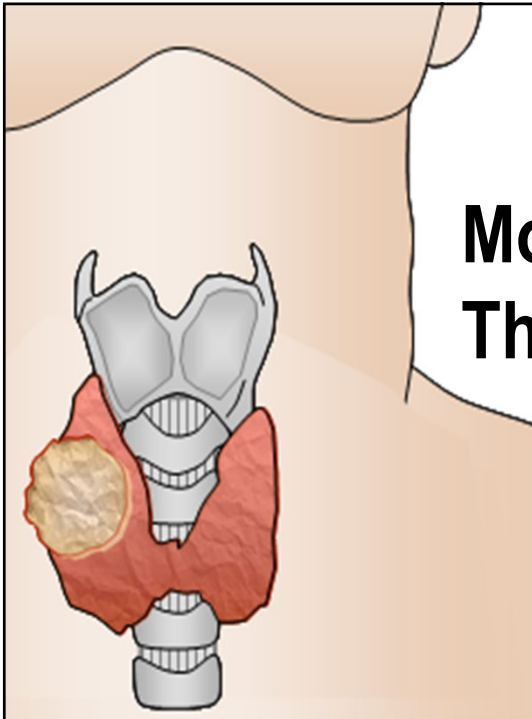




Molecular Testing in Thyroid FNA Specimens

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Program Director, Cytopathology Fellowship
Associate Professor of Pathology
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Beth Israel Lahey Health
Beth Israel Deaconess
Medical Center



HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

Objectives

- 1. Key molecular changes**
- 2. Purpose of molecular testing**
- 3. Testing platforms**

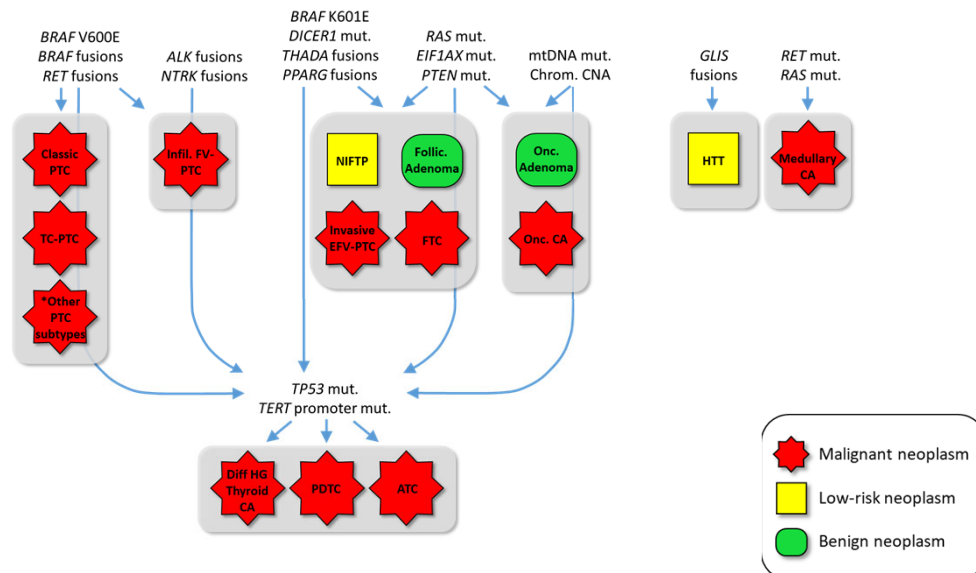
Objectives

1. Key molecular changes

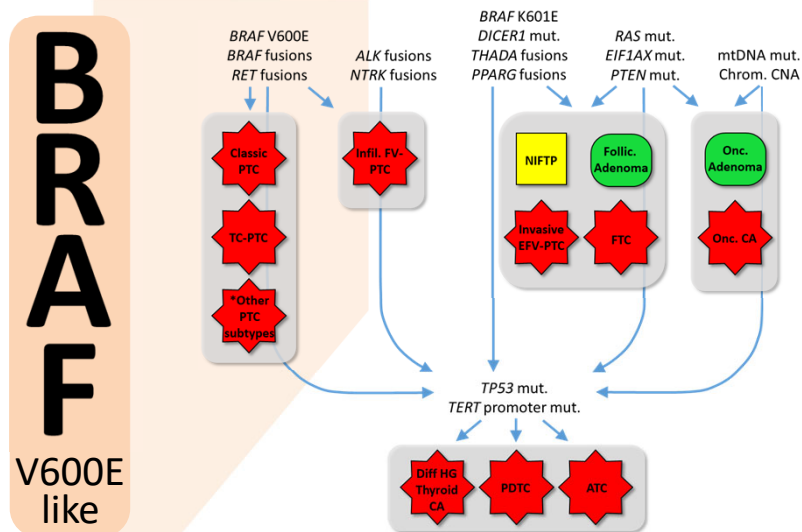
2. Purpose of molecular testing

3. Testing platforms

Tumor genotype is associated with phenotype



Tumor genotype is associated with phenotype



BRAF^{V600E}-like tumors

BRAF V600E mutation

RET fusions

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

BRAF fusions

NTRK1/3 fusions*



BRAF^{V600E}-like tumors

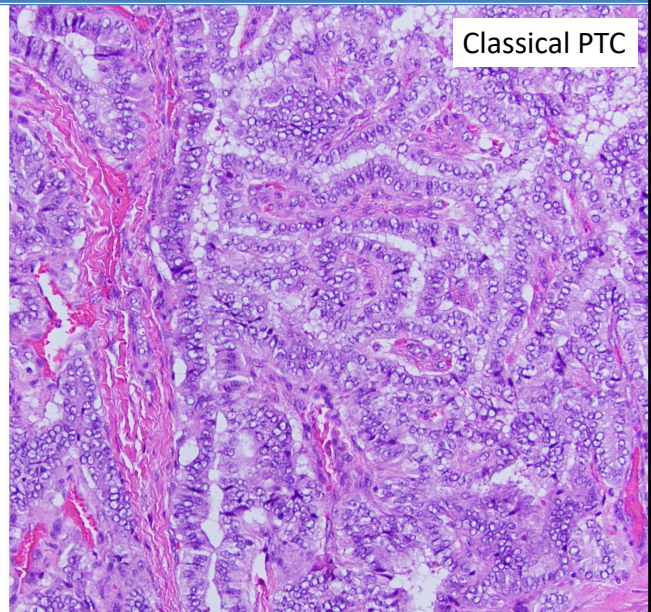
***BRAF* V600E** mutation

RET fusions

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BRAF fusions

NTRK1/3 fusions*



BRAF^{V600E}-like tumors

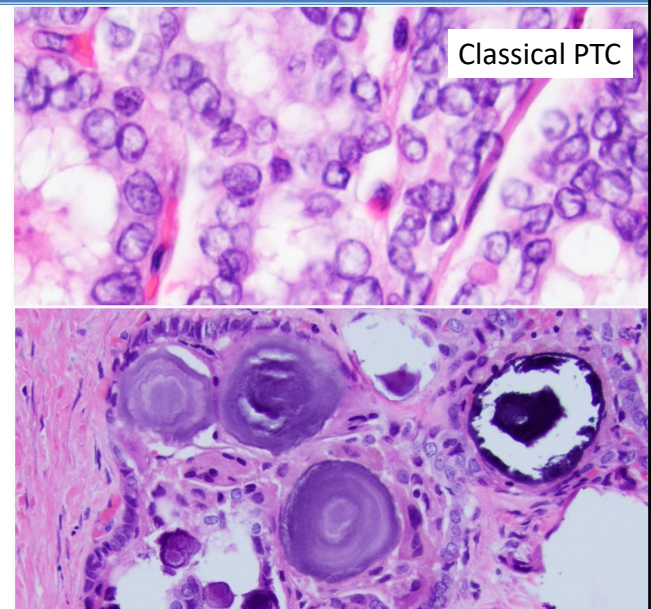
***BRAF* V600E** mutation

RET fusions

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BRAF^{V600E}-like tumors

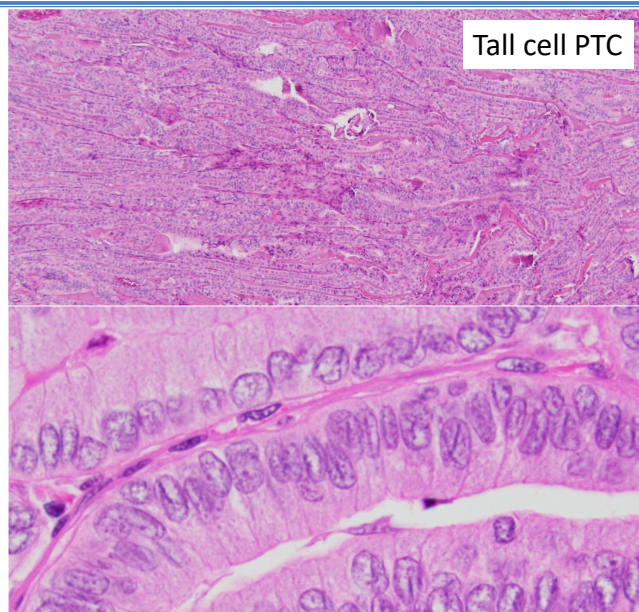
BRAF V600E mutation

RET fusions

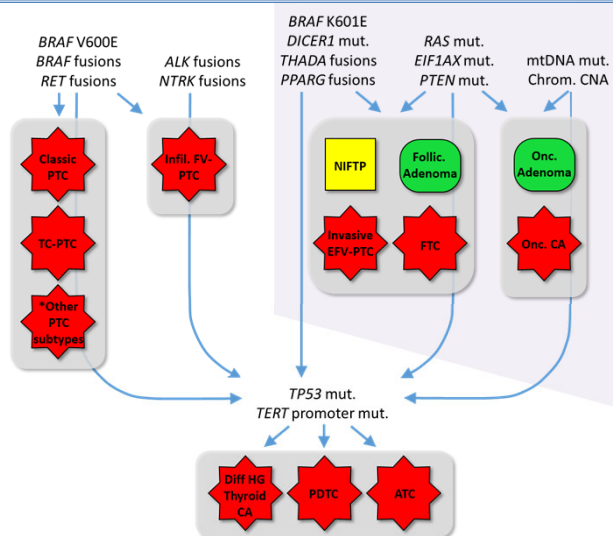
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- *NCOA4::RET* (RET-PTC3)

BRAF fusions

NTRK1/3 fusions*

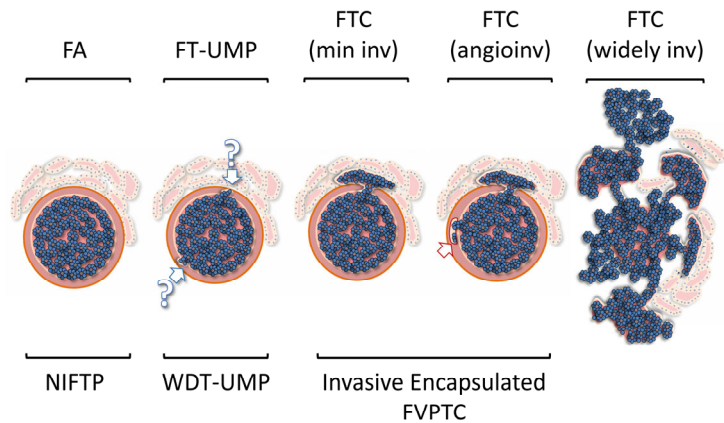


Tumor genotype is associated with phenotype



RAS-like tumors

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



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

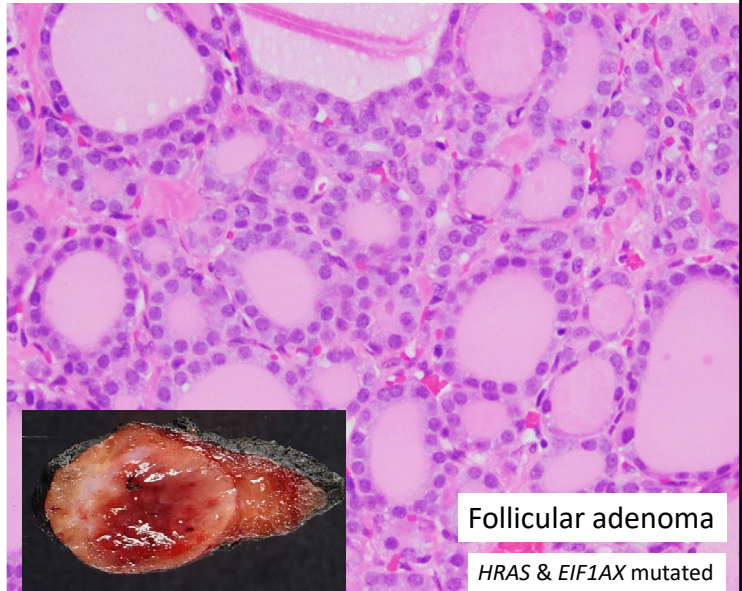


Follicular adenoma

HRAS & *EIF1AX* mutated

RAS-like tumors

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

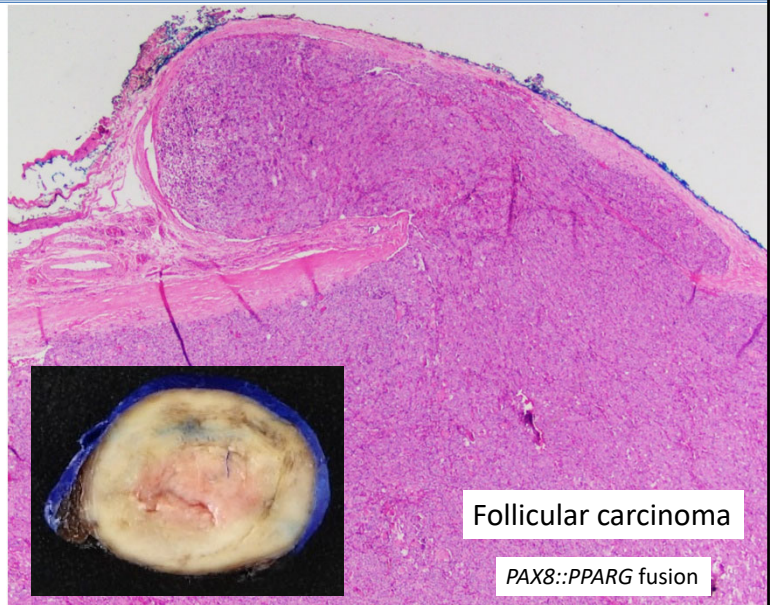


Follicular adenoma

HRAS & *EIF1AX* mutated

RAS-like tumors

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BRAF K601E mutation
DICER1 mutation
EIF1AX mutation
PTEN mutation
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THADA fusions

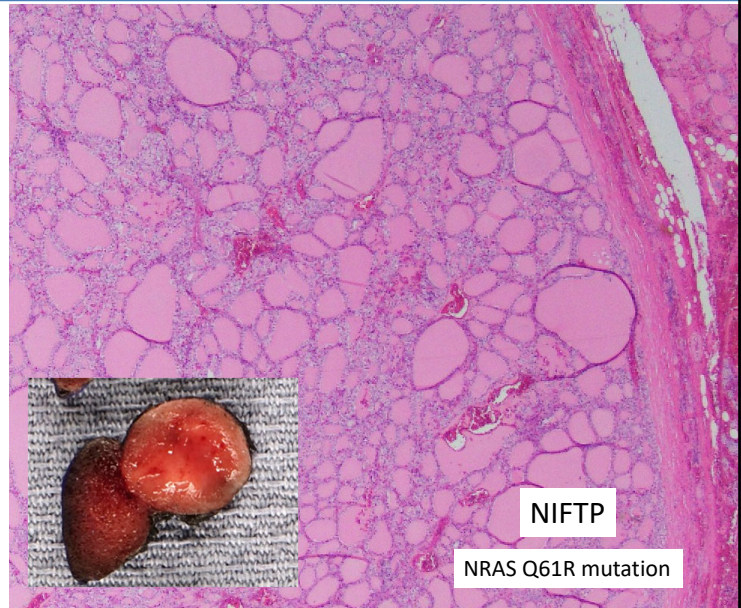


Follicular carcinoma

PAX8::PPARG fusion

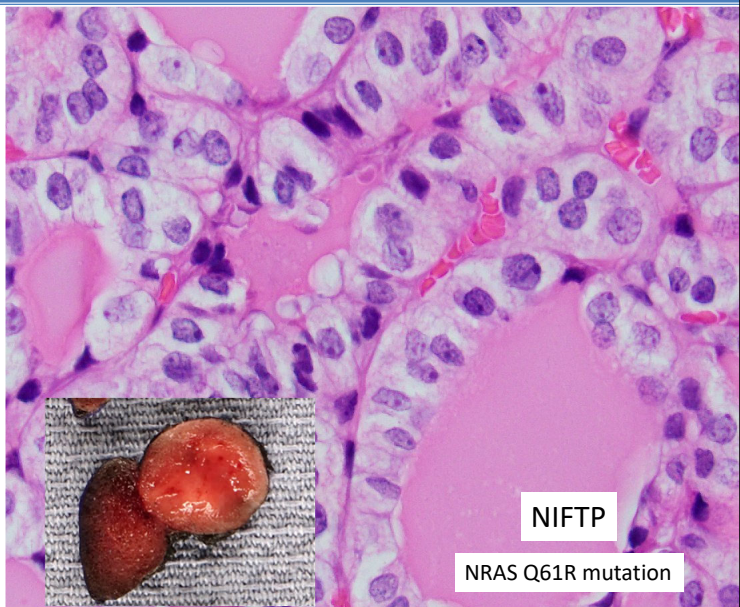
RAS-like tumors

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



RAS-like tumors

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





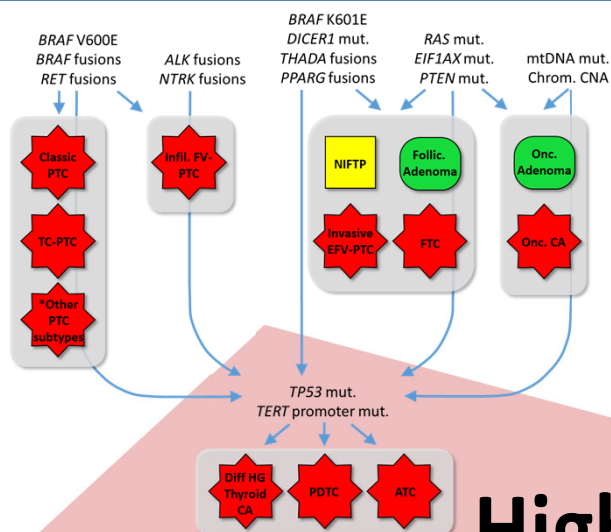
BRAF^{V600E}-like vs RAS-like tumors

Very high
probability of
cancer

Intermediate
probability of
cancer

	BRAF ^{V600E} -like	RAS-like
Mutations	<i>BRAF V600E</i>	<i>H/K/NRAS</i> <i>BRAF K601E</i> <i>DICER1</i> <i>EIF1AX</i> <i>PTEN</i>
Gene fusions	<i>RET</i> <i>BRAF</i> <i>NTRK1/3</i>	<i>PAX8::PPARG</i> <i>THADA</i>
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

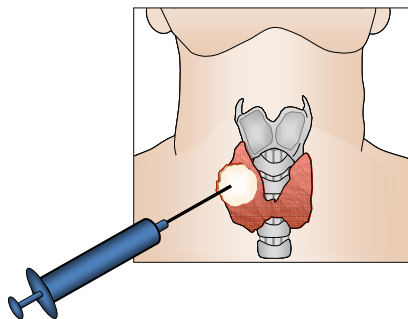


High risk
of adverse outcomes

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*

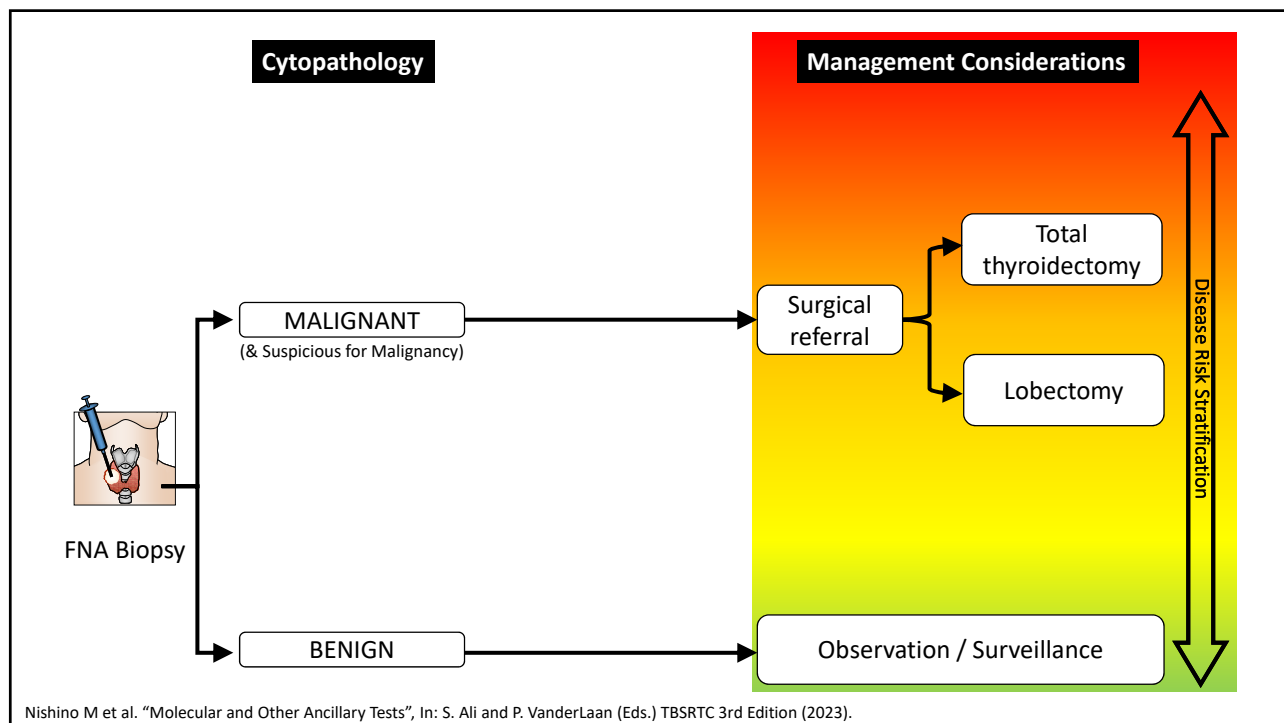
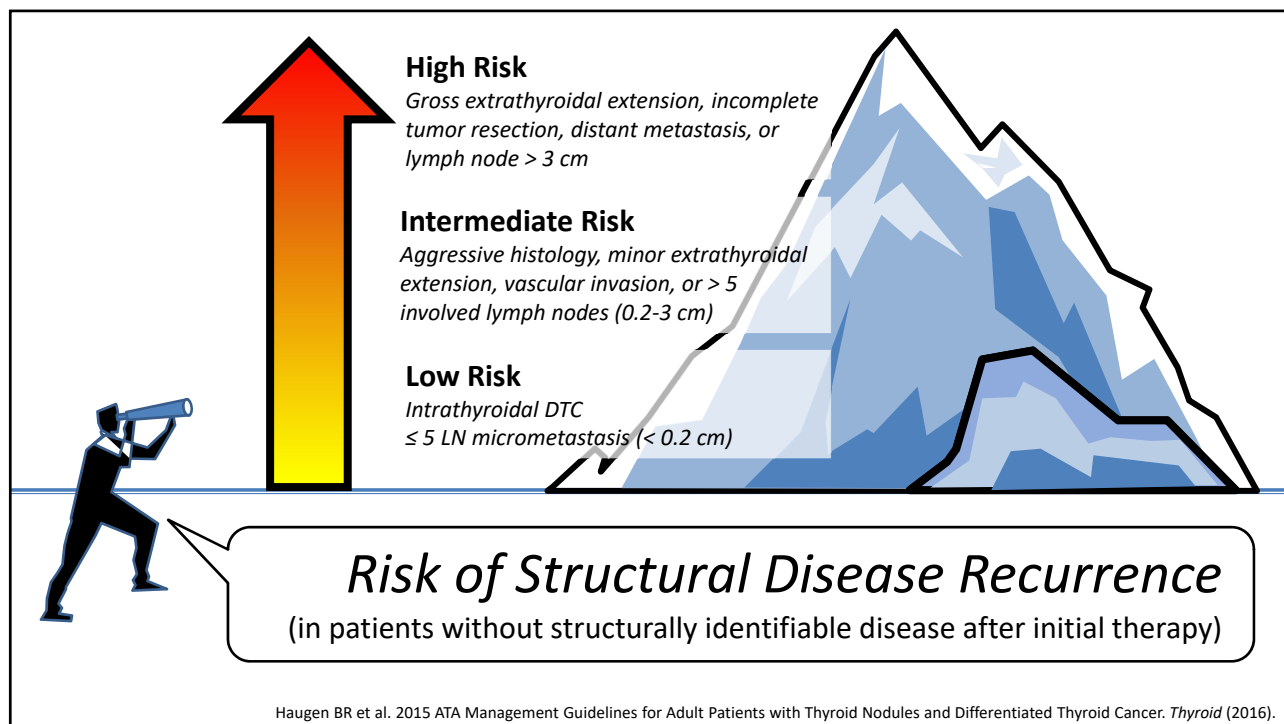


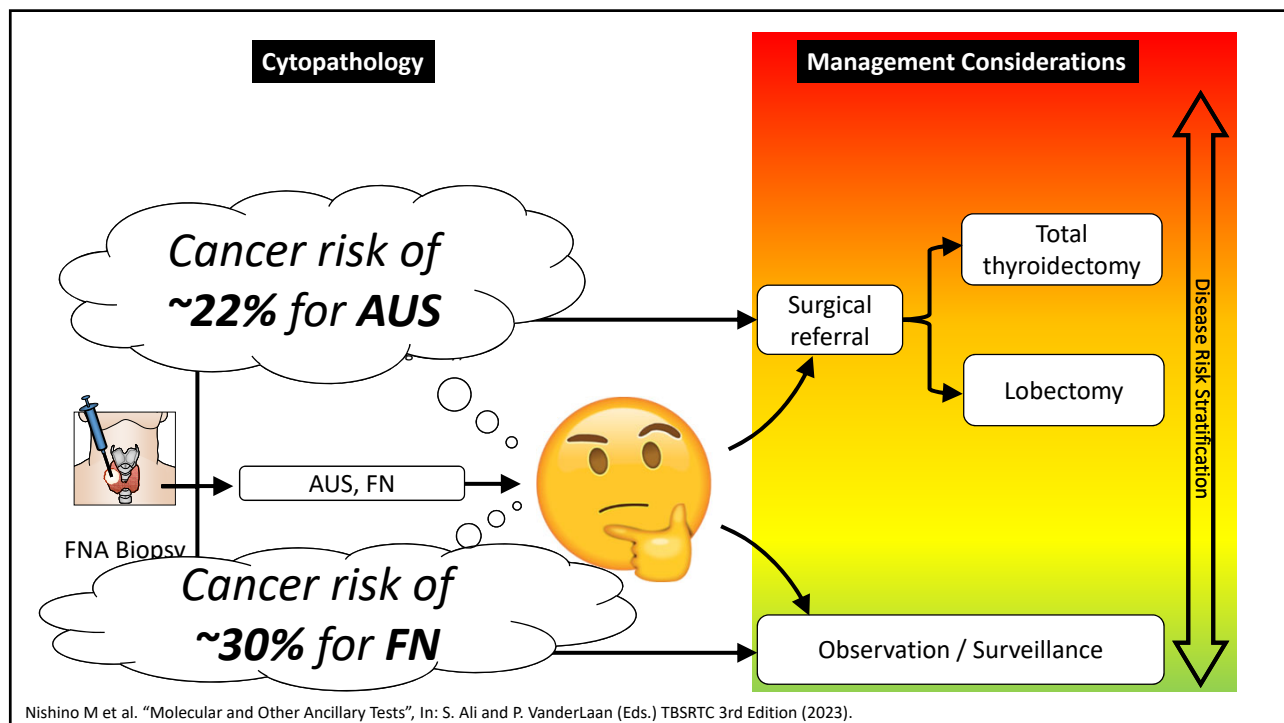
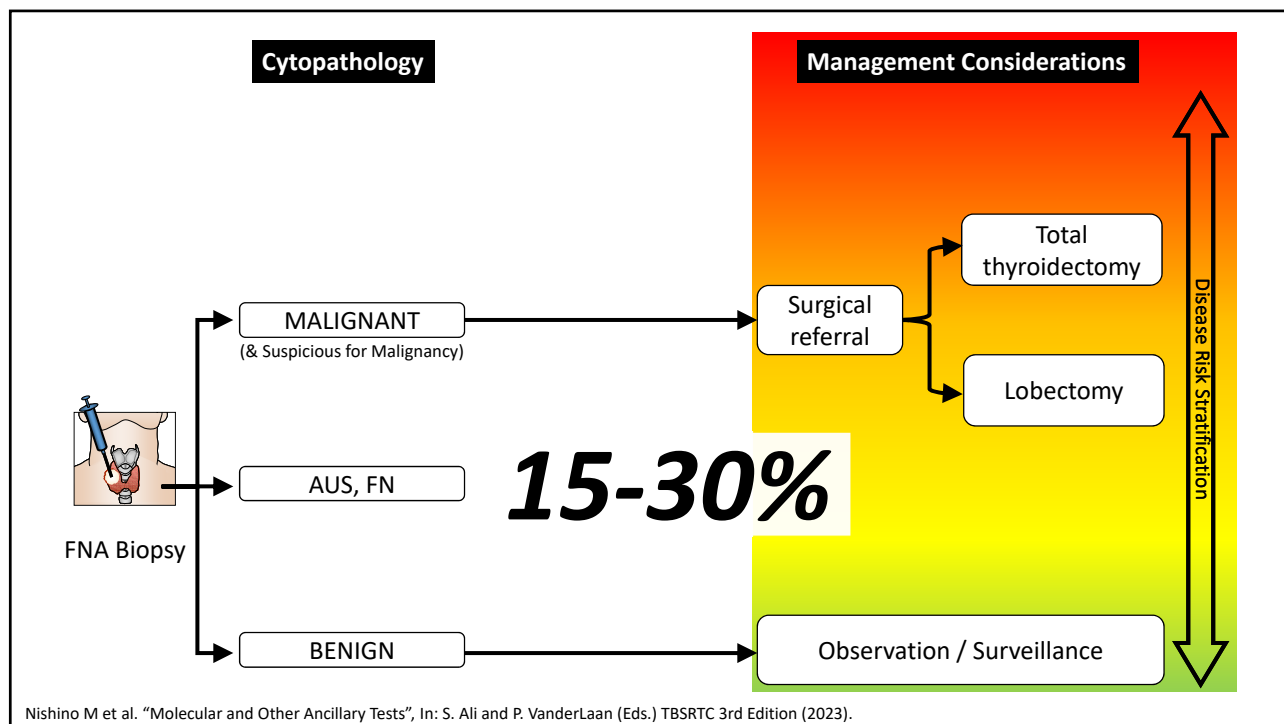
Risk of structural
disease recurrence

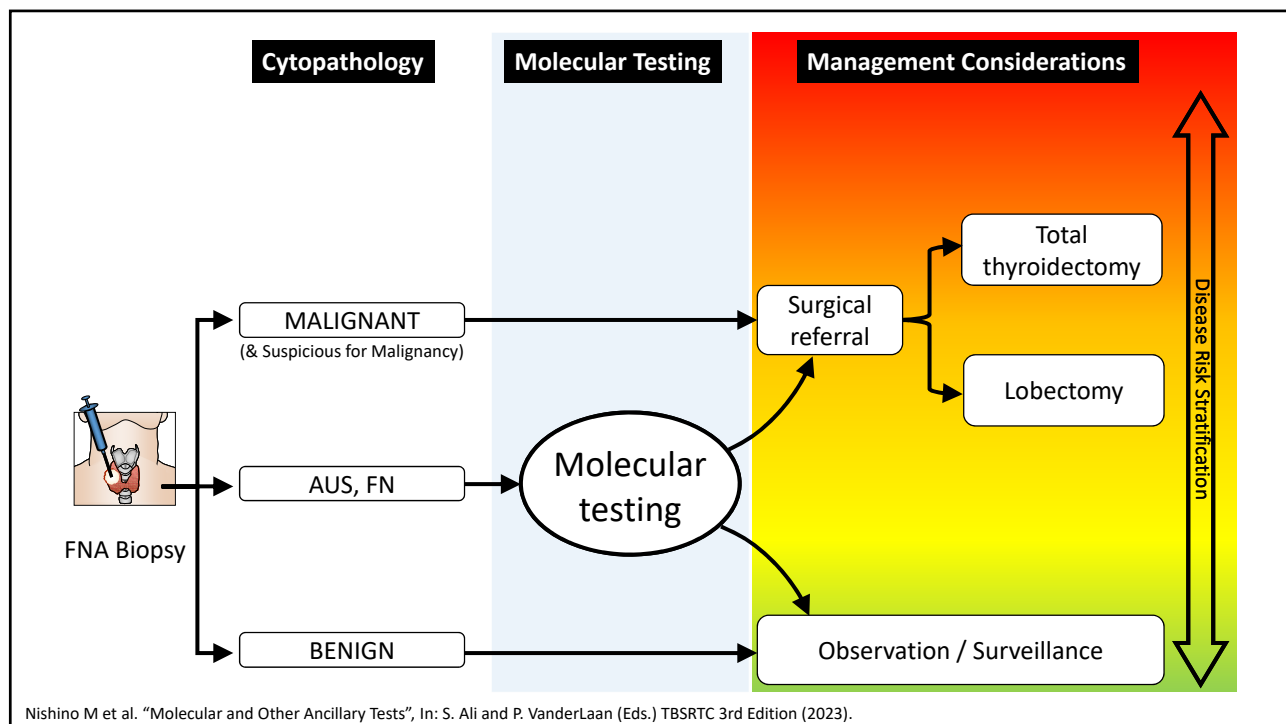


Bethesda System and *Risk of Malignancy*

FNA Diagnosis	Risk of CA or NIFTP (%)	Suggested Management
I. Nondiagnostic	13	Repeat FNA with U/S
II. Benign	4	Clinical & U/S surveillance
III. Atypia of Undetermined Significance	22	Repeat FNA, MT, or lobectomy, surveillance
IV. Follicular Neoplasm / Oncocytic Neoplasm	30	MT or lobectomy
V. Suspicious for Malignancy	74	MT, lobectomy, or near-total thyroidectomy
VI. Malignant	97	Lobectomy or near-total thyroidectomy



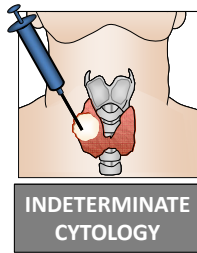




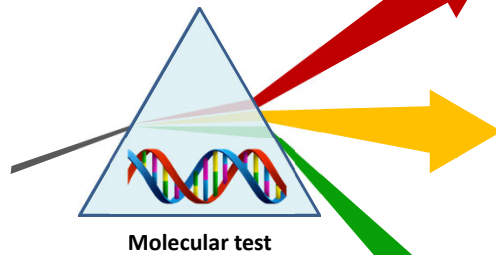
Objectives

1. Key molecular changes
2. Purpose of molecular testing
- 3. Testing platforms**

Molecular tests help risk-stratify cytologically indeterminate nodules



(AUS, FN)



High probability for malignancy
(Consider lobectomy vs total thyroidectomy)

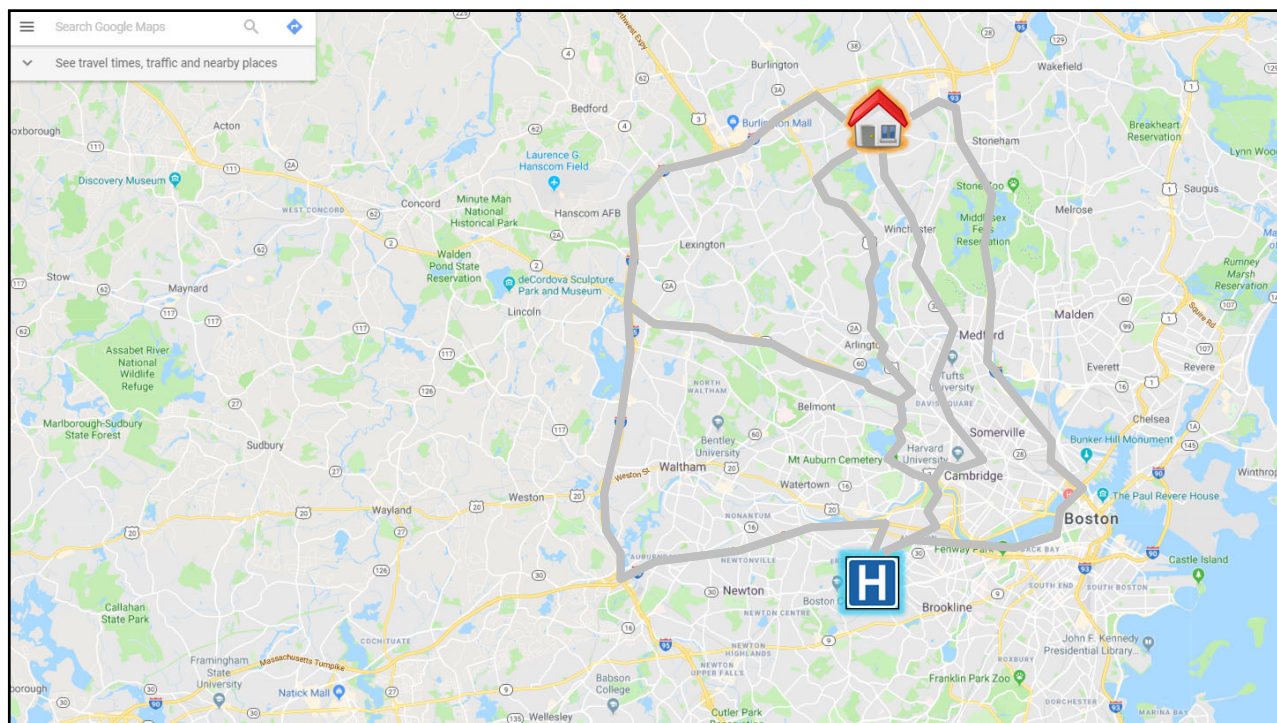
Intermediate probability
(Consider lobectomy)

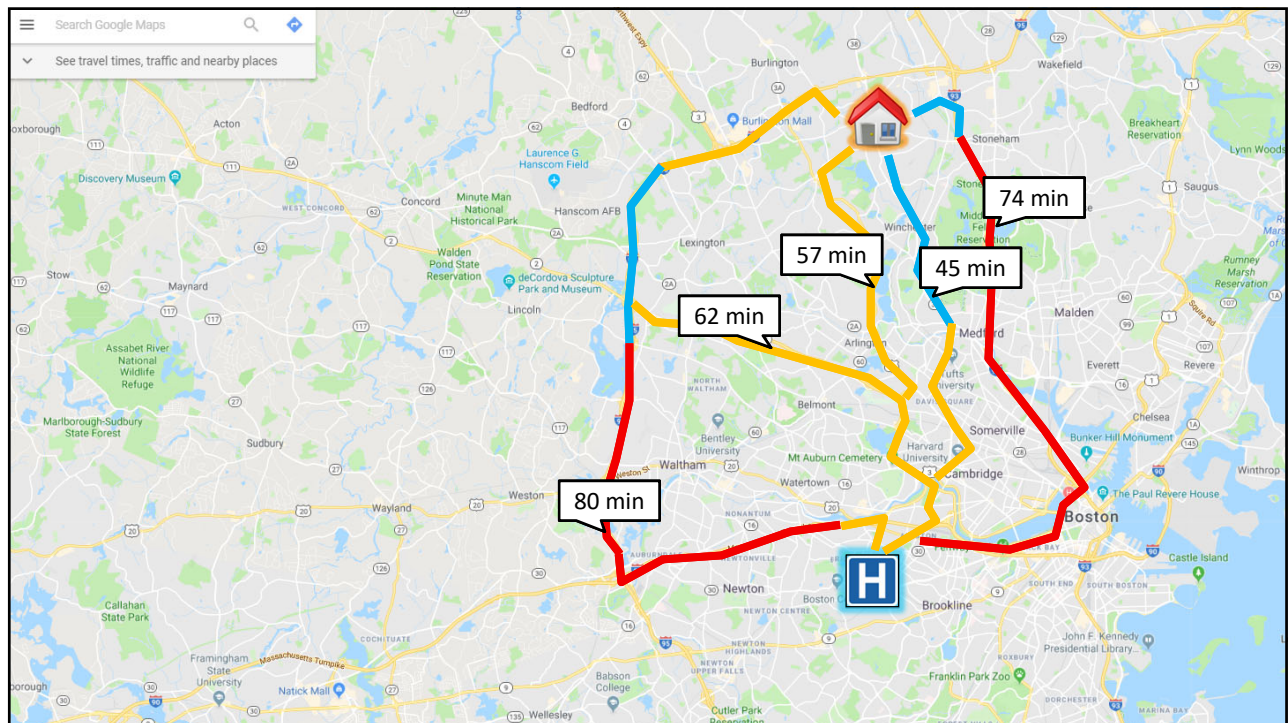
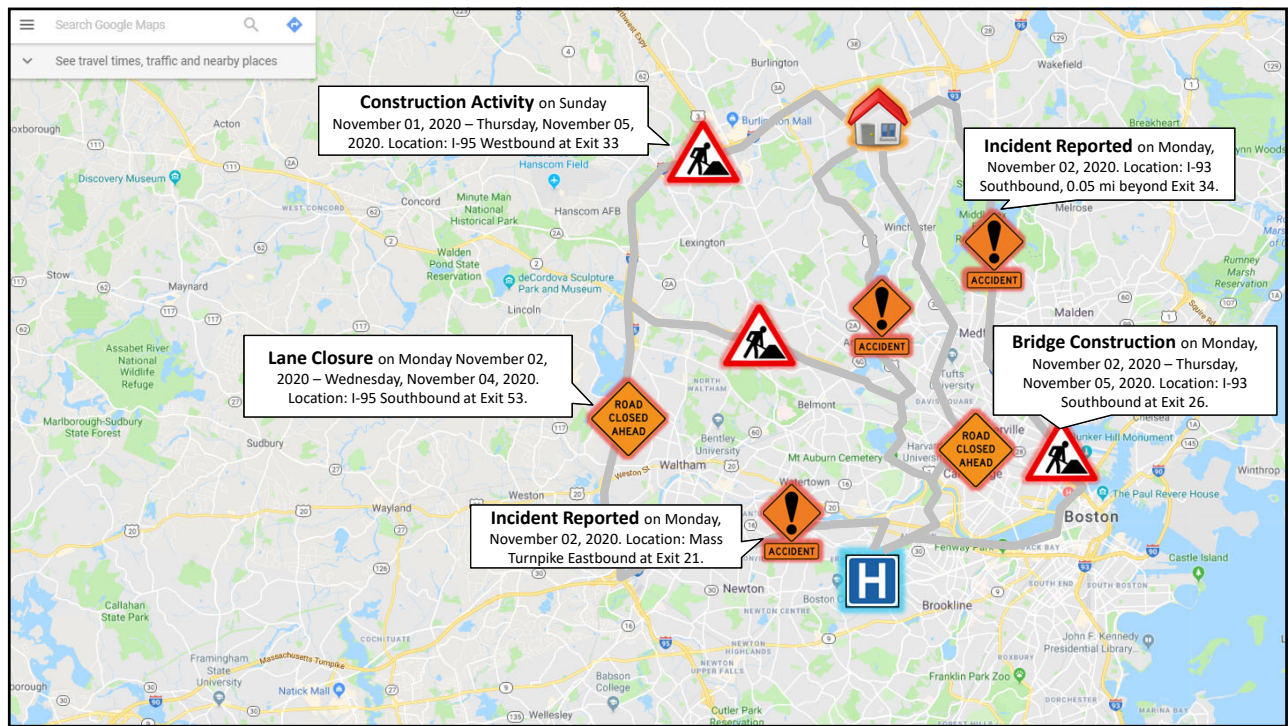
Low probability for malignancy
(Risk of malignancy <5%, safe to follow clinically)

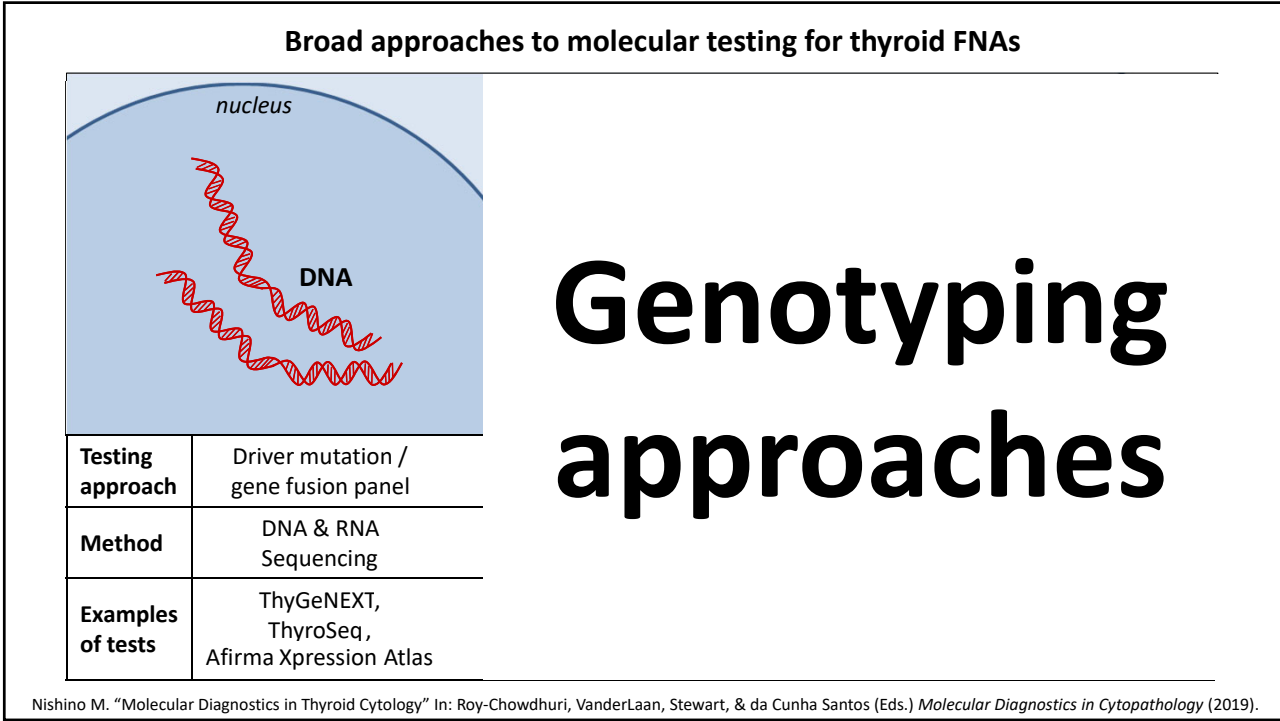
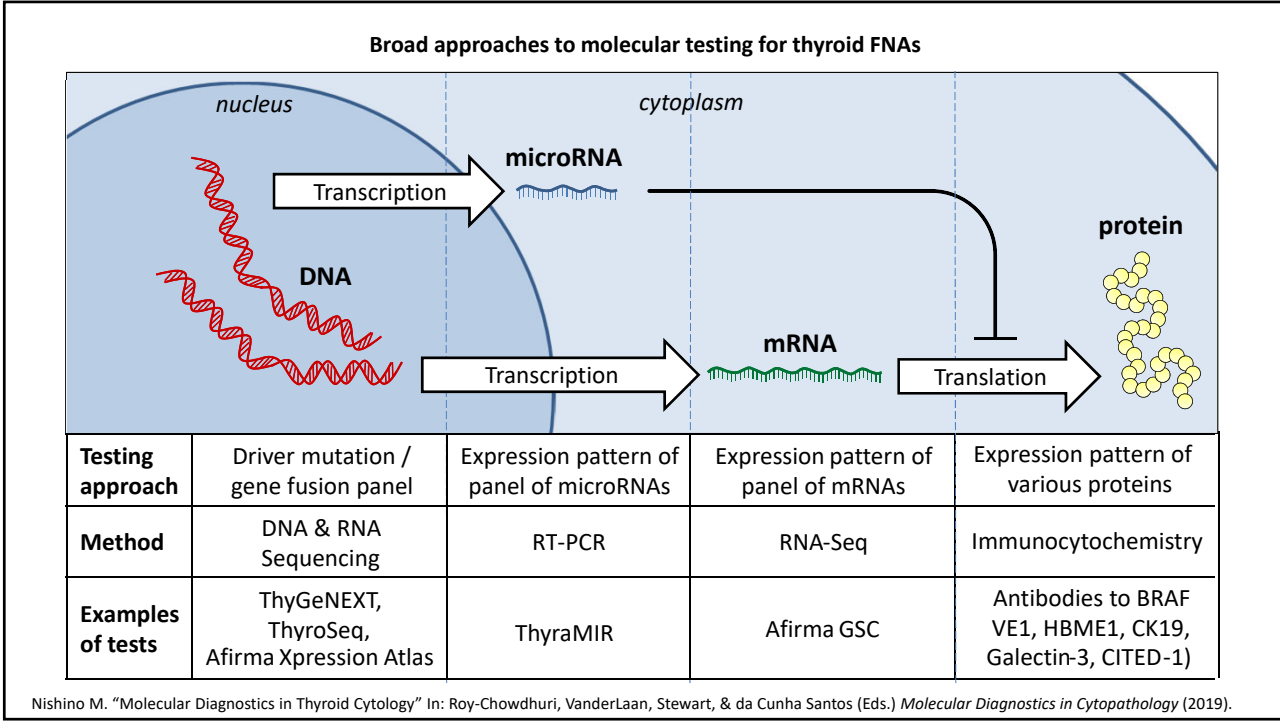
Nishino M, Bellevicine C, Baloch Z. J. *Mol Pathol* 2021, 2(2):135-146.

Molecular tests for cytologically indeterminate thyroid nodules in the USA

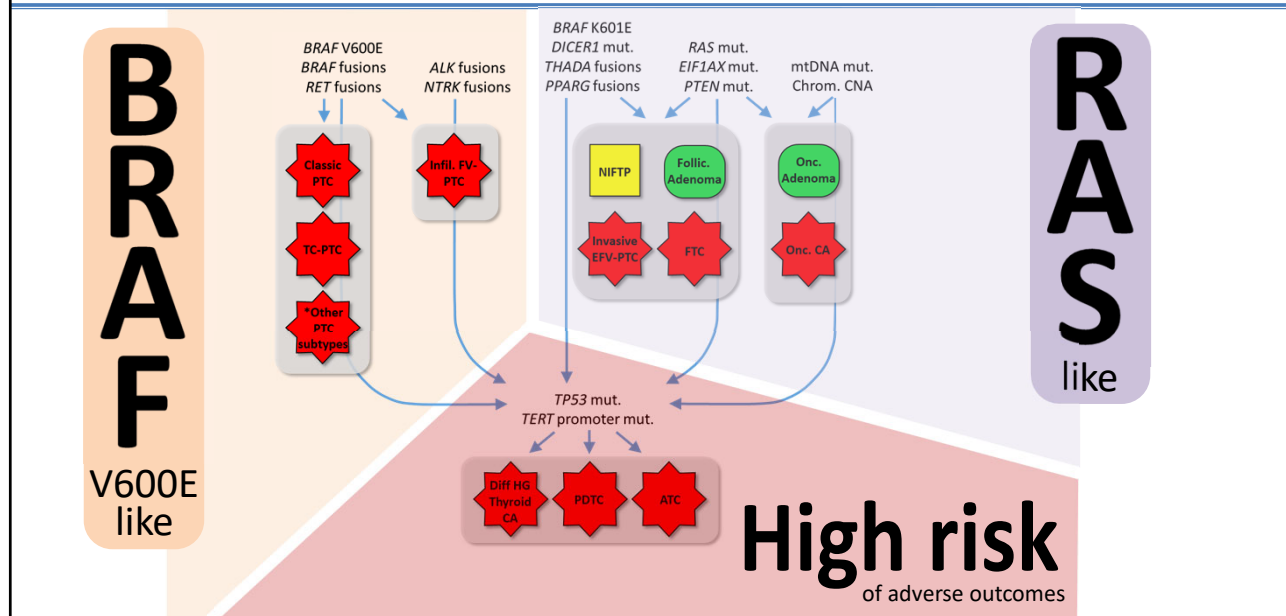
- **ThyroSeq GC** (UPMC / Sonic Healthcare USA)
- **ThyGeNEXT / ThyraMIR** (Interpace Diagnostics)
- **Afirma GSC** (Veracyte)



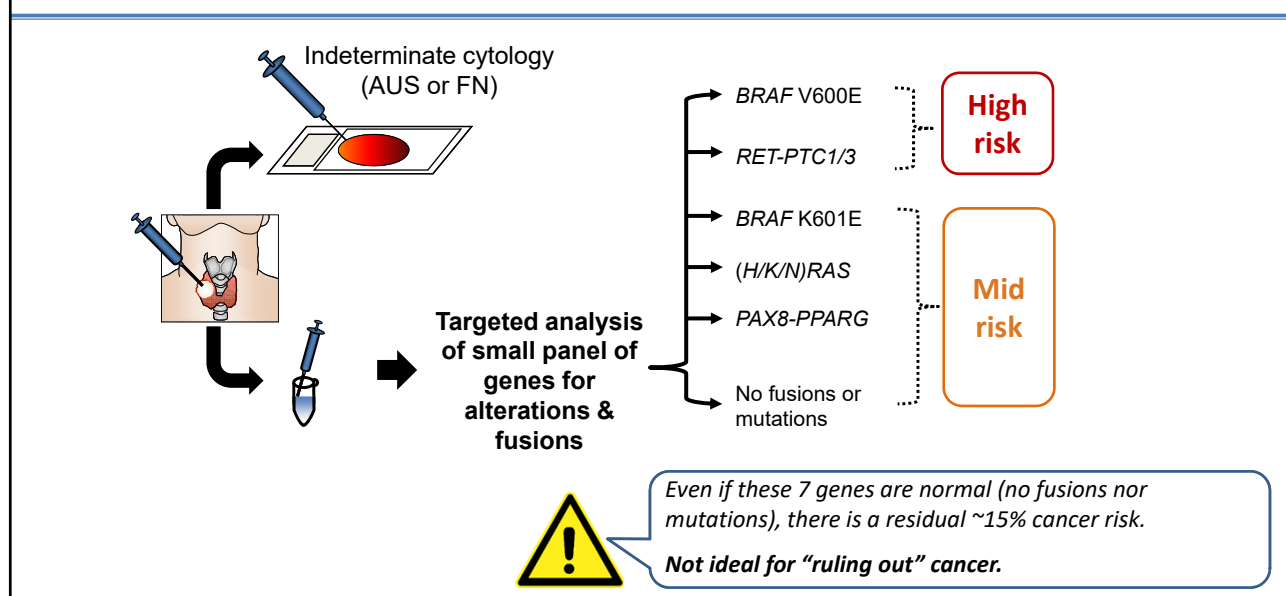




Tumor genotype is associated with phenotype



Early mutation/fusion panels



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

ThyGeNEXT / ThyraMIR

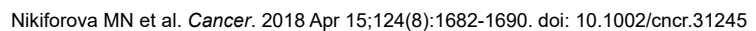
Combines the mutation / fusion panel with a ***11-miRNA expression classifier*** with high NPV

ThyroSeq

Vastly broadens the mutation / fusion panel to increase the sensitivity of the test

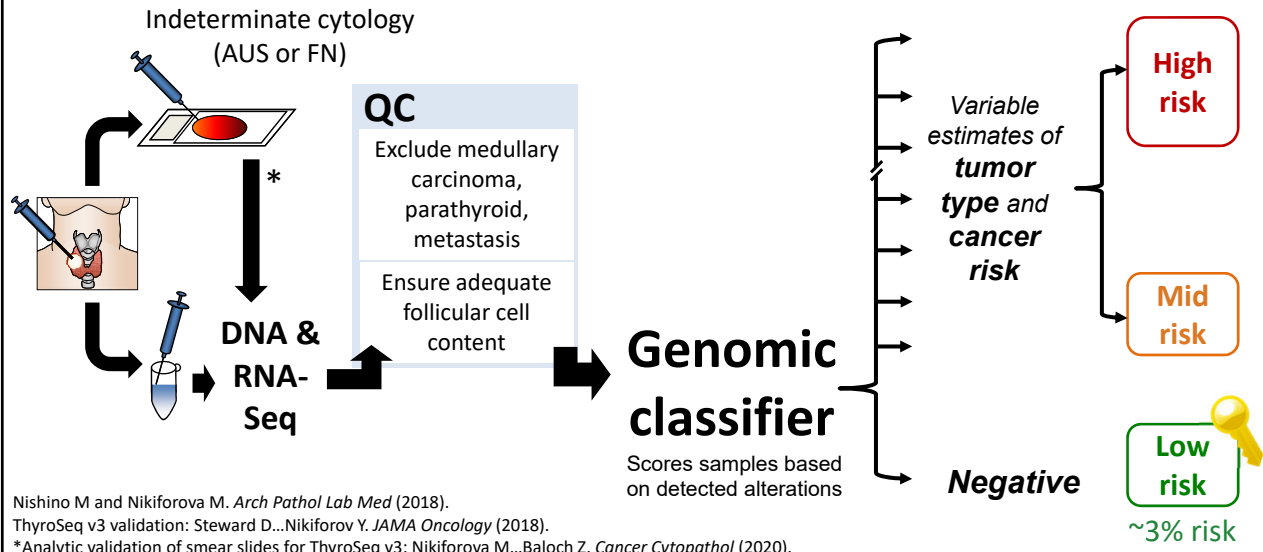
ThyroSeq

ThyroSeq v3



Nikiforova MN et al. *Cancer*. 2018 Apr 15;124(8):1682-1690. doi: 10.1002/cncr.31245

ThyroSeq v3



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

ThyGeNEXT / ThyraMIR

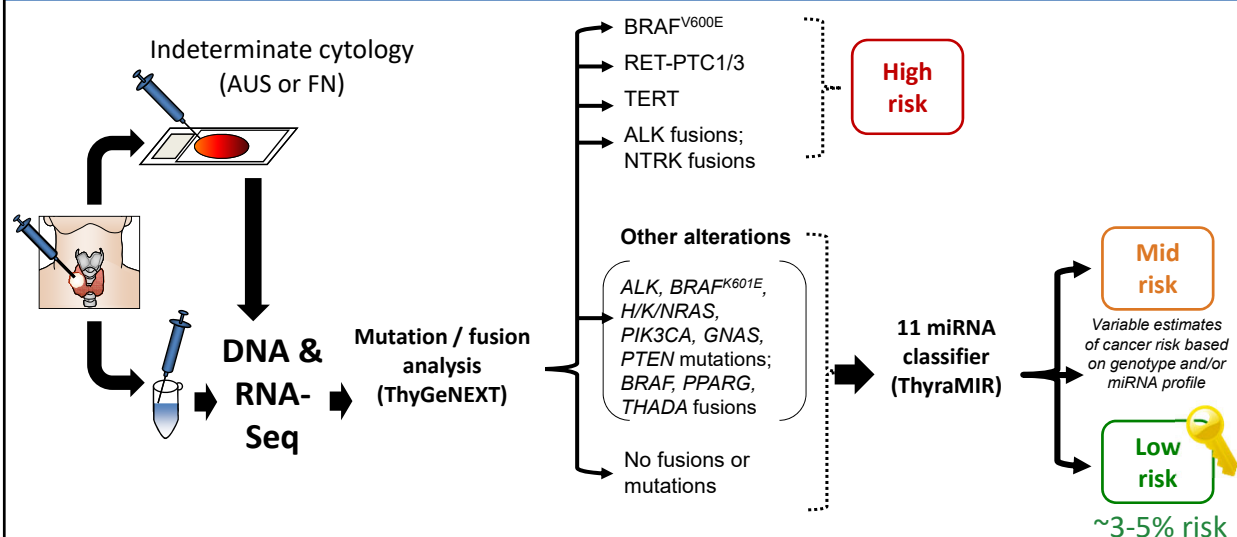
Combines the mutation / fusion panel with a **11-miRNA expression classifier** with high NPV

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ThyGeNEXT / ThyraMIR

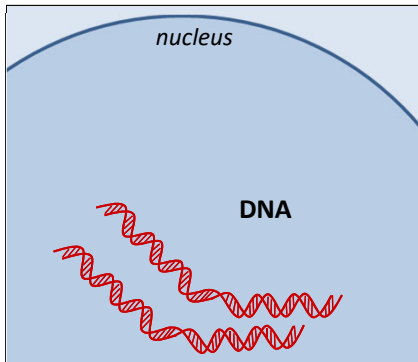
ThyGeNEXT / ThyraMIR



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

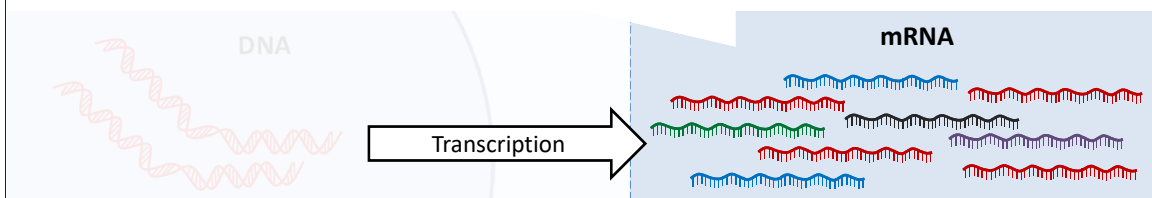


Genotyping approaches

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

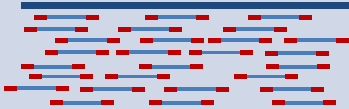
Broad approaches to molecular testing for thyroid FNAs

Gene-expression approaches

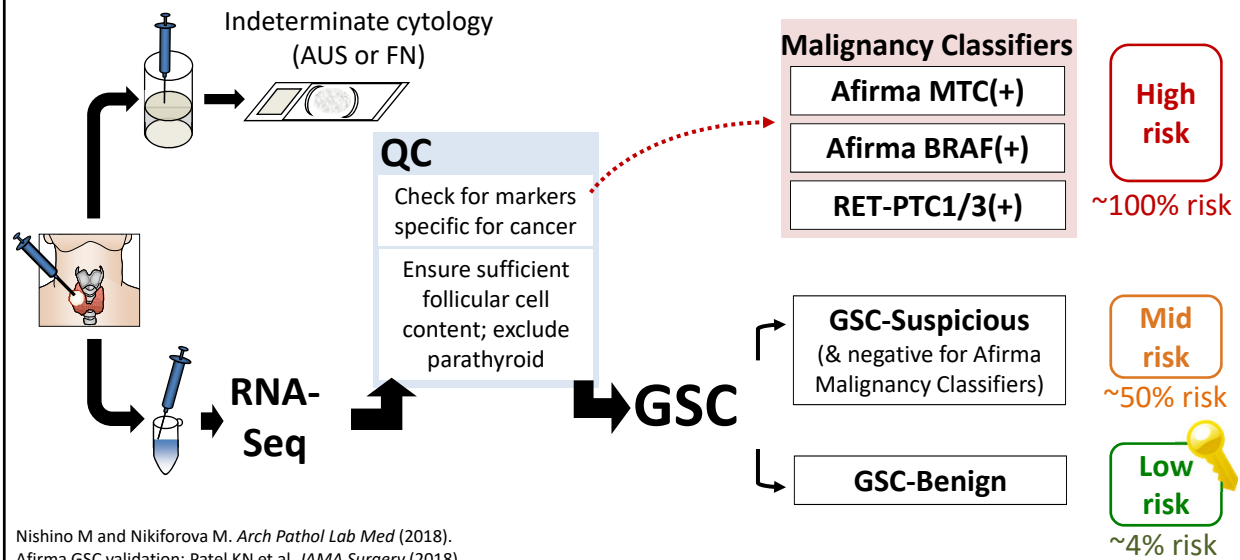


Testing approach	Driver mutation / gene fusion panel	Expression pattern of panel of mRNAs
Method	DNA & RNA Sequencing	RNA-Seq
Examples of tests	ThyGeNEXT, ThyroSeq, Afirma Xpression Atlas	Afirma GSC

Afirma GSC

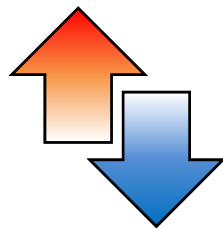
Afirma Gene Sequencing Classifier (GSC)	
Platform	High-throughput sequencing of RNA (RNA-Seq) 
Measures expression pattern of . . .	1,115 “core” genes (10,196 genes analyzed overall)
Analysis by . . .	Machine-learning algorithms that classify samples in a binary manner: “Benign” vs. “Suspicious”

Afirma Gene Sequencing Classifier (GSC)



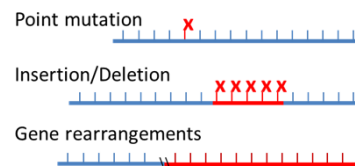
Afirma's RNA sequencing provides *expression and sequence* data

Gene expression levels



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

Sequencing data

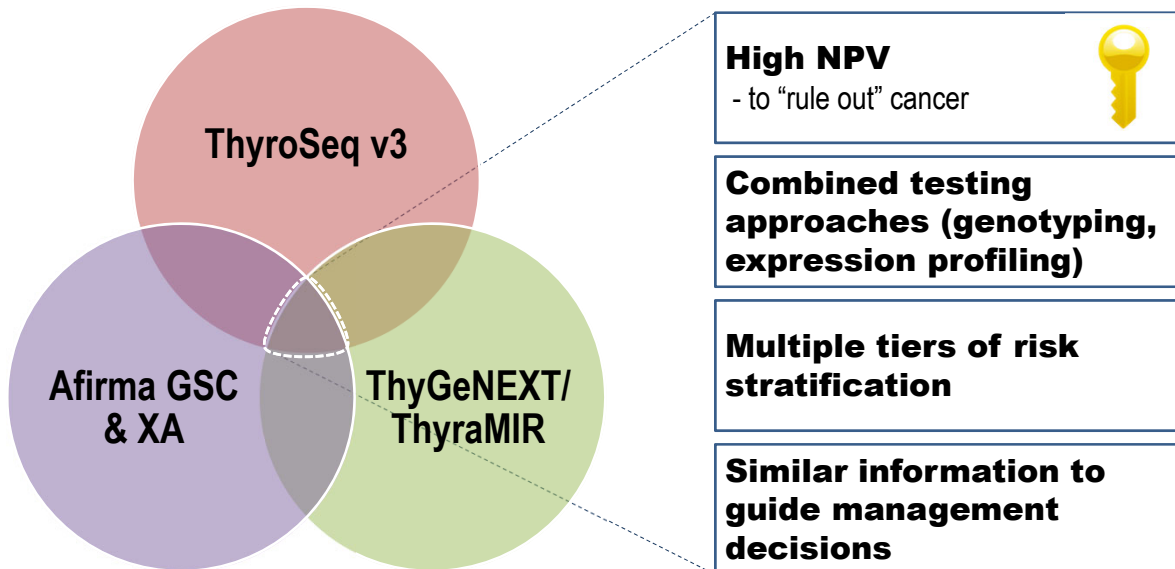


- 761 variants (including BRAF mutations)
- 130 fusions (including RET-PTC1/3*)

Xpression Atlas

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

Common themes . . .



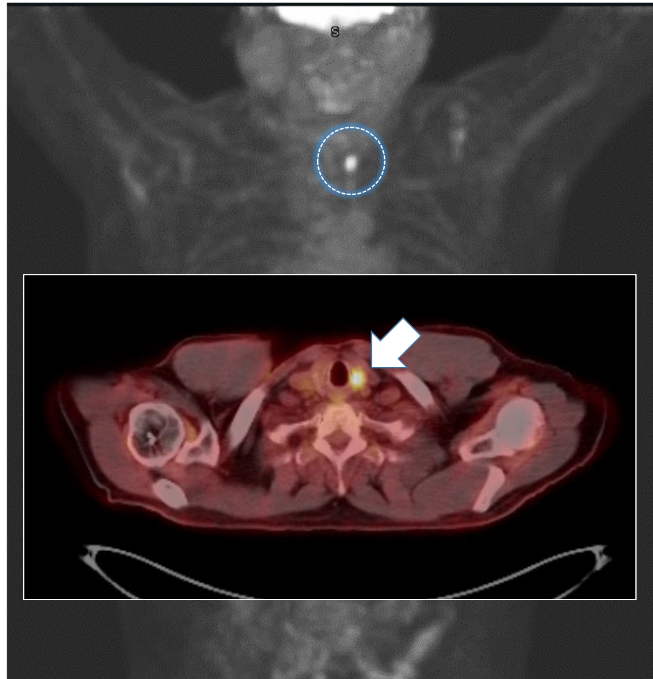
Case Study

70s / M

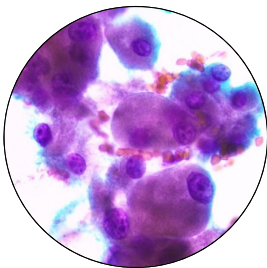
L thyroid nodule

Incidentally found on PET imaging for lung nodule.

U/S: 1.3 cm, hypoechoic, solid



FNA & ThyroSeq testing



**Follicular Neoplasm,
Oncocytic Type
(Bethesda IV)**

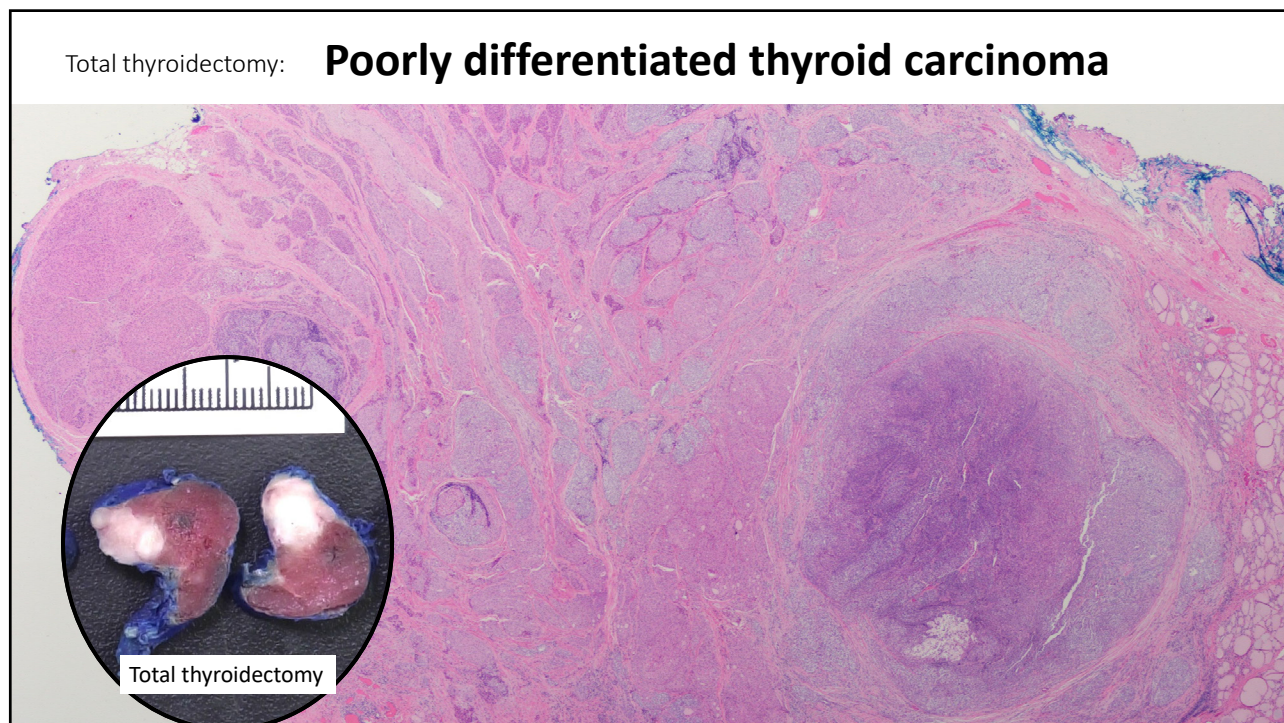
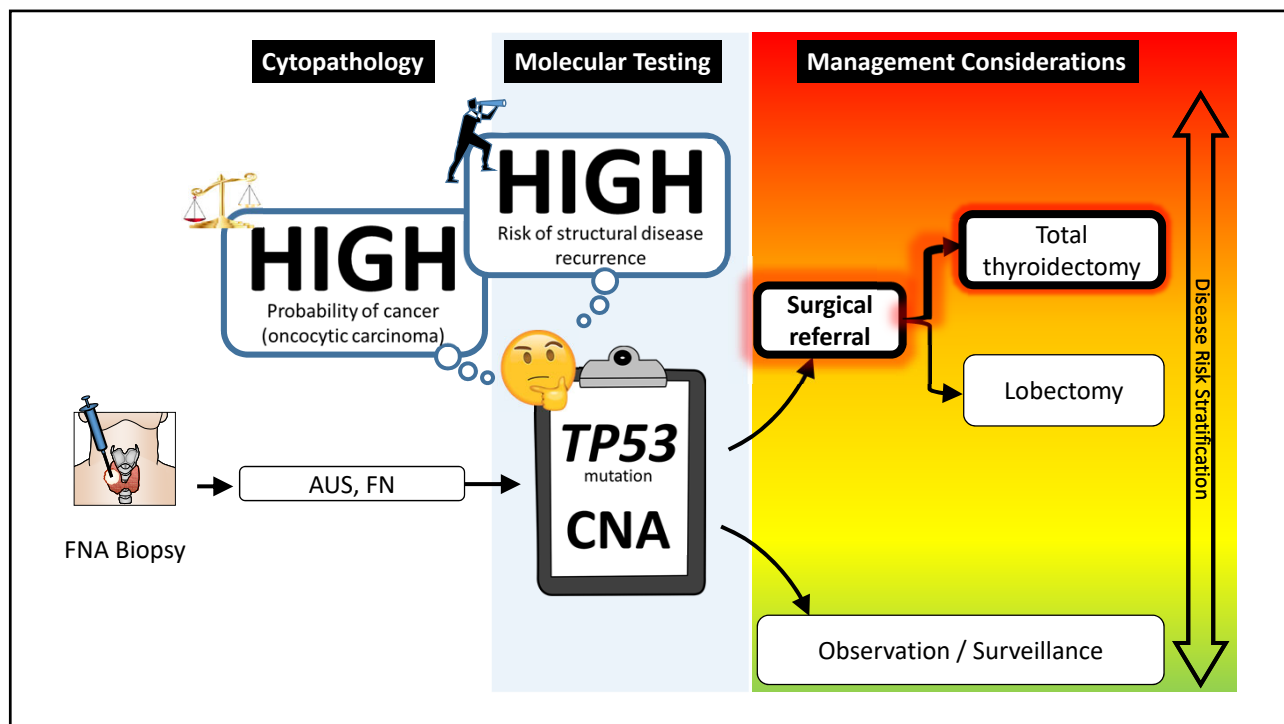
THYROSEQ[®] V3 GC RESULTS SUMMARY

LEFT MID THYROID FNA

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

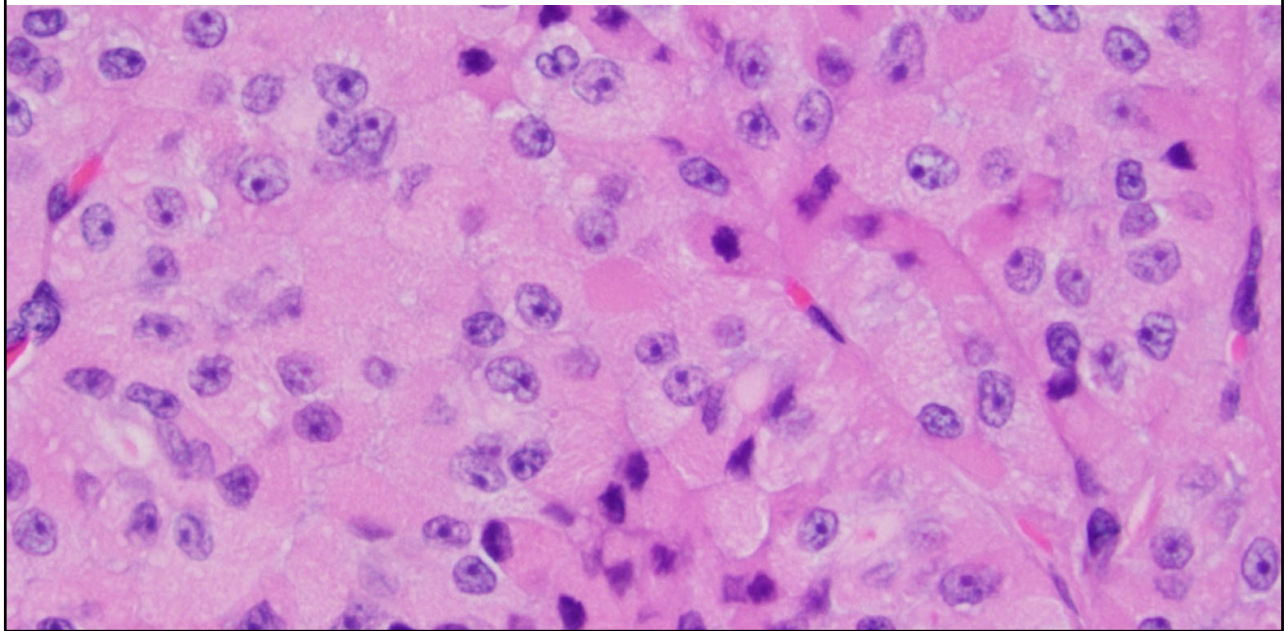
INTERPRETATION

- 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 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.



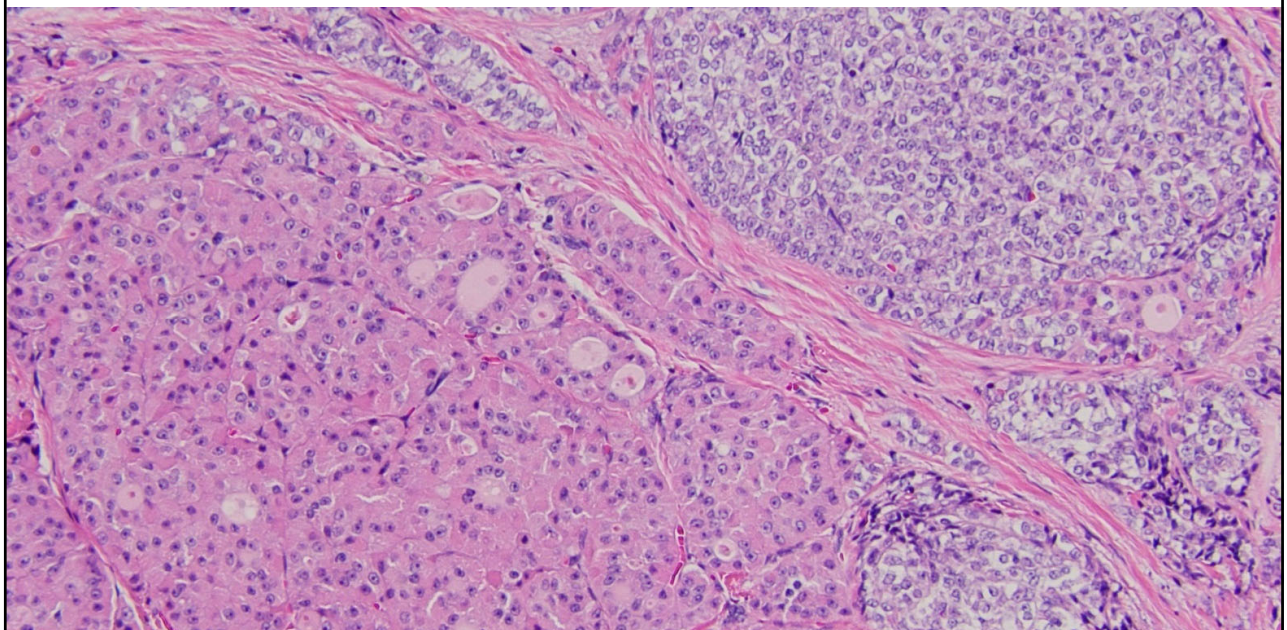
Total thyroidectomy:

Poorly differentiated thyroid carcinoma



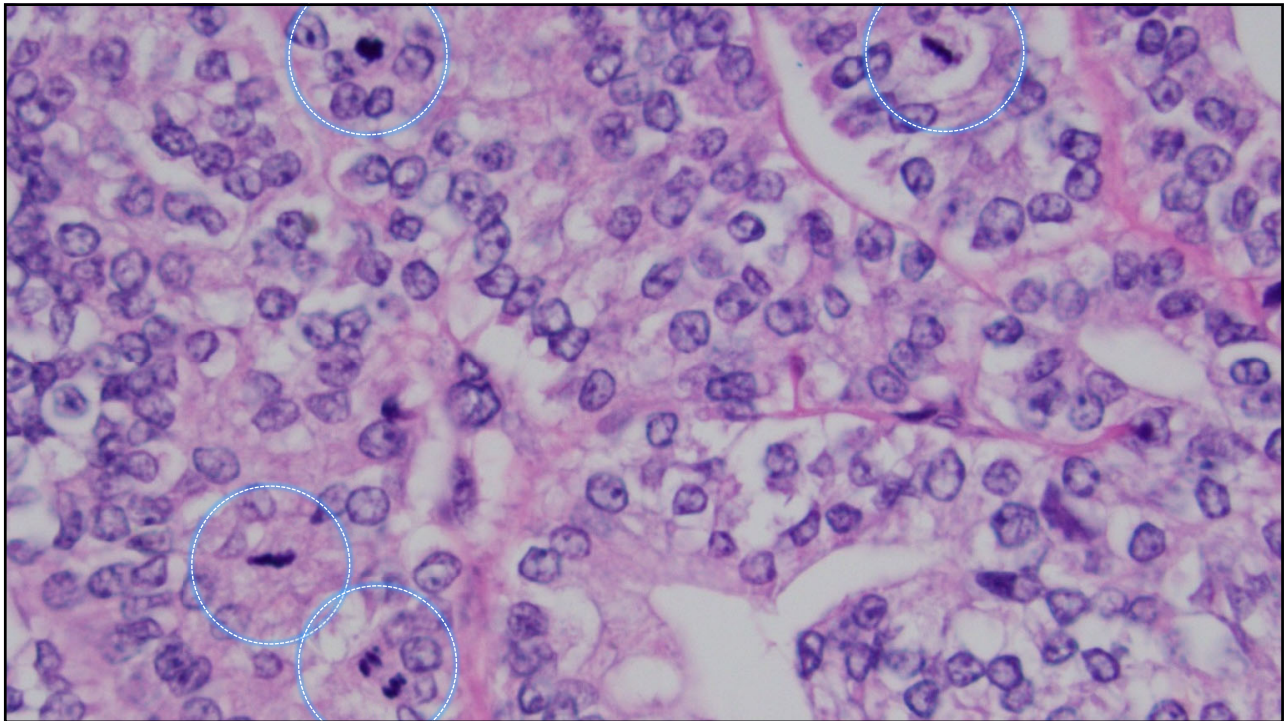
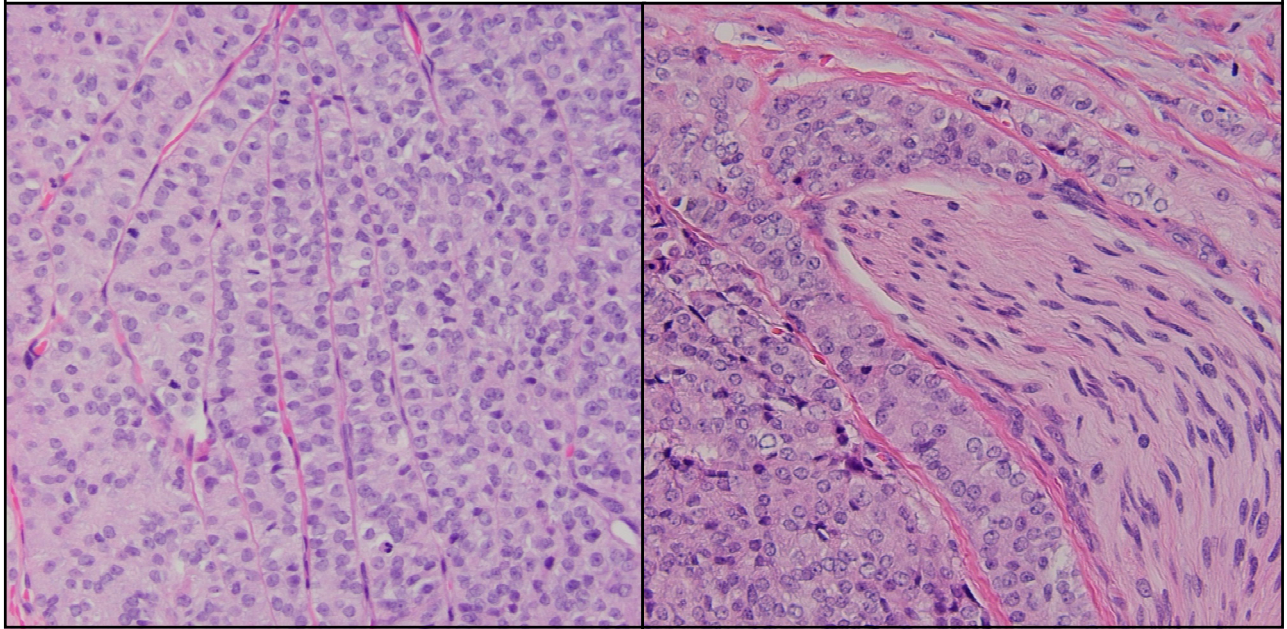
Total thyroidectomy:

Poorly differentiated thyroid carcinoma



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

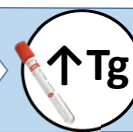
Total thyroidectomy:
Poorly diff thyroid CA



Radioactive
iodine

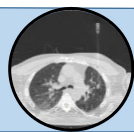
2
years

↑Lung nodules
on serial imaging



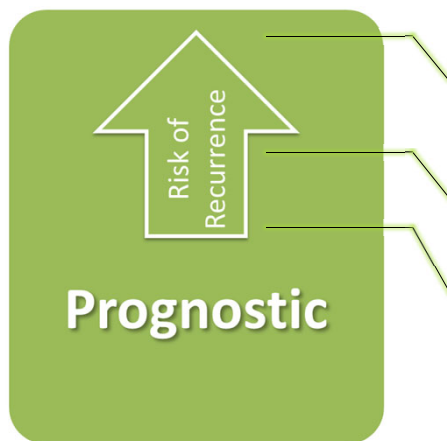
↑serum
thyroglobulin

Lung biopsy:
Metastatic PDTC

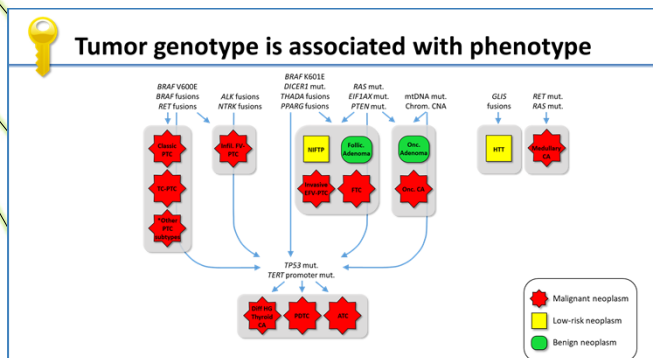


Emerging roles for molecular testing in thyroid FNA specimens

Emerging* uses for thyroid FNA molecular testing

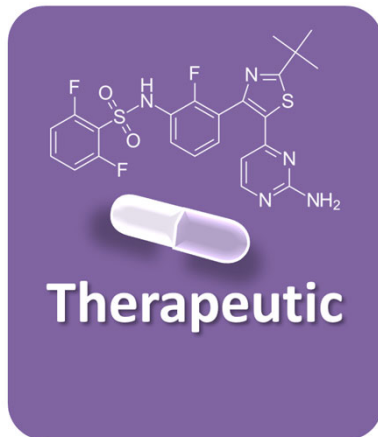


Prognostication of tumors based on molecular profiles



*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

*for FNAs classified as Bethesda V or VI

Emerging* uses for thyroid FNA molecular testing

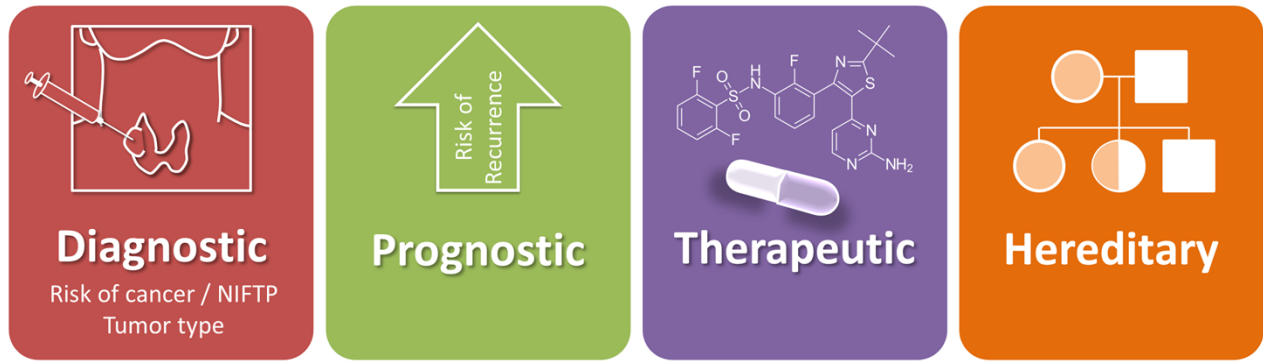


(Incidental) identification of germline mutations suggestive of hereditary cancer syndromes

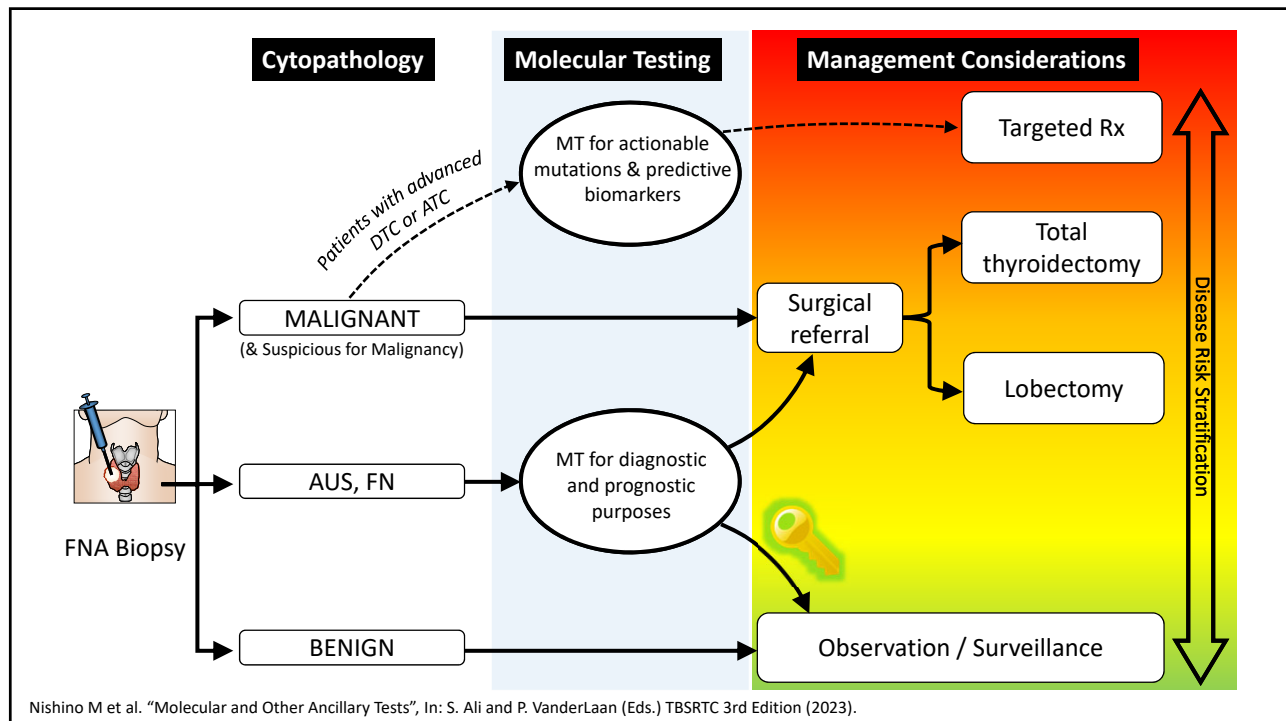
RET	Multiple Endocrine Neoplasia Type 2
PTEN	Cowden Syndrome
APC	Familial Adenomatous Polyposis
PRKAR1A	Carney Complex
DICER1	DICER1 Syndrome

*for FNAs classified as Bethesda V or VI

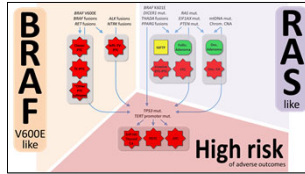
Current and emerging* uses for thyroid FNA molecular testing



*for FNAs classified as Bethesda V or VI



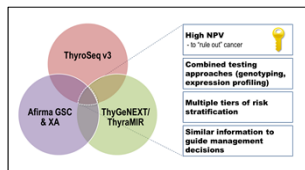
Summary



Key molecular changes



Purpose of molecular testing



Testing platforms