

Thyroid Tumor Markers – The old and the new –

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Thanks

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- **Christine Snozek, PhD**
- **Bryan McIver, MBBS, PhD**
- **Alicia Algeciras-Schminich, PhD**
- **Kendall Cradic, MS**
- **Dragana Milosevic, MS**



Outline

- **Types of thyroid cancer covered in this talk**
- Why we need thyroid cancer markers
- Thyroglobulin (Tg) – THE current thyroid cancer marker
- Some new trends in Tg testing
- Novel thyroid cancer markers and why they are needed



What we will cover

- **Follicular cell-derived, differentiated thyroid cancer**
 - Papillary thyroid carcinoma (PTC)
 - Follicular thyroid carcinoma (FTC)
 - Hürthle cell carcinoma (HCC)



What we will not cover

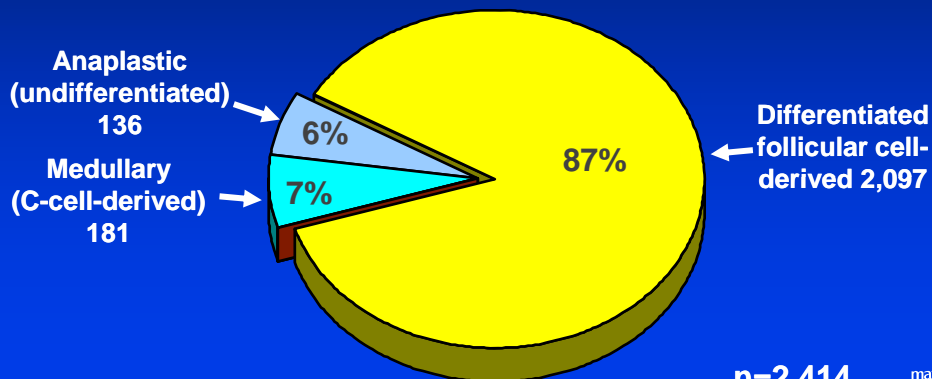
- **Follicular cell-derived, undifferentiated thyroid cancer**
 - Anaplastic thyroid carcinoma (ATC)
- **Non-follicular thyroid carcinoma**
 - C-cell tumors – medullary thyroid carcinoma (MTC)
 - Rare other tumors, e.g. lymphomas, squamous cell carcinomas, metastases to the thyroid, ...



Why cover only differentiated follicular cell-derived thyroid carcinomas?

Thyroid cancer morphotype distribution, Mayo Clinic Rochester 1940-90

Differentiated follicular cell-derived cancers = ~90% of cases



Modified picture, original courtesy Dr. McIver



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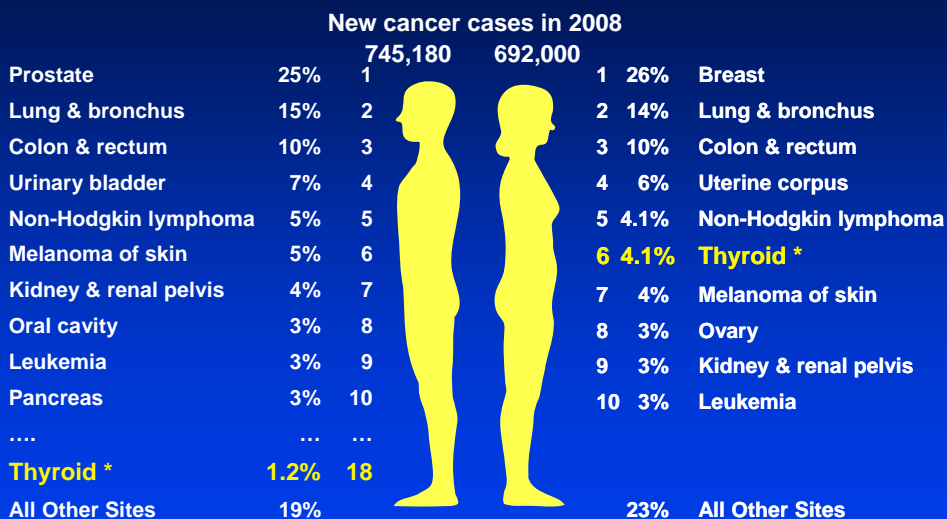


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 - Women 6th
 - Men 18th



Thyroid cancer – incidence rates compared to other cancers (excl. non-melanoma skin cancer)



* : ~80% of thyroid cancer cases are PTC

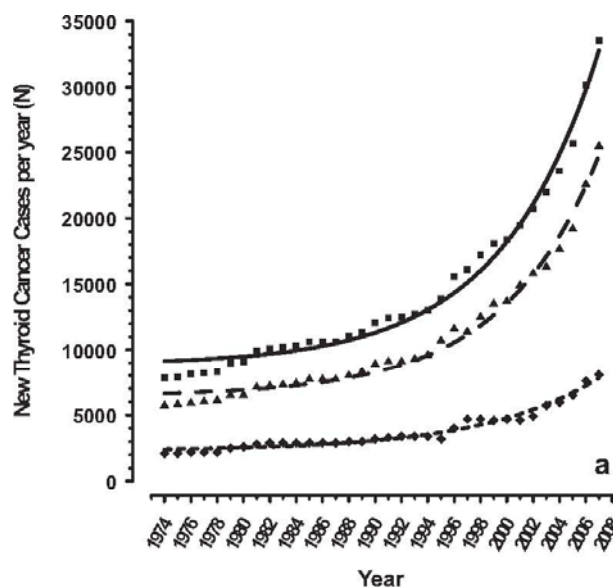


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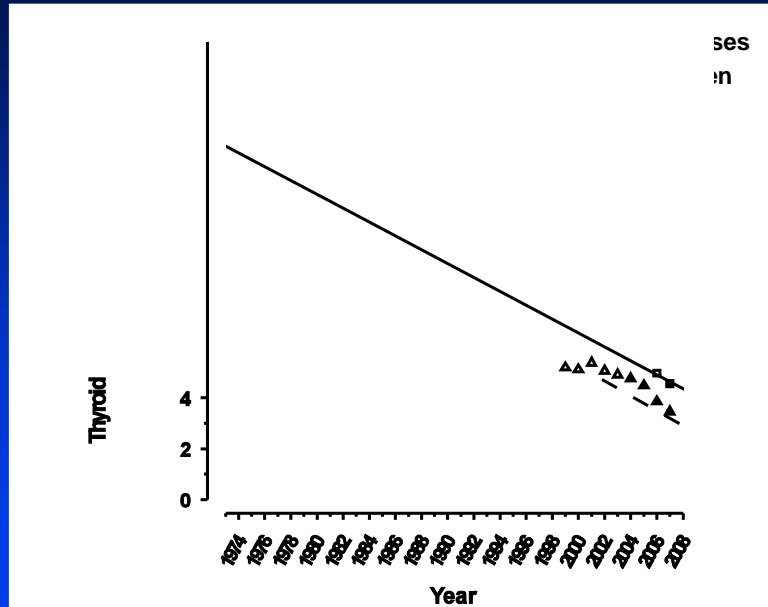
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New US thyroid cancer cases 1974-2006



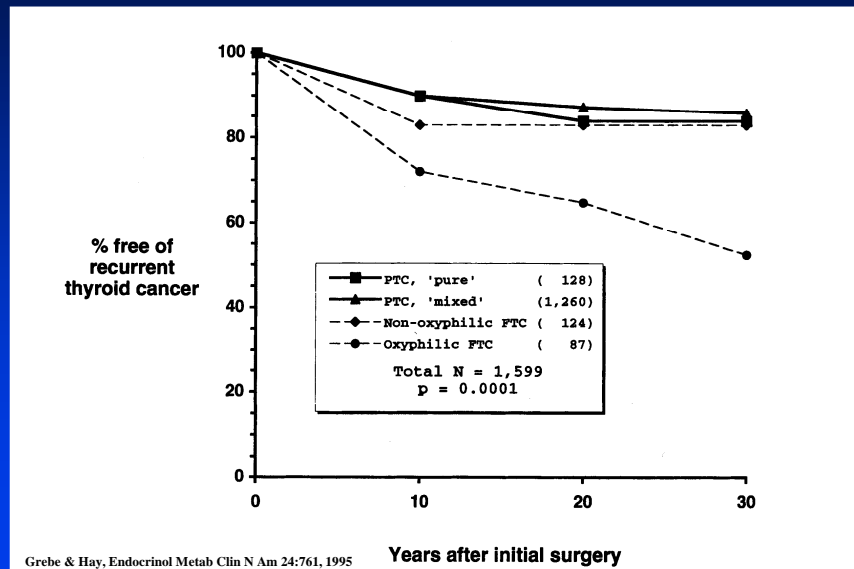
Thyroid Cancer Deaths (% of new cases)



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- **Thyroid cancer incidence rates continue to increase, while mortality falls**
- **Thyroid cancer patients continue to suffer morbidity from recurrences over many decades**

Thyroid cancers continue to recur over several decades



Why we need thyroid tumor markers

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- Depending on the morphotype *15% to 50% of patients will suffer a recurrence in their lifetime*
- **Need for prolonged (over decades) sensitive, non-invasive and specific detection of tumor recurrence in an ever increasing patient population**



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- **Not tumor specific**
 - **Circulating levels correlate with thyroid size**
 - **Elevated in conditions of disordered/excessive thyroid growth or glandular destruction**
 - **Goitre**
 - **Graves' disease, thyroiditis**



Tg measurements: current practice guidelines – assays & testing process

- **Perform at least one stimulated Tg measurement in each patient**
- **Use Tg assays with sensitivity (<1 ng/mL)**
- **Use Tg assays calibrated to IS CRM-457**
- **Use the same Tg assay over time in individual patients**
- **Measure TgAb in all samples tested for Tg, to identify samples that might have false low Tg measurements**
 - **Use direct quantitative TgAb assays, rather than Tg recovery test or semi-quantitative tests**



Tg measurements: current practice guidelines – indicators of recurrence

- **Serum Tg should be undetectable in athyrotic thyroid cancer patients (total thyroidectomy +/- RRA)**
 - **Unstimulated or stimulated (thyroid hormone withdrawal or rhTSH) Tg >2 ng/mL suspicious of recurrence, but positive predictive value only 5-10%**
 - **Unstimulated or stimulated Tg >10 ng/mL highly predictive of persistent/recurrent disease**



Tg measurements - limitations

- **Residual benign thyroid tissue**
 - Each 1 g of residual tissue contributes to serum Tg levels:
 - ~0.5 ng/mL if serum TSH <0.1 mIU/L
 - ~1 ng/mL if serum TSH ≥0.1 mIU/L



Tg measurements - limitations

- **Residual benign thyroid tissue**
- **Autoantibody interferences – false low Tg**
 - ~25% of thyroid cancer patients have anti thyroglobulin auto-antibodies (TgAb)
 - “True” Tg conc. (usually) impossible to determine in TgAb+ patients
 - Very “rough” relationship between TgAb concentration and likelihood of false low Tg
 - TgAb levels <4-6 kU/L unlikely to cause false low Tg
 - TgAb ≥6-10 kU/L: ~30% incidence of false low Tg
 - TgAb ≥50-100 kU/L: >70% incidence of false low Tg



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- **Residual benign thyroid tissue**
- **Autoantibody interferences – false low Tg**
- **Calibration differences or reagent lot-to-lot variability**
- **Poor low-end sensitivity of some assays can necessitate stimulated Tg testing**



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Recent Changes in Practice - Tg

- **Unstimulated Tg testing**



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- **Unstimulated Tg testing – the traditional argument used against it:**
“ ... consensus is that an undetectable Tg measurement during thyroid hormone suppression is misleading in a large proportion of patients ... ” (Mazzaferri et al., JCEM 88:1433, 2003)



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Is this true?



Stimulated versus unstimulated Tg – a look at the facts

784 patients with unstimulated serum Tg <1 ng/mL or <2 ng/mL (4 papers each)



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21 (2.6%) had metastatic disease that might have been missed by physical exam or neck ultrasound



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What happens if you use a more sensitive assay???



Recent Changes in Practice - Tg

• Unstimulated Tg testing

- Assays with a functional sensitivity of $\leq 0.1-0.2$ ng/mL obviate the need for stimulated Tg testing in most athyrotic patients without detectable TgAb
 - Supported by >10 studies
 - Positive and negative predictive value (compared to stimulated Tg) of 90-100%
 - False negative rate (=stimulated Tg >2 ng/mL) in each study <3%, 2 studies had no “false negatives”
 - 40-130 stimulated Tg tests required to detect a single potentially “false negative” suppressed Tg (~90% of these turn out to be false positive)



Recent Changes in Practice - Tg

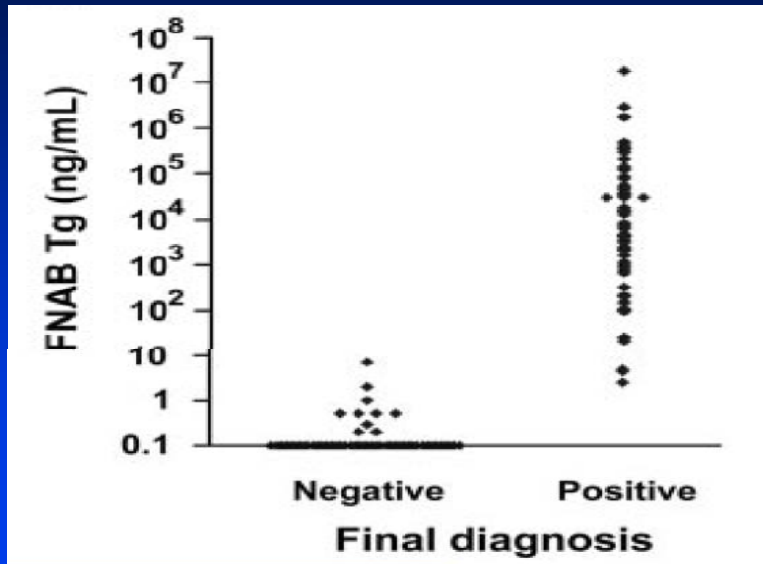
• Unstimulated Tg testing

• Tg testing in body fluids/biopsies

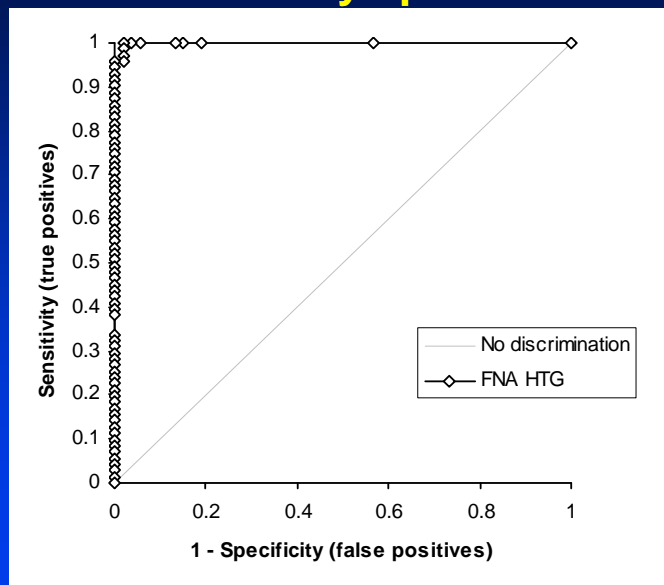
- >15 studies support utility of Tg measurement on fine needle aspiration biopsy (FNAB) needle washings
- Tg measurement in needle washes of lymph node FNABs has higher diagnostic rate than cytology (>99% versus 80-90%)
- Diagnostic agreement of cytology and Tg for samples that are diagnostic by both methods: >90% in all studies
- Sensitivity and specificity >90% (near 100% in several studies)



Clinical performance of Tg measurements in needle washes of lymph node FNAB



ROC-curve for Tg measurements on needle washes of lymph node FNABs



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Future challenges – the changing epidemiology of thyroid cancer

- **Thyroid cancer incidence is increasing exponentially, mortality is declining**
- **Lifetime risk of dying from thyroid for a new thyroid cancer patient will fall below 4%**
- **Many future thyroid cancer patients might not undergo complete thyroid ablation**
- **Tg reference ranges, linked to residual thyroid tissue and TSH levels, will have to be established**
- **Tumor specific thyroid cancer markers (e.g. tumor specific mutations) are likely to become increasingly important**

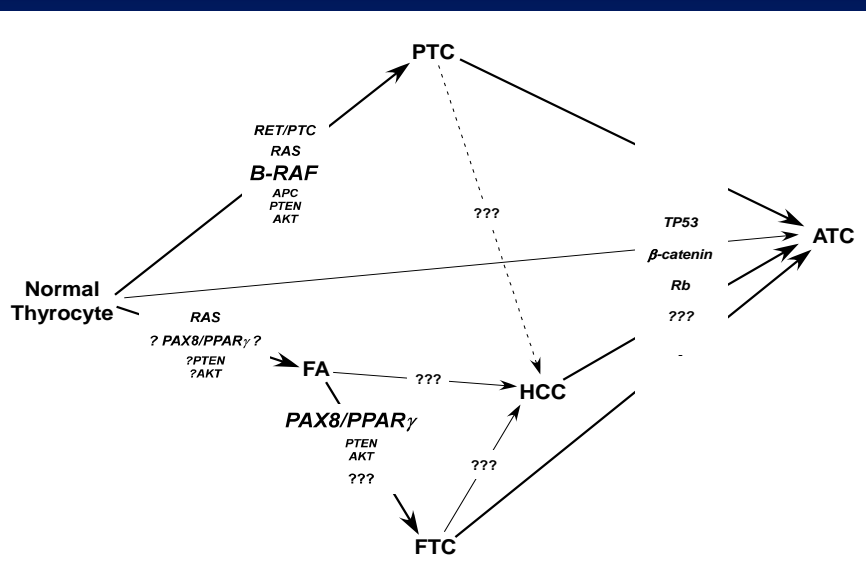


The case for tumor specific markers

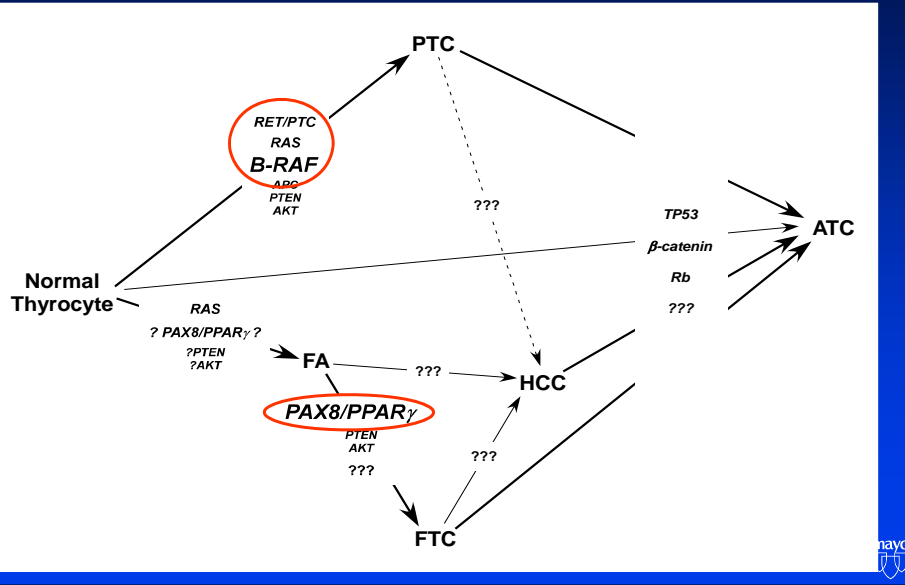
- Specific for the presence of residual or recurrent disease, rather than for the presence of thyroid-derived tissue
- Might not be affected by autoantibodies to thyroid antigens, particularly if the marker is nucleic acid based
- Could be fairly specific for thyroid-derived malignancy, if based on genetic changes that have a very high prevalence in thyroid tumors, compared to other human malignancies



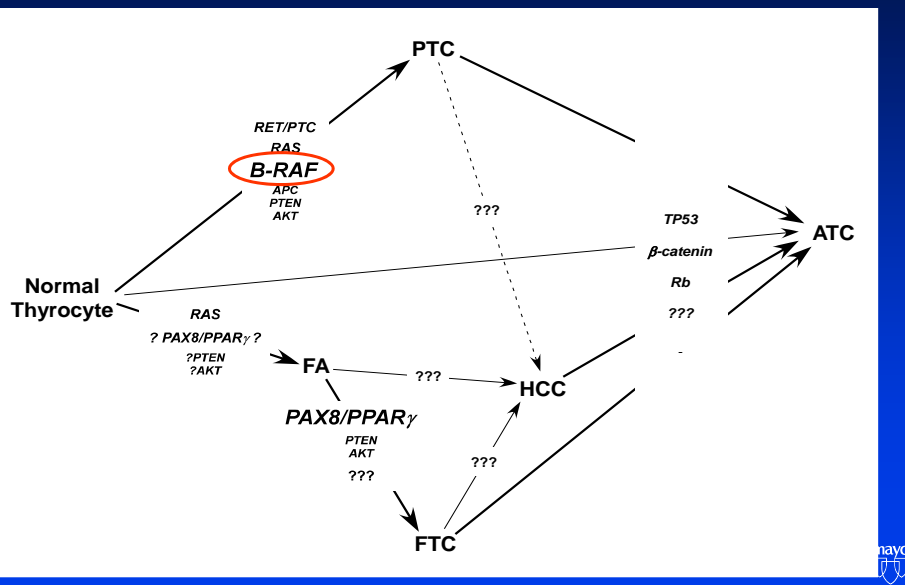
Somatic tumor-genetic changes in thyroid carcinoma – potential diagnostic targets



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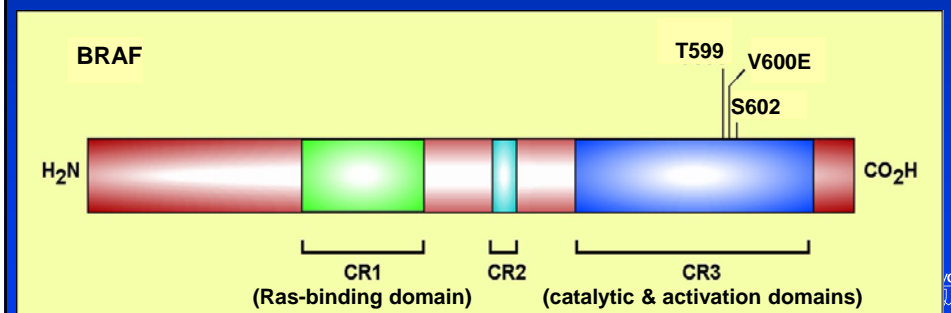


Somatic tumor-genetic changes in thyroid carcinoma – potential diagnostic targets

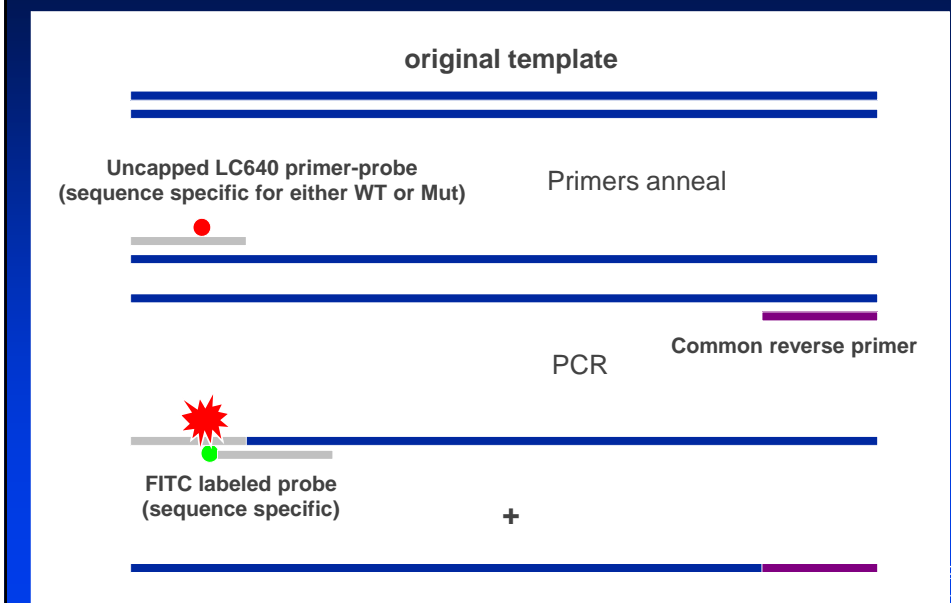


BRAF 1799 T>A (V600E) mutation

- Accounts for >90% of *BRAF* mutations in melanoma & papillary thyroid cancer
- Not found in normal tissue
- T>A change at NT1799 subs. Glutamic acid for Valine
- Results in constitutively active BRAF
- Occurs in 40-80% of melanomas and PTC; < 10% most other cancers

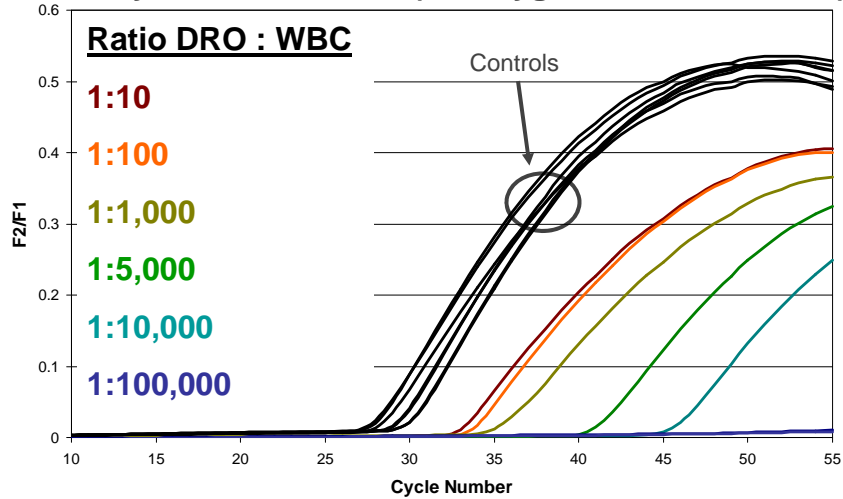


BRAF 1799T>A assay – allele specific realtime PCR



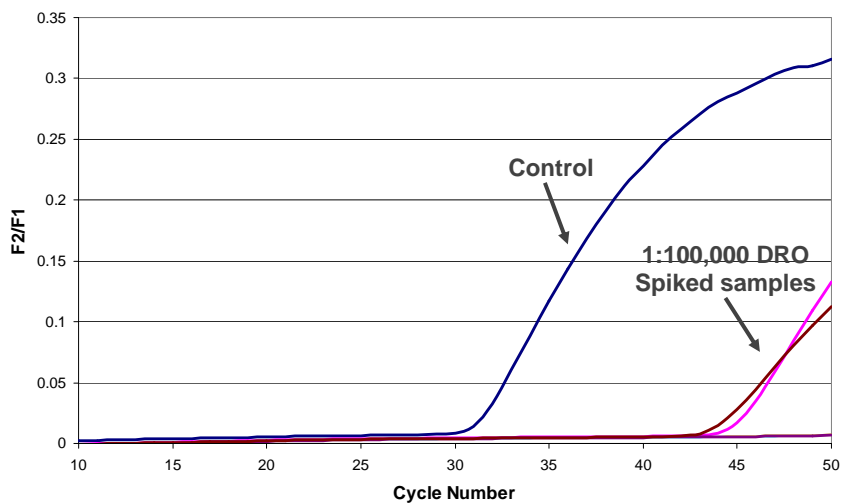
Detection sensitivity for *BRAF* 1799T>A positive cells in blood – single reaction

Blood with defined white blood cell count spiked with DRO thyroid cancer cells (hemizygous *BRAF*1799T>A)

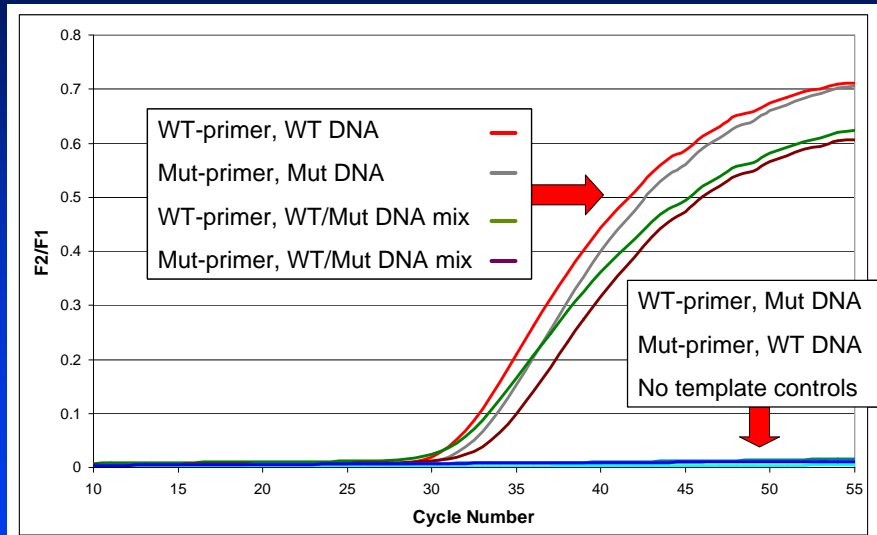


Detection sensitivity for *BRAF* 1799T>A positive cells in blood – multiple reactions

DRO-WBC mix of 1:100,000 re-run in 5 replicates



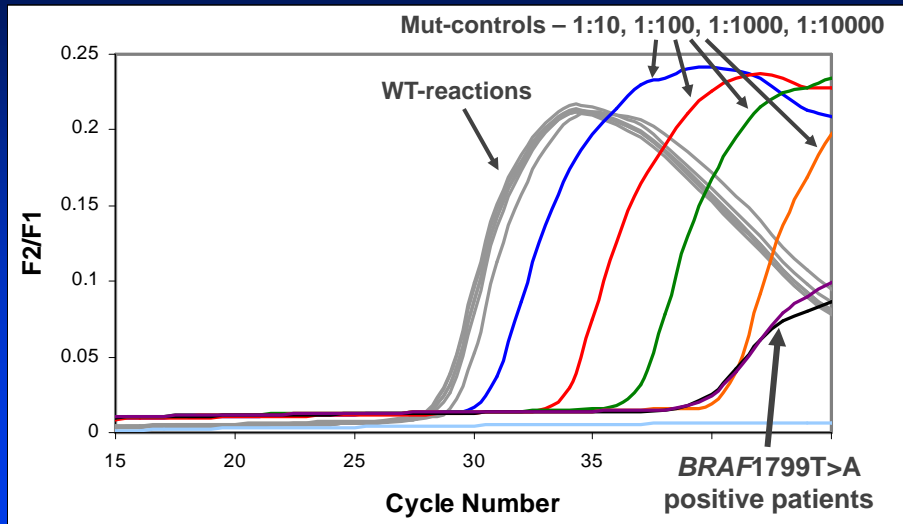
Demonstrating primer-probe specificity



Circulating *BRAF*1799T>A - The definitive study in thyroid cancer -

- **Prospective blood collection from Mayo Clinic Rochester thyroid cancer patients attending for follow-up between 5/2004 and 12/2006**
 - Adults scheduled for routine blood draw were offered participation
 - 193 patients enrolled
 - 173 PTC
 - 56 had archival tumor tissue available
 - 20 non-PTC thyroid cancers (FTC, HCC, MTC)
 - 19 had archival tumor tissue available
- **Complete staging and follow-up for all patients**
- **DNA extracted and frozen, *BRAF*1799T>A and *BRAF*-WT assays run in batches of 5-6 patients, blinded to patient data.**
- **Results correlated with clinical data after ≥ 2 year follow-up (12/2008) & archival tissue *BRAF* genotyped**

Detection of *BRAF* 1799T>A in blood samples of PTC patients



Prevalence of circulating *BRAF*1799T>A in thyroid cancer

• PTC

- Circulating *BRAF*1799T>A + 20/173 (11.6%)
- Archival tissue *BRAF*1799A>T + 42/56 (75%)
 - Archival +, circulating + 8/42 (19%)
 - Archival +, circulating - 34/42 (81%)
 - Archival -, circulating + 1/14 (7%)
 - Archival -, circulating - 13/14 (93%)

• Non-PTC

- Circulating *BRAF*1799T>A + 0/20 (0%)
- Archival tissue *BRAF*1799T>A + 0/19 (0%)



Copy numbers in BRAF1799T>A positive patients

<i>BRAF</i> ^{T1799A} haploid copy numbers per <i>BRAF</i> wild-type diploid copies	Samples per category (N)	Time interval between initial treatment and blood draw (y; median, range)
≥1:1,000	1	0.1
<1:1,000 – 1:10,000	5	4.8, 0.2-14.7
<1:10,000 – 1:100,000	5	11.6, 0.2-26.5
<1:100,000	9	0.8, 0.8-15.8



BRAF-status correlates with PTC disease status at time of blood draw

	AFD	AWD
<i>BRAF</i>-WT*	111	30
<i>BRAF</i>1799T>A*	10	8

* RDX and UK patients excluded.

AWD RR for *BRAF*1799T>A vs. *BRAF*-WT: 2.55
(95% CI: 1.08-5.99; 2-tail p<0.04)



***BRAF*-status and Tg**

- 9 *BRAF*1799A>T positive patients had Tg levels >0.1 ng/mL.
- 1 *BRAF*1799A>T positive patient had a likely false low Tg (<0.1 ng/mL), based on TgAB levels of 66 kIU/L
- No difference between *BRAF*-WT and *BRAF*1799A>T positive patients regarding proportion of patients with Tg >0.1 ng/mL
- Among TgAB negative patients, *BRAF* testing did not detect any recurrences that would not have been detected by Tg >0.1 ng/mL



Conclusions about the utility of detection of circulating *BRAF*1799T>A

- Detected in approximately 20% of patients with a *BRAF*1799T>A mutation in their primary tumor
 - Some negative correlation with time since treatment
- Occurs rarely, if ever, in the absence of a *BRAF*1799T>A mutation in the primary
- Correlates with disease status and is concordant with Tg status in TgAB negative patients
- Does not add substantial value over Tg in TgAB negative patients without thyroid tissue – this conclusion might change in patients with sizable remnant glands
- Potentially adds value in TgAB positive patients



Additional assays that have already developed in the lab

HRAS
KRAS
NRAS
RET/PTC1
RET/PTC2
RET/PTC3
PAX8/PPAR γ



Some “far off” stuff we are working on

- **Circulating micro RNA in thyroid cancer**
- **Individual patient “tumor genetic profile” at diagnosis**
- **Linking genetic changes with drug responses (kinase inhibitors, PPAR γ agnoists, conventional chemotherapy drugs)**



Thank You

Questions?

