Can Molecular Biology Improve the Diagnosis of Thyroid Nodules?

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INTRODUCTION

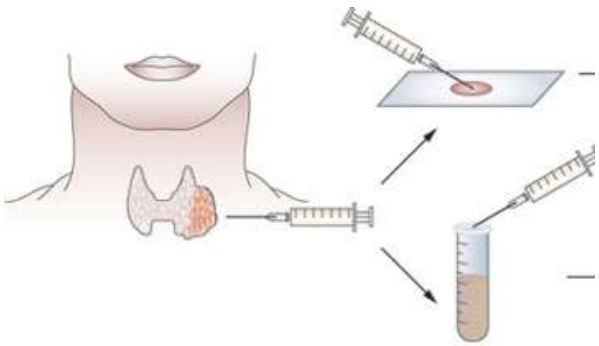
- The assessment of thyroid nodules is a common clinical problem
  - Thyroid nodules are common in the adult population
  - Thyroid cancers :
    - 5-15% of thyroid nodules examined by ultrasound and FNA
    - 1% of all cancers

- Challenge :
  - accurately diagnose cancer in these nodules
  - avoid unnecessary thyroid surgery for benign disease

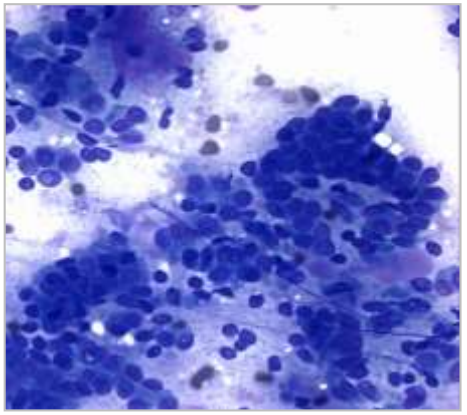
Yeung, Oncologist 2008; Hegedus, The New England Journal of Medicine 2004; DeLellis R, et al., WHO 2004

Fine-Needle Aspiration (FNA)

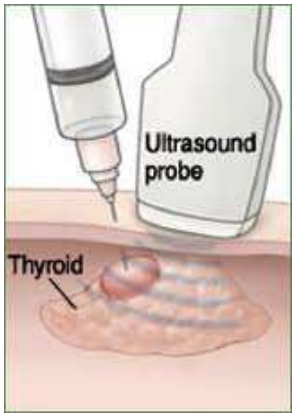
- Gold standard diagnosis method for thyroid nodules



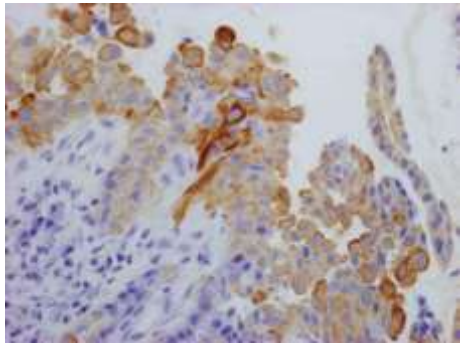
Staining



Cytological diagnosis



Fixation
=
FFPE
cell block

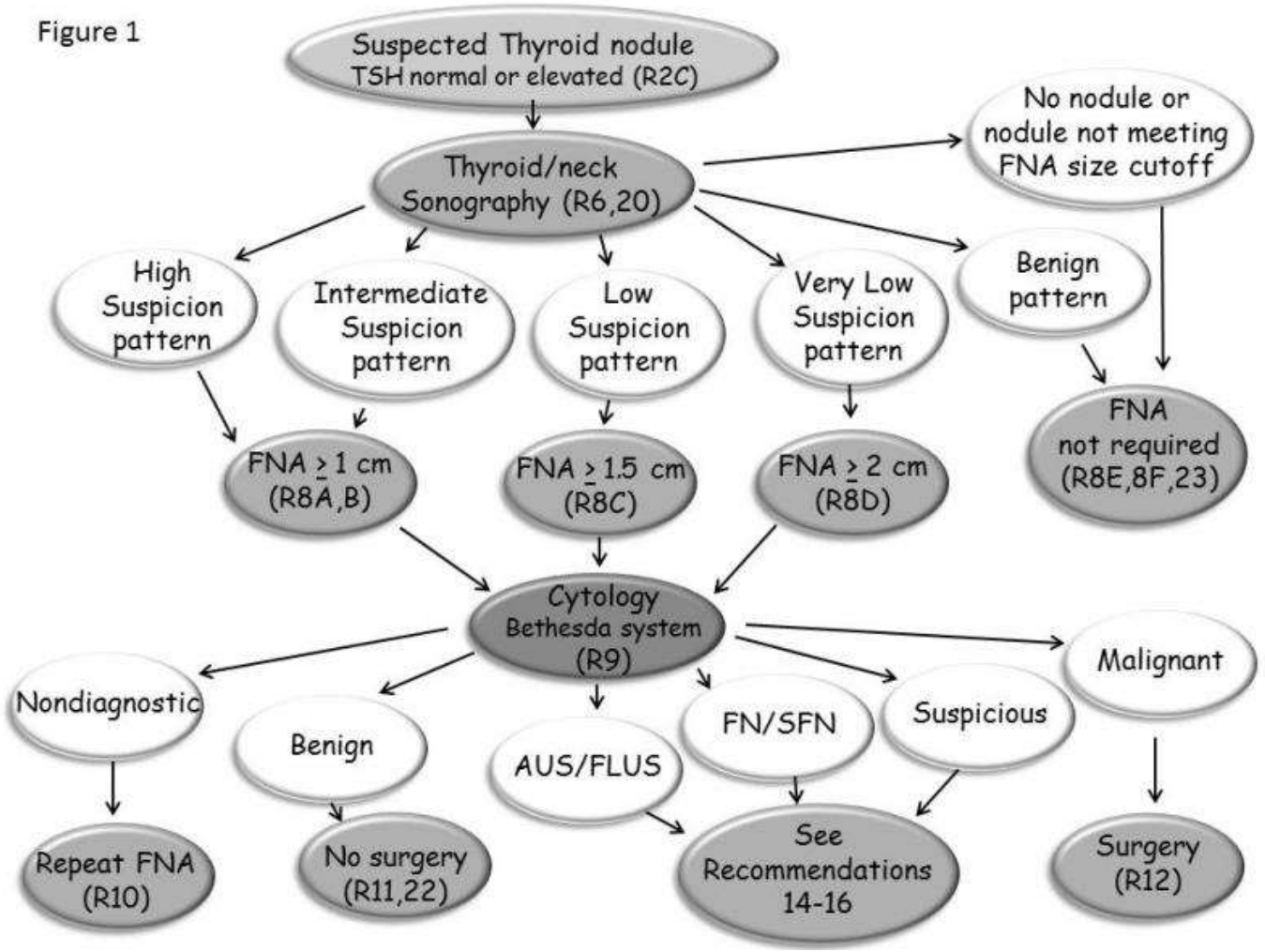


FINE-NEEDLE-ASPIRATION (FNA)

- **FNA = gold standard** to differentiate benign nodules from malignant nodules
  - Sensitivity: 94% - Specificity: 98,5%
  - Positive predictive value: 97-99,7%
  - Negative predictive value 90-98%

- **Intrinsic limitation** in differentiating benign from malignant follicular lesions which results in an *indeterminate cytological diagnosis*

FNA FOR PATIENT MANAGEMENT AND SURGERY SELECTION : 2015 ATA GUIDELINES



Haugen et al., Thyroid, 2016

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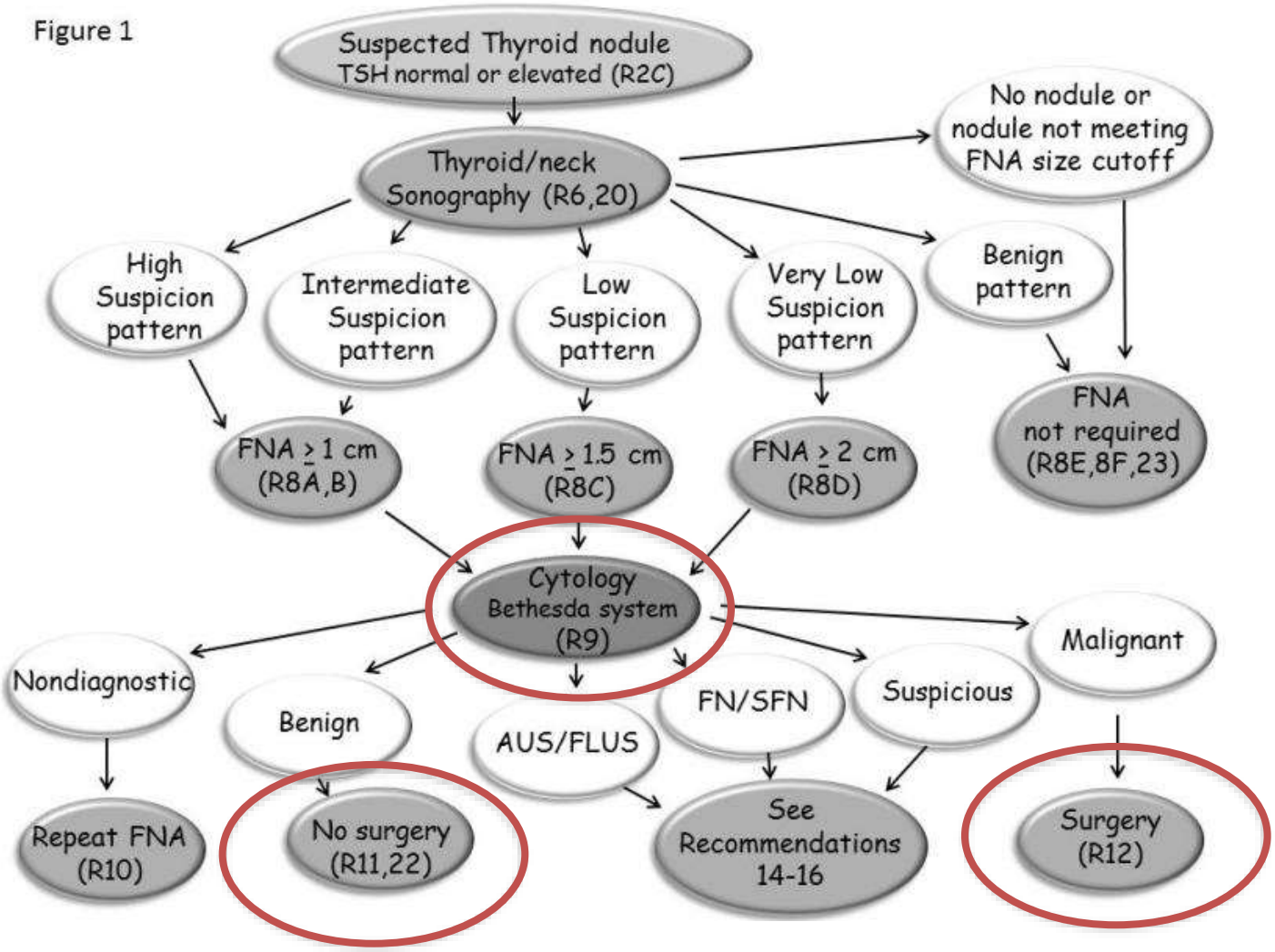
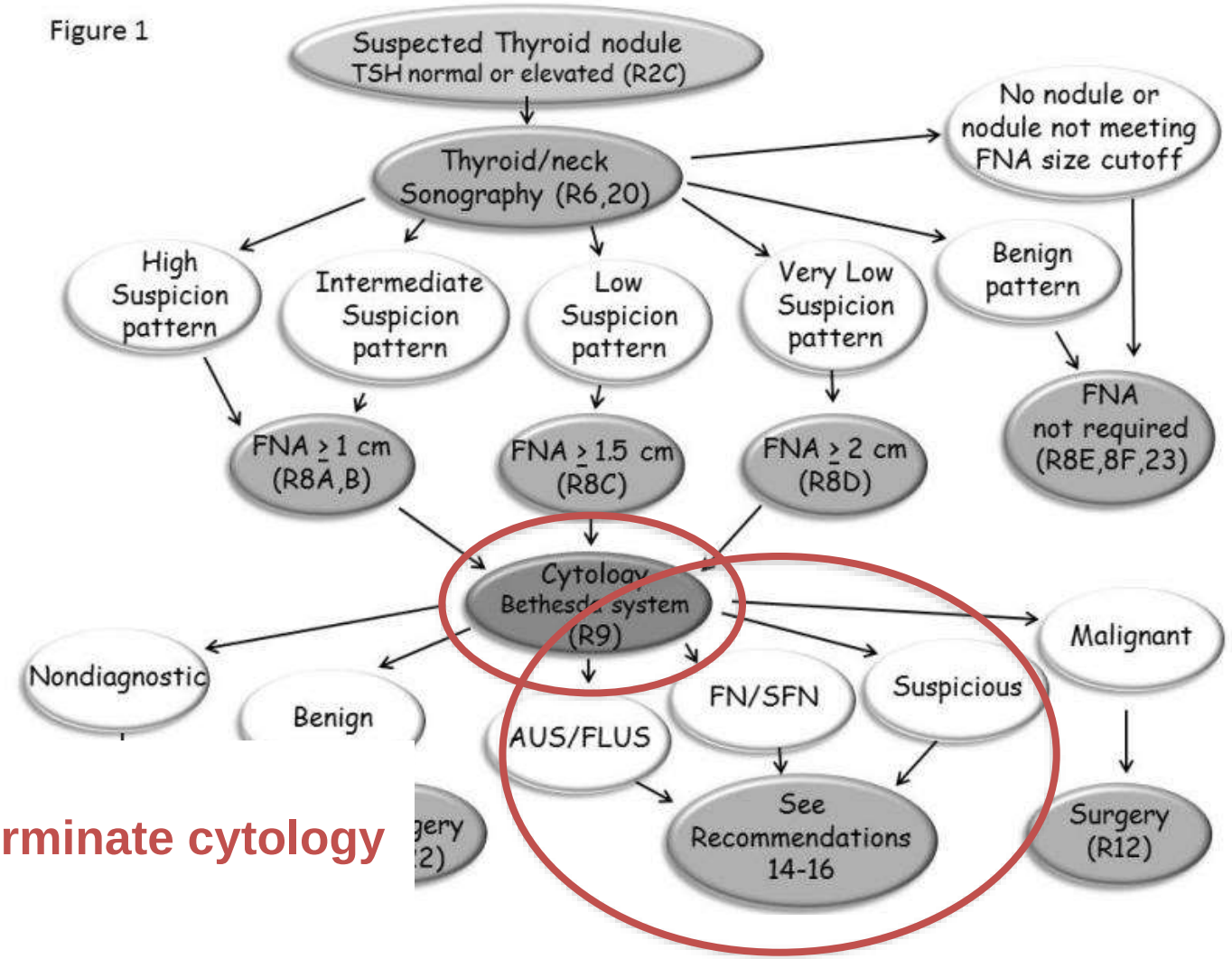


Haugen et al., Thyroid, 2016

FNA FOR PATIENT MANAGEMENT AND SURGERY SELECTION : 2015 ATA GUIDELINES



Indeterminate cytology

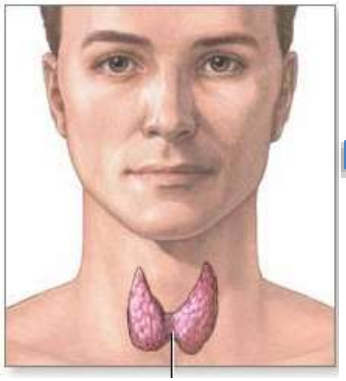
Haugen et al., Thyroid, 2016

Classification	Categories		Expected incidence	Cancer risk
Bethesda[1]	III : AUS		<7%	5-15%
	IV: follicular neoplasm +/- Hurtle cell		6-11%	15-30%
	V: suspicious for malignancy		2-8%	60-75%
Royal college of Pathologist[2]	Thy3a: atypia of undetermined significance or follicular lesion of undetermined significance			5-15%
	Thy3f: follicular neoplasm or suspicious for a follicular neoplasm			15-30%
	Thy 4: suspicious for malignancy			60-75%
SIAPEC[3]	TIR3:inconclusive/indeterminate (follicular proliferative)	TIR3A (low-risk indeterminate lesion)	4,8%	<10%
		TIR3B (high-risk indeterminate lesion)	5,4%	15-30%
	TIR 4: probably malignant		3,1%	60-80%
Japan Thyroid association[4]	3:indeterminate	A1: follicular neoplasm favor benign		5-15%
		A2: follicular neoplasm borderlines		15-30%
		A3: follicular neoplasm favor malignant		40-60%
	4: Malignancy suspected (not conclusive for malignancy)			80%
Proliferation grading system[5]	PF1 : probably benign		8%	7,7%
	PF2 : indeterminate		15%	17,7%
	PF3:suspicious for malignancy		5%	45,7%

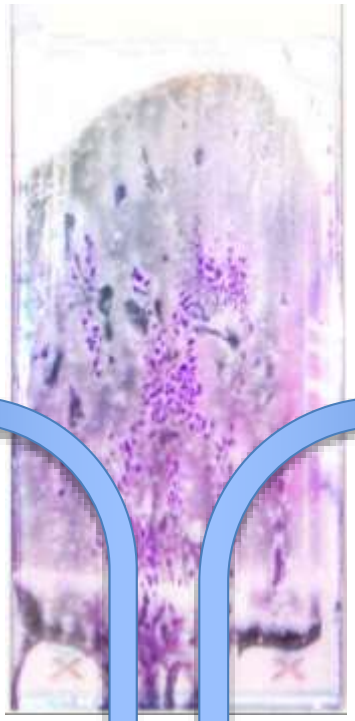
[1]Bethesda 2010 Thyroid cytopathology
[2]The Royal College of Pathologists (2009) Guidance on the Reporting of Thyroid Cytology Specimens.
[3]Fadda Pathologica. 2010
[4]Kakudo 2014
[5]Rorive 2010

How to improve the management of patients with indeterminate cytology?

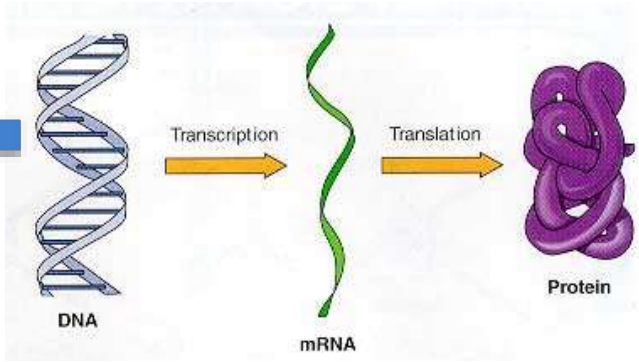
HOW TO IMPROVE THE MANAGEMENT OF PATIENTS WITH INDETERMINATE CYTOLOGY?



Clinical data

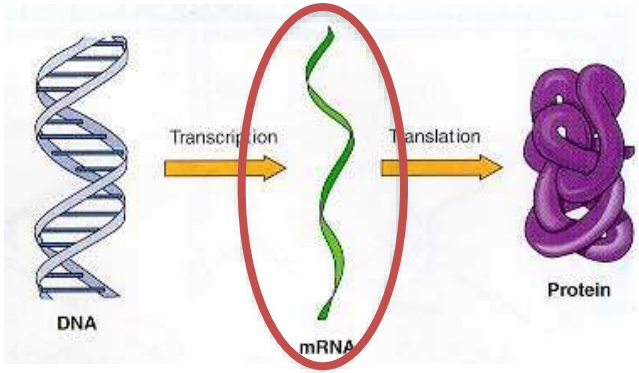


Surgery ?



Biomarkers

How to improve the management of patients with indeterminate cytology?



GENE EXPRESSION

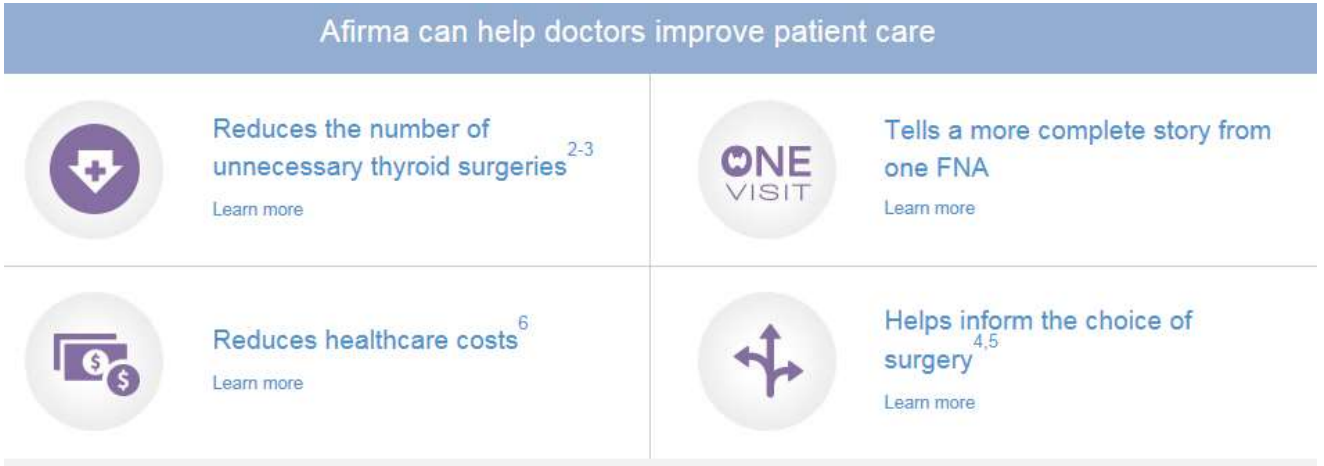
Gene expression + thyroid cytology:

+/- 5300 hits

(Pubmed 10/2020)

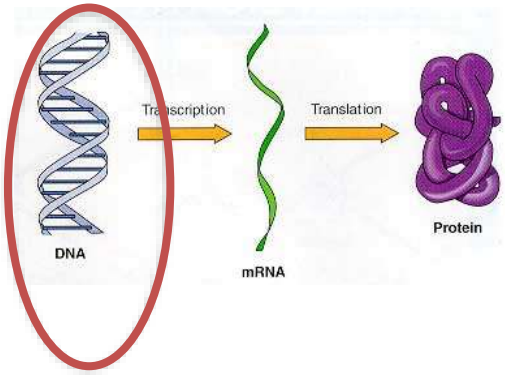
- Afirma – mRNA expression of 142 genes (USA)
- Rule-out test : exclude malignancy
  - Sensitivity : 92%/Specificity :

Afirma Thyroid FNA Analysis



Alexander NEJM 2012

How to improve the management of patients with indeterminate cytology?

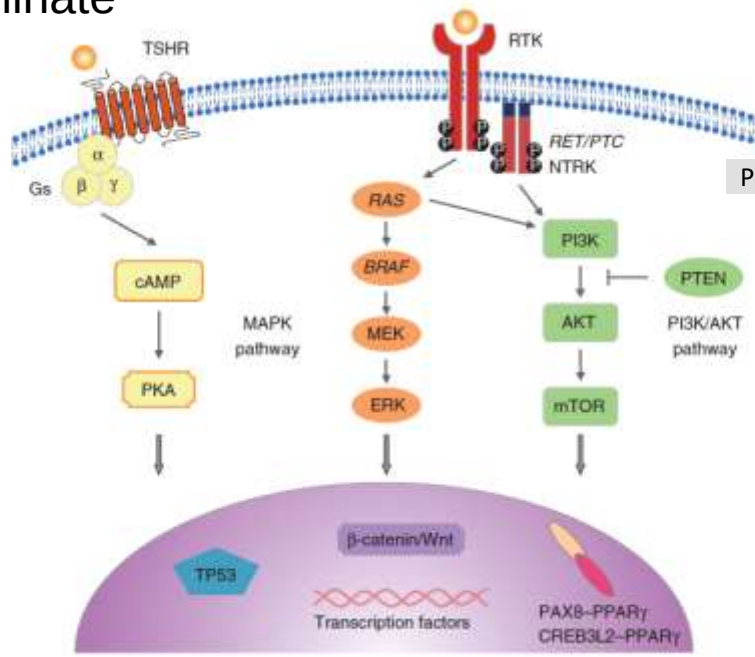


GENOMIC ALTERATIONS

Gene mutation + indeterminate thyroid FNA:

+/- 1900 hits

(Pubmed 10/2020)



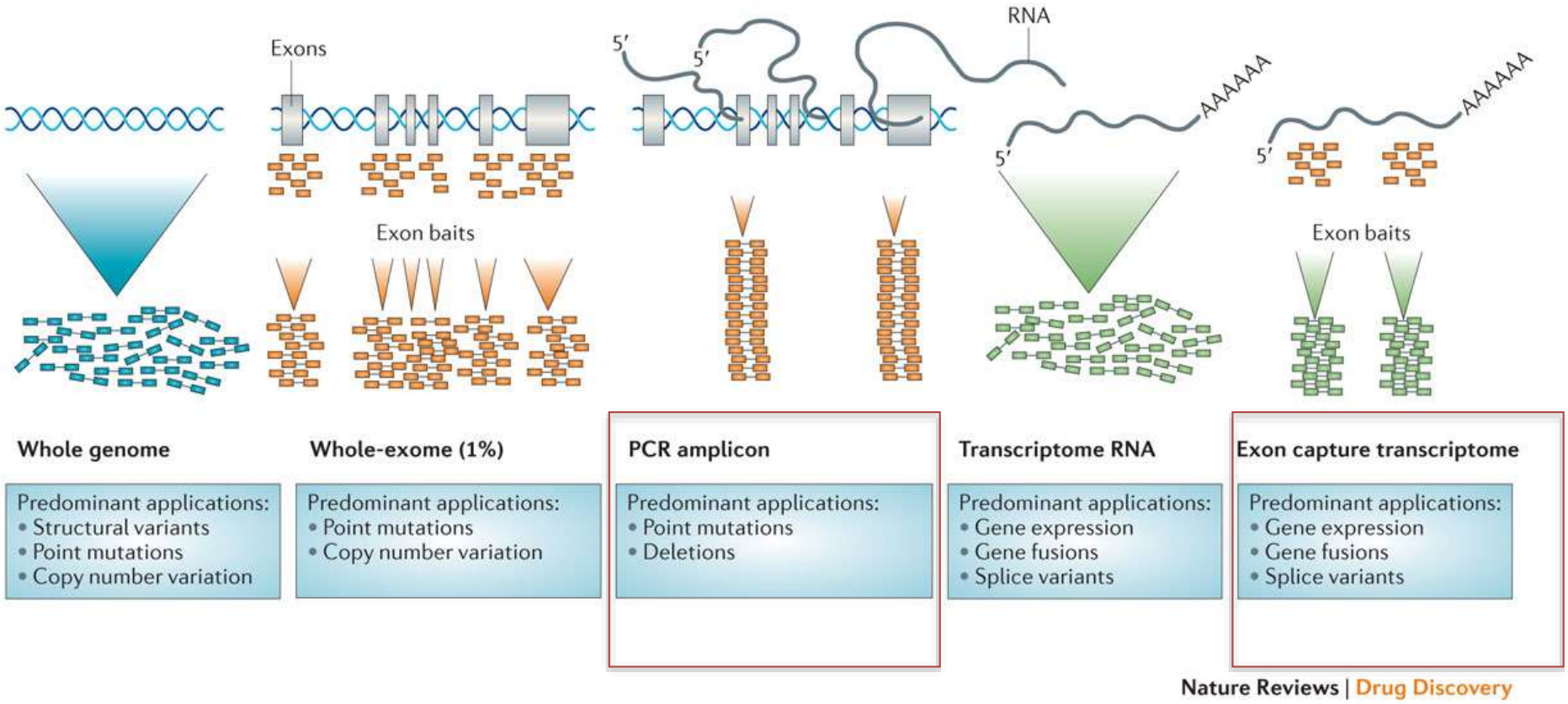
Histological Type	Prevalence (%)
Follicular carcinoma	
RAS	40-50
PAX8/PPARγ	30-35
PIK3CA	< 10
PTEN	< 10
Papillary carcinoma	
BRAF	40-60
RET/PTC	10-20
RAS	10-20
TRK	< 5
Anaplastic carcinoma	
TP53	50-80
CTNNB1	50-70
RAS	20-40
BRAF	20-40
PIK3CA	10-20
PTEN	5-15
AKT1	5-10
Poorly differentiated carcinoma	
RAS	20-40
TP53	20-30
CTNNB1	10-20
BRAF	10-20
PIK3CA	5-10
AKT1	5-10

Hsiao, Endocr Relat Cancer, 2014
Xing, Nat Rev Cancer, 2013

GUIDELINES

- ATA 2015 (Haugen, Alexander, *et al.,* Thyroid. Jan 2016)
  - There is currently **no single optimal molecular test** that can definitively rule in or rule out malignancy in all cases of indeterminate cytology, and long-term outcome data proving clinical utility are needed.
  - AUS/FLUS + FN/SFN : molecular testing **may be used** to supplement malignancy risk assessment
- European thyroid association 2017 (Paschke R, et al. Eur Thyroid J. 2017)
  - For c*ytologically indeterminate nodules* **consider, if available**, the detection of *BRAF* and *RET/PTC*, and, possibly, *PAX8/PPARG* and *RAS* mutations. The significance of detecting *RAS* mutations needs to be clarified.
  - The substitution of classic mutation detection methods for thyroid FNAs with the tNGS approach is most promising, although it has not been confirmed in other laboratories. Larger tNGS mutation panels have the potential to become a simultaneous rule-in and rule-out test if NPVs >95% can be confirmed.

Next Generation Sequencing: Applications in oncology



= targeted NGS

Original Article

Highly Accurate Diagnosis of Cancer in Thyroid Nodules With Follicular Neoplasm/Suspicious for a Follicular Neoplasm Cytology by ThyroSeq v2 Next-Generation Sequencing Assay

Yuri E. Nikiforov, MD, PhD[1]; Sally E. Carty, MD[2]; Simon I. Chiosea, MD[1]; Christopher Coyne, MD[3]; Umamaheswar Duvvuri, MD[4]; Robert L. Ferris, MD, PhD[4]; William E. Gooding, MS[5]; Steven P. Hodak, MD[3]; Shane O. LeBeau, MD[3]; N. Paul Ohori, MD[1]; Raja R. Seethala, MD[1]; Mitchell E. Tublin, MD[6]; Linwah Yip, MD[2]; and Marina N. Nikiforova, MD[1]

143 patients with known surgical outcome

THYROID
Volume X, Number X, 2015
DOI: 10.1089/thy.2015.0305

ORIGINAL STUDY

95 patients with known surgical outcome

Impact of the Multi-Gene ThyroSeq Next-Generation Sequencing Assay on Cancer Diagnosis in Thyroid Nodules with Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance Cytology

Yuri E. Nikiforov,[1] Sally E. Carty,[2] Simon I. Chiosea,[1] Christopher Coyne,[3] Umamaheswar Duvvuri,[4] Robert L. Ferris,[4] William E. Gooding,[5] Shane O. LeBeau,[3] N. Paul Ohori,[1] Raja R. Seethala,[1] Mitchell E. Tublin,[6] Linwah Yip,[2] and Marina N. Nikiforova[1]

Nikiforov, Cancer 2014
Nikiforov, Thyroid 2015

Point Mutations	
NRAS	*RET*
HRAS	*TSHR*
KRAS	*AKT1*
BRAF	*TP53*
PIK3CA	*GNAS*
PTEN	*CTNNB1*

+ 2014 : TERT

+ 2015: EIF1AX

Gene Rearrangements
RET/PTC
PAX8/PPARG
NTRK1
NTRK3
ALK
THADA

FNA Cancer risk :

2014 : 27%

2015 : 23%

FNA & Test + Cancer risk

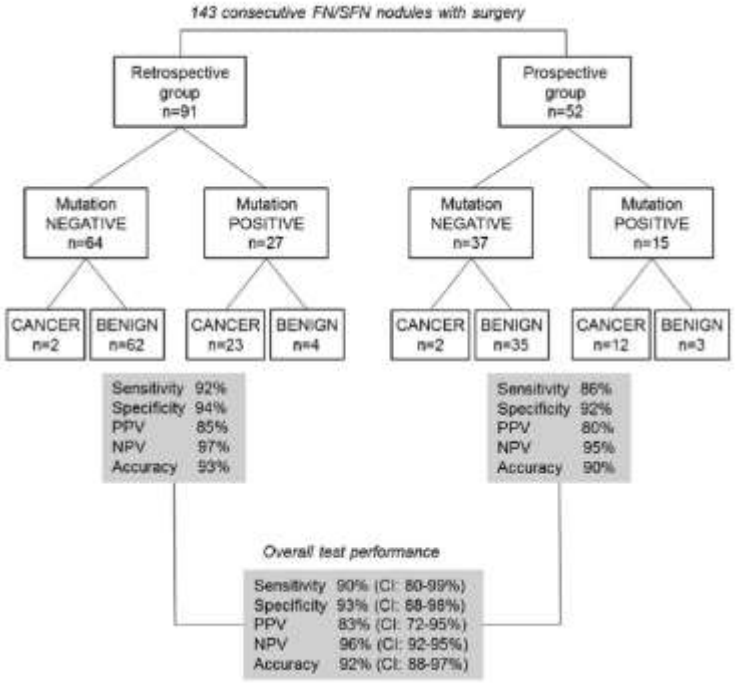
2014 : 83%

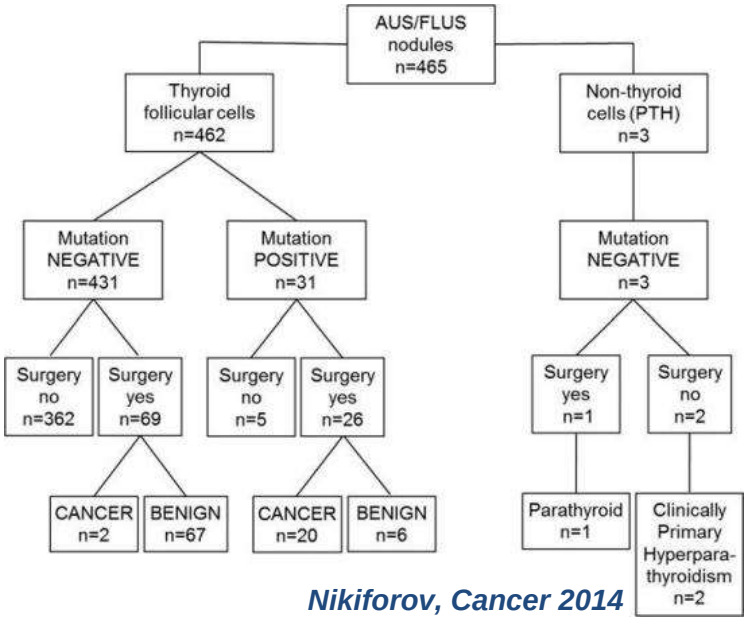
2015 : 77%

FNA & test – Cancer risk

2014 : 4%

2015 : 3%

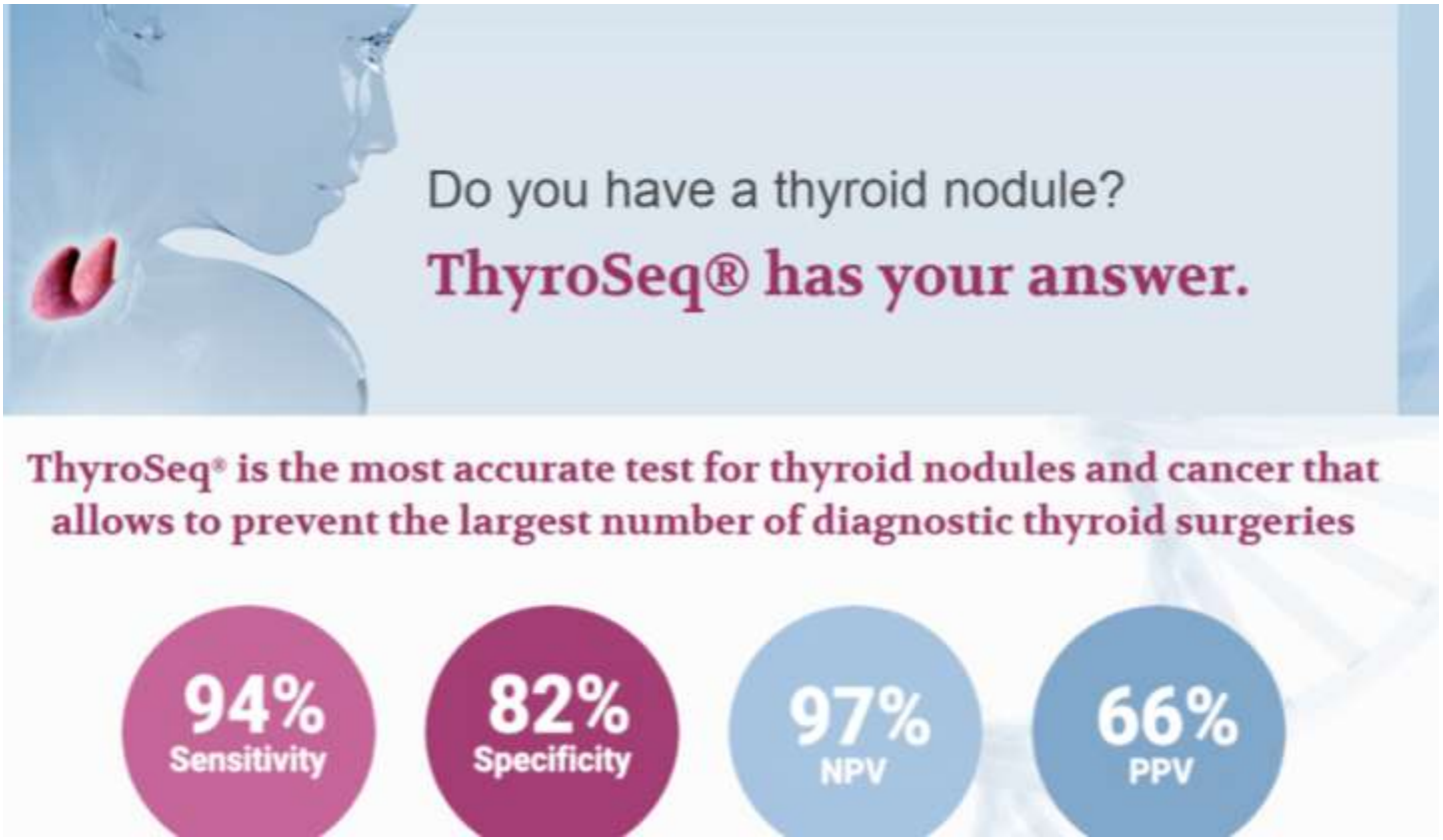


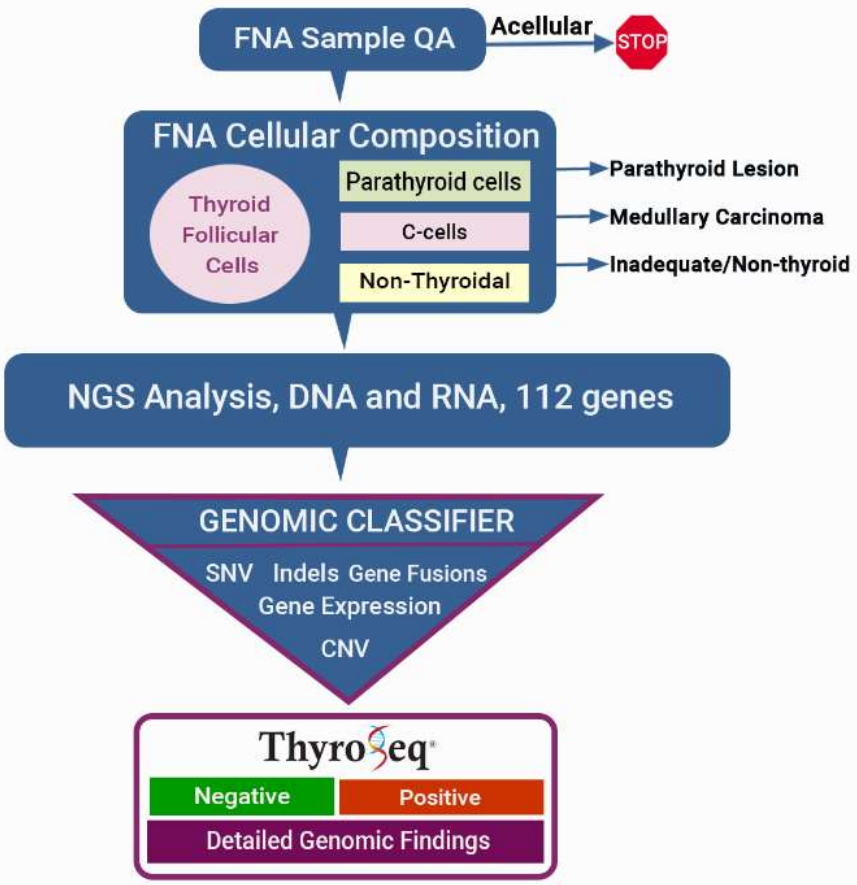


Nikiforov, Cancer 2014

Nikiforov, Thyroid 2015

- Thyroseq + thyroid cytology (10/2020) :
  - 48 results





ThyroSeq test consists of several steps.

- It starts with the assessment of FNA sample cellularity. This is a quality assurance (QA) step that determines if the provided sample has sufficient number of cells to proceed with the analysis. If the number of cells is below the required limit, the test is cancelled and no charges are posted.
- Next, cellular composition of the sample is evaluated. This step assures that the provided sample has an adequate proportion of thyroid follicular cells. It also allows accurate detection of c-cells (MTC), parathyroid cells, and other non-thyroidal cells.
- Then, the generated next generation sequencing data on 112 genes are processed using an in-house bioinformatic pipeline that applies a complex algorithm to estimate cancer probability in the tested nodule. The algorithm was built based on cancer probability associated with each genetic alteration and their combination and validated in a prospective, multicenter, double-blind study.
- Next, the test results and findings are reviewed by a board-certified pathologist who verifies all findings and

- 112 genes
  - 12,135 single nucleotide variations (SNVs) and insertions/deletions (indels) (COSMIC hotspots),
  - more than 120 gene fusion (GF) types,
  - abnormal gene expression alterations (GEAs) of 90 genes,
  - copy number alterations (CNAs) in 10 genomic regions in FNA samples and in up to 27 genomic regions in tissue samples.

Nikiforova, Cancer 2018

- to assign a value to each detected genetic alteration based on the strength of association with malignancy:
  - 0 (no association with cancer),
  - 1 (low cancer probability),
  - 2 (high cancer probability).

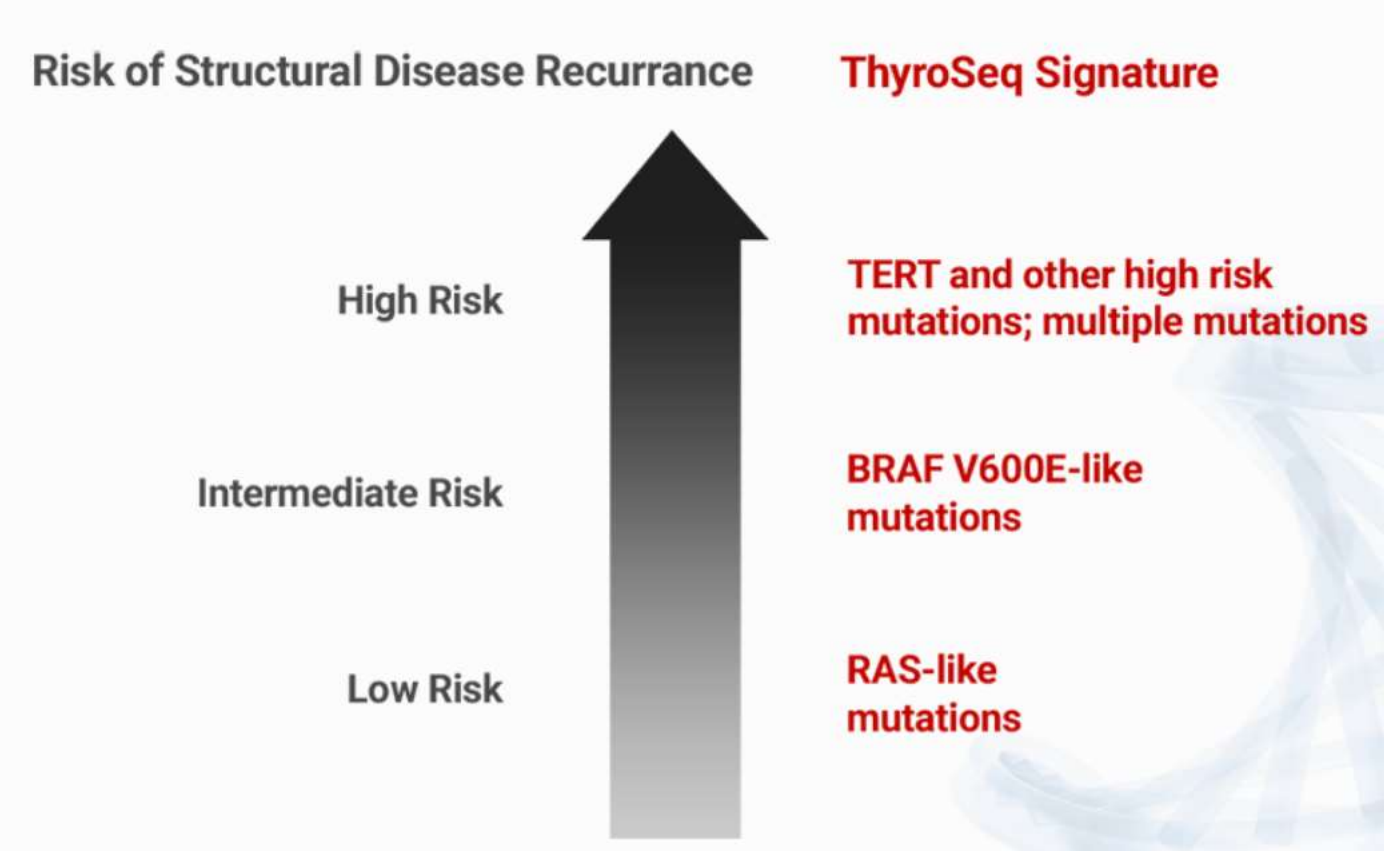
Nikiforova, Cancer 2018

- The values were derived from
  - (i) extensive literature and searchable database review (TCGA, cBioPortal, COSMIC, etc.),
  - (ii) in-house database of >1000 thyroid surgical and FNA samples with known surgical outcome,
  - (iii) RNA-Seq analysis of thyroid cancer tissue and FNA samples
  - (iv) CytoScan analysis of 17 thyroid cancer tissue samples.

Nikiforova, Cancer 2018

- Genomic Classifier =sum of individual values of all detected alterations,
  - scores 0 and 1: test negative
  - scores 2 and above : test positive.

Nikiforova, Cancer 2018


Risk of Structural Disease Recurrance
ThyroSeq Signature
High Risk
TERT and other high risk mutations; multiple mutations
Intermediate Risk
BRAF V600E-like mutations
Low Risk
RAS-like mutations

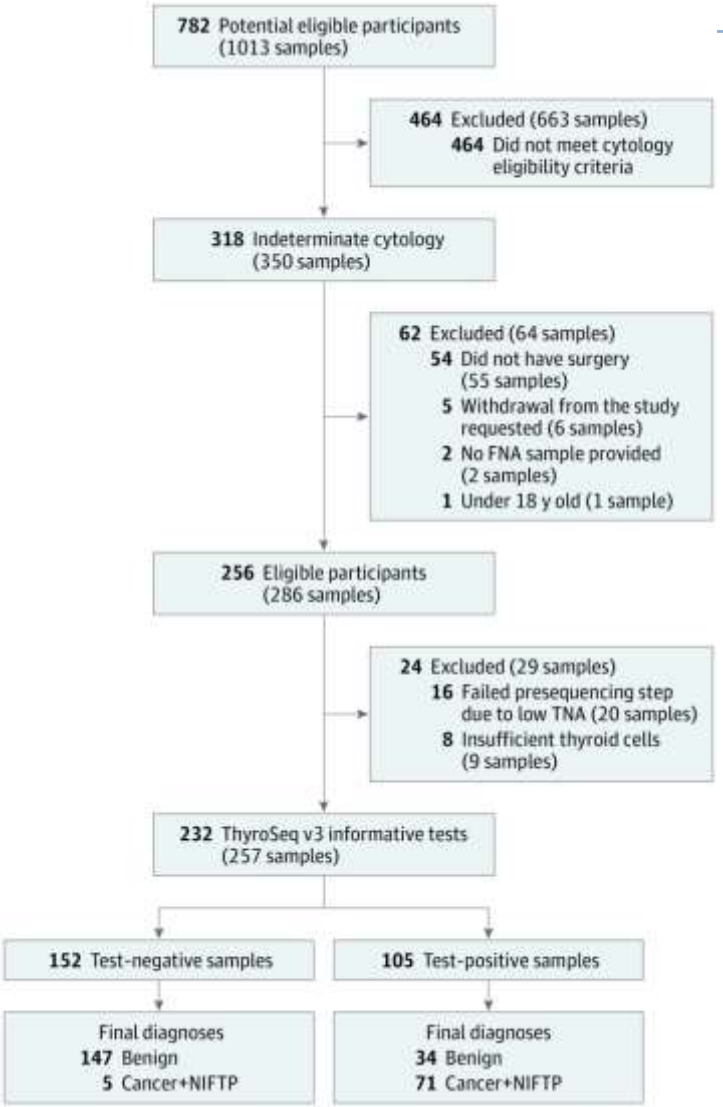
Multicenter Study > JAMA Oncol. 2019 Feb 1;5(2):204-212. doi: 10.1001/jamaoncol.2018.4616.

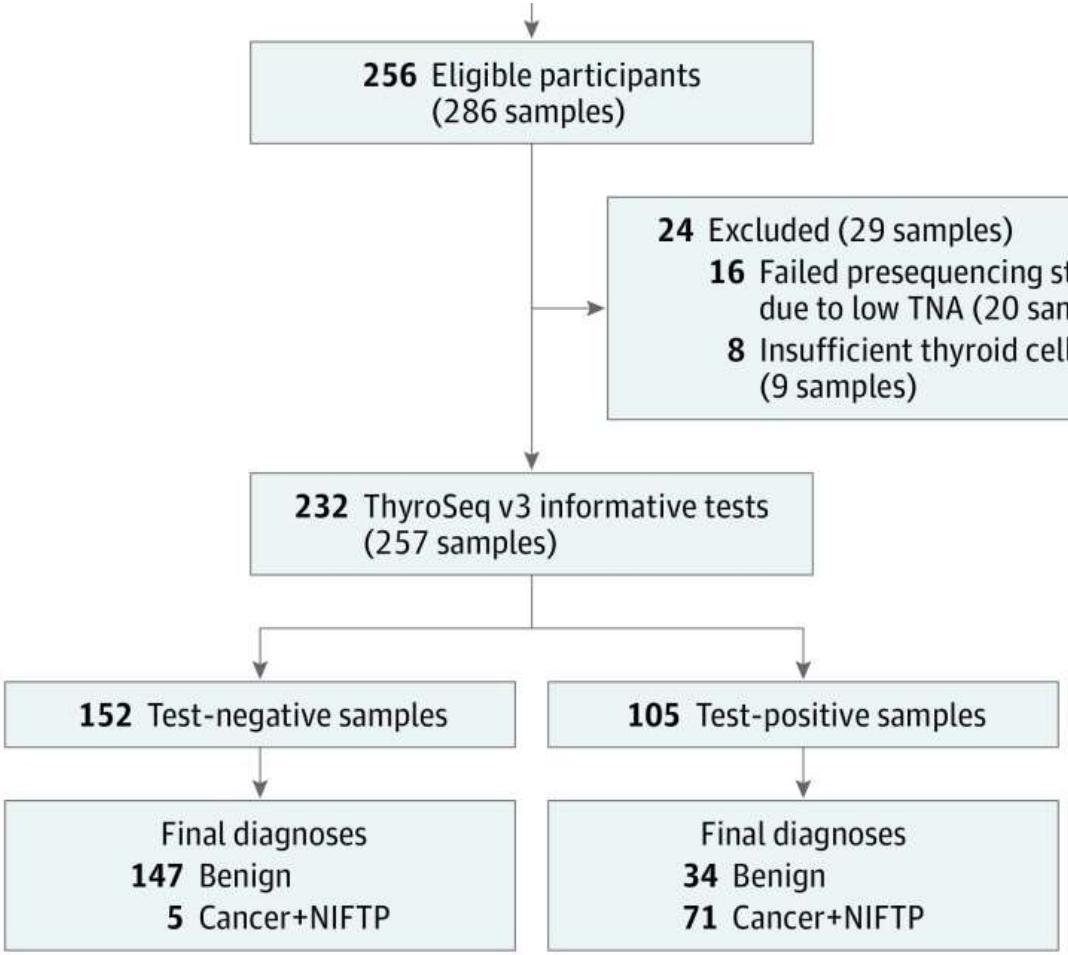
Performance of a Multigene Genomic Classifier in Thyroid Nodules With Indeterminate Cytology: A Prospective Blinded Multicenter Study

David L Steward [1], Sally E Carty [2], Rebecca S Sippel [3], Samantha Peiling Yang [4], Julie A Sosa [5 6], Jennifer A Sipos [7], James J Figge [8], Susan Mandel [9], Bryan R Haugen [10], Kenneth D Burman [11], Zubair W Baloch [12], Ricardo V Lloyd [13], Raja R Seethala [14], William E Gooding [15], Simio I Chiosea [11], Cristiane Gomes-Lima [11], Robert L Ferris [16], Jessica M Folek [8], Raheela A Khawaja [7], Priya Kundra [11], Kwok Seng Loh [17], Carrie B Marshall [18], Sarah Mayson [10], Kelly L McCoy [2], Min En Nga [19], Kee Yuan Ngiam [20], Marina N Nikiforova [14], Jennifer L Poehls [21], Matthev D Ringel [7], Huaitao Yang [22], Linwah Yip [2], Yuri E Nikiforov [14]

Hôpital Erasme ULB

prospective, blinded, multicenter clinical validation study





10%

61% of the nodules yielding a negative test result

→ only 3% residual cancer risk in these nodules (rate similar to that of benign cytology)

→ Up to 61% of patients with indeterminate cytology thyroid nodules may avoid diagnostic surgery by undergoing multigene genomic classifier testing.

Table 1.

Performance of the Genomic Classifier Test in Cytologically Indeterminate Thyroid Nodules

Performance Across the Entire Cohort (n = 257; Disease Prevalence 30%)

Result	Cancer+NIFTP (n = 76)	Benign (n = 181)	Test performance, % (95% CI)
Positive	71	34	Sensitivity, 93 (86-97)
			Specificity, 81 (75-86)
Negative	5	147	NPV, 97 (93-99)
			PPV, 68 (58-76)

cancer probabilities varying from 59% to 100%

Table 3.

Probability of Cancer/NIFTP in Specific Molecular Alteration Groups

Group	Molecular Alterations, No.	Prevalence in Test-Positive Samples, No. (%)	Histopathologic Diagnosis, %		Cancer Type/NIFTP (%)
			Cancer/NIFTP	Benign	
High-risk group	*TERT* (and *HRAS*) (1) *TP53* (and *MEN1*) (1)	2 (2)	100	0	Papillary carcinoma (50) Follicular carcinoma (50)
BRAF-like group	*BRAF V600E* (9) *NTRK3* fusions (2) *RET* fusions (1) *BRAF* fusions (1)	13 (12)	100	0	Classical papillary carcinoma (92) Follicular variant papillary carcinoma (8)
RAS-like group	*NRAS* (21) *HRAS* (18) *KRAS* (8) *BRAF K601E* (3) *PTEN* (1) *IDH2* (1) *DICER1* (1) *PPARG* fusions (4) *THADA* fusions (4)	60 (57)	62	38	Follicular variant papillary carcinoma (22) Papillary carcinoma, other variants (17) Follicular carcinoma (9) Hürthle cell carcinoma (5)
Copy number alterations group	Copy number alterations	22 (21)	59	41	Hürthle cell carcinoma (32) Follicular variant papillary carcinoma (14) Papillary carcinoma, other variants (9) NIFTP (5)
Gene expression alterations group	Gene expression alterations	8 (8)	75	25	Classical papillary carcinoma (37) NIFTP (13) Other cancers (MTC, mRCC) (25)

100% : TERT, TP53, BRAF V600E, fusions NTRK3, BRAF or RET

Abbreviations: mRCC, metastatic renal cell carcinoma; MTC, medullary thyroid carcinoma; NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features.

LIMITATIONS SIMILAR : NOT APPLIED IN DAILY PRACTICE → WHY?

- Time
- Cost
- Commercial companies
- Availability

ERASME EXPERIENCE : CUSTOM THYROID SPECIFIC PANEL

- Creation and validation of 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

→NTRK3

→BRAF

→ALK

26 genes for SNV and 93 gene fusions

APPLICATION

- FNA : indeterminate cytology
- NGS : BRAF V600E mutation

Diagnosis ?

- → PTC

APPLICATION

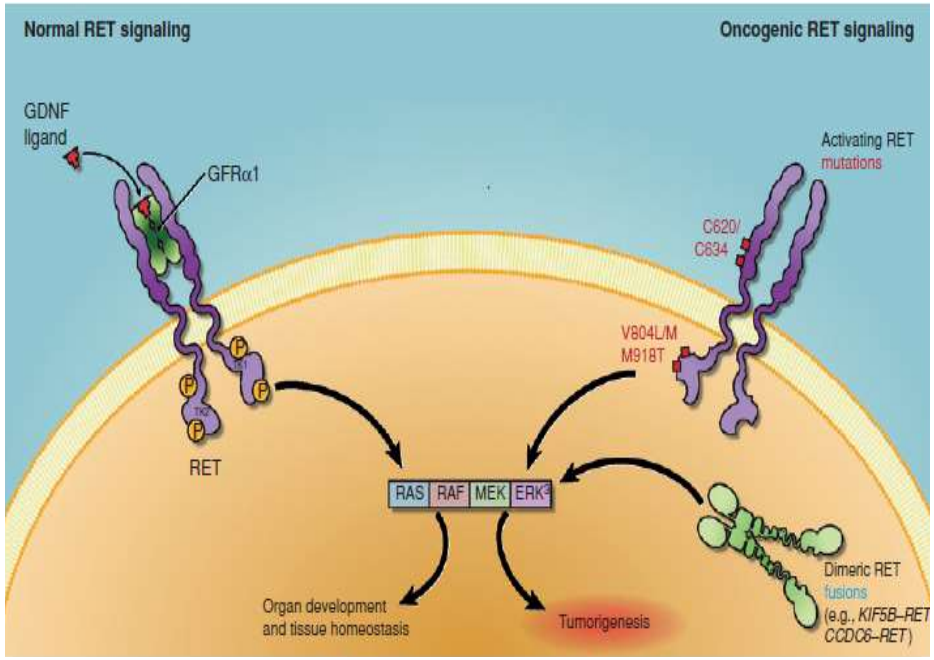
- FNA : indeterminate cytology
- NGS : RET M918T

- Diagnosis?

- → MTC

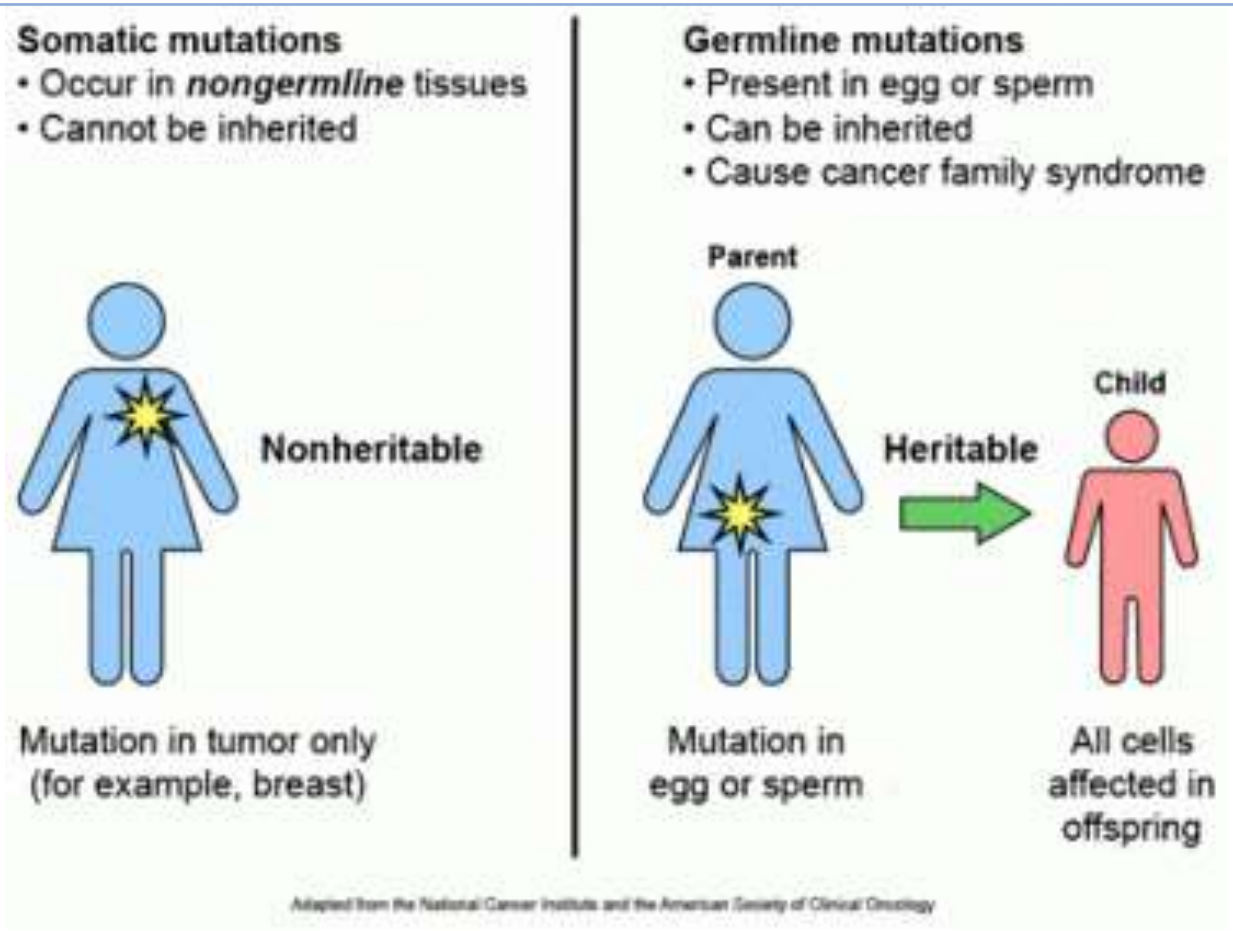
RET

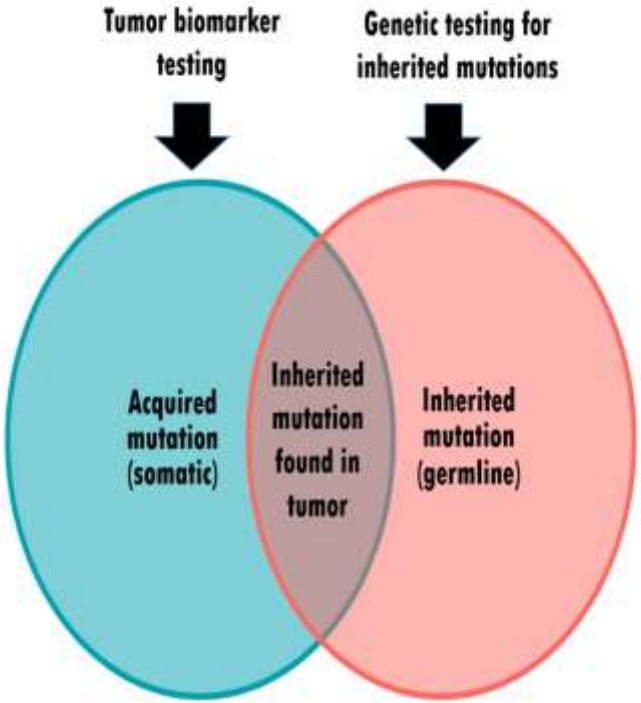
- Transmembrane receptor with tyrosine kinase activity
- Oncogenic alterations → constitutive activation
  - Rearrangements → fusion : papillary thyroid carcinoma and NSCLC (somatic)
  - Mutations : medullary thyroid cancer (somatic or germline), multiple endocrine neoplasia 2 (MEN2 - germline)



Subbiah et al. Cancer Discovery 2020

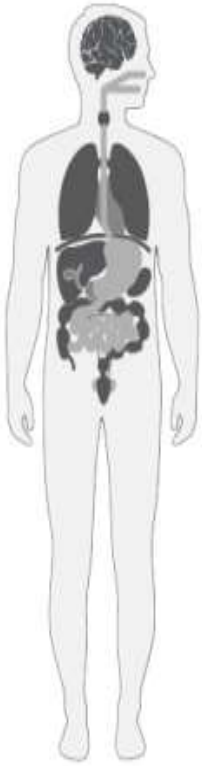
RAPPEL : GERMLINE OR SOMATIC MUTATION



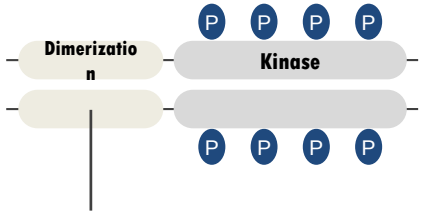


A GENE MAY HAVE MULTIPLE METHODS OF ONCOGENIC ACTIVATION: *RET*

RET fusions are found in many tumour types

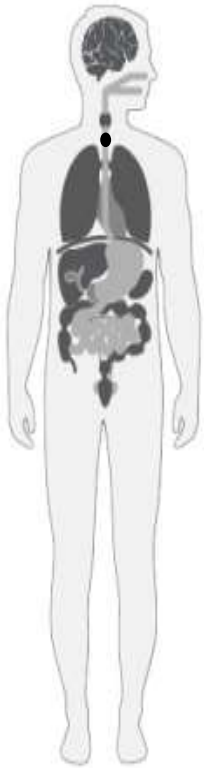


Non-small cell lung cancer (NSCLC) (2%)
Papillary and other thyroid cancers (10%-20%)
Pancreatic cancer (<1%)
Salivary gland cancer (<1%)
Spitz tumours (<1%)
Colorectal cancer (<1%)
Ovarian cancer (<1%)
Myeloproliferative disorders (<1%)

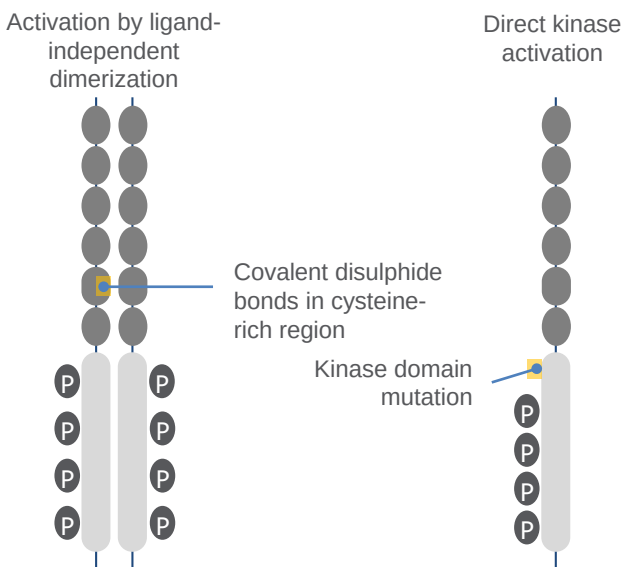


KIF5B (most common in lung cancer)
CCDC6 or NCOA4 (most common in thyroid cancer)

RET-activating mutations are found in medullary thyroid cancer (MTC)



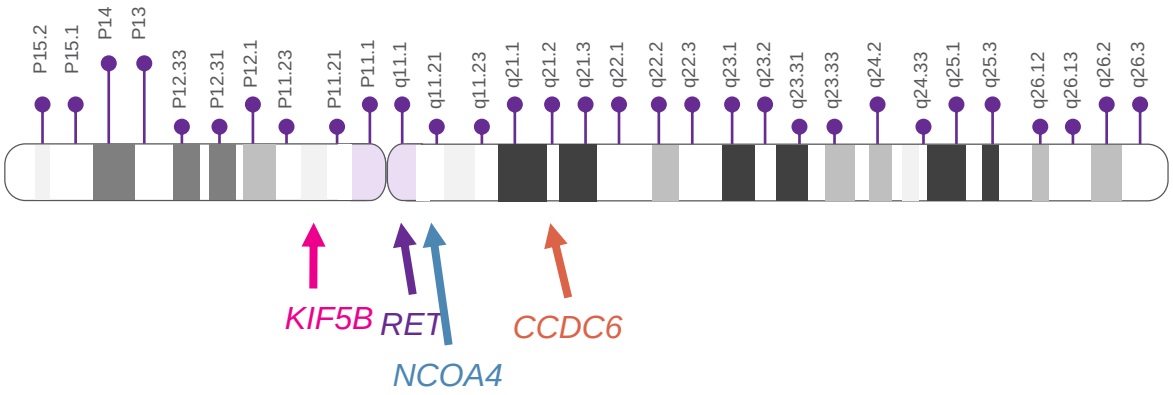
MTC
Sporadic (>60%)
Hereditary (>90%)

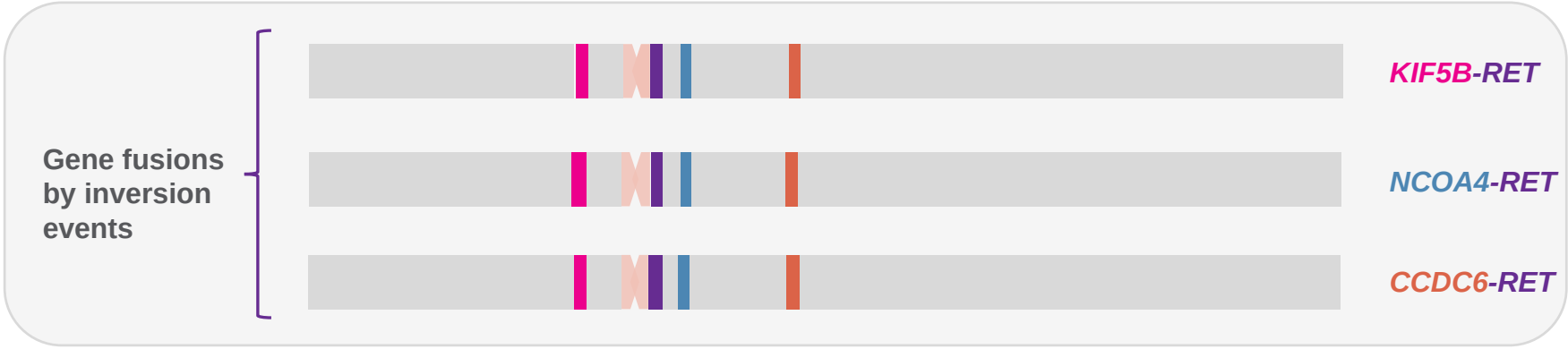


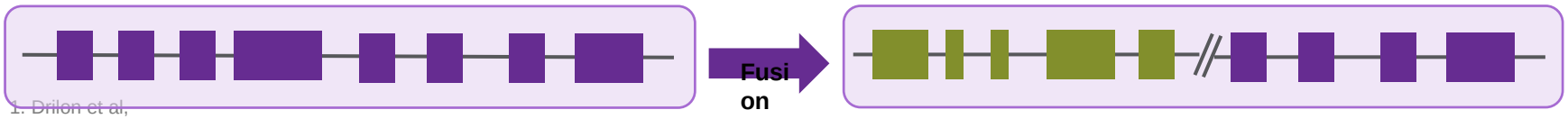
Most common mutation: *RET* M918T

RET=rearranged during transfection.
Drilon A, et al. Oral presentation. ASCO 2018. Abstract 102.

FUSIONS IN *RET* GENE ARE ONCOGENIC DRIVERS IN NSCLC AND THYROID CANCER; INTRA-CHROMOSOMAL INVERSIONS GENERATE THE COMMON *RET* FUSIONS[1]

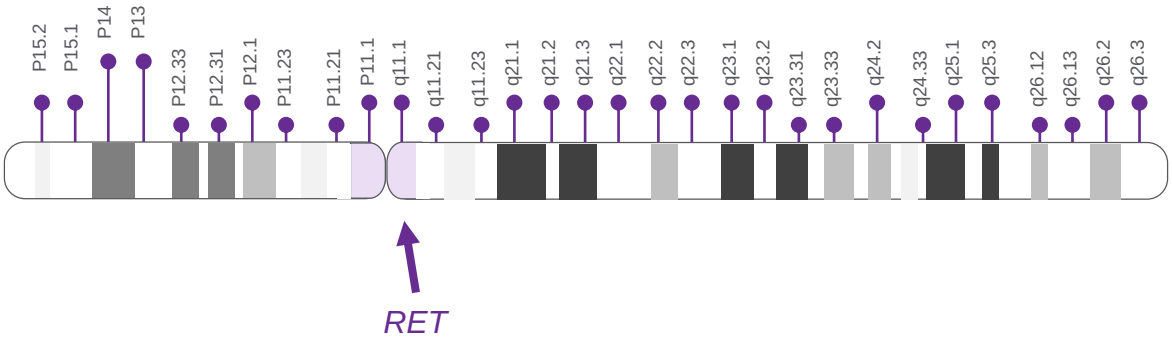






1. Drilon et al, 2018;15(3):151-167.

DELETIONS AND TRANSLOCATIONS ARE OTHER MECHANISMS THAT CAN GENERATE FUSIONS[1]



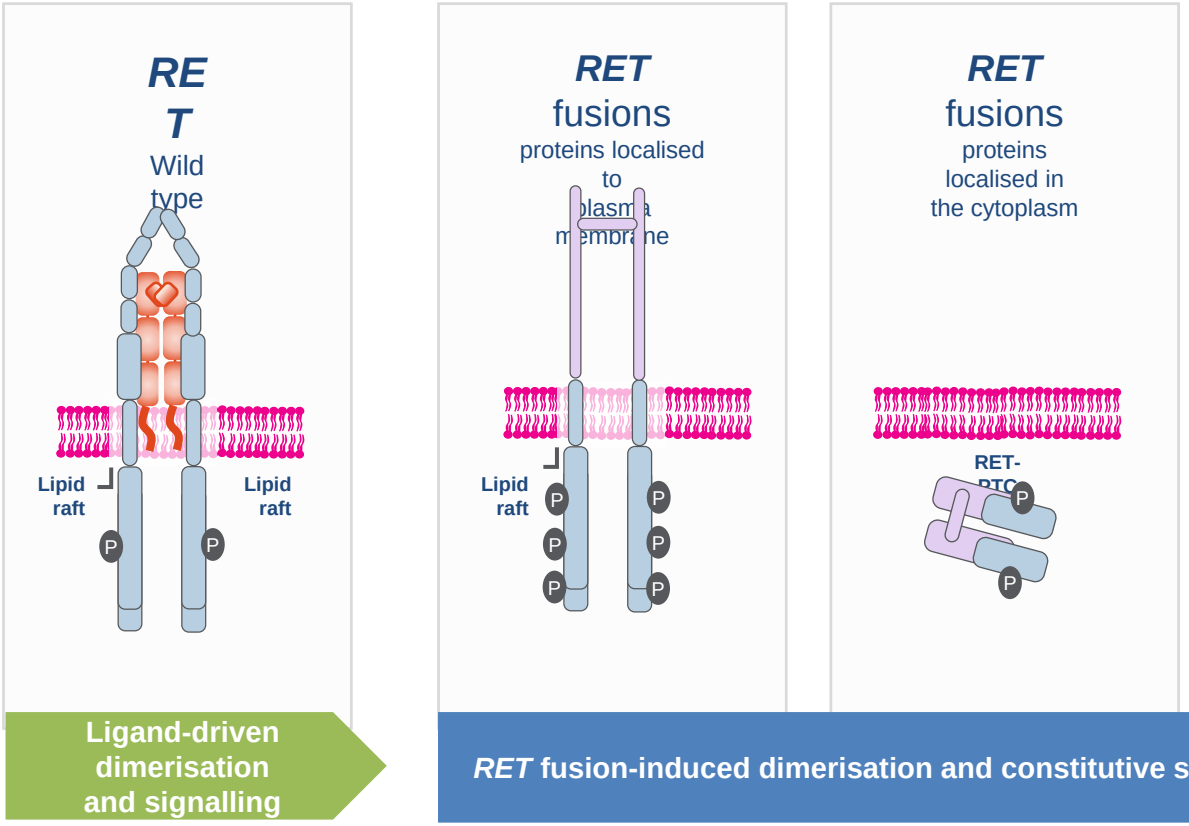
Gene fusion by deletion

Gene fusion by translocation between two different chromosomes

1. Drilon, et al. *Nat Rev Clin Ocol*. 2018;15(3):151-167.

RET FUSIONS LEAD TO CONSTITUTIVE ACTIVATION OF SIGNALLING; CELLULAR LOCALISATION DEPENDS ON FUSION PARTNERS AND BREAKPOINTS[1,2]

RET FUSIONS ARE FOUND IN MANY TUMOUR TYPES



Non-small cell lung cancer (NSCLC) (2%)[3,4]

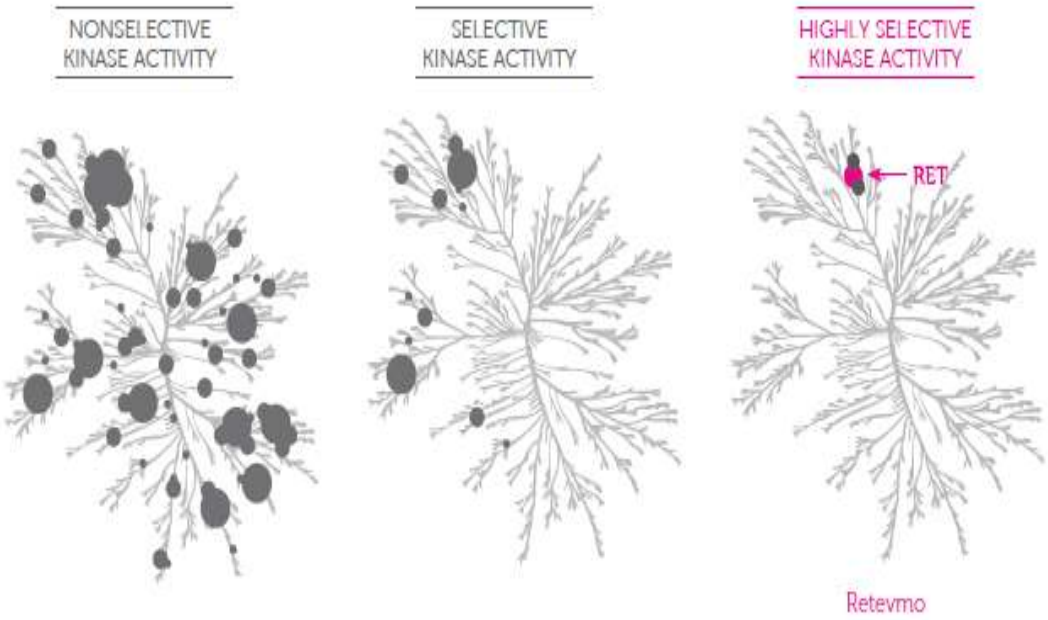
Papillary and other thyroid cancers (10–20%) [3,4]

Other solid tumours: <1% [3,4]

RET=rearranged during transfection.

Adapted from: 1. Mulligan LM. *Nat Rev Cancer*. 2014;14:173-186 and 2. Drilon A, et al. *Nat Rev Clin Oncol* 2018;15:151-167; 3. Belli C, et al. *Ann Oncol*. 2020;32:337-350; 4. Yang S, et al. *Clin Cancer Res*. 2021;27(5):1316-1328.

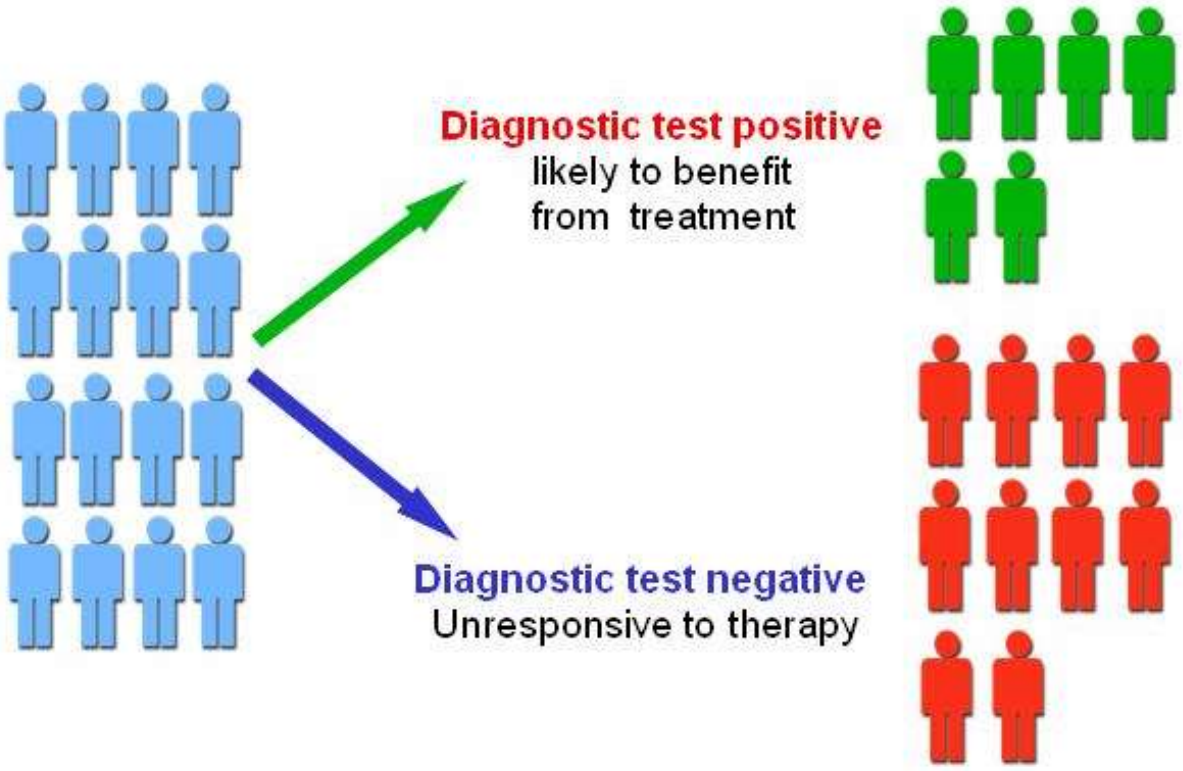
RETSEVMO (SELPERCATINIB) IS A POTENT AND SELECTIVE *RET* INHIBITOR



- Selpercatinib was at least 250-fold more selective for RET relative to other kinases[1]
- Selpercatinib caused significant cytotoxicity in human cancer cell lines that harbored endogenous, clinically relevant *RET* gene alterations (IC_{50} 1-10 nM) and was much less cytotoxic against human cancer cell lines without *RET* alterations (IC_{50} 100-10,000 nM)
- Selpercatinib strongly inhibited the *in vitro* growth of 4 cell lines harboring *RET* gene alterations, with EC_{50} values less than 10 nM
  - In contrast, Selpercatinib had 60- to 1300-fold less inhibitory anti-proliferative activity against 83 human cancer cell lines that lacked alterations in the endogenous *RET* gene

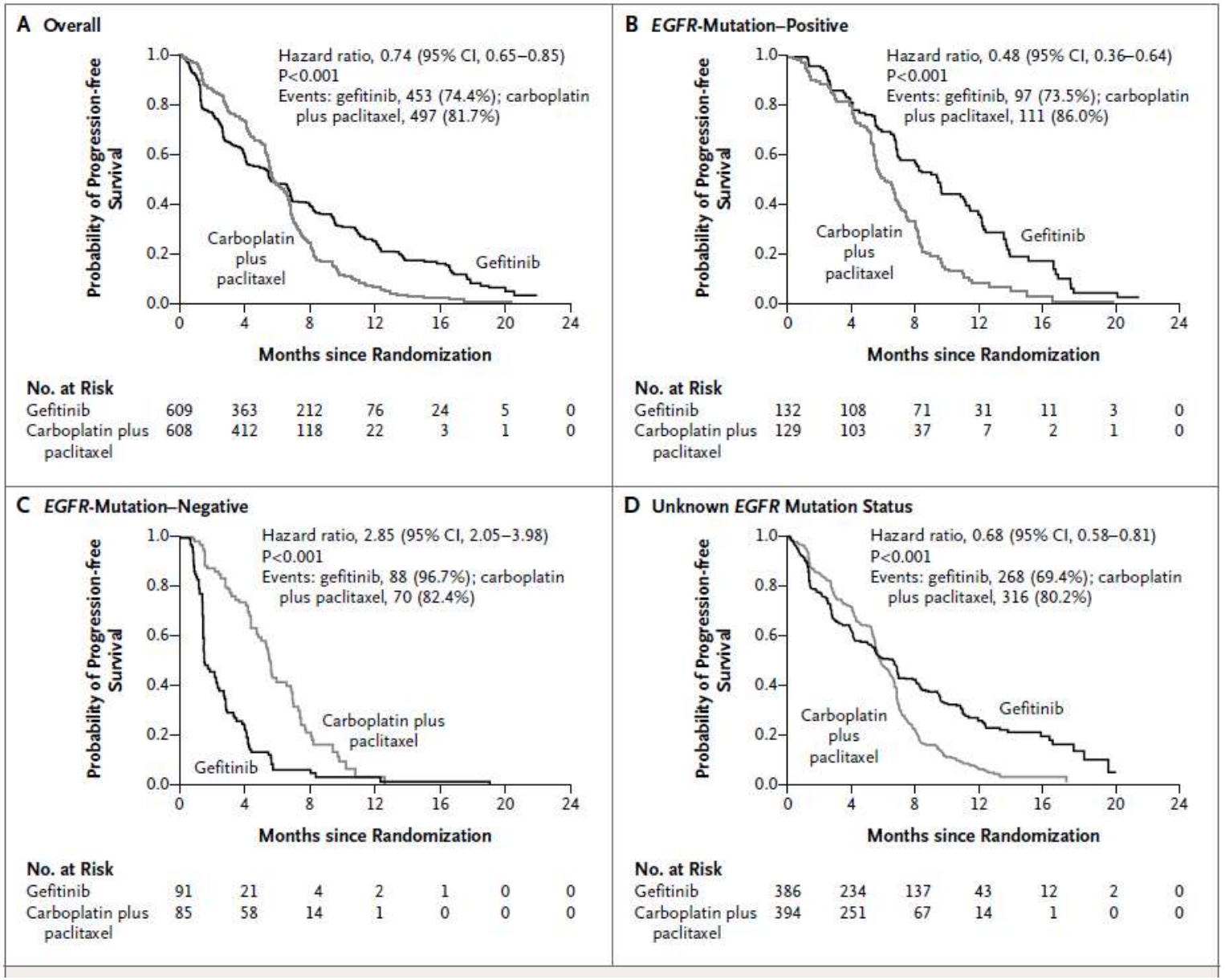
EC_{50}=half-maximal effective concentration; IC_{50}=half maximal inhibitory concentration; nM=nanomolar; RET=rearranged during transfection.

1. Drilon AE, et al. ASCO 2018. Abstract 102. **2.** Drilon A et al. IASLC 2017. Abstract 10955. **3**. Gainor J, et al. ASCO 2019. Oral presentation

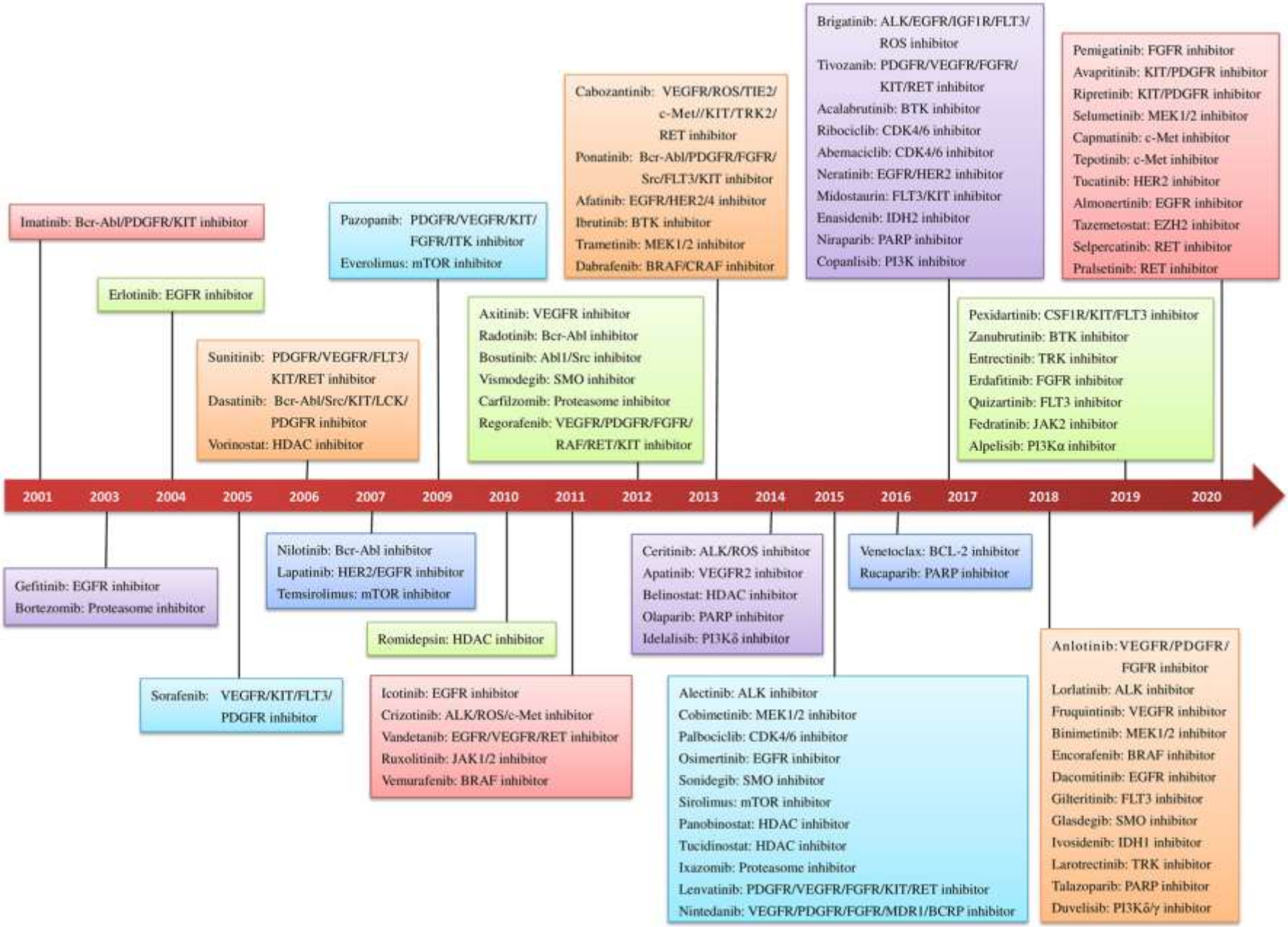


The right drug, the right dose for the right patient at the right time

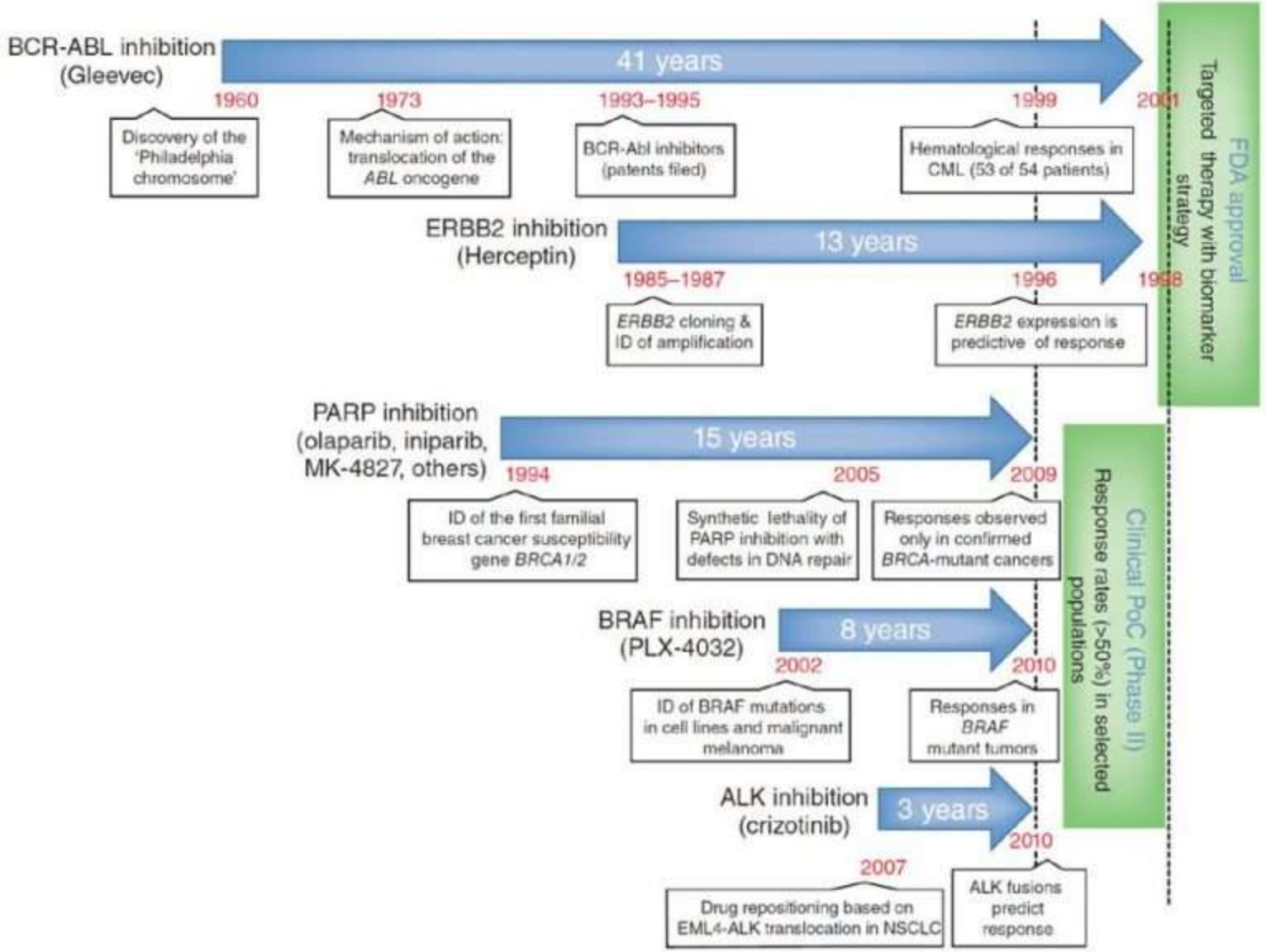
Stricker – Semin Oncol 2011



Mock, NEJM, 2009



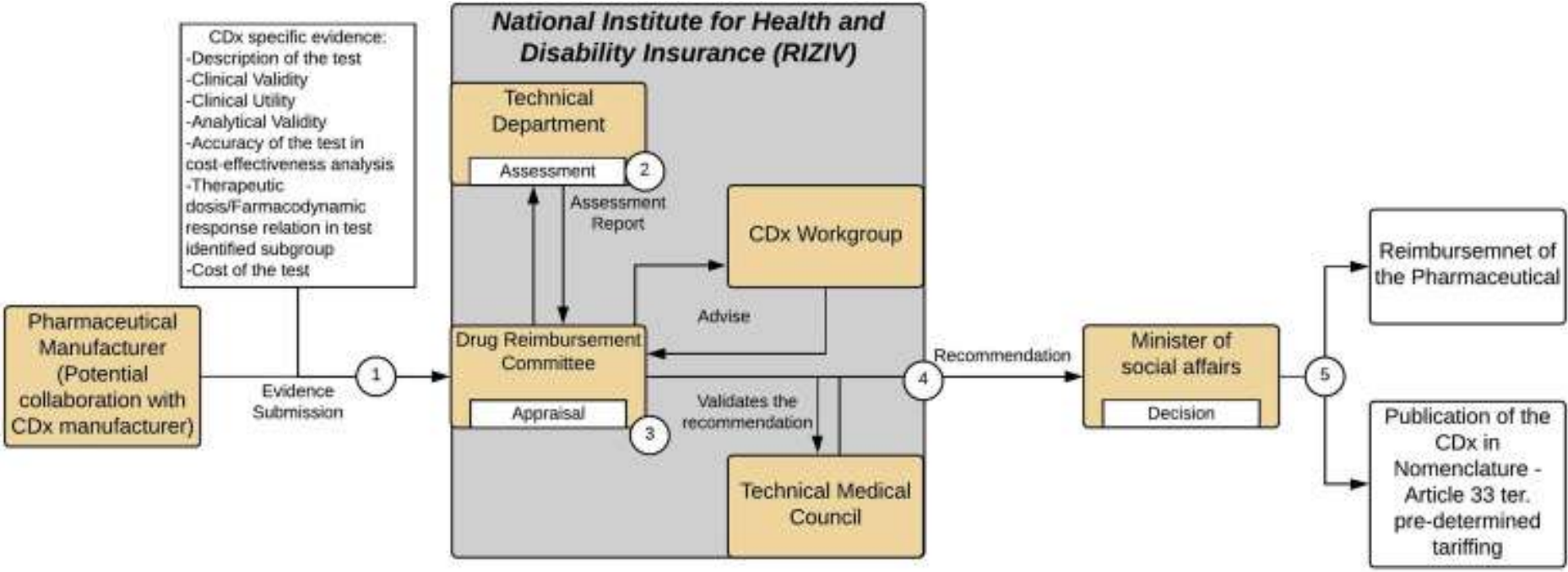
Zhong et al. Signal Transduction and Targeted Therapy- 2021



http://www.genomesop.com/genome-assembly/

- Between August 1, 2020 and July 31, 2021, the FDA has approved 10 new anticancer therapeutics that are directed against particular molecules or genetic mutations
- → Companion diagnostic

COMPANION DIAGNOSTIC REIMBURSEMENT PROCEDURE OF BELGIUM



Govaerts et al. Value Health 2020

Liste des marqueurs prédictifs qui peuvent être attestés selon l'article 33ter de l'annexe à l'arrêté royal du 14 septembre 1984 établissant la nomenclature des prestations de santé en matière d'assurance obligatoire soins de santé et indemnités

Tumeurs solides

Cancer gastrique

Pseudocode/ID	Libellé	Code art 33ter / niveau
594252 - 594263	Analyse d'une amplification du gène HER2 en cas d'adénocarcinome métastatique primaire de l'estomac ou de la jonction oeso-gastrique	594090 - 594101 (Niveau 3 diagnostique)

Cancer colorectal

Pseudocode/ID	Libellé	Code art 33ter / niveau
594274 - 594285	Analyse du statut mutationnel des gènes RAS en cas de carcinome colorectal métastatique primaire	594053 - 594064 (Niveau 2 diagnostique)

Cancer du poumon

Pseudocode/ID	Libellé	Code art 33ter / niveau
594311 - 594322	Dépistage d'une mutation EGFR activatrice en cas de cancer du poumon non squameux non à petites cellules primaire avancé (non résécable ou métastatique)	594053 - 594064 (Niveau 2 diagnostique)
594333 - 594344	Analyse du réaménagement du gène ALK en cas de carcinome du poumon non squameux non à petites cellules ALK positif (IHC) avancé (non résécable ou métastatique)	594090 - 594101 (Niveau 3 diagnostique)
594355 - 594366	Analyse du réaménagement du gène ROS1 en cas de cancer du poumon non squameux non à petites cellules ROS1 positif (IHC) avancé (non résécable ou métastatique)	594090 - 594101 (Niveau 3 diagnostique)
594370 - 594381	Dépistage d'une mutation EGFR T790M en cas de progression pendant ou après le traitement avec une TKI EGFR d'un cancer du poumon non squameux non à petites cellules avancé (non résécable ou métastatique)	594016 - 594020 (Niveau 1 diagnostique)
595070 – 595081	Analyse du statut mutationnel du gène BRAF V600 en cas de cancer du poumon non à petites cellules primaire avancé (non résécable ou métastatique)	594016 - 594020 (Niveau 1 diagnostique)
595136 - 595140	Détection d'une fusion du gène RET en cas de cancer du poumon non squameux non à petites cellules avancé (non résécable ou métastatique)	594090 - 594101 (Niveau 3 diagnostique)

Mélanome

Pseudocode/ID	Libellé	Code art 33ter / niveau
594392 - 594403	Analyse du statut mutationnel du gène BRAF V600 en cas de mélanome primaire avancé (non résécable ou métastatique)	594016 - 594020 (Niveau 1 diagnostique)
594296 – 594300	Analyse du statut mutationnel du gène BRAF V600 en cas de mélanome de stade III résécable	594016 - 594020 (Niveau 1 diagnostique)

Tumeur stromale gastro-intestinale

Pseudocode/ID	Libellé	Code art 33ter / niveau
594414 - 594425	Analyse de la mutation PDGFRA D842V en cas de tumeur stromale gastro-intestinale	594016 - 594020 (Niveau 1 diagnostique)

Cancer du sein

Pseudocode/ID	Libellé	Code art 33ter / niveau
594436 - 594440	Analyse de l'amplification du gène HER2 en cas de cancer du sein non métastatique	594090 - 594101 (Niveau 3 diagnostique)
594451 - 594462	Analyse de l'amplification du gène HER2 en cas de cancer du sein métastatique	594090 - 594101 (Niveau 3 diagnostique)

Tumeur Solide avancée quelle que soit l'histologie

Pseudocode/ID	Libellé	Code art 33ter / niveau
594952 – 594963	Détection d'une fusion du gène NTRK1 chez une tumeur TRK-positive (ICH) tumeur solide avancée.	594053 - 594064 (Niveau 2 diagnostique)
594974 - 594985	Détection d'une fusion du gène NTRK2 chez une tumeur TRK-positive (ICH) tumeur solide avancée.	594053 - 594064 (Niveau 2 diagnostique)
594996 - 595000	Détection d'une fusion du gène NTRK-3 chez une tumeur TRK-positive (ICH) tumeur solide avancée.	594053 - 594064 (Niveau 2 diagnostique)

Tumeur solide avancée avec une prévalence élevée de fusions de gènes NTRK

Pseudocode/ID	Libellé	Code art 33ter / niveau
595011 - 595022	Détection d'une fusion du gène NTRK (ou NTRK1, ou NTRK2, ou NTRK3) d'une tumeur solide avancée du type cancer du sein sécrétoire, cancer de la glande salivaire sécrétoire, fibrosarcome infantile congénital, nephrome mesoblastique congénital.	594053 - 594064 (Niveau 2 diagnostique)

Carcinome de la thyroide

Pseudocode/ID	Libellé	Code art 33ter / niveau
595151 - 595162	Détection d'une mutation RET (probablement) pathogène en cas de carcinome médullaire avancé (non résécable ou métastatique) de la thyroïde	594090 - 594101 (Niveau 3 diagnostique)

Affections hématologiques : phase diagnostique

Leucémie myéloïde aiguë

Pseudocode/ID	Libellé	Code art 33ter / niveau
594834 - 594845	Dépistage des mutations FLT3-TKD en cas de leucémie myéloïde aiguë	594016 - 594020 (Niveau 1 diagnostique)
594856 - 594860	Dépistage des mutations FLT3-ITD en cas de leucémie myéloïde aiguë	594053 - 594064 (Niveau 2 diagnostique)
595033 – 595044	Dépistage des mutations FLT3-TKD en cas de leucémie myéloïde aiguë en rechute ou réfractaire	594016 594020 Niveau 1 diagnostique
595055 – 595066	Dépistage des mutations FLT3-ITD en cas de leucémie myéloïde aiguë en rechute ou réfractaire	594053 594064 Niveau 2 diagnostique

CONVENTION NGS

INAMI Institut national d'assurance maladie-invalidité

Presse Plan du site Agenda Glossaire Liens utiles Travailler à

Rechercher

Accueil Thèmes Professionnels Nomenclature Documents Programmes web Publications Statistiques L'INAMI

⚠ Les formulaires destinés aux dispensateurs de soins se trouvent sur notre site web. Depuis ce 1er juin 2018, nous n'envoyons donc ...

Accueil ▸ Professionnels ▸ Établissements et services de soins ▸ Laboratoires ▸ Oncologie: Remboursement des tests de biologie moléculaire par « next generation sequencing » (NGS)

- Circulaires aux laboratoires
- Les laboratoires agréés et leurs prestations
- Oncologie: Remboursement des tests de biologie moléculaire par « next generation sequencing » (NGS)

Programmes associés

- Outil d'inscription en ligne PITTER

Pages associées

- Laboratoires médicaux

Oncologie: Remboursement des tests de biologie moléculaire par « next generation sequencing » (NGS)

Lors d'un traitement personnalisé contre le cancer, la technique innovante du « Next Generation Sequencing » (NGS) permet de déterminer rapidement les séquences d'un ensemble de gènes, simultanément.

Une étude pilote a pour but d'introduire cette technologie dans notre système de santé. Dans ce cadre, les réseaux NGS d'hôpitaux et de laboratoires ayant signé une convention avec nous peuvent bénéficier d'un remboursement plus élevé pour les tests de diagnostic moléculaire en oncologie et hémato-oncologie effectués par NGS.

En plus d'optimiser la qualité et l'échange d'expertise, nous souhaitons aussi optimiser les délais et le rapport coût-efficacité dans les réseaux NGS.

CONVENTION NGS

- La convention NGS a comme objectifs :
  - d'introduire de manière contrôlée le NGS dans le diagnostic en routine clinique
  - de garantir l'accès à cette nouvelle technologie dans un cadre médical où des formations sont prévues
  - d'assurer une exécution uniforme et qualitativement optimale du NGS (depuis le moment du prélèvement jusqu'au rapport final) grâce à la création d'une collaboration multidisciplinaire au sein des réseaux NGS formés d'hôpitaux et de laboratoires qui ont une expertise en oncologie médicale, en anatomopathologie, en biologie clinique et en analyse génomique
  - l'enregistrement des données NGS au niveau national
  - de créer des centres de référence qui constitueraient chacun, une plate-forme de connaissances techniques et cliniques au sein de laquelle, principalement, la biologie clinique, l'anatomopathologie et la génétique concluraient des accords mutuels et seraient intégrées comme partenaires équivalents.

https://www.inami.fgov.be/fr/professionnels/etablissements-services/laboratoires/Pages/oncologie-remboursement-biologie-moleculaire-ngs.aspx

Annexe 2 'Indications ComPerMed pour NGS chez des cancers tumeurs solides

Test NGS « standard of care » sur ADN ou ARN pour l'analyse de tumeurs malignes

Ce test NGS doit permettre:

- l'indication ou la contre-indication d'une thérapie ciblée remboursée en Belgique
- et/ou de poser un diagnostic lié à l'application de guidelines
- et/ou de déterminer un pronostic pour autant que celui-ci interfère avec la prise en charge du patient

Le test NGS doit être réalisé sur matériel tumoral et doit inclure, pour chaque indication, au minimum, les gènes et les régions suivantes :

- **<u>Carcinome du poumon</u> remplissant un des critères suivants:**
  - Carcinome non squameux (présence d'un composant ADC ou carcinome peu différencié pour lequel un ADC ne peut être exclu)
  - Carcinome squameux chez un patient n'ayant jamais/peu fumé
  - Progression sous thérapie ciblée*

Hôpital Erasme ULB

* En cas de progression avant 1 an, un test NGS est permis après un avis favorable d'une COM (consultation oncologique multidisciplinaire). Une vérification à postériori du dossier est possible.

BRAF (exon 15 (codon 600))	***thérapie***
EGFR (exon 18, exon 19, exon 20, exon 21),	***thérapie***
KRAS (exon 2 (codons 12,13), exon 3 (codons 59, 61), exon 4 (codons 117, 146))	***pronostic***
MET exon 14 skipping	***thérapie***
HER2 (exon 20)	***thérapie***

- **<u>Carcinome du poumon pour lequel aucune mutation driver n'est trouvée (avec NGS ou autre technique moléculaire)</u>**

Recherche des fusions pour lesquelles les gènes suivants sont impliqués:

ALK	***thérapie***
MET exon 14 skipping.	***thérapie***
NTRK1, NTRK2, NTRK3	***thérapie***
RET	***thérapie***
ROS1	***thérapie***

Si un panel RNA-seq est réalisé, contrairement à ce qui est indiqué au point c du chapitre VIII des médicaments à propos du cancer du poumon - ALK et ROS1, aucune IHC ne peut être facturée pour les gènes répertoriés, à l'exception de ALK.

Dans le cas où un panel RNA-seq est réalisé, ROS1 et ALK sont facturés sous le code 594090-594101 de l'article 33ter selon le tableau de financement de l'annexe 4. Les marqueurs(NTRK1, NTRK2, NTRK3, RET) ne peuvent pas être facturés avec d'autres codes de l'article 33bis ou 33ter.

GUIDELINES BELGES

FR NL EN

ComPerMed

NGS Guidelines | Algorithmes | Projets | Organisation | Contact

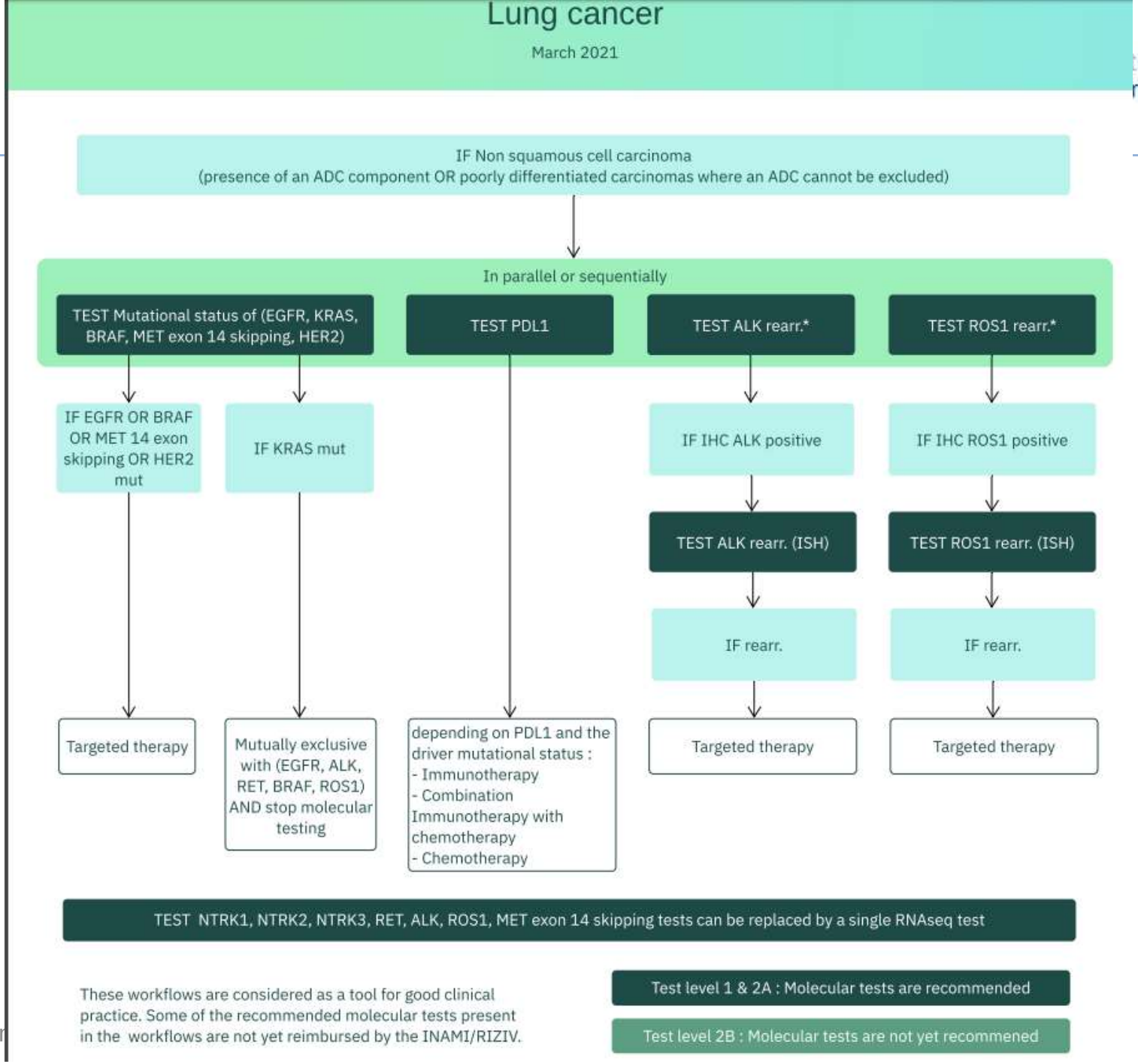
La Commission de Médecine Personnalisée (ComPerMed) est un comité qui **rassemble l'expertise scientifique belge dans le domaine.** Le séquençage de l'ADN par le « Next -Generation Sequencing » (NGS) permet de **personnaliser le traitement** et d'**optimiser la prise en charge** des patients cancéreux. À l'heure actuelle, les applications de la médecine personnalisée s'observent en oncologie et en hémato-oncologie principalement.

La création de ComPerMed fin 2015 par le Centre du Cancer est **une des actions concrètes du roadbook** (Roadbook "Médecine personnalisée", introduction du Next-Generation Sequencing dans le diagnostic de routine en oncologie et hémato-oncologie) publié en octobre 2015 pour faire face à l'évolution rapide et la complexité technique des analyses par NGS.

Les **principaux objectifs** du ComPerMed sont :

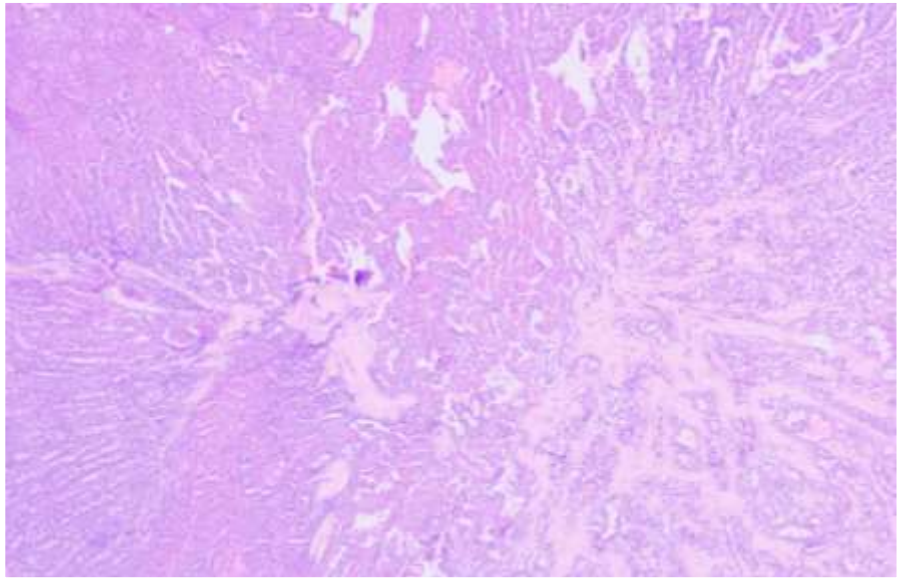
- d'élaborer des guidelines techniques permettant d'assurer la qualité des tests moléculaires utilisés en oncologie et hémato-oncologie et plus particulièrement des tests NGS.
- de définir quels biomarqueurs moléculaires (ADN), au minimum doivent être analysés par NGS pour chaque type de tumeur (solide et hémato). Le choix de ces gènes est basé sur les évidences scientifiques de(s) utilité(s) clinique(s) spécifique(s) pour le type de tumeur en question.
- d'élaborer, pour chaque type de tumeurs, de bonnes pratiques cliniques sont établies sous forme d'un algorithme schématisant les différents tests moléculaires à réaliser en routine clinique.
- d'évaluer les nouvelles technologies « omics » dans le cadre d'une utilisation en clinique en (hémato)-oncologie.

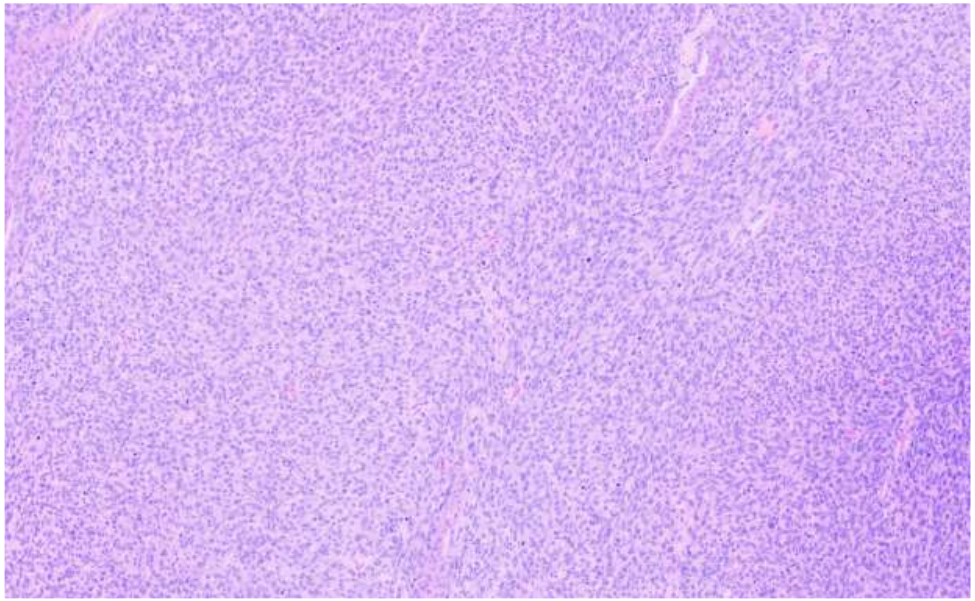
https://www.compermed.be/

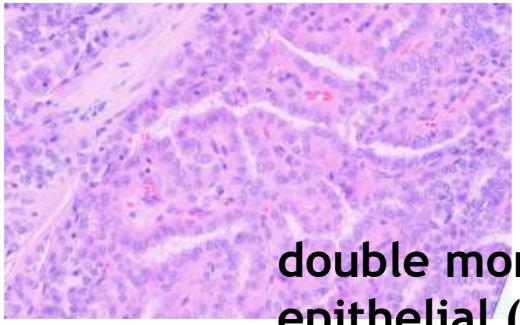

Lung cancer
March 2021
IF Non squamous cell carcinoma
(presence of an ADC component OR poorly differentiated carcinomas where an ADC cannot be excluded)
In parallel or sequentially
TEST Mutational status of (EGFR, KRAS, BRAF, MET exon 14 skipping, HER2)
TEST PDL1
TEST ALK rearr.*
TEST ROS1 rearr.*
IF EGFR OR BRAF OR MET 14 exon skipping OR HER2 mut
IF KRAS mut
IF IHC ALK positive
IF IHC ROS1 positive
TEST ALK rearr. (ISH)
TEST ROS1 rearr. (ISH)
IF rearr.
IF rearr.
Targeted therapy
Mutually exclusive with (EGFR, ALK, RET, BRAF, ROS1) AND stop molecular testing
depending on PDL1 and the driver mutational status :
- Immunotherapy
- Combination Immunotherapy with chemotherapy
- Chemotherapy
Targeted therapy
Targeted therapy
TEST NTRK1, NTRK2, NTRK3, RET, ALK, ROS1, MET exon 14 skipping tests can be replaced by a single RNAseq test
These workflows are considered as a tool for good clinical practice. Some of the recommended molecular tests present in the workflows are not yet reimbursed by the INAMI/RIZIV.
Test level 1 & 2A : Molecular tests are recommended
Test level 2B : Molecular tests are not yet recommened

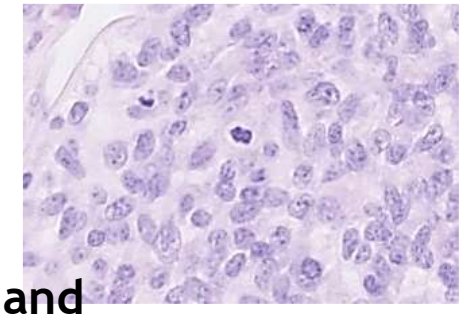
CASE REPORT

- **A 80 y.o. female with a past medical history of a right hemithyroidectomy presented with shortness of breath. Multiple hypermetabolic pleural nodules associating pleuresia were found.**



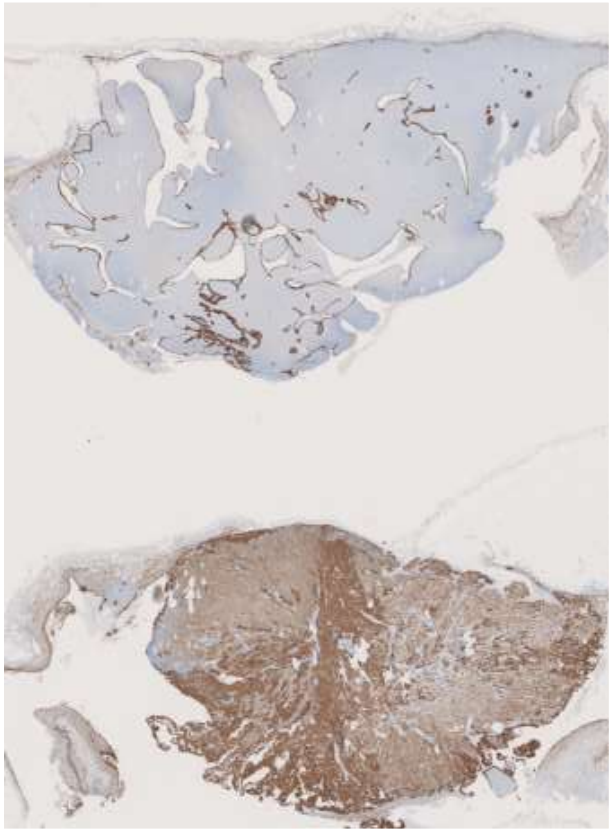


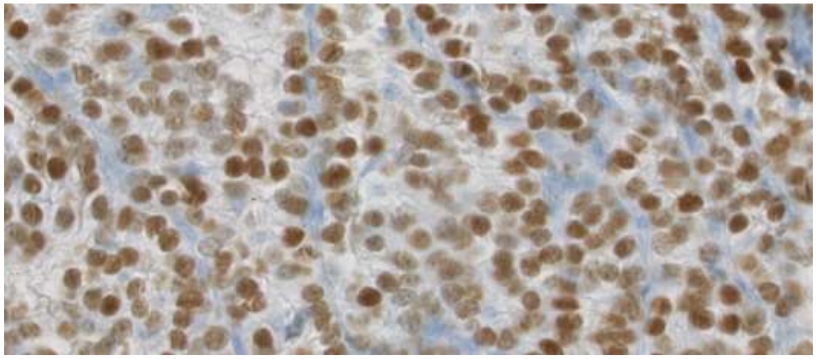


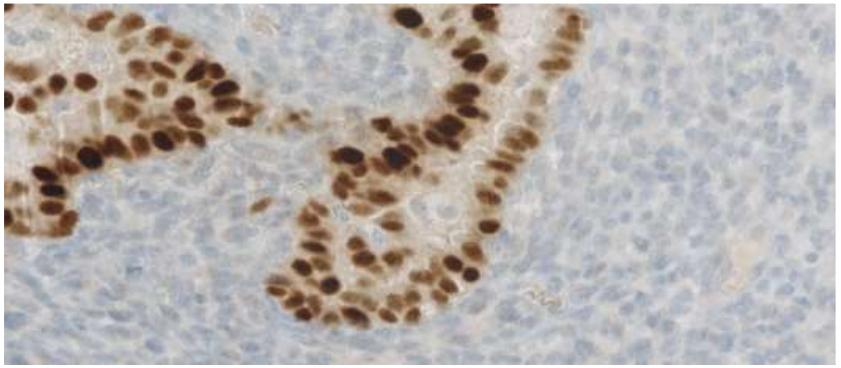


double morphological component: epithelial (papillary architecture), and sarcomatoid (spindle-cell architecture)

AE1/AE3, TTF-1, PAX8







pleural metastasis of a <u>thyroid carcinosarcoma</u>

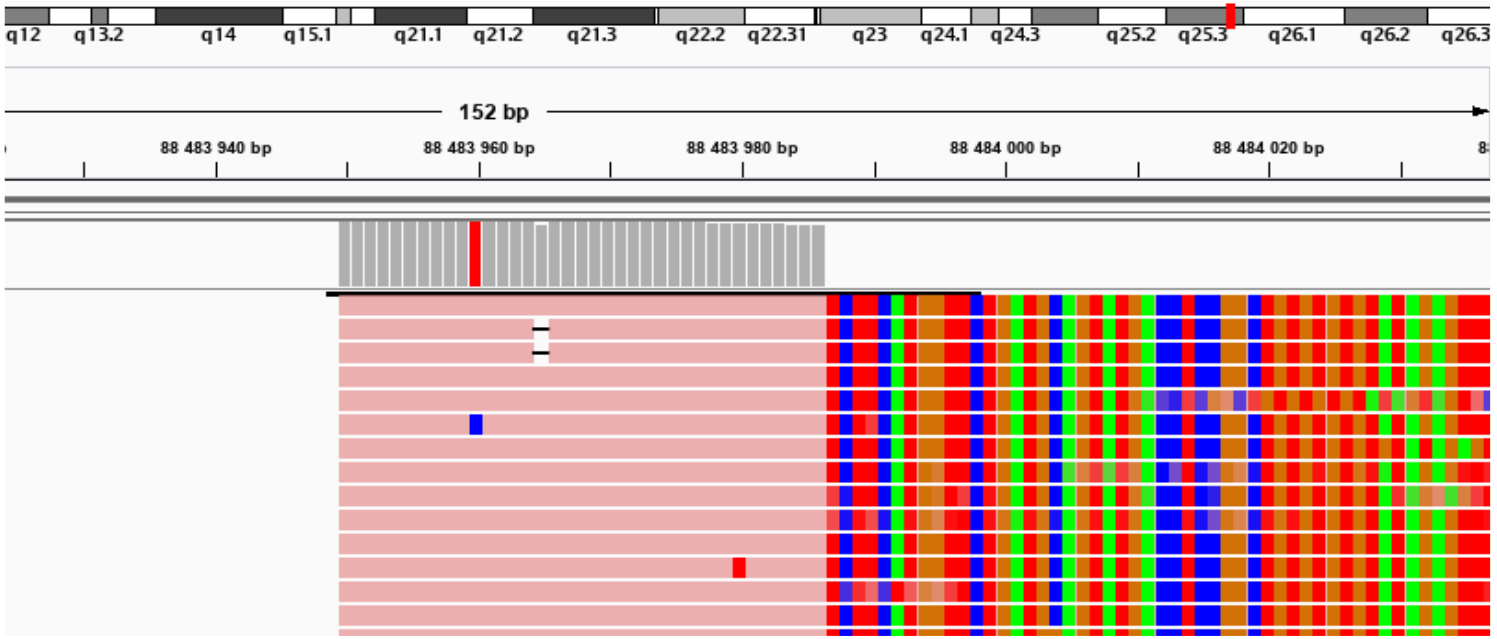
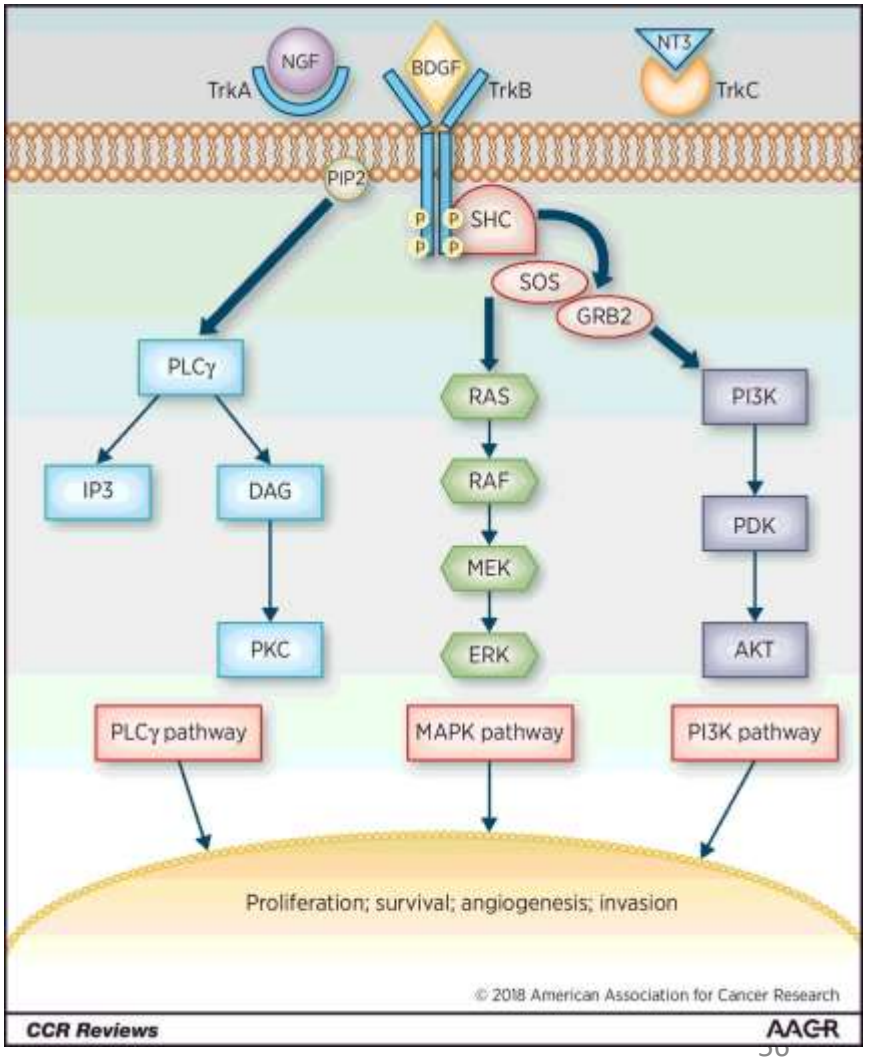


Fig.3: ETV6-NTRK3 RNA fusion transcript, aligned on chr.15

NTRK gene fusions

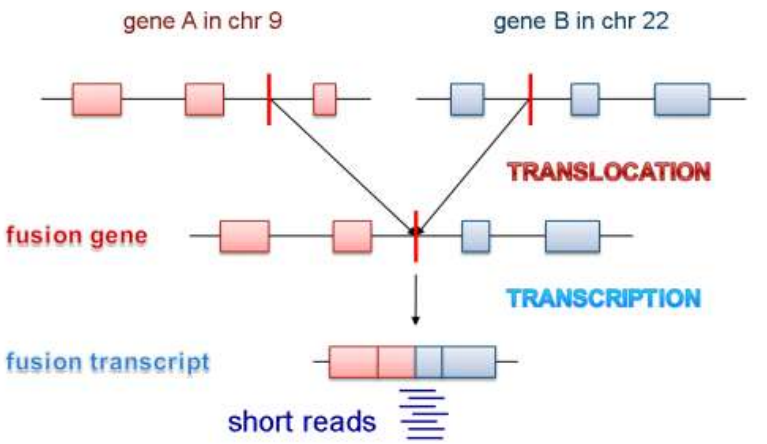
NTRK : NEUROTROPHIC TROPOMYOSIN-RELATED KINASE

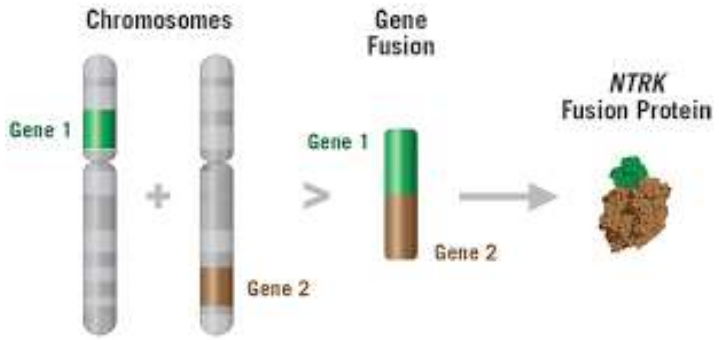
- 3 genes → 3 proteins
  - NTRK 1,2,3, → TrKA, B, C
- Tyrosine kinase receptor
- Neural development
- After embryogenesis, expression limited to nervous system, testes, smooth muscle

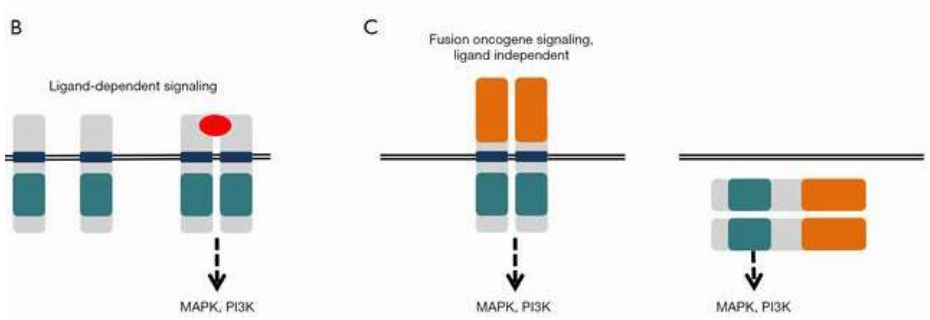


NTRK GENE FUSION

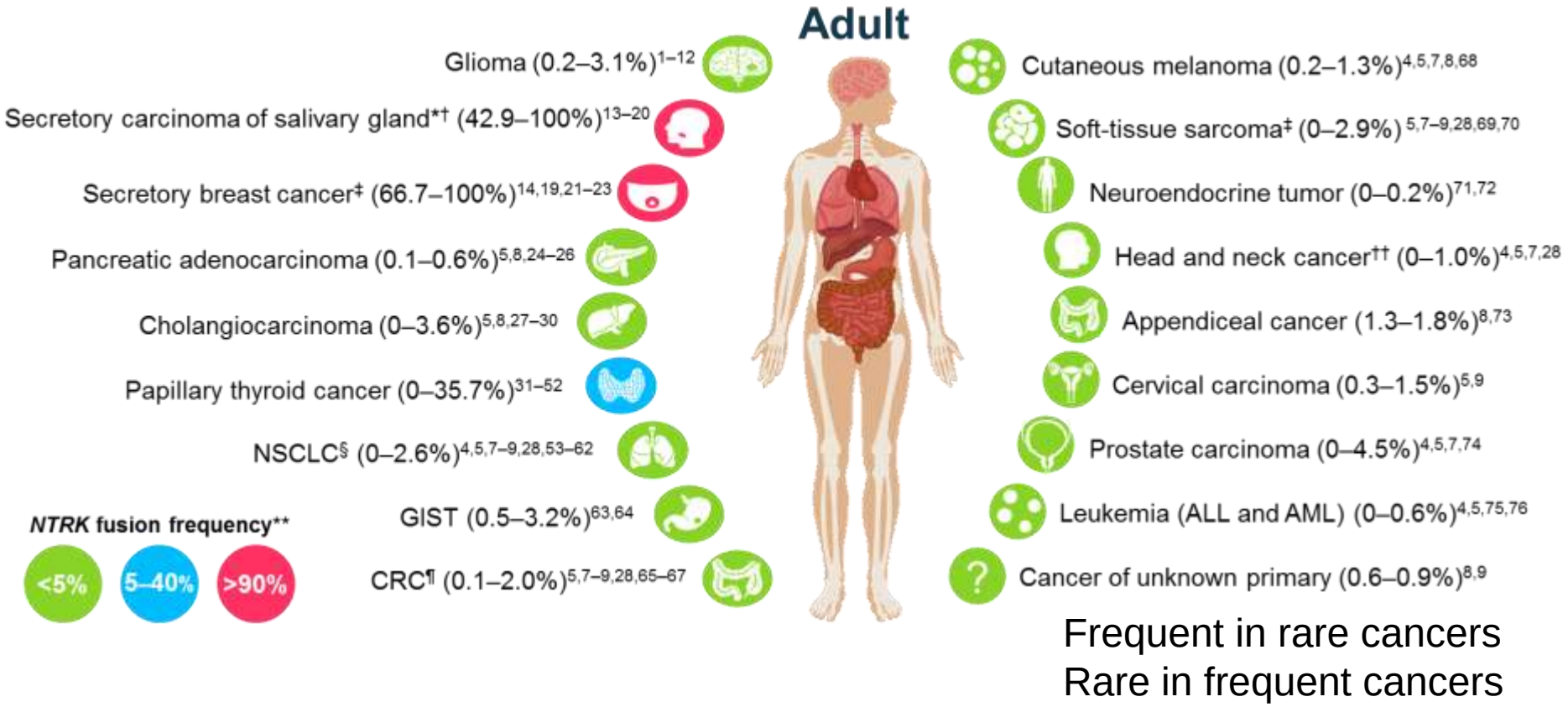
- 3’ of NTRK – 5’ fusion partner
- → chimeric gene and protein
- Overexpression and constitutive activation (ligand independant)
- Oncogenic properties







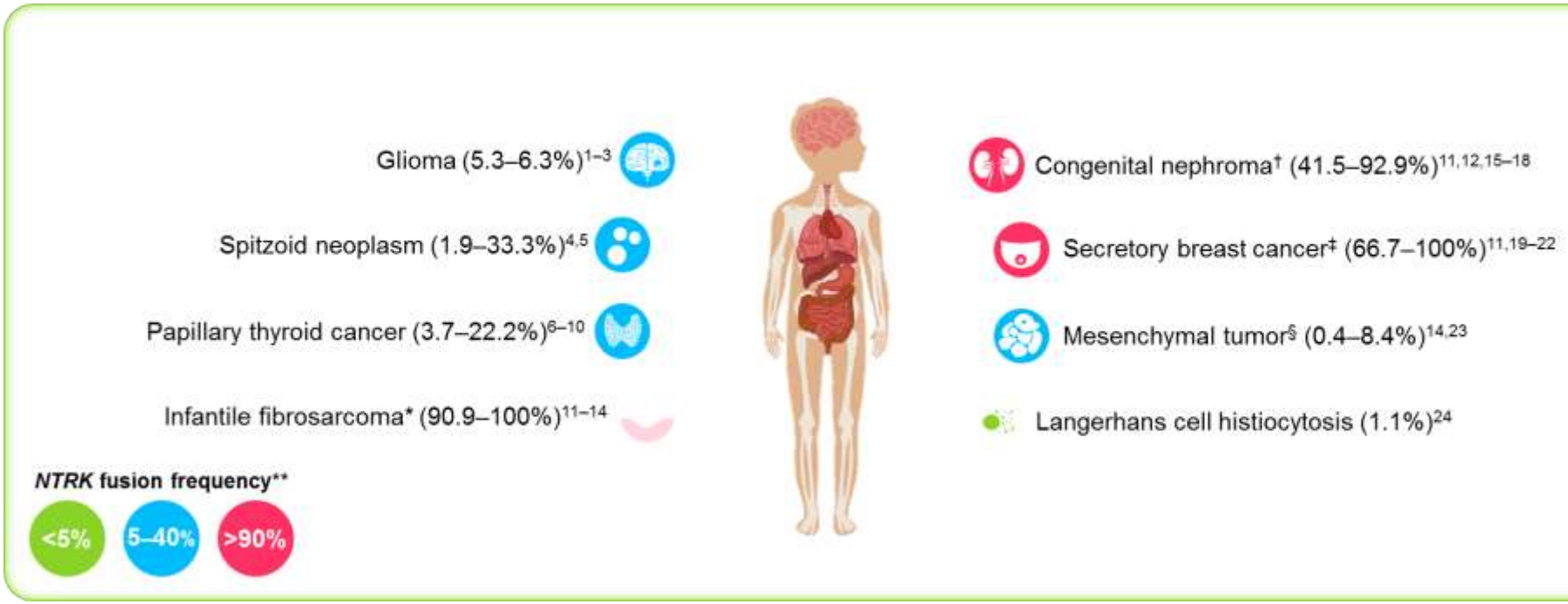
NTRK GENE FUSIONS OCCUR IN A RANGE OF ADULT TUMOR TYPES



Frequent in rare cancers
Rare in frequent cancers

1. Ferguson SD, et al. J Neuropathol Exp Neurol. 2018;77:437–442// 2. Jones DT, et al. Nat Genet. 2013;45:927–932 //3. Qaddoumi, et al. Acta Neuropathol. 2016;131:833–845//4. Yoshihara K, et al. Oncogene. 2015;34:4845–4854 //5. Okamura R, et al. JCO Precis Oncol. 2018;2:1–20 //6. Subramaniam DS, et al. J Clin Oncol. 2017;35:2019//7. Stransky N, et al. Nat Comms. 2014;5:4846 //8. Zehir A, et al. Nat Med. 2017;23:703–713 //9. Gatalica Z, et al. Mod Pathol. 2019;32:147–153 //10. Zheng Z et al. Nat Med. 2014;20:1479–1484//11. Kim J et al. PLoS One. 2014;9:e91940//12. Frattini V et al. Nat Genet. 2013;45:1141–1149//13. Malpartida JC, et al. J Clin Oncol. 2017;35:e23141 //14. Serrano-Arevalo ML, et al. Med Oral Patrol Cir Bucal. 2015;20:e23–e29 //15. Krings G, et al. Mod Pathol. 2017;30:1086–1099 //16. Skálová A, et al. Am J Surg Pathol. 2010;34:599–608 //17. Ito Y, et al. Am J Surg Pathol. 2015;39:602–610 //18. Bishop JA, et al. Hum Pathol. 2013;44:1982–1988 //19. Skálová A, et al. Am J Surg Pathol. 2016;40:3–13 //20. Church AJ, et al. Mod Pathol. 2018;31:463–473//21. Guilmette J, et al. Hum Pathol. 2019;83:50–58//22. Tognon C, et al. Cancer Cell. 2002;2:367–376 //23. Diallo R, et al. Verh Dtsch Ges Pathol. 2003;87:193–203 //24. Osako T, et al. Histopathology. 2013;63:509–519 //25. Lowery MA, et al. Clin Can Res. 2017;23:6094–6100 //26. Pishvaian MJ, et al. Clin Can Res. 2018;24:5018–5027 //27. Singhi AD, et al. J Clin Oncol. 2018;36:2982//28. Ross JS, et al. Oncologist. 2014;19:235–242 //29. Ling Q, et al. Ann Oncol. 2018;29:mdy269.073 //30. Lowery MA, et al. Clin Can Res. 2018;24:4154–4161 //31. Westphalen CB, et al. Clin Transl Oncol. 2019. [Epub ahead of print] doi: 10.1007/s12094-018-02030-6//32. Bounacer A, et al. Br J Can. 2000;82:308–314//33. Pfeifer A, et al. Genes Chromosomes Cancer. 2019; doi: 10.1002/gcc.22737. [Epub ahead of print] //34. Brzezianska E, et al. Mutat Res. 2006;599:26–35 //35. Wajwalku W, et al. Jpn J Can Res. 1992;83:671–675 //36. Bongarzone I, et al. Clin Can Res. 1998;4:223–228 //37. Bongarzone I, et al. J Clin Endocrinol Metab. 1996;81:2006–2009 //38. Sassolas G, et al. Thyroid. 2012;22:17–26//39. Mushiolt TJ, et al. Surgery. 2000;128:984–993//40. Leeman-Neill RJ, et al. Cancer. 2014;120:799–807//41. Bastos AU, et al. Eur J Endocrinol. 2018;178:83–91 //42. Liu RT, et al. Clin Endocrinol. 2006;63:461–466 //43. Kitamura Y, et al. J Hum Genet. 1999;44:96//44. Lee MY, et al. Cancer Res Treat. 2017;49:906–914//45. Lee SE, et al. Thyroid. 2017;27(6):802–810 //46. Lu Z, et al. Oncotarget. 2017;8(28):45784–45792//47. Park K, et al. USCAP Meeting. 2018;98:235//48. Pesenti C, et al. Endocrine. 2018;61:36–41//49. Sánchez Güemi I SM, et al. ECC. 2018;29:73 //50. Cancer Genome Atlas Research Network. Cell. 2014;159:676–690 //51. Smallridge RC, et al. J Clin Endocrinol Metab. 2014;99:E338–E347 //52. Costa V, et al. Oncotarget. 2015;6:11242–11251 //53. Beimfohr C, et al. Int J Cancer. 1999;80:842–847//54. Benayed R, et al. J Clin Oncol. 2018;36:12076 //55. Nakamura A, et al. Ann Oncol. 2017;28:mdx671.004//56. Wang W, et al. J Thorac Oncol. 2018;13:S719 //57. Farago AF, et al. JCO Precis Oncol. 2018;2018//58. Varella-Garcia M, et al. J Thorac Oncol. 2015;10:S248//59. Chen MW, et al. Ann Oncol. 2017;28:vii16//60. Blakely CM, et al. J Clin Oncol. 2016;34:e23079 //61. Ou S, et al. IASLC. 2017;12:S1848 //62. Schrock AB, et al. J Thorac Oncol. 2018;13:1312–1323 //63. Suh JH, et al. Oncologist. 2016;21:684–691 //64. Brenca M, et al. J Pathol. 2016;238:543–549//65. Shi E, et al. J Transl Med. 2016;14:339//66. Park DY, et al. Oncotarget. 2018;9:37262–37270 //67. Créancier L, et al. Cancer Letters. 2015;365:107–111 //68. Hechtman JF, et al. Mol Cancer Res. 2016;14:295–301//69. Lezcano C, et al. Am J Surg Pathol. 2018;42:1052–1058//70. Boddu S, et al. JCO Precis Oncol. 2018;1–8 //71. Ravi V, et al. Cancer Res. 2017;77:P2-12-01//72. Vasseur R, et al. Clin Breast Cancer. 2018 [Epub ahead of print] doi: 10.1016/j.clbc.2018.09.001 //73. Sigal DS, et al. Oncotarget. 2018;9:35809–35812//74. Braghiroli, et al. J Clin Oncol. 2016;34:4102//75. Ikeda S, et al. Cancer Biol Ther. 2018:1–8//76. Roberts KG, et al. New Engl J Med. 2014;371:1005–1015//77. Taylor J, et al. J Clin Invest. 2018;128:3819–3825.//78. Cocco E, et al. Nat Rev Clin Oncol. 2018;15:731–747

NTRK GENE FUSIONS OCCUR IN A RANGE OF PEDIATRIC TUMOR TYPES



†Includes fibrosarcoma, solitary fibrous tumor, hemangioma, schwannoma, histiocytoma, primitive neuroectodermal tumor, inflammatory myofibroblastic tumor, lipofibromatosis, primitive myxoid mesenchymal tumor of infancy, fibrous hamartoma of infancy, myofibroma/myofibromatosis, low-grade fibromyxoid sarcoma, synovial sarcoma, spindle-cell rhabdomyosarcoma, malignant nerve sheath tumor, fibrosarcomatous dermatofibrosarcoma protuberans, and nodular fasciitis.

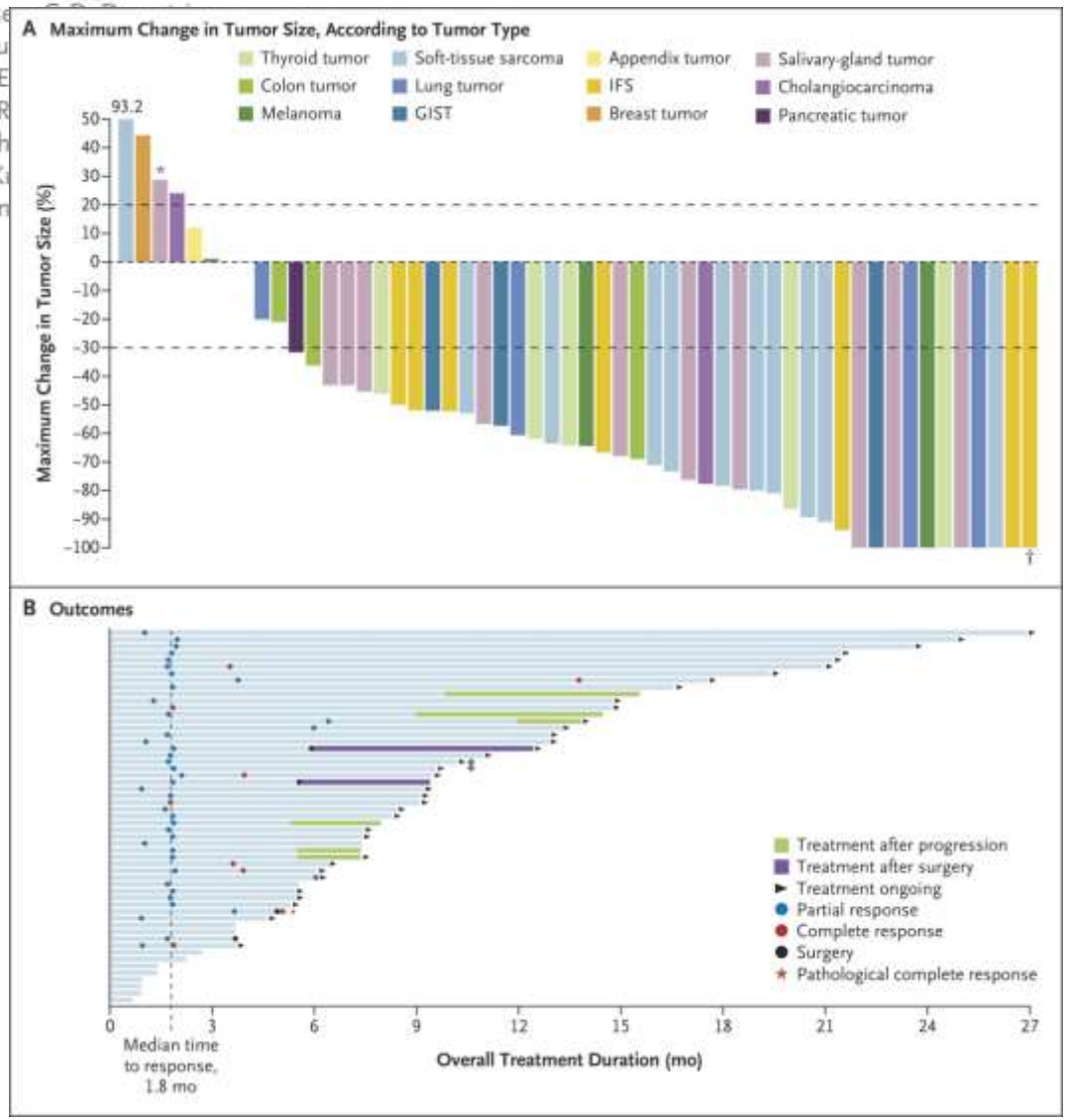
1. Okamura R, et al. JCO Precis Oncol. 2018;2:1–20// 2. Wu G, et al. Nat Genet. 2014;46:444–450//3. Tauziede A, et al. Virchows Archiv. 2018;473:PS-16-009//4. Wu G et al. Mod Pathol. 2016;29:359–369//5. Wang L, et al. J Mol Diagn. 2017;19:387–396//6. Prasad ML, et al. Cancer. 2016;122:1097–1107//7. Cordioli MI, et al. Thyroid. 2017;27:182–188 //8. Ricarte-Filho JC, et al. J Clin Invest. 2013;123:4935–4944 //9. Potter SL, et al. Cancer Research. 2018;78:A36// 10. Picarsic JL, et al. Pediatr Dev Pathol. 2016;19:115–122 //11. Malpartida JC, et al. J Clin Oncol. 2017;35:e23141//12. Church AJ, et al. Mod Pathol. 2018;31:463–473//13. Rubin BP, et al. Am J Pathol. 1998;153:1451–1458 //14. Bourgeois JM, et al. Am J Surg Pathol. 2000;24:937–946//15. Hung YP, et al. Histopathology. 2018;73:634–644 //16. Argani P, et al. Mod Pathol. 2000;13:29–36//17. Knezevich SR, et al. Cancer Research. 1998;58:5046–5048//18. El Demellawy D, et al. Pathology. 2016;48:47–50 //19. Vokuhl C, et al. Pediatr Blood Cancer. 2018;65:e26925 //20. Bayindir P, et al. Pediatr Radiol. 2009;39:1066–1074 //21. Krings G, et al. Mod Pathol. 2017;30:1086–1099//22. Tognon C, et al. Cancer Cell. 2002;2:367–376//23. Diallo R, et al. Verh Dtsch Ges Pathol. 2003;87:193–203//24. Osako T, et al. Histopathology. 2013;63:509–519//25. Pavlick D, et al. Pediatr Blood Cancer. 2017;64:e26433//26. Cai J, et al. Int J Cancer. 2019;144:117–124//27. Cocco E, et al. Nat Rev Clin Oncol. 2018;15:731–747.

ORIGINAL ARTICLE

Hôpital Erasme ULB

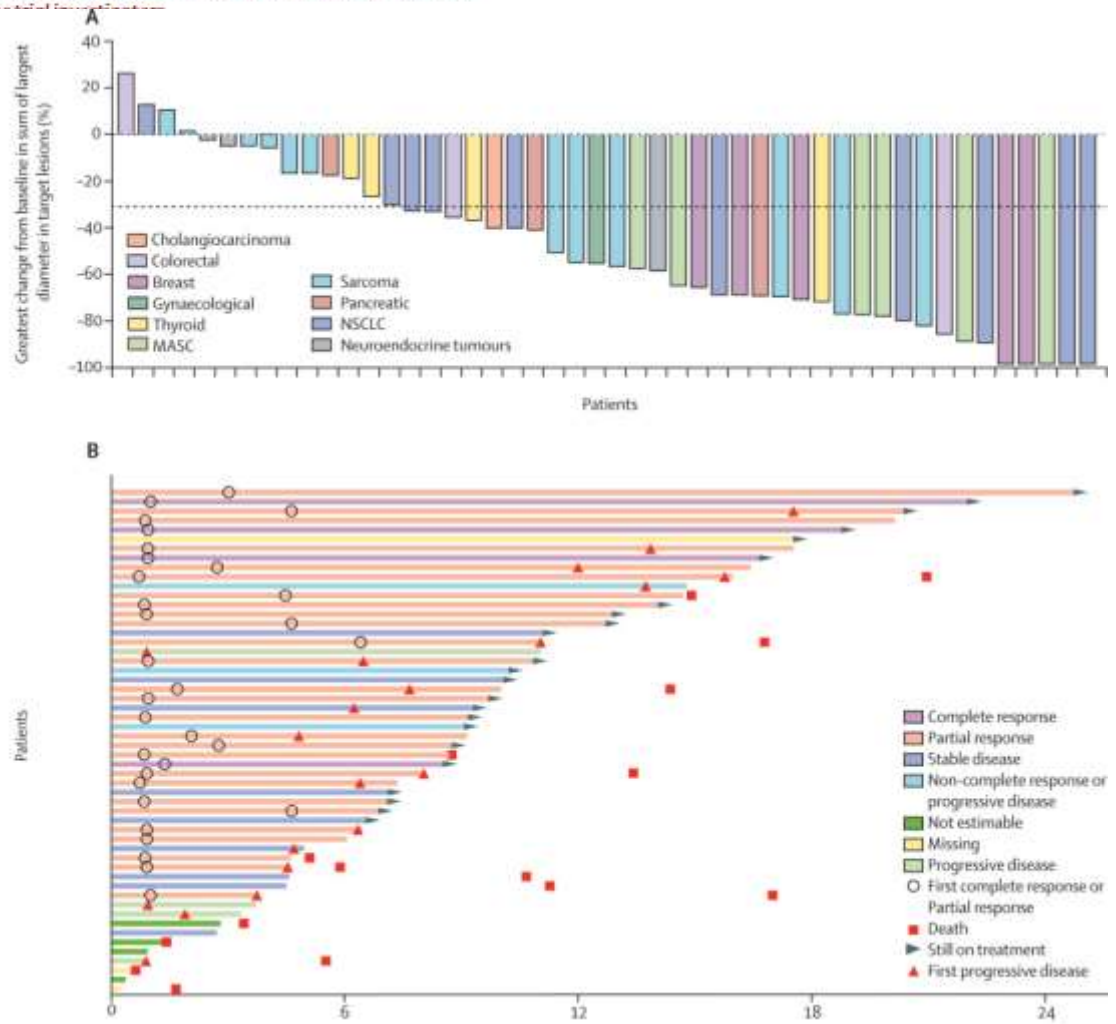
Efficacy of Larotrectinib in *TRK* Fusion–Positive Cancers in Adults and Children

A. Drilon, T.W. Laetsch, S. Kummar, S.G. DuBois, U.N. Lasse
M. Nathenson, R.C. Doebele, A.F. Farago, A.S. Pappo, B. Tu
M.S. Brose, L. Mascarenhas, N. Federman, J. Berlin, W.S. E
J. Deeken, V. Boni, R. Nagasubramanian, M. Taylor, E.R
F. Meric-Bernstam, D.P.S. Sohal, P.C. Ma, L.E. Raez, J.F. Hech
M. Ladanyi, B.B. Tuch, K. Ebata, S. Cruickshank, N.C. K
D.S. Hawkins, D.S. Hong, and D.M. Hyman

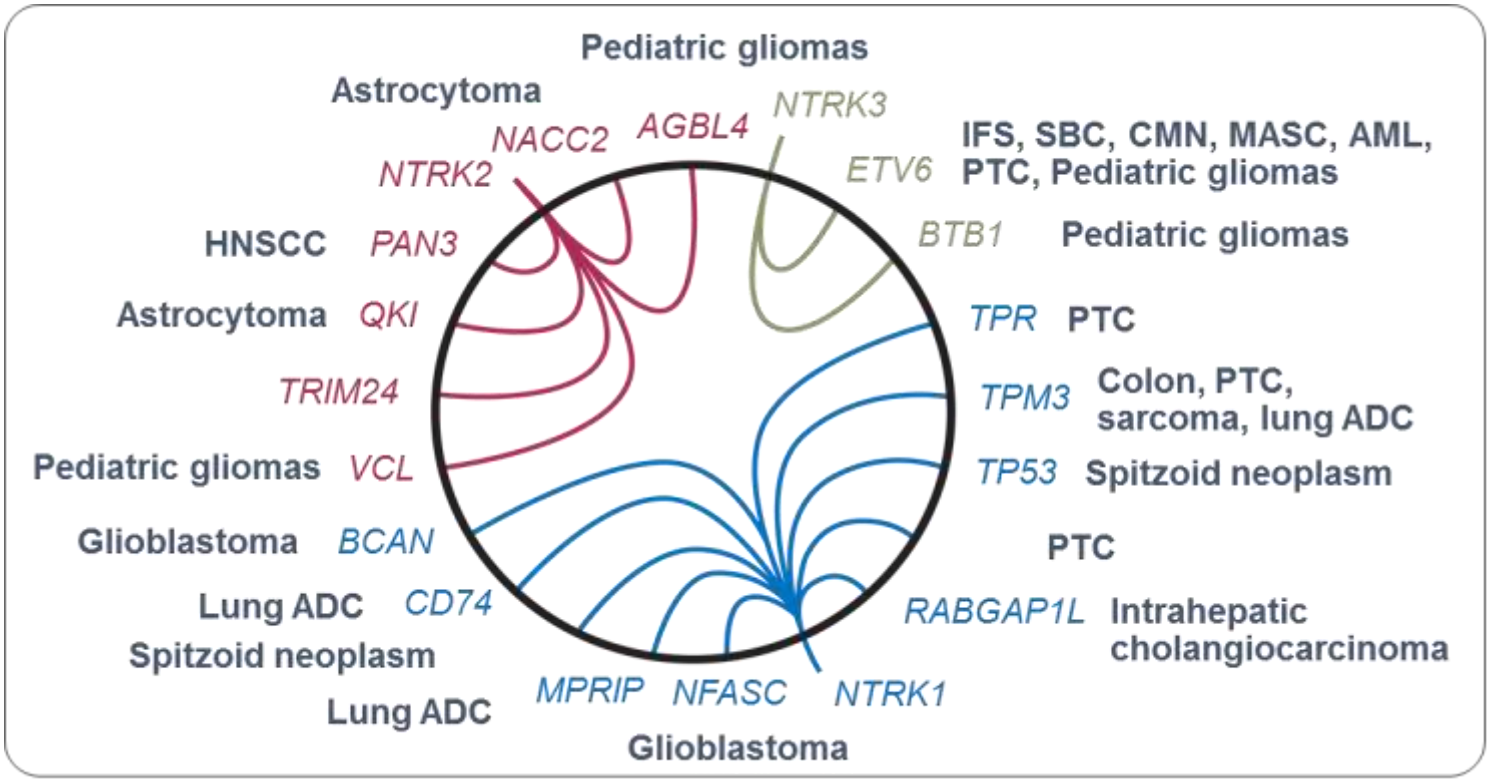


Entrectinib in patients with advanced or metastatic *NTRK* fusion-positive solid tumours: integrated analysis of three phase 1–2 trials

Robert C Doebele, Alexander Drilon*, Luis Paz-Ares, Salvatore Siena, Alice T Shaw, Anna F Farago, Collin M Blakely, Takashi Seto, Byung Chul Cho, Diego Tosi, Benjamin Besse, Sant P Chawla, Lyudmila Bazhenova, John C Krauss, Young Kwang Chae, Minal Barve, Ignacio Garrido-Laguna, Stephen V Liu, Paul Conkling, Thomas John, Marwan Fakih, Darren Sigal, Herbert H Loong, Gary L Buchschacher Jr, Pilar Garrido, Jorge Nieva, Conor Steuer, Tobias R Overbeck, Daniel W Bowles, Elizabeth Fox, Todd Riehl, Edna Chow-Maneval, Brian Simmons, Na Cui, Ann Johnson, Susan Eng, Timothy R Wilson, George D Demetri, on behalf of the trial investigators*



NTRK GENE FUSIONS OCCUR WITH MULTIPLE FUSION PARTNERS

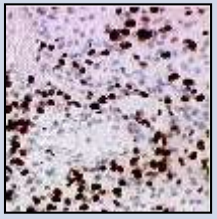
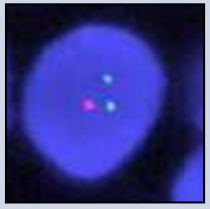


Optimal screening methods should detect all fusions, regardless of tumor type or fusion partner

ADC, adenocarcinoma; CMN, congenital mesoblastic nephroma; HNSCC, head and neck squamous cell cancer; IFS, infantile fibrosarcoma; SBC, secretory breast cancer; MASC, mammary analogue secretory carcinoma; PTC, papillary thyroid cancer.

1. Vaishnavi A, et al. *Cancer Discov.* 2015;5(1):25-34. **2.** Stransky N, et al. *Nat Commun*. 2014;5:4846.

TOOLBOX OF THE MOLECULAR PATHOLOGIST

	IHC	FISH	PCR	NGS
				
Target	Protein	Gene	Gene DNA/RNA	Gene panel DNA/RNA
Precision	+	++	+	+++
Hands on time	-	+++	+	++
Cost	+-++	++	+-++	+++-++++
TAT	48h	10 days	10 days	10 days
NTRK	Yes	Yes	Yes	Yes

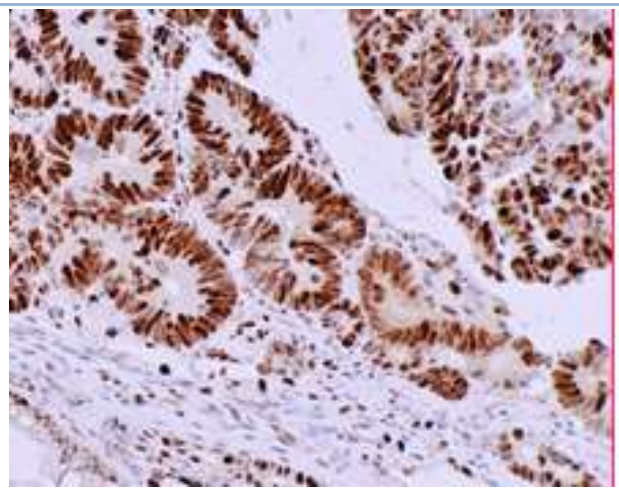
CONSIDERATIONS FOR DETECTING NTRK GENE FUSIONS

- >150 different fusions described in the literature
- Inconsistent breakpoints and fusion partners
- Large intronic regions
- NTRKs are endogenously expressed in som tissues

→

- DNA panels lack of sensitivity
- IHC may lack specificity
- FISH requires at least 3 assays
- RT-PCR only useful to detect 1 specific fusion

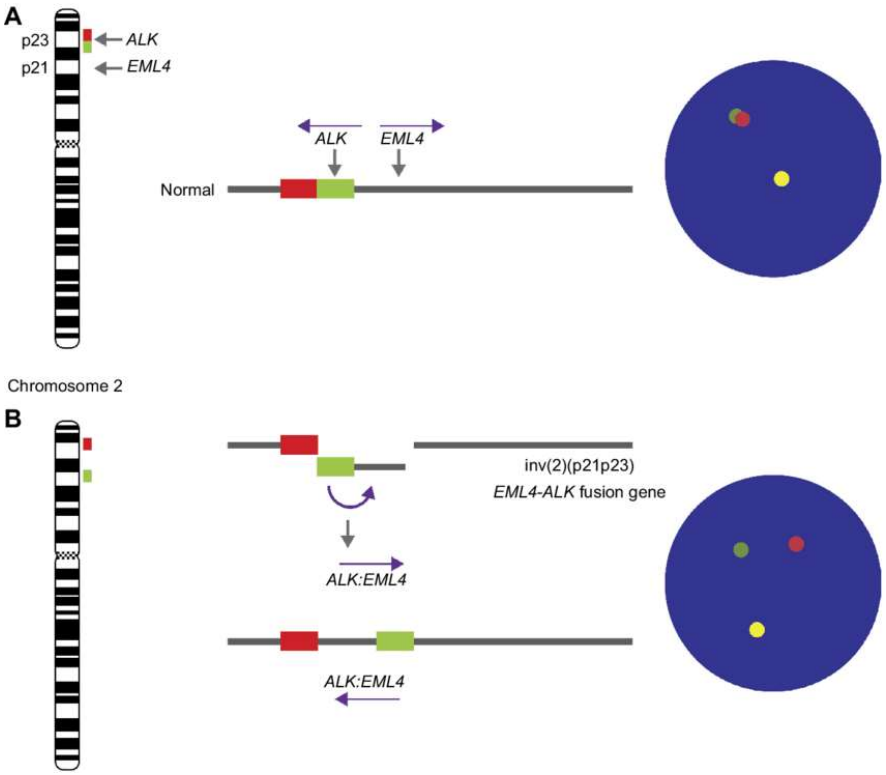
IDENTIFYING *NTRK* GENE FUSIONS: IHC



- Pan TRK : TrKA, B, C
- Internal control : nerves
- Nuclear, membrane or cytoplasm
- Advantages
  - Good screening method :
    - sensitivity 95-100%; specificity : 93-100%
  - Widely available
  - Short TAT
  - Low cost
  - Detect only transcribed and translated fusion proteins
  - Limited material
- Limitations
  - Preanalytical issues
  - Can be positive without gene fusion (muscle or neuronal differentiation)
  - Interpretation challenging in tissues such as CNS (physiological expression)
  - Non standardized interpretation
  - Some false negative (NTRK3)
- Confirmatory test is necessary

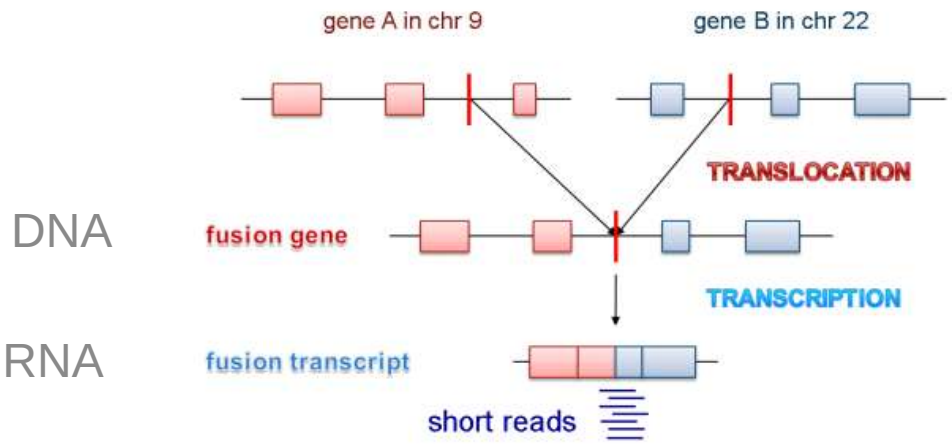
IDENTIFYING *NTRK* GENE FUSIONS: FISH

- Break-apart probes
- Advantages
  - Novel fusions can be detected
  - Sensitivity :94% specificity : 96%
- Limitations
  - 3 probes
  - Expensive
  - Time consuming
  - Interpretation can be challenging
- Useful for common fusions in cancers that have high frequency



ESMO guidelines, Kirchner et al. 2020

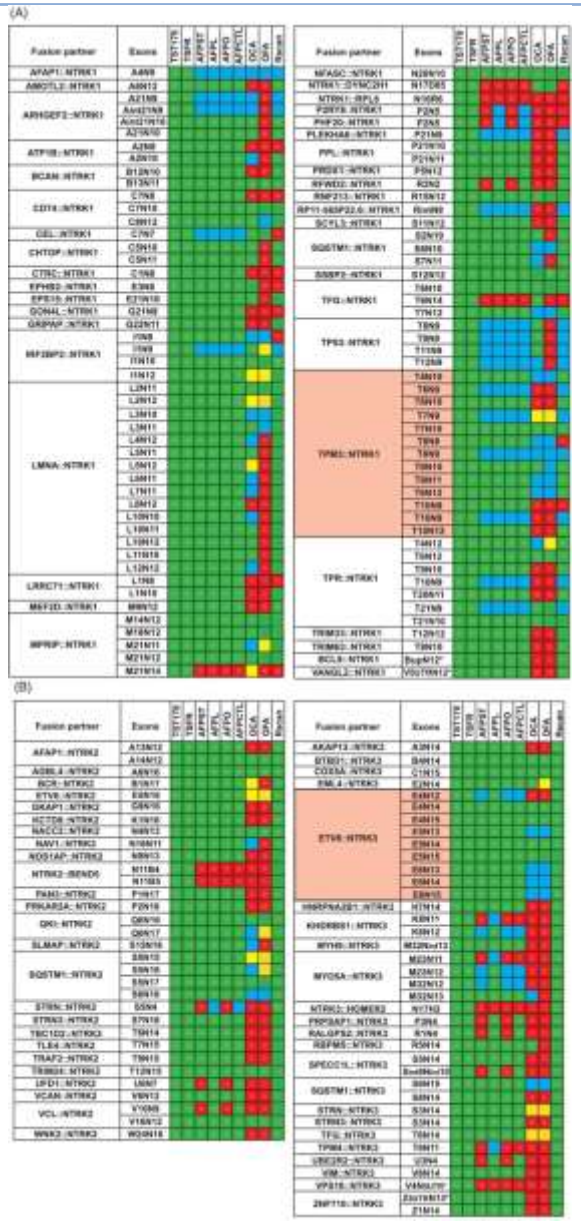
IDENTIFYING *NTRK* GENE FUSIONS: DNA NGS



- Need to sequence introns
  - Reduced sensitivity
- Detect fusions of unknown functional significance
  - Confirmation method

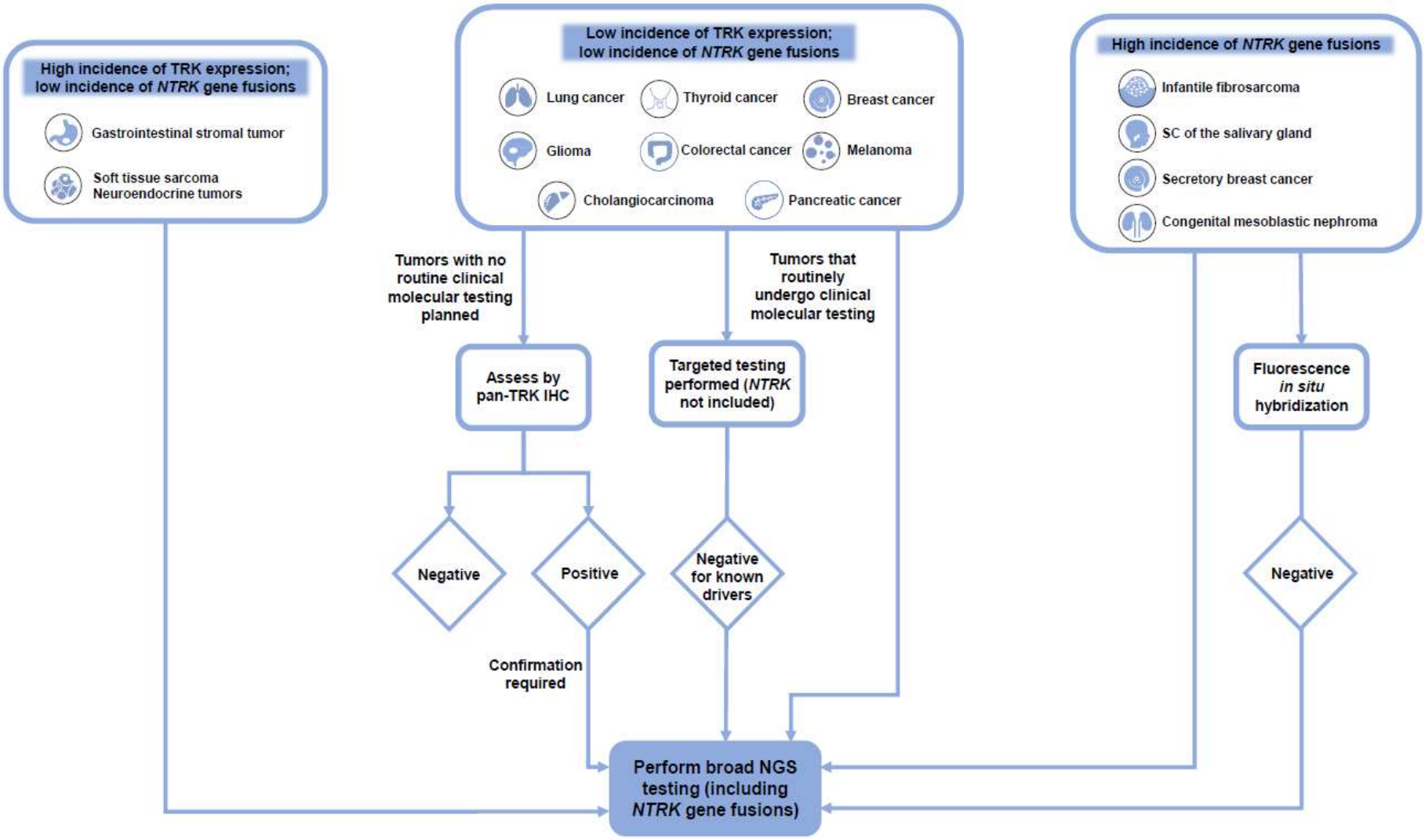
IDENTIFYING *NTRK* GENE FUSIONS: RNA NGS

- Detected transcribed fusions
  - Number of detected fusions can vary
- No need to sequence introns
  - Better sensitivity than DNA panels
- RNA input varies depending on the technologies from 20 to 200ng
- Sensitivity : 95,3% / specificity : 100%
- → preferred method to detect or confirm NTRK gene fusions.



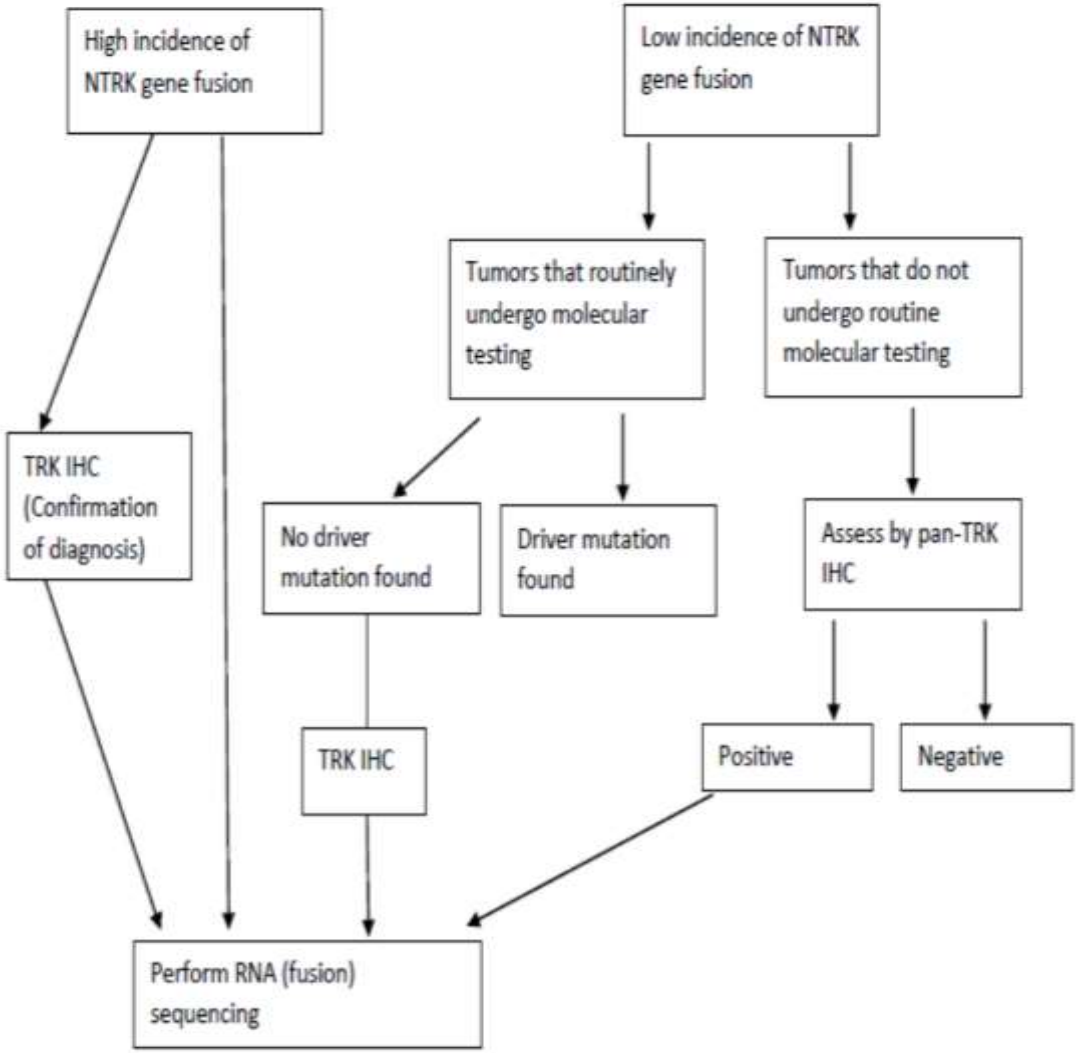
ESMO guidelines, Kirchner et al. 2020

NTRK ALGORITHME



Hsiao, Zehir, Aisner, Sireci, and Hamilton -J MolDiagnostics

NTRK ALGORITHME



Pauwels et al. BJMO 2021

A Case Report

Andrei LEUCĂ[1], Jennifer FALLAS[1], Claude VAN CAMPENHOUT[1], Marie-Paule VAN CRAYNEST[1], Jean-Christophe NOEL[1], Isabelle SALMON[1], Calliope MARIS[1], Nicky D'HAENE[1]
[1]Erasme University Hospital, Brussels, Belgium

CASE REPORT

A 76 y.o. female patient presented with abdominal pain and hematochezia. The colonoscopy revealed an ulcerated rectal tumour.

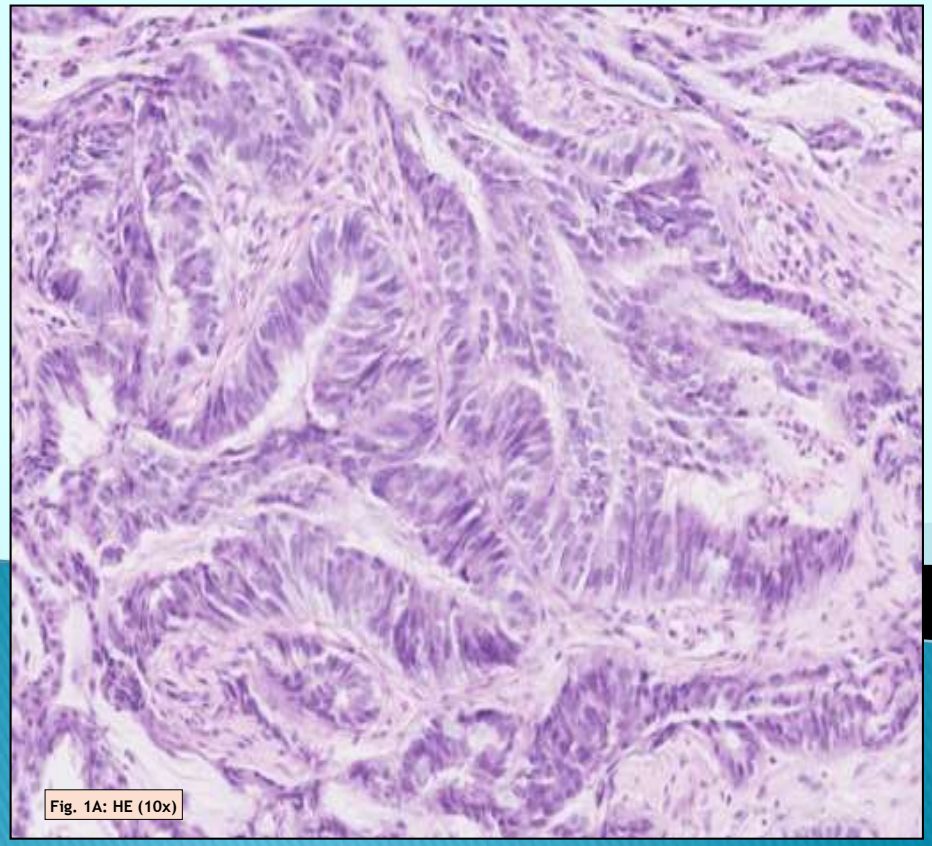


Fig. 1A: HE (10x)

CASE REPORT

A 76 y.o. female patient presented with abdominal pain and hematochezia. The colonoscopy revealed an ulcerated rectal tumour. The biopsy showed a moderately differentiated adenocarcinoma (Fig. 1A).
A peritoneal extension was described radiologically, therefore a Next Generation Sequencing (NGS) analysis (Colon & Lung Cancer panel, Thermo Fischer Scientific) was performed and showed no RAS gene mutation. However, the mutation profile (Table 1) of the rectal tumour revealed similarities with a molecular test performed on pT1 N0 R0 endometrial adenocarcinoma diagnosed 3 years before (Fig.1B).

Rectal tumour: NGS colon & lung cancer panel (22 genes, *Thermo Fischer Scientific*)	Endometrial tumour: NGS gynaecologic panel (17 genes)
p.Y375C (*FGFR2*)	
p.S252W (*FGFR2*)	
p.E542G (*PIK3CA*)	p.T576delT (*PIK3R1*)
p.N401S (*FBXW7*)	p.R130G (*PTEN*)

Additionnal *immunohistochemistry* performed on the rectal tumour showed a positivity for PAX8 antibody and lack of expression of the SATB2 protein (Fig.3).

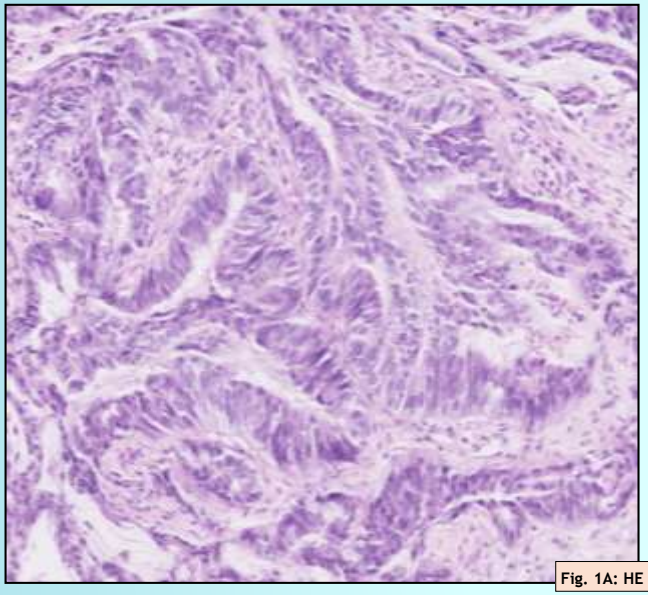


Fig. 1A: HE (10x)

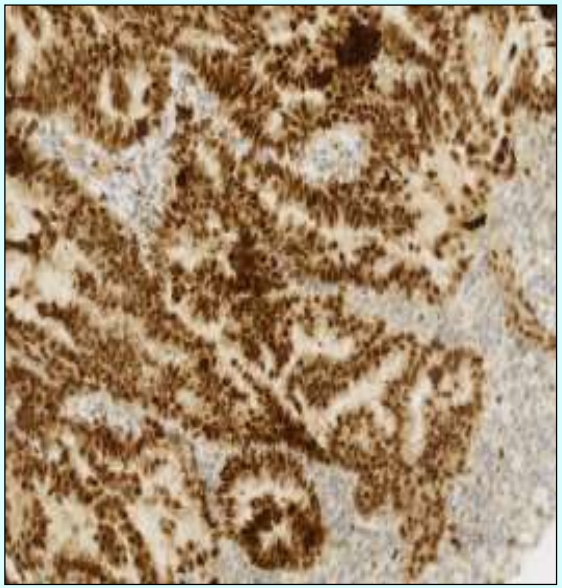


Fig. 3A: PAX8 (10x)

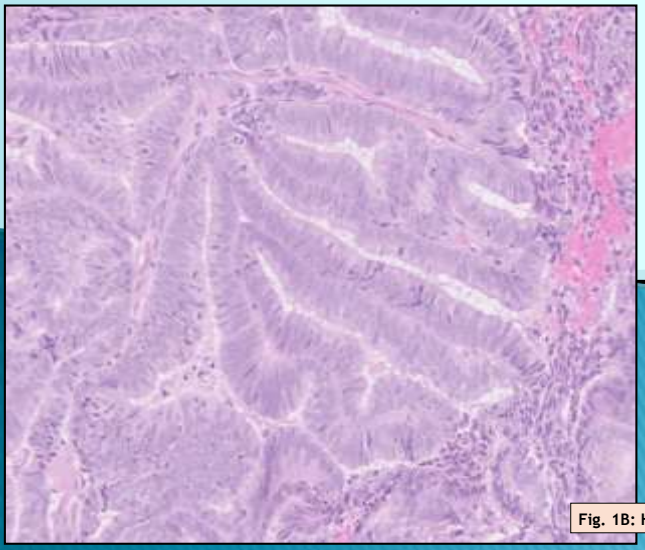


Fig. 1B: HE(10x)

Fig. 3B: SATB2 (10x)

CONCLUSION

Our final diagnosis was a rectal metastasis of the previous endometrial carcinoma

Pathologie moléculaire et cancer colorectal

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

Introduction

- ✓ Colorectal Cancer (CRC) is the **second most common cancer in Europe** and is responsible for **12% of cancer deaths**
  - ✓ In 2012, in Europe there were an estimated 447 000 new cases of CRC with 215 000 deaths

- ✓ **One out of four** patients has metastasis at initial diagnosis (Stage IV) and **one out of two** develops metastasis

- ✓ Current therapeutical guidelines for **stage IV** patients include a combination of **chemotherapy, a cytotoxic regimen and biological targeted agents** (such as anti-EGFR cetuximab and panitumumab)

Ferlay et al. Eur J Cancer. 2013

MOLECULAR BIOMARKERS IN CRC

Molecular In My Pocket™...

ONCOLOGY: *Molecular Biomarkers of Colorectal Cancer*

Samples to Test: Metastatic or recurrent tumor is preferable if available and adequate*; primary tumor is an acceptable alternative. **Sample Types to Test:** Formalin fixed paraffin embedded tissue (FFPE) or other type of specimens (*e.g.*, cytology). * *Lynch syndrome screening is recommended for all primary colorectal cancers.*

www.AMP.org

NCCN National Comprehensive Cancer Network®

NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)

Colon Cancer

Version 3.2021 — September 10, 2021

Biomarker	Specific Alterations/ Alternative Names	Indications	Result Interpretation/ Significance	Assay Techniques
KRAS	Mutations in codons 12, 13 of exon 2; codons 59, 61 of exon 3; codons 117, 146 of exon 4	Consideration of anti-EGFR therapy Should be performed in all patients with metastatic CRC	Patients with these mutations should not be treated with panitumumab and cetuximab Significant PFS advantage for adding anti-EGFR therapy for *KRAS* WT tumors compared to chemotherapy alone	NGS, pyrosequencing, Sanger sequencing, genotyping, PCR-based assays
NRAS	Mutations in codons 12, 13 of exon 2; codons 59, 61 of exon 3; codons 117, 146 of exon 4	Consideration of anti-EGFR therapy Should be performed in all patients with metastatic CRC	Patients with these mutations should not be treated with panitumumab and cetuximab	NGS, pyrosequencing, Sanger sequencing, genotyping, PCR-based assays
BRAF	*BRAF* V600; V600E, V600K	Prognostic stratification	Poorer PFS and OS compared to *BRAF* WT patients	NGS, pyrosequencing, Sanger sequencing, genotyping, PCR-based assays
		Consideration of anti-EGFR therapy	Unlikely response to panitumumab and cetuximab unless given with a BRAF inhibitor (2)	
		In MMRd tumors with MLH1 loss	Presence of mutation strongly favors sporadic tumor; the presence of BRAF mutations does not exclude the risk of Lynch Syndrome	
NTRK	Fusions	Therapy selection	Predicts response to larotrectinib (2)	NGS, pyrosequencing, FISH, IHC, PCR-base assays
MSI/ MMR	Loss of *MLH1, PMS2, MSH2, MSH6* expression and/or MSI-high status	Lynch syndrome screening	Consideration of genetic counseling and germline testing (in the absence of *BRAF* mutation or *MLH1* promoter methylation)	IHC, PCR-based assays
	MSI-high	Therapy selection (stage II patients)	Improved prognosis and no benefit from 5-FU adjuvant therapy Consideration of immune checkpoint inhibitor therapy	
***MLH1* promoter methylation**	Methylation of *MLH1* promoter	MLH1 loss by IHC	Presence of *MLH1* promoter methylation in a setting of *MLH1* loss suggests sporadic origin	Methylation assays

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NCCN Guidelines Version 3.2021
Colon Cancer

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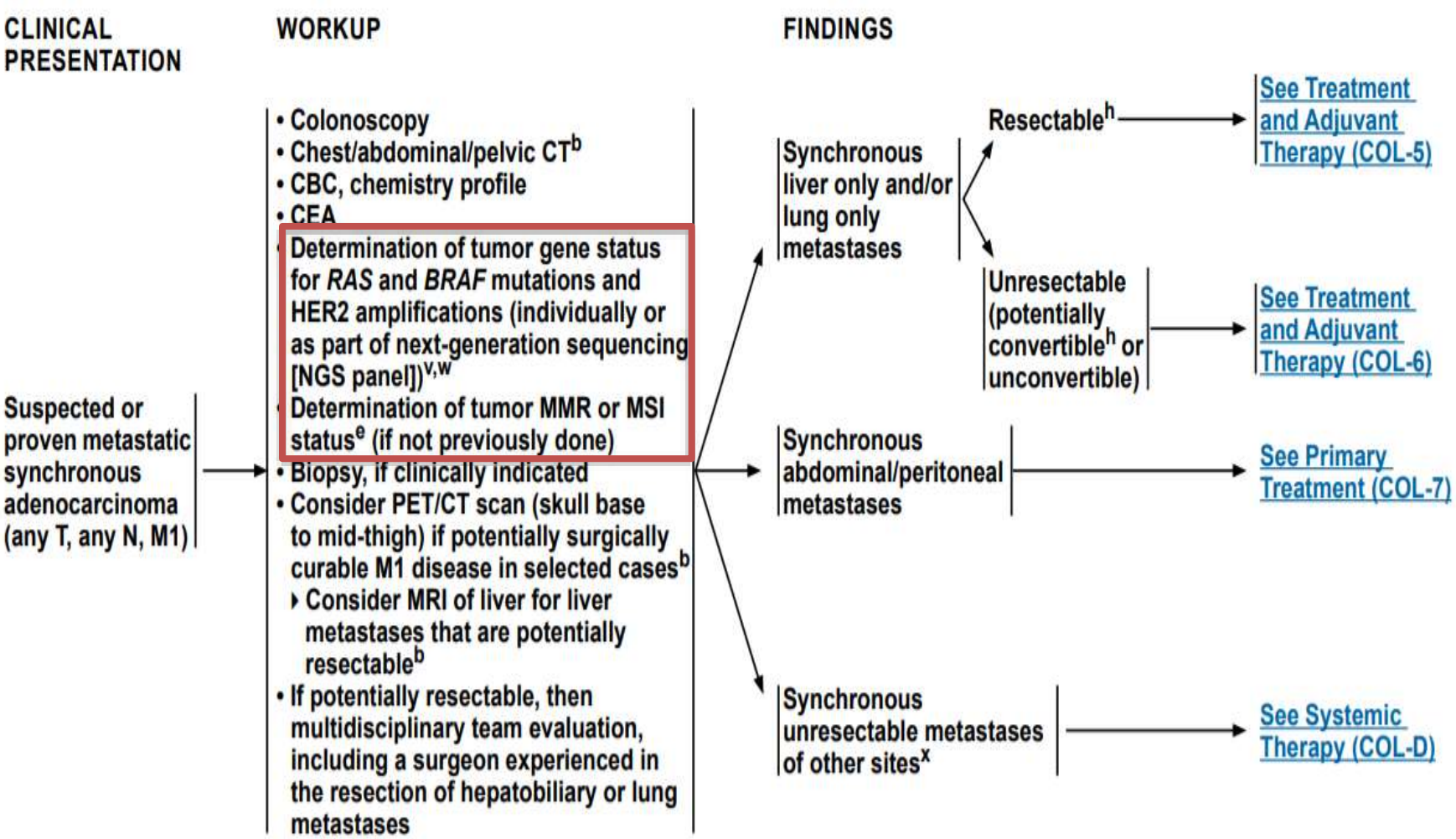


PRINCIPLES OF PATHOLOGIC REVIEW

Microsatellite Instability or Mismatch Repair Testing

- Universal mismatch repair (MMR)[a] or microsatellite instability (MSI)[a] testing is recommended in all newly diagnosed patients with colon cancer. See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal.

KRAS, *NRAS*, and *BRAF* Mutation Testing

- All patients with metastatic colorectal cancer should have tumor tissue genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations individually or as part of an NGS panel. Patients with any known *KRAS* mutation (exon 2, 3, 4) or *NRAS* mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab.[53-55] *BRAF* V600E mutation makes response to panitumumab or cetuximab highly unlikely unless given with a *BRAF* inhibitor.[56-58]

HER2 Testing

- Diagnostic testing is via immunohistochemistry, fluorescence in situ hybridization (FISH), or NGS.
- Positive by immunohistochemistry is defined as: 3+ staining in more than 50% of tumor cells. 3+ staining is defined as an intense membrane staining that can be circumferential, basolateral, or lateral. Those that have a HER2 score of 2+ should be reflexed to FISH testing.[62-64] HER2 amplification by FISH is considered positive when the HER2:CEP17 ratio is ≥2 in more than 50% of the cells.[62-64] NGS is another methodology for testing for HER2 amplification.[65]
- Anti-HER2 therapy is only indicated in HER2-amplified tumors that are also *RAS* and *BRAF* wild type.

NTRK Fusions

- *NTRK* fusions are extremely rare in colorectal carcinomas.[66] The overall incidence is approximately 0.35% in a cohort of 2314 colorectal carcinomas, with *NTRK* fusions confined to those tumors that are pan-wild type *KRAS, NRAS*, and *BRAF*. In one study of 8 colorectal cancers harboring *NTRK* fusions, 7 were found in the small subset that were dMMR (MLH-1)/MSI-H.[67] These data support limiting the subpopulation of colorectal cancers that should be tested for *NTRK* fusions to those with wild type *KRAS, NRAS, BRAF*, and arguably to those that are MMR deficient (dMMR)/MSI-H.[67]
- *NTRK* inhibitors have been shown to have activity ONLY in those cases with *NTRK* fusions, and NOT with *NTRK* point mutations.

CONCLUSIONS : MOLECULAR TESTING ET CRC

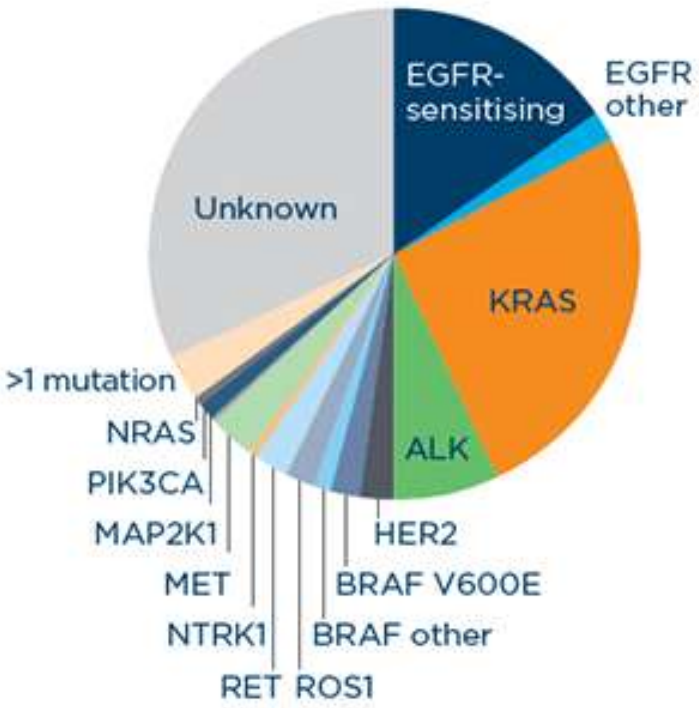
- Tous les patients avec un CRC
  - MSI : valeur diagnostique, pronostique et/ou prédictive

- Tous les patients avec un CRC métastatique
  - NGS : status RAS et BRAF + emerging biomarkers
    - Valeur pronostique et/ou prédictive

Lung cancers

TARGETED THERAPY

DRIVER MUTATIONS IN LUNG ADENOCARCINOMA

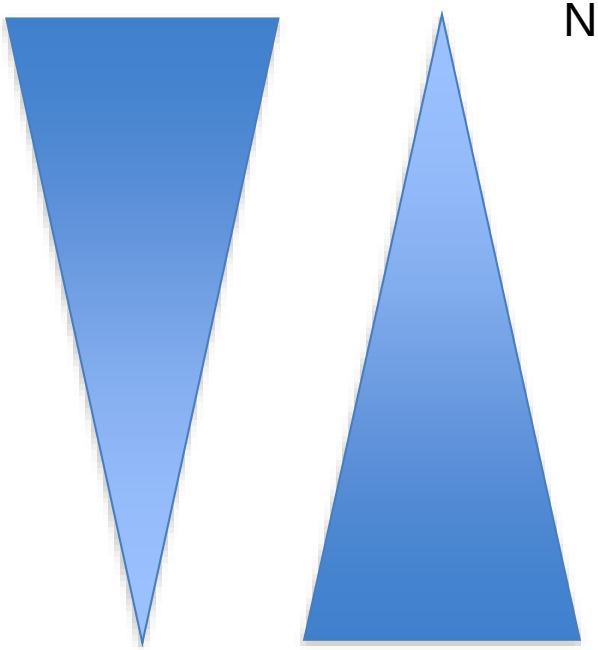


Driver mutations in lung adenocarcinoma	
EGFR-sensitizing	15%
EGFR other	2%
KRAS	25%
ALK	7%
HER2	2%
BRAF V600E	2%
BRAF other	1%
ROS1	2%
RET	2%
NTRK1	0–5%
MET	3%
MAP2K1	0–5%
PIK3CA	1%
NRAS	0–5%
>1 mutation	3%
Unknown	31%

INTRODUCTION

Sample size

Number of biomarkers to test

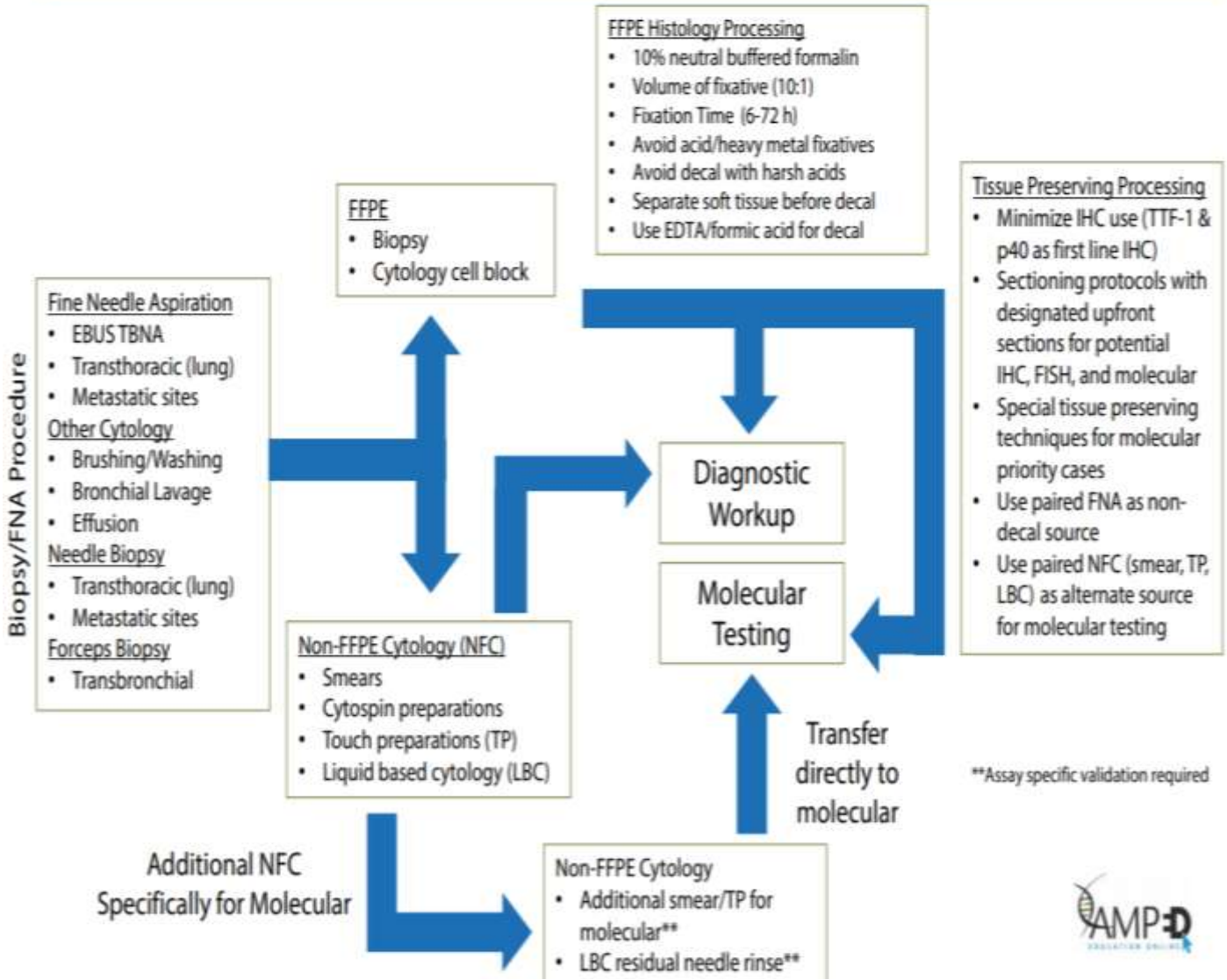


Less invasive procedures

EGFR
ALK
ROS1
PDL-1

KRAS, BRAF, MET, ERBB2, RET,….

ONCOLOGY: *Molecular Testing in NSCLC – Laboratory Aspects in Small Specimen Processing*



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GUIDELINES INTERNATIONAUX

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SPECIAL ARTICLE

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

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Prepared by the Association for Molecular Pathology Training and Education Committee
For More Educational Resources: *www.amp.org/AMPEducation*

Molecular In My Pocket…

ONCOLOGY: *Molecular Biomarkers of Lung Cancer*

What to test:

Tumor Stage – Advanced-stage (stages IIIb and IV) or recurrent lung cancer: Consideration of testing early-stage patients (based on institutional policy); in particular, *EGFR* mutation testing on diagnostic biopsy or post-surgical resection specimens for use in making adjuvant treatment decisions in stage IB to IIIA non-small cell lung cancer (NSCLC).

Histology – Adenocarcinomas, large cell, or NSCLC not otherwise specified: Consideration of testing for squamous cell carcinoma.

Materials – Formalin-fixed paraffin-embedded tissue (FFPE); fresh, frozen, or alcohol-fixed tissue; any type of cytology specimen with adequate cellularity and appropriate validation. Macro/microdissection encouraged for tumor enrichment*

https://www.amp.org/education/education-resources/molecular-in-my-pocket-guides/

Biomarker	Specific Alterations	Indications	Result Interpretation Significance	Assays Techniques*
Must Test (Broad Molecular Profiling Recommended) **				
EGFR	Exons 18-21 (p. L858R; exon 19 deletions)	Consideration of therapy with EGFR-targeted tyrosine kinase inhibitors (TKI)	Responsiveness to EGFR-targeted TKIs	NGS, PCR-based assays
	Exon 20 insertions	Consideration of therapy with EGFR-targeted TKIs	Primary resistance to EGFR-targeted TKI therapy (with some exceptions)	
	T790M	Progression after treatment with early generation EGFR-targeted TKIs	Consideration of third-generation EGFR-targeted therapy (osimertinib)	
ALK	*ALK* rearrangements	Consideration of therapy with targeted inhibitors	Predicts response to alectinib, brigatinib, lorlatinib (also ceritinib, crizotinib)	FISH, IHC, NGS, RT-PCR††
ROS1	*ROS1* rearrangements	Consideration of therapy with targeted inhibitors	Predicts response to ceritinib, crizotinib	FISH†, RT-PCR††, NGS†††; IHC as a screening with FISH or molecular confirmation of positive IHC results
BRAF	p.V600E	Consideration of therapy with targeted inhibitors	Predicts response to BRAF/MEK inhibitors (dabrafenib-trametinib)	NGS, Sanger sequencing, PCR-based assays, IHC after extensive validation
KRAS**	Codon 12, 13, 61, and 146	Consideration of therapy with targeted inhibitors	Predicts response to sotorasib (*KRAS* G12C); Diminished likelihood of another targetable oncogenic alteration	NGS, PCR-based assays
MET	Exon 14 skipping variants	Consideration of therapy with targeted inhibitors	Predicts response to capmatinib, crizotinib	NGS†††
RET	*RET* rearrangements	Consideration of therapy with targeted inhibitors	Predicts response to selpercatinib, pralsetinib (also cabozantinib, vandetanib)	FISH†, RT-PCR††, NGS†††
NTRK1/2/3	*NTRK* rearrangements	Consideration of therapy with targeted inhibitors	Predicts response to larotrectinib, entrectinib	FISH, IHC, RT-PCR††, NGS†††

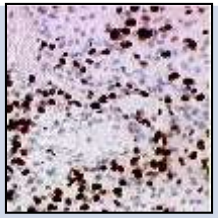
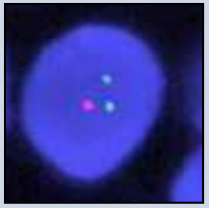
Emerging Biomarkers				
***ERBB2* (HER2)**	*ERBB2* (HER2) mutations (in frame insertions in exon 20, substitutions at codon S310, & amplification)	Consideration for a clinical trial with ERBB2 (HER2)-targeted therapy	Predicts response to ERBB2-targeted therapy (afatanib, TDM1)	NGS, PCR-based methods
MET	High- level amplification	Consideration for a clinical trial with MET targeted therapy	Predicts response to crizotinib Secondary resistance to EGRF-targeted TKIs	FISH, NGS

Cell-Free Plasma DNA (Liquid Biopsy):

Considerations: Cell-free tumor DNA testing should not be used in lieu of a histologic tissue diagnosis. Cell-free DNA testing may have very high specificity, but low sensitivity (up to 30% false-negative rate).

When to Use: When a patient is unfit for invasive tissue biopsy or diagnostic biopsy is insufficient for molecular analysis. Follow-up tissue analysis should be planned for all patients in which an oncogenic driver is not found

Assay Techniques: NGS, PCR

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