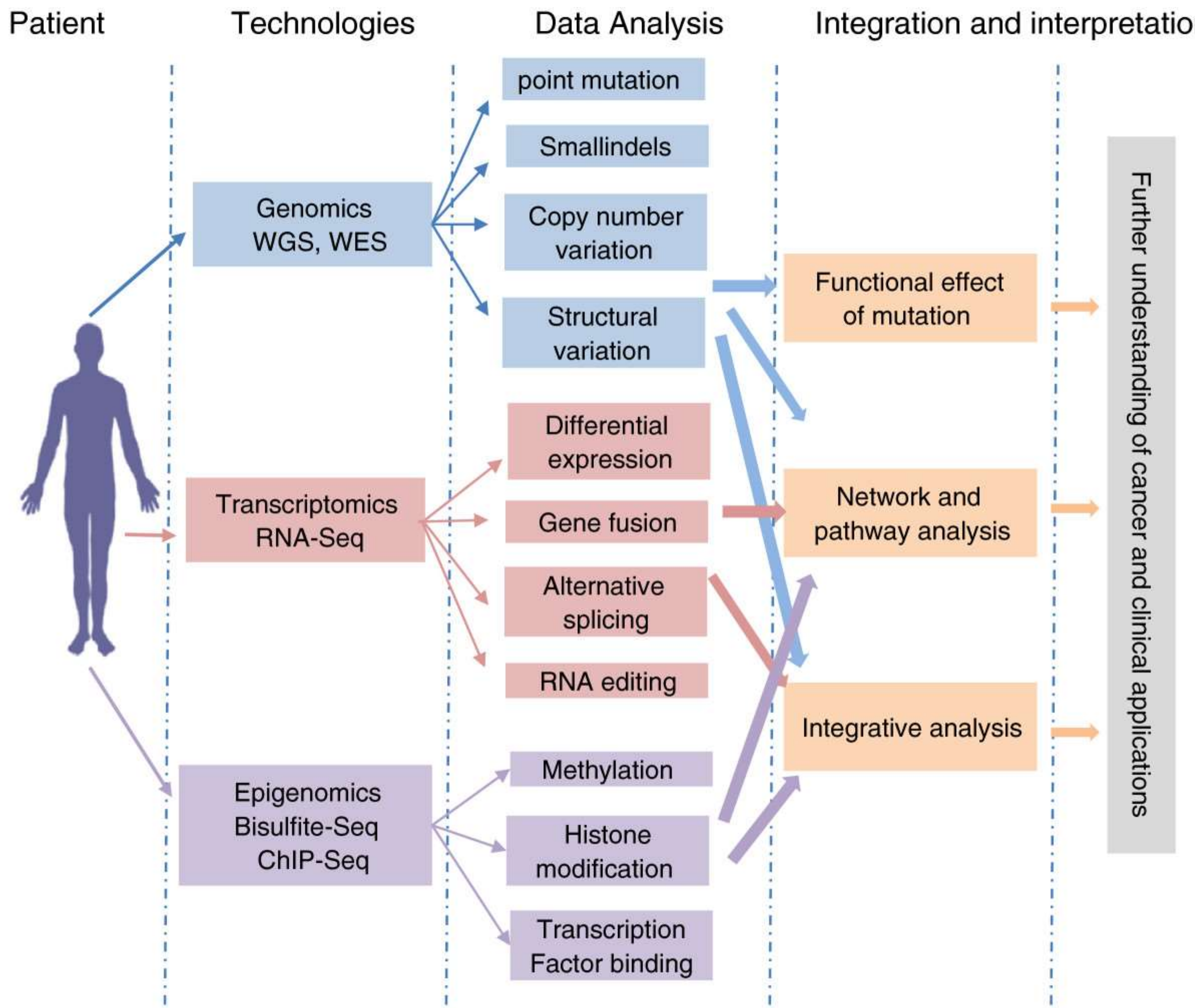
```
#how many probes per island
summary(as.numeric(islands))
# Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
# 1.00    5.00   10.00   10.59   15.00   90.00
```

Fragmentomics



Trends in Molecular Medicine

GECP
lung cancer research



Patient

Technologies

Data Analysis

Integration and interpretation

Genomics
WGS, WES

Transcriptomics
RNA-Seq

Epigenomics
Bisulfite-Seq
ChIP-Seq

point mutation

Smallindels

Copy number variation

Structural variation

Differential expression

Gene fusion

Alternative splicing

RNA editing

Methylation

Histone modification

Transcription Factor binding

Functional effect of mutation

Network and pathway analysis

Integrative analysis

Further understanding of cancer and clinical applications

Methods based on Polymerase Chain Reaction
NGS - 2nd generation

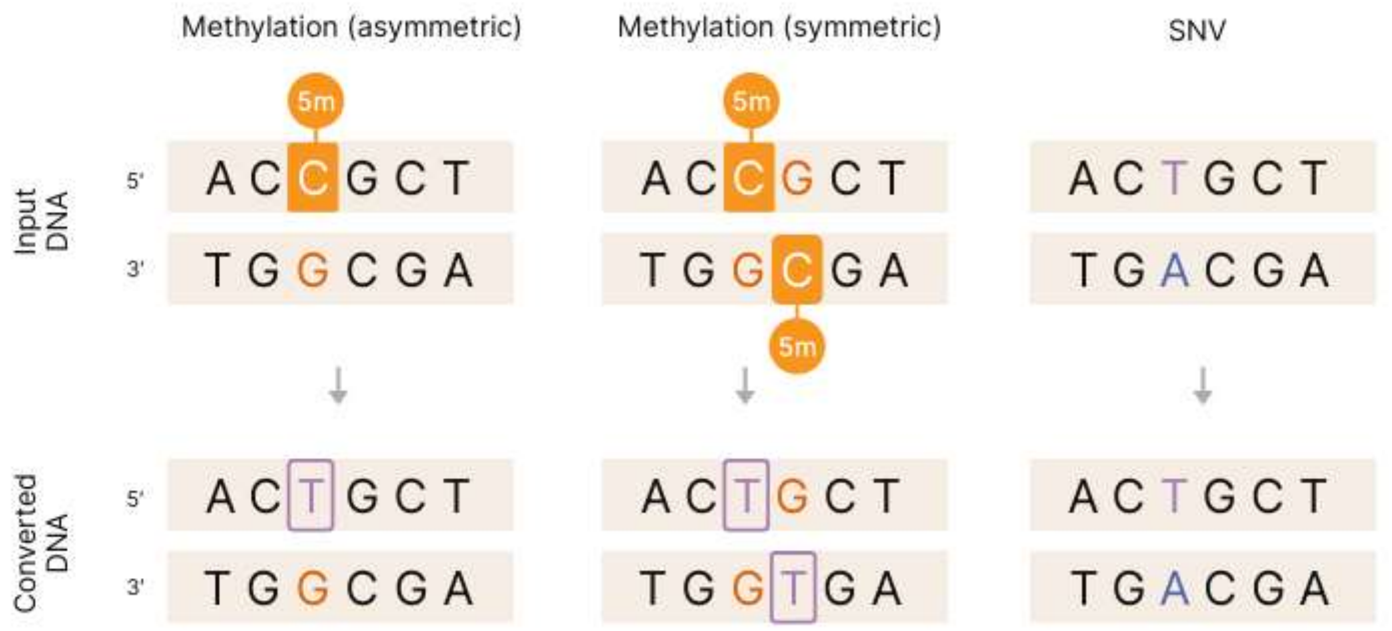
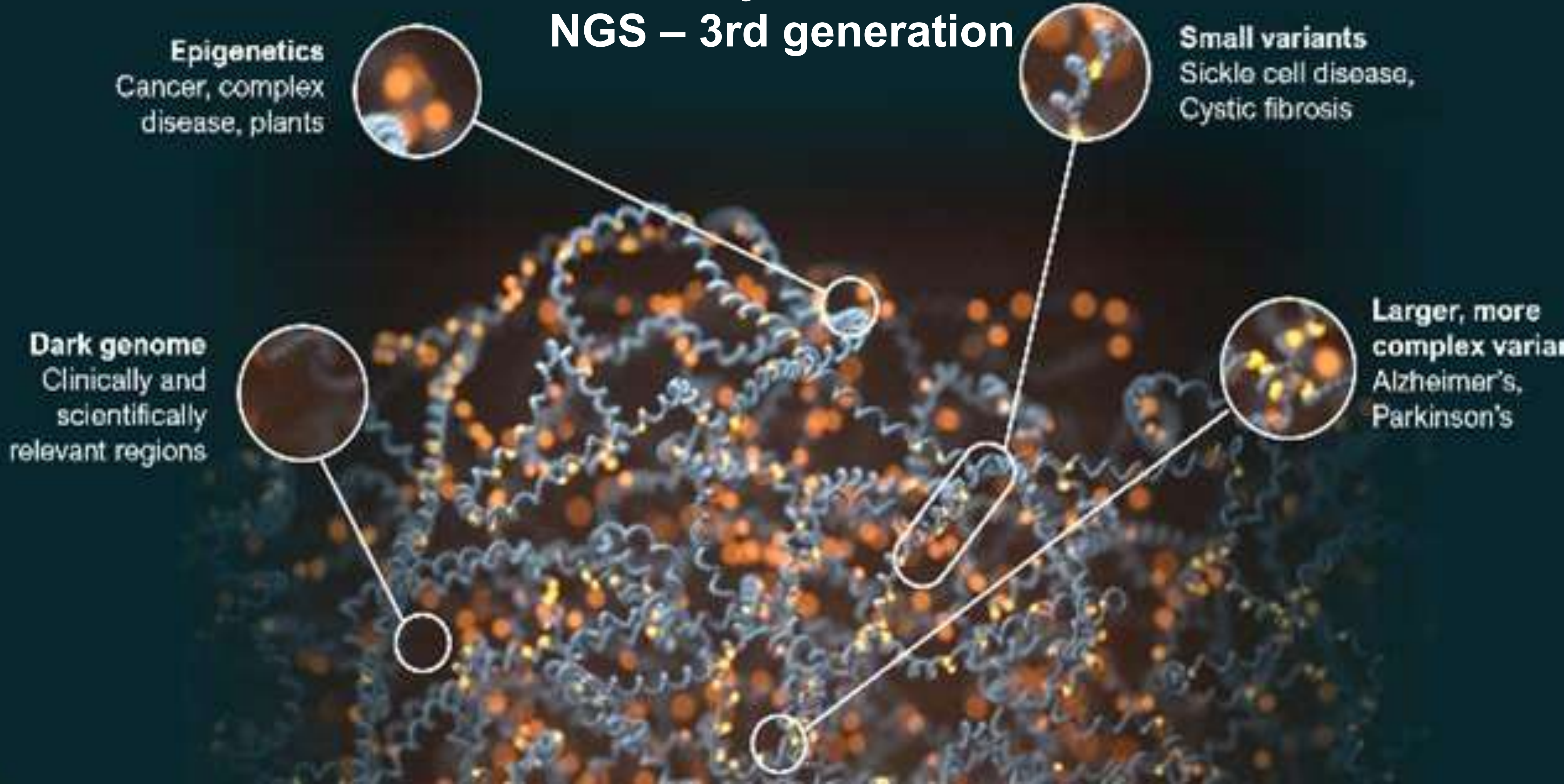


Figure 5: DRAGEN algorithms distinguish methylation events from SNVs

Innovative DRAGEN algorithms leverage the complementary strand sequence to accurately discern between methylation and small variant calls in the same read. For 5mC converted to T, the complementary base will be G, whereas for a C-to-T genomic variant, the complementary base will be A. Duplex UMI collapsing further enables single-molecule resolution to detect symmetric and asymmetric methylation at CpG sites.

Methods without Polymerase Chain Reaction
NGS – 3rd generation

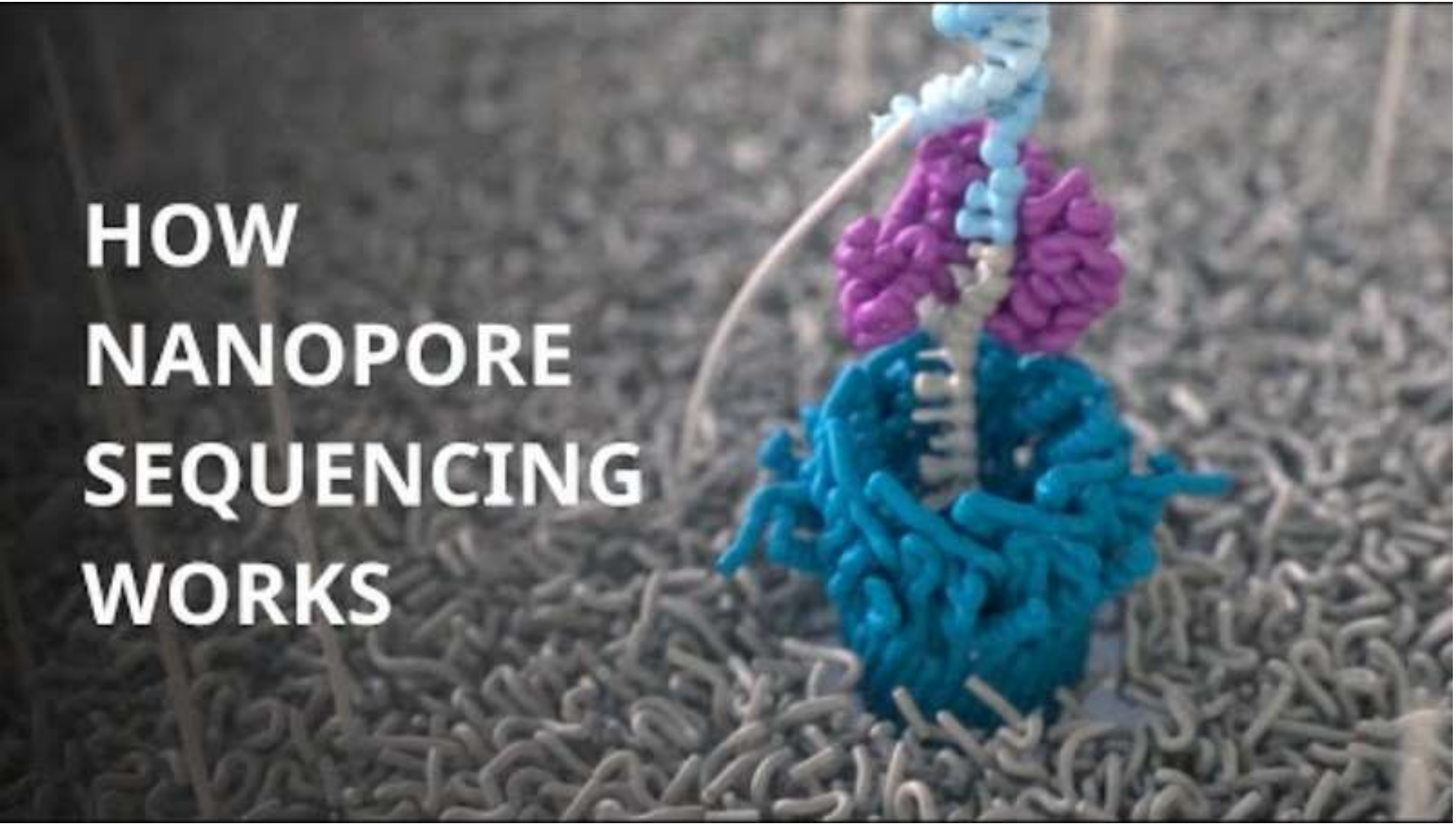


Epigenetics
Cancer, complex disease, plants

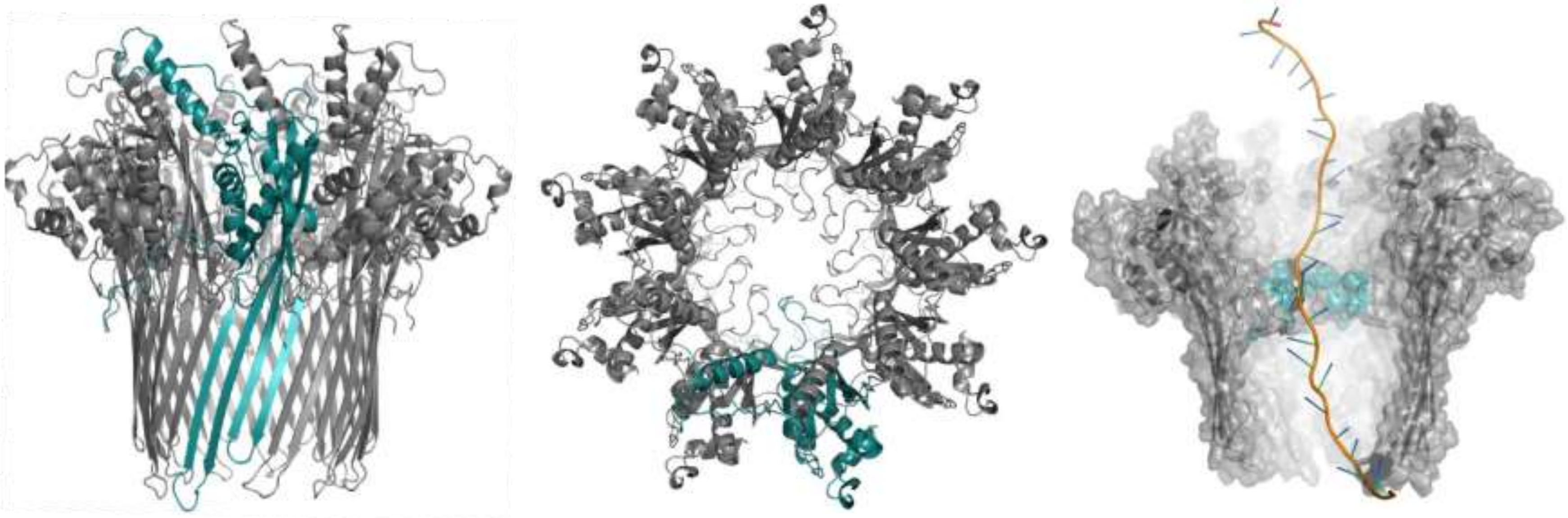
Small variants
Sickle cell disease, Cystic fibrosis

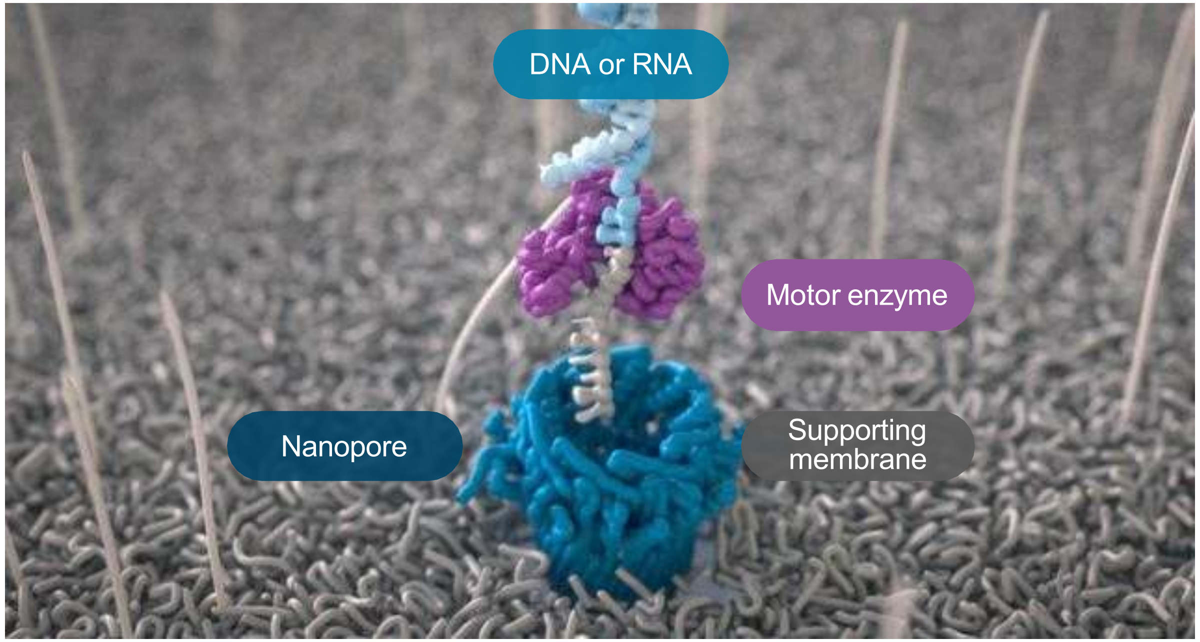
Dark genome
Clinically and scientifically relevant regions

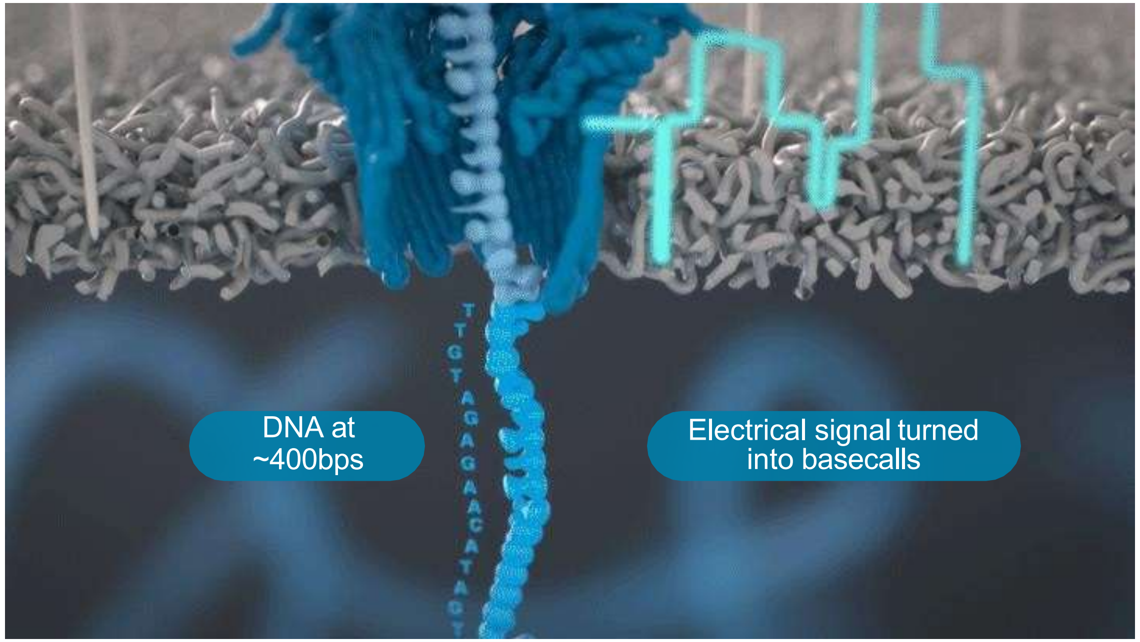
Larger, more complex varia
Alzheimer's, Parkinson's


HOW
NANOPORE
SEQUENCING
WORKS

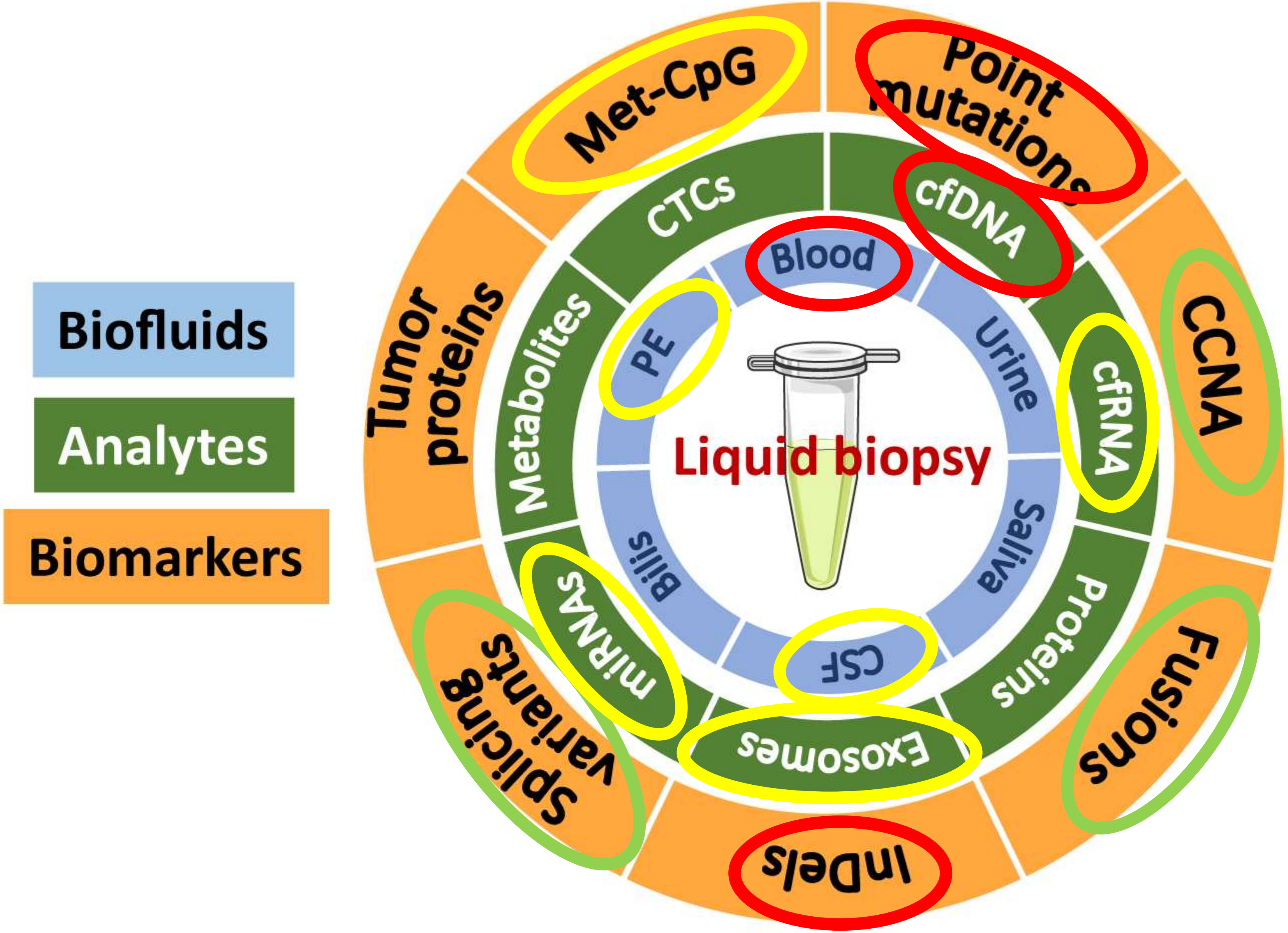
nanopore

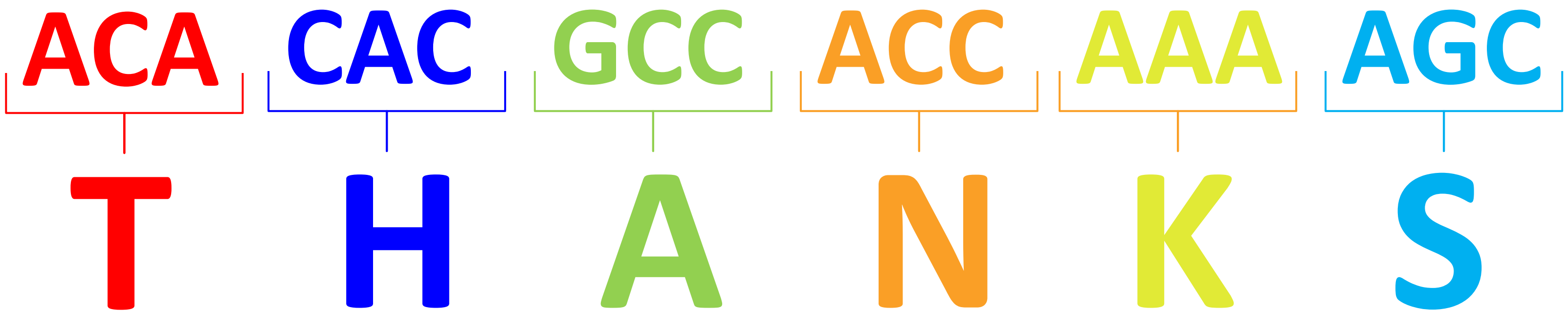



DNA or RNA
Motor enzyme
Nanopore
Supporting membrane


DNA at ~400bps
Electrical signal turned into basecalls

GeCP
lung cancer research





Dr. Valeria Denninghoff

- Liquid Biopsy and Cancer Interception Group - Center for Genomics and Oncology Research - Pfizer - University of Granada - Regional Government of Andalusia (GENyO) – Granada – Spain
- Provincial Intercenter Unit of Pathological Anatomy of the province of Granada (UPIGAP), San Cecilio University Hospital / ibs.Granada, Spain
- Molecular Pathology Lab - University of Buenos Aires (UBA) - National Council for Scientific and Technical Research (CONICET) - Ministry of Science, Technology, and Innovation – Argentina

*Member of the Molecular Working Group – Pathology Committee - IASLC
*Member of the Biomarker Working Group – Pathology Committee – IASLC
*Co-Chair of thel Membership Committee – ISLB
*Founder Member – AIPM (AMP)