# Next Generation Sequencing for Thyroid Cancer Diagnosis and Treatment

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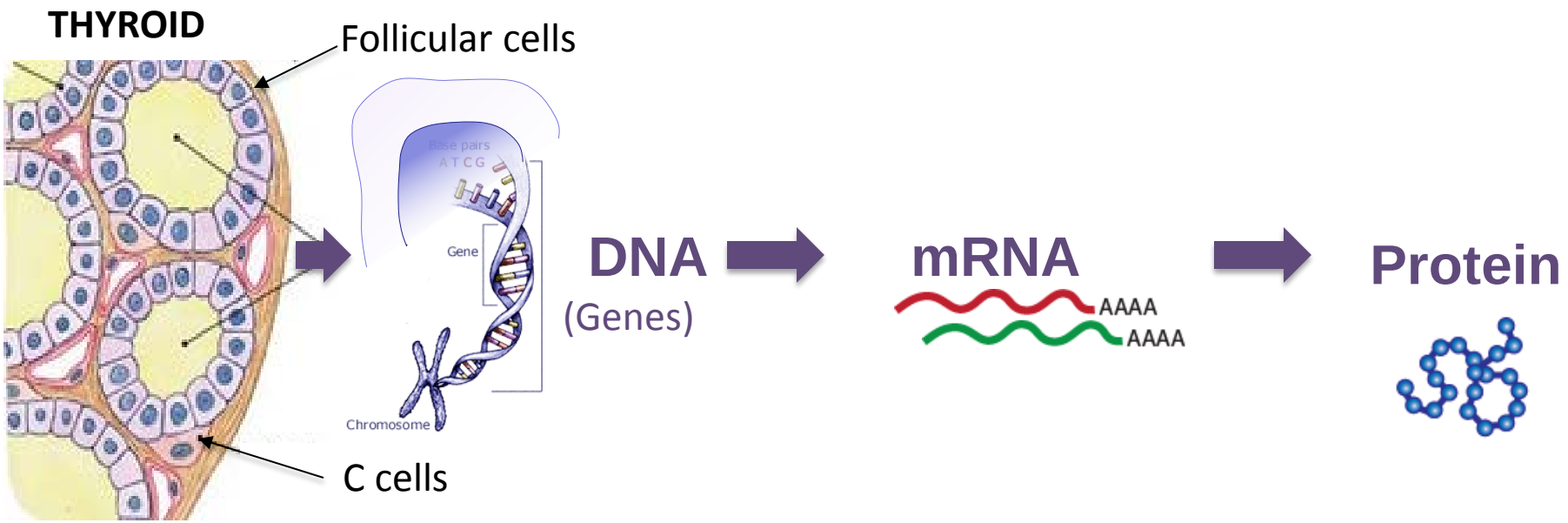
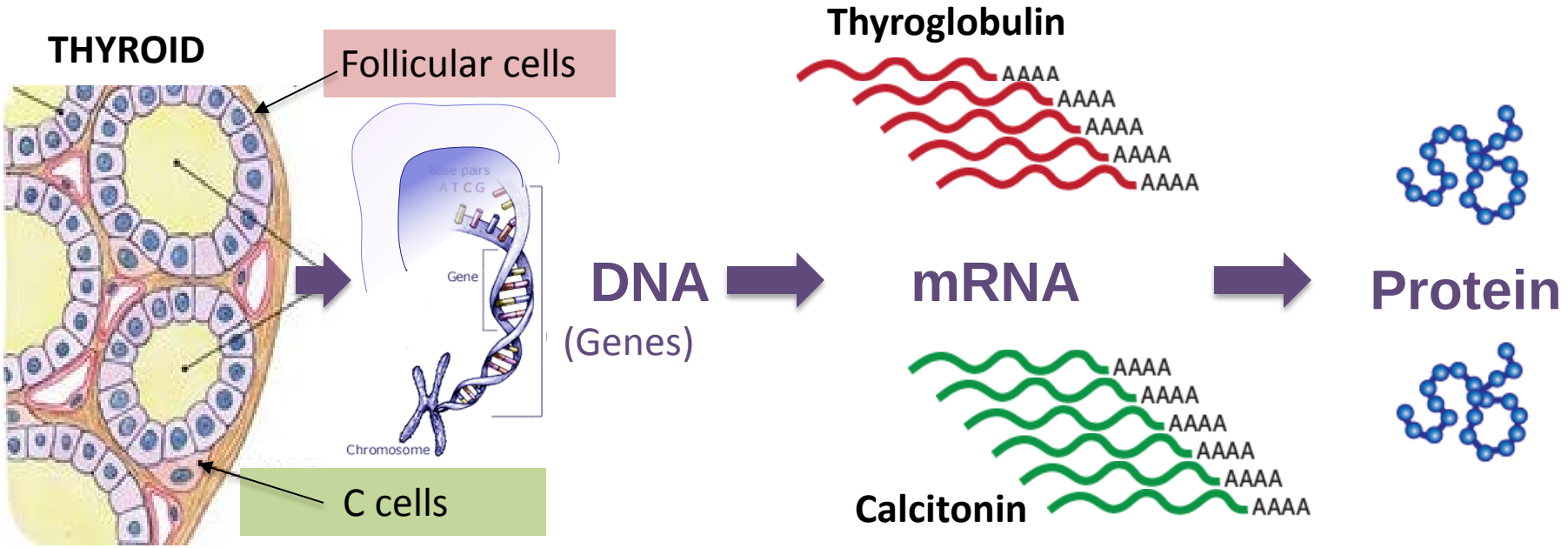
University of Pittsburgh Medical Center

MGP
UPMC LIFE CHANGING MEDICINE

# Principles of Molecular Biology of Thyroid Cell

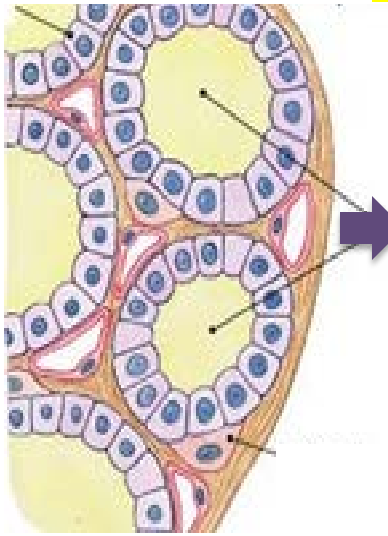


# Normal Thyroid Cells

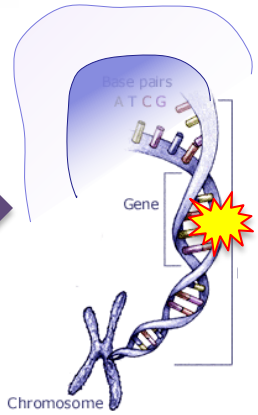


# Thyroid Tumors

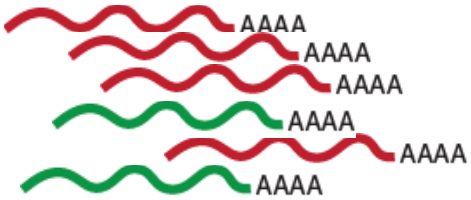
**THYROID**

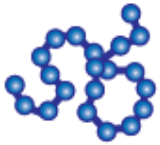


**Thyroid Tumor**




**DNA** (Genes) → **mRNA** → **Protein**



- *Mutations*
- *Chromosomal variations*
- *Gene fusions*
- *Alterations in gene expression*

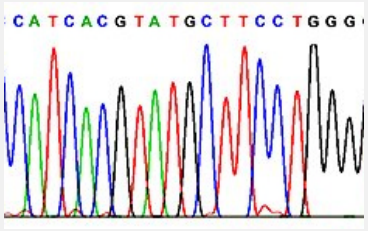
**MOLECULAR TECHNIQUES**

**Next Generation Sequencing**

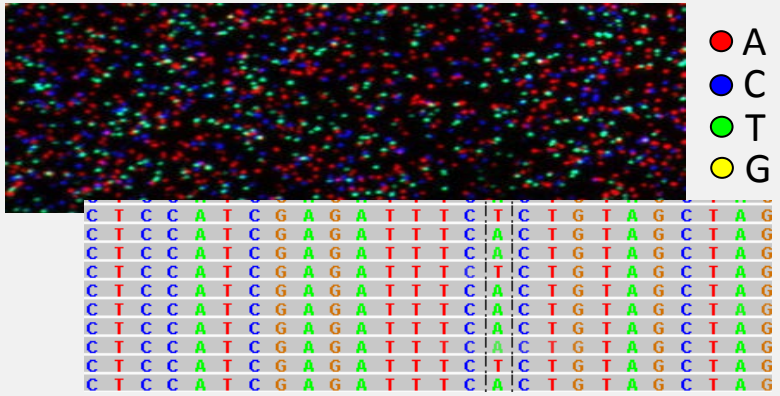
**Gene Expression Profiling**

# Genomic Revolution:
# *Next Generation Sequencing*

Sanger Sequencing

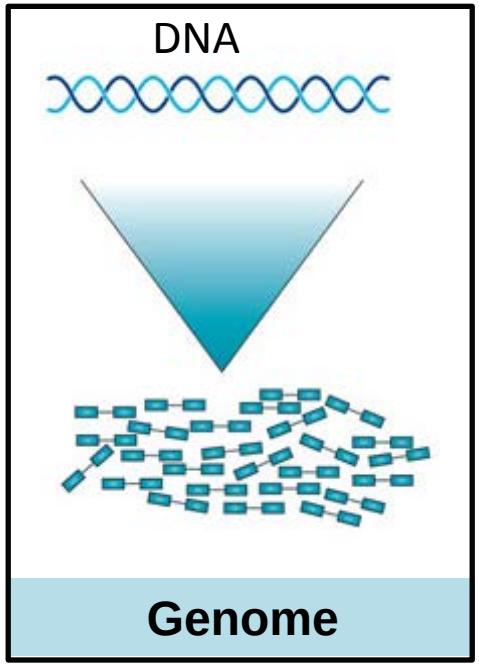


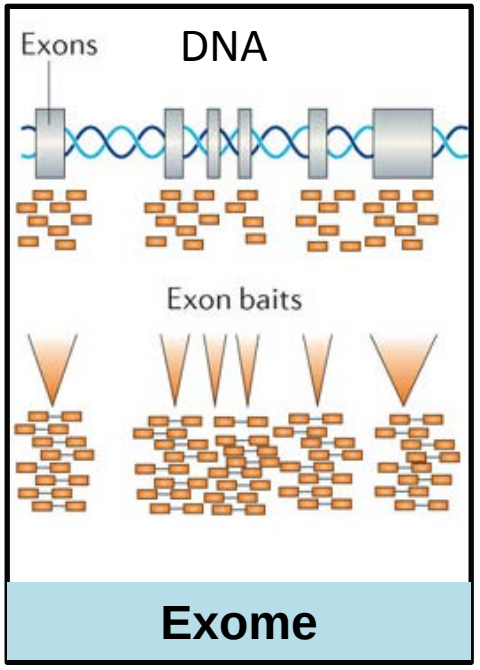

Next Generation Sequencing

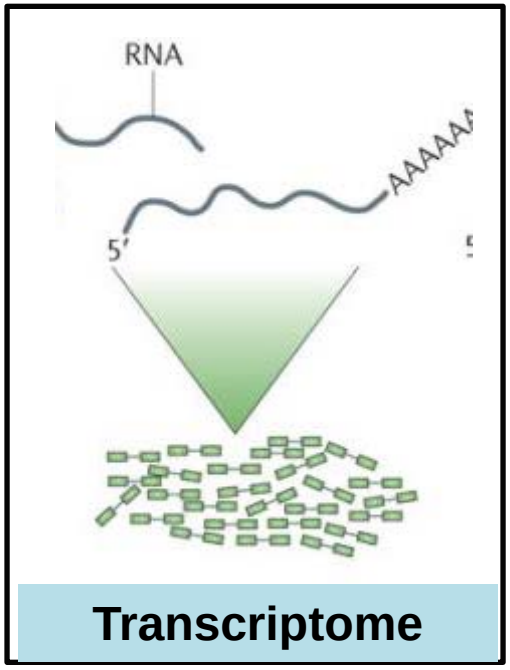



- Interrogates multiple regions of genome at once
- Sequence DNA/RNA in massively parallel configuration

# Next Generation Sequencing Approaches



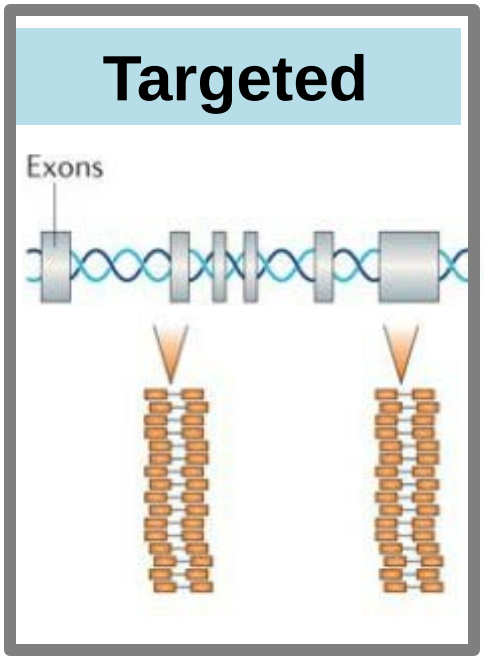




- Discovery tool
- Expensive, time consuming, complex BI analysis and results interpretation

*Adopted from Simon et al. Nature 2013*

# Next Generation Sequencing Approaches




- Sequencing of multiple preselected genes or gene regions
- Used in clinical practice

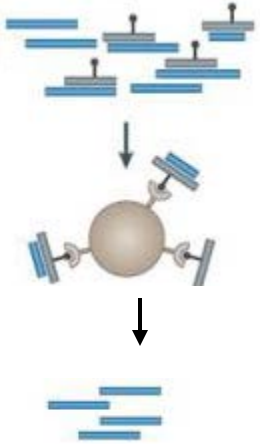
# Next Generation Sequencing Approaches

**Hybrid Capture** ← **Targeted** → **Amplification**

Exons

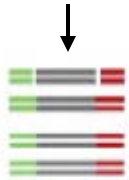
## DNA/RNA isolation

Fragmentation
Hybridization to probes



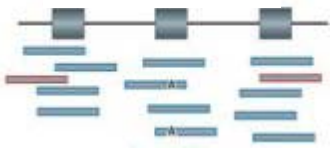
## NGS library preparation

Amplification



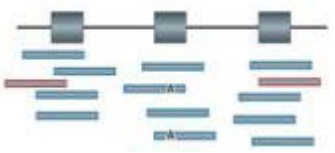
Emulsion PCR

Sequencing

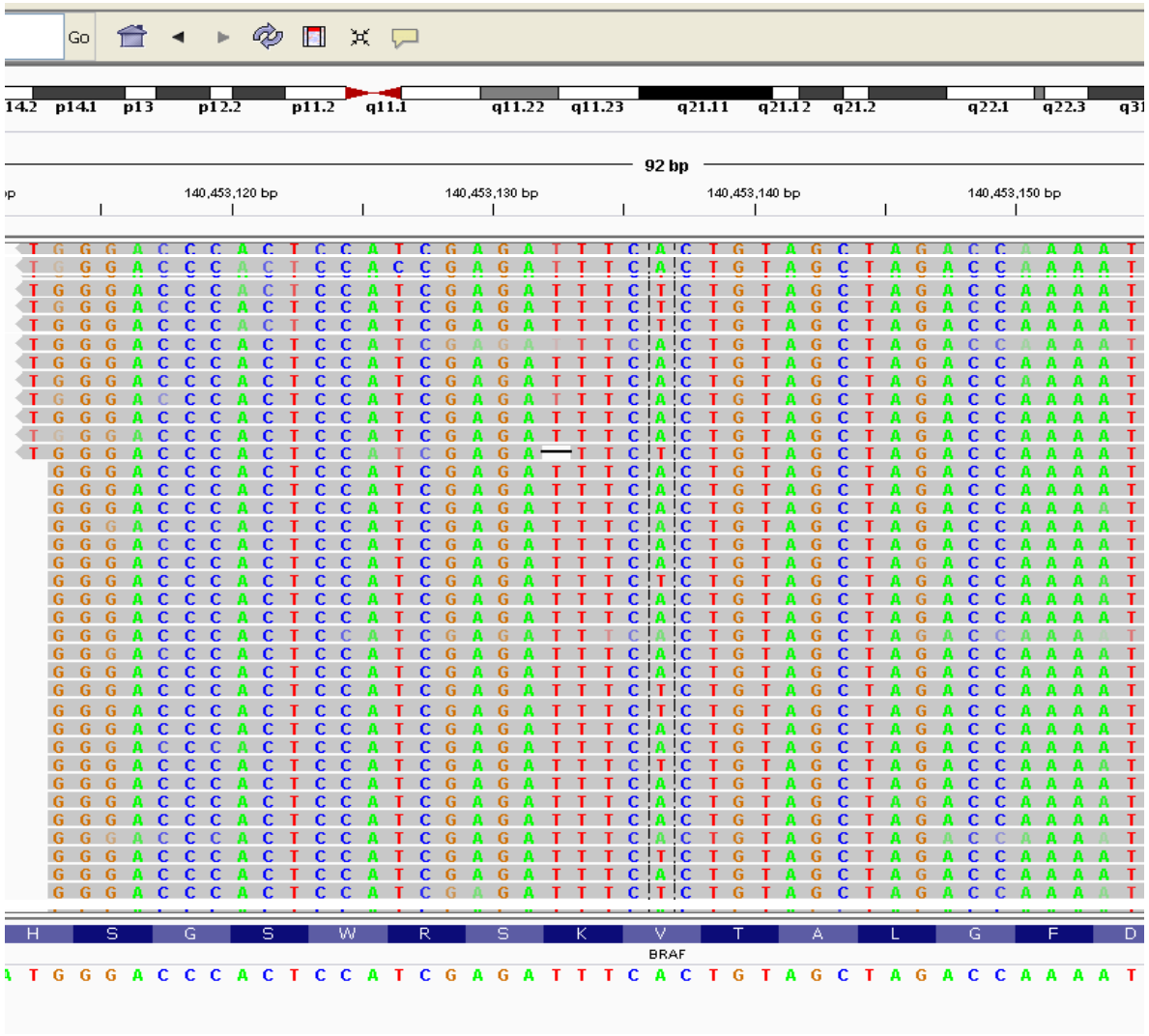


## Sequencing, BI Analysis

Sequencing



# NGS Sequence Analysis



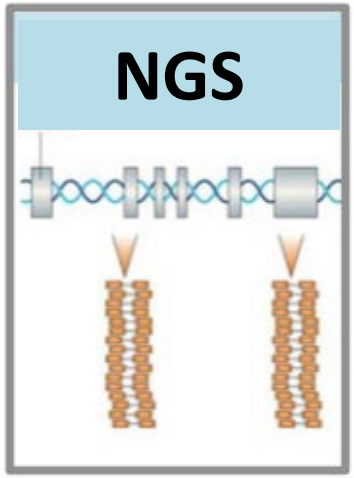

*Sequence Reads*

*Reference Human Genome*

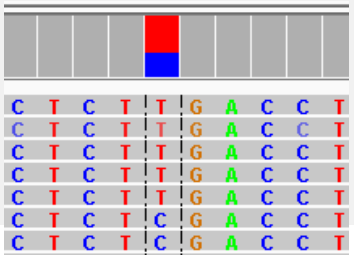
*Depth of sequencing or depth of coverage:* *Number of times genome position is sequenced*

# Advantages of NGS

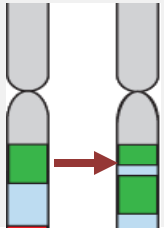
# Technology that allows for detection of all types of genetic alterations in a single workflow



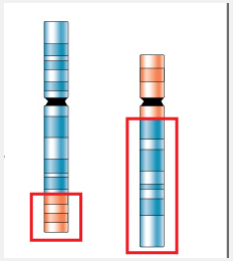

**Point mutations Indels**



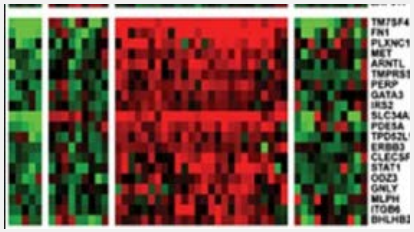
**Copy number alteration**



**Gene fusions**



**Gene expression**

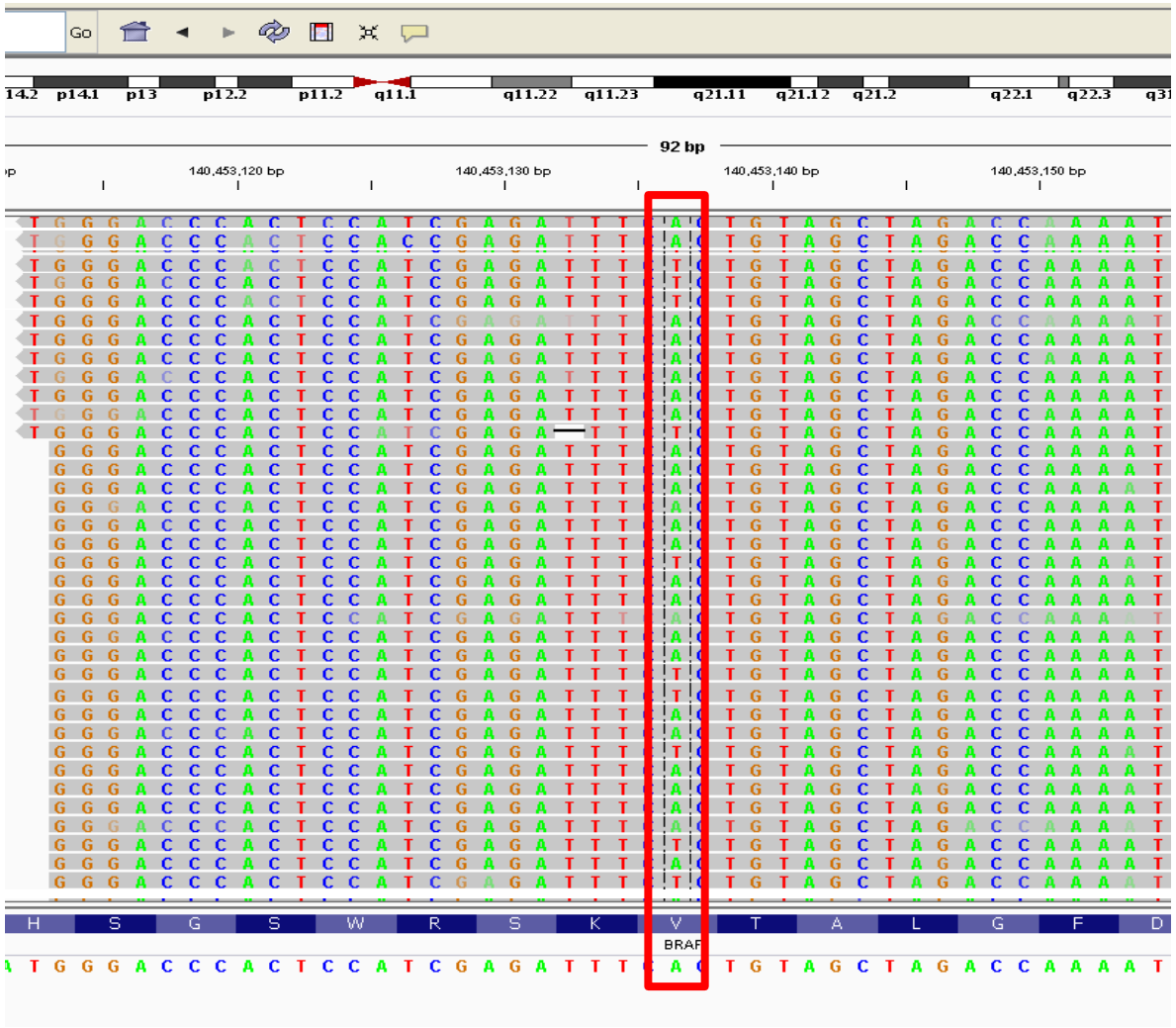


Adopted from Simon et al. Nature 2013

# Allows quantitation (mutation frequency, gene expression)

*NGS allows to quantitate number of sequencing reads with mutation*

*BRAF V600E* 24% of alleles or 48% of cells with heterozygous mutation



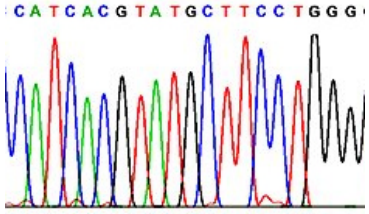

*Sequence Reads*

*Depth of sequencing or depth of coverage:* *Number of times genome position is sequenced*

*Reference Human Genome*

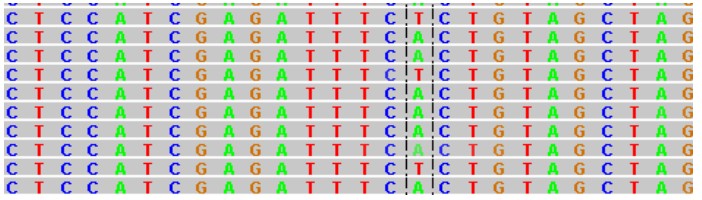
- Requires small amount of DNA/RNA (5-50 ng)
- Works on any type of material: FNA (fresh or fixed slides), FFPE tissue, blood
- Highly sensitive detection of genetic alterations

**Sanger Sequencing**




**15-20% of alleles**
**or**
**30-40% of cells**

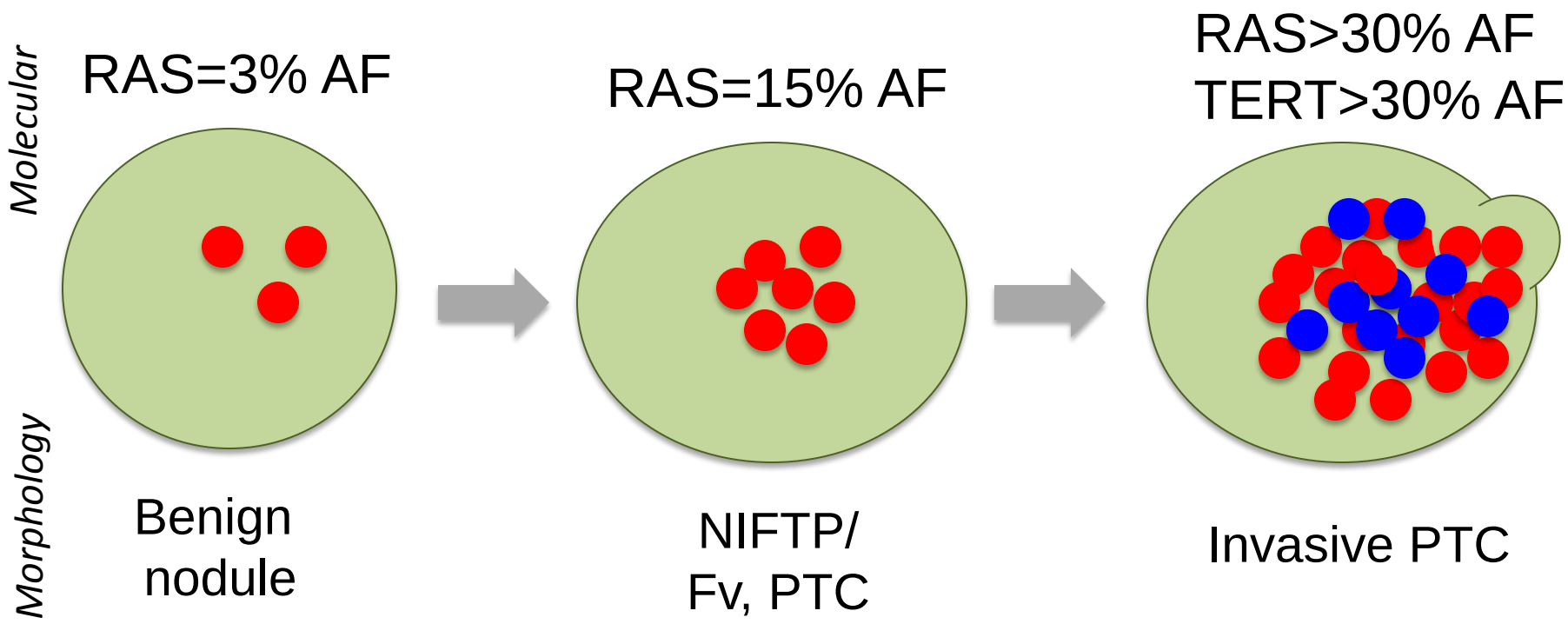
**NGS**



**3-5% of alleles**
**or**
**6-10% of cells**

Allows to overcome limited sampling of the lesion, can be used for early detection of cancer

# Allows quantitation (mutation frequency, gene expression)

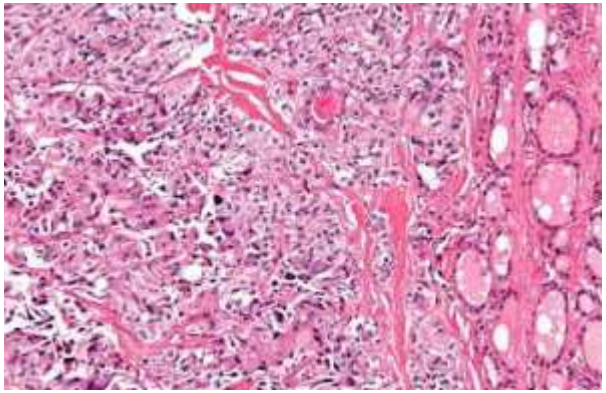



**Observation, Mutation monitoring** | **Lobectomy** | **Total thyroidectomy**

Molecular monitoring of mutant clone expansion and disease progression, personalized management

- Detects both germ line and somatic variants
- Provides genetic mechanism of disease

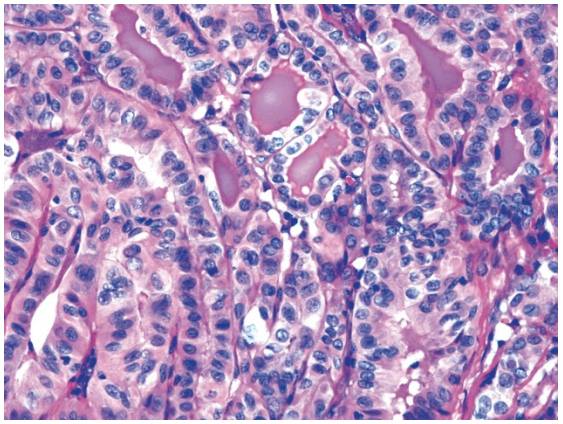
Medullary Thyroid Carcinoma



RET p.M918T
mutation

Germ line or somatic

Papillary Thyroid Carcinoma



RET/CCDC6 (RET/PTC1)
fusion

Somatic

# Clinical Applications of NGS in Thyroid

# Diagnostics

## Diagnosis of benign or malignant thyroid nodules in FNA samples with indeterminate cytology

Ion AmpliSeq™

- *Cancer hot spot panel (ThermoFisher Scientific)*
- *50 genes*

ThyroSeq®
Thyroid Genomic Classifier

- *Thyroid specific panel (UPMC)*
- *56 genes*

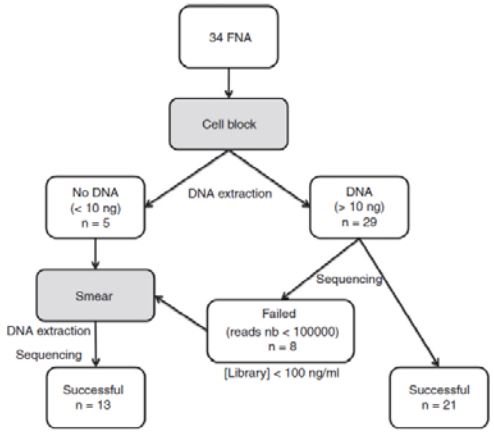
Histopathology

*Histopathology* 2015, 66, 215–224. DOI: 10.1111/his.12461

### Next-generation sequencing improves the diagnosis of thyroid FNA specimens with indeterminate cytology

Marie Le Mercier,[1] Nicky D'Haene,[1] Nancy De Nève,[1] Oriane Blanchard,[1] Caroline Degand,[1] Sandrine Rorive[1,2] & Isabelle Salmon[1,2]

[1]*Department of Pathology, Erasme University Hospital, Université Libre de Bruxelles (ULB), Brussels, Belgium, and* [2]*DIAPath, Center for Microscopy and Molecular Imaging (CMMI), Académie Universitaire Wallonie-Bruxelles, Gosselies, Belgium*




JCEM ONLINE

Advances in Genetics—Endocrine Research

### Targeted Next-Generation Sequencing Panel (ThyroSeq) for Detection of Mutations in Thyroid Cancer

Marina N. Nikiforova, Abigail I. Wald, Somak Roy, Mary Beth Durso, and Yuri E. Nikiforov

Cancer

Explore this journal >

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, Sally E. Carty MD, Simon I. Chiosea MD, Christopher Coyne MD, Umamaheswar Duvvuri MD, Robert L. Ferris MD, PhD, William E. Gooding MS, Steven P. Hodak MD, Shane O. LeBeau MD, N. Paul Ohori MD, Raja R. Seethala MD, Mitchell E. Tublin MD, Linwah Yip MD, Marina N. Nikiforova MD

# ThyroSeq® v.2

- 14 genes for mutations, >1000 hotspots
- 42 fusion types, 16 genes for expression
- Ion Torrent/Proton based targeted NGS

## DNA Library

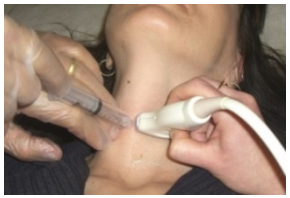
| GENE MUTATIONS | |
|---|---|
| BRAF | RET |
| NRAS | TSHR |
| HRAS | AKT1 |
| KRAS | TP53 |
| PIK3CA | GNAS |
| PTEN | CTNNB1 |
| TERT | EIF1AX |

**5-10 ng DNA/RNA**

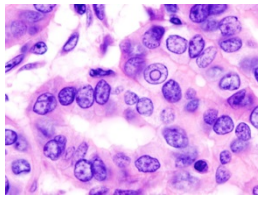
## RNA Library

| GENE FUSIONS GENE EXPRESSION | |
|---|---|
| RET | PPARG |
| NTRK1 | NTRK3 |
| BRAF | ALK |
| PGK | KRT7 |
| TG | TTF1 |
| NIS | PTG |
| CALCA | OTHER |

# ThyroSeq v2® Genomic Classifier



FNA



TISSUE

DNA
RNA

## Analytical Pipeline

DNA/RNA NGS Library Preparation

Library Enrichment

Sequencing Bioinformatics

ThyroSeq®
Thyroid Genomic Classifier

## Interpretation Pipeline

Variants Annotation

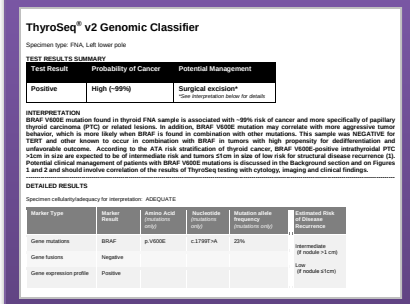
UPMC Database

Thyroid Cancer Risk Assessment

Cytology diagnosis

Mutations Gene expression

### Reporting



**ThyroSeq® v2 Genomic Classifier**

Specimen type: FNA, Left lower pole

TEST RESULTS SUMMARY

| Test Result | Probability of Cancer | Potential Management |
|---|---|---|
| Positive | High (~99%) | Surgical excision* *See interpretation below for details |

INTERPRETATION

BRAF V600E mutation found in thyroid FNA sample is associated with ~99% risk of cancer and more specifically of papillary thyroid carcinoma (PTC) or related lesions. In addition, BRAF V600E mutation may correlate with more aggressive tumor behavior, which is more likely when BRAF is found in combination with other mutations. This sample was NEGATIVE for TERT and other known to occur in combination with BRAF in tumors with high propensity for dedifferentiation and unfavorable outcome. According to the ATA risk stratification of thyroid cancer, BRAF V600E-positive intrathyroidal PTC >1cm in size are expected to be of intermediate risk and tumors ≤1cm in size of low risk for structural disease recurrence (1). Potential clinical management of patients with BRAF V600E mutations is discussed in the Background section and on Figures 1 and 2 and should involve correlation of the results of ThyroSeq testing with cytology, imaging and clinical findings.

DETAILED RESULTS

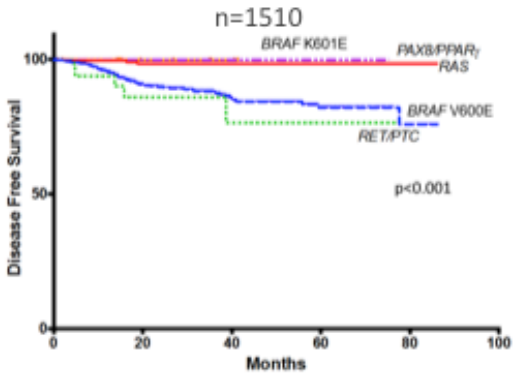
Specimen cellularity/adequacy for interpretation: ADEQUATE

| Marker Type | Marker Result | Amino Acid (mutations only) | Nucleotide (mutations only) | Mutation allele frequency (mutations only) | Estimated Risk of Disease Recurrence |
|---|---|---|---|---|---|
| Gene mutations | BRAF | p.V600E | c.1799T>A | 23% | Intermediate (if nodule >1 cm) |
| Gene fusions | Negative | | | | |
| Gene expression profile | Positive | | | | Low (if nodule ≤1cm) |

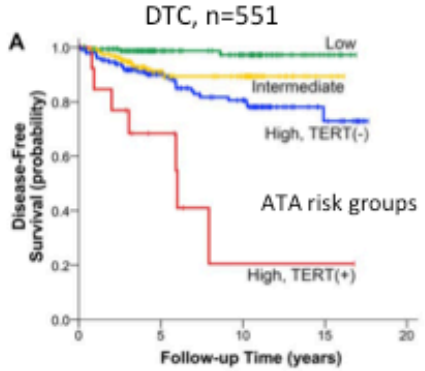
# Clinical Applications of NGS in Thyroid

## Prognostic

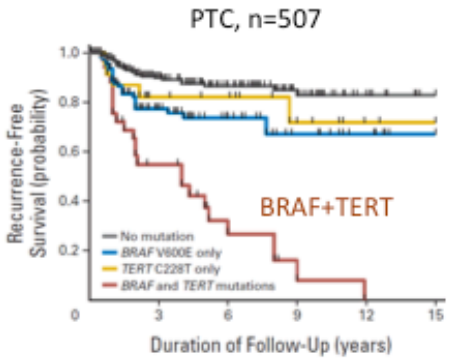
Prediction of clinical outcome based on genetic alterations (e.g. BRAF+TERT or RAS+TERT = aggressive disease)



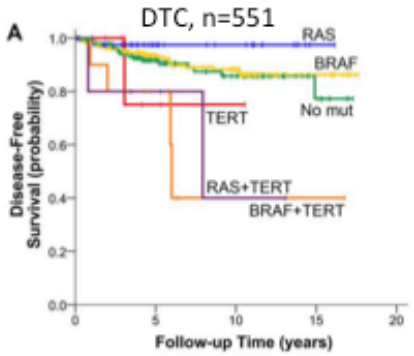

Yip L et al. *Ann Surg* (2015)




Song YS et al. *Cancer* (2016)




Xing M et al. *JCO* (2014)




Song YS et al. *Cancer* (2016)

# Multiple Mutations by ThyroSeq® v2

THYROID
Volume 25, Number 12, 2015

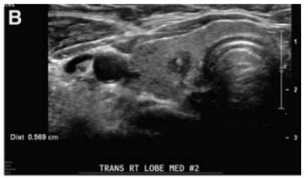
DOI: 10.1089/thy.2015.0278

**CASE STUDIES, and PATIENTS WITH REMARKABLE FEATURES OR RARE DISORDERS**

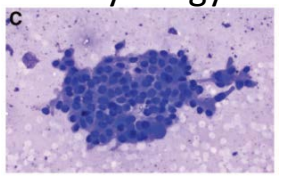
## Multiple Mutations Detected Preoperatively May Predict Aggressive Behavior of Papillary Thyroid Cancer and Guide Management—A Case Report

Rupendra T. Shrestha,[1] Arivarasan Karunamurthy,[2] Khalid Amin,[3] Yuri E. Nikiforov,[2] and M. Luiza Caramori[1]

0.6 cm nodule



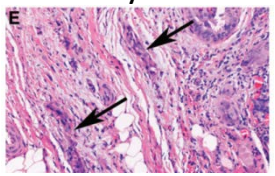

AUS cytology




BRAF+/TERT+
AKT1+/PIK3CA+

| Gene | cDNA | Protein | Allelic Frequency (%) |
|---|---|---|---|
| BRAF | c.1799T>A | p.V600E | 37% |
| PIK3CA | c.3140A>G | p.H1047R | 21% |
| AKT1 | c.49G>A | p.E17K | 6% |
| TERT | c.1-124C>T | - | 77% |

mPTC with extrathyroid ext




ThyroSeq® Thyroid Genomic Classifier

## Multiple High-Risk Mutations Detected in Thyroid FNA Samples are Associated With Aggressive Cancer

Marina N. Nikiforova[1], Linwah Yip[1], Umamaheswar Duvvuri[1], Simion Chiosea[1], Daniel B. Kuriloff[2], Nicla Borrelli[1], Steven Hodak[3], Carlos Urmacher[4], Yuri E. Nikiforov[1]
[1] University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA; [2]New York Head & Neck Institute, New York, NY; [3]New York University Langone Medical Center, New York, NY; [4]CBLPath, Inc., Rye Brook, NY

- **35 (55%) BRAF + Another HR mutation**
- **18 (29%) RAS + Another HR mutation**
- **3 (5%) Other Multiple HR mutations**

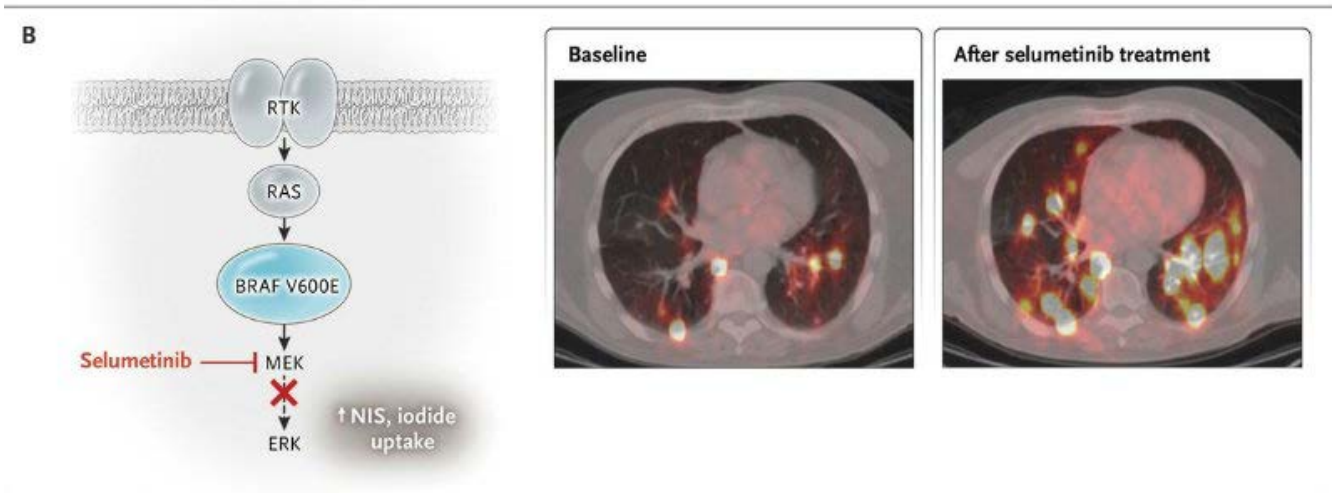
→ **55 (98%) Thyroid Cancer** →

**51 (93%) Cancers with Aggressive Features:**
- Extrathyroidal extension (55%)
- Vascular invasion (53%)
- Lymph node macrometastasis (47%)
- Poorly differentiated/anaplastic carcinoma areas (14%)
- Distant metastasis (8%)

# Clinical Applications of NGS in Thyroid

## Therapeutic

Identify potential markers (BRAF, RET, HRAS, PPARG, ALK, NTRK) for targeted chemotherapy




Fagin J and Wells S. N Engl J Medicine 2016; 375:1054-1067

# Clinical Applications of NGS in Thyroid

## Therapeutic

Identify potential markers (BRAF, RET, HRAS, PPARG, ALK, NTRK) for targeted chemotherapy

*Pan-cancer NGS Panels:*

**Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT)**

*341 genes*

FOUNDATION MEDICINE®

*315 genes*

*Thyroid NGS Panel:*

ThyroSeq®

Thyroid Genomic Classifier

# Summary

- Next-gen sequencing allows simultaneous analysis for multiple genomic alterations with high accuracy and sensitivity
- It requires minimal amount of DNA and RNA and can be performed on FNA and paraffin tissue samples
- Quantitate mutation frequency,  gene fusion transcript, and gene expression, can be used for monitoring of mutant clone and disease progression
- Used diagnostically in FNA samples with indeterminate cytology and in surgically removed samples for prognostication and treatment of patients with thyroid cancer