Welcome to the 50th anniversary of
the French Society of Clinical Cytology

Looking at the Past and into the Future
of Cytopathology

22th November 2017
Convention Centre "Palais des Congrès", Paris, France

Cytopathology in the future: New worlds to conquer

Prof. Fernando Schmitt
Department of Pathology and Oncology, Medical Faculty of Porto University
Head of Molecular Pathology Unit, IPATIMUP
General-Secretary of the International Academy of Cytology

No financial disclosures


STAR TREK
EXPLORING NEW WORLDS
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TOMORROW'S

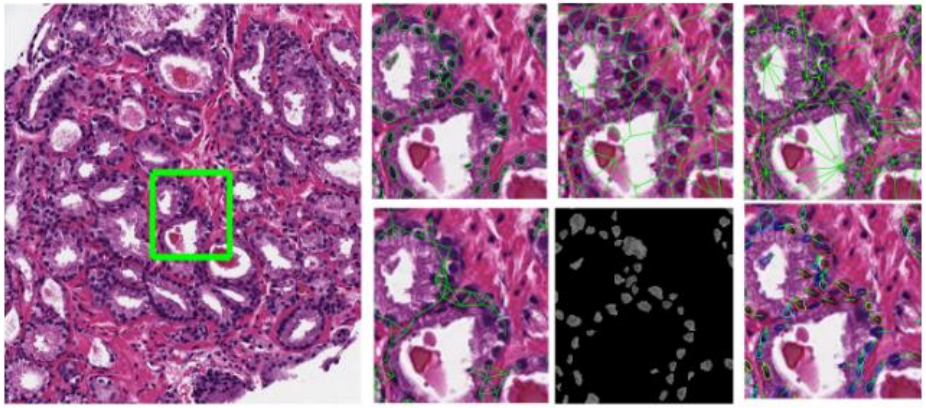
TECHNOLOGICAL CHANGE IS ACCELERATING TODAY AT AN UNPRECEDENTED SPEED AND COULD CREATE A WORLD WE CAN BARELY BEGIN TO IMAGINE.

WE LIVE IN A MIND-BLOWINGLY DIFFERENT WORLD THAN OUR GRANDPARENTS.

AlphaGo, Deep Learning, and the Future of the Human Microscopist

Scott R. Granter, MD; Andrew H. Beck, MD, PhD; David J. Papke Jr, MD, PhD

Digital Pathology And Artificial Intelligence In The Precision Medicine Era



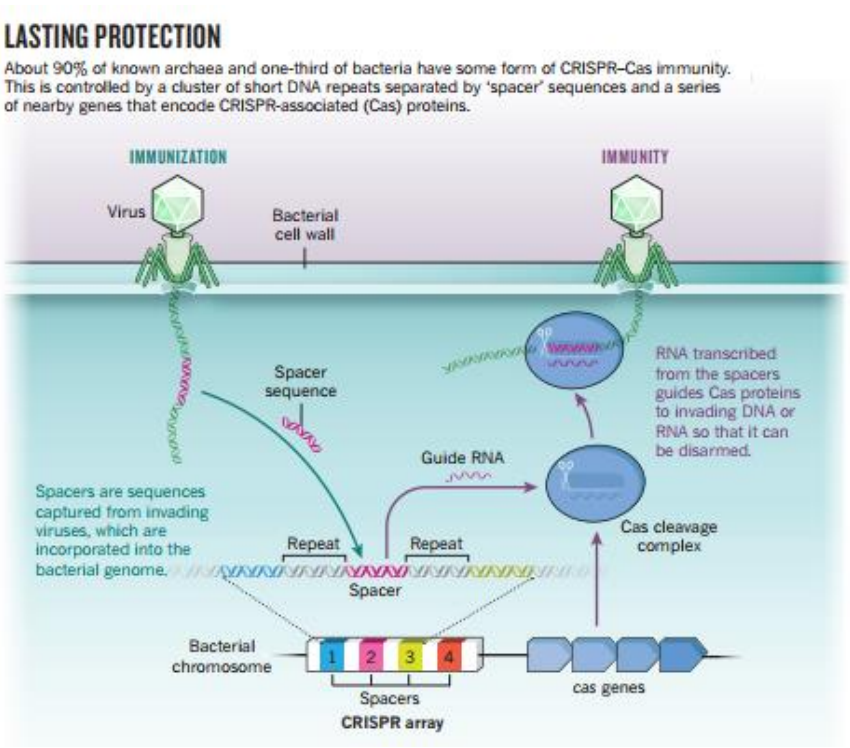
Oncology and Genomics

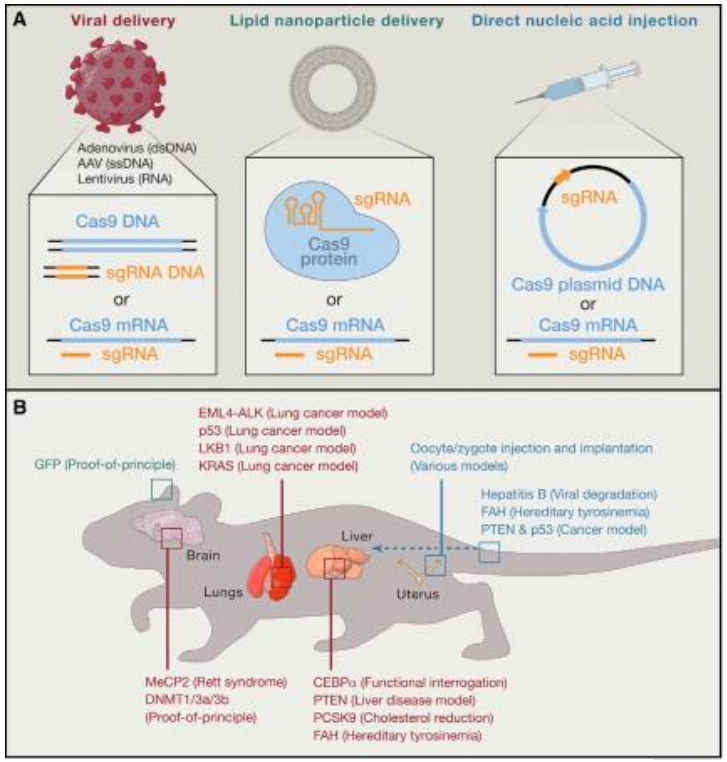
Watson for Oncology

Spend less time searching literature and the EMR, and more time caring for patients. Watson can provide clinicians with evidence-based treatment options based on expert training by Memorial Sloan Kettering (MSK) physicians.

Tomorrow's children

What would genome editing really mean for future generations?





BIOTECHNOLOGY

CRISPR fixes embryo error

Gene-editing experiment in human embryos pushes scientific and ethical boundaries.

Gynecological Cytology:
Too Old to be a Pop Star but Too Young to Die

*Correspondence to: Fernando C. Schmitt, M.D., Ph.D., F.I.A.C., IPATIMUP, Rua Roberto Frias s/n, 4200 Porto, Portugal.
E-mail: fschmitt@ ipatimup.pt
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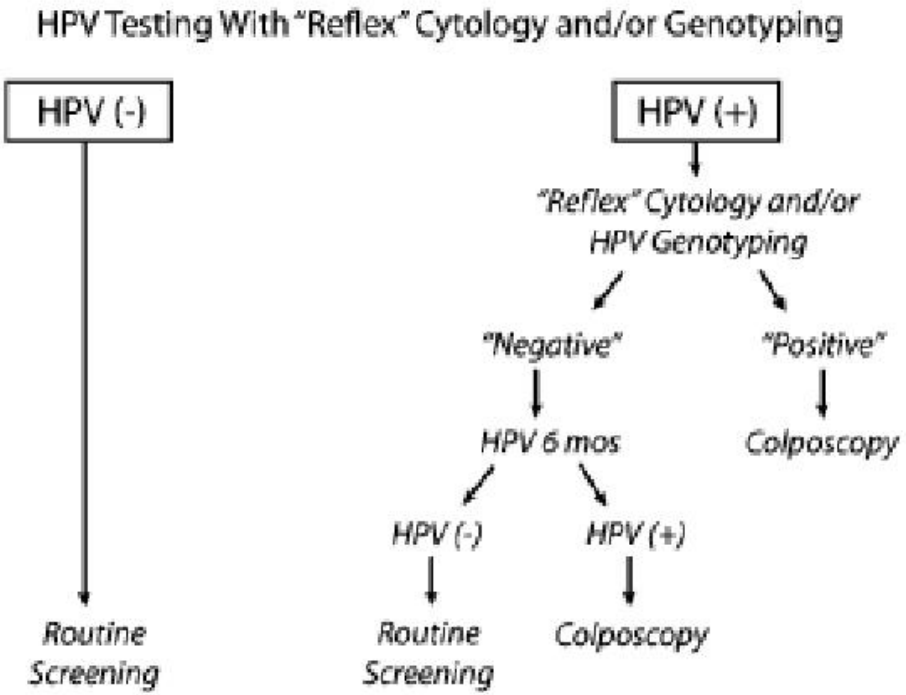
The British rock composer Ian Anderson wisely described a history of a *rocker* with his old idiosyncrasies in a world in which the paradigms are constantly transformed: "Now he's too old to *Rock'n'Roll* but he's too young to die." It is an inherent situation where the "time waits for no one." In the science scenario this is not so different. The innovations in methods and equipments continuously improve and replace old techniques. The expected results of this progressing process are to enhance the quality of results.[6]

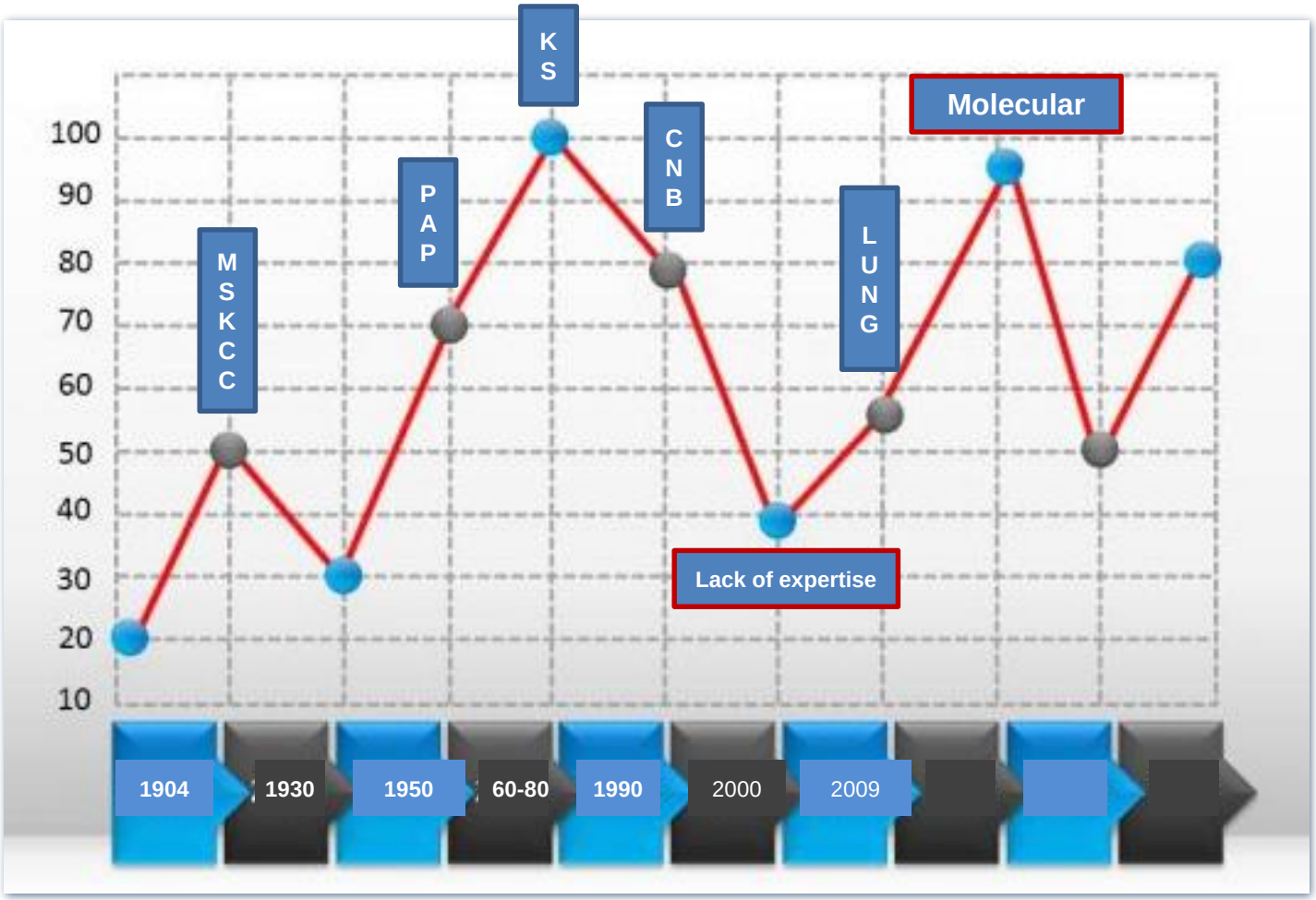
Gynecological cytology

To old to be a popstar but too young to die

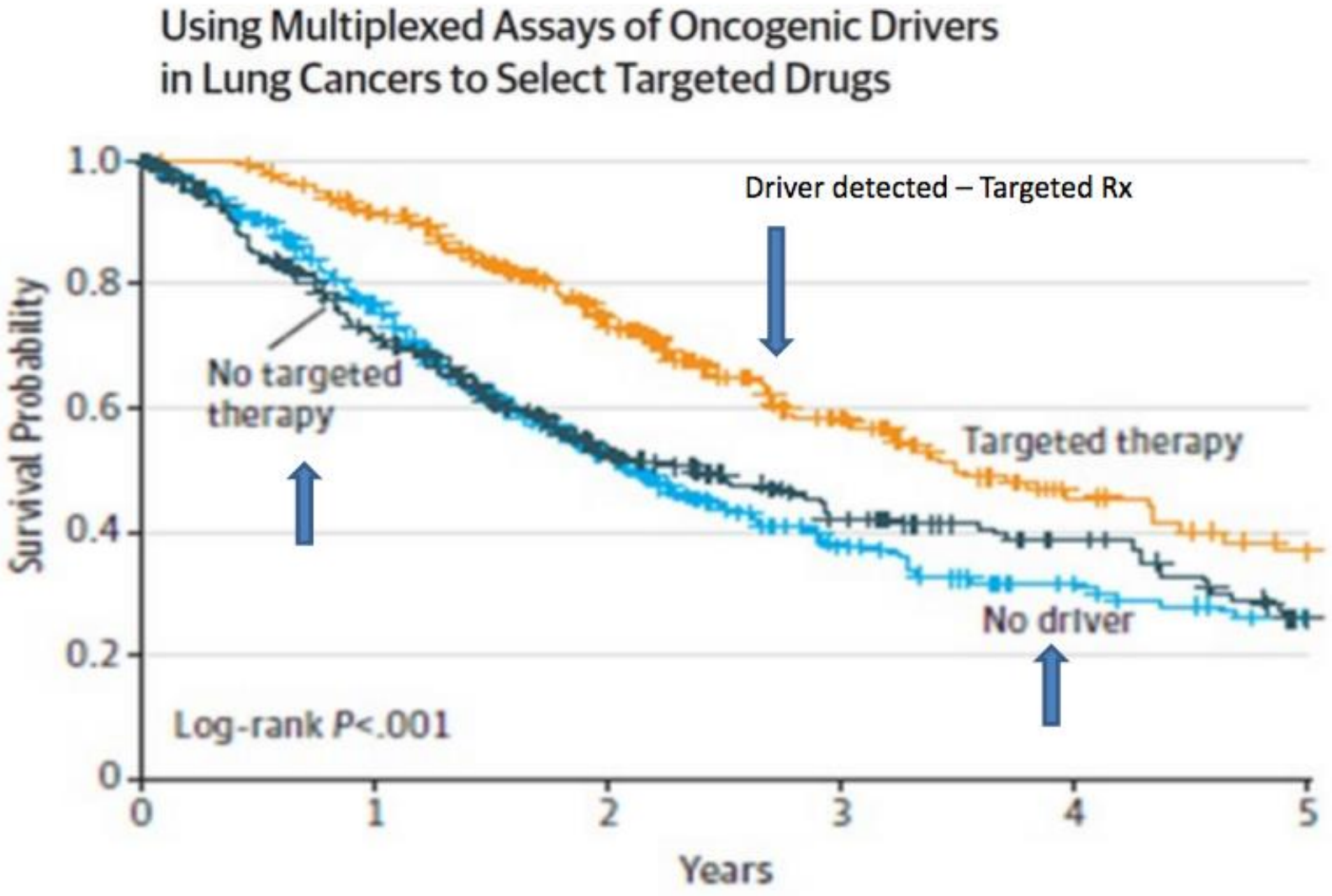
Longatto-Filho A & Schmitt FC. Diagn Cytopathol 35: 672, 2007



Ups And Downs of Cytology



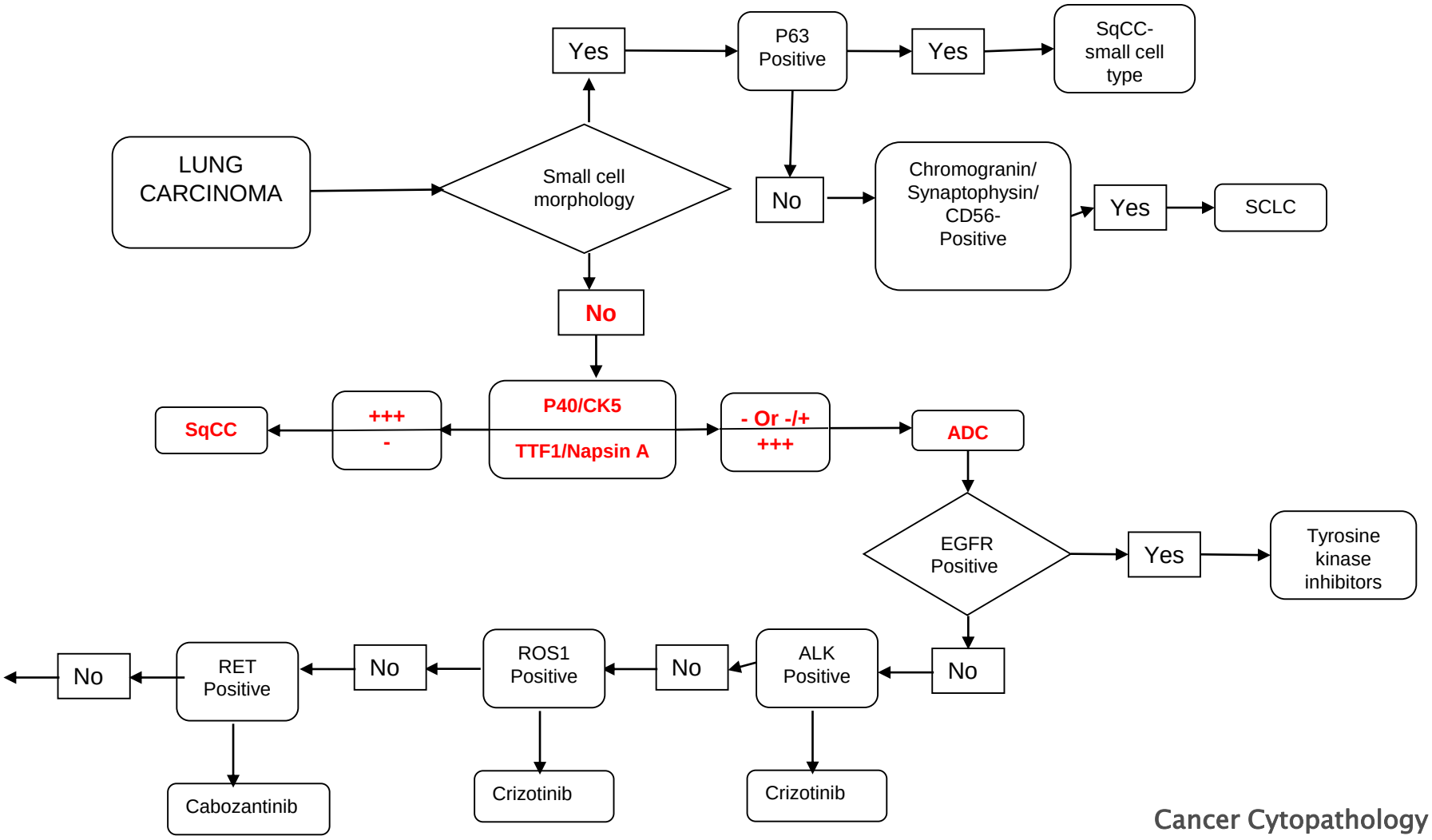
It is worthwhile finding an Actionable genetic alteration in Lung cancer



Kris MG et al. JAMA 2014; 311, 1998-2006

Role of Ancillary Studies in Fine-Needle Aspiration From Selected Tumors

Fernando Schmitt, MD, PhD[1,2] and Helena Barroca, MD[3]



International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma

Journal of Thoracic Oncology • Volume 6, Number 2, February 2011

PATHOLOGY CONSIDERATIONS FOR GOOD PRACTICE

- Small biopsy and cytology samples should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.

KEY POINTS TO USE MOLECULAR TECHNIQUES IN CYTOLOGY

- Collect good and well-preserved material.
- Validate molecular studies on cytological material.
- Control the cases morphologically.

Schmitt FC. Cytopathology 2011


R+C

How Technology Is Reshaping the Practice of Nongynecologic Cytology

Frontiers of Cytology Symposium

Moderator and Editor
Marluce Bibbo, M.D., Sc.D., F.A.S.C.P., F.I.A.C.

Participants
Måns Åkerman, M.D., Ph.D., F.I.A.C., **Venancio A. F. Alves**, M.D., Ph.D., **Lukas Bubendorf**, M.D., M.I.A.C., **Terence J. Colgan**, M.D., F.R.C.P.C., F.C.A.P., M.I.A.C., **Hitoshi Itoh**, C.T.I.A.C., M.I.A.C., **Kusum Kapila**, M.D., F.A.M.S., F.R.C.Path., F.I.A.C., **Ruth L. Katz**, M.D. M.I.A.C., **Gerardine Mitchell**, B. App. Sc. (Medical Technology), A.S.C., M.A.I.M.S., **Nicholas J. Mulvany**, M.B., B.Ch., B.A.O., D.C.P., F.I.A.C., F.R.C.P.A., **Joseph F. Nasuti**, M.D., F.C.A.P., F.A.S.C.P., **Wai-Kuen Ng**, M.B.B.S., F.R.C.P.A., F.H.K.C.Path, F.H.K.A.M.(Pathology), F.I.A.C., **Robert Y. Osamura**, M.D., F.I.A.C., **José P. Schalper**, M.D., M.I.A.C., **Fernando Carlos Schmitt**, M.D., Ph.D., F.I.A.C., **Akihiko Serizawa**, C.T.I.A.C., **Alain P. Verhest**, M.D., Ph.D., F.I.A.C., and **Philippe Vielh**, M.D., Ph.D., M.I.A.C.

[Acta Cytol 2007;51:123–152]

Commentary

ACTA CYTOLOGICA

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Expectations and Projections for the Future of Nongynecolgical Cytology 10 Years Ago: Did They Materialize and How Did We Do?

Fernando C. Schmitt[a, b] Philippe Vielh[c]

[a]Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), and [b]Department of Pathology and Oncology, Medical Faculty, University of Porto, Porto, Portugal; [c]Laboratoire National de Santé, Dudelange, Luxembourg

1. *The Role of Morphology*. For several decades, cytopathologists have practiced diagnostic cytopathology of nongynecologic specimens based on morphologic features. Do you think morphologic features will still play an important role 10 years from now?

- All participants predicted that morphology would still play an important role 10 years later.
- In 2017 and in the next years morphology still will be the cornerstone for diagnosis in cytopathology.
- The success of molecular techniques still depends on a good qualitative and quantitative evaluation of the cytological material.

Acta Cytologica 2017;61:373–407

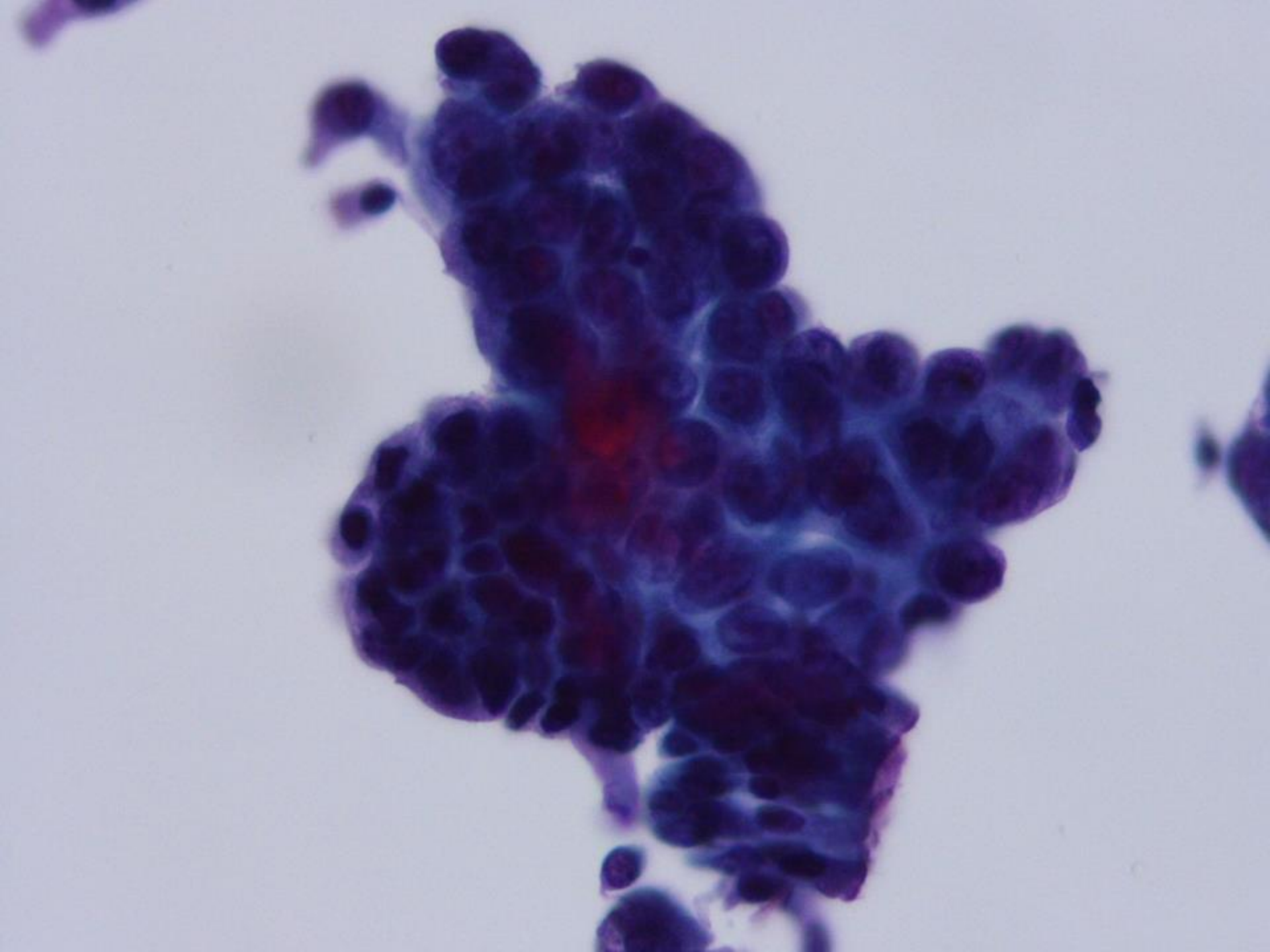
EDITORIAL DOI:10.1111/j.1365-2303.2011.00941.x

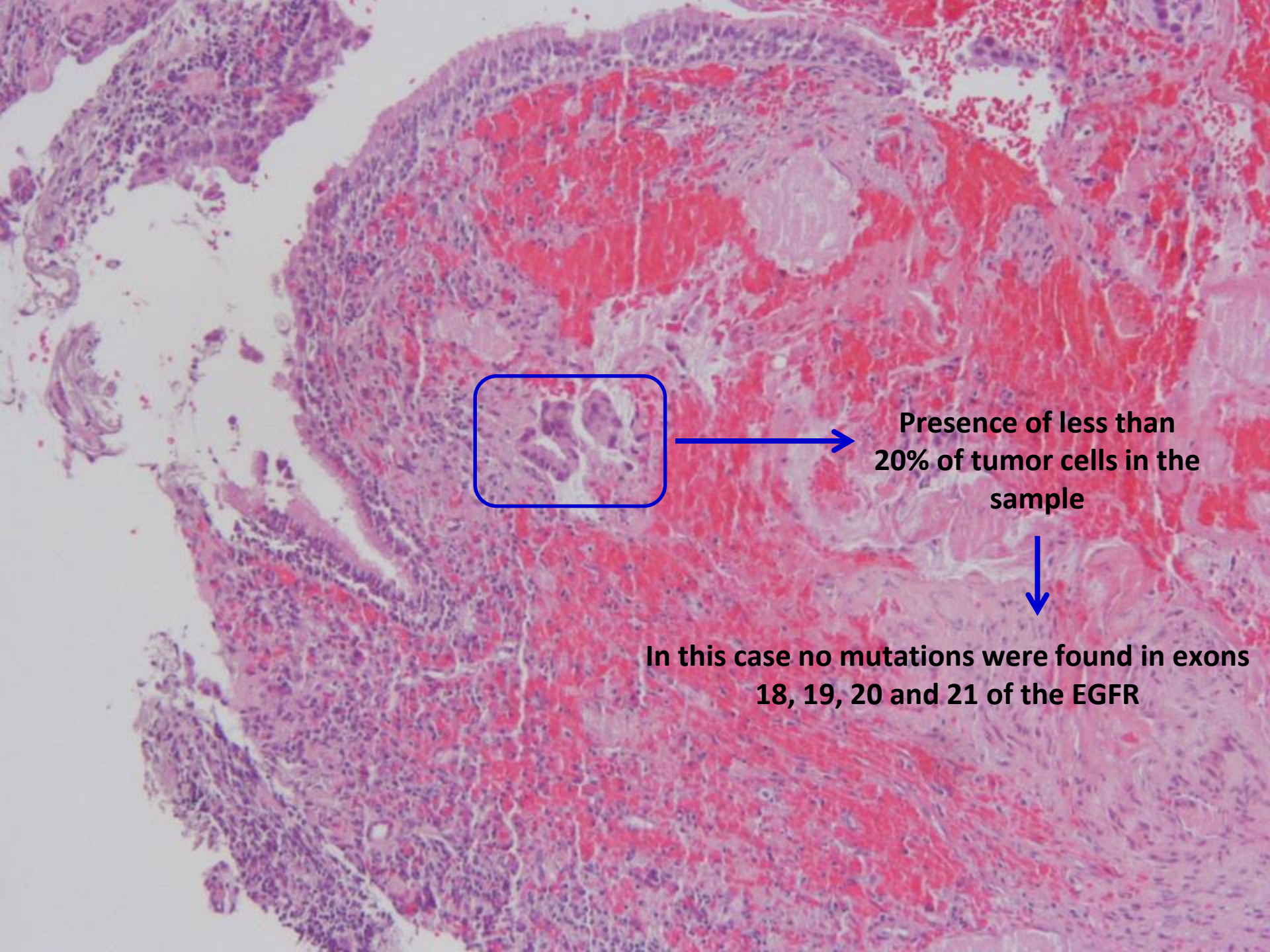
Molecular cytopathology and flow cytometry: pre-analytical procedures matter

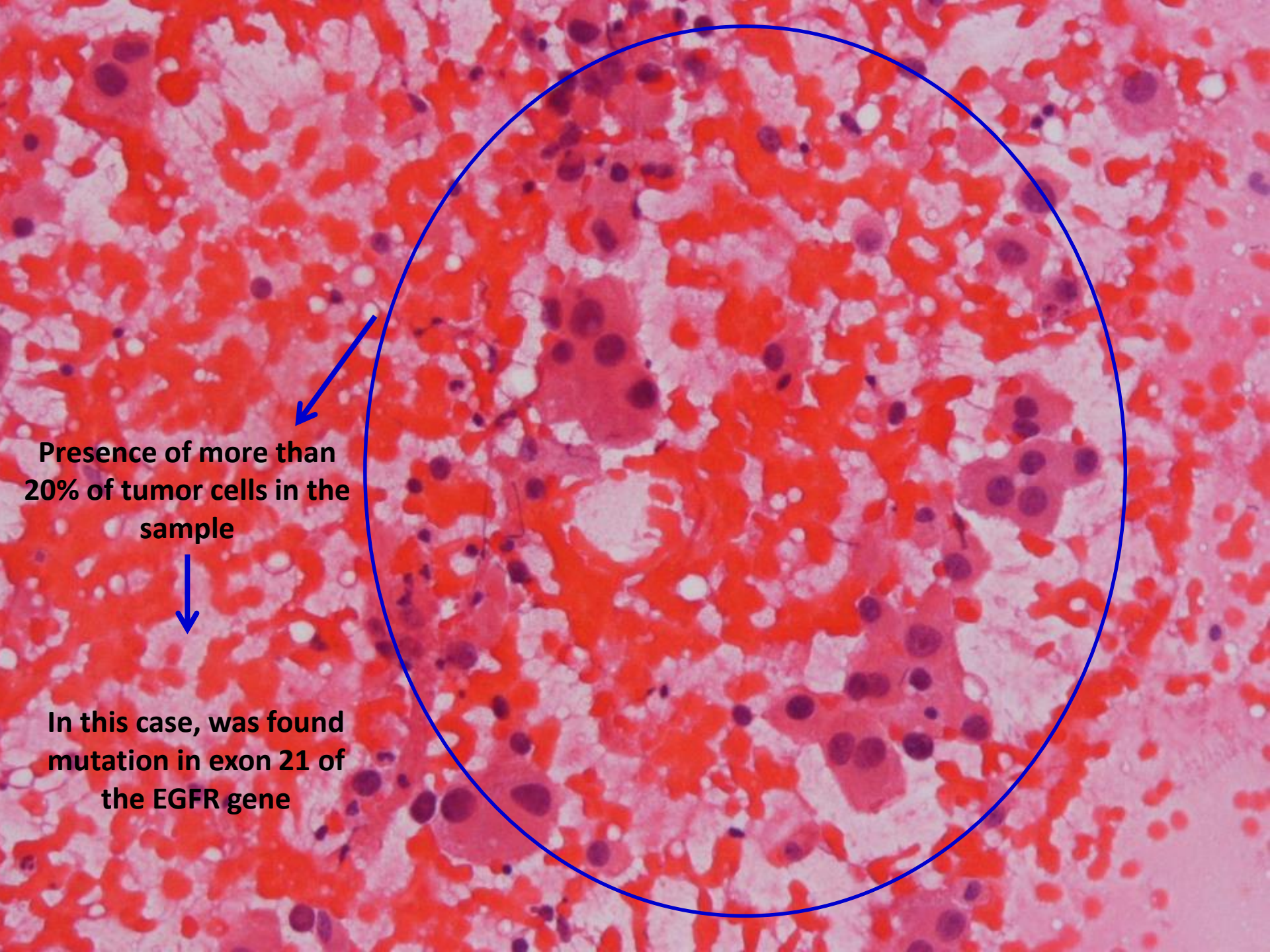
- Morphological control is one of the most crucial issues in the application of molecular techniques on cytology.

- The role of cytopathologist is mandatory in the collection and selection of cells.

Schmitt FC. Cytopathology 2011




Presence of less than 20% of tumor cells in the sample
In this case no mutations were found in exons 18, 19, 20 and 21 of the EGFR


Presence of more than 20% of tumor cells in the sample
In this case, was found mutation in exon 21 of the EGFR gene

Tumour cell content: Which molecular test?

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

ONCOMINE FOCUS ASSAY

20%

50%

2. *The Role of Imaging Techniques.* Fine-needle aspiration (FNA) under the guidance of several imaging techniques has become an indispensable component of the workup of many lesions. The issues discussed under this topic were: Who performs FNA? What is the role of core biopsy? Can the imaging techniques replace cytology in the diagnosis?

- Guided FNA is usually performed by radiologists and interventional radiology has grown exponentially in the last decade. Pathologists, clinicians and surgeons still performs aspirations, mainly under US-guided.

- EBUS expanded significantly allowing obtain material from lung, mediastinal lymph nodes and GI-tract creating new opportunities for cytology with the practice of ROSE.

- The role of core biopsy expanded in breast, prostate and soft-tissue and in many situations complements FNA.

- Until now no imaging technique replace morphological diagnosis.

Acta Cytologica 2017;61:373–407

3. *The Role of Immunocytochemistry.* Immunocytochemistry (ICC) has become an important adjunct for cytological diagnosis; however, the use of different preparations and fixation were a problem for standardization. Questions about the current and future roles of this technique and the challenges were discussed.

- Despite collaborative studies, improvements in the technique with automation and better quality control, we still do not have universal standardization for the application of ICC in cytology. From the different types of preparation the most used is cellblocks.

- Most of diagnostic and prognostic tests using immuno 10 years ago are still in use, however one of the markers that is extremely useful today for the subtyping of NSCLC, p40 appeared in the last 10 years.

- None of the panelists predicted the screening role of ICC for molecular tests as ALK and ROS-1 antibodies used to select cases for searching translocations in these genes.

Acta Cytologica 2017;61:373–407

Immunocytochemistry in Europe: results of the European Federation of Cytology Societies (EFCS) inquiry

F. Schmitt*, B. Cochand-Priollet†, M. Toetsch‡, B. Davidson§, A. Bondi¶ and P. Vielh**

Cytopathology 2011, **22**, 238–242

- This survey showed wide variation in the use of ICC among different laboratories across the Europe.
- The variability in material and fixatives is a major factor preventing standardization of some procedures using ICC.
- Currently, ICC methods have been refined and high quality reagents and automation are more widely available, so it is expected that technical problems will be reduced in the near future.

Multinational study of oestrogen and progesterone receptor immunocytochemistry on breast carcinoma fine needle aspirates

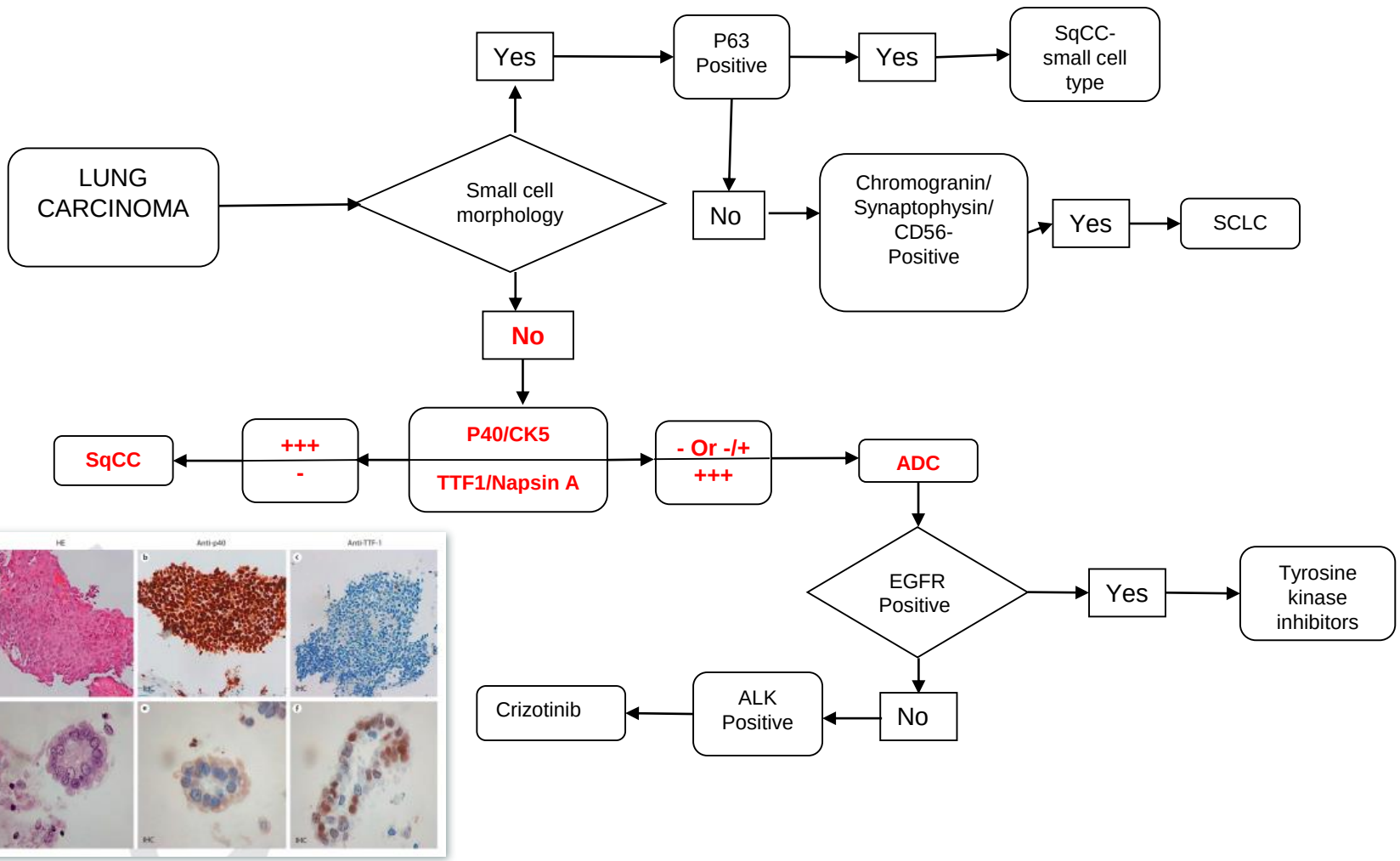
Ž. P. Marinšek*, N. Nolde*, I. Kardum-Skelin†, R. Nizzoli‡, B. Önal§, T. Rezanko¶, E. Tani**, K. T. Ostović††, P. Vielh‡‡, F. Schmitt§§ and G. Kocjan¶¶

Cytopathology 2012

- Cytospins and monolayer preparations were superior to direct smears for the evaluation.
- Methods of fixation and antigen retrieval were the key points in the staining process.
- While it was not possible to prove the superiority of a single fixation protocol, the usefulness of antigen retrieval (heat-induced) was clearly demonstrated.

Role of Ancillary Studies in Fine-Needle Aspiration From Selected Tumors

Fernando Schmitt, MD, PhD[1,2] and Helena Barroca, MD[3]



Review

ACTA CYTOLOGICA

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p40: A p63 Isoform Useful for Lung Cancer Diagnosis – A Review of the Physiological and Pathological Role of p63

Ana Rita Nobre[a, b] André Albergaria[a, c] Fernando Schmitt[a, c]

No p40, no squamous

Table 1. Distribution of TTF-1, p63, and p40 in lung cancer

	Histological diagnosis					SE	SP	PPV	NPV	Ref.
	ADC	SCC	LCC/SC	ADSC	LCL					
TTF-1	115/150 (77)	0/50 (0)	n.a.	n.a.	n.a.	0.77	1.00	1.00	0.59	45
p63	27/150 (18)	50/50 (100)	n.a.	n.a.	n.a.	1.00	0.82	0.65	1.00	
p40	0/150 (0)	50/50 (100)	n.a.	n.a.	n.a.	1.00	1.00	1.00	1.00	
TTF-1+/p63+	26/180 (14)	0/50 (0)	n.a.	n.a.	n.a.	0.14	1.00	1.00	0.25	
TTF-1+/p63–	115/180 (64)	0/50 (0)	n.a.	n.a.	n.a.	0.64	1.00	1.00	0.43	
TTF-1–/p63+	4/180 (2)	50/50 (100)	n.a.	n.a.	n.a.	1.00	0.98	0.93	1.00	
TTF-1–/p63–	35/180 (19)	0/50 (100)	n.a.	n.a.	n.a.	0.19	1.00	1.00	0.26	
TTF-1+/p40+	0/180 (0)	0/50 (0)	n.a.	n.a.	n.a.	0.00	1.00	0.00	0.22	
TTF-1+/p40–	141/180 (78)	0/50 (0)	n.a.	n.a.	n.a.	0.78	1.00	1.00	0.56	
TTF-1–/p40+	0/180 (0)	50/50 (100)	n.a.	n.a.	n.a.	1.00	1.00	1.00	1.00	
TTF-1–/p40–	39/180 (22)	0/50 (0)	n.a.	n.a.	n.a.	0.00	0.78	0.00	0.74	
TTF-1	28/30 (93)	0/10 (0)	0/1 (0)	3/5 (60)	n.a.	0.93	1.00	1.00	0.83	46
p63	9/30 (30)	10/10 (100)	1/1 (100)	5/5 (100)	n.a.	1.00	0.70	0.53	1.00	
p40	5/30 (17)	10/10 (100)	0/1 (0)	5/5 (100)	n.a.	1.00	0.83	0.67	1.00	
TTF-1+/p63+	8/30 (27)	0/10 (0)	0/1 (0)	3/5 (60)	n.a.	0.27	1.00	1.00	0.31	
TTF-1+/p63–	20/30 (67)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.67	1.00	1.00	0.50	
TTF-1–/p63+	1/30 (3)	10/10 (100)	1/1 (100)	2/5 (40)	n.a.	1.00	0.97	0.91	1.00	
TTF-1–/p63–	1/30 (3)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.00	0.97	0.00	0.74	
TTF-1+/p40+	4/30 (14)	0/10 (0)	0/1 (0)	3/5 (60)	n.a.	0.13	1.00	1.00	0.28	
TTF-1+/p40–	24/30 (80)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.80	1.00	1.00	0.63	
TTF-1–/p40+	1/30 (3)	10/10 (100)	0/1 (0)	2/5 (40)	n.a.	0.53	0.97	0.91	0.76	
TTF-1–/p40–	1/30 (3)	0/10 (0)	1/1 (100)	0/5 (0)	n.a.	0.00	0.97	0.00	0.74	
p63	74/237 (31)	81/81 (100)	n.a.	n.a.	82/152 (54)	1.00	0.69	0.52	1.00	47
p40	7/205 (3)	81/81 (100)	n.a.	n.a.	0/152 (0)	1.00	0.97	0.92	1.00	
TTF-1	51/66 (77)	0/24	3/12	0/1	n.a.	0.77	1.00	1.00	0.62	49
p63	13/66 (20)	24/24 (100)	7/12	1/1 (100)	n.a.	1.00	0.80	0.65	1.00	
p40	1/29 (3)	15/15 (100)	3/12 (25)	1/1 (100)	n.a.	1.00	0.97	0.94	1.00	
Napsin-A	11/29 (38)	0/15 (0)	1/12 (8)	0/1 (0)	n.a.	0.38	1.00	1.00	0.45	

Integrative data of the expression of TTF-1, p63, and p40 in subtypes of lung cancer and comparison of the sensibility and specificity of these markers for SCC. Values in parentheses are percentages. ADSC = Adenosquamous carcinoma; LCC = large cell carcinoma; LCL = large cell lymphoma; n.a. = not assessed; NPV = negative predictive value; PPV = positive predictive value; SC = sarcomatoid carcinoma; SE = sensitivity; SP = specificity; Ref. = reference.

Detection of ALK-Positive Non–Small-Cell Lung Cancers on Cytological Specimens

High Accuracy of Immunocytochemistry with the 5A4 Clone

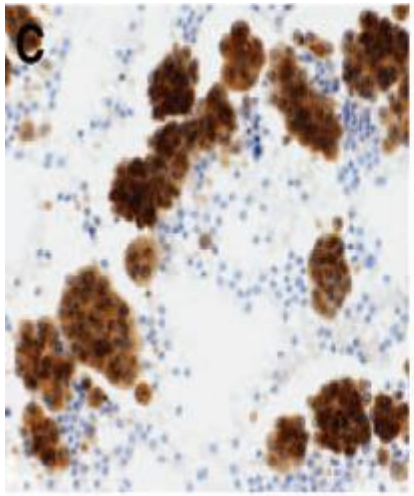
Spasenija Savic, MD, Beata Bode, MD,† Joachim Diebold, MD,‡ Ivo Tosoni, MD,§ Audrey Barascud,* Betty Baschiera,* Bruno Grilli,* Michelle Herzog,* Ellen Obermann, MD,* and Lukas Bubendorf, MD**

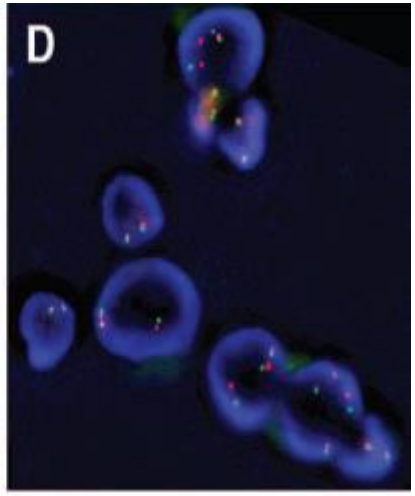
J Thorac Oncol. 2013;8: 1004-1011

TABLE 1. Correlation between ALK ICC-Staining Intensity and the Final ALK FISH Results

	FISH		
ICC-Staining Intensity	**Positive (%)**	**Negative (%)**	**Total**
0	1 (6.7)	24 (96.0)	25
1+	0	1 (4.0)	1
2+	2 (13.3)	0	2
3+	12 (80)	0	12
Total	15	25	40

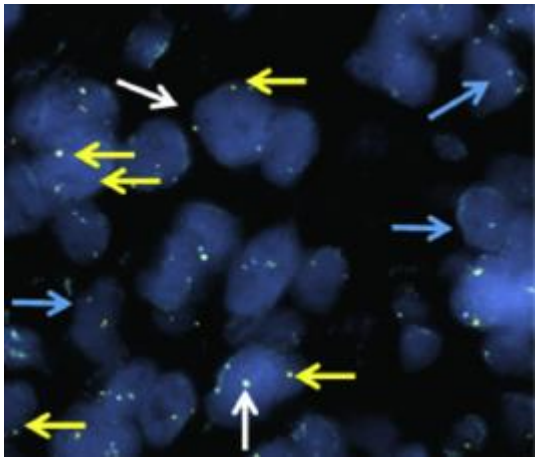
ICC, immunocytochemistry; FISH, fluorescence in situ hybridization.

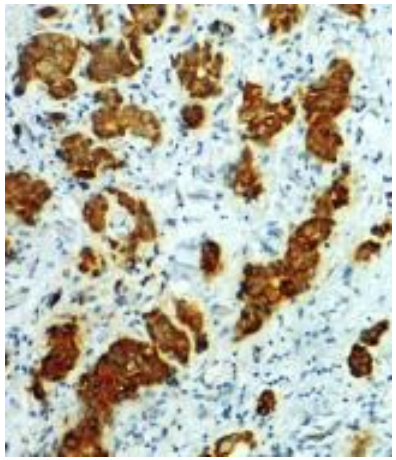


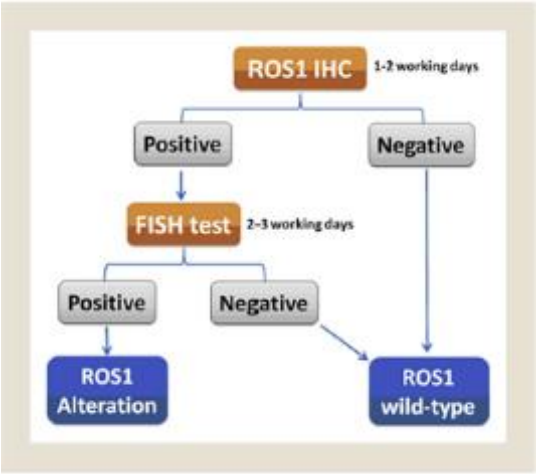


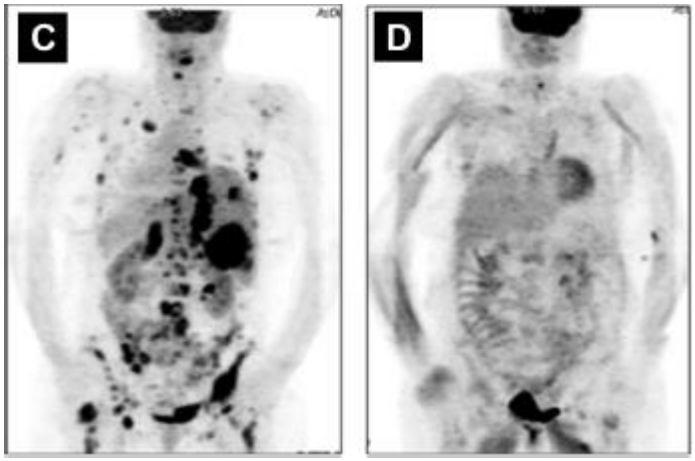
Dramatic Response to Crizotinib in *ROS1* Fluorescent In Situ Hybridization- and Immunohistochemistry-Positive Lung Adenocarcinoma: A Case Series

Rita Chiari,[1] Fiamma Buttitta,[2] Daniela Iacono,[1] Chiara Bennari,[1] Giulio Metro,[1] Alessia Di Lorito,[2] Manuela Iezzi,[2] Marcello Tiseo,[3] Francesca Mazzoni,[4] Federico Cappuzzo,[5] Antonio Marchetti,[2] Lucio Crinò[1]









4. *The Role of Clinical Cytogenetics.* Clinical applications of fluorescence in situ hybridization (FISH) have been growing in the last decade.

- Although some authors predicted the expansion of use of FISH, in this last decade, its use in cytology is practically the same.

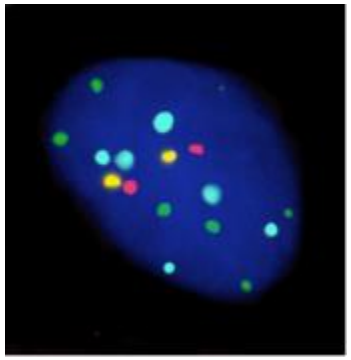
- Indications for using the UroVysion kit in urinary cytology is still under debate as well as its role in the diagnosis of effusions and pancreatobiliary cytology. Like 10 years ago, FISH is used for the detection of translocations in sarcomas and lymphomas, but is limited to some specialized centers.

- The prediction that FISH analysis would be fully automatized, with specialized fluorescence microscopes and multiple filters for multiprobe FISH will become the norm was not accurate and it is not in regular use in nonhematological solid tumours.

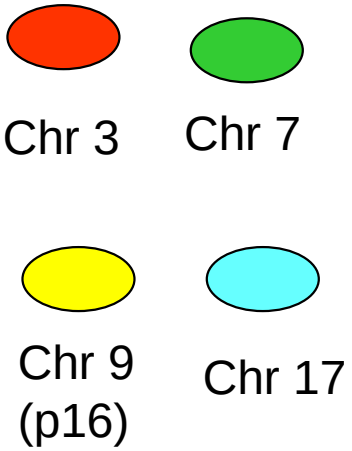
- Interestingly, one of the most common use of FISH in cytology labs nowadays, the search for ALK translocations in lung cancer was not predicted at that time.

Acta Cytologica 2017;61:373–407

Utility of Multiprobe FISH

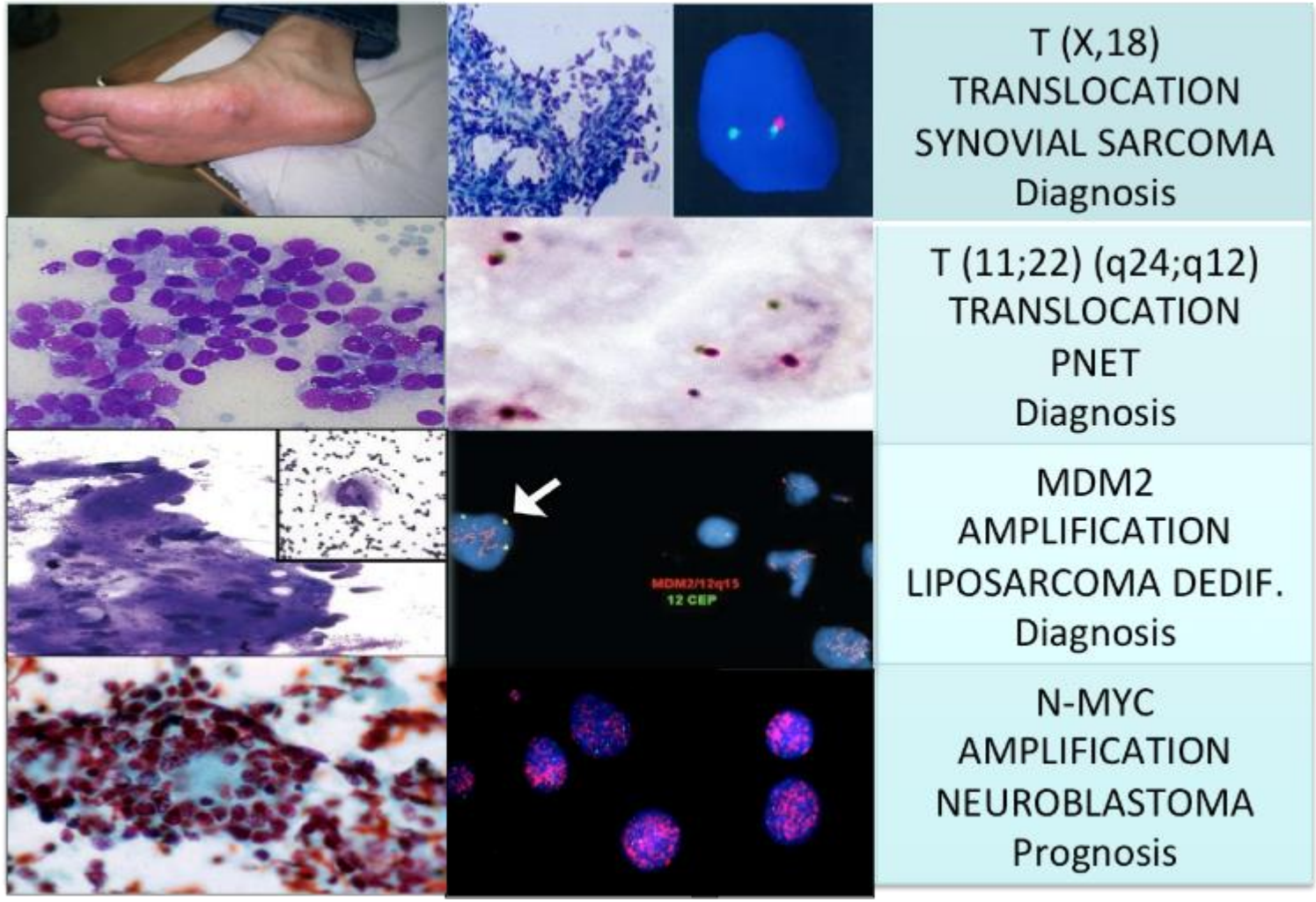


- UroVysion looks at the ploidy status for chromosomes 3, 7, 17 and the 9p21 locus.
- 9p21 deletion in malignant mesothelioma.
- FISHing for pancreatobiliary tract malignancy in endoscopic brushings enhances the sensitivity of routine cytology.

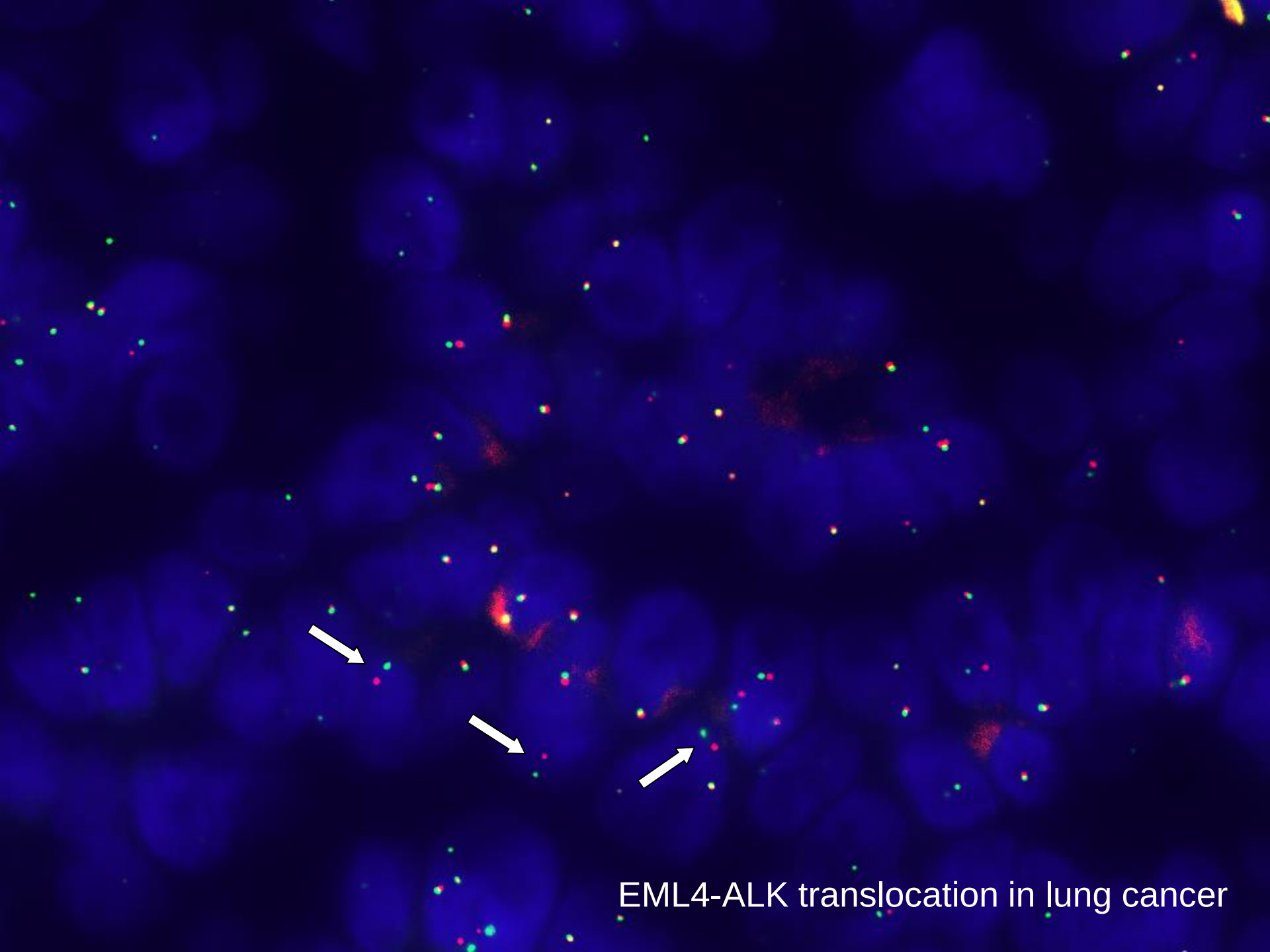


Bubbendorf L . Acta Cytol 2011

MOLECULAR TECHNIQUES
SOFT TISSUE FNA



Schmitt F & Barroca H, 2011


EML4-ALK translocation in lung cancer

5. *The Role of Molecular Techniques.* A variety of molecular assays for the detection of gene mutations, amplifications, and DNA instability have demonstrated clinical utility in cytology. The main topics discussed were the applications of these techniques, the question if target therapies boost the use of molecular techniques in cytology, and the prediction of advances and challenges in their implementation.

- Ten years ago, the use of molecular techniques to detect EGFR in lung cancer, KRAS in colon cancer and cKIT in GISTs was just starting.

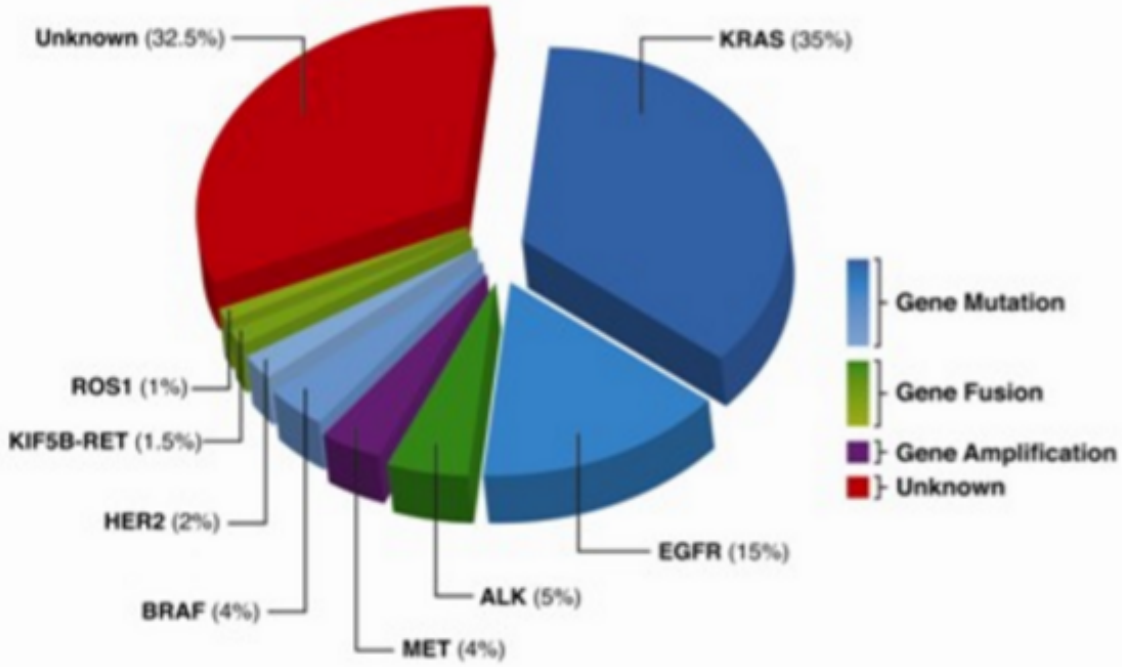
- Today, in almost 50% of lung cancers the only material available for molecular studies that guide therapy is cytology. This changed the paradigm of using small biopsies and cytology samples for diagnosis, maximizing their use for molecular applications.

- Curiously, a technique that is revolutionizing cancer genomics, next-generation sequencing was not mentioned at that time. This technology is rapidly replacing the classical use of PCR and Sanger sequencing, allowing to study not only panel of genes but also RNA alterations.

Acta Cytologica 2017;61:373–407

Oncogene 'drivers' in Adenocarcinoma

Kerr KM. J Clin Pathol 2013;66:832–838



ROS1 fusion genes

1.1-2.6% Adenocarcinomas
Fusion correlates with protein (IHC)
Sensitive to crizotinib

BRAF mutations

2.5-4% Adenocarcinomas
Incl V600E other V600
Smoking vs non-smoking
Vemurafenib

KRAS mutations

25-35% Adenocarcinomas
MEK inhibition?

HER2 mutations

1-2% Adenocarcinomas
Mutually exclusive of EGFR, KRAS
Traztuzamab?

NTRK1 fusion

MPRIP-NTRK1 and *CD74-NTRK1*
3.3% of 'onco-negative' adenocarcinomas
Trk inhibitors exist
Vaishnavi A et al. Nat Med 2013; 19, 1469-72

RET fusion genes

~1% Adenocarcinomas
Vandetanib & others

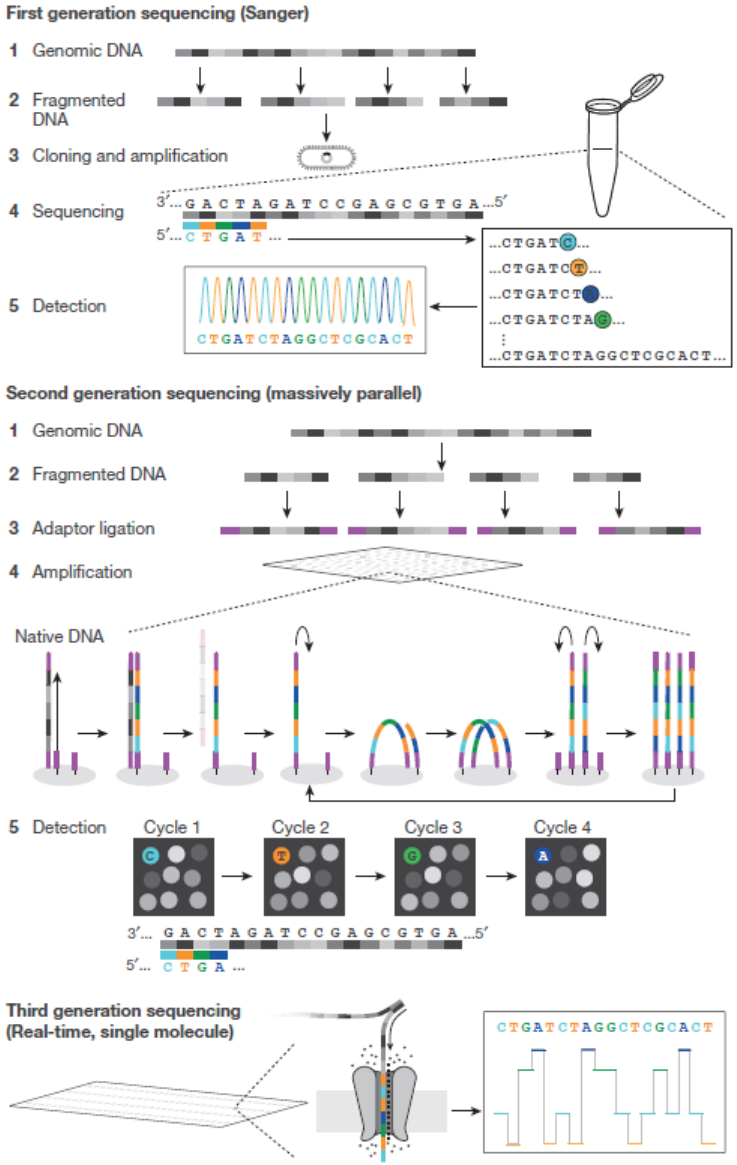
CD74-NRG1 fusion

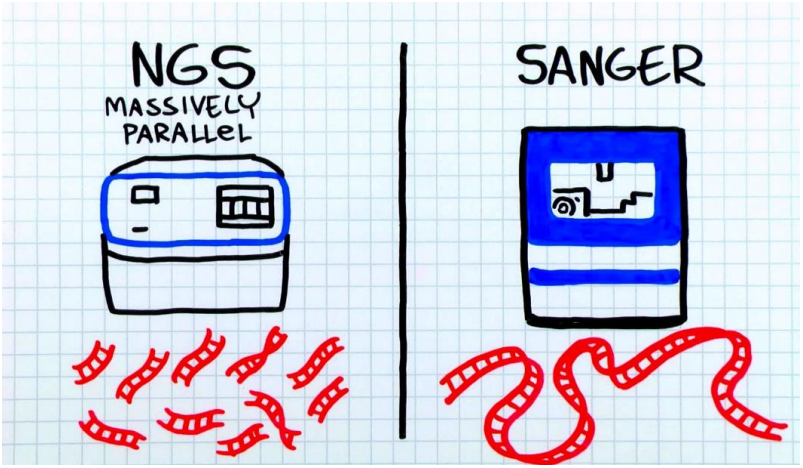
Search in 'onco-negative' adenocarcinomas
ERBB3 and PI3K-AKT pathway activation
Mucinous adenocarcinomas
Potential therapeutic target
Fernandez-Cuesta L et al. Can Disc 2014; jan30 epub

MET upregulation

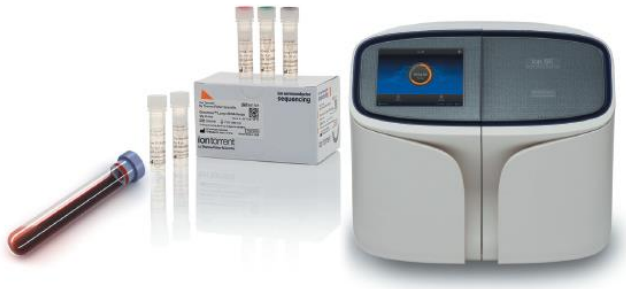
4% amplification, ~50% overexpression
Biomarker issues
Failed trials

Multi-test single assay

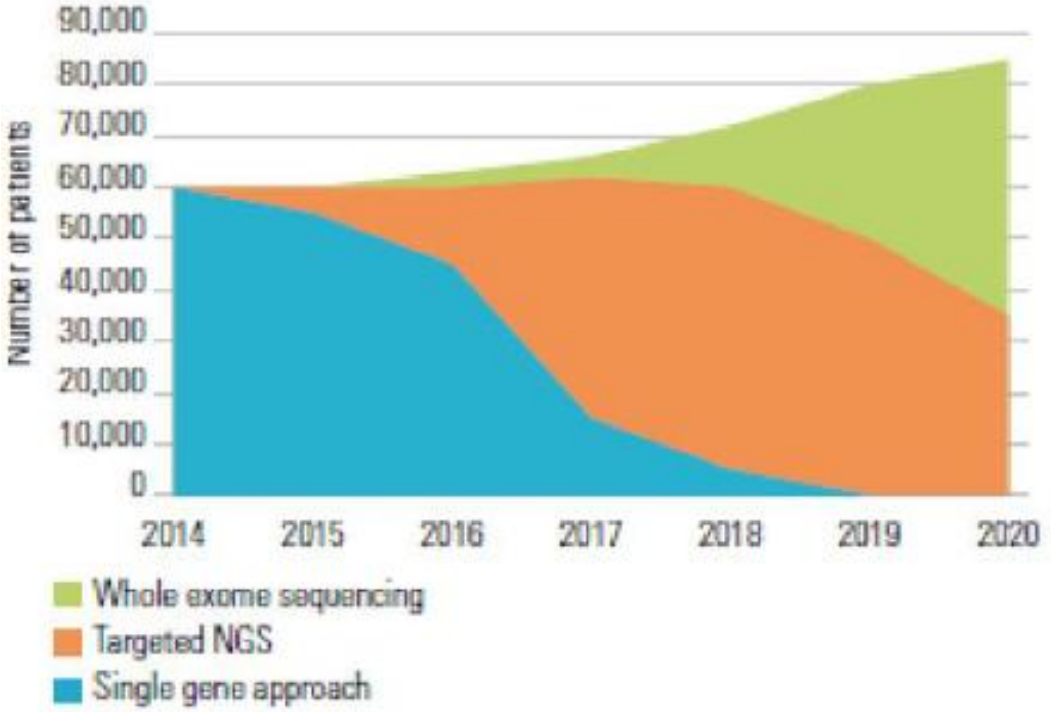




Hotspot genes		Copy number variants	Fusion drivers
35 genes		19 genes	23 genes
DNA			RNA
AKT1	*JAK1*	*ALK*	*ABL1*
ALK	*JAK2*	*AR*	*ALK*
AR	*JAK3*	*BRAF*	*AKT3*
BRAF	*KIT*	*CCND1*	*AXL*
CDK4	*KRAS*	*CDK4*	*BRAF*
CTNNB1	*MAP2K1*	*CDK6*	*EGFR*
DDR2	*MAP2K2*	*EGFR*	*ERBB2*
EGFR	*MET*	*ERBB2*	*ERG*
ERBB2	*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



NGS in oncology



INCa Analysis as cited in KCE Report on NGS (2015)

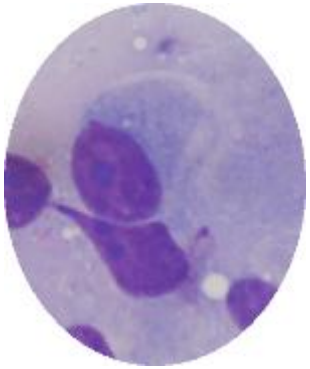
Common commercial targeted and comprehensive sequencing panels for solid tumours

Platform/panel parameters	Ion AmpliSeq™ Cancer Hotspot Panel v2	Ion AmpliSeq™ Comprehensive Cancer Panel	TruSeq Amplicon Cancer Panel	TruSight™ Tumor Sequencing Panel
NGS platform, Manufacturer	Ion Torrent, Life Technologies	Ion Proton, Life Technologies	MiSeq or NextSeq, Illumina	MiSeq or NextSeq, Illumina
Sample type	FFPE, cytology	FFPE, cytology	FFPE, cytology *	FFPE, cytology
DNA input	10 ng	40 ng	250 ng	30–300 ng
Genes in the panel	50	409	48	26
Library preparation	Multiplexed PCR	Multiplexed PCR	Multiplexed probe-based capture	Multiplexed probe-based capture
Amplicon size	111–187 bp	125–175 bp	170–190 bp	165–195 bp
Number of amplicons	207	~16,000	212	174
Sequencing technique	Semiconductor sequencing	Semiconductor sequencing	Fluorescence based sequencing-by-synthesis	Fluorescence based sequencing-by-synthesis
Sequencing quality cutoff	AQ 20	AQ 20	Q30	Q30

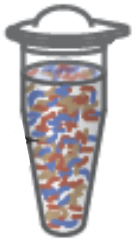
* only specimens with high cellularity meeting minimum DNA threshold.

Massive parallel sequencing workflow

FNA



gDNA



10ng of gDNA
(2000 cells)

739 known mutation in 46 oncogenes and tumor suppressor genes

ABL1	EGFR	HRAS	NOTCH1	SMARCB1
AKT1	ERBB2	IDH1	NPM1	SMO
ALK	ERBB4	JAK2	NRAS	SRC
APC	FBXW7	JAK3	PDGFRA	STK11
ATM	FGFR1	KDR	PIK3CA	TP53
BRAF	FGFR2	KIT	PTEN	VHL
CDH1	FGFR3	KRAS	PTPN11	
CDKN2A	FLT3	MET	RB1	
CSF1R	GNAS	MLH1	RET	
CTNNB1	HNF1A	MPL	SMAD4	

Ultra-high Multiplex PCR → Phosphorilation → Adapter ligation → Nick-translation and amplification

Construct library

3.5 h

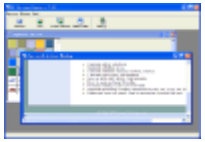
Prepare template

4 h

Run sequence

3 h

Analyze data



2 h

Cytology specimens are suitable for NGS

- Smears (both PAP & Diff-Quik), cell blocks and LBC.

- Studies have been successful using:
  - different sequencing platforms (Illumina & Life Technologies)
  - different enrichment strategies (PCR amplification & hybridization capture)

- Cytology specimens used for NGS capable of:
  - Identifying targets for therapeutic agents
  - Increase accuracy of diagnoses

Advantages and disadvantages of the cytology preparations used for NGS

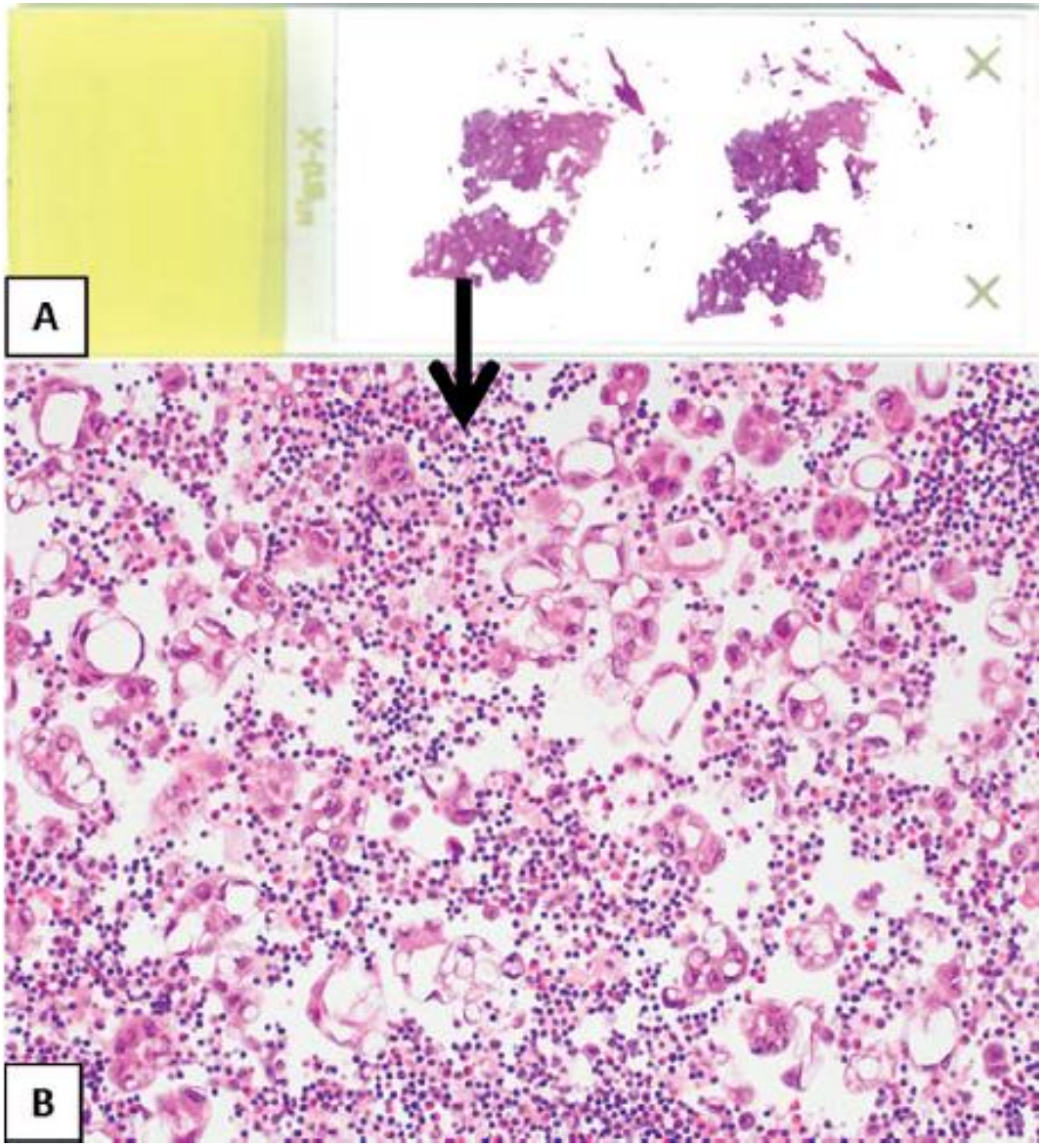
Cytology specimen	Advantages	Disadvantages
Direct smear	• Immediate assessment for adequacy • High quality nucleic acid • Whole cells with whole nuclei • Superior tumor mapping in samples with low tumor fraction	• Sacrificing slide from archival material (medicolegal issues – use of digital pathology) • Additional validation
LBC	• Standardized procession with optimal preservation of the cells • High quality nucleic acid • Whole cells with whole nuclei	• Lack of immediate assessment • Additional validation • Nucleic acid retrieval may be variable based on preservative/fixative
Cell block	• Ease of acquisition • Multiple serial sections • Standardized validation in most molecular labs	• Lack of immediate assessment • Frequently suboptimal cellularity • Nucleic acid may be suboptimal because of formalin fixation • Partial nuclei on standard 4- to 5-micron sections

Pre-analytical factors that affects NGS analysis in cytology

- Specimen cellularity.
- Type of preparation.
- Type of fixative and stains.
- Type of glass slides.
- Tumour fraction.
- DNA yield
- Input DNA

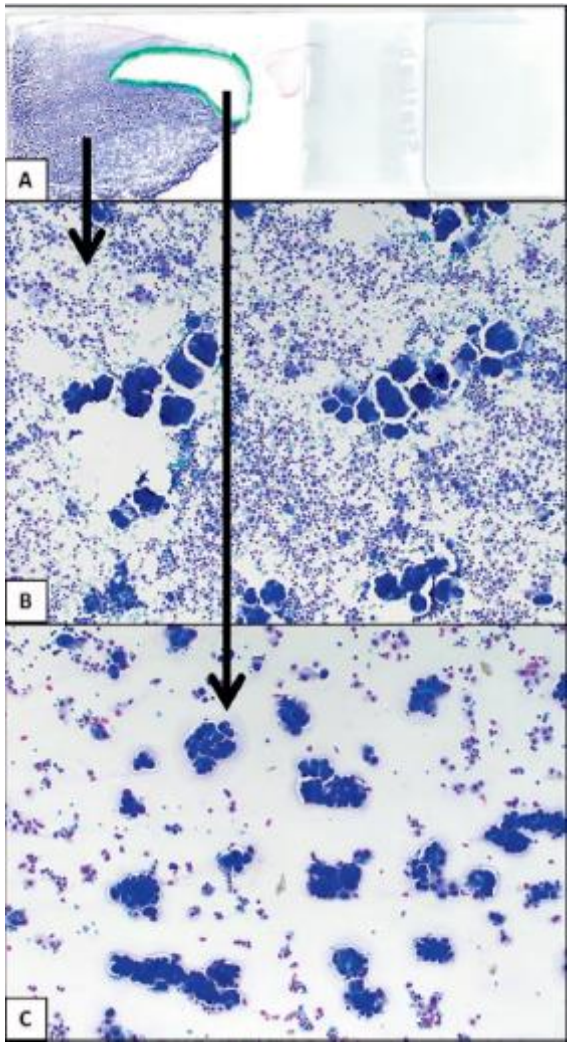
Chen H. et al. Cancer 2015

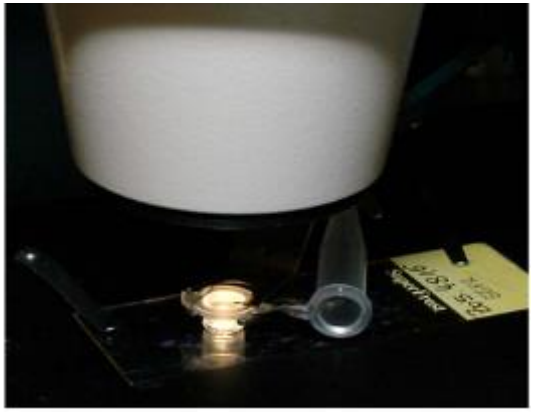
High-cellularity/low tumor fraction

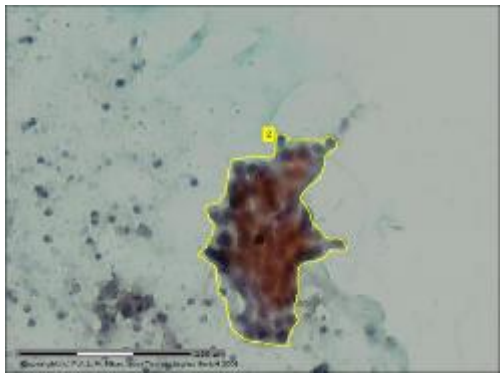


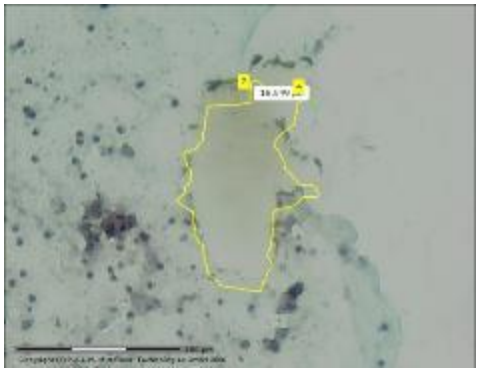
Roy-Chowdhuri et al. Arch Pathol Lab Med 2016

Tumor mapping in cases with high-cellularity/low tumor fraction







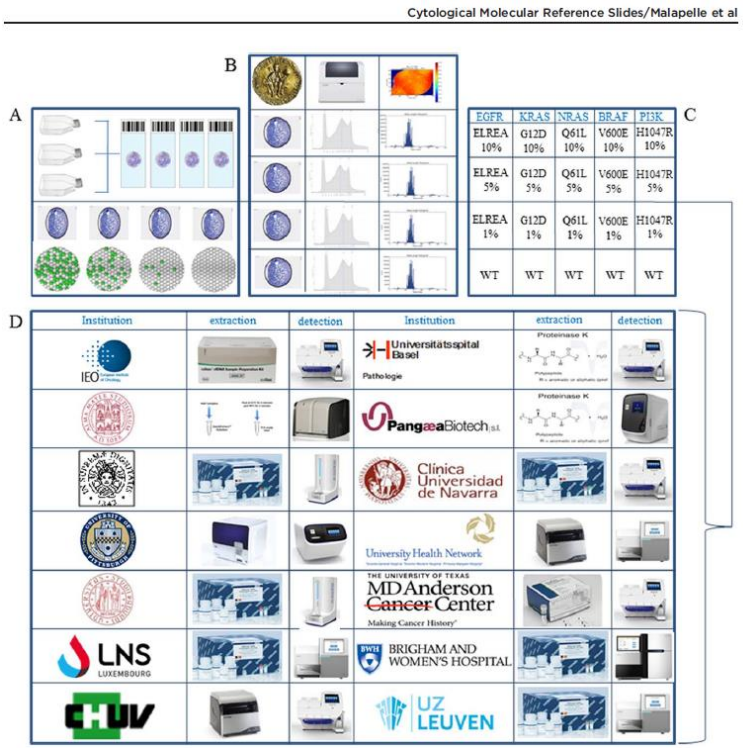



cytology
assays
consistency
reproducibility
multiplex
gene

Consistency and Reproducibility of Next-Generation Sequencing and Other Multigene Mutational Assays: A Worldwide Ring Trial Study on Quantitative Cytological Molecular Reference Specimens

Umberto Malapelle, Clara Mayo-de Las Casas, Miguel A. Molina, Rafael Rosell, Spasenija Savic, Michel Bihl, Lukas Bubendorf, Manuel Salto-Tellez, Dario de Biase, Giovanni Tallini, David H. Hwang, Lynette M. Sholl, Rajyalakshmi Luthra, Sinchita Roy-Chowdhuri, Birgit Weynand, Sara Vander Borght, Edoardo Missiaglia, Massimo Bongiovanni, Daniel Stieber, Philippe Vielh, Fernando Schmitt, Alessandra Rappa, Massimo Barberis, Francesco Pepe, Pasquale Pisapia, Nicola Serra, Elena Vigliar, Claudio Bellevicine, Matteo Fassan, Massimo Rugge, Carlos E. de Andrea, Maria D. Lozano, Fulvio Basolo, Gabriella Fontanini, Marina N. Nikiforova, Yuri E. Nikiforov, Suzanne Kamel-Reid, Gilda da Cunha Santos, Giancarlo Troncone for the Molecular Cytopathology Meeting Group

This interlaboratory ring trial study shows that next-generation sequencing and other multigene mutational assays are robust and accurate with cytological samples. In particular, the performance of laboratories using next-generation sequencing is excellent, regardless of the platform or gene panel type.



RESEARCH ARTICLE

Multi-Center Evaluation of the Fully Automated PCR-Based Idylla™ KRAS Mutation Assay for Rapid KRAS Mutation Status Determination on Formalin-Fixed Paraffin-Embedded Tissue of Human Colorectal Cancer

Jérôme Solassol[1], Julie Vendrell[1], Bruno Märkl[2], Christian Haas[2], Beatriz Bellosillo[3], Clara Montagut[4], Matthew Smith[5], Brendan O'Sullivan[5], Nicky D'Haene[6], Marie Le Mercier[6], Morten Grauslund[7], Linea Cecilie Melchior[7], Emma Burt[8], Finbarr Cotter[8], Daniel Stieber[9], Fernando de Lander Schmitt[10], Valentina Motta[11], Calogero Lauricella[11], Richard Colling[12], Elizabeth Soilleux[12], Matteo Fassan[13], Claudia Mescoli[13], Christine Collin[14], Jean-Christophe Pagès[14], Peter Sillekens[15]*

- Integrated system
  - DNA extraction
  - Multiplex RealTime PCR
- Up to 30 molecular targets in a single cartridge
- High Sensitivity (DNAzymes)
- Minimal hands-on time
- Access on-demand

TEST RESULT REPORT

idylla

Sample ID			
Sample type	FFPE tissue		
Cartridge ID	02258115		
Test type	KRAS	TTP Version	1.0
Lot ID	00002258	Expiration date	27 Dec 2015

Instrument serial number	SER717
Instrument software version	20140711.1149
Console software version	3.0.0.180
Test request completed	23 Nov 2015 (08:18)
Test started	23 Nov 2015 (08:20)
Test ended	23 Nov 2015 (10:28)
Test status	Released result: Automatic 23 Nov 2015 (10:28)
Operator	Stieber

Test Result (1) In Vitro Diagnostic Medical Device. For use in diagnostic procedures.

Idylla™ KRAS Mutation Test

KRAS GENOTYPE	MUTATION DETECTED IN KRAS CODON 12
Mutation	G12R
Protein	p.Gly12Arg
Nucleotide Change	c.34G>C

Error
None

Comments/Annotations

Stieber, 23 Nov 2015 (08:18)
Patho Nummer = 25562-14

PLOS ONE | DOI:10.1371/journal.pone.0163444 September 29, 2016

One of the most important discussions still deserve reflections

"It is important that we cytologists take an active role in the adoption and application of molecular techniques, since we are able to interpret the results in the light of cytomorphology. If we do not act, others will take over."

"The advance of molecular techniques poses two challenges for the cytology laboratory: deciding when to adopt a new test and deciding who should perform and interpret it."

"The cytology laboratory will likely find itself squeezed between rising expectations for advances in molecular medicine and the limitation imposed by good evidence-based practice and funding."

Acta Cytologica 2017;61:373–407

Ten years on….

- Cytopathologists and pathologists, in general, still do not play an active role in the adoption and application of molecular techniques.

- There is no doubt that many things changed and there are more pathologists involved in molecular pathology. Molecular tumor boards start to be a reality.

- However there is a gap between the fast implementation of the technology and the slow engagement of the pathologists. In some laboratories, it is possible to see pathologists working actively together with molecular biologists and scientists deciding with them the implementation of new tests and sign out of the reports.

- Funding is still a problem but many test used are tailored to specific treatments and in many cases it is possible to demonstrate the financial advantages to implement the tests.

Acta Cytologica 2017;61:373–407

Molecular techniques in cytopathology practice

F C Schmitt,[1,2] A Longatto-Filho,[3,4] A Valent,[5] P Vielh[5]

J Clin Pathol 2008;**61**:258–267. doi:10.1136/jcp.2006.044347

Why is cytopathology not more molecular ?

- ***Science***: lack of more good molecular markers
- ***Specimens***: pre-analytical variability
- ***Tests:*** lack of analytically and clinically validated tests
- ***Culture:*** resistance, reluctance, unfamiliarity and inexperience from cytotechnologists, pathologists and others…

Julius Caesar crosses Rubicon

Romans under Julius Caesar crossed the Rubicon River from Gaul into Italy in 49 B.C. Caesar, in breaking Roman law by leading his army back across the river towards Rome, uttered "alea iacta est" ("the die is cast”). Thus the expression "crossing the Rubicon" has come to suggest an action that cannot be undone.

“Alea jacta est”
(The die has been cast)

- Caesar cross the Rubicon and constructed a new era for the Romans that still influence the occidental culture.

- Molecular and genomic techniques need to become a significant component of the training curriculum of pathology. Without our commitment, it is inevitable that these gaps will be filled by other medical specialties.

- Now is the time to engineer a core expertise in pathology that meets the future’s needs, and this opportunity cannot be missed or we run the risk of being one of the last generation of pathologists.

Living in the past or crossing the Rubicon? Longatto-Filho A & Schmitt F. Acta Cytol 2010

What's next?

Fluids (blood, urine, CSF)?

- Promises: Easily accessible sample, unlimited number of collections
- Challenges: Nowhere near sensitive as advertised
- Reality: It is happening

WES, WGS, Epigenetics (DNA, RNA, MIRs), Proteinomics, Pharmacogenetics

- Promises: Rare diseases; predict unusual responses
- Challenges: Price (WGS), reproducibility, QC
- Reality: It is happening

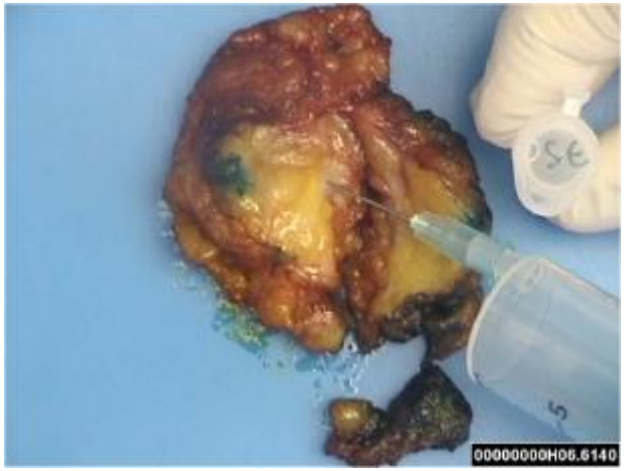
(CYTO)PATHOLOGY IS CHANGING….

- Introduction of FNA for sampling tumours is important because there are changes in clinical practice. For example, the small size of breast cancers diagnosed in the screening can limited the sampling without compromising current diagnostic standards.

Fine-Needle Aspiration as an Alternative Method for Frozen Tissue Banking of Breast Cancer

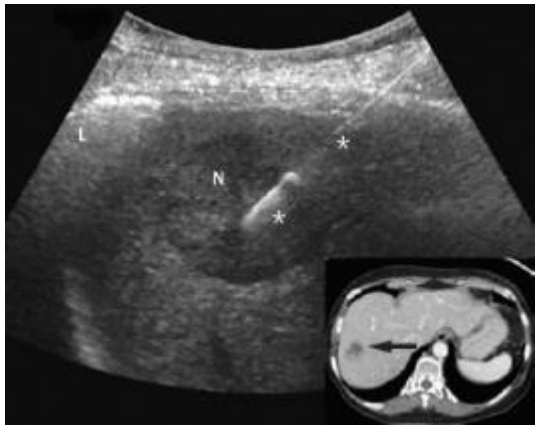
Catarina Eloy, M.D.*
Isabel Amendoeira, M.D.
Fernando C. Schmitt, M.D., Ph.D., F.I.A.C.

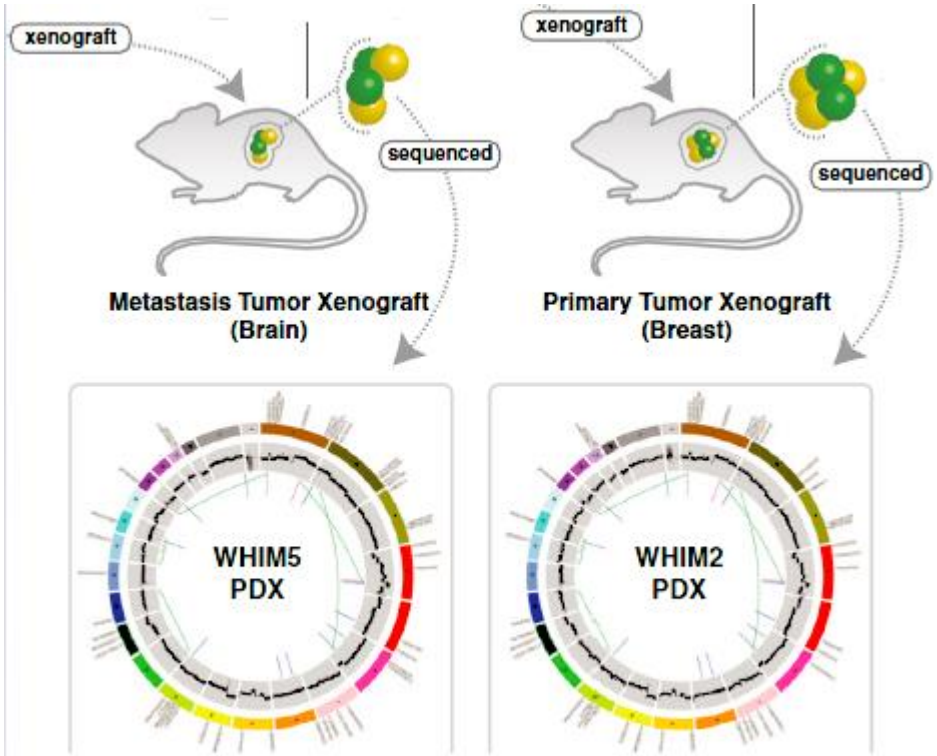
Diagnostic Cytopathology, Vol 37, No 1



(CYTO)PATHOLOGY IS CHANGING….

PDX

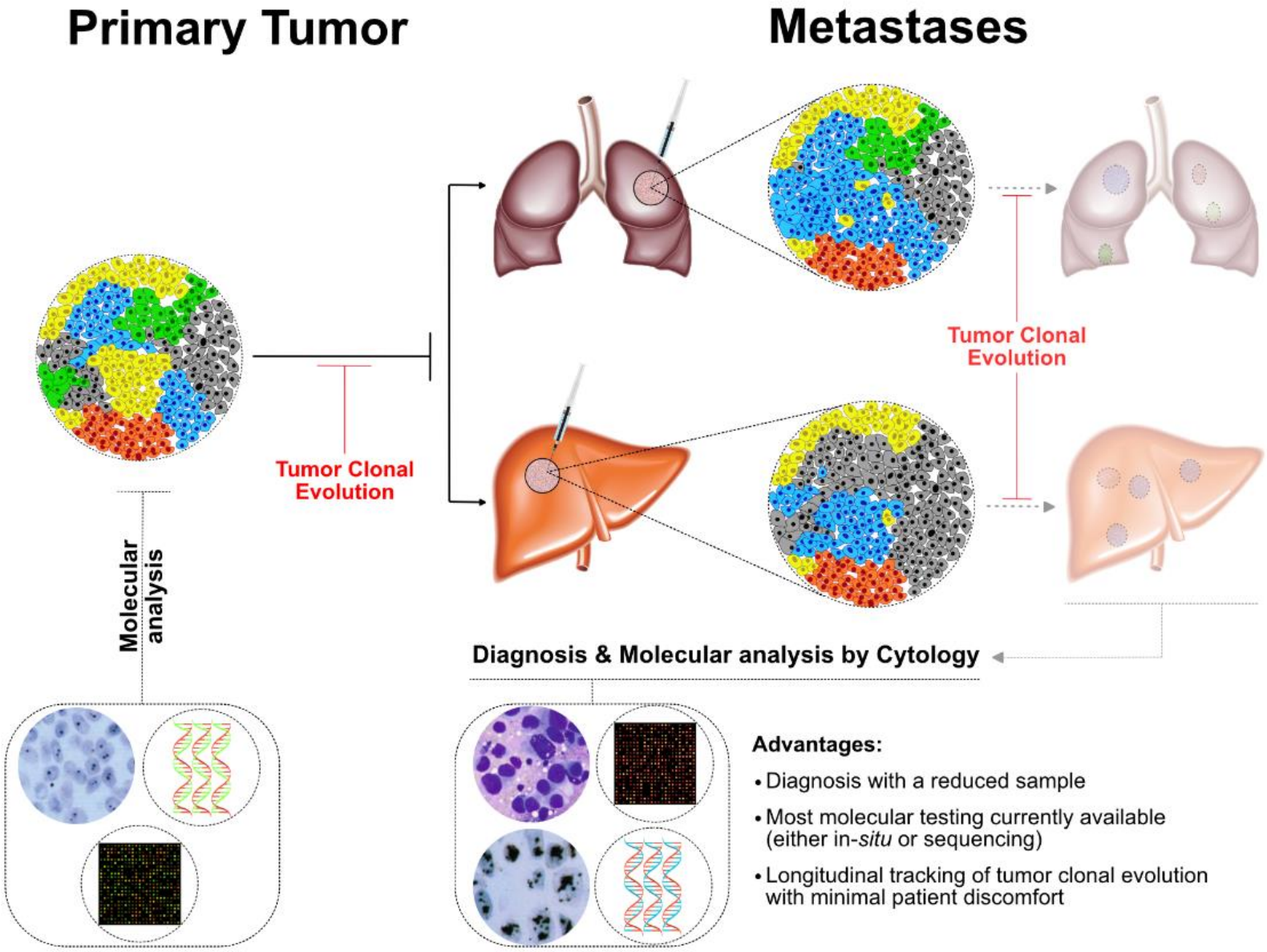




Using Mice to Treat (Wo)men: Mining Genetic Changes in Patient Xenografts to Attack Breast Cancer

Steffi Oesterreich,[1,*] Adam M. Brufsky,[1] and Nancy E. Davidson[1]
[1]Women's Cancer Research Center, University of Pittsburgh Cancer Institute and UPMC Cancer Center, Magee-Womens Hospital and Magee-Womens Research Institute, Departments of Pharmacology and Chemical Biology and Medicine, University of Pittsburgh, Pittsburgh 15213, USA

Cell Reports *4*, 1116–1130, September 26, 2013

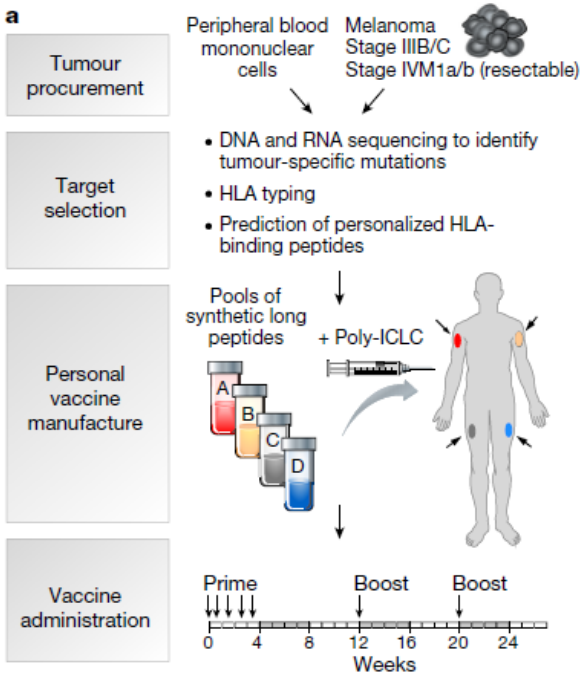


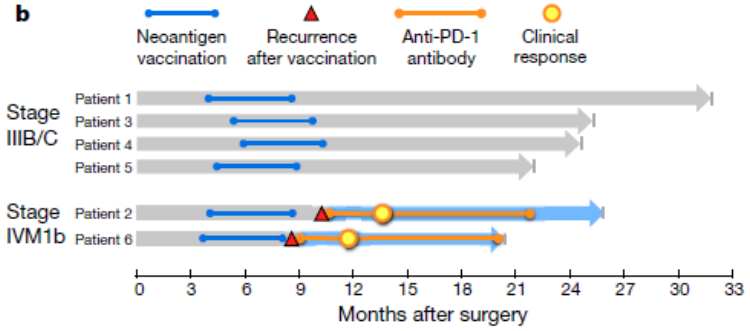
Beca F & Schmitt F. 2017

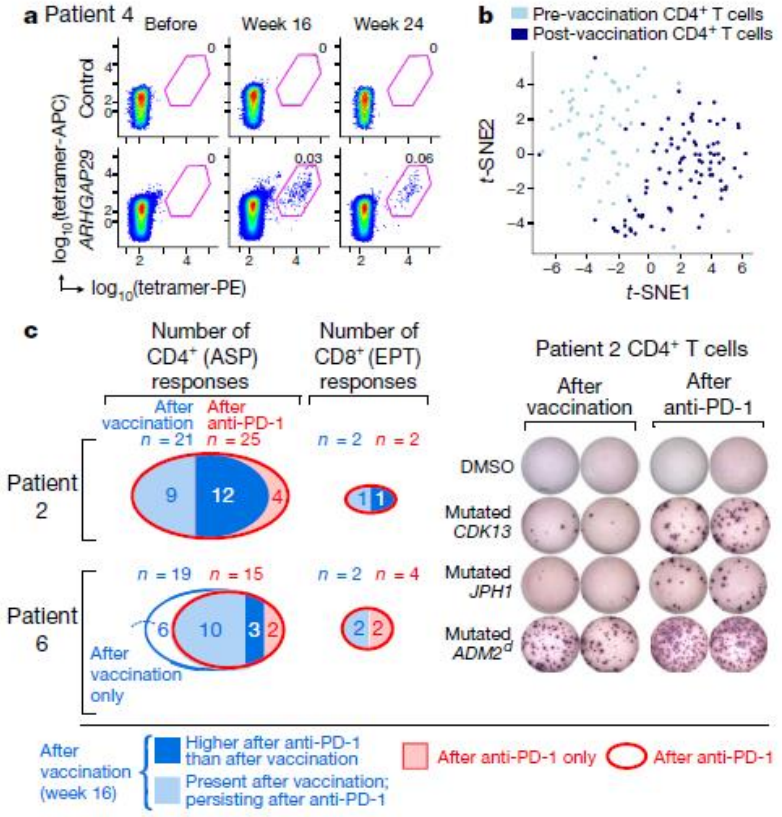
An immunogenic personal neoantigen vaccine for patients with melanoma

doi:10.1038/nature22991

Patrick A. Ott[1,2,3]*, Zhuting Hu[1]*, Derin B. Keskin[1,3,4], Sachet A. Shukla[1,4], Jing Sun[1], David J. Bozym[1], Wandi Zhang[1], Adrienne Luoma[5], Anita Giobbie-Hurder[6], Lauren Peter[7,8], Christina Chen[1], Oriol Olive[1], Todd A. Carter[4], Shuqiang Li[4], David J. Lieb[4], Thomas Eisenhaure[4], Evisa Gjini[9], Jonathan Stevens[10], William J. Lane[10], Indu Javeri[11], Kaliappanadar Nellaiappan[11], Andres M. Salazar[12], Heather Daley[1], Michael Seaman[7], Elizabeth I. Buchbinder[1,2,3], Charles H. Yoon[3,13], Maegan Harden[4], Niall Lennon[4], Stacey Gabriel[4], Scott J. Rodig[9,10], Dan H. Barouch[3,7,8], Jon C. Aster[3,10], Gad Getz[3,4,14], Kai Wucherpfennig[3,5], Donna Neuberg[6], Jerome Ritz[1,2,3], Eric S. Lander[3,4], Edward F. Fritsch[1,4]†, Nir Hacohen[3,4,15] & Catherine J. Wu[1,2,3,4]







Commentary

ACTA CYTOLOGICA

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Published online: July 11, 2017

Expectations and Projections for the Future of Nongynecolgical Cytology 10 Years Ago: Did They Materialize and How Did We Do?

Fernando C. Schmitt[a, b] Philippe Vielh[c]

[a]Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), and [b]Department of Pathology and Oncology, Medical Faculty, University of Porto, Porto, Portugal; [c]Laboratoire National de Santé, Dudelange, Luxembourg

- After 10 years, we can conclude exactly as Dr Bibbo conclude the original discussion: “Since molecular biology results are meaningful only when interpreted with proper morphologic correlation, it is important to standardize molecular techniques and organize education in molecular biology for pathologists”.

First Tissue-Agnostic Drug Approval Issued

DOI: 10.1158/2159-8290.CD-NB2017-078 Published July 2017

The PD-1 inhibitor pembrolizumab received accelerated approval for adult and pediatric patients with solid tumors that are mismatch repair–deficient or microsatellite instability–high. This is the first time the FDA has greenlighted a drug based not on tumor type, but on a common biomarker.

ABOUT THE TEST:

FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related genes.

PATIENT RESULTS

- 6 genomic findings
- 2 therapies associated with potential clinical benefit
- 0 therapies associated with lack of response
- 9 clinical trials

TUMOR TYPE: EYE INTRAOCULAR MELANOMA

Genomic Alterations Identified†

GNAQ Q209L
MYC amplification – equivocal*
BAP1 W5*
PARK2 N52fs*29

Additional Findings†

Microsatellite status MS-Stable
Tumor Mutation Burden TMB-Low; 3 Muts/Mb

Additional Disease-relevant Genes with No Reportable Alterations Identified†

BRAF
KIT
NRAS

† For a complete list of the genes assayed and performance specifications, please refer to the Appendix
* See Appendix for details

THERAPEUTIC IMPLICATIONS

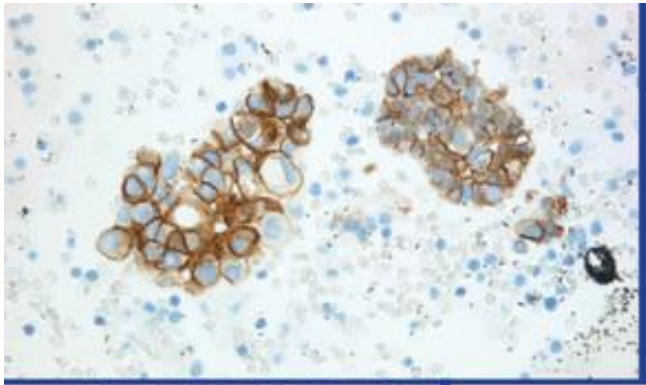
Genomic Findings Detected	FDA-Approved Therapies (in patient's tumor type)	FDA-Approved Therapies (in another tumor type)	Potential Clinical Trials
GNAQ Q209L	Cobimetinib Trametinib	None	Yes, see clinical trials section
MYC amplification - equivocal	None	None	Yes, see clinical trials section
BAP1 W5*	None	None	None
Microsatellite status MS-Stable	None	None	None

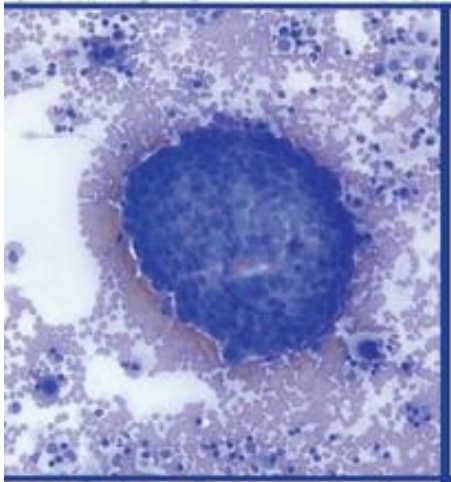
For more comprehensive information please log on to the Interactive Cancer Explorer™
To set up your Interactive Cancer Explorer account, contact your sales representative or call 1-888-988-3639.
For further information and assistance please call Roche Customer Care: +351 214 257 003

Electronically Signed by Julia A. Elvin, M.D., Ph.D. | Jeffrey S. Ross, M.D., Medical Director | 05 October 2017
Foundation Medicine, Inc. / 1-888-988-3639

Sample Preparation: 150 Second St., 1st Floor, Cambridge, MA 02141 / CLIA: 22D2027531
Sample Analysis: 150 Second St., 1st Floor, Cambridge, MA 02141 / CLIA: 22D2027531

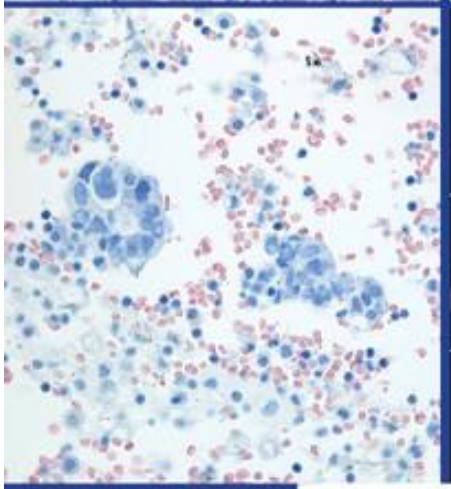

CONFEITARIA

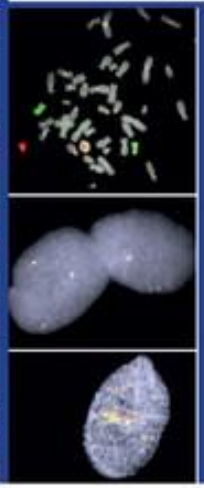




MOLECULAR PATHOLOGY

A Practical Guide for the Surgical Pathologist and Cytopathologist





EDITED BY:
John M. S. Bartlett
Abeer Shaaban
Fernando Schmitt

CAMBRIDGE Medicine

CONCLUSIONS

- Small samples obtained by minimally invasive methods exploited morphological and molecular information are the model biopsies of the future.

- Various techniques using different types of cytological samples have shown good results, with similar or higher accuracy when compared with surgical specimens.

- Presently, with the rapid development of personalized treatment each tumor should be tested for available biomarkers and every effort should be made to spare the tissue for molecular testing.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