From Cytology to Molecular Profiling and the Use of NGS

Dr Haene Nicky, MD PhD
Department of Pathology
Erasme Hospital – Université Libre de Bruxelles (ULB)
Belgium

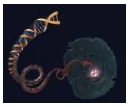
Introduction

Disclosure

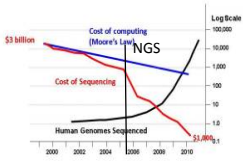
- Consultation fees/ Advisory boards:
  - Pfizer
  - Astra Zeneca
  - Biocartis
  - MSD
  - Boerhingher
  - BMS

The sequencing explosion

- Sequencing : determining the order of nucleotide bases
- 1990 – 2003: 1st sequencing of human genome
  - Lasted 13 years
  - Cost 3 billion $ (1$/base)
- now: <2 days



The Sequencing Explosion



Thousands of cancer genomes are already available

Discovery of new driver cancer genes

Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

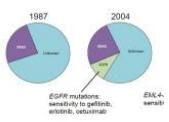
Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology

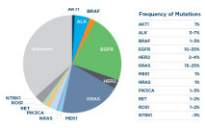
- 3 categories of genes
  - « must test » : EGFR, ALK, ROS1
  - Expanded panel : BRAF, MET, RET, ERBB2, KRAS
  - Investigational
- Choice
  - Comprehensive cancer panel (categories 1-2) for all appropriate patients
  - Targeted testing for EGFR, ALK, ROS1 for all appropriate patients
    - Expanded panel for patients who are suitable candidates for clinical trials
  - Multiplexed sequencing panels are preferred over multiple single-gene tests beyond EGFR, ALK, ROS1

 Lindeman et al. Arch Pathol Lab Med 2018

Introduction

- Molecular testing represents a paradigm shift in lung cancer diagnosis and is now a standard of care.
- International guidelines recommend testing for EGFR mutations and ALK and ROS1 rearrangements to guide patient selection for therapy





Kerr et al. Annal Oncol 2014; Lindeman et al. Arch Pathol Lab Med 2018

Sample size

Number of biomarkers to test

Less invasive procedures

EGFR
ALK
ROS1
PDL-1

KRAS, BRAF, MET, ERBB2, RET,....

Cytological samples

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Samples by aspiration

EBUS/ EUS

FNA

Samples of body fluids, broncho-alveolar lavages,...

Pleural effusion

Smears

FFPE cell block

LBC

Cytospin

Cytological diagnosis

Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology

Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology

- 2013 : Expert Consensus Opinion: Cytologic samples are also suitable for EGFR and ALK testing, with cell blocks being preferred over smear preparations.
- 2018 : Recommendation: Pathologists may use either cell blocks or other cytologic preparations as suitable specimens for lung cancer biomarker molecular testing
  - Excellent performance of smear preparations
  - Needs validation !

CYTOLOGICAL SAMPLES

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- Variety and versatility of specimen preparation
  - Different fixation types
  - Different staining types
    - Smears
      - Air-dried → Diff-Quik
      - Alcohol-fixed → Papanicolaou
  - → needs standardization
  - → needs validation for each type of sample

TOOLBOX OF THE MOLECULAR PATHOLOGIST

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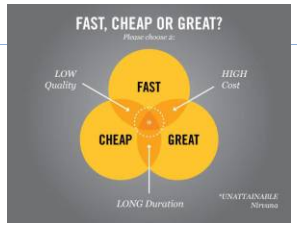
	IHC	FISH	(RT-)PCR	NGS
				
Target	Protein	RNA/DNA	RNA/DNA	RNA/DNA
Precision	+	++	+	+++
Hands on time	-	+++	+	++
Cost	+-++	++	+-++	+++-++++
TAT	+	++	++	++-+++



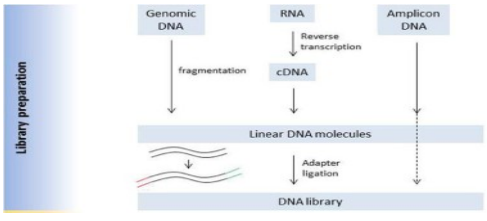
NEXT GENERATION SEQUENCING

- Definition :

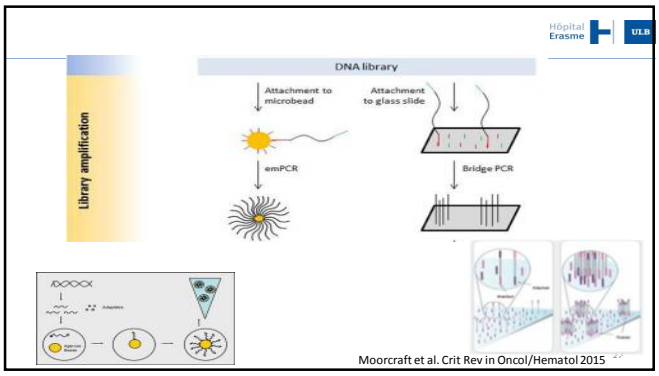
  Technologies that have the ability to massively sequence millions of DNA templates in parallel

DNA Library → Clonal amplification → sequencing

	IHC	FISH	(RT-)PCR	NGS
				
EGFR	NOT	/	+	+
ALK	+	+	(+)	(+)
ROS1	Screening method Confirmation needed	+	+	+
Expanded panel	/	/	(+)	+ preferred



Moorcraft et al. Crit Rev in Oncol/Hematol 2015 16



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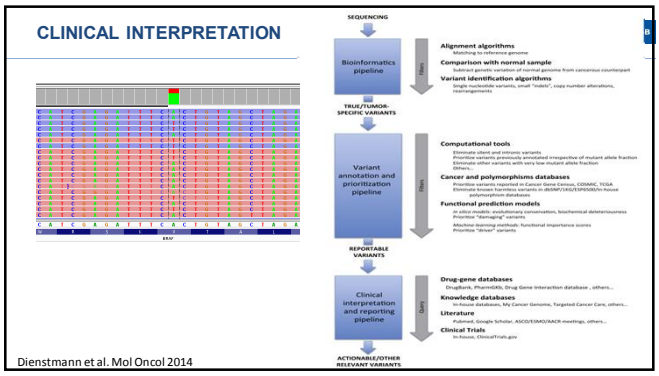
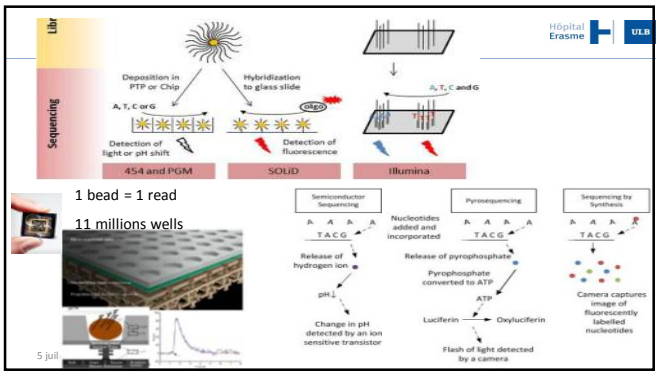
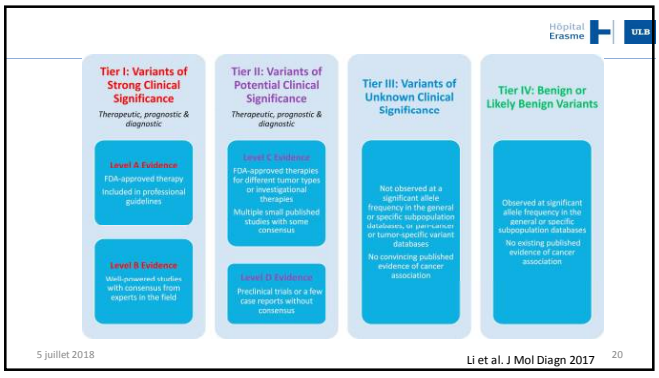


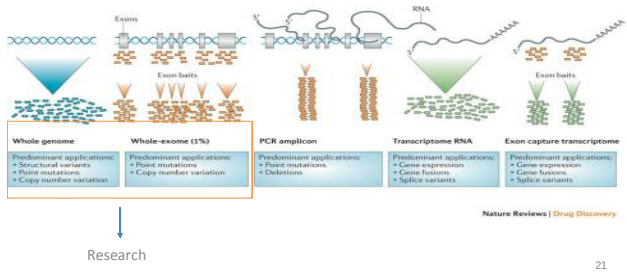
Dienstmann et al. Mol Oncol 2014





Li et al. J Mol Diagn 2017

Next Generation Sequencing: Applications in oncology



Research

21

Targeted DNA Sequencing

- Only the target of interest → gene panels
- Applications → Daily practice
  - Tumor molecular diagnostic -> Known Hotspots of several genes
    - Commercial panels
    - Custom panel
- Design flexibility
  - Coverage
  - Number of amplicons (targeted region)
  - Number of patients -> barcodes

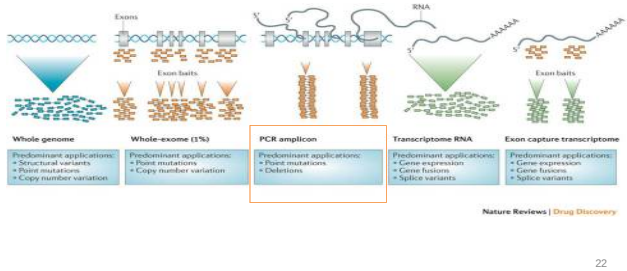
-> 100 amplicons, 10 patients, 1000x coverage

-> 1000 amplicons, 1 patient, 1000x coverage

-> 1000 amplicons, 10 patients, 100x coverage

23

Next Generation Sequencing: Applications in oncology



22

Targeted DNA Sequencing

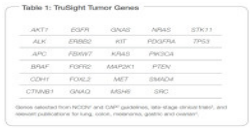


Table 1: TruSight Tumor Genes

AKT1	EGFR	GNAS	NRAS	STK11
ALK	ERBB2	KIT	PDGFRA	TP53
APC	FBXW7	KRAS	PIK3CA	
BRAF	FGFR2	MAP2K1	PTEN	
CDH1	FOXL2	MET	SMAD4	
CTNNB1	GNAQ	MSH6	SRC	

Genes selected from NCCN® and CAP® guidelines, late-stage clinical trials*, and relevant publications for lung, colon, melanoma, gastric and ovarian.

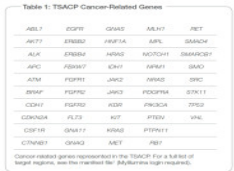


Table 1: TSACP Cancer-Related Genes

ABL1	EGFR	GNAS	MLH1	RET
AKT1	ERBB2	HNF1A	MPL	SMAD4
ALK	ERBB4	HRAS	NOTCH1	SMARCB1
APC	FBXW7	IDH1	NPM1	SMO
ATM	FGFR1	JAK2	NRAS	SRC
BRAF	FGFR2	JAK3	PDGFRA	STK11
CDH1	FGFR3	KDR	PIK3CA	TP53
CDKN2A	FLT3	KIT	PTEN	VHL
CSF1R	GNA11	KRAS	PTPN11	
CTNNB1	GNAQ	MET	RB1	

Cancer related genes represented in the TSACP. For a full list of target regions, see the manifest file (MyIllumina login required).

The Ion AmpliSeq™ Cancer Panel targets 50 genes

ABL1	EZH2	JAK3	PTEN
AKT1	FBXW7	IDH2	PTPN11
ALK	FGFR1	KDR	RB1
APC	FGFR2	KIT	RET
ATM	FGFR3	KRAS	SMAD4
BRAF	FLT3	MET	SMARCB1
CDH1	GNA11	MLH1	SMO
CDKN2A	GNAS	MPL	SRC
CSF1R	GNAQ	NOTCH1	STK11
CTNNB1	HNF1A	NPM1	TP53
EGFR	HRAS	NRAS	VHL
ERBB2	IDH1	PDGFRA	
ERBB4	JAK2	PIK3CA	

Ion AmpliSeq™ Colon & Lung Panel = 22 genes

AKT1	ERBB2	KRAS	PTEN
ALK	ERBB4	MAP2K1	SMAD4
BRAF	FBXW7	MET	STK11
CTNNB1	FGFR1	NOTCH1	TP53
DDR2	FGFR2	NRAS	
EGFR	FGFR3	PIK3CA	

24

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The Journal of Molecular Diagnostics, Vol. 15, No. 5, September 2013

Routine Clinical Mutation Profiling of Non–Small Cell

ELSEVIER

the Journal of Molecular Diagnostics

jmd.amjpathol.org

See relate

Valida t
Clinica nd Lung

Targeted, High-Depth, Next-Generation Sequencing of Cancer Genes in Formalin-Fixed, Paraffin-Embedded and Fine-Needle Aspiration Tumor Specimens

Andrew G. Hadd,* Jeff Houghton,* Ashish Choudhary,* Sachin Sah,* Liangjing Chen,* Adam C. Marko,* Tiffany Sanford,* Kalyan Buddavarapu,* Julie Krosting,* Lana Garmire,* Dennis Wylie,* Rupali Shinde,* Sylvie Beaudenon,* Erik K. Alexander,† Elizabeth Mambo,* Alex T. Adai,* and Gary J. Latham*

From Asuragen, Inc., Austin, Texas; and the †Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts*

From the Departments of Pathology and Immunology, and Genetics,† Genomics and Pathology Services, Washington University School of Medicine, St. Louis, Missouri*

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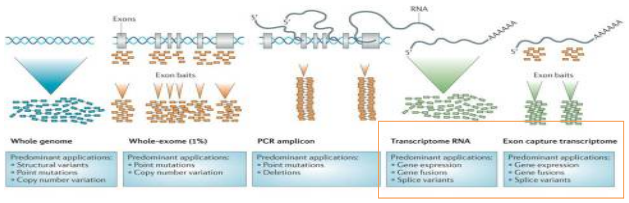
Next Generation Sequencing: Applications in oncology



Nature Reviews | Drug Discovery

27

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- Validation of targeted NGS for solid tumors using the different platforms.
  - FFPE
  - Cytology
- 96 to 100% concordance between NGS and conventional testing
- NGS outperforms Sanger sequencing
  - Sensitivity
  - DNA input
  - Time
  - Cost

D'Haene, PlosOne 2015; Le Mercier Histopathology 2015; Cottrell. J Mol Diagn. 2014; Hadd J Mol Diagn. 2013; Singh J Mol Diagn. 2013;de Biase PLoS One ;Tops BMC cancer. 2015; Endris J Mol Diagn. 2013; Deeb Arch pathol lab med. 2015

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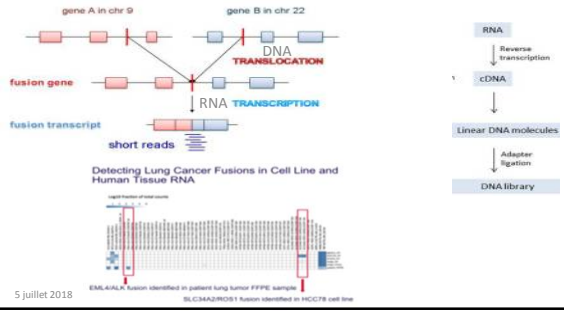
RNA seq for gene fusion detection



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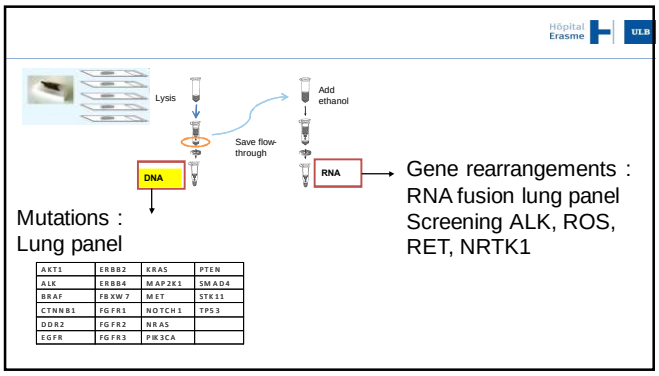
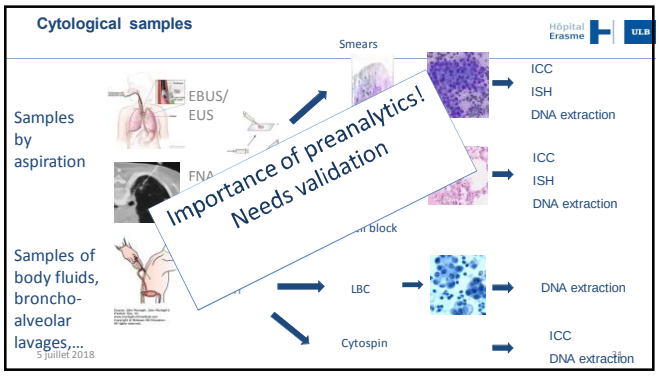




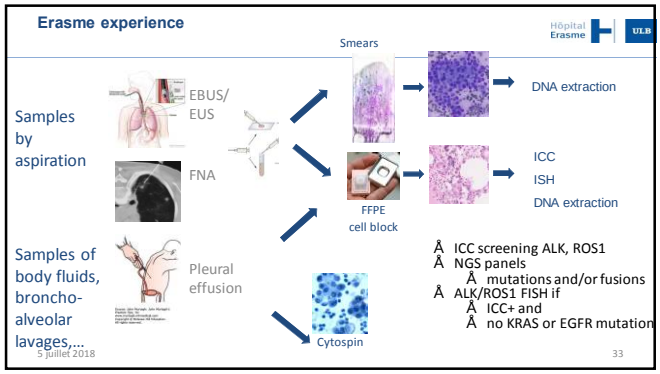
Molecular testing and cytological samples

- Different types of samples
- Different methods of testing

Molecular testing and cytological samples

- Different types of samples
- Different methods of testing
- Smears and cytospins
  - Not formalin-fixed
    - Higher ï quality DNA
    - ! ICC : Antibodies optimized for FFPE can failed on alcohol-fixed samples.

Erasme experience



NGS: Erasme experience

- Colon and Lung Panel
  - Since 2014: +/- 2000 lung samples tested from >20 different labs
  - Successful analysis in 94%
    Starting material :
    - Cytological samples : 16% : cell blocks (+/-90%) and smears (+/-10%)

NGS: Erasme experience

- Platform : Ion Torrent ï PGM
- Reagents and Kits : Life technologies (Ion Torrent)
  - 2 Panels :
  - AmpliSeq™ Colon and Lung Panel v2 (22 genes, 92 primer pairs)
  - AmpliSeq™ Lung Fusion Panel v2 (ALK, ROS1, RET, NTRK1)
- Accreditation ISO15189 of the 2 panels

NGS: Erasme experience

Workflow
2 runs per week
Collection of samples (Thursday)
DNA extraction (Friday)
Library prep (Monday)
Clonal amplification + sequencing (Tuesday)
Analysis + reporting : Wednesday

Agreement with some centers to receive samples Wednesday or Thursday
→ one week workflow

Applications for other cytological samples

- Thyroid FNA
  - Creation and validation of a thyroid specific panel

Thyroid gene panel

BRAF	KRAS	AKT1
RET	PIK3CA	TERT
NRAS	CTNNB1	IDH1
HRAS	TP53	PIK3CA
PTEN	AXIN1	CDKN2A
EGFR	EIF1AX	PRKAR1A
APC	CDH1	FLT3
SMAD4	VHL	GNAS
TSHR	RASAL1	PPM1D
CHEK2		

Thyroid fusion panel :

→RET/PTC

→PAX8/PPARG

→NTRK1

→ THADA

Conclusions

- Requirements for clinical testing include
  - the test must be performed on routine samples with low DNA content
  - the test results must be delivered rapidly
  - the test results must be accurate and facilitate clinical decision making.
- Validation of the methods
  - Preanalytics

Conclusions

- Molecular testing represents a paradigm shift in lung cancer diagnosis and is now a standard of care.
- New challenge for the pathologist : optimization of available tumor tissue or cells !
- Prioritization of testing
  - Choice of the best method related to the available samples
  - Introduction of methods that test multiple biomarkers
    - Next generation sequencing

Algorithms of testing

Acknowledgments

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