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Molecular Genetics of Thyroid Cancer and their Influence in the Diagnosis and Management of this Disease

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ABSTRACT

The majority of thyroid neoplasms originate from the thyroid epithelial follicular cells, while 3% to 5% of neoplasms arise from the C cells or parafollicular cells. Differentiated thyroid cancer (DTC), which derives from these follicular cells, includes papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), oncocytic cell carcinoma (formerly known as Hürthle Cell Carcinoma/OCC), poorly differentiated carcinoma (insular carcinoma), and anaplastic thyroid carcinoma (ACC/undifferentiated). These thyroid tumors comprise the majority, more than 90% of the cases, of all thyroid neoplasms. Of all these subtypes, ATC is the rarest and is characterized by its extremely poor prognosis. Likewise, poorly differentiated carcinoma is characterized by its aggressive behavior and its unfavorable prognosis. Recent advances in our comprehension of the molecular genetics of thyroid cancer have been made by identification of targetable genetic alterations in its pathogenesis. This paper will undergo an extensive review of molecular pathways that lead to the development of thyroid cancer, their implications in the diagnosis, surgical management, and adjuvant treatment.

Keywords: Thyroid Cancer, Thyroid Neoplasms, Molecular Genetics of Thyroid Cancer

The preponderance of thyroid neoplasms originate from the thyroid epithelial follicular cells, while 3% to 5% of neoplasms arise from the C cells or parafollicular cells [1]. Differentiated thyroid cancer (DTC), which derives from these follicular cells, includes papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), oncocytic cell carcinoma (formerly known as Hürthle Cell Carcinoma / OCC), poorly differentiated carcinoma (insular carcinoma), and anaplastic thyroid carcinoma (ACC / undifferentiated) [1-7]. These thyroid tumors comprise the majority, more than 90% of the cases, of all thyroid neoplasms. Of all these subtypes, ATC is the rarest and is characterized by its extremely poor prognosis. Likewise, poorly differentiated carcinoma is characterized by its aggressive behavior and its unfavorable prognosis.

Between the year 2010 and 2014, 63,229 patients per year were diagnosed with thyroid cancer. Of these 89.4% had PTC, 4.6% had FTC, 2.0% had OCC, 1.7% had medullary thyroid carcinoma (MTC), and 0.8% had ATC [1, 8]. A follicular adenoma is a benign tumor (clonal neoplasm) that may serve as a precursor lesion for some follicular carcinomas. Less-differentiated thyroid cancers, namely poorly differentiated carcinomas, and

anaplastic carcinomas, can develop de novo, although many of them arise through the process of a stepwise dedifferentiation of papillary and follicular carcinomas (Figure 1).

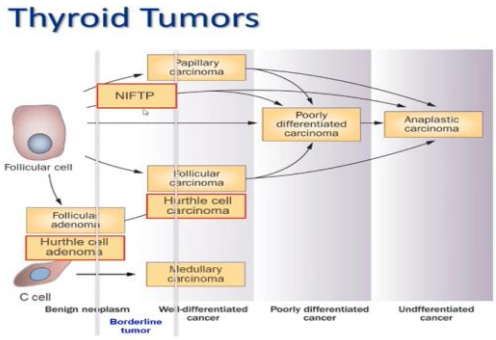


Figure 1: Stepwise dedifferentiation of papillary and follicular carcinomas.

Thyroid nodules are a major health problem worldwide. Studies have shown that the prevalence of palpable thyroid nodules is roughly 5% in women and 1% in men living in parts of the world with sufficient iodine [1,3-5,9,10]. In contrast, high-resolution ultrasound can detect thyroid nodules in around 19% to 68% of randomly selected people, with higher frequencies in women and the elderly [1,3,5,10]. The clinical significance of thyroid nodules lies in the need to exclude thyroid cancer, which occurs anywhere between 7% and 15% of the cases, depending on age, gender, radiation exposure history, and family history [1,3].

The discovery in 1953 of the double helix structure of deoxyribonucleic acid (DNA), by James Watson and Francis Crick marked a milestone in the history of science and gave rise to modern molecular genetics that has rapidly advanced and has help to identify the driver mutations in in many cancers, including thyroid neoplasms: RET / PTC, RAS, TP53, RET, TSHR, GNAS, PTEN, APC, TRK, CTNNB1, PAX8-PPARG, BRAF, AKAP9-BRAF, AKT1, TERT, ETVS-NTRK3, DICER1, EIFF1AX, STRN-ALK, MEN1, VCL-FGRF2, TGF-MET, THADA-IGF2BP3, MAP2K1, PAX8-GLIS3, PAX8-GLIS1 (Figure 2). Analogous to other malignant neoplasms, the initiation and progression of thyroid cancer occurs through steady accumulation of multiple genetic and epigenetic alterations, including activating and inactivating somatic mutations, alteration in gene expression patterns, microRNA (miRNA) dysregulation, and aberrant gene methylation [7].

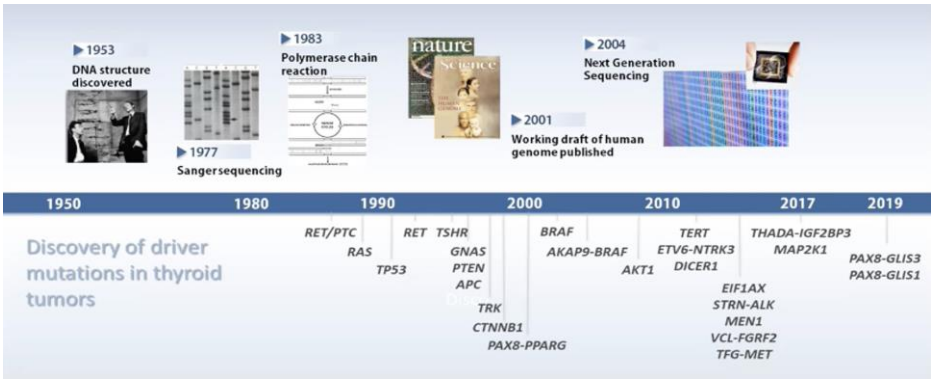


Figure 2: Progress in the Understanding of Cancer Genetics.

Most of the data that has been gathered is associated with somatic mutations, many of which occur early in the transformation process and are crucial for cancer development. In thyroid cancer, vital genes are commonly

mutated via two separate molecular mechanisms: point mutations or chromosomal rearrangements [7]. Point mutations are the result of single nucleotide change within the DNA chain, which activates or inactivates a protein, whereas chromosomal rearrangements represent a large-scale genetic abnormality with breakage and fusion of parts of the same chromosome or different chromosomes [7].

The molecular pathogenesis of thyroid cancer is relatively simple with two molecular pathways, the mitogen-activated protein kinase (MAPK) pathway and phosphatidylinositol 3 - kinase - protein kinase B (PI3K-AKT) pathway (Figure 3). These pathways are activated in most thyroid cancers via distinct molecular mechanisms mentioned previously, like point mutations (single nucleotide variant) where a single nucleotide is changed which activates or inactivates a protein, gene fusions were parts of two different genes located in the same or different chromosome are fused to each other that results in the generation of chimeric protein, and copy number alterations (CNA) that have been recently accepted as a driver mutation for oncocytic cell tumors [11]. Activation of the MAPK pathway is a critical step for tumor initiation. The mutated genes that impact these pathways encode the cell-membrane receptor tyrosine kinases RET and NTRK1 and intracellular signal transducers RAS and BRAF. These mutually exclusive mutations (they do not overlap with each other because they activate the same pathway and only one event is sufficient to activate the process for cancer development), occur in roughly 70% to 75% of patients with PTC and are associated with specific clinical, pathologic, and biological tumor characteristics (Table 1) (15-18). In FTC, in addition to mutations of RAS, another common event is PAX8 / PPARG rearrangement. Thyroid cancer progression and dedifferentiation involves a number of additional mutations that affect the PI3K-AKT pathway and other cell signaling pathways.

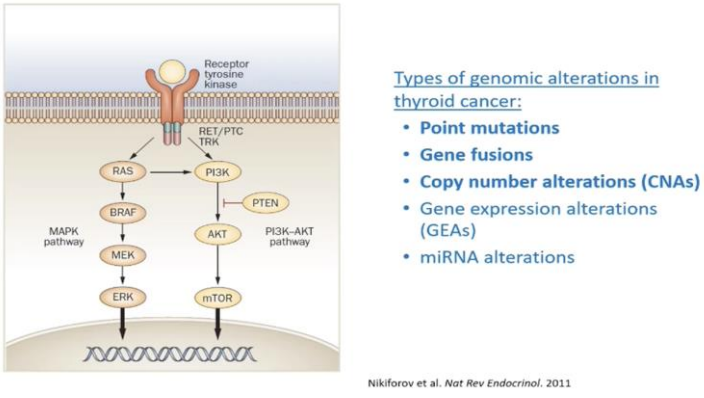
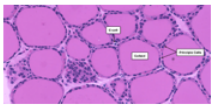
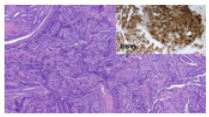
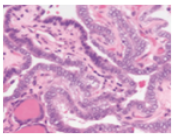
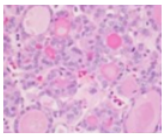


Figure 2: Molecular Pathogenesis of Thyroid Cancer.

Table 1: Molecular pathways in PTC and typical histological presentation and clinical-pathological features linked with specific mutations.

Normal Thyroid	BRAF	RET / PTC	RAS
	 • PTC: 75% (29% to 83%) • 62% V600E BRAF • Tall cell and classic PTC • Infiltrative: Common Extrathyroidal Extension • Spread to lymph node first • Higher Stage at Presentation • Higher Rate of Tumor Recurrence • Propensity for Dedifferentiation • Prone to loss markers of thyroid differentiation **early** in the disease process	 • PTC: 20% (2.5% 73%) • Younger Age at Presentation • Classic Histology PTC • Association with Radiation Exposure • Frequent Lymph Node Metastasis • Lower Stage at Presentation	 • PTC: 15% • Invasive encapsulated follicular variant of papillary thyroid carcinoma (IEFVPTC) • Nuclear Features of PTC may or not be present • More Frequent Encapsulation • Less Frequent Lymph Node Metastasis • More Frequent Distant Metastasis

PTC is one of the best molecularly understood cancers with more that 97% of the driver mutations identified. One of the main goals of the cancer genome atlas research network study (TCGA) thyroid project was to detect cancer-initiating mutations, i.e., driver mutations, in those cases that lacked the well-known PTC driver mutations (BRAF V600E, point mutations of RAS genes, and gene fusions involving RET and NTRK) [12]. These cases are referred to by computational biologists as "dark matter" cases. These very important studies under the umbrella of NCI and NIH analyzed 496 PTCs that permitted several analyses that showed that approximately 75% of all PTC developed through the molecular mechanism of point mutation, the most common been the BRAF V600E mutation, the second most common the RAS mutation, followed by the TERT mutation, that constituted a small percentage of tumors but was found to be a marker of aggressive disease [12]. Roughly 15% of PTC develop through the mechanism of gene fusions, the most common been RET / PTC, NTRK 1/3, ALK, BRAF, PAX8 / PPARG. The RET / PTC, NTRK 1/3, ALK have very important therapeutic implications for advanced thyroid cancer because of the availability of targeted inhibitors with low toxicity and high efficacy for the management of these tumors. Roughly 7% of PTC develop exclusively from the molecular mechanism of copy number alterations (CNA) (Figure 4).

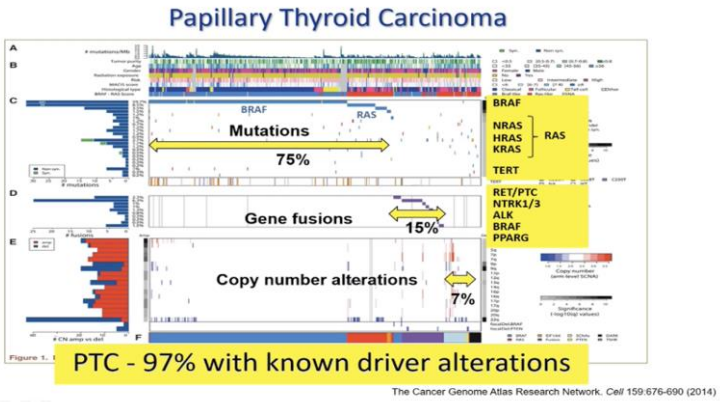


Figure 3: Cancer-initiating mutations in PTC [12].

Roughly 45% of PTC have a mutation in the BRAF gene, making it the most frequently known genetic alteration in PTC [13-15]. Practically all mutations involve nucleotide 1799 and result in a valine-to-glutamate substitution at residue 600 (V600E) [14]. This point mutation leads to constitutive activation of BRAF kinase, resulting in a constant phosphorylation of MEK and downstream effectors of the MAPK pathway (Figure 2) [16]. Other mechanisms of BRAF activation in PTC include K601E point mutation, small in-frame insertions or deletions surrounding codon 600 [17,18], and AKAP9-BRAF rearrangement, which is more common in PTC associated with radiation exposure [19].

BRAF V600E mutation is the prevailing mutation in PTC with classical histology and in the tall cell variant but is rare in follicular variant tumors [14,20]. In multiple studies, the existence of the BRAF mutation has been linked with aggressive tumor biology such as advanced stage at presentation, extrathyroidal extension, recurrence, and lymph node or distant metastases [21,22]. BRAF V600E mutation is an independent predictor of tumor recurrence, even in patients with stage I to stage II disease [23,24]. The ability of thyroid cancers to trap radioiodine has been identified to be decreased in tumors with the BRAF V600E mutations and this may lead to treatment failures of the recurrent disease, which is due to the dysregulation of the function of sodium iodide symporter (NIS) and other genes metabolizing iodide in the thyroid follicular cells [23,25-27]. In thyroid nodules, the V600E BRAF mutation is limited to PTC and poorly differentiated and ATC arising from PTC [21,22,28]. Consequently, the identification of the BRAF V600E mutation in FNA cytology of thyroid nodules practically confirms the diagnosis of PTC [20,29]. Testing for the BRAF mutation is very useful diagnostically in thyroid FNA samples with indeterminate results, as it can help to determine the diagnosis of PTC in a important portion of these biopsies [30].

Pathologically PTC is one disease and biologically at a minimum PTC has two significantly different groups based on multiple type of analysis like gene expression alterations, microRNA alterations, DNA methylation, transcriptomics (Figure 5) [12, 31]. The Cancer Genome Atlas (TCGA) study on PTC highlighted that PTC can be grouped into BRAFV600E-like and RAS-like tumors. BRAFV600E-like tumors also harbor RET fusions, while RAS-like tumors show RAS mutations, the BRAFK601E, and PPARG and THADA fusions [31]. The V600E BRAF–like PTC are usually classic PTC and tall cell subtype of PTC. Clinically when we look at the differentiation score, which is the expression of genes involved in iodine metabolism and synthesis, BRAF tumors loose expression of thyroid differentiation markers while RAS-like tumors retain them, almost at the level of a normal thyroid cell [31]. This is important therapeutically because one of the most efficient management options in thyroid cancer is iodine therapy. RAS-like tumors are usually follicular variant PTC. Usually all BRAF mutations are BRAF-like and all RAS mutations are RAS-like, but all other type of molecular alterations can be hidden in either BRAF-like and RAS-like. For example, BRAF K601E mutations is found in RAS-like tumors.

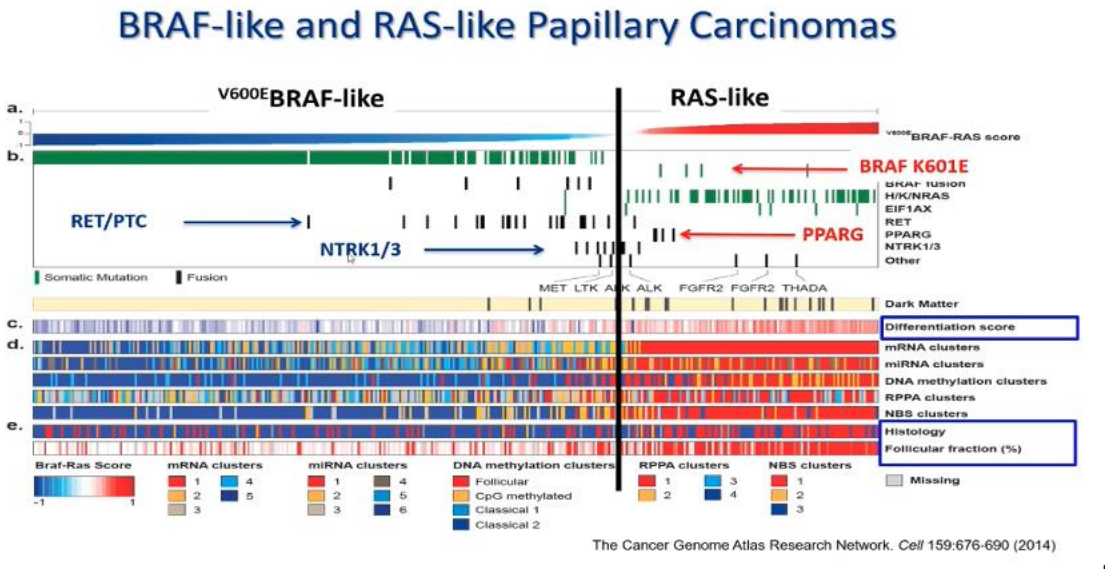


Figure 4: Molecular Subtypes of Papillary Thyroid Carcinomas [32].

The second most common type of thyroid cancer is FTC [1]. Fundamentally all follicular carcinomas are RAS-like tumors. There profile is different from classic PTC because they do not have BRAF mutations, and most of them have RAS and RAS-like mutations. Yoo S.K et al, from Korea showed that the genetic profile of follicular carcinomas is very similar to follicular adenomas because they are related tumors [33]. Most FTC originate from a FA and eventually break through the capsule and become carcinomas. In encapsulated follicular variant of PTC their molecular profile is much closer to a FA and FTC than to classic PTC. Infiltrative follicular variant of PTC has a molecular profile that is more like classic PTC than FTC [33]. The biologic difference between follicular pattern RAS-like tumors and classic PTC is the infiltrative growth pattern (Figure 6). The difference between these tumors is not only phenotypically based on gross pattern, but also based on biological and clinical differences, because follicular pattern RAS-like tumors retain avidity to radioactive iodine. BRAF-like tumors (classic PTC and infiltrative follicular variant of PTC) have the classic features of PTC, they are infiltrative, they spread to lymph nodes first and later to distant sites, and they lose the expression of genes associated with thyroid differentiation. RAS-like tumors (FA, FTC, NIFTP, and invasive encapsulated follicular variant of PTC) may or may not have nuclear features of PTC, they are encapsulated, they spread to distant sites (rarely to lymph nodes), and they retain expression of genes associated with thyroid differentiation.

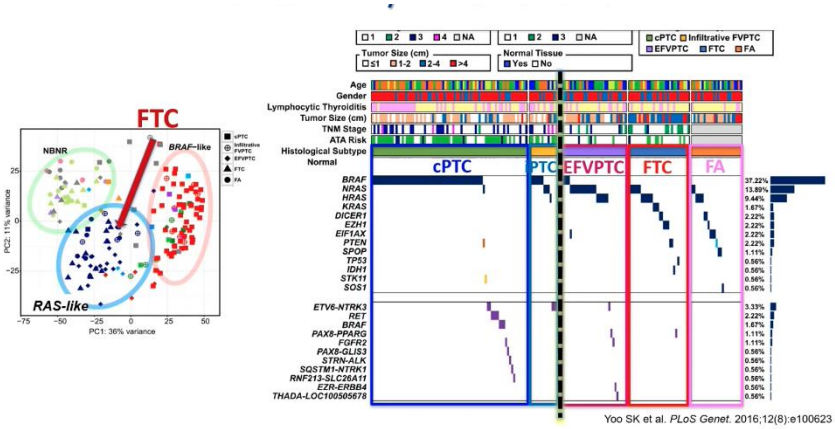


Figure 5: Graph exemplifying the difference between follicular pattern RAS-like tumors and classic PTC, which is the infiltrative growth pattern [33].

The RAS genes (HRAS, KRAS and NRAS) encode for the interconnected G-proteins that play a critical role in the intracellular transduction of signals arising from cell membrane receptors [34]. RAS protein in its inactive state, is bound to guanosine diphosphate (GDP), upon activation, it releases GDP and binds guanosine triphosphate (GTP), thus activating the MAPK and other signaling pathways, such as PI3K/AKT. Typically, the activated RAS / GTP protein becomes promptly inactive due to its intrinsic GTPase activity and the action of cytoplasmic GTPase-activating proteins [11]. Point mutations in the domains of the RAS gene either increase its affinity for GTP (mutations in codons 12 and 13) or inactivate its autocatalytic GTPase activity (mutation in codon 61) [22,35,36]. The consequence of this is that the mutant protein becomes permanently switched in the active position and continuously activates its downstream targets. Mutations in the RAS genes are believed play an early role in the cellular transformation and may predispose to the progression benign tumors to malignant tumors [37].

Point mutations involving the specific sites (codons 12, 13 and 61) of the NRAS, HRAS or KRAS genes are identified in roughly 10% to 20% of PTC [30,38]. PTC holding RAS mutation invariably have follicular variant histology; this mutation also correlates with significantly less prominent nuclear features of PTC, more common thyroid encapsulation, and a lower rate of lymph node metastases [20,36,37]. A few studies have linked RAS mutations with PTC that have a more aggressive behavior, such as a higher frequency of distant metastases [39]. Mutations in the RAS gene are not limited to PTC and also found in other benign and malignant thyroid neoplasms, as well as in tumors from other tissues. RAS gene mutations are identified in approximately 20% to 40% of follicular thyroid adenomas (FTA), 40% to 50% of FTC, and 20% to 40% of ATC [30,38]. When a FNA cytology is indetermined and the molecular profile identifies a RAS mutation the risk of malignancy varies between the type of mutation, HRAS = 71%, NRAS = 63%, and KRAS = 33% [40].

The concept of progression from benign to malignant tumors is supported by the molecular profile shared by these different tumors [33]. More evidence supporting this concept of cancer progression is the similar morphology that these lesions have, along with experimental mouse data showing very similar results. Pathologist observing thyroid nodules have detected this step wise progression (Figure 7) [41]. The nodule in Figure 6 developed from a single cell driven by the RAS mutation, it continued to grow and grow, it eventually

forms a capsule, looking microscopically like a benign adenoma (goiter), then it continues to progress, it accumulates more genetic alterations (micro mRNA, and it involves other molecular pathways), it becomes a tumor, it eventually breaks through the capsule, and it will give you invasive encapsulated follicular variant of PTC. If an FNA is performed of area A it would come back as Bethesda II, in area B it would come back as a Bethesda IV, and in area C as Bethesda V. This has changed the practice of cytopathology with molecular testing helping us understand better the biological nature of these tumors [41].

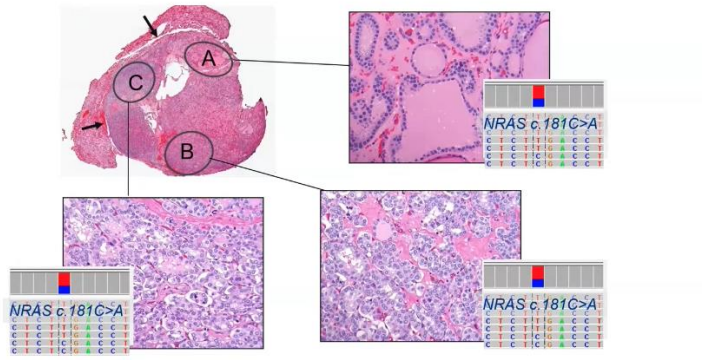
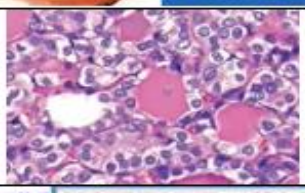
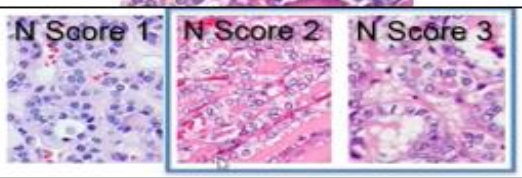


Figure 6 : Molecular Changes Precede Histological Changes [41]. The tumor measures 2.5 cm, it has a thin capsule (black arrows) and shows an area of flat epithelial cells lining the follicles representing a benign thyroid goiter (tumor area A). Microfollicular areas with well-developed nuclear features of PTC are seen in tumor area B representing a probable NFTP. Tumor area C has separation artifact with the formation of papillary structures with nuclear features of PTC. Molecular studies of each section will show NRAS mutation.

As mentioned previously follicular tumors develop through a multistep process where they can be identified via cytopathology at different stages in their development. These does not occur in BRAF-like tumors, where they develop from the early stages as microcarcinomas. The problem is that in clinical practice we want the tumors to be classified in a binary distribution (benign or malignant), which is very difficult because of the multistep process of their development. NIFTP has partially resolved this problem (NIFTP would be a seen in the later stages of this multistep process) [42]. The diagnostic features of NIFTP include follicular architecture, nuclear features of PTC, formation of aa capsule with lack of invasion [42]. This entity was previously known as encapsulated follicular variant of papillary carcinoma [42]. The histologic criteria of NIFTP are depicted in Table 2. NIFTP showed be viewed as a borderline malignant tumor (equivalent to carcinoma in situ) and if no invasion is identified on the final pathology the risk of recurrence is very low (less than 1%) [42]. This lesion still requires surgical resection by a minimalistic approach usually is sufficient (thyroid lobectomy). The molecular characteristics of NIFTP include RAS and RAS-like mutations, BRAF V600E and TERT mutations should not be identified in this lesion. NIFTP is considered a precursor lesion for invasive encapsulated follicular variant of PTC (EFVPTC). The introduction of NIFTP did not resolve the uncertainty in the pathological diagnosis of PTC. A study from four different institution (Memorial Sloan Kettering Cancer Center - MSKCC, Moffit Cancer Center - MCC, Cedar Sinai Medical Center in Los Angeles - CSMC, Mount Sinai Health System in New York - MSHS) with RAS-positive thyroid nodules by Marcardis et al [43], identified a wide variation in the prevalence of NIFTP in resected indeterminate thyroid nodules across several institutions, ranging from 5% to 46%. At MSKCC, the most common overall and non-malignant diagnosis in RAS-mutated nodules was NIFTP. This was significantly greater than the NIFTP rate at the other three institutions (MCC,

CSMC, MSHS) where the most common overall and benign diagnosis was follicular adenoma / nodular hyperplasia [43]. Significant variations in the rates NIFTP (5% to 13%) and follicular adenoma / nodular hyperplasia (63% to 85%) in the other three institutions (MCC, CSMC, MSHS) also occurred. This reflects the same issue that some thyroid nodules may be identified at stages in their development where the nuclear features of PTC are clearly absent, some at stages where the nuclear features are clearly present, while others can be identified at different stages in the development of the nuclear features of PTC (in some of these stages the nuclear features of PTC are not fully expressed). Depending on where the pathologist draws the line in this continuum more or less thyroid nodules, will be called cancers or NIFTP, leading to the difficulty in making a diagnosis (Figure 8).

Table 2: Revised Diagnostic Criteria for NIFTP [44]

Diagnostic Criteria of NIFTP	
Encapsulation or clear demarcation	
Follicular growth pattern: • No well-formed papillae • No psammoma bodies • Less than 30% solid / trabecular / insular growth pattern	
Nuclear features of PTC (nuclear score 2 to 3)	
No vascular or capsular invasion	
No aggressive histology: • No tumor necrosis or high mitotic activity	
Secondary Criteria: • Lack of **BRAF V600E mutation** detected by molecular assays or immunohistochemistry • Lack of BRAF V600E-like mutations or other high-risk mutations (TERT, TP53)	

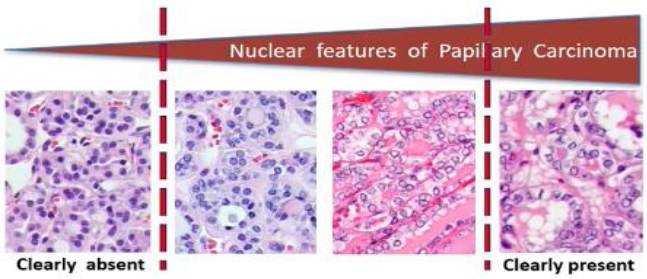


Figure 7: Different Stages in the Development of PTC [43].

BRAF-like tumors comprise classic PTC or infiltrative FVPTC. These tumors morphologically are characterized by having nuclear features of PTC, they have an infiltrative growth pattern, clinically spread to regional lymph nodes first and later to distant metastatic sites [32]. BRAF-like tumors are prone to lose markers of thyroid differentiation at early stages in their development and therefore should be treated surgically from the very beginning, usually with a total thyroidectomy with or without a central compartment neck dissection. RAF-like tumors include follicular adenomas and follicular carcinomas. These two tumors have a very similar morphology, and the only difference between them is the capsular invasion or vascular invasion. NIFTP are not invasive but have a follicular growth pattern with nuclear features of PTC [32]. Invasive encapsulated FVPTC develop from a NIFTP tumor, they are characterized by having a thick capsule, nuclear features of PTC, replacement of the thyroid lobe with cancer cells, smooth borders, with a pushing growth pattern (not infiltrative), and they retain for longer periods of time the markers of thyroid differentiation [32].

In the 2022 the WHO classification changed the designation of Hürthle cell carcinoma (HCC) to oncocytic thyroid carcinoma (OCA) [45]. Besides the terminology, the histopathological criteria for diagnosis of OCAs and their subclassification into minimally invasive, angioinvasive, and widely invasive type have remained unchanged [45]. Oncocytic cell tumors, oncocytic adenoma (OA) or OCA are tumors composed by more than 75% cells showing the typical appearance of oncocytes, that is large size, deeply eosinophilic (very pink), very granular cytoplasm due to accumulation of mitochondria, and large nuclei with prominent nucleoli [45-47]. Many of these tumors have mitochondrial DNA mutations, which are unlikely to be oncogenic in nature. One very important genetic characteristic is that OCA have profound chromosomal copy number (CNA) alterations, also known as near haploidization (near-haploid genome) [47]. Most human chromosomes have in every cell two alleles, one paternal and one maternal, in OCA a phenomenon called uniparental disomy (UPD) is present, in which both members of a chromosome pair are inherited from one parent, and the other parent's chromosome for that pair is missing [47]. This alteration is highly characteristic of oncocytic cell tumors (both benign OA and malignant OCA). In a study from Doerfler et al [48], found CNA in roughly 80% of OCA but also identified CNA in 38% of OA (secondary mutations can occur). It is not a surprise that we find CNA in OA because they are a precursor lesion for OCA. In this situation pathologist must be very diligent in looking at the entire capsule in order not to miss an OCA.

Dedifferentiated thyroid cancer is characterized by having progressive accumulation of mutations that can occur as early and late events (Figure 9) [49]. We are identifying a multi-step progression (not from benign to malignant) but from differentiated thyroid cancer to poorly differentiated thyroid cancer [49]. The mutational burden, which means the more mutations accumulated will lead to an overall worse prognosis. Poorly differentiated carcinoma is a rare thyroid tumor that arises from the follicular cells and is characterized by a partial loss of thyroid differentiation and has a less favorable prognosis in comparison with well-differentiated papillary or follicular carcinomas. Anaplastic (undifferentiated) carcinoma represents the most undifferentiated type of thyroid tumors. In thyroidectomy samples of poorly differentiated and anaplastic carcinomas, it is not infrequent to find areas of well-differentiated papillary, conventional follicular or oncocytic carcinomas [49].

This hints that these tumors may represent distinct steps in the stepwise progression from a well-differentiated carcinoma derived from follicular cells to a poorly differentiated carcinoma terminating in an anaplastic carcinoma [50]. In agreement with such a progression, some molecular alterations, considered to be early events in thyroid carcinogenesis (i.e., mutations of RAS and BRAF), are found in tumors with all levels of dedifferentiation, whereas others, late events (i.e., TERT and TP53 mutations) occur with increasing frequency in tumors that have progressively lost thyroid differentiation [49]. This progression can be seen histologically (Figure 9).

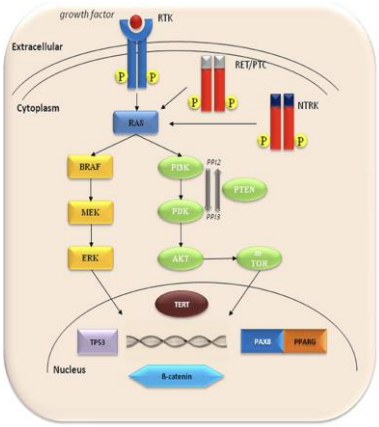
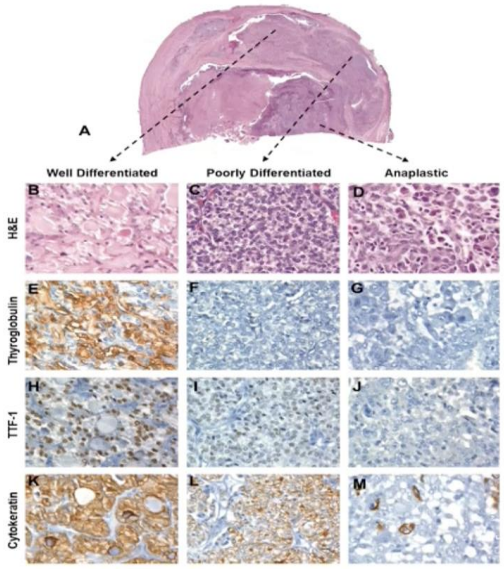


Figure 8: Genetics of dedifferentiated thyroid cancer [30].

Figure 9: The image depicts one tumor that has areas of well deafferented thyroid cancer, poorly differentiated thyroid cancer and anaplastic thyroid cancer. The well differentiated tumors preserver all markers differentiation (thyroglobulin, TTF-1, and cytokeratin) compared to the ATC which losses these markers [50].



Among the most common mutations found in human cancers are inactivating point mutations in the TP53 tumor suppressor gene [30]. In thyroid tumors, point mutations of the TP53 gene are a late event, reported in roughly 60% to 80% of ATC and 15% to 30% of poorly differentiated thyroid cancer (PDTC), but are rarely seen in FTC and PTC [30]. Most of these inactivating mutations involve exons 5 to 8 of the gene that alter its DNA binding properties. p53 inactivation in thyroid cells is not only responsible for accelerated tumor growth but is

also associated with the progressive loss of thyroid differentiation markers [30]. In fact, the recovery of wild-type p53 expression in cultured thyroid ATC cells leads to the reduction in proliferation rate, re-expression of thyroid-specific genes (e.g., TPO, PAX-8), and re-acquisition of the ability to respond to thyroid-stimulating hormone stimulation. This alludes that the progressive loss of differentiation in PDTC and ATC is mediated, at least in part, by the inactivation of the p53 gene. It also hints that the restoration of TP53 function could possibly act as a therapeutic approach for these highly aggressive tumors [30]. Indeed, viral TP53 gene therapy has been tested in preclinical and clinical trials for various cancer types and is under evaluation for ATC [30].

A cytoplasmic protein called β-catenin is encoded by the CTNNB1 gene and is an important intermediate in the wingless (Wnt) signaling pathway [30]. Point mutations in exon 3 of CTNNB1 have been found in approximately 25% of PDTC and in 66% of ATC, but not in well-differentiated carcinomas. The majority of tumors carrying this mutation also showed an aberrant nuclear expression of the protein determined by immunohistochemical analysis, although there was no full correlation between these findings.

Point mutations of the RAS genes have been reported in 18% to 27% of PDTC and in 50% to 60% of ATC [30]. It is very likely that RAS mutations stimulate genomic instability in the affected thyroid cells and predisposes them to accumulation of additional genetic abnormalities, such as mutations of the TP53 gene [30]. BRAF gene mutations are identified in approximately 15% of PDTC and 20% of ATC. This provides more evidence that these tumors represent distinct steps in the stepwise progression from a well-differentiated carcinoma derived from the follicular cells to a PTDC, culminating in an ATC [50].

The classic diagnostic features that help to distinguish hyperplastic thyroid nodules from adenomas are depicted in table 3. None of these histopathologic features can truly differentiate between hyperplastic nodules and follicular adenomas. The only true biological difference is that follicular adenomas are monoclonal and hyperplastic nodules are polyclonal. The hyperplastic nodule is formed by multiple thyroid cells dividing several times (a million cells dividing multiple times) until a nodule is formed (no genetic alteration) and an adenoma develops from a single cell that acquires a genetic mutation (like a RAS mutation) followed multiple / hundreds of divisions until a follicular adenoma develops (clonal gene mutation). These nodules have a greater chance of secondary events that may lead to cancer development. Identifying a clonal (somatic) molecular alteration is diagnostic of a neoplasm (adenoma or carcinoma) irrespective of microscopic appearance.

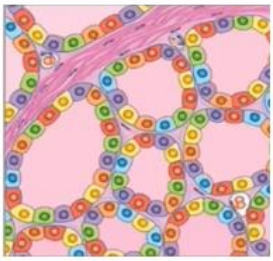
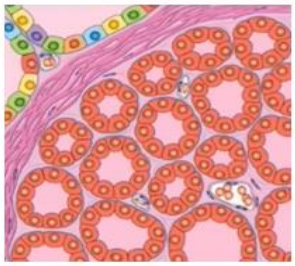
Hyperplastic Nodule	Follicular Adenoma
Multiple Lesions	Solitary lesion
Variable Degree of Encapsulation	Well-developed capsule
Variable cells in the nodules without compression and similar to those outside nodule	Uniform lesion with compression of adjacent dissimilar thyroid
Polyclonal cell population	*Monoclonal cell population*
 **No genetic alteration**	 **Clonal gene mutation**

Table 3: Histopathologic features that help to distinguish hyperplastic thyroid nodules from follicular adenomas.

The vast majority of thyroid nodules are non-neoplastic / non-clonal and are grouped as hyperplastic nodules. Hyperplastic nodules have a very low rate of progression to thyroid cancer because they have no mutations. Neoplastic thyroid tumors can be divided into two or three molecular pathways (Figure 10): BRAF V600E-like and RAS-like or BRAF V600E-like, RAS-like, and non-BRAF V600-E / non-RAS-like based on the mutational and gene expression profiles [31,33]. The BRAF V600E group is generally represented by PTC. The BRAF V600E-like molecular profile includes the BRAF V600E mutation and gene fusions involving BRAF, RET, and neurotrophic receptor tyrosine kinase 1/3 (NTRK1/3). RAS-like molecular profiles include NRAS, HRAS, KRAS, EIF1AX, enhancer of zeste 1 polycomb repressive complex 2 subunit (EZH1), Dicer 1, ribonuclease III (DICER1), phosphatase and tensin homolog (PTEN) mutations, BRAF K601E, and If the three-group molecular classification is applied, PAX8: PPARG gene fusion and mutations of EIF1AX, EZH1, IDH1, SOS1, SPOP, DICER1, and PTEN genes are classified as a non-BRAF V600E-/ non-RAS-like group. Encapsulated, well circumscribed thyroid neoplasm with a predominant follicular growth pattern generally has a RAS-like molecular profile [45]. High grade is histologically defined as the presence of ≥ 5 mitoses per 2 mm2 and / or tumor necrosis.

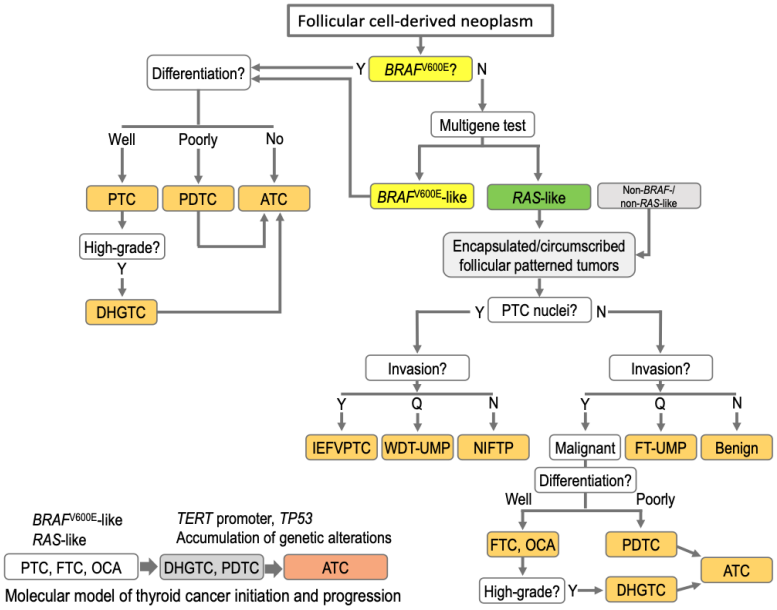


Figure 10: Molecular classification and histopathological correlates in follicular cell-derived neoplasms [45].

yes; N, no; Q, questionable; PDTC, poorly differentiated thyroid carcinoma; ATC, anaplastic thyroid carcinoma; DHGTC, differentiated high-grade thyroid carcinoma; IEFVPTC, invasive encapsulated follicular variant of papillary thyroid carcinoma; WDT-UMP, well-differentiated tumor of uncertain malignant potential; NIFTP, non-invasive follicular thyroid neoplasm with papillary-like nuclear features; FT-UMP, follicular tumor of uncertain malignant potential; FTC, follicular thyroid carcinoma; OCA, oncocytic carcinoma of the thyroid; TERT, telomerase reverse transcriptase; TP53, tumor protein p53; PAX8, paired box 8.

Thyroid cancer risk stratification based on the American Thyroid Association (ATA) modified classification of risk of structural disease recurrence (postoperative classification) is very important because it drives almost all decisions about thyroid cancer management: active surveillance, lobectomy vs completion thyroidectomy, use of radioactive iodine, and close follow-up vs longer interval follow-up (Figure 11) [51].

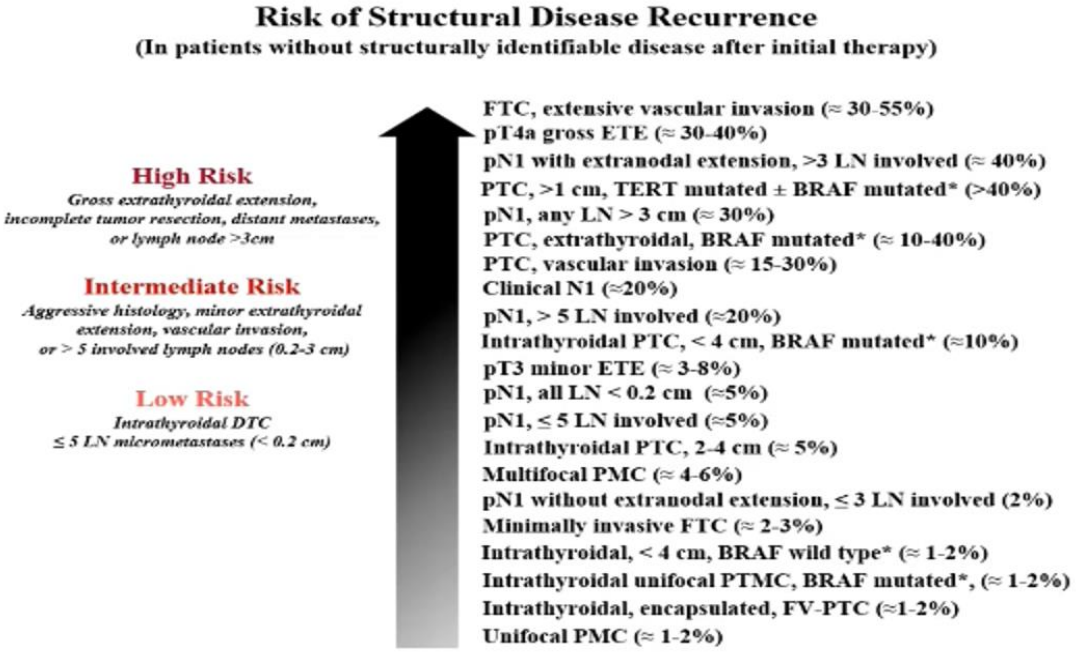


Figure 11: American thyroid association (ATA) modified classification of risk of structural disease recurrence [51].

We have molecular markers that can help us predict the behavior of thyroid neoplasms like solitary RAS mutations are seen in low-risk tumors, solitary BRAF mutations are seen in intermediate risk tumors, and RAS+TERT or BRAF+TERT are seen in high risk tumors, and solitary TERT mutations located in between intermediate risk and high risk tumors (Figure 12) [52,53]. Today in parallel to the postoperative classification from the ATA we are beginning to understand the molecular profile of low risk, intermediate risk, and high-risk thyroid tumors, helping us come up with a molecular markers that can help us risk stratify thyroid cancer in the preoperative and postoperative setting (Figure 13) [51,54,55].

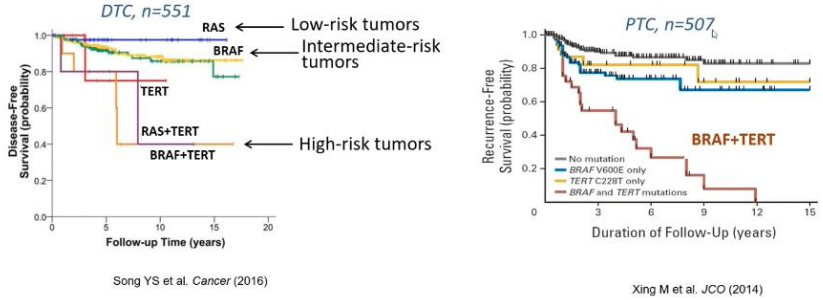


Figure 12: Molecular markers that can help us predict the behavior of thyroid neoplasms [52,53].

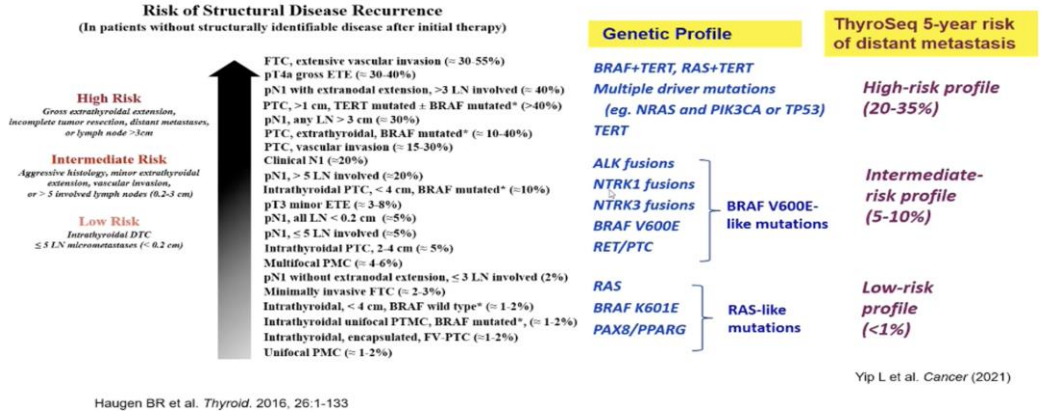


Figure 13: Molecular Markers for Thyroid Cancer Risk Stratification.

We can use molecular testing to help us individualize patient management, at our institution we use the ThyroSeq V3 GC (Figure 14). In patients who have a negative result with no molecular alterations most of the nodules will be hyperplastic nodules (they are not neoplastic / clonal nodules) for this reason, observation is a good management option. Patients with low risk / low level alterations have true clonal neoplasms, most of them will be benign, but some of them may progress to malignant tumors. Active surveillance is a good management option. Primary treatment options for thyroid neoplasms are determined by preoperative risk assessment that with molecular studies / markers will help us bridge the gap with postoperative risk assessment tools, allowing us to choose the appropriate treatment before embarking definitive therapy. An operation (total thyroidectomy vs thyroid lobectomy) is indicated for most patients with thyroid cancer, the extent of which (lobectomy, total thyroidectomy with or without central neck dissection, and extended resection) depends on patient and disease characteristics both histopathologic and molecular (Figure 14) [20, 56]. Radioactive iodine (RAI) is indicated to prevent and treat recurrent disease for patients with FTC and PTC who have undergone total thyroidectomy [57]. Nevertheless, roughly 60% to 70% of patients with PDTC and metastatic DTC eventually have thyroid cancer that develops resistance to RAI (RAI-refractory [RAI-R]). Life expectancy for patients with RAI-R thyroid cancer is 3 to 5 years [58].

With the increase in the understanding of the molecular origin of thyroid cancer, an increasing selection of treatment options have been developed that are FDA-approved either for specific tumor types harboring specific therapeutic targets, or in a disease agnostic manner focused solely on the therapeutic target. Important information may be obtained from genomic testing of thyroid cancer tissues that can influence management. DTC, even when it metastasizes to distant sites (lung or bone), often remain radiographically inactive for prolonged periods of time adopting a path similar to the primary tumor. Over time, these tumors may begin to progress more rapidly, as evidenced by shorter thyroglobulin or radiographic “doubling times” [59,60]. By contrast, PDTC and more rapidly growing primary thyroid cancers often portend more rapidly growing distant metastases. If treatment with TSH suppression therapy and RAI (even if there is uptake) do not slow progression of measurable metastatic lesions, consideration of use of non-targeted or targeted systemic therapies is recommended for patients who do not have contraindications for such therapy (Table 4).

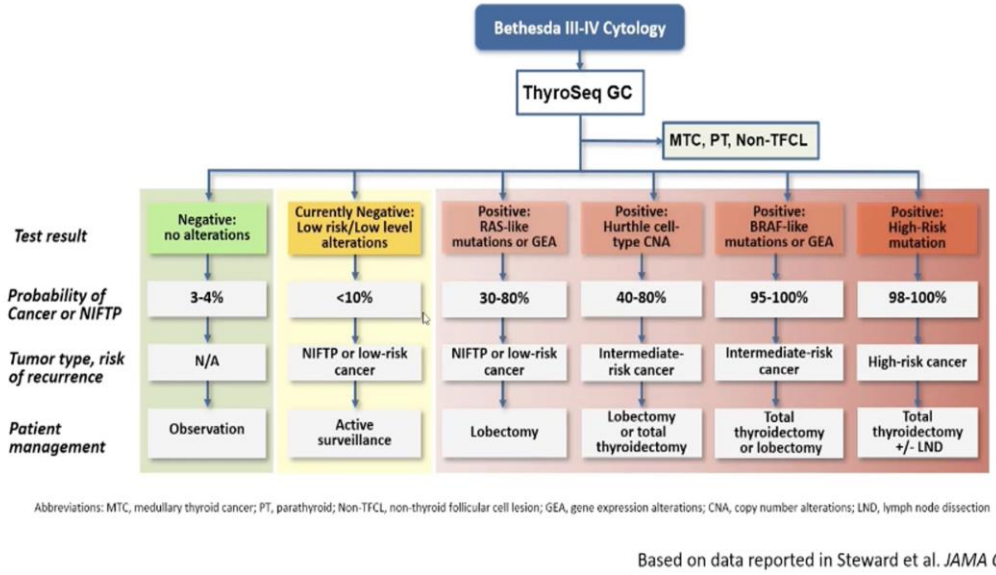


Figure 14: Individualized patient management informed by ThyroSeq testing [56].

Genetic Alteration	Tumor Type	Available Targeted Therapy
BRAF V600E	PTC, ATC	Vemurafenib, Debrafenib + Trametinib
HRAS	PTC, FTC, PDTC, ATC	Farnesyltransferase inhibitor tipifarnib
PAX8 / PPARG	FTC	Pioglitazone
ALK Fusions	PTC, ATC, PDTC	Crizotinib, Certinib
NTRK1/2/3 Fusions	PTC, ATC, PDTC	Entrectinib, Labrotectinib
RET	MTC, PTC	Vandetanib, Cabozantinib, Selpercatinib

Table 4: Molecular Markers for Management of Advanced Thyroid Cancer [61].

CONCLUSION

Thanks to the rapidly evolving field of molecular genetics the molecular drivers for most subtypes of thyroid tumors have been discovered. Thyroid nodules carrying clonal / somatic mutations are neoplasms (adenomas and carcinomas) and are not hyperplasia. Many thyroid tumors develop from pre-existing benign or borderline neoplasms through a multistep process. The molecular alterations identified in thyroid tumors define their biological properties, tumor linage, and clinical behavior. Molecular testing the management of thyroid nodules allows for a safe avoidance of diagnostic surgeries, offers prognostic information perioperatively to help tailor the operation to be recommended and detects therapeutic targets for advanced thyroid cancer.

The molecular profiling of WDTC is a rapidly evolving field, and it is one that holds great promise in individualizing the management of this disease when used in conjunction with current, established criteria. With continued research the development of predictive models that combine genetic data with the clinical and cytopathologic findings will allow for accurate preoperative risk assessment, helping to guide and individualize management options.

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