Molecular Testing in Thyroid FNA Specimens

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HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

Objectives

1. **Key molecular changes**

2. **Purpose of molecular testing**

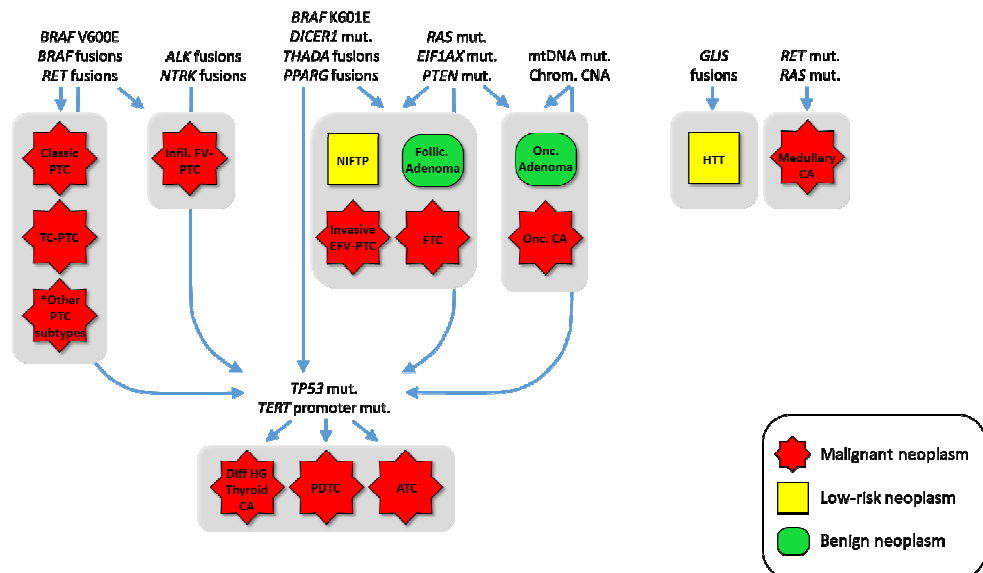**3.** **Testing platforms**

Objectives

1. **Key molecular changes**

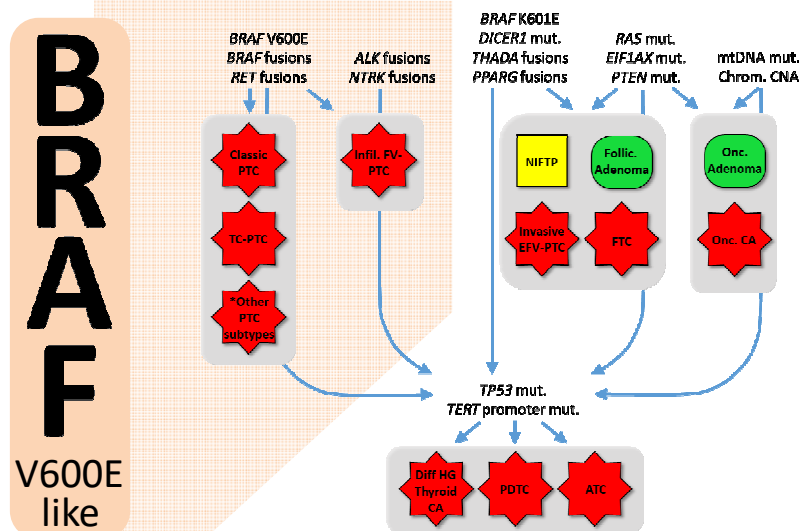**2.** Purpose of molecular testing

3. Testing platforms

Tumor genotype is associated with phenotype

Nishino M et al. Figure prepared for "Molecular and Other Ancillary Tests", TBSRTC 3rd Ed (2023).

Tumor genotype is associated with phenotype

BRAF^{V600E}-like tumors

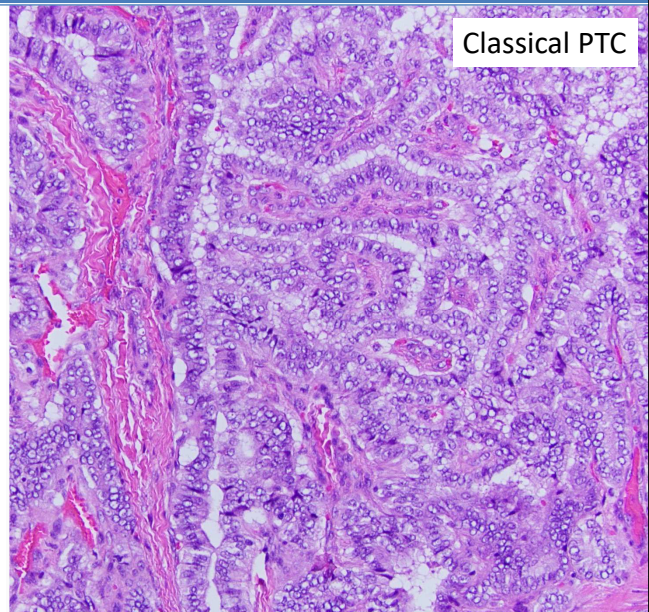**BRAF V600E** mutation

RET fusions
- *CCDC6::RET* (RET-PTC1)
- *NCOA4::RET* (RET-PTC3)

ALK fusions
BRAF fusions
NTRK1/3 fusions
MET fusions

BRAF^(V600E)-like tumors

BRAF V600E mutation

RET fusions
- *CCDC6::RET* (RET-PTC1)
- *NCOA4::RET* (RET-PTC3)

ALK fusions

BRAF fusions

NTRK1/3 fusions

MET fusions

Classical PTC

BRAF^(V600E)-like tumors

BRAF V600E mutation

RET fusions
- *CCDC6::RET* (RET-PTC1)
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ALK fusions

BRAF fusions

NTRK1/3 fusions

MET fusions

Classical PTC
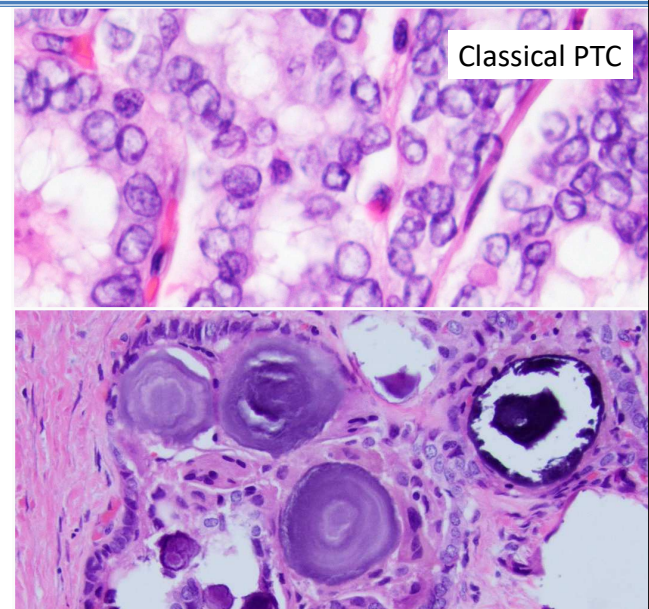
BRAF^{V600E}-like tumors

BRAF V600E mutation

RET fusions
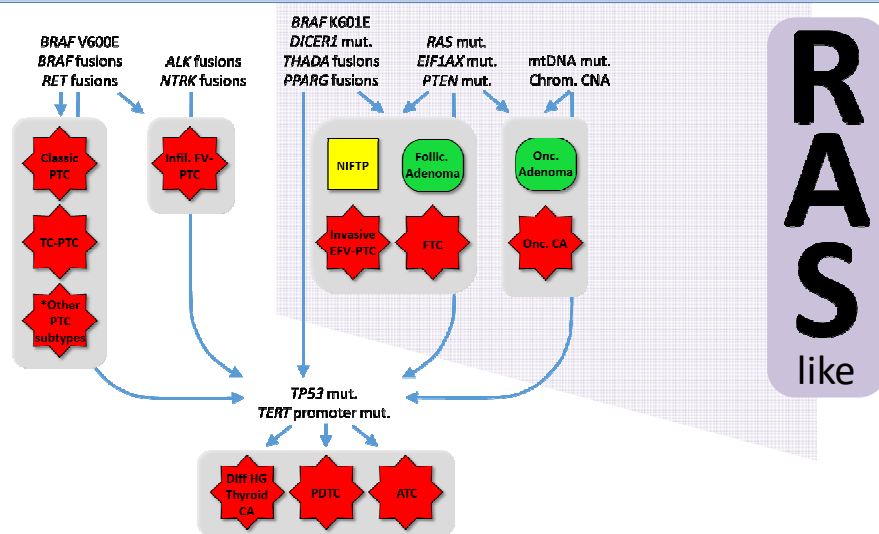- *CCDC6::RET* (RET-PTC1)
- *NCOA4::RET* (RET-PTC3)

ALK fusions
BRAF fusions
NTRK1/3 fusions
MET fusions

Tall cell PTC
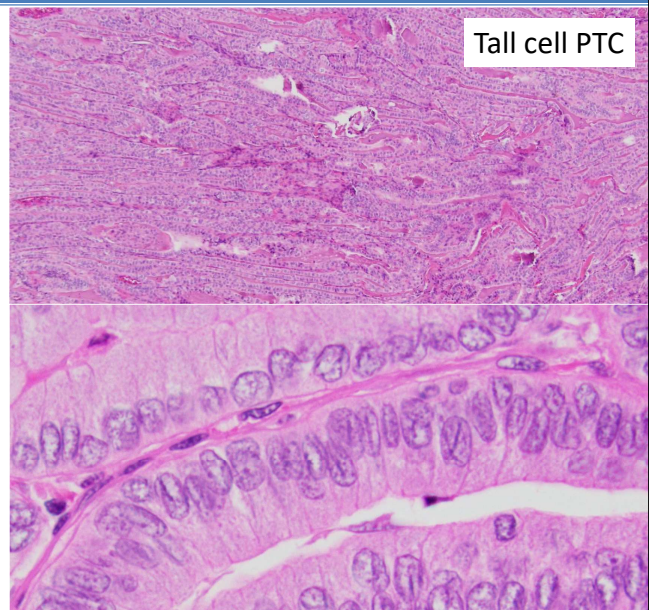
Tumor genotype is associated with phenotype

RAS-like tumors

H/N/KRAS mutation
BRAF K601E mutation
DICER1 mutation
EIF1AX mutation
PTEN mutation
PAX8::PPARG fusion
THADA fusions

FA   FT-UMP   FTC (min inv)   FTC (angioinv)   FTC (widely inv)

NIFTP   WDT-UMP   Invasive Encapsulated FVPTC
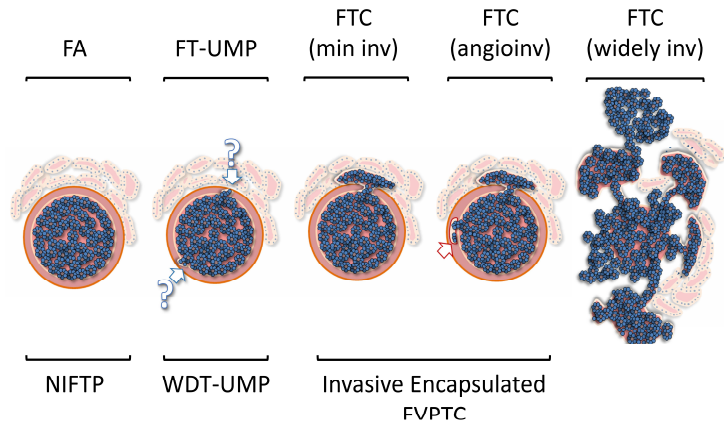
Ohori NP and Nishino M. *Adv Anat Pathol* 2022.

RAS-like tumors

H/N/KRAS mutation
BRAF K601E mutation
DICER1 mutation
EIF1AX mutation
PTEN mutation
PAX8::PPARG fusion
THADA fusions

Follicular adenoma

HRAS & *EIF1AX* mutated

RAS-like tumors

H/N/KRAS mutation
BRAF K601E mutation
DICER1 mutation
EIF1AX mutation
PTEN mutation
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H/N/KRAS mutation
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PAX8::PPARG fusion
THADA fusions

Follicular carcinoma

PAX8::PPARG fusion

RAS-like tumors
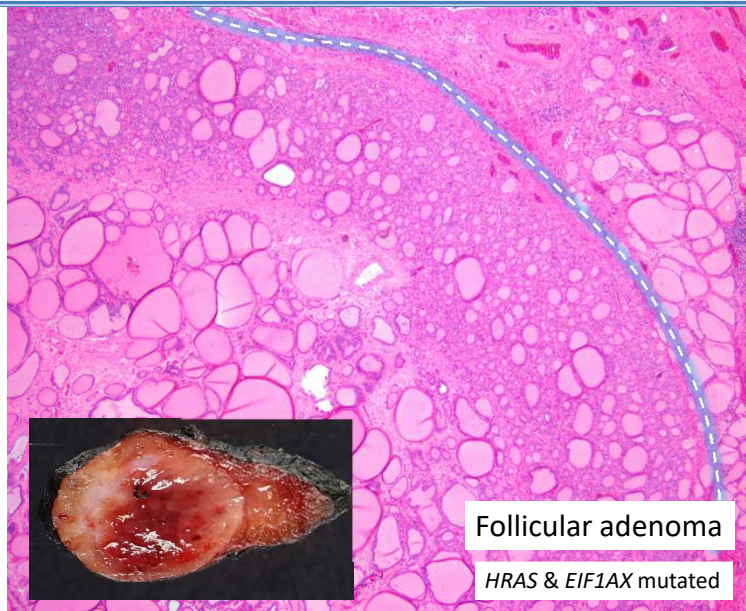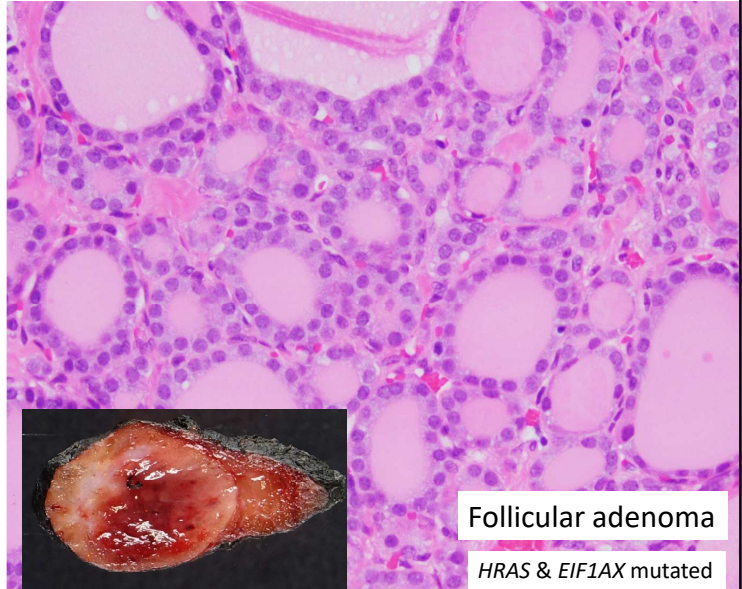
H/N/KRAS mutation
BRAF K601E mutation
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EIF1AX mutation
PTEN mutation
PAX8::PPARG fusion
THADA fusions

NIFTP

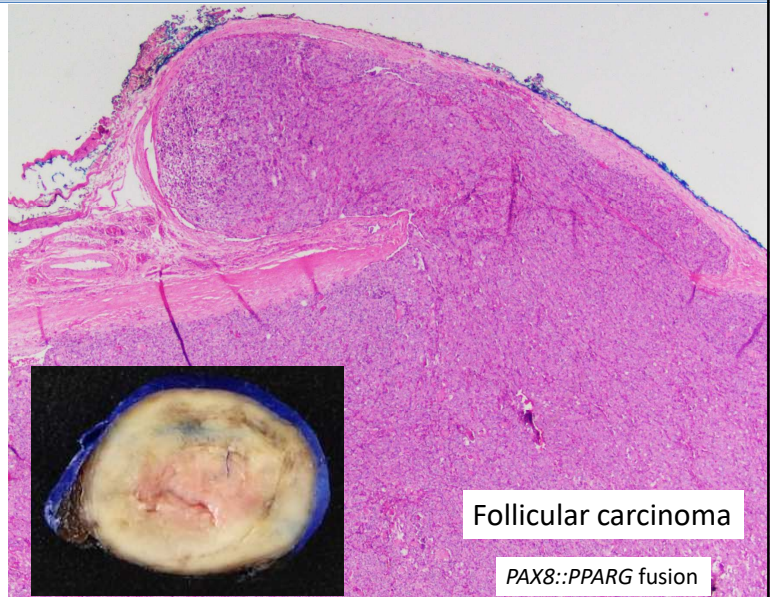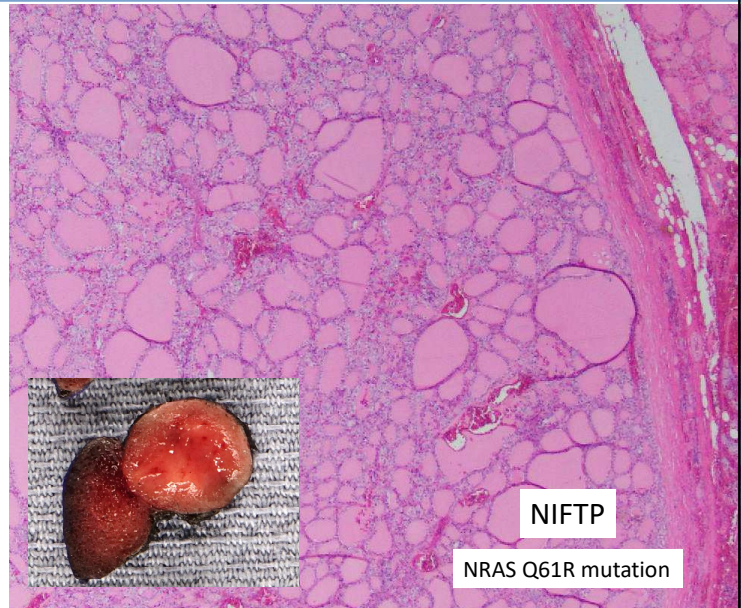NRAS Q61R mutation

BRAF^{V600E}-like vs RAS-like tumors

Very high probability of cancer

Intermediate probability of cancer

	BRAF^{V600E}-like	RAS-like	
Mutations	***BRAF* V600E**	*H/K/NRAS* *BRAF K60LE* *DICER1*	*EIF1AX* *PTEN*
Gene fusions	*RET* *ALK* *BRAF* *NTRK1/3* *MET*	*PAX8:PPARG* *THADA*	
Benign or pre-malignant tumors	*{none}*	Follicular adenoma NIFTP	
Malignant tumors	**Classical PTC** **Tall cell PTC**	Follicular carcinoma Invasive EFV-PTC	

Tumor genotype is associated with phenotype

BRAF V600E
BRAF fusions
RET fusions

ALK fusions
NTRK fusions

BRAF K601E
DICER1 mut.
THADA fusions
PPARG fusions

RAS mut.
EIF1AX mut.
PTEN mut.

mtDNA mut.
Chrom. CNA

Classic PTC — TC-PTC — *Other PTC subtypes

Infil. FV-PTC

NIFTP — Follic. Adenoma — Invasive EFV-PTC — FTC

Onc. Adenoma — Onc. CA

TP53 mut.
TERT promoter mut.

Diff HG Thyroid CA — PDTC — ATC
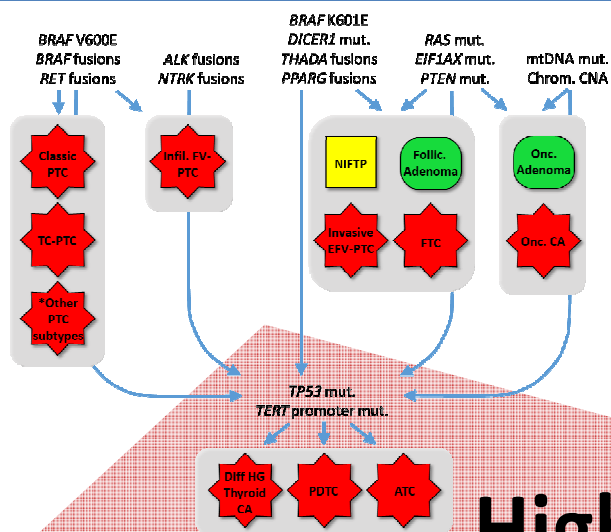
High risk of adverse outcomes

Objectives

1. Key molecular changes

2. Purpose of molecular testing

3. Testing platforms

FNA helps **risk-stratify** thyroid nodules
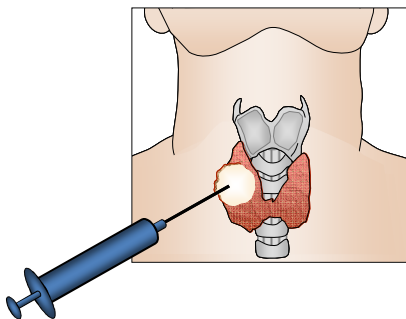
Fine needle aspiration biopsy

Diagnostic / therapeutic surgery

Clinical follow-up

2

types of "risk"

Risk of *malignancy*

Risk of structural *disease recurrence*

Bethesda System and *Risk of Malignancy*

FNA Diagnosis	Risk of CA or NIFTP (%)	Suggested Management
I. Nondiagnostic	5-10	Repeat FNA with U/S
II. Benign	0-3	Clinical & U/S surveillance
III. Atypia of Undetermined Significance	10-30	Repeat FNA, MT, or lobectomy
IV. Follicular Neoplasm / Oncocytic Neoplasm	25-40	MT or lobectomy
V. Suspicious for Malignancy	50-75	Lobectomy or total thyroidectomy
VI. Malignant	97-99	Lobectomy or total thyroidectomy

Baloch ZW et al. "Overview of Diagnostic Terminology and Reporting" In: S. Ali and E. Cibas (Eds.) *TBSRTC* 2nd Edition, 2018.

High Risk
Gross extrathyroidal extension, incomplete tumor resection, distant metastasis, or lymph node > 3 cm

Intermediate Risk
Aggressive histology, minor extrathyroidal extension, vascular invasion, or > 5 involved lymph nodes (0.2-3 cm)

Low Risk
Intrathyroidal DTC
≤ 5 LN micrometastasis (< 0.2 cm)

Risk of Structural Disease Recurrence
(in patients without structurally identifiable disease after initial therapy)

Haugen BR et al. 2015 ATA Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid* (2016).

Cytopathology

Management Considerations

FNA Biopsy

MALIGNANT
(& Suspicious for Malignancy)

BENIGN

Surgical referral

Total thyroidectomy

Lobectomy

Observation / Surveillance

Disease Risk Stratification

Nishino M et al. Figure prepared for "Molecular and Other Ancillary Tests", TBSRTC 3rd Ed (2023).

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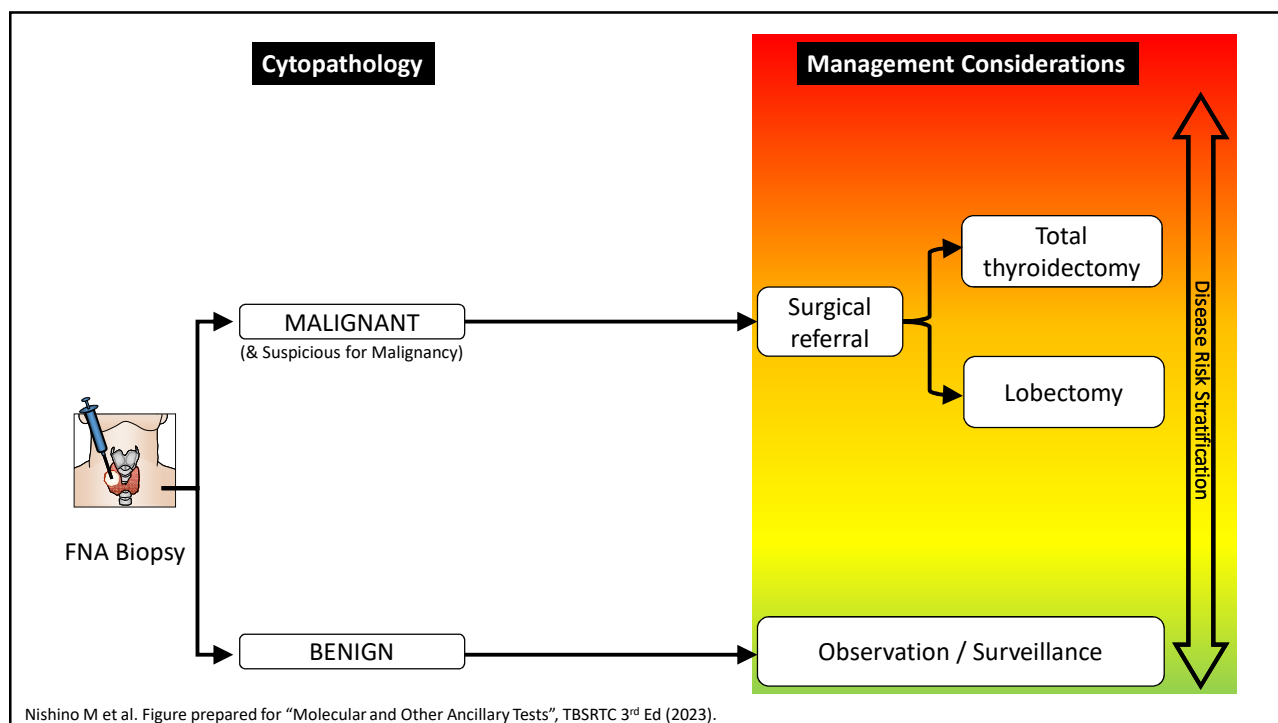Nishino M et al. Figure prepared for "Molecular and Other Ancillary Tests", TBSRTC 3rd Ed (2023).

Objectives

1. Key molecular changes

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3. **Testing platforms**

Molecular tests help risk-stratify cytologically indeterminate nodules

INDETERMINATE CYTOLOGY

(AUS, FN)

Molecular test
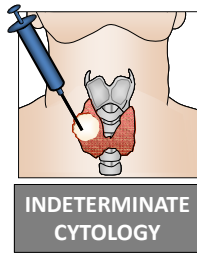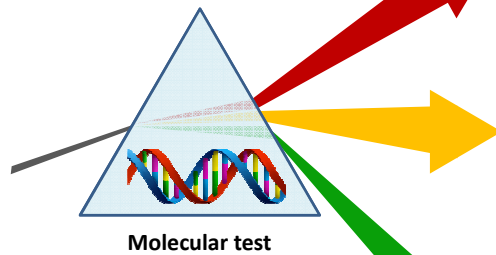
High probability for malignancy
(Consider lobectomy vs total thyroidectomy)

Intermediate probability
(Consider lobectomy)

Low probability for malignancy
(Risk of malignancy <5%, safe to follow clinically)

Nishino M, Bellevicine C, Baloch Z. *J. Mol Pathol* 2021, 2(2):135-146.

Molecular tests for cytologically indeterminate thyroid nodules in the USA

- **ThyroSeq GC** (UPMC / Sonic Healthcare USA)

- **ThyGeNEXT / ThyraMIR** (Interpace Diagnostics)

- **Afirma GSC** (Veracyte)

Photo Credit: Matt Stone / Boston Herald

Construction Activity on Sunday November 01, 2020 – Thursday, November 05, 2020. Location: I-95 Westbound at Exit 33

Incident Reported on Monday, November 02, 2020. Location: I-93 Southbound, 0.05 mi beyond Exit 34.

Lane Closure on Monday November 02, 2020 – Wednesday, November 04, 2020. Location: I-95 Southbound at Exit 53.

Bridge Construction on Monday, November 02, 2020 – Thursday, November 05, 2020. Location: I-93 Southbound at Exit 26.

Incident Reported on Monday, November 02, 2020. Location: Mass Turnpike Eastbound at Exit 21.

74 min

57 min

45 min

62 min

80 min

Broad approaches to molecular testing for thyroid FNAs

Testing approach	Driver mutation / gene fusion panel	Expression pattern of panel of microRNAs	Expression pattern of panel of mRNAs	Expression pattern of various proteins
Method	DNA & RNA Sequencing	RT-PCR	RNA-Seq	Immunocytochemistry
Examples of tests	ThyGeNEXT, ThyroSeq, Afirma Xpression Atlas	ThyraMIR	Afirma GSC	Antibodies to BRAF VE1, HBME1, CK19, Galectin-3, CITED-1)

Nishino M. "Molecular Diagnostics in Thyroid Cytology" In: Roy-Chowdhuri, VanderLaan, Stewart, & da Cunha Santos (Eds.) *Molecular Diagnostics in Cytopathology* (2019).

Broad approaches to molecular testing for thyroid FNAs

Testing approach	Driver mutation / gene fusion panel
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Examples of tests	ThyGeNEXT, ThyroSeq, Afirma Xpression Atlas

Genotyping approaches

Nishino M. "Molecular Diagnostics in Thyroid Cytology" In: Roy-Chowdhuri, VanderLaan, Stewart, & da Cunha Santos (Eds.) *Molecular Diagnostics in Cytopathology* (2019).
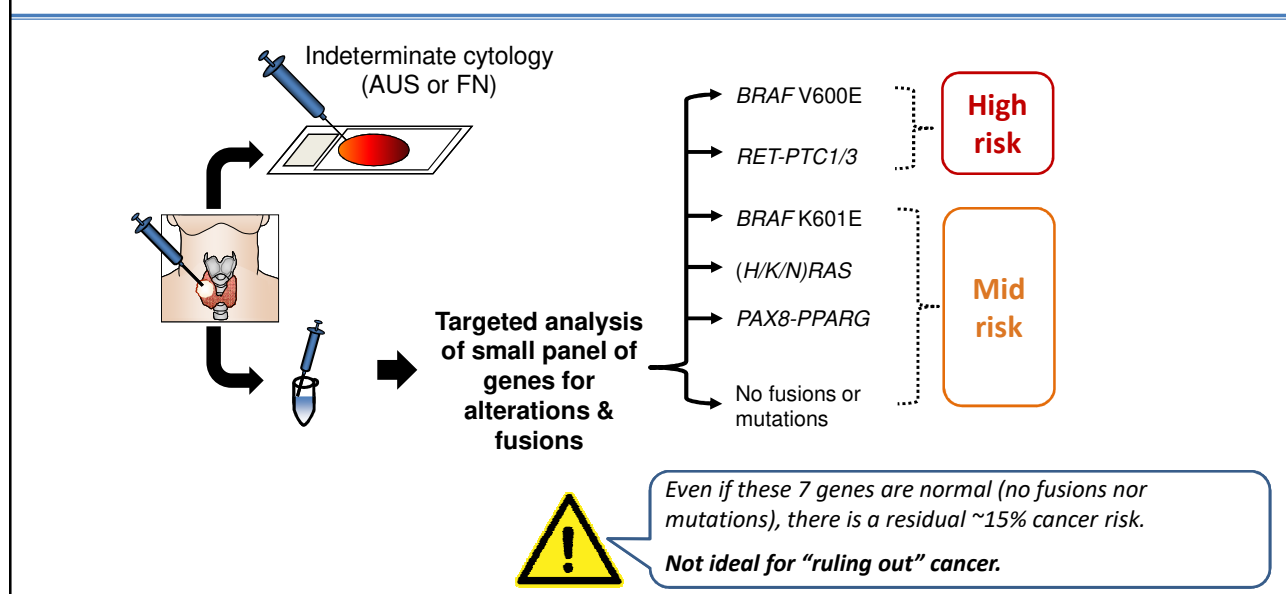
Tumor genotype is associated with phenotype

Early mutation/fusion panels

How can we improve the NPV of the driver mutation/fusion panel?
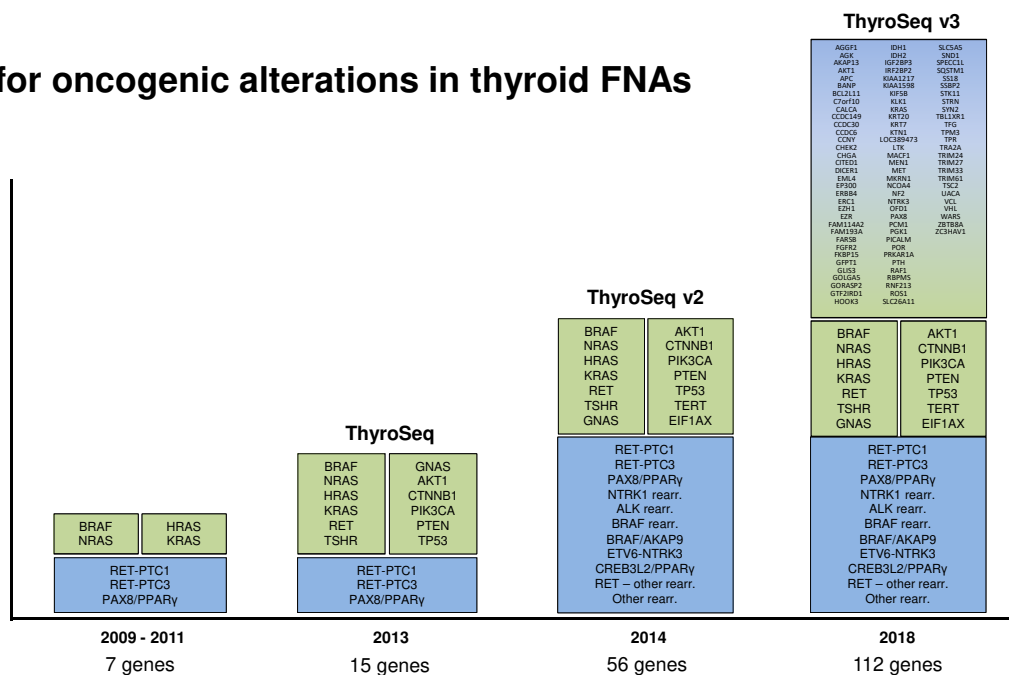
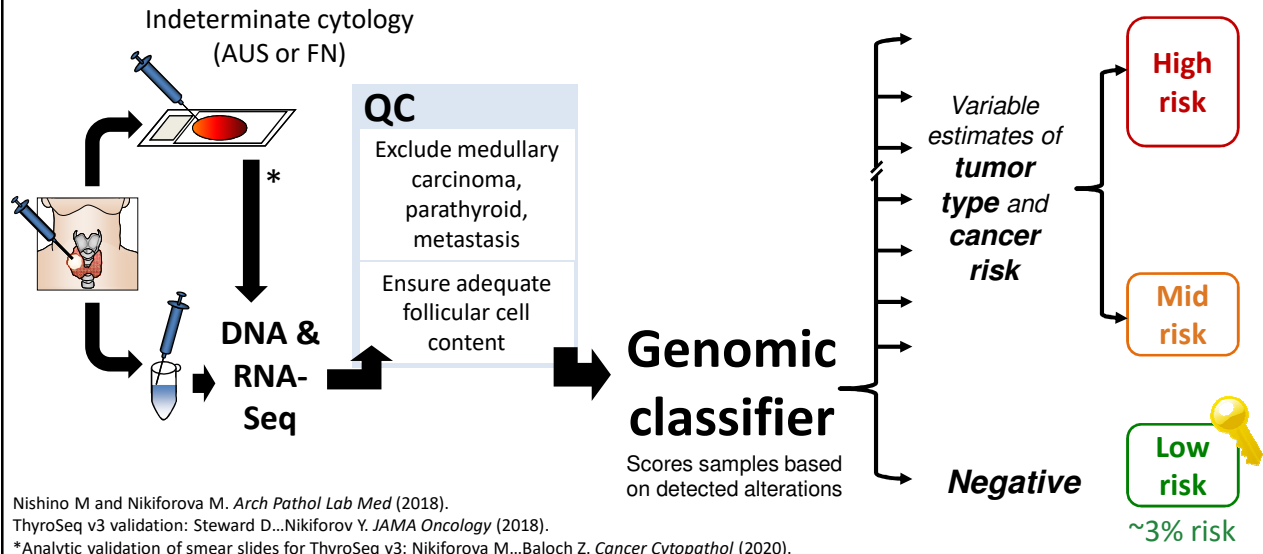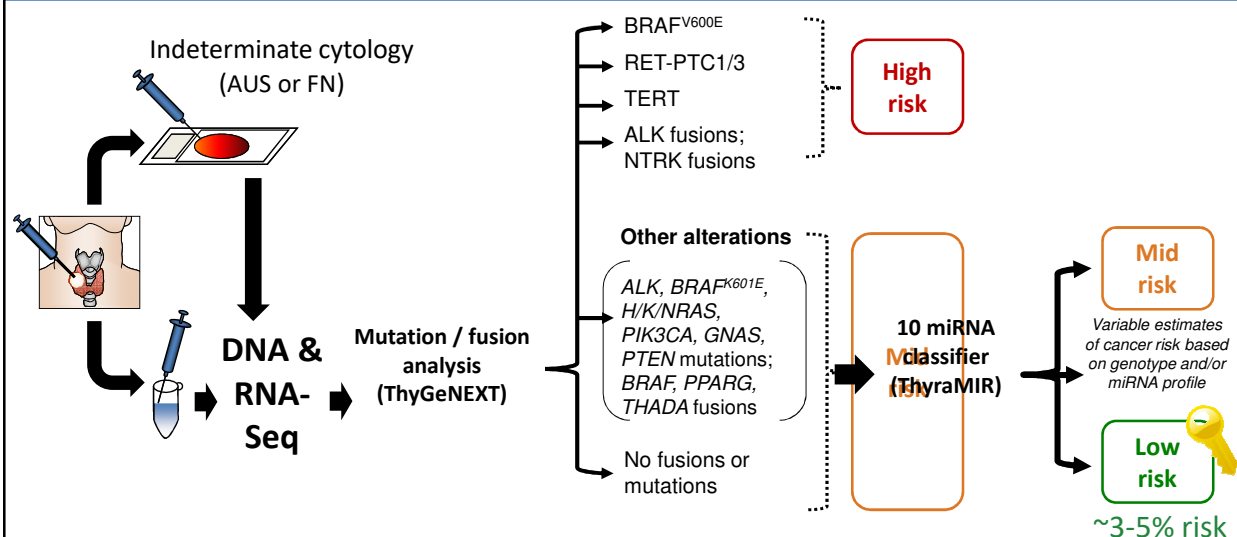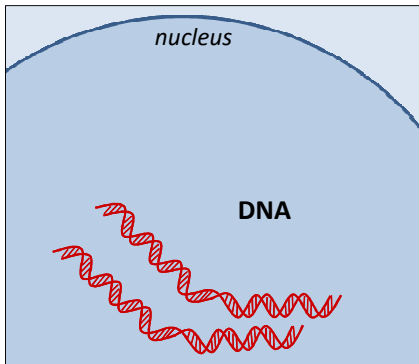| ThyGeNEXT / ThyraMIR	ThyroSeq
Combines the mutation / fusion panel with a *10-miRNA expression classifier* with high NPV	*Vastly broadens* the mutation / fusion panel to increase the sensitivity of the test

ThyroSeq

Tests for oncogenic alterations in thyroid FNAs

ThyroSeq v3

AGGF1	IDH1	SLC5A5
AGK	IDH2	SND1
AKAP13	IGF2BP3	SPECC1L
AKT1	IRF2BP2	SQSTM1
APC	KIAA1217	SS18
BANP	KIAA1598	SSBP2
BCL2L11	KIF5B	STK11
C7orf10	KLK1	STRN
CALCA	KRAS	SYN2
CCDC149	KRT20	TBL1XR1
CCDC30	KRT7	TFG
CCDC6	KTN1	TPM3
CCNY	LOC389473	TPR
CHEK2	LTK	TRA2A
CHGA	MACF1	TRIM24
CITED1	MEN1	TRIM27
DICER1	MET	TRIM33
EML4	MKRN1	TRIM61
EP300	NCOA4	TSC2
ERBB4	NF2	UACA
ERC1	NTRK3	VCL
EZH1	OFD1	VHL
EZR	PAX8	WARS
FAM114A2	PCM1	ZBTB8A
FAM193A	PGK1	ZC3HAV1
FARSB	PICALM	
FGFR2	POR	
FKBP15	PRKAR1A	
GFPT1	PTH	
GLIS3	RAF1	
GOLGA5	RBPMS	
GORASP2	RNF213	
GTF2IRD1	ROS1	
HOOK3	SLC26A11	

ThyroSeq v2

BRAF	AKT1
NRAS	CTNNB1
HRAS	PIK3CA
KRAS	PTEN
RET	TP53
TSHR	TERT
GNAS	EIF1AX

BRAF	AKT1
NRAS	CTNNB1
HRAS	PIK3CA
KRAS	PTEN
RET	TP53
TSHR	TERT
GNAS	EIF1AX

RET-PTC1
RET-PTC3
PAX8/PPARγ
NTRK1 rearr.
ALK rearr.
BRAF rearr.
BRAF/AKAP9
ETV6-NTRK3
CREB3L2/PPARγ
RET – other rearr.
Other rearr.

RET-PTC1
RET-PTC3
PAX8/PPARγ
NTRK1 rearr.
ALK rearr.
BRAF rearr.
BRAF/AKAP9
ETV6-NTRK3
CREB3L2/PPARγ
RET – other rearr.
Other rearr.

ThyroSeq

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

RET-PTC1
RET-PTC3
PAX8/PPARγ

BRAF	HRAS
NRAS	KRAS

RET-PTC1
RET-PTC3
PAX8/PPARγ

2009 - 2011	**2013**	**2014**	**2018**
7 genes	15 genes	56 genes	112 genes

ThyroSeq v3

- **Targeted high-throughput sequencing** of DNA and RNA
- **112** genes analyzed for **12,135** single-nucleotide variants and insertions/deletions.
- **120+** gene fusions.
- Gene expression alterations of **19** genes.
- Copy number alterations in **10** genomic regions

ThyroSeq v3

Indeterminate cytology
(AUS or FN)

QC

Exclude medullary carcinoma, parathyroid, metastasis

Ensure adequate follicular cell content

DNA & RNA-Seq

*

Genomic classifier

Scores samples based on detected alterations

Variable estimates of **tumor type** *and* **cancer risk**

Negative

High risk

Mid risk

Low risk

~3% risk

Nishino M and Nikiforova M. *Arch Pathol Lab Med* (2018).
ThyroSeq v3 validation: Steward D…Nikiforov Y. *JAMA Oncology* (2018).
*Analytic validation of smear slides for ThyroSeq v3: Nikiforova M…Baloch Z. *Cancer Cytopathol* (2020).

How can we improve the NPV of the driver mutation/fusion panel?

ThyGeNEXT / ThyraMIR	ThyroSeq
Combines the mutation / fusion panel with a ***10-miRNA expression classifier*** with high NPV	*Vastly broadens* the mutation / fusion panel to increase the sensitivity of the test

ThyGeNEXT / ThyraMIR

Indeterminate cytology
(AUS or FN)

DNA & RNA-Seq

Mutation / fusion analysis (ThyGeNEXT)

BRAF^{V600E}
RET-PTC1/3
TERT
ALK fusions; NTRK fusions

High risk

Other alterations

ALK, BRAF^{K601E}, H/K/NRAS, PIK3CA, GNAS, PTEN mutations; BRAF, PPARG, THADA fusions

No fusions or mutations

10 miRNA classifier (ThyraMIR)

Mid risk

Variable estimates of cancer risk based on genotype and/or miRNA profile

Low risk

~3-5% risk

Nishino M and Nikiforova M. *Arch Pathol Lab Med* (2018).
ThyGeNEXT/ThyraMIR validation: Lupo MA et al. *Diagn Cytopathol* (2020).

Broad approaches to molecular testing for thyroid FNAs

Testing approach	Driver mutation / gene fusion panel
Method	DNA & RNA Sequencing
Examples of tests	ThyGeNEXT, ThyroSeq, Afirma Xpression Atlas

Genotyping approaches
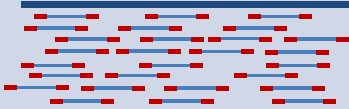
Broad approaches to molecular testing for thyroid FNAs

Gene-expression approaches

Transcription
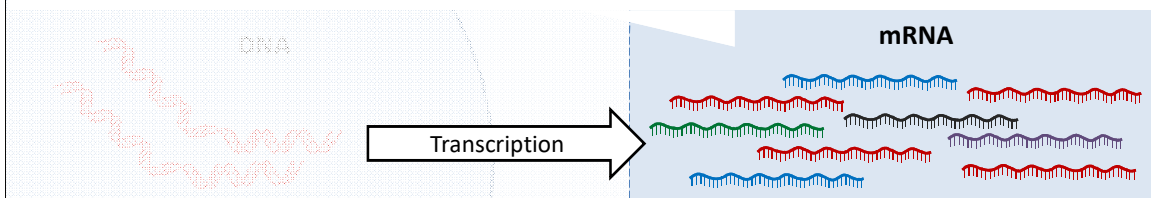
Testing approach	Driver mutation / gene fusion panel	Expression pattern of panel of mRNAs
Method	DNA & RNA Sequencing	RNA-Seq
Examples of tests	ThyGeNEXT, ThyroSeq, Afirma Xpression Atlas	Afirma GSC

Afirma GSC

Afirma Gene Sequencing Classifier (GSC)	
Platform	**High-throughput sequencing of RNA (RNA-Seq)**
Measures expression pattern of . . .	**1,115** "core" genes (10,196 genes analyzed overall)
Analysis by . . .	**Machine-learning algorithms** that classify samples in a binary manner: "Benign" vs. "Suspicious"

Afirma Gene Sequencing Classifier (GSC)

Indeterminate cytology
(AUS or FN)

QC

Check for markers specific for cancer

Ensure sufficient follicular cell content; exclude parathyroid

RNA-Seq

GSC

Malignancy Classifiers
- Afirma MTC(+)
- Afirma BRAF(+)
- RET-PTC1/3(+)

High risk
~100% risk

GSC-Suspicious
(& negative for Afirma Malignancy Classifiers)

Mid risk
~50% risk

GSC-Benign

Low risk
~4% risk

Nishino M and Nikiforova M. *Arch Pathol Lab Med* (2018).
Afirma GSC validation: Patel KN et al. *JAMA Surgery* (2018)

Afirma's RNA sequencing provides *expression* <u>and</u> *sequence* data

Gene expression levels

Sequencing data

Point mutation

Insertion/Deletion

Gene rearrangements

- Benign vs Suspicious results (Afirma GSC)
- BRAF V600E mutation (Afirma BRAF)*
- Medullary carcinoma (Afirma MTC)*

- 761 variants (including BRAF mutations)
- 130 fusions (including RET-PTC1/3*)

Xpression Atlas
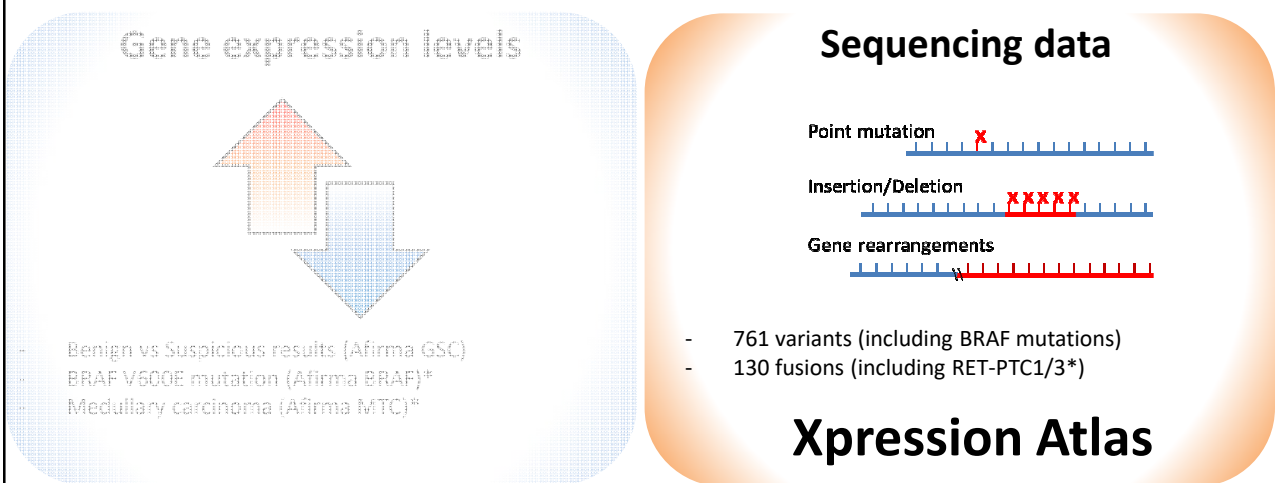
*High specificity for cancer & part of the "Afirma Malignancy Classifiers"

Common themes . . .

High NPV
 - to "rule out" cancer

Combined testing approaches (genotyping, expression profiling)

Multiple tiers of risk stratification

Similar information to guide management decisions

ThyroSeq v3

Afirma GSC & XA

ThyGeNEXT/ ThyraMIR
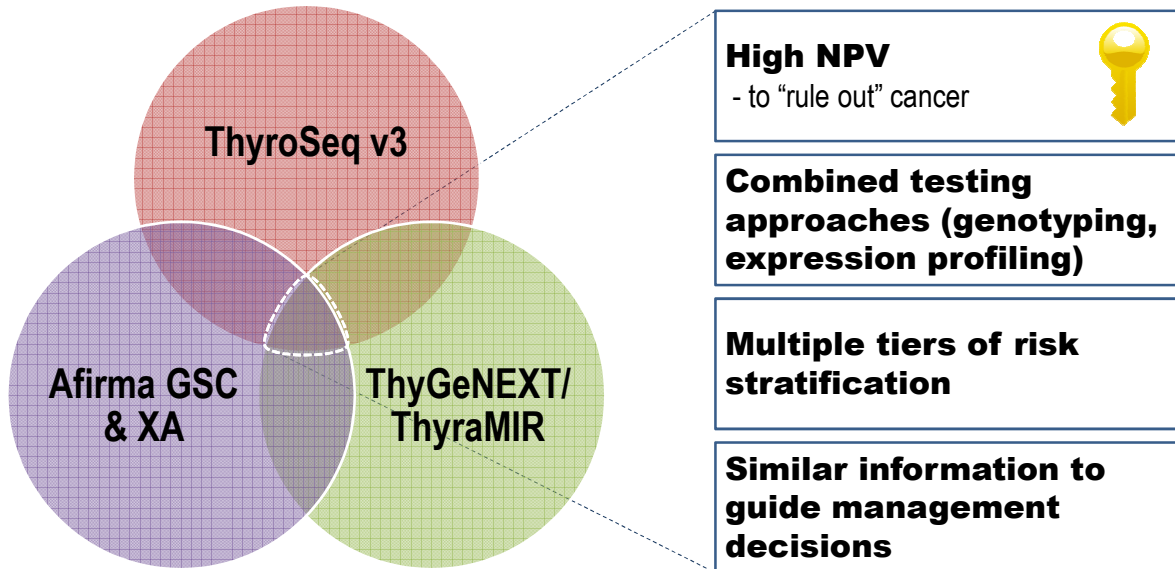
Case Study

70s / M

L thyroid nodule

Incidentally found on PET imaging for lung nodule.

U/S: 1.3 cm, hypoechoic, solid

FNA & ThyroSeq testing
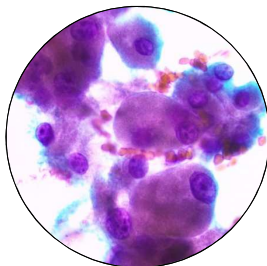
Follicular Neoplasm, Oncocytic Type (Bethesda IV)

THYROSEQ® V3 GC RESULTS SUMMARY
LEFT MID THYROID FNA

Test Result	Probability of Cancer	Potential Management
POSITIVE	Intermediate-high (~80%)	Surgical excision * *See interpretation below for details

INTERPRETATION
- TP53 mutation and chromosomal copy number alterations were identified in this sample.
- This molecular signature is associated with ~80% probability of cancer, more often Hurthle cell (oncocytic) carcinoma, whereas the rest of the nodules are expected to be follicular adenomas.
- In a setting of cancer, TP53 mutations are associated with a high risk for disease recurrence.
- Correlation of the test result with imaging and other clinical data is recommended to define the most appropriate patient management.
- Patient management decisions must be based on the independent medical judgment of the treating physician. Molecular test results should be taken into consideration in conjunction with all relevant imaging and clinical findings, patient and family history, as well as patient preference.
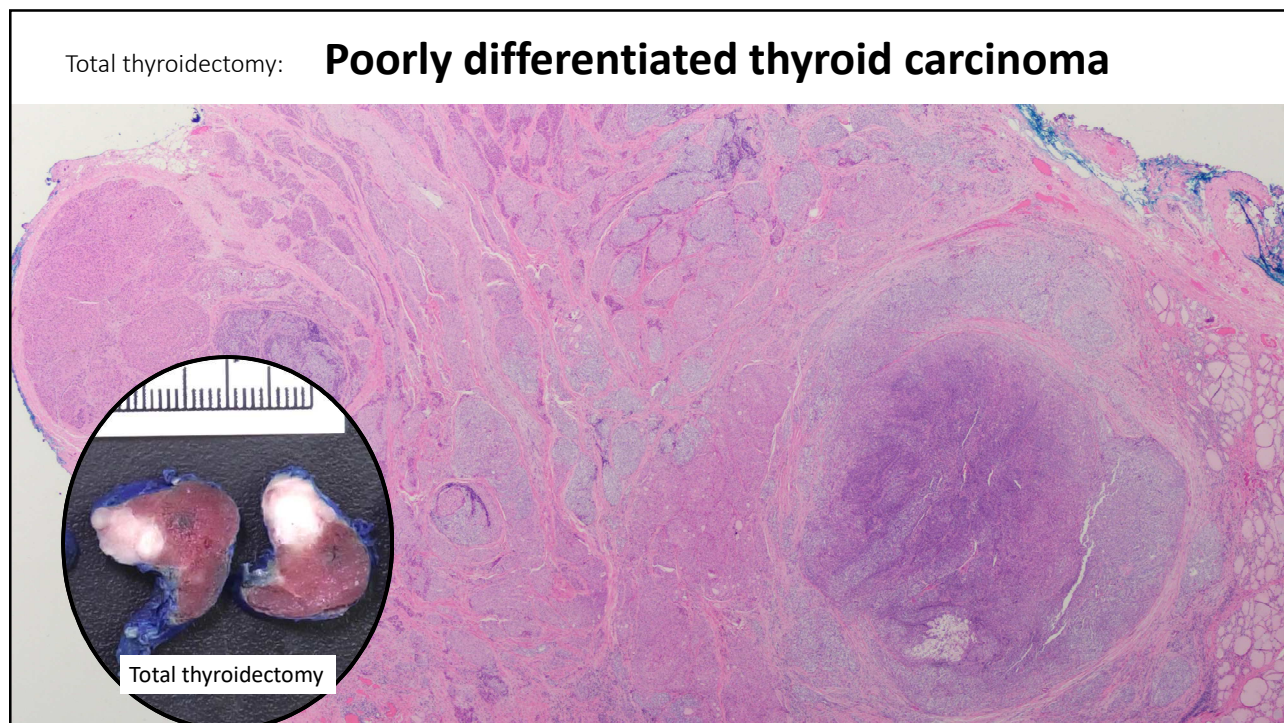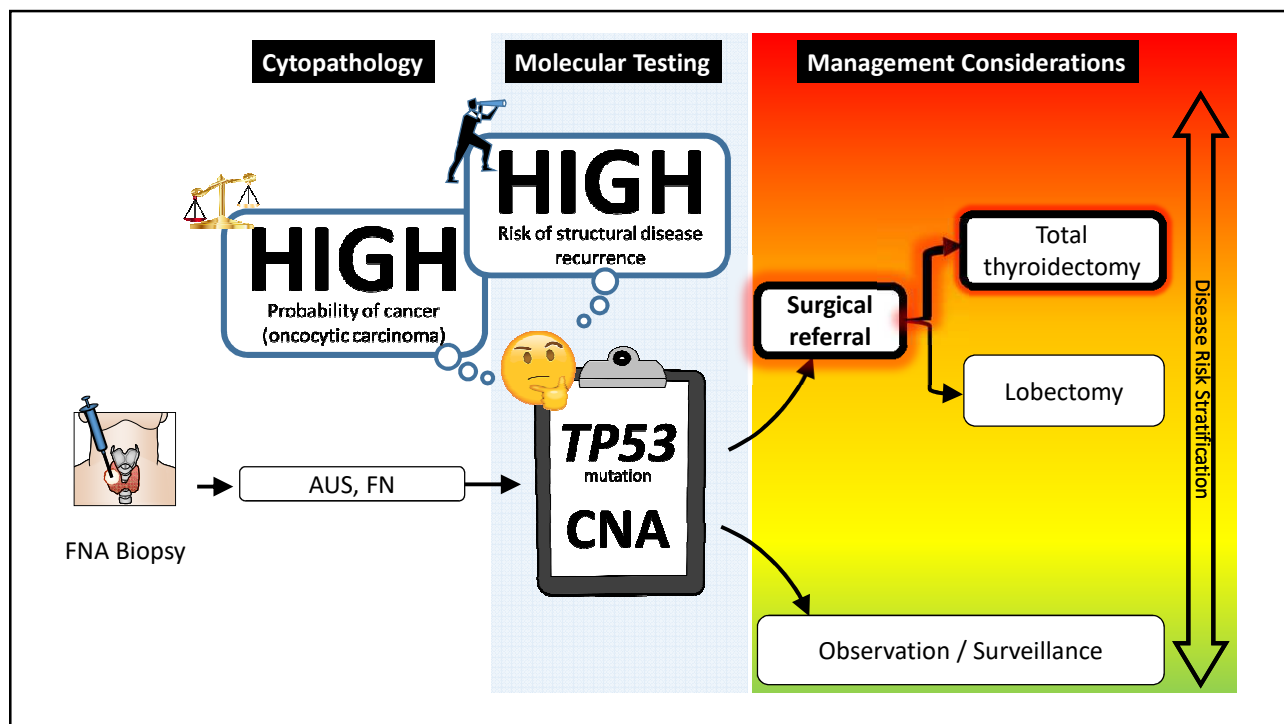
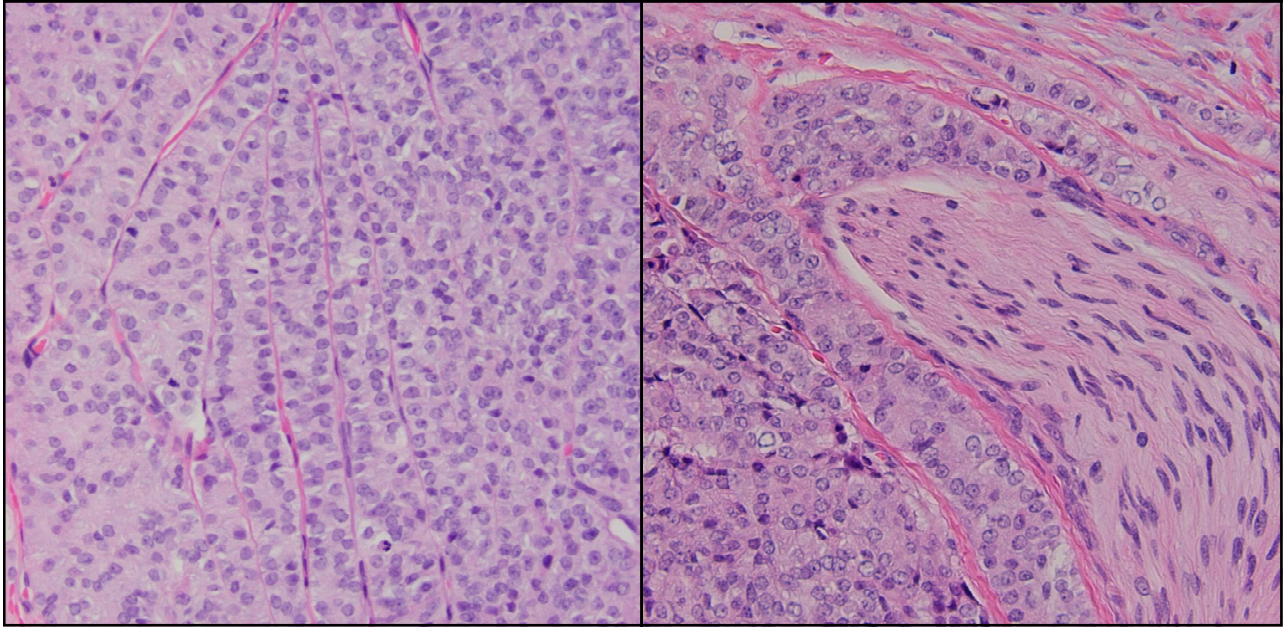Total thyroidectomy: **Poorly differentiated thyroid carcinoma**
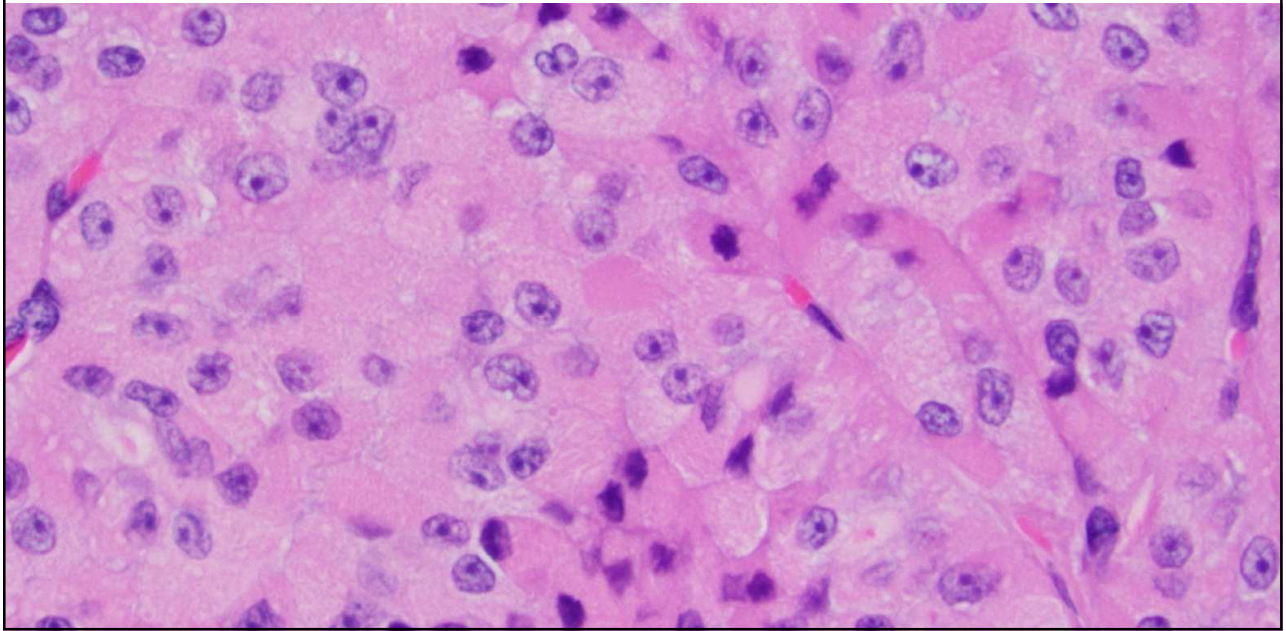
Total thyroidectomy

Total thyroidectomy: **Poorly differentiated thyroid carcinoma**

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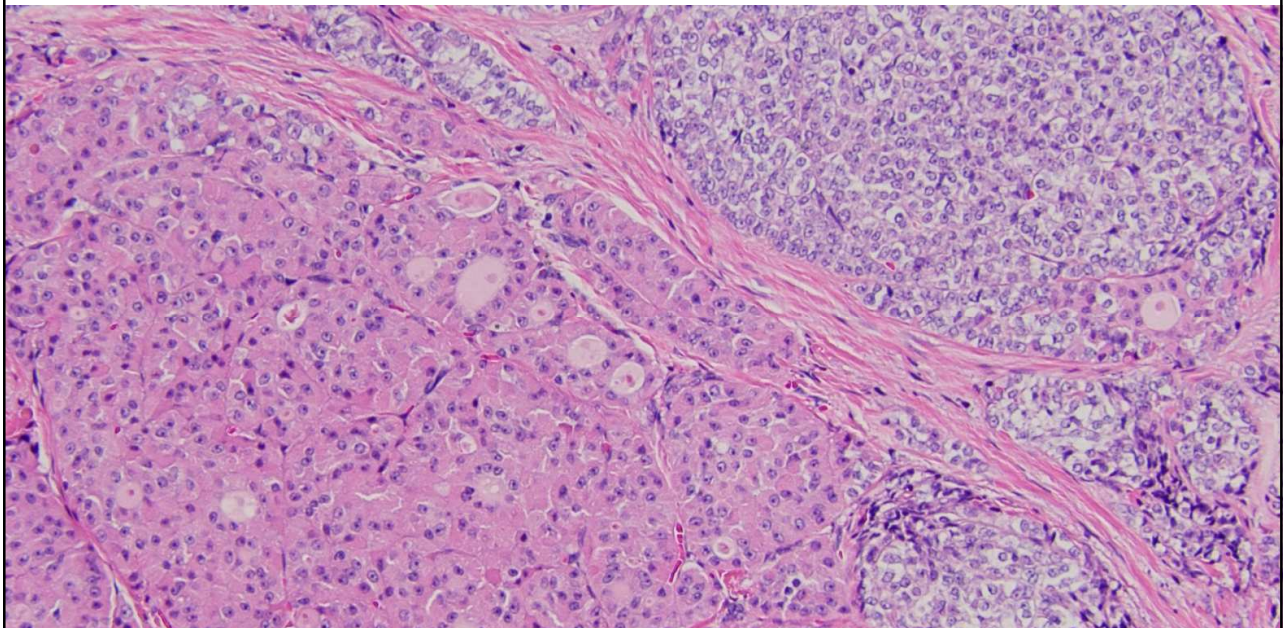
Total thyroidectomy: **Poorly differentiated thyroid carcinoma**
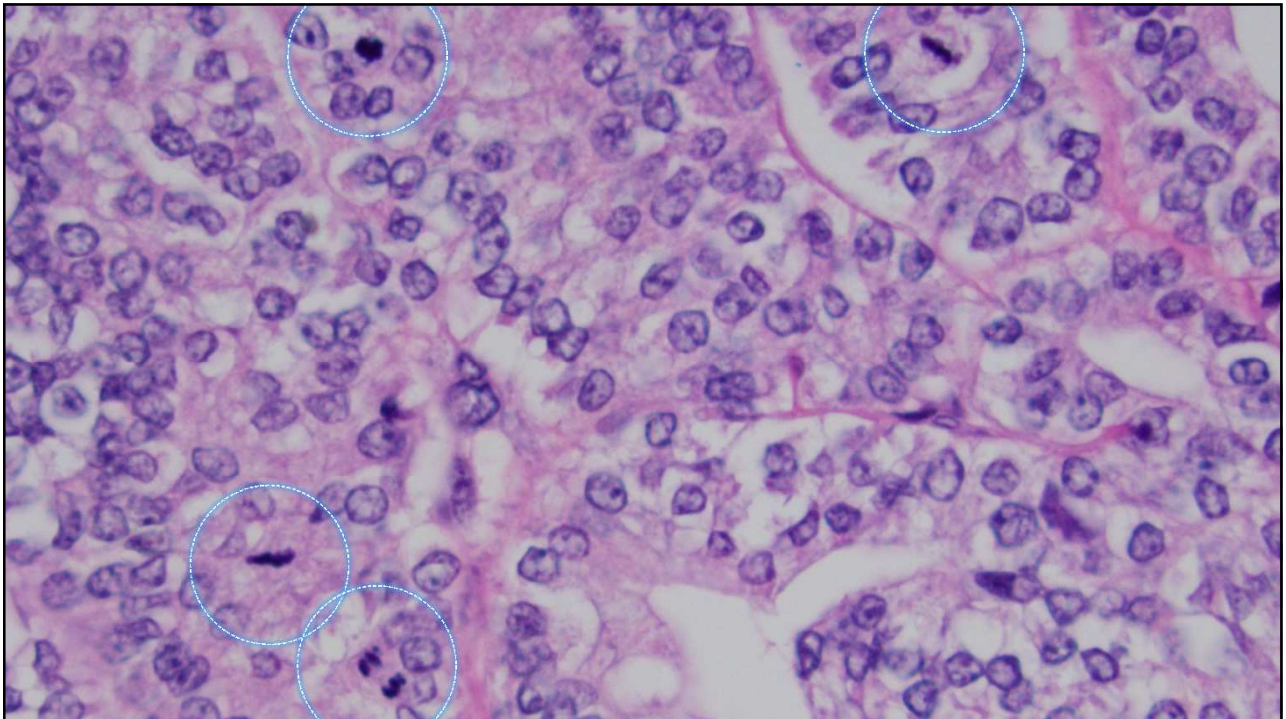
POORLY DIFFERENTIATED CARCINOMA, arising from oncocytic carcinoma (1.1 cm).

- Margins positive.
- Perineural invasion present.
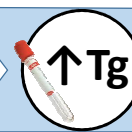- Lymphovascular invasion present.

Disease recurrence

Total thyroidectomy:
Poorly diff thyroid CA

Radioactive iodine

2 years

↑Lung nodules on serial imaging

↑Tg

↑serum thyroglobulin

Lung biopsy:
Metastatic PDTC
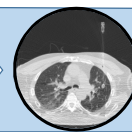
Emerging roles for molecular testing in thyroid FNA specimens

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Emerging* uses for thyroid FNA molecular testing
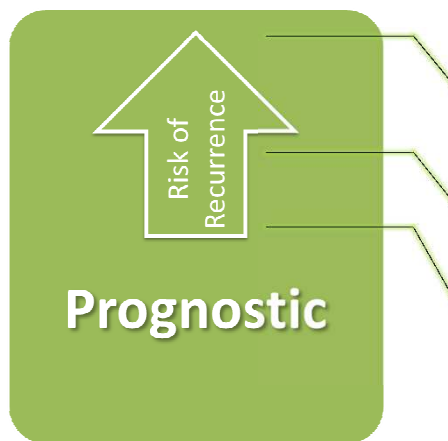
Prognostication of tumors based on molecular profiles

Prognostic — Risk of Recurrence
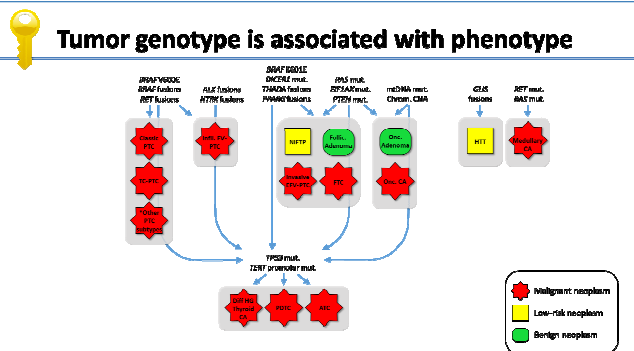
*for FNAs classified as Bethesda V or VI

Emerging* uses for thyroid FNA molecular testing

Systemic therapies or clinical trials tailored to molecular profile

BRAF V600E	Dabrafenib
RAS mutation	Selumetinib, Trametinib
mTOR mutation	Everolimus
RET fusion	Selpercatinib, Praseltinib
NTRK fusion	Larotrectinib, Repotrectinib, Entrectinib
ALK fusion	Crizotinib, Repotrectinib, Entrectinib
ROS1 fusion	Repotrectinib, Entrectinib

Therapeutic

*for FNAs classified as Bethesda V or VI

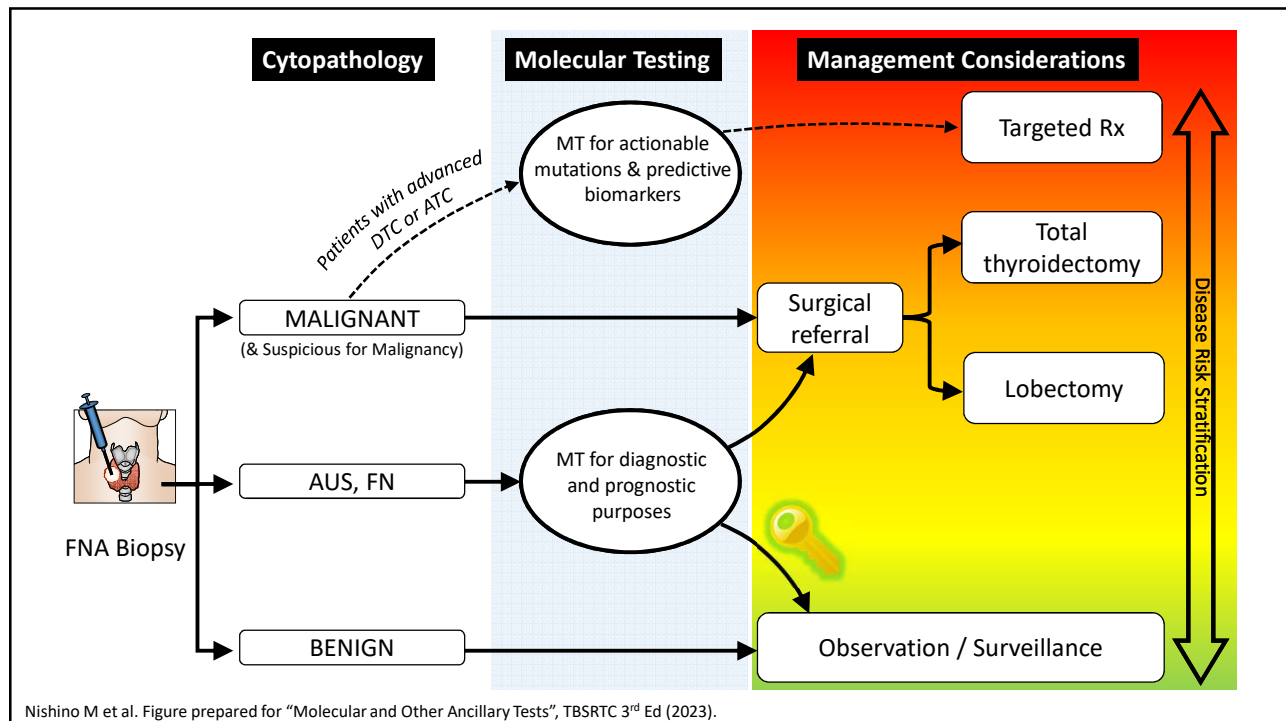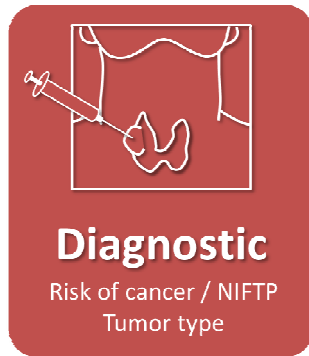# Emerging* uses for thyroid FNA molecular testing
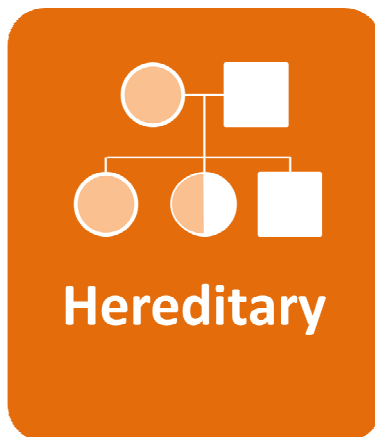
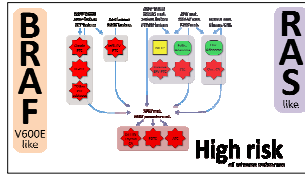**(Incidental) identification of germline mutations suggestive of hereditary cancer syndromes**

RET	Multiple Endocrine Neoplasia Type 2
PTEN	Cowden Syndrome
APC	Familial Adenomatous Polyposis
PRKAR1A	Carney Complex
DICER1	DICER1 Syndrome

Hereditary

*for FNAs classified as Bethesda V or VI

Current and emerging* uses for thyroid FNA molecular testing

Diagnostic
Risk of cancer / NIFTP
Tumor type

Nishino M et al. Figure prepared for "Molecular and Other Ancillary Tests", TBSRTC 3rd Ed (2023).
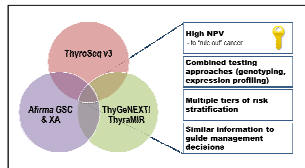
Summary

Key molecular changes

Purpose of molecular testing

Testing platforms