3º ENCONTRO NACIONAL DE INVESTIGAÇÃO CLÍNICA & INOVAÇÃO BIOMÉDICA

21 MAIO | ISCTE LISBOA

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fct Fundação para a Ciência e a Tecnologia
Infarmed Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.
apifarma Associação Portuguesa da Indústria Farmacêutica
Health Cluster Portugal
PtCRIN Portuguese Clinical Research Infrastructure Network
iscte Instituto Universitário de Lisboa

3° ENCONTRO NACIONAL DE INVESTIGAÇÃO CLÍNICA & INOVAÇÃO BIOMÉDICA

Avanços da Medicina Personalizada no Contexto da Inovação Biomédica

"Biomarcadores e Medicina Personalizada" uma estratégia para todos

FERNANDO SCHMITT

Professor of Pathology and Oncology, Medical Faculty of Porto University
Director, RISE (Health Research Network)
Head of Molecular Pathology Unit, IPATIMUP
President of The International Academy of Cytology

25 ANOS A DESCOBRIR O CANCRO ipatimup

IAC 1957

RISE Health Research Network From the Lab to the Community

U.PORTO FM UP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

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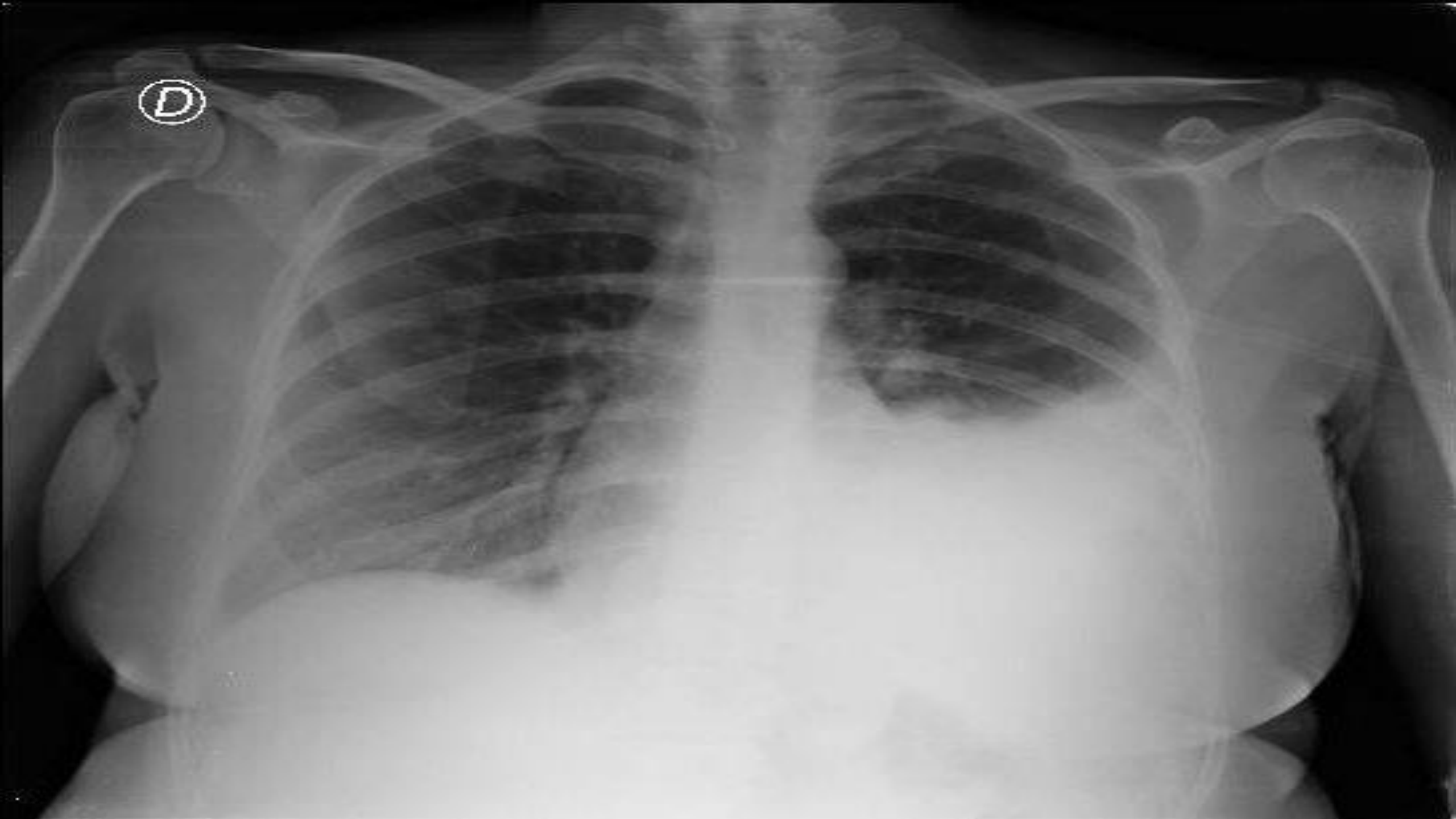
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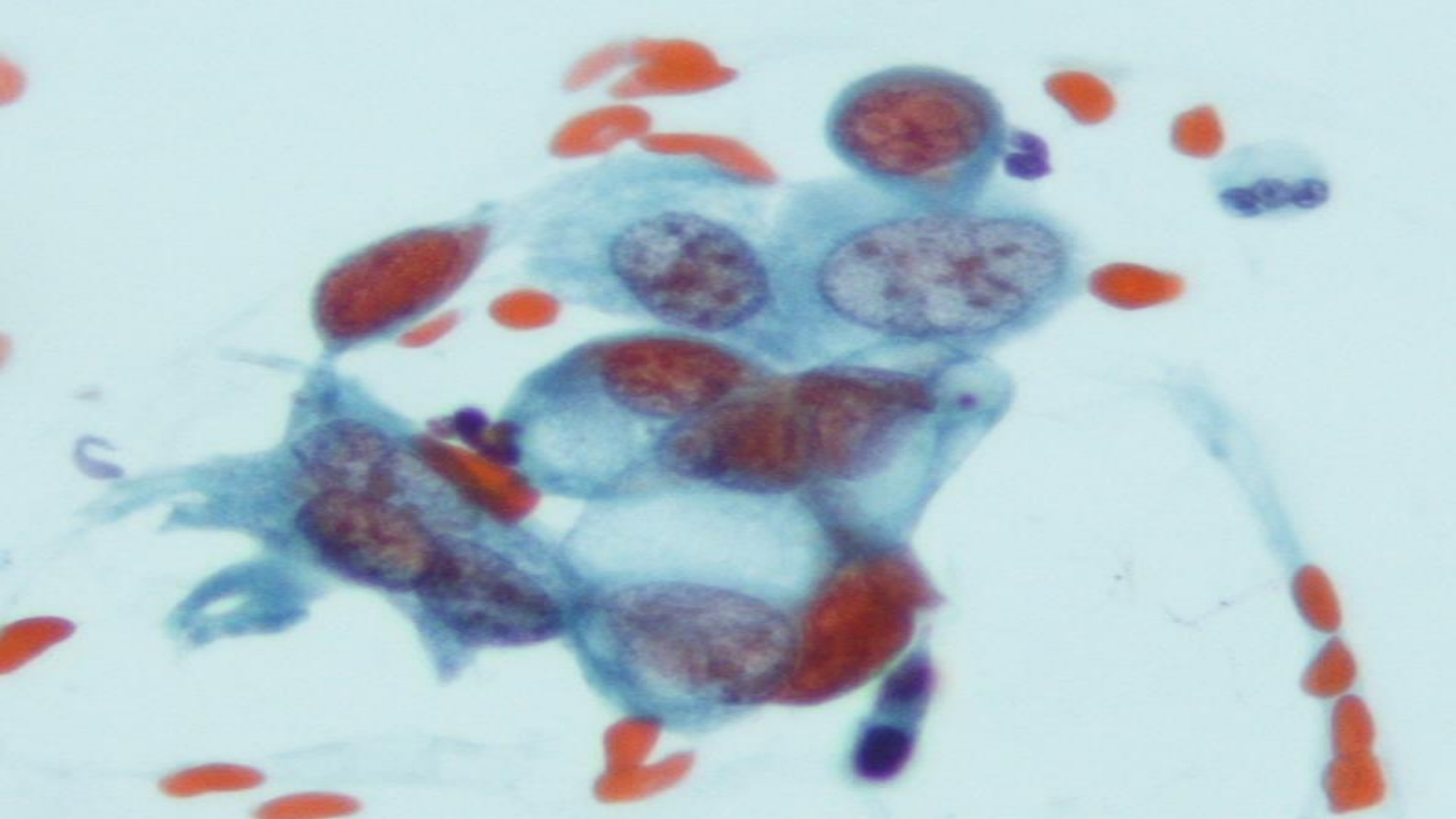
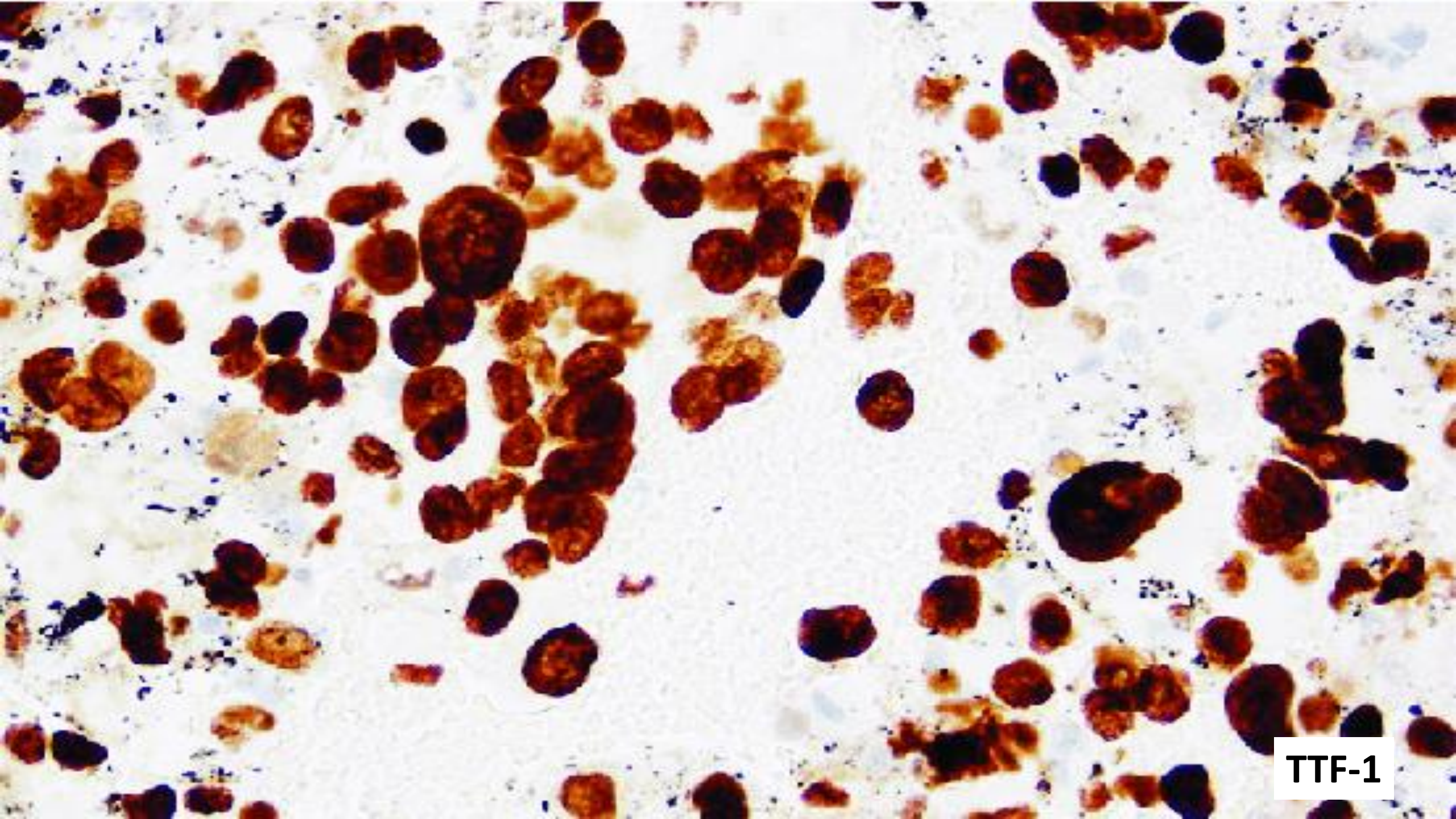


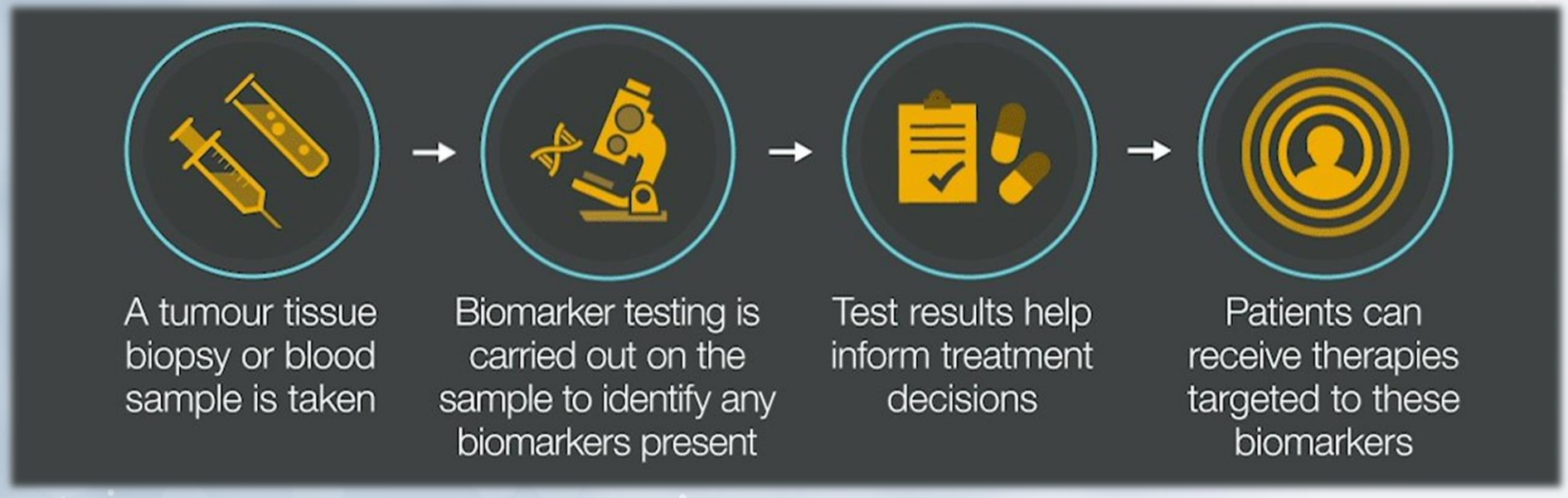

TTF-1

What to do now?

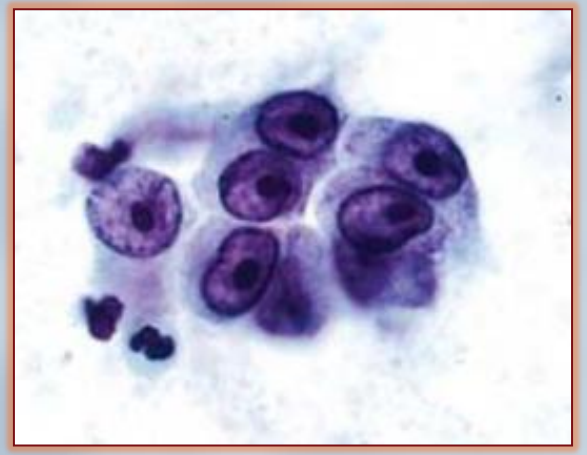
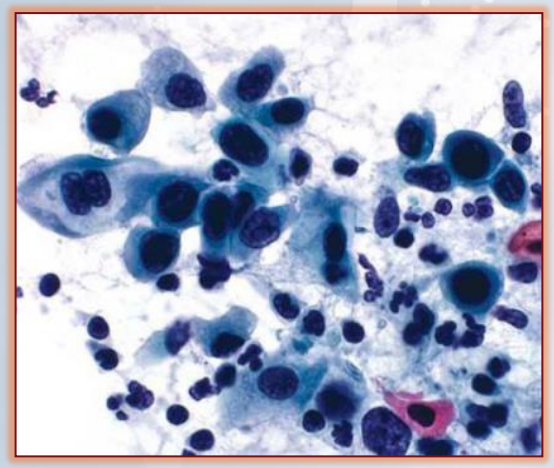
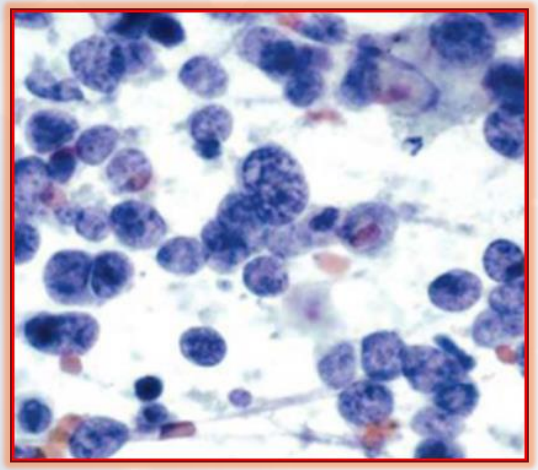
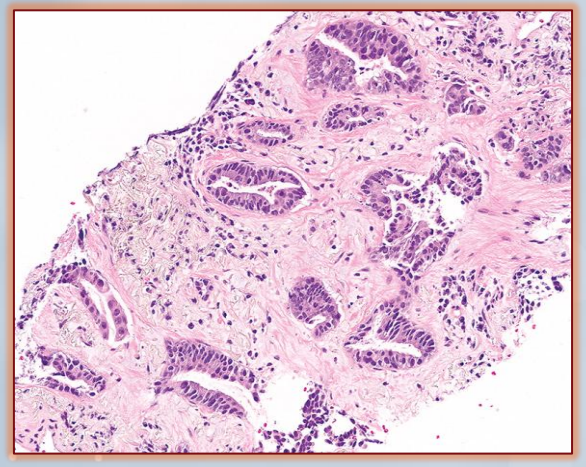
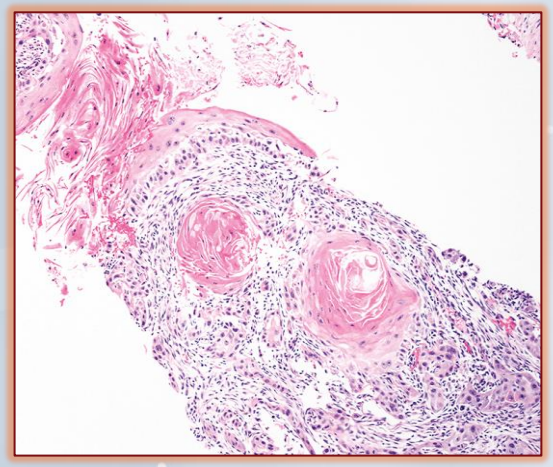
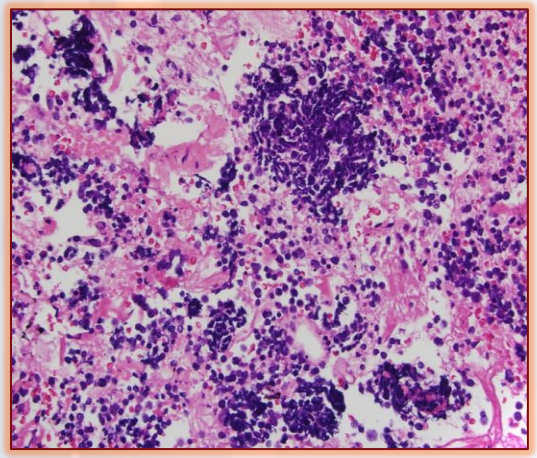
In addition to clinical considerations, the decision depends on:

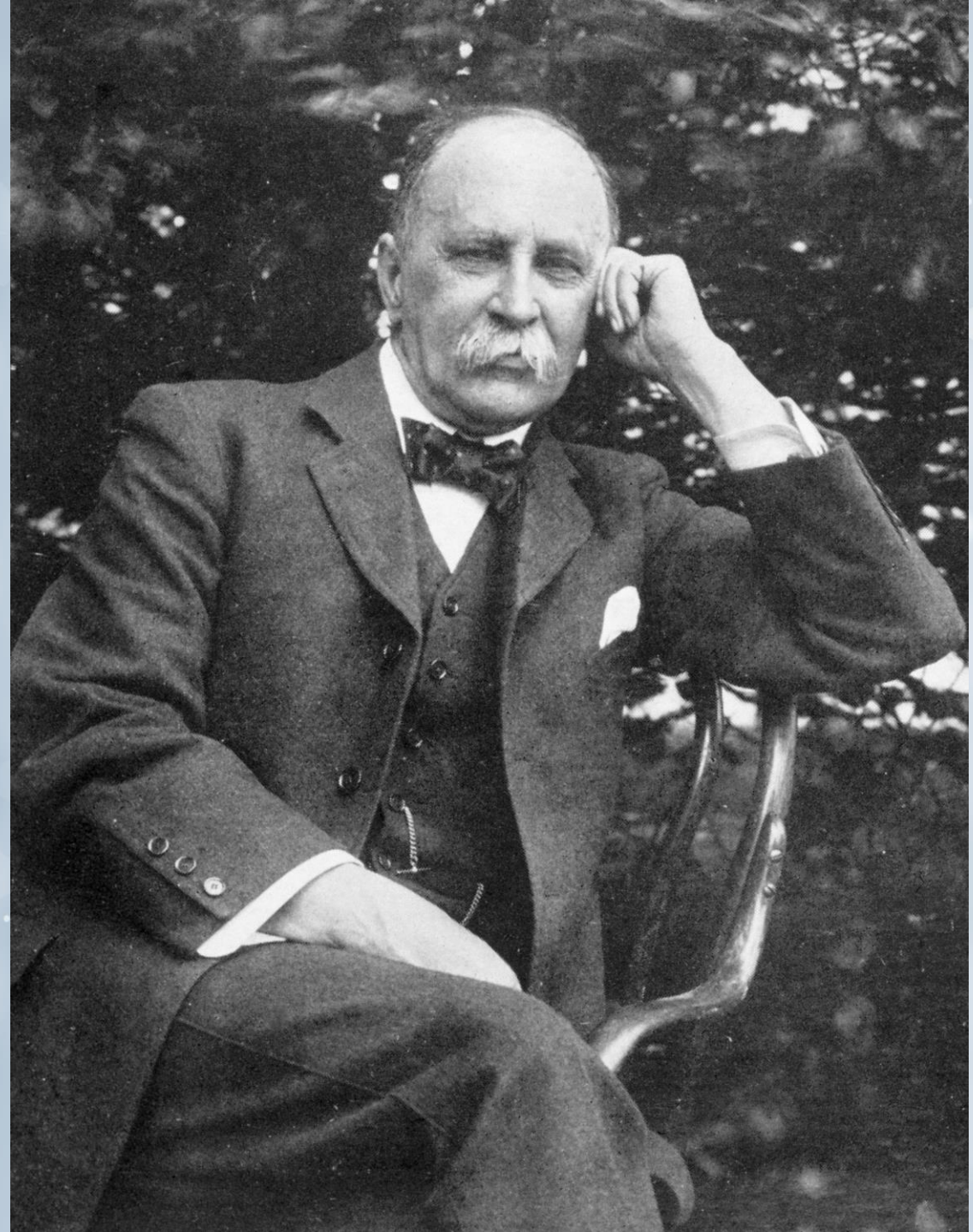
- The patient´s location: continent, country, region
- Availability of good material for analysis
- Accessibility of tests
- Financial circumstances
- Availability of treatment

Precision Medicine and Biomarkers



Cancer diagnosis is morphological

ADENOCARCINOMA	SqCC	SCC
		
		

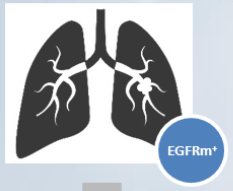


" The GOOD physician treats the DISEASE; the GREAT physician treats the PATIENT with the disease."

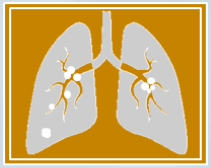
Sir William Osler

EGFR T790M mutation testing should be performed in patients with NSCLC who have progressed on 1st/2nd generation EGFR TKIs

Patient diagnosed with
EGFR–sensitising mutation positive NSCLC



1st/2nd generation EGFR TKI



EGFR T790M mutation testing should be performed in EGFR mutant advanced NSCLC patients that have progressed on treatment with 1st / 2nd generation EGFR TKIs

Pathology is Evolving to Meet Patient Needs and Be a Central Driver of Personalized Healthcare

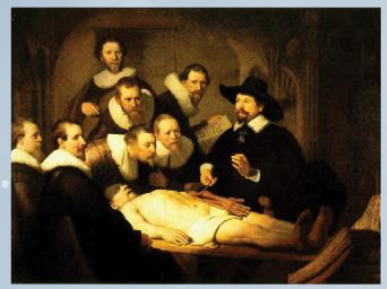
Gross Pathology

Antonio Benivieni
(1443-1502):
First autopsy

Giovanni Morgagni
(1682-1771):
Correlated patient symptoms to autopsy findings

John Hunter
(1728-1793):
Devised method for preserving tissue

Bichat
(1771-1802):
"Father of modern pathology"



Cellular Pathology

Leeuwenhoek
(1632-1723):
Developed 1st microscope

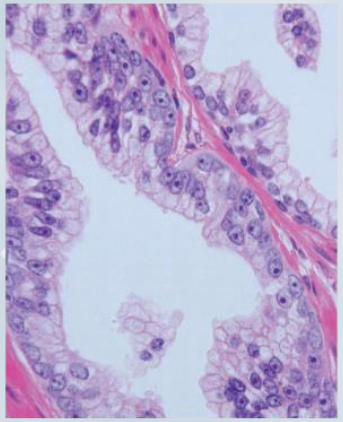
Virchow
(1821-1905):
Recognized that diseases arise from alterations within tissues and cells

Morphologic Pathology

Morphologic classification of cancer

Pathologists provide diagnostic and prognostic information

Hematoxylin and eosin is 'primary stain' for all cases

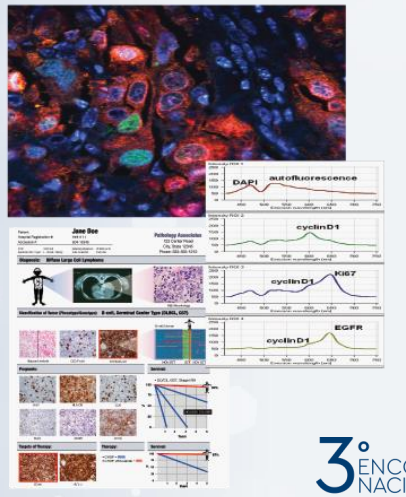


Molecular/Predictive Pathology

Comprehensive molecular tumor profiling

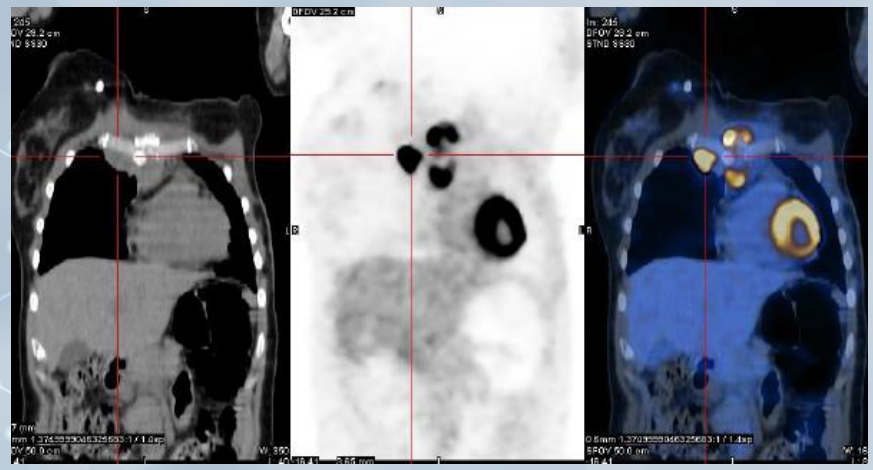
Pathologists provide personalized medicine/predictive biomarker information

Proteomic and genomic data in the context of morphology



Precision Oncology/Biomarkers
More biology from smaller samples

Smaller tumours/targets



Smaller samples

Cytology or blood sample

More biology

Hotspot genes		Copy number variants	Fusion drivers
35 genes		19 genes	23 genes
DNA			RNA
AKT1	*JAK1*	*ALK*	*ABL1*
ALK	*JAK2*	*AR*	*ALK*
AR	*JAK3*	*BRAF*	*AKT3*
BRAF	*KIT*	*CCND1*	*AXL*
CDK4	*KRAS*	*CDK4*	*BRAF*
CTNNB1	*MAP2K1*	*CDK6*	*EGFR*
DDR2	*MAP2K2*	*EGFR*	*ERBB2*
EGFR	*MET*	*ERBB2*	*ERG*
ERBB2	*MTOR*	*FGFR1*	*ETV1*
ERBB3	*NRAS*	*FGFR2*	*ETV4*
ERBB4	*PDGFRA*	*FGFR3*	*ETV5*
ESR1	*PIK3CA*	*FGFR4*	*FGFR1*
FGFR2	*RAF1*	*KIT*	*FGFR2*
FGFR3	*RET*	*KRAS*	*FGFR3*
GNA11	*ROS1*	*MET*	*MET*
GNAQ	*SMO*	*MYC*	*NTRK1*
HRAS		*MYCN*	*NTRK2*
IDH1		*PDGFRA*	*NTRK3*
IDH2		*PIK3CA*	*PDGFRA*
			PPARG
			RAF1
			RET
			ROS1

Specimen with lung carcinoma

NE Differentiation

Positive for NE markers

No NE Differentiation

+Pan Keratin
-p40,TTF1, Napsin A

Squamous Carcinoma
+p40

...ıe out metastasis

Treatment

Target Therapy

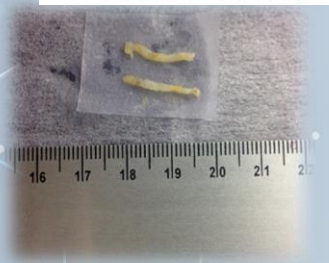
≤ 21 calendar days

Pre-laboratory				Intra-laboratory			Post-laboratory
Diagnosis of non-squamous NSCLC	Pathologist initiates comprehensive biomarker testing	Selection by pathologist of appropriate block for biomarker testing	Receipt of sample in the testing laboratory	Comprehensive biomarker testing	Biomarker testing results	Generation of summary biomarker report	Receipt of report by ordering physician
≤ 3 days*				≤ 10 days*			≤ 24 hours*

*business days

BRAF, HER2 Status?
Agnostic Markers?
Answer to ImmunoTx?

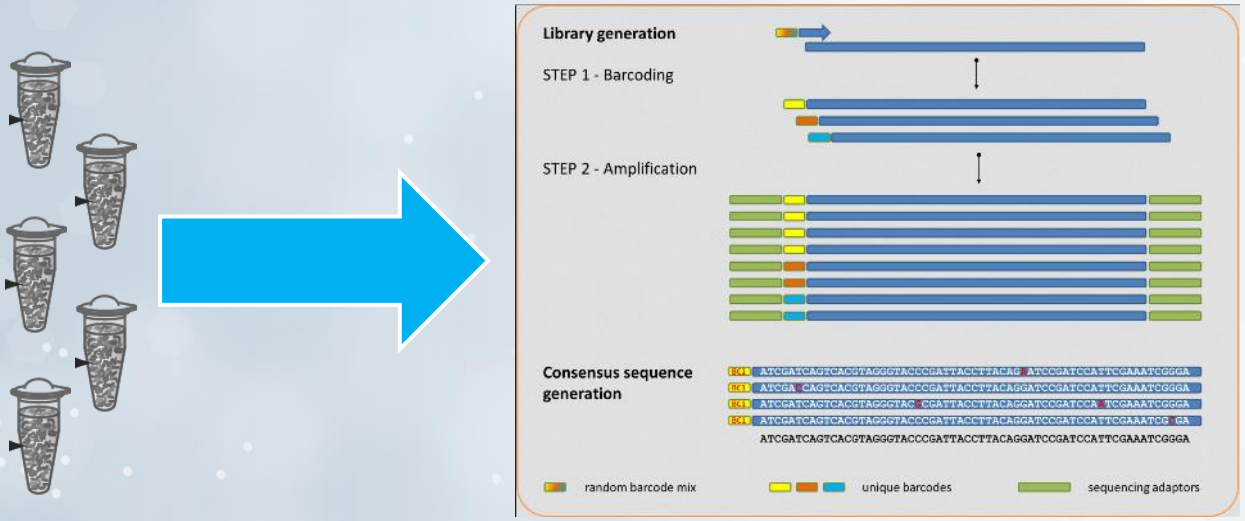
Chemotherapy



Cheema, P. K., et al. "Consensus recommendations for optimizing biomarker testing to identify and treat advanced EGFR-mutated non-small-cell lung cancer." *Current Oncology* 27.6 (2020): 321-329

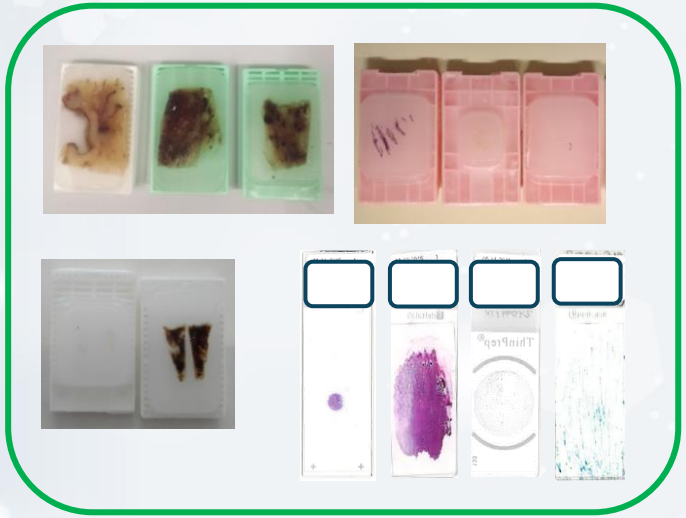
Which technology should be used for mutation testing?

- Any validated.
- However, multiplexed genetic testing with NGS is strongly recommended over single-gene testing.
  - Cheaper
  - Quicker
  - Sample-friendly
  - Sensitive

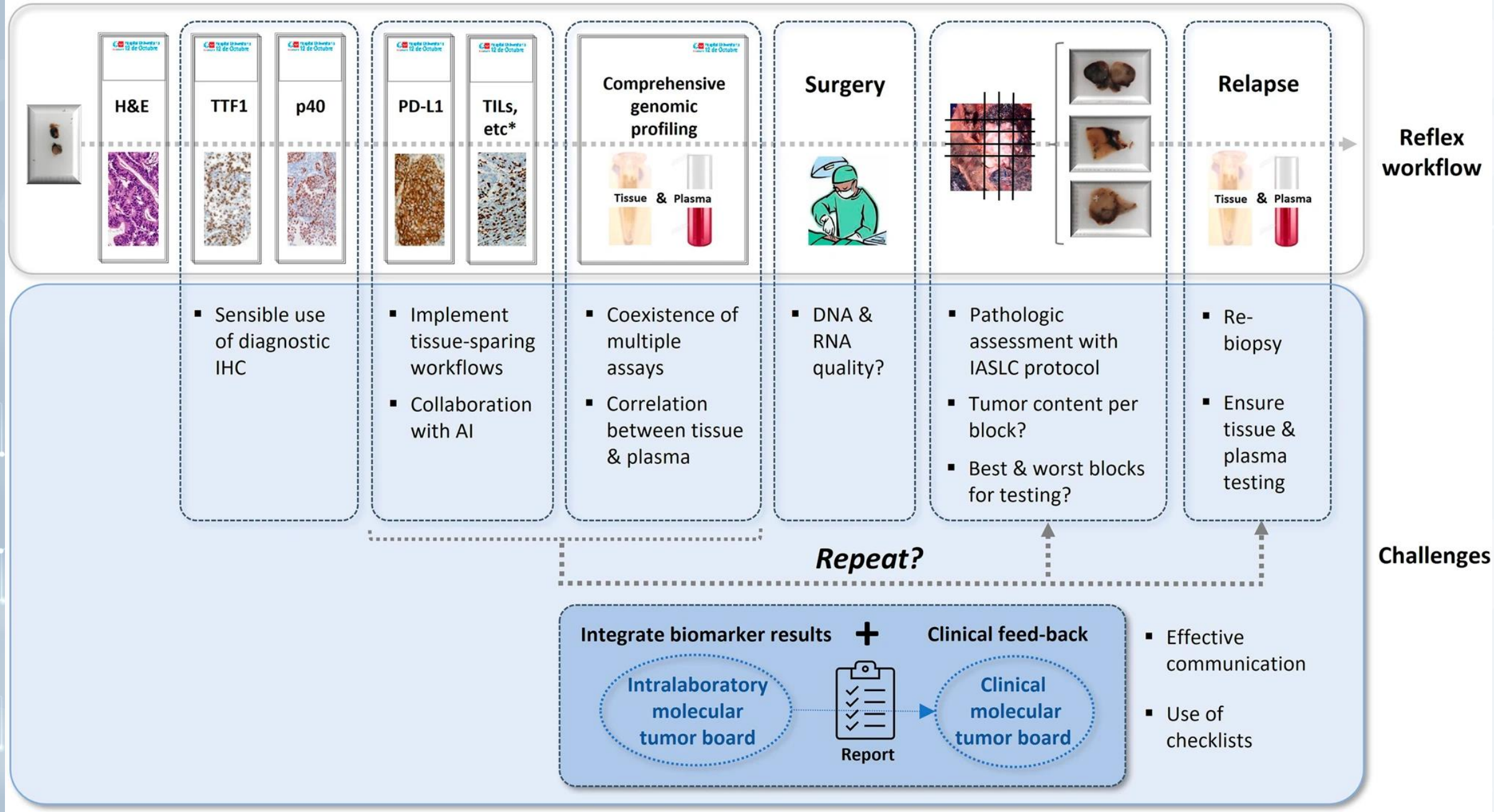


What type of sample can be used at the diagnosis stage?

- Surgical specimens
- Tissue biopsies
- Cytology specimens, with adequate cellularity.



- Plasma samples (liquid biopsies), if other sample is not available or if re-biopsy is not feasible.


H&E
TTF1
p40
PD-L1
TILs, etc*
Comprehensive genomic profiling
Tissue & Plasma
Surgery
Relapse
Tissue & Plasma
Reflex workflow
Sensible use of diagnostic IHC
Implement tissue-sparing workflows
Collaboration with AI
Coexistence of multiple assays
Correlation between tissue & plasma
DNA & RNA quality?
Pathologic assessment with IASLC protocol
Tumor content per block?
Best & worst blocks for testing?
Re-biopsy
Ensure tissue & plasma testing
Repeat?
Challenges
Integrate biomarker results
+
Clinical feed-back
Intralaboratory molecular tumor board
Report
Clinical molecular tumor board
Effective communication
Use of checklists

ROLE OF PATHOLOGIST

- Pre-analytics
- Diagnosis and select of correct material (quality/quantity).
- Use of ”in situ” based techniques.
- Integrated Molecular Pathology – Translation to the clinicians.
- Education.

Schmitt FC. Cytopathology 2011, 2019
Roy-Chowdhuri S. Arch Pathol Lab Med 2016

We need to actively participate in the process of Specimen Acquisition

- ✓ Good communication with clinicians, lab technicians, cytotechs, nurses…
- ✓ Providing a clear order form
- ✓ Using image-guided procedure for better yield
- ✓ Optimizing technique for best diagnostic yield (FNA sampling technique, needle size, number of passes…)
- ✓ ROSE

Go where the fresh material is…

WORKFLOW OF ROSE

MOLECULAR CYTOPATHOLOGY

Molecular/biomarker testing in lung cytology: A practical approach

Fernando Schmitt MD, PhD, FIAC[1,2,3] | Maria D. Lozano MD, PhD, MIAC[4,5,6]

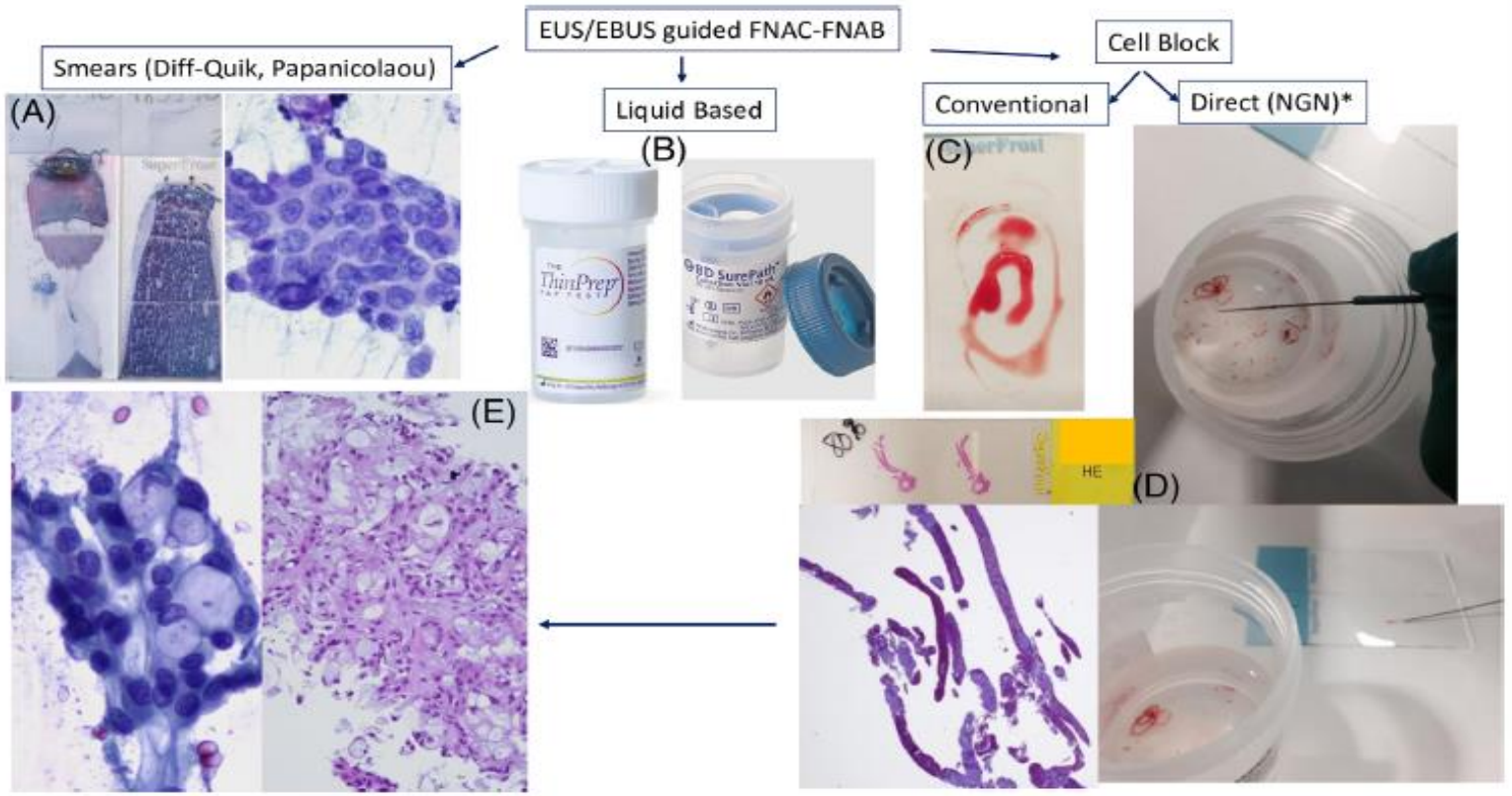


FIGURE 1 Diversity of types of cytological samples obtained from minimally invasive procedures. (A) Smears; (B) liquid based cytology; (C) conventional cell block; (D) direct cell block using new generation needles*; (E) cyto-histological correlations

TABLE 1 Type of cytology samples, fixatives, and ICC results and efficiency

Type of cytological samples	Fixative	Results
Cellblock	Formalin	Comparable results to surgical samples/ biopsies
Pap-stained smears	Alcohol 96°	Comparable results to surgical samples/ biopsies
Unstained smears	Alcohol 96°	Slightly lower but OK
DQ and air-dried smears	No fixative	High rate of false—Low intensity of immunostaining
Liquid based	Methanol-based fixatives	High rate of false—Low intensity of immunostaining

Tissue management in precision medicine: What the pathologist needs to know in the molecular era

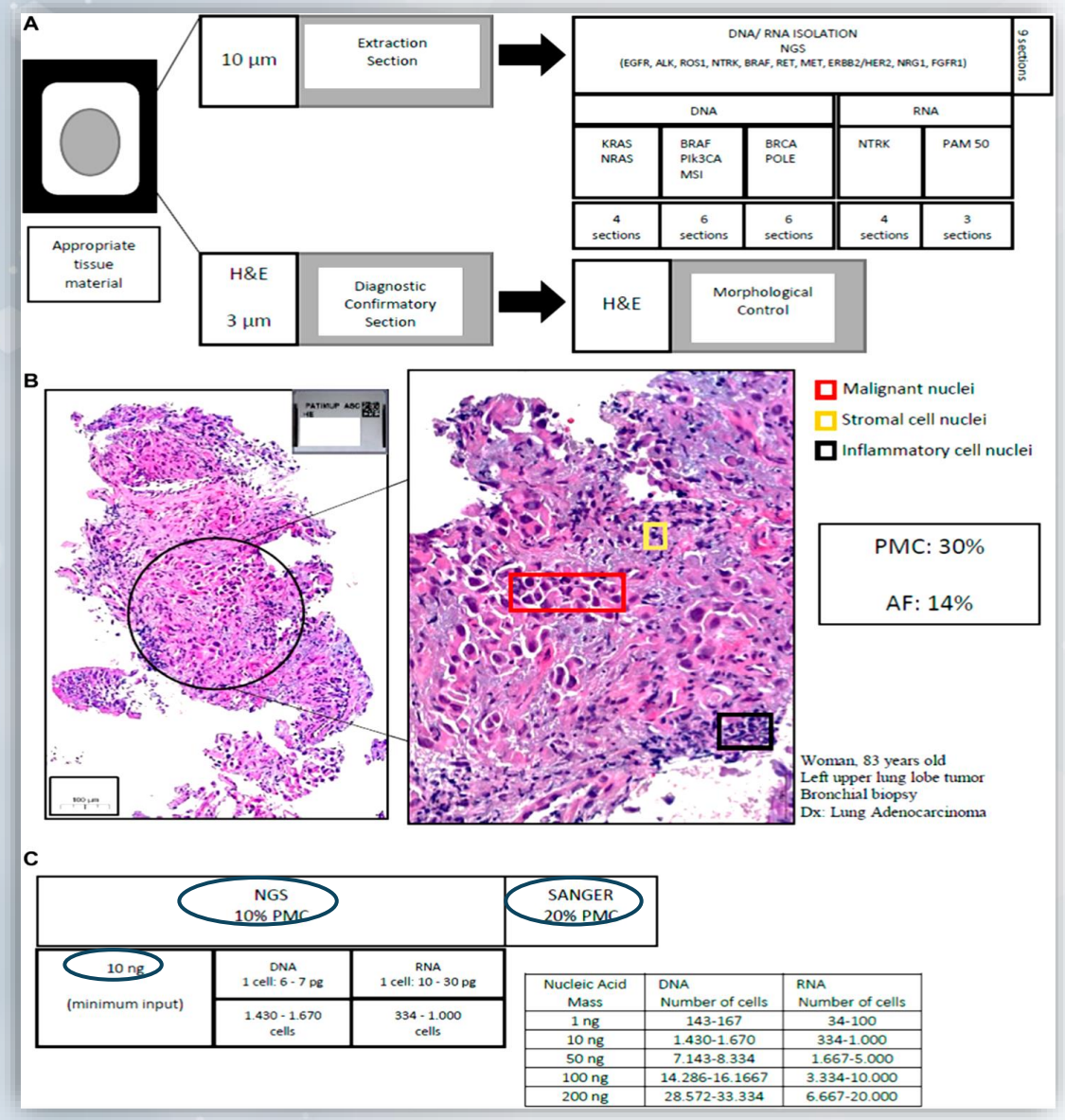
Ricella Souza da Silva[1], Regina Pi Fernando Schmitt[1,2,3]*

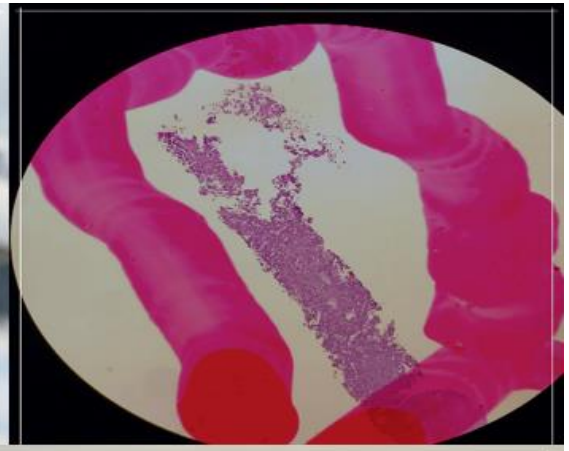
frontiers | Frontiers in Molecular Biosciences
PUBLISHED 26 October 2022
DOI 10.3389/fmolb.2022.983102

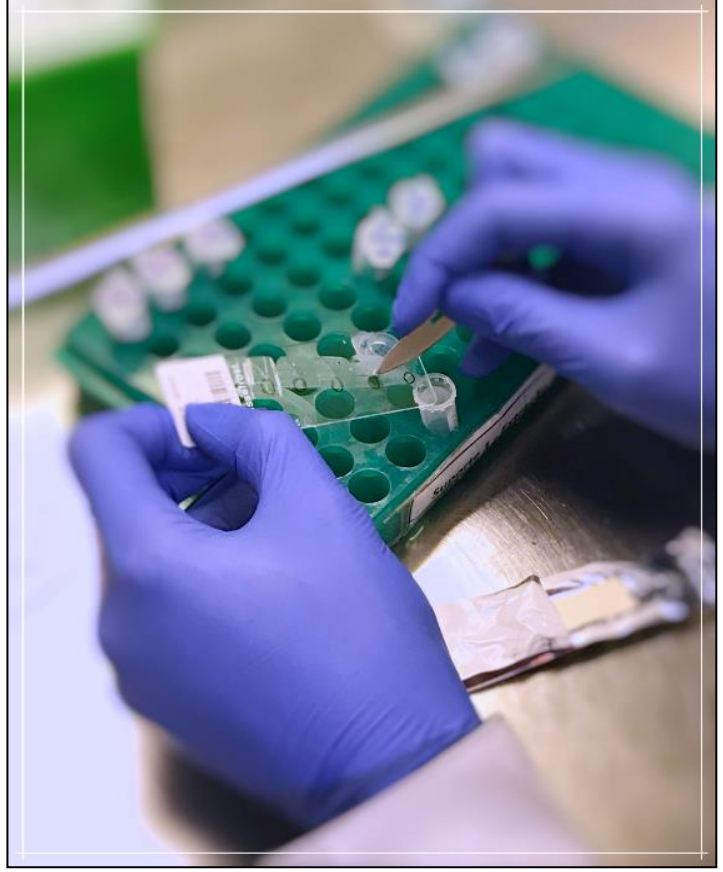
Morphological control for molecular testing

- **Fraction of malignant cells greater than 10%–20% is considered a lower acceptable limit for molecular methods.**
- **In cytology material samples with <100 cells are not suitable for NGS; 100–2000 low levels; 2000-5000 intermediary levels; >5000 cells are suitable for any NGS, including large panels.**

- The coverslip is removed using xylene if crystal coverslip is used or acetone if plastic and the selected cells on the slide are transferred into buffer medium for nucleic acid extraction without need of destaining the slides.
- Single Slide: Scanning and digital archiving is mandatory to ensure medical-legal issues







If it is possible to choose

Molecular analysis on the most Recent Sample

Avoid tissue sample with scarce material: history of larger immune staining and/or molecular test

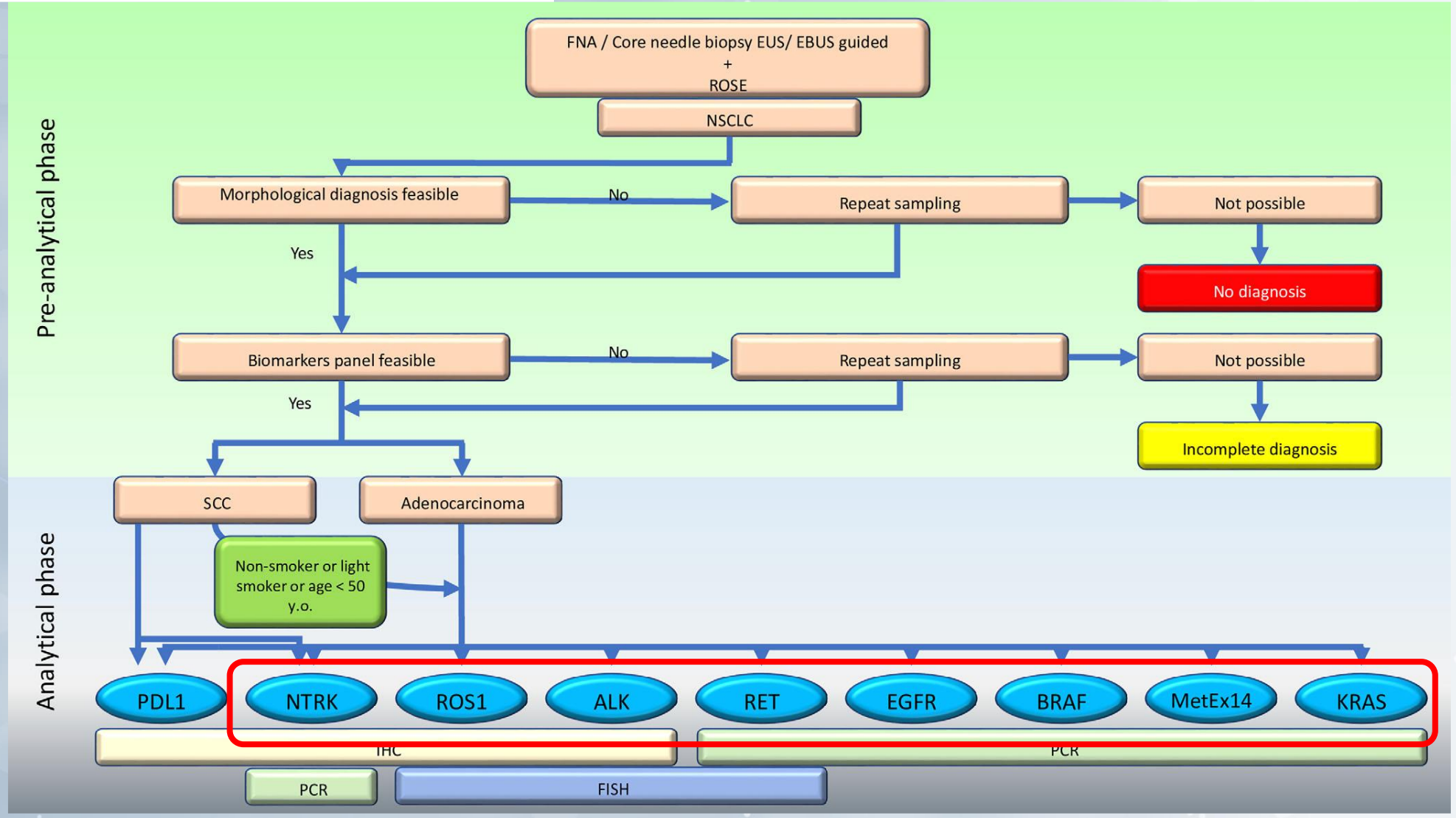
AIM

Samples with the highest amount of material

MOLECULAR CYTOPATHOLOGY

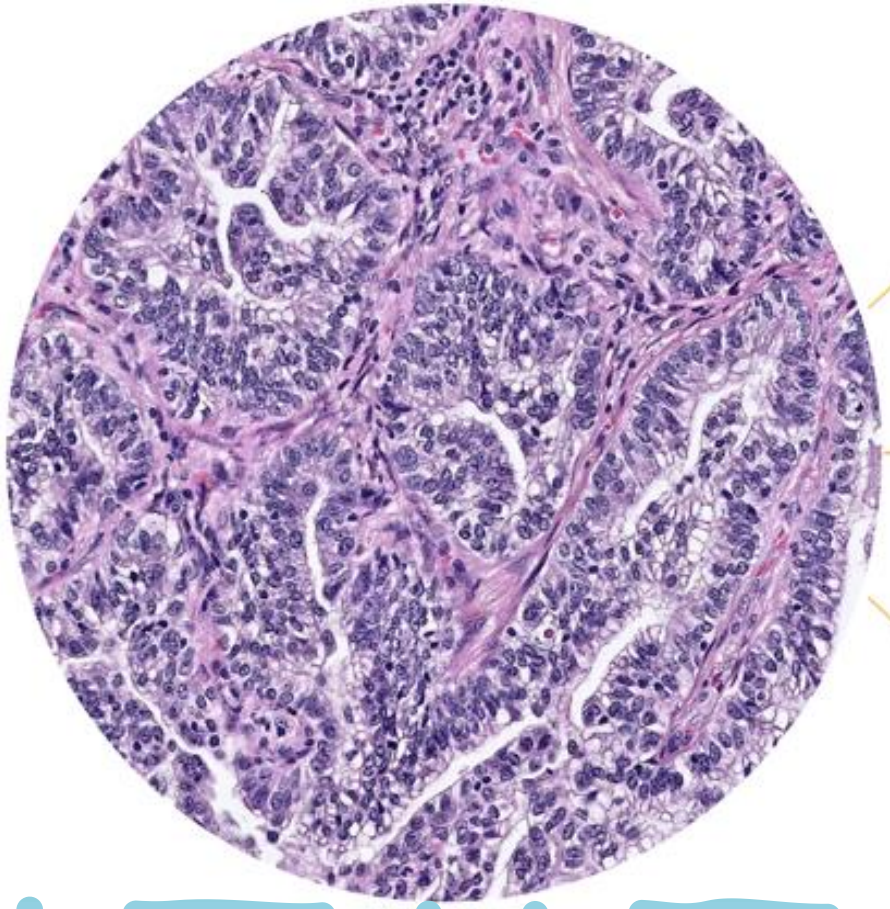
Molecular/biomarker testing in lung cytology: A practical approach

Fernando Schmitt MD, PhD, FIAC[1,2,3] | Maria D. Lozano MD, PhD, MIAC[4,5,6]



NGS

NSCLC: options for first-line therapy

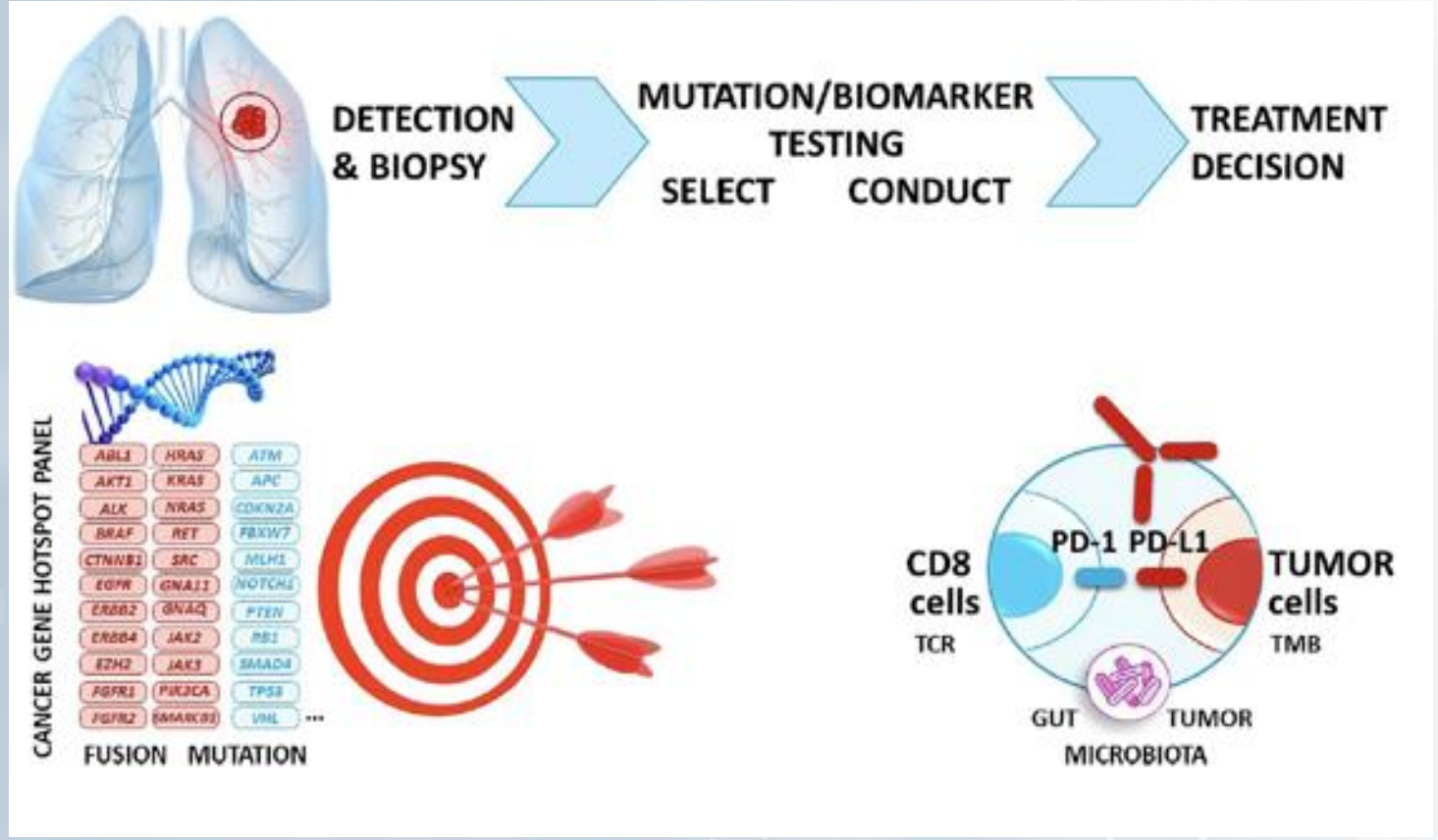


Targeted Therapy
- EGFR
- ALK
- ROS1
- BRAF V600E
- MET ex 14 skipping
- RET
- NTRK

ICI monotherapy
- If no driver mutation *and*
- PD-L1 IHC TPS ≥50%

Chemo + ICI
- If no driver mutation *and*
- PD-L1 IHC TPS <50%

COMBINED BIOMARKERS FOR TARGETED THERAPY AND IMMUNOTHERAPY IN THORACIC ONCOLOGY



Virchows Archiv
https://doi.org/10.1007/s00428-023-03651-1

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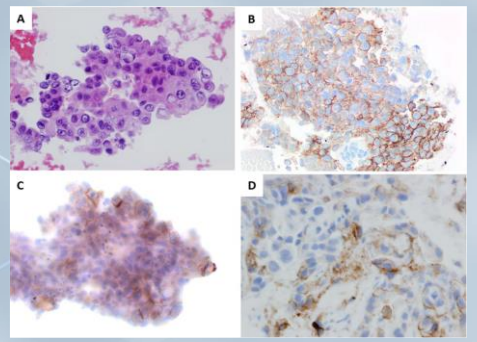
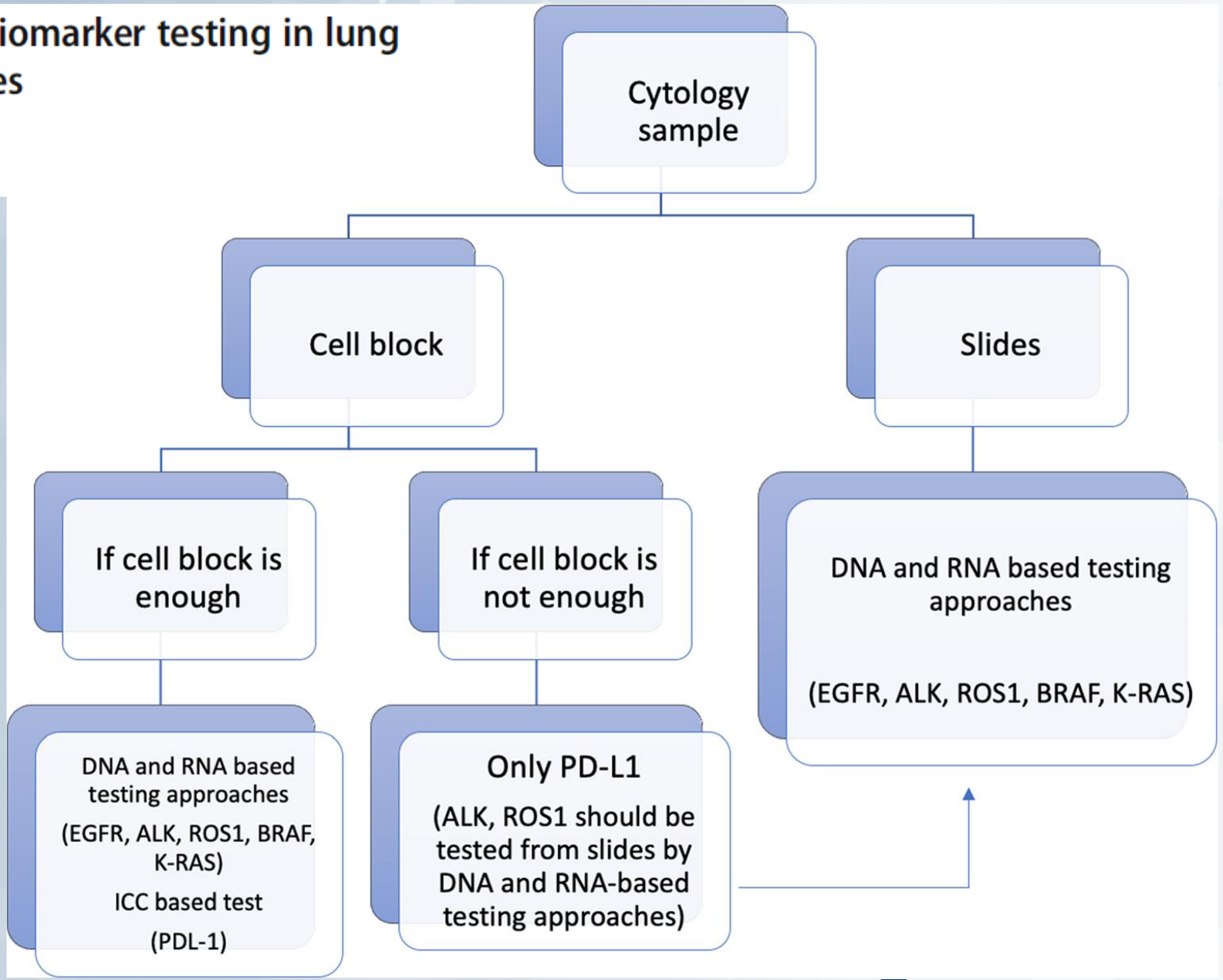
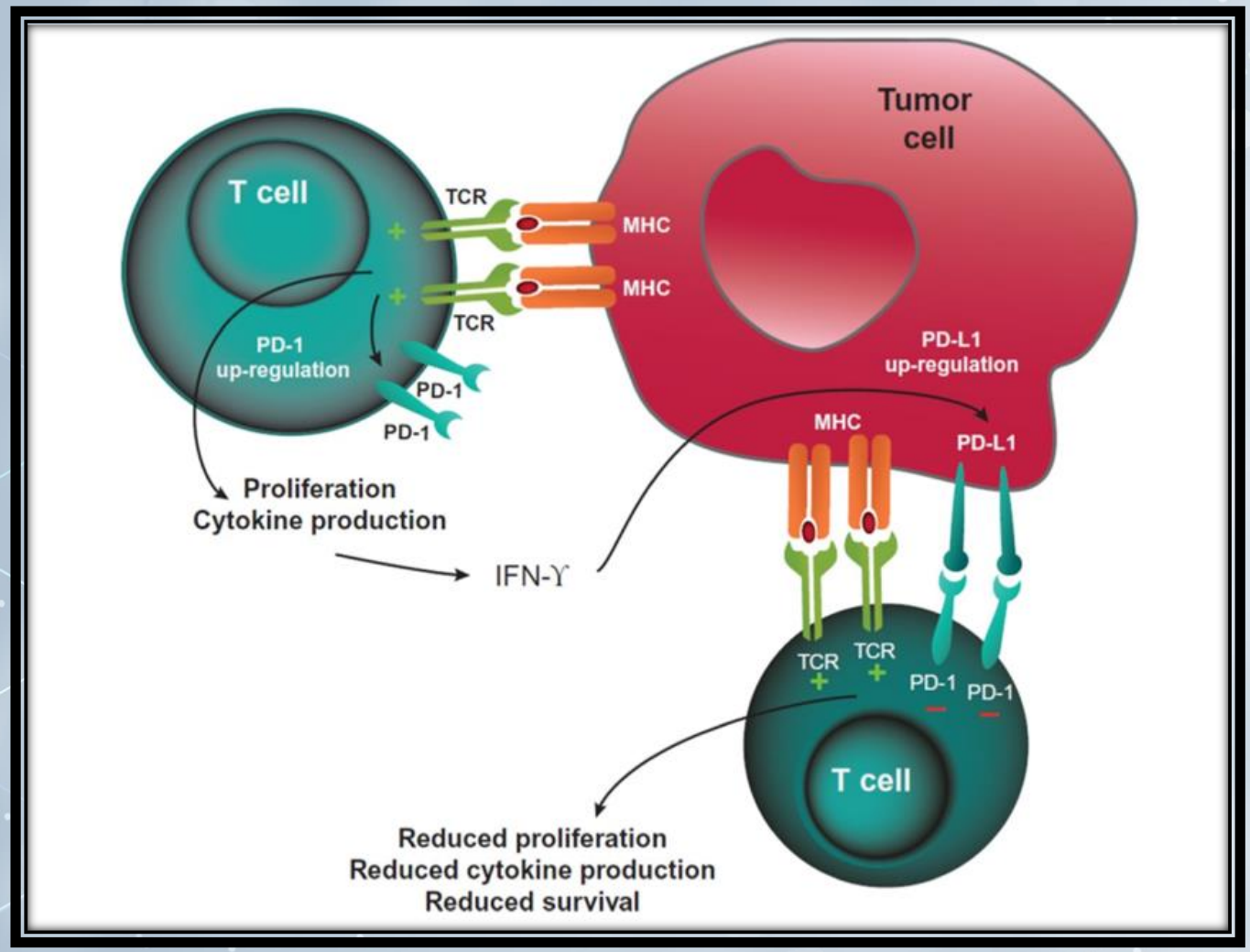
Cytology samples and molecular biomarker testing in lung cancer—advantages and challenges

Sule Canberk [1,2,3] • Marianne Engels [4]





PD-1 mediated inhibition of T cells



- Anti-PD-1 therapy blocks PD-1 on the T cell, thus, the tumor cell cannot use PD-L1 to inactivate the T cell.
- Anti-PD-1 therapy enables T cells to stay active and attack the tumor.

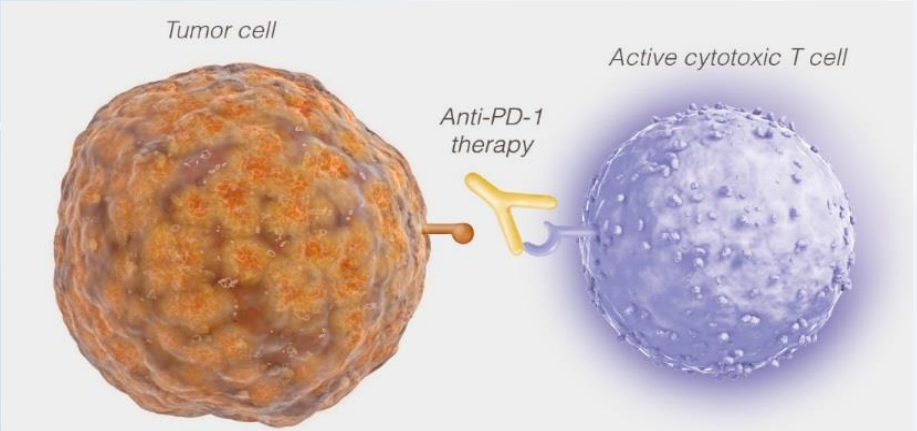


Figure 3: Blocking the PD-1/PD-L1 interaction helps to enable active T cells and tumor cell death and elimination.

Curr. Oncol. **2022**, *29*, 479–489.

Predictive biomarkers for targeted therapies and immunotherapies in non-small cell lung cancer

Mandatory and highly recommended molecular biomarkers			Emerging molecular biomarkers			Exploratory and potential molecular biomarkers		
MANDATORY ESCAT I	*ALK*	Fusion Mutation as a mechanism of resistance	Predictive biomarkers for TARGETED THERAPY ESCAT III	*BRAC1* *BRAC2*	Mutation	Predictive biomarkers for TARGETED THERAPY	*TP53* *RB1*	Mutation
	BRAF	V600E mutation		*FGFR*	Fusion Mutation		*RBM10*	Mutation
	EGFR	Common mutation: Del19, L858R Uncommon mutation: G719X, L861Q, S7681 T790M mutation		*HER2, HER3* *B7-H3* *CEACAM5* *MET, TROP2*	Protein expression		*AKT* *CTNNB1* *JAK2/3*	Mutation
	MET	Mutation exon 14 skipping		*PI3KA*	Mutation		*NRAS*	Mutation
	NTRK	Fusion		*NRG1*	Fusion		*HRAS*	Mutation
	RET	Fusion	Predictive biomarkers for IMMUNO THERAPY	*KEAP1*	Mutation	Predictive biomarkers for IMMUNO THERAPY	High **TCR** clonality	
	ROS1	Fusion Mutation as a mechanism of resistance		*MTAP*	Protein expression		High **CD8** density	
Highly RECOMMENDED ESCAT II	*EGFR*	Exon 20 Insertion		*NOTCH*	Mutation		High **dNLR/LIPI**	
	HER2	Mutations		*STK11*	Mutation			
	KRAS	G12C mutation		*SMARCA4*	Mutation		Adequate **microbiota** Gut and tumor DNA, metabolites, products	
	MET	Amplification		*TMB*	Mutations			

Virchows Archiv
https://doi.org/10.1007/s00428-023-03651-1

Personalized Medicine and Biomarkers
Key Features

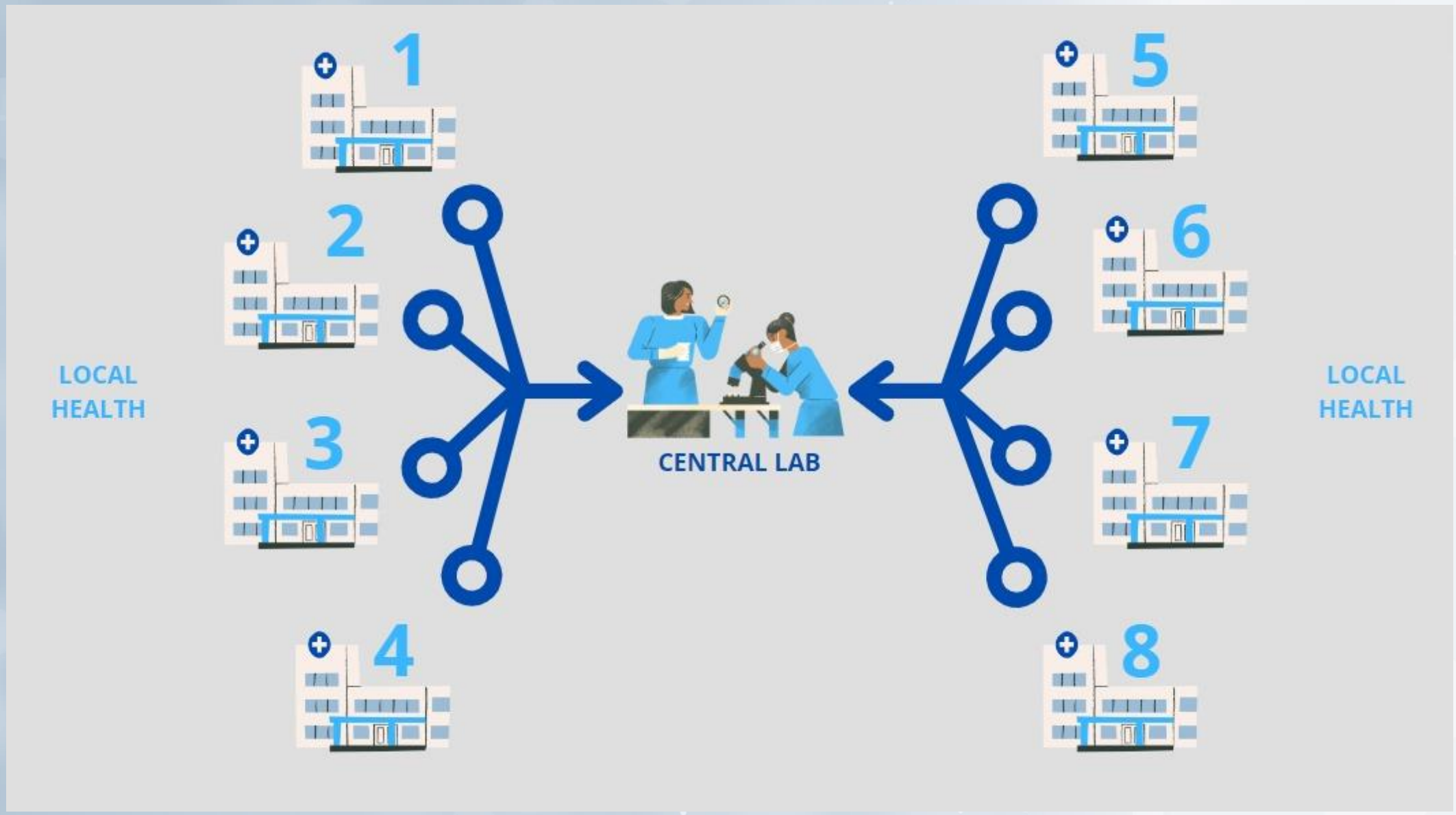
Good material: quality and quantity

Availability of tests: broad panels and high-quality

Financial sustainability

High level of expertise. Highly qualified human resources

Clinical Interaction (Molecular Tumour Boards)


1
2
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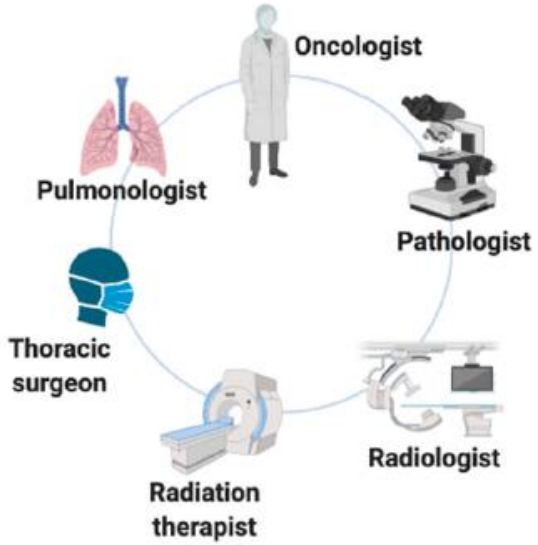
LOCAL HEALTH
LOCAL HEALTH
CENTRAL LAB

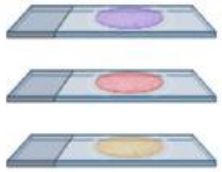

Toronto
General Hospital
University Health Network

REFLEX TEST

1. Multidisciplinary session



2. Tissue selection

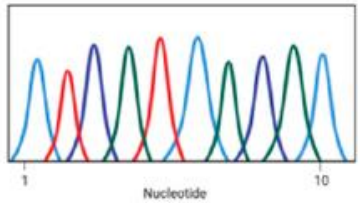


Cellular density → increased success rate

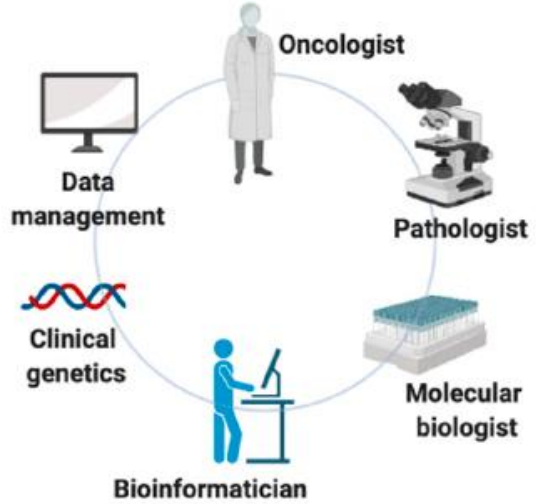
Necrosis and Fibrosis → decreased cellular density

Tumor cell content → optimum 40%, minimum 20-10 %

3. Sequencing



4. Molecular Tumor Board



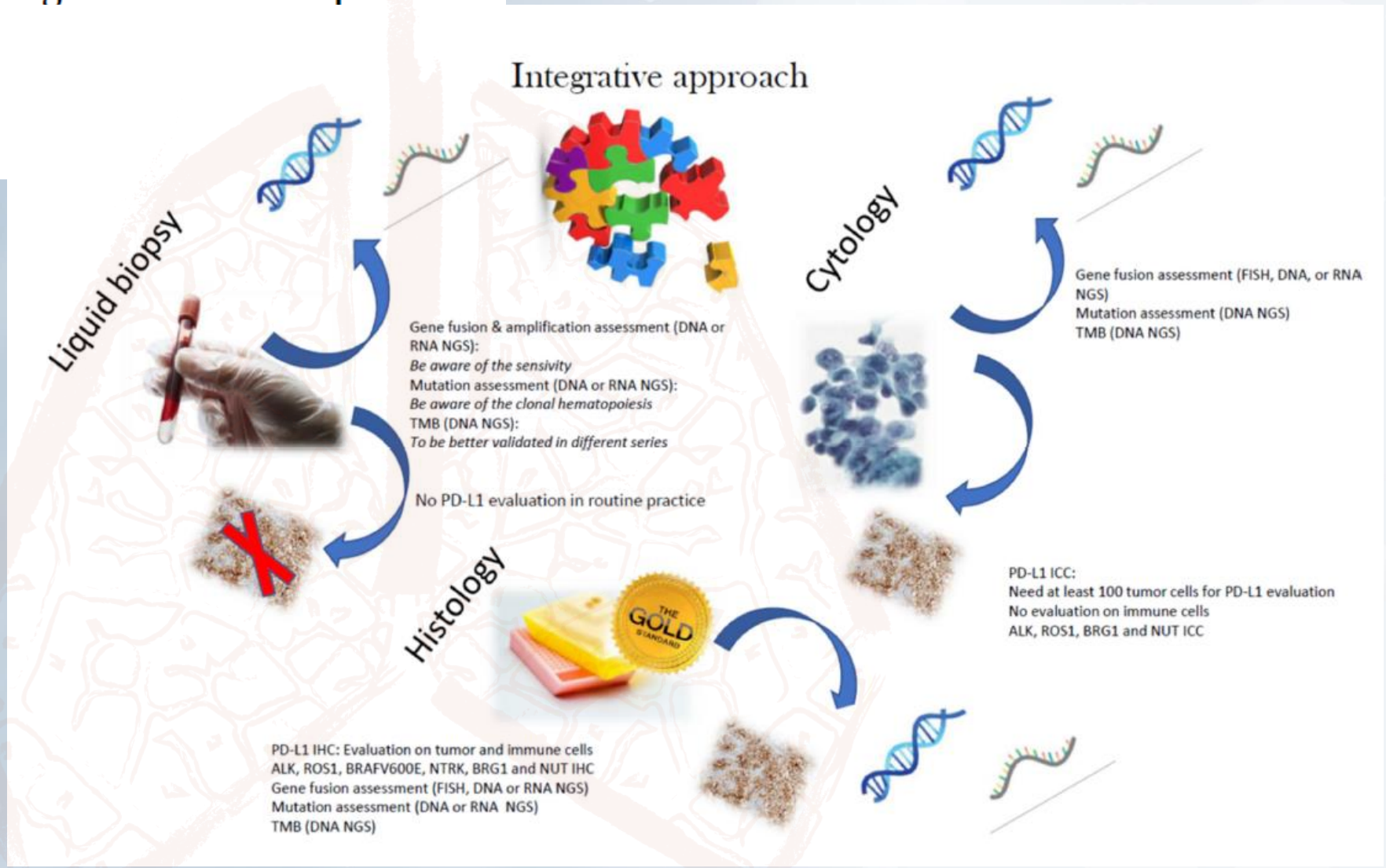
5. Report curation

Lazzari, Chiara, et al. "Next generation sequencing in non-small cell lung cancer: pitfalls and opportunities." *Diagnostics* 10.12 (2020): 1092

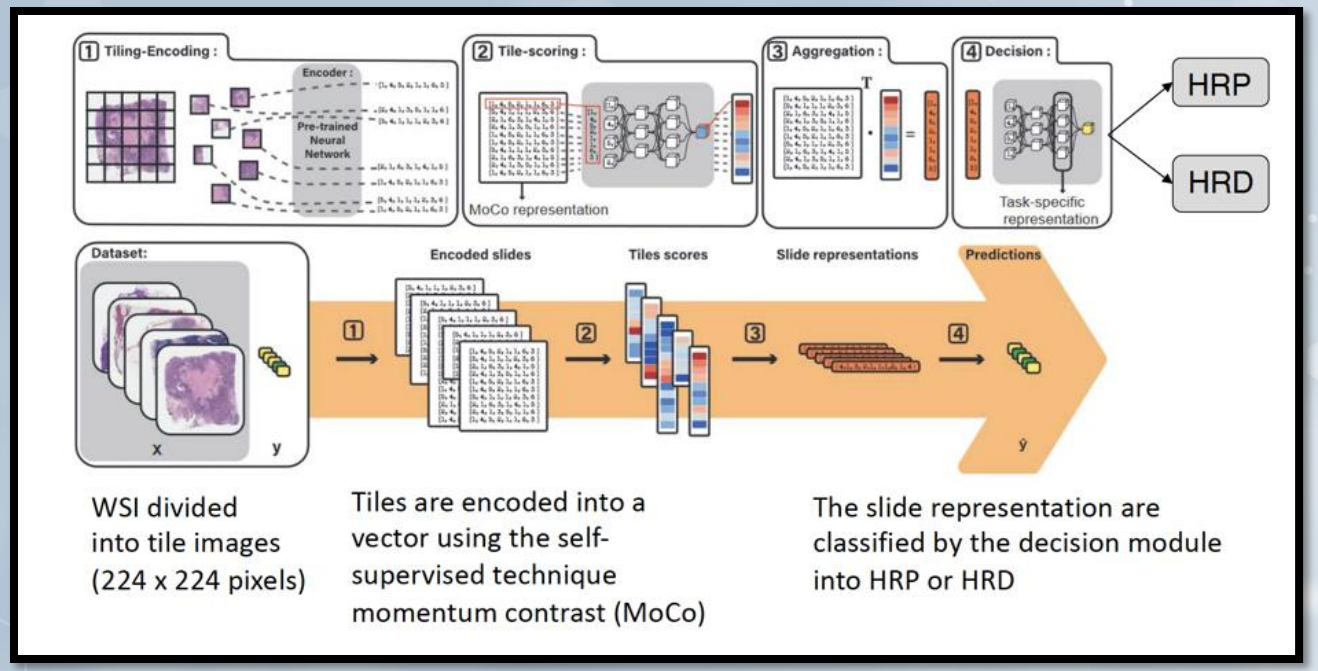
Review

What Is New in Biomarker Testing at Diagnosis of Advanced Non-Squamous Non-Small Cell Lung Carcinoma? Implications for Cytology and Liquid Biopsy

Paul Hofman [1,2]



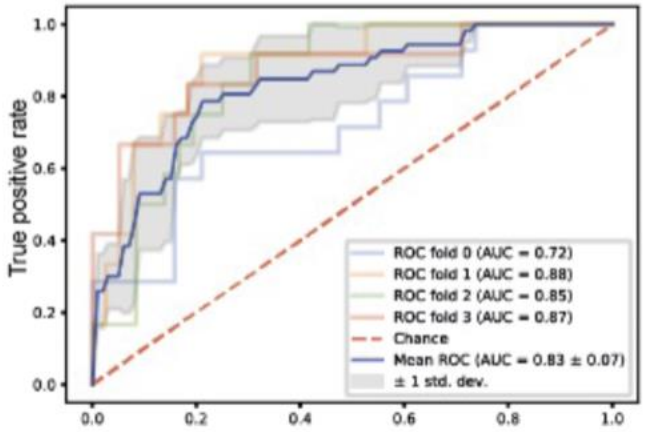
Virchows Archiv
https://doi.org/10.1007/s00428-023-03651-1



Anne Vincent-Salomon
Department of Pathology
Institut CURIE Paris, France

Artificial Intelligence and Breast Cancer Diagnostics

251 Luminal WSI (188 HRD, 63 HRP)
With no technical bias



AUC = 0.83

Sensitity: 88%
Specificity: 57%
Positive predictive value: 86%

	Ground Truth = genomic status Luminal (n= 251)	
AI Prediction	HRD	HRP
HRD	166	27
HRP	22	36
Total	188	63

Conclusions of this study

- Homologous recombination deficiency is predictable from H&E slides with high accuracy

Opening the black box for pathologists is important !

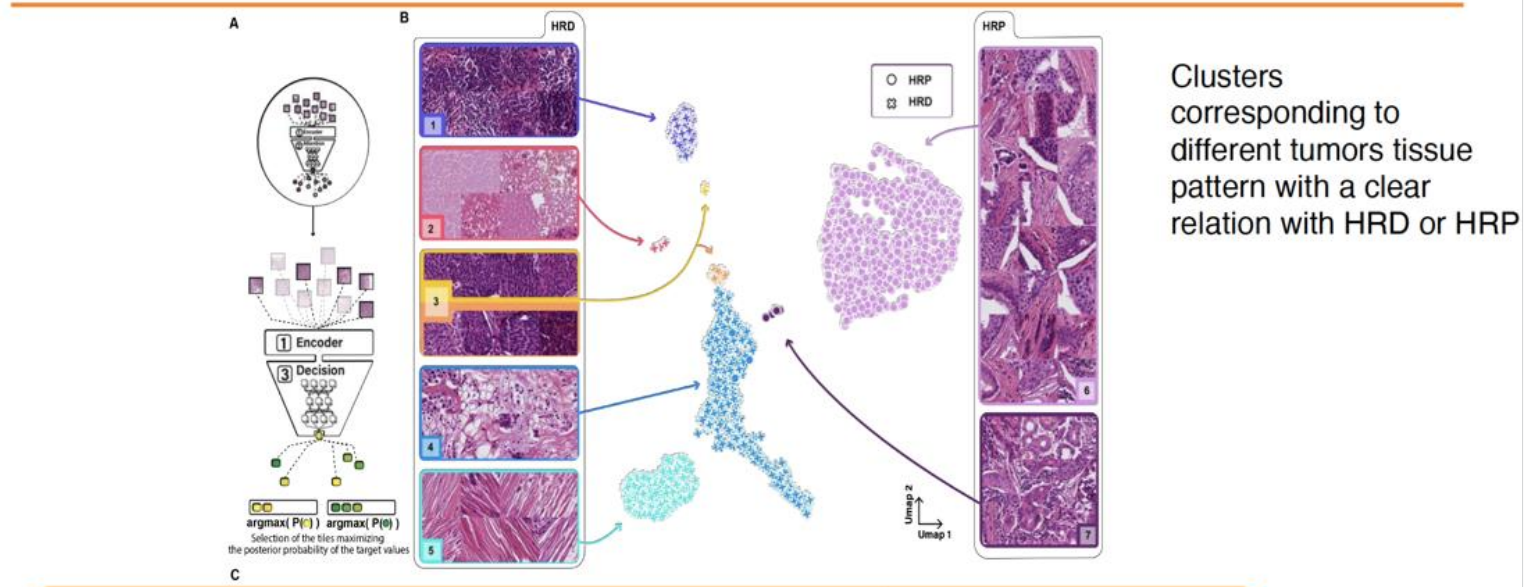
It means to visualize the TILE features that weighted in the algorithm

- To understand the features that weighted the most to predict the label
- To pave the path of MACHINE TEACHING !

Anne Vincent-Salomon
Department of Pathology
Institut CURIE Paris, France

Artificial Intelligence and Breast Cancer Diagnostics

Visualization of TILE features that weighted in the algorithm



Clusters corresponding to different tumors tissue pattern with a clear relation with HRD or HRP

1. High density of Tumor Infiltrating Lymphocytes TILs
2. Hemorragic suffusion associated to necrotic tissue
3. Basal/hyperchromatic carcinomatous cells with nuclear atypia
4. Adipose tissue with inflammatory changes associated with clear tumor cells
5. Laminated fibrosis
6. Low tumor cell density and clear spaces around cell nests
7. Clear space surrounding apocrine cell nests

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ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN CANCER HORIZONS

REVIEW

Druggable targets meet oncogenic drivers: opportunities and limitations of target-based classification of tumors and the role of Molecular Tumor Boards

R. Danesi[1], S. Fogli[1†], S. Indraccolo[2], M. Del Re[1], A. P. Dei Tos[3], L. Leoncini[4], L. Antonuzzo[5], L. Bonanno[6], V. Guarneri[6,7], A. Pierini[8], G. Amunni[9*] & P. Conte[6,7]

Precision Medicine, to be **P**recise, needs **P**athology

Table 1. Biomarkers and available drugs

Name of marker	Druggable/actionable alterations	Tumor type	Predictive value, LoE (e.g. available drugs)	FDA-approved liquid biopsy CDx test
EGFR/ErbB1	Mutations (e.g. L858R, ex19del, T790M)	NSCLC	1 (gefitinib, erlotinib, afatinib, osimertinib, dacomitinib)	Yes
HER2/ErbB2	Amplification	Breast	1 (trastuzumab, T-DM1, trastuzumab + pertuzumab, lapatinib, neratinib)	No
	Amplification	Esophagogastric	1 (trastuzumab)	No
	Point mutations (V659E)	NSCLC	3A (lapatinib)	No
c-Met	ex14 skipping mutations, amplification	NSCLC	1 (crizotinib, capmatinib, savolitinib*, tepotinib)	No
RET	Fusion	NSCLC	1 (selpercatinib, pralsetinib), 2A (cabozantinib), 3A (vandetanib)	No
ALK	Fusion	NSCLC	1 (crizotinib, alectinib, ceritinib, lorlatinib), 3A (brigatinib)	Yes (alectinib)
	Mutations (L1196M, L1196Q)	Soft tissue sarcoma	2A (crizotinib, ceritinib)	No
ROS1	Fusion, mutation	NSCLC	1 (crizotinib, entrectinib)	No
NTRK	Fusion	All tumors	1 (larotrectinib, entrectinib)	No
c-Kit	Mutations (e.g. 449_514mut), deletions (e.g. D419del)	GIST	1 (imatinib, sunitinib, regorafenib), 2A (sorafenib)	No
		Thymic tumors	2A (sunitinib)	No
		Melanoma	2A (imatinib)	No
PDGFR		GIST	2A (imatinib, dasatinib)	No
		Leukemia, myelodysplasia	1 (imatinib)	No
FGFR1		LSCC	3A (erdafitinib)	No
		NSCLC	3A (AZD4547)	No
FGFR2		Bladder, cholangiocarcinom	1 (erdafitinib, pemigatinib)	No
	Amplification	Breast	3A (dovitinib)	No
FGFR3	Fusion, mutation	Bladder	1 (erdafitinib)	No
RAS	Wild-type	CRC	1 (cetuximab, panitumumab)	No
BRAF	Mutations (e.g. V600E)	Melanoma	1 (vemurafenib, dabrafenib, trametinib, combo), 3A (trametinib)	No
		NSCLC	1 (dabrafenib + trametinib)	No
		Histiocytosis	3A (cobimetinib)	No
	Mutation (V600E)	CRC	1 (encorafenib + cetuximab)	Yes
	Fusions	Ovarian	3A (trametinib, cobimetinib)	No
MEK	Mutations	Melanoma, NSCLC, ovarian, histiocytic disorder	3A (trametinib, cobimetinib, selumetinib)	No
mTOR	Mutations (e.g. E2014K)	Bladder, RCC	3A (everolimus, temsirolimus)	No
AKT	Mutation (E17K)	Breast, ovarian	3A (capivasertib)	No
PTEN	Homozygous deletions, loss-of-function mutations	Breast	2A (capivasertib)	No
PIK3CA	Mutations	Breast	1 (alpelisib)	Yes
CDK4	Amplification	Soft tissue sarcoma	2A (palbociclib)	No
IDH1	Mutations	AML, cholangiocarcinoma	1-3A (ivosidenib)	No
IDH2	Mutations	AML	1 (enasidenib)	No
BRCA1/2 and ATM	Mutations (somatic)	Breast	1 (olaparib, talazoparib, rucaparib)	No
	Mutations (somatic)	Ovarian, prostate	1 (rucaparib, olaparib)	Yes
ERα	Mutations (e.g. E380Q)	Breast	2A (fulvestrant)	No
MSI-H	Not applicable	All	1 (pembrolizumab)	Yes
TML	Not applicable	Multiple tumor types	1 (pembrolizumab, nivolumab)	No

SAMPLES
FFPE
CYTOLOGY

- Cooperating is tougher than competing.

25 ANOS A DESCOBRIR O CANCRO
ipatimup

RISE
Health Research Network
From the Lab to the Community

U.PORTO

FM UP

FERNANDO SCHMITT

RISE – HEALTH RESEARCH NETWORK

THANK YOU
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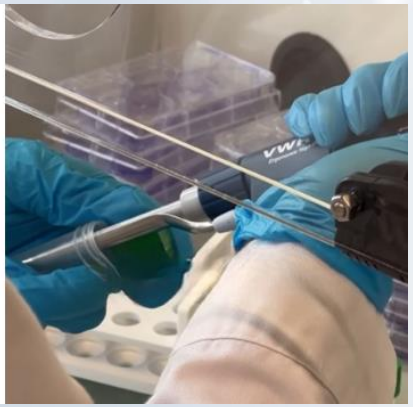

3° ENCONTRO NACIONAL DE INVESTIGAÇÃO CLÍNICA & INOVAÇÃO BIOMÉDICA