An Integrated Model of Morphologic and Molecular Signatures for the Classification of Papillary Thyroid Carcinoma

by

Karen Ester Gomez Hernandez

A thesis submitted in conformity with the requirements for the degree of Master of Science

Institute of Medical Science
University of Toronto

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Abstract

While it is appreciated that the $BRAF^{V600E}$ mutation is associated with classical papillary thyroid carcinoma (PTC) and mutations in $RAS$ are associated with follicular variant papillary thyroid carcinoma (FVPTC), there is overlap with reports of approximately 20% $BRAF^{V600E}$-mutated FVPTCs. We hypothesize that the discordance between morphologic and molecular classification stems from the application of less rigid histopathological criteria to define variants of PTC, namely, diagnosing FVPTC without excluding scattered true papillary structures. To address this question, we examined the molecular signatures of PTCs assessed by expert thyroid pathologists who classify these neoplasms based on strict morphologic criteria. Applying these criteria we noted a striking segregation of the $BRAF^{V600E}$ mutation to the group of classical PTCs as well as an association between classical architecture and adverse clinicopathological features. Integration of stricter morphologic criteria with molecular signatures should provide a robust platform for clinical applications.
Acknowledgments

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I want to acknowledge here Dr. Sylvia Asa, Dr. Ozgur Mete and Dr. Daniel Winer for their expert review of the surgical pathology specimens included in this body of work. Their expertise made this project possible.
Finally, I would like to thank my friend and colleague Dr. Zoe Lysy for supporting me through this challenging process and for being family when my own could not be here.

“A mi Padre, quien ha hecho y hará”
Contributions

Re-review of Tumor Samples-Ozgur Mete, Sylvia Asa

Photomicrographs of Papillary Thyroid Carcinoma-Ozgur Mete

Supervision-Shereen Ezzat (first and second year), Ozgur Mete (second and third year)

Co-Supervision-Sylvia Asa (first and second year)

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<th>Term</th>
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<tbody>
<tr>
<td>Akt</td>
<td>Akt-serine/threonine kinase also known as protein kinase B</td>
</tr>
<tr>
<td>AJCC</td>
<td>American Joint Committee on Cancer</td>
</tr>
<tr>
<td>BAD</td>
<td>BCL2 associated agonist of death</td>
</tr>
<tr>
<td>BCL2</td>
<td>apoptosis regulator BCL2</td>
</tr>
<tr>
<td>BRAF</td>
<td>B-Raf</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>cN1</td>
<td>clinical lymph node metastasis</td>
</tr>
<tr>
<td>DUSP</td>
<td>dual specific phosphatases</td>
</tr>
<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
</tr>
<tr>
<td>EIF1AX</td>
<td>eukaryotic translation initiation factor 1A, X-linked</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular regulated mitogen-activated protein kinase</td>
</tr>
<tr>
<td>ETE</td>
<td>extrathyroidal extension</td>
</tr>
<tr>
<td>FAP</td>
<td>familial adenomatous polyposis</td>
</tr>
<tr>
<td>FVPTC</td>
<td>follicular variant papillary thyroid carcinoma</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>NIFTP</td>
<td>noninvasive follicular thyroid neoplasm with papillary-like nuclear features</td>
</tr>
<tr>
<td>NFPTC</td>
<td>nuclear features of papillary thyroid carcinoma</td>
</tr>
<tr>
<td>NTRK</td>
<td>neurotrophin tyrosine kinase receptor</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>PI3K</td>
<td>phosphatidylinositol 3-kinase</td>
</tr>
<tr>
<td>PTC</td>
<td>papillary thyroid carcinoma</td>
</tr>
<tr>
<td>p90</td>
<td>p90 ribosomal s6 kinase</td>
</tr>
<tr>
<td>RET</td>
<td>rearranged during transfection</td>
</tr>
<tr>
<td>SEER</td>
<td>Surveillance, Epidemiology, and End Results</td>
</tr>
<tr>
<td>STK11</td>
<td>serine/threonine kinase 11</td>
</tr>
<tr>
<td>TCGA</td>
<td>The Cancer Genome Atlas</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumor, Node, and Metastasis</td>
</tr>
<tr>
<td>TSC2</td>
<td>tuberous sclerosis 2 protein</td>
</tr>
<tr>
<td>TSH</td>
<td>thyroid stimulating hormone also known as thyrotropin</td>
</tr>
<tr>
<td>UHN</td>
<td>University Health Network</td>
</tr>
<tr>
<td>WDTC</td>
<td>well-differentiated thyroid carcinoma</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WT</td>
<td>wild-type</td>
</tr>
</tbody>
</table>
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Appendix 8. Copyright agreement with Elsevier
1 Rationale

Thyroid carcinoma is a term that describes malignant neoplasms arising from the follicular epithelium (WDTC which comprises PTC and follicular carcinoma, poorly differentiated thyroid carcinoma, and anaplastic thyroid carcinoma) or the parafollicular cells (medullary thyroid carcinoma). This neoplasm is the most frequent endocrine malignancy with data from the year 2012 showing an estimated age-standardized world population incidence rate of 6.10/100,000 women and 1.90/100,000 men (Ferlay 2012). Moreover, the thyroid carcinoma incidence rate is the most rapidly increasing rate amongst all major cancers in Canada and worldwide (Pellegriti, Frasca et al. 2013, Canadian Cancer Society’s Advisory Committee on Cancer Statistics 2015). This increased incidence can be mainly explained by a rise in the diagnosis of WDTCs (Chen, Jemal et al. 2009), particularly PTC (Davies and Welch 2014, Mao and Xing 2016) which accounts for approximately 90% of all thyroid carcinoma cases (Mao and Xing 2016). Within WDTC, the highest rate of increase has occurred in tumors measuring <1cm in size (Chen, Jemal et al. 2009), and the two predominant PTC morphological variants, i.e. classical PTC and FVPTC (Davies and Welch 2014, Mao and Xing 2016). The proportion of these two major PTC variants has changed over the past decades (Ceresini, Corcione et al. 2012, Jung, Little et al. 2014, Mao and Xing 2016). Our understanding of the genomics and proteomics of PTC has also changed over the last few years and it has allowed for the identification of molecular subtypes (Giordano, Kuick et al. 2005, Cancer Genome Atlas Research 2014). For example, PTCs with papillary (classical) architecture [even if it comprises a small percentage of a PTC with a predominant follicular pattern (Jakubowski and Hunt 2009)] feature a
predominantly $BRAF^{V600E}$ signature while FVPTCs are more frequently associated with a $RAS$ signature (Cancer Genome Atlas Research 2014, Yip, Nikiforova et al. 2015) (Figure 1).

**Figure 1.** Cartoon illustrating the association between papillary thyroid carcinoma architecture and its predominant molecular signatures. Papillary thyroid carcinomas with exclusive follicular architecture (growth pattern) commonly harbor mutations in $RAS$ (left) while those with predominant papillary architecture (middle) frequently harbor the $BRAF^{V600E}$ mutation. Tumors with mixed architecture of a dominant follicular pattern (right) also frequently harbor the $BRAF^{V600E}$ mutation (Jakubowski and Hunt 2009). In this latter setting the mutation is present in both the areas of papillary and follicular growth suggesting that the term follicular variant papillary thyroid carcinoma should be reserved for tumors that completely lack papillae (Jakubowski and Hunt 2009). *Predominant driver mutations in follicular variant papillary thyroid carcinoma and **classical papillary thyroid carcinoma.
In parallel, it has also become apparent that these molecular/histologic subtypes harbor distinct clinical behavior (Xing, Alzahrani et al. 2015, Shi, Liu et al. 2016). However, there are controversies regarding both the histologic diagnosis of FVPTC and the potential clinical implications of the $BRAF^{V600E}$ mutation.

Firstly, there is lack of universal agreement on the histological criteria to distinguish FVPTC from other follicular neoplasms. Moreover, how these criteria are applied varies. Even among expert thyroid pathologists, there is a significant interobserver and intraobserver variation in the recognition of nuclear features of PTC (NFPTC) (Hirokawa, Carney et al. 2002, Lloyd, Erickson et al. 2004, Elsheikh, Asa et al. 2008). To complicate matters further, there is also no consensus on the amount of follicular architecture that must be met to distinguish FVPTC from a classical PTC (tumor “dominated” by follicular architecture with “virtually” no papillae, up to 99%, or 100%) (DeLellis 2004, Liu, Singh et al. 2006, Baloch and LiVolsi 2014, Mete and Asa 2016). The latter perhaps partly explains why there seems to be significant overlap between the $BRAF^{V600E}$ signature and PTC subtypes with approximately 20% (0%-62.5%) of FVPTCs harboring this mutation (Trovisco, Soares et al. 2005, Di Cristofaro, Marcy et al. 2006, Fugazzola, Puxeddu et al. 2006, Lee, Lee et al. 2007, Eloy, Santos et al. 2011, Chakraborty, Narkar et al. 2012, Jung, Im et al. 2012, Nam, Jung et al. 2012, Fernandez, Piccin et al. 2013, Lee, Jung et al. 2013, Min, Lee et al. 2013, Park, Kim et al. 2013, Rossi, Martini et al. 2015, Walts, Mirocha et al. 2015, Xing, Alzahrani et al. 2015, Onder, Ozturk Sari et al. 2016). It should also be noted that earlier this year it was recommended that a particularly low-risk subgroup of FVPTCs be given a different name to avoid overtreatment of these lesions (Nikiforov, Seethala et al. 2016). Specifically, the term “noninvasive well-demarcated/encapsulated FVPTCs” (Nikiforov, Seethala et al. 2016). Although, one of the recommendations on the diagnostic criteria for was that tumors could -arbitrarily- harbor up to 1% papillae (Nikiforov, Seethala et al.
2016), some expert thyroid pathologists involved in the development of this new nomenclature prefer to reserve the term for neoplasms completely lacking papillary growth (personal communication with S.A. and O.M, Baloch and LiVolsi 2014).

Secondly, it is unclear whether the $BRAF^{V600E}$ status of PTC adds to the already well-defined high-risk clinicopathological features including older age, distant metastasis, large tumor size, extrathyroidal extension (ETE), angioinvasion and clinical neck lymph nodal involvement (Ruegemer, Hay et al. 1988, DeGroot, Kaplan et al. 1990, Hay, Bergstralh et al. 1993, Dean and Hay 2000, Davies and Welch 2006, Edge 2010, Sipos and Mazzaferri 2010, Cheng, Serra et al. 2011, Mete and Asa 2011, Randolph, Duh et al. 2012, Momesso and Tuttle 2014, Haugen, Alexander et al. 2016). This is particularly relevant because the management of PTC is currently tailored according to the risk of disease-specific recurrence and mortality that is ascertained from staging and scoring systems based on clinicopathological features but not routinely on $BRAF^{V600E}$ status (Haugen, Alexander et al. 2016).

In this body of work, I aimed to determine whether an association exists between tumor tissue architecture, somatic mutational profile, and selected high-risk clinicopathological features (i.e. predictive of recurrence and/or mortality) of PTC at the time of resection. The unique feature of our institution (University Health Network) is its expertise in thyroid cancer pathology diagnosis and therefore we aimed to couple this with the evolving knowledge of somatic mutational profiles. The presence of a near exclusive association between tissue architecture and distinct molecular signatures would further (Jakubowski and Hunt 2009, Cancer Genome Atlas Research 2014) support a PTC classification system that distinguishes tumors with any papillary growth pattern from those that are devoid of it. In addition, it would serve as a unique platform for a study of prognosis.
1.1 **Hypothesis**

The application of strict morphologic criteria to classify PTC identifies two main subgroups of tumors with distinct molecular signatures and biologic behavior.

1.2 **Objectives**

1. To determine whether the mutational profile of PTC is associated with tumor architecture as classified by expert thyroid pathologists at the University Health Network.

2. To assess whether the mutational profile and tumor architecture of PTC is associated with high-risk clinicopathological features at the time of surgery as classified by current criteria and reviewed by expert thyroid pathologists at the University Health Network.
CHAPTER 2
LITERATURE REVIEW

2 Papillary Thyroid Carcinoma

2.1 Definition and Classification

The thyroid gland is composed of two distinct groups of hormone producing cells: calcitonin-producing parafollicular neuroendocrine cells and thyroid hormone-producing follicular cells (Asa and Mete 2013). While malignant tumors arising from parafollicular C cells are singularly classified as medullary thyroid carcinomas, follicular cell-derived malignant neoplasms are a heterogeneous group that varies widely in terms of clinical behavior and structural follicular cell differentiation from the generally indolent WDTCs to aggressive poorly differentiated thyroid carcinomas and the rapidly lethal anaplastic (undifferentiated) carcinomas (Table 1) (DeLellis 2004).

Table 1. Histological classification of thyroid carcinoma of follicular cell origin

<table>
<thead>
<tr>
<th>Well differentiated thyroid carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Papillary thyroid carcinoma</td>
</tr>
<tr>
<td>1.a. Variants based on architecture (pattern of growth): classical, follicular, diffuse sclerosing, solid, cribriform-morular, macrofollicular, villous, radial scar-like, cystic</td>
</tr>
<tr>
<td>1.b. Variants based on cytology: columnar cell, clear cell, oncocytic, tall cell, hobnail cell, spindle cell</td>
</tr>
<tr>
<td>1.c. Variants based on stroma: papillary thyroid carcinoma with fascitis-like stroma, adipose stroma, or myxoid-mucinous stroma</td>
</tr>
<tr>
<td>1.d. Variant based on size: microcarcinoma (occult, latent, microtumor)</td>
</tr>
<tr>
<td>1.e. Variant based on architecture, cytology, and stroma: Warthin-like variant</td>
</tr>
</tbody>
</table>
Table 1. Continuation

2. Follicular thyroid carcinoma

2.e. Variants based on cytology: oncocytic, clear cell

2.f. Variants based on degree of invasiveness: minimally invasive, widely invasive

Anaplastic thyroid carcinoma

Variants and main histological growth patterns: paucicellular variant, rhabdoid variant, lymphoepithelioma-like variant, carcinosarcoma variant, spindle cell pattern, pleomorphic giant cell pattern, squamoid pattern

Italicized, predominant variants of papillary thyroid carcinoma.

\(^a\)This classification is based on the 2004 World Health Organization Classification of Thyroid Carcinoma (DeLellis 2004), the 2014 American College of Pathology Protocol for the Examination of Specimens From Patients with Carcinomas of the Thyroid Gland (Seethala 2014), Mete and Asa’s classification of papillary thyroid carcinoma variants according to cytology stroma, size, and architecture (Mete and Asa 2016), and the American Thyroid Association Guidelines for Management of Patients with Anaplastic Thyroid Cancer (Smallridge, Ain et al. 2012).

\(^b\)Classical papillary thyroid carcinoma is also referred to as usual or conventional papillary thyroid carcinoma.

\(^c\)The 2004WHO classification requires that these tumors have virtually no papillae which implies that rare papillae are permitted.

\(^d\)These variants are recognized as more aggressive than classical papillary thyroid carcinoma in the current American Thyroid Association Management Guidelines for Adult Patients with Differentiated Thyroid Carcinoma (Haugen, Alexander et al. 2016).

\(^e\)These variants have been shown to harbor more aggressive behavior in some but not all studies (Nikiforov, Erickson et al. 2001, Chang, Kim et al. 2014, Malandrino, Russo et al. 2016).

Originally, WDTCs were classified into two broad categories based on their pattern of growth (architecture) (Mc, Morgan et al. 1954, Meissner and Warren 1968, Asa, Giordano et al. 2015). Namely, these tumors were categorized into those that formed arborizing papillae (PTC) and those that formed follicles that recapitulate normal thyroid architecture (follicular thyroid carcinoma) (Mc, Morgan et al. 1954, Meissner and Warren 1968, Asa, Giordano et al. 2015). These morphologic criteria have evolved over time and currently the diagnostic hallmark of PTC is its characteristic nuclei that are clear (“Orphan-Annie eyed”, ground glass), enlarged, crowded, overlapping one another, and irregularly contoured including the presence of grooves, pseudoinclusions and an oval shape (Hapke and Dehner 1979, Chan and Saw 1986, DeLellis 1993, DeLellis 2004, Elsheikh, Asa et al. 2008, LiVolsi 2011, Mete and Asa 2012) (Figure 2). In
contrast, follicular carcinomas are recognized by the lack of these nuclear features and they are distinguished by their follicular architecture and presence of invasive growth (please refer to Table 2) (DeLellis 1993, DeLellis 2004, LiVolsi 2011, Sobrinho-Simões, Eloy et al. 2011, Baloch and LiVolsi 2014). This historical shift by no means implies that architecture is not relevant to the modern classification of WDTC. Indeed, the pattern of tumor growth is critical to distinguish PTC subtypes (Table 1). In addition, PTCs are subclassified according to their cell morphology, characteristics of the stroma, and tumor size (DeLellis 2004, Mete and Asa 2016) (Table 1).

Figure 2. Nuclear features of papillary thyroid carcinoma. The presence of nuclear membrane irregularities in enlarged nuclei is the hallmark of papillary thyroid carcinoma. The nuclear membrane irregularities result from folding of nuclear membranes into themselves. The deep nuclear membrane invaginations with cytoplasmic content give rise to intranuclear pseudoinclusions. The white arrow indicates an intranuclear inclusion while the black arrow illustrates grooves giving the appearance of coffee bean to the nucleus.
2.1.1 Overview of Epidemiology

Thyroid carcinoma is the most frequent endocrine malignancy (Ferlay 2012) with an incidence rate that is the most rapidly growing rate amongst all major cancers in Canada and worldwide (Liu, Semenciw et al. 2001, Pellegriti, Frasca et al. 2013, Canadian Cancer Society’s Advisory Committee on Cancer Statistics 2015). It was estimated that last year, 4,820 Canadian women and 1,407 Canadian men were diagnosed with this tumor making it the 5th and 16th most frequent cancer diagnosis in females and males respectively (Canadian Cancer Society’s Advisory Committee on Cancer Statistics 2015). The increased incidence of thyroid cancer can be largely explained by a rise of PTC diagnoses, which account for approximately 90% of thyroid cancer cases (Davies and Welch 2014, Mao and Xing 2016). At the same time, classical PTC and FVPTC account for nearly 99% of PTC morphologic variants with classical PTC being the principal subtype (Mao and Xing 2016).

Most cases of thyroid carcinoma follow an indolent clinical course. In the United States, data from the (SEER) Program showed an overall 5- year survival rate of 98.1% for this carcinoma over 2006-2012 (SEER Program 1975-2013), which is similar to the estimated survival rate for Canadian patients (Canadian Cancer Society’s Advisory Committee on Cancer Statistics 2015). Furthermore, although incidence rates in America have been rising on average 4.5% each year over 2004-2013, the number of deaths per 100,000 (age-adjusted) has remained the same (SEER Program 1975-2013). Prognosis, however, varies depending on several factors that include the extent of disease, the patient’s age, and PTC histological type (Wu, Young et al. 2000, Sampson, Brierley et al. 2007, Sipos and Mazzaferri 2010, Shi, Liu et al. 2016). Patients with PTC of any size confined to the thyroid have a 10-year cancer-specific survival that is >98% whether they receive treatment or not (Davies and Welch 2010). Conversely, subjects >60
years old who present with distant metastasis have a 3-year survival rate of 60% (Sampson, Brierley et al. 2007). Patients with tall cell PTCs are also at higher risk of mortality (24.61 deaths per 1000 person-years, 95% CI: 18.34 –33.35 vs. 5.87 deaths per 1000 person-years, 95% CI: 4.37–7.88 for classical PTC) (Shi, Liu et al. 2016). These data help to explain why there has been such an effort to identify clinical, radiological, pathological, or molecular features that would allow for the selection of the relatively few cases in which more aggressive treatment and more frequent follow-up is warranted.

### 2.1.2 Overview of Diagnosis and Initial Treatment

Patients with PTC generally present with a thyroid nodule that is either discovered incidentally or clinically evident on physical exam (Malone, Zagzag et al. 2014). The presumptive diagnosis of PTC relies on the cytological evaluation of the thyroid nodule cells; however, in some instances where cytological features are indeterminate molecular diagnostic tools may be utilized (Alexander, Kennedy et al. 2012, Hsiao and Nikiforov 2014, Nikiforov, Carty et al. 2014). For example, detection of a *BRAF* mutation in any of the categories of indeterminate cytology has a high specificity and positive predictive value for carcinoma detection (Hsiao and Nikiforov 2014).

Treatment of PTC is principally aimed at removing the primary tumor/clinical lymph node metastasis and reducing the risk of disease recurrence and metastatic spread while minimizing treatment-related morbidity and allowing for accurate staging and risk stratification of disease (Haugen, Alexander et al. 2016). For patients with low-risk disease (low probability of recurrence/distant metastasis/mortality) surgery alone is generally sufficient. In contrast, those who have high-risk disease, including those with suspected or known distant metastasis, benefit from a strategy that involves surgery, radioactive iodine, and thyroid hormone suppressive
therapy (Haugen, Alexander et al. 2016). Similarly, patients with intermediate-risk disease may also require triple therapy (Haugen, Alexander et al. 2016).

2.2 Selected Histologic Variants of Papillary Thyroid Carcinoma

Before briefly describing some of the variants of PTC it is important to mention that the criteria used to diagnose these subtypes as well as how they are applied vary among pathologists. Also, whether complete tumor submission and examination is performed or not might be a determining factor in the detection of scant papillary growth or invasive features. These aspects may affect the ultimate classification of PTC and consequently could account for some of the discrepancies in the literature regarding both clinical outcomes and molecular signatures.

2.2.1 Variants Based on Architecture

2.2.1.1 Classical Variant

According to the SEER data, classical PTC is the most frequent PTC subtype accounting for 61% of cases (Mao and Xing 2016). Morphologically, this tumor is characterized by the presence of arborizing papillae with fibrovascular cores that are lined by cells with NFPTC (LiVolsi 2011) (Figure 2 and Figure 3c). However, classical PTCs may harbor other growth patterns, mainly follicular (LiVolsi 2003, DeLellis 2004). It is crucial to note that although some pathologists allocate PTCs with scattered papillary structures and predominant follicular growth to the FVPTC category, thyroid pathologists at the University Health Network (UHN) classify a tumor with any true fibrovascular core-containing papillae as classical PTCs (Mete and Asa 2016) (Figure 2d).
Clinicopathological studies of PTC have shown that classical PTCs are more likely to be associated with ETE, lymph nodal metastasis, advanced Tumor, Node, Metastasis (TNM) stages, disease recurrence, and mortality than FVPTCs (Passler, Prager et al. 2003, Burningham, Krishnan et al. 2005, Santos and Bugalho 2016, Shi, Liu et al. 2016). However, other authors have found comparable survival between these two predominant PTC subtypes (Zidan, Karen et al. 2003, Lang, Lo et al. 2006, Ito, Hirokawa et al. 2008). This discrepancy may be partly explained by the use of different criteria to define PTC variants. That is, when tumors with papillae are included in the FVPTC group, this may result in an artificial rise of adverse clinicopathological features within that category.

2.2.1.2 Follicular Variant

As shown in Figures 3a and 3b and the cartoons in Table 2, follicular tumors recapitulate normal thyroid architecture and can be classified into follicular adenomas, follicular carcinomas or FVPTCs. The term FVPTC arose in the 1960s/1970s when it was recognized that the presence of NFPTC in a subgroup of “follicular carcinomas” was sufficient to predict a clinical behavior similar to that of PTC (Lindsay 1960, Cuello, Correa et al. 1969, Franssila 1973, Chem and Rosai 1977). Namely, it identified tumors that frequently involved cervical lymph nodes but generally lacked angioinvasion and distant metastatic spread (Lindsay 1960, Cuello, Correa et al. 1969, Franssila 1973, Chem and Rosai 1977). Subsequently, the 1988 World Health Organization (WHO) classification of thyroid carcinomas and the 1992 Armed Forces Institute of Pathology Atlas of Tumor Pathology established that WDTC with NFPTC and a follicular growth pattern should be classified as FVPTC (Table 2) and not as follicular carcinomas (Hedinger, Sobin et al. 1988, Rosai, Carcangiu et al. 1992).
Figure 3. Normal thyroid and predominant variants of papillary thyroid carcinoma. (a). Normal thyroid architecture (b). The exclusive follicular architecture of a follicular variant papillary thyroid carcinoma is illustrated (c). A classical papillary thyroid carcinoma displaying fibro-vascular core-containing papillae is illustrated (d). A classical papillary thyroid carcinoma displaying focal classical architecture (enclosed within the black square) in the background of predominant follicular growth. The area with focal classical architecture is illustrated in this photomicrograph.

Although the classification of follicular neoplasms seems straightforward (Table 2), there have been several challenges associated with it. Firstly, distinguishing follicular adenomas from FVPTCs can be extremely challenging when NFPTC are not well developed or focally expressed, particularly in the absence of invasion (Table 2) (Nakamura, Erickson et al. 2006, Elsheikh, Asa et al. 2008). Furthermore, invasive follicular-patterned carcinomas with subtle NFPTC may be classified by some pathologists as FVPTC while others may diagnose these
tumors as follicular carcinomas (Lloyd, Erickson et al. 2004, Elsheikh, Asa et al. 2008). These challenges are evident even among expert pathologists in whom the interobserver and intraobserver variability in the diagnosis of FVPTC is surprisingly high (Hirokawa, Carney et al. 2002, Lloyd, Erickson et al. 2004, Elsheikh, Asa et al. 2008). Secondly, the term FVPTC groups two clinically heterogeneous tumor types: 1. noninvasive encapsulated or noninvasive well-demarcated/unencapsulated FVPTCs, and 2. invasive FVPTCs (Liu, Singh et al. 2006) (Table 2). The former tumors tend to remain localized to the thyroid gland and as such are not associated with recurrent disease or mortality (Rivera, Ricarte-Filho et al. 2010, Howitt, Jia et al. 2013, Xu and Ghossein 2015, Nikiforov, Seethala et al. 2016) while the latter commonly result in regional lymph nodal metastatic disease. Indeed, in order to incentivize conservative management of noninvasive encapsulated or well-demarcated FVPTCs, a group of expert pathologists recently proposed the term “noninvasive follicular thyroid neoplasm with papillary-like nuclear features” (NIFTP) to describe these very low-risk follicular-patterned thyroid neoplasms (Nikiforov, Seethala et al. 2016). Thirdly, although a FVPTC diagnosis is conventionally rendered when the tumor has virtually no papillae (2004 WHO classification) (DeLellis 2004), molecular studies have shown that the presence of even a small percentage of true papillae is associated with a distinct molecular signature suggesting that a “pure” FVPTC is one that is completely devoid of any papillae (Jakubowski and Hunt 2009). Based on this crucial finding, currently at the UHN the diagnosis of FVPTC (Mete and Asa 2016) and NIFTP mandates complete absence of true papillae. This contrasts one of the current criteria for the diagnosis of NIFPT permitting up to 1% of papillary growth (Nikiforov, Seethala et al. 2016).
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Distinct morphologic features</th>
<th>Cartoon</th>
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</thead>
<tbody>
<tr>
<td>Follicular adenoma</td>
<td>Follicular growth pattern distinct from surrounding thyroid parenchyma, lack of invasive growth(^a), and NFPTC</td>
<td><img src="image1" alt="Image" /></td>
</tr>
<tr>
<td>Follicular carcinoma</td>
<td>Follicular growth pattern, invasive growth(^a), and lack of NFPTC</td>
<td><img src="image2" alt="Image" /></td>
</tr>
<tr>
<td>Encapsulated noninvasive FVPTC(^c)</td>
<td>Follicular growth pattern(^b), NFPTC, tumor capsule completely surrounding the tumor, and no evidence invasion into thyroid capsule, surrounding thyroid parenchyma, nerves or vessels</td>
<td><img src="image3" alt="Image" /></td>
</tr>
<tr>
<td>Encapsulated invasive FVPTC</td>
<td>Follicular growth pattern(^b), NFPTC, invasive growth(^b), and presence of a tumor capsule</td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td>Well-demarcated noninvasive unencapsulated FVPTC(^c)</td>
<td>Follicular growth pattern(^b), NFPTC, lack of a tumor capsule but well-demarcated tumor without any invasion of surrounding thyroid parenchyma, nerves, or vessels</td>
<td><img src="image5" alt="Image" /></td>
</tr>
<tr>
<td>Unencapsulated infiltrative FVPTC</td>
<td>Follicular growth pattern(^b), NFPTC, lack of a tumor capsule, and invasive growth with infiltration of normal thyroid parenchyma</td>
<td><img src="image6" alt="Image" /></td>
</tr>
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</table>

Light burgundy oval, normal thyroid tissue; brown area: follicular epithelial neoplasm without nuclear features of papillary thyroid carcinoma; dark burgundy area, follicular epithelial neoplasm with follicular architecture and nuclear features of papillary thyroid carcinoma; darker burgundy line, tumor capsule; elliptical shape with black borders; blood vessel. NFPTC, nuclear features of papillary thyroid carcinoma.

\(^a\) Tumor capsular invasion, thyroid parenchyma invasion, or angioinvasion.

\(^b\) >99% follicular architecture with virtually no papillae is conventionally required to make a follicular variant of papillary thyroid carcinoma (FVPTC) diagnosis, however at the University Health Network complete absence of true papillae with a 100% follicular architecture.

\(^c\) Nikiforov et al. (Nikiforov, Seethala et al. 2016) recently proposed the term “noninvasive follicular thyroid neoplasm with papillary-like nuclear features” to describe these tumors.
On average, FVPTCs account for nearly 29% of PTC cases in the United States (Mao and Xing 2016). Interestingly, although the incidence rate of classical PTC has increased more than FVPTC in this country (Mao and Xing 2016), a group from the University of Pittsburg reported that the incidence of FVPTC in their institution rose from 25.5% of PTC cases in the year 2000 to 56.6% of all PTCs in 2009 (Jung, Little et al. 2014). Relative to classical PTC, FVPTCs as a whole (including the heterogeneous mix of invasive and noninvasive tumors) have been previously shown to have less adverse clinicopathological features (lower prevalence of ETE, lymph node metastasis, and more advanced TNM stages) (Shi, Liu et al. 2016). Moreover, patients with FVPTCs have been shown to have a more favorable long-term prognosis (higher disease recurrence-free probability and disease-specific patient survival), particularly if they are ≥45 years old (Shi, Liu et al. 2016). In other studies, however, although FVPTCs were associated with less ETE and lymph nodal metastasis than classical PTCs, long-term prognosis was not significantly better for patients within the FVPTCs group (Passler, Prager et al. 2003, Zidan, Karen et al. 2003, Burningham, Krishnan et al. 2005, Ito, Hirokawa et al. 2008, Yu, Schneider et al. 2013). In one unique study where FVPTC was associated with recurrent disease, the diagnostic criteria to render a FVPTC diagnosis permitted the presence of papillary architecture (Grogan, Kaplan et al. 2013). If current diagnostic criteria were to be applied to these cases, a subgroup of FVPTCs would have been allocated to the classical PTC category. This example highlights a crucial matter: clinicopathological correlations in PTC should always be interpreted cautiously and after careful consideration of the criteria used to define PTC and its variants.

2.2.1.3 Macrofollicular Variant

These tumors are comprised of follicular architecture with macrofollicular growth that is predominant or exceeding 50% of the tumor volume (Albores-Saavedra, Gould et al. 1991,
DeLellis 2004). The combination of this pattern of growth and bland cytological features imply that these tumors are sometimes difficult to differentiate from benign nodules (Fukushima, Ito et al. 2009, Yeo, Bae et al. 2014). Although, there are a few publications that have described the clinicopathological characteristics of macrofollicular variant PTC (Albores-Saavedra, Gould et al. 1991, Fukushima, Ito et al. 2009, Erol, Makay et al. 2014, Yeo, Bae et al. 2014), the number of patients is insufficient to ascertain the clinical implications of this diagnosis. Indeed, this variant is rare accounting for <0.5% of PTC cases (Ito, Hirokawa et al. 2008).

2.2.1.4 Diffuse Sclerosing Variant

This tumor is characterized by diffuse thyroid involvement with stromal fibrosis, squamous metaplasia, dense lymphocytic infiltration, and psammoma bodies (Caplan, Wester et al. 1997, DeLellis 2004, Pillai, Gopalan et al. 2015). Compared to classical PTCs, diffuse sclerosing variant PTCs harbor a higher risk of ETE, lymph nodal involvement, and distant metastasis (Malandrino, Russo et al. 2016). The risk of persistent/recurrent disease is also comparatively high (Kim, Park et al. 2016) except when the treatment strategy homogeneously includes routine administration of radioactive iodine post-surgical resection (Malandrino, Russo et al. 2016). Interestingly, diffuse sclerosing variant of PTC is exclusively seen in association with chronic lymphocytic thyroiditis (Hashimoto thyroiditis). Furthermore, this variant of PTC (as well as the solid variant discussed below) is overrepresented in the pediatric population as well as in patients exposed to Chernobyl fallout (Nikiforov, Rowland et al. 1997, Lam, Lo et al. 2005, Koo, Hong et al. 2009, Pillai, Gopalan et al. 2015).
2.2.1.5 Solid Variant

The solid variant of PTC accounts for 0.23-12.8% of PTC cases (Nikiforov, Erickson et al. 2001, Chang, Kim et al. 2014, Xing, Alzahrani et al. 2015). To classify a tumor as a solid variant of PTC some experts require at least 50% solid architecture (LiVolsi 2011) while others prefer 70% (Nikiforov, Erickson et al. 2001) or even any amount exceeding 30% (Mete and Asa 2016). The reason this definition is clinically relevant is that variant of PTC was previously identified in the WDTC management guidelines as an aggressive form of PTC (American Thyroid Association Guidelines Taskforce on Thyroid, Differentiated Thyroid et al. 2009). However, it must be recognized that there are discordant reports in the literature. For example, Nikiforov et al. (Nikiforov, Erickson et al. 2001) found that in twenty patients with solid variant PTC (4 children/adolescents and 16 adults from 1962-1989) harboring ≥70% solid architecture, death or distant metastasis were more frequent events than in classical PTC cases matched by age, tumor size, and follow-up (M=18.7 years). Similarly, Mizukami et al. (Mizukami, Noguchi et al. 1992) found that their predominantly adult cohort of patients with trabecular PTC [some solid PTCs have a trabecular appearance (Lastra, LiVolsi et al. 2014)] harboring ≥50% trabecular architecture had a lower survival rate than classical and FVPTC cases (follow-up of at least five years). In contrast, Chang et. al. (Chang, Kim et al. 2014) showed that their patients with solid variant PTC harboring ≥50% solid architecture had no distant metastasis or deaths after a mean follow-up of 2 years.

It is noteworthy that the prognostic implications of focal solid growth in PTC have not been systematically investigated. There is, however, one study where approximately 6.8% of PTCs showed focal solid growth and this finding was not associated with prognosis (Carcangiu, Zampi et al. 1985).
2.2.1.6 Cribriform-Morular Variant

This variant of PTC was initially recognized in patients with familial adenomatous polyposis (FAP) (Harach, Williams et al. 1994). Indeed, cribriform-morular variant PTC accounts for up to 90% of PTC cases in FAP (Perrier, van Heerden et al. 1998, Feng, Milas et al. 2015) while it only represents 0.16% of all PTC cases (Tomoda, Miyauchi et al. 2004, Levy, Hui et al. 2014). Conversely, 16.7% to 42.9% of patients with this variant of PTC will be discovered to have FAP (Tomoda, Miyauchi et al. 2004, Levy, Hui et al. 2014). Histologically, this carcinoma is characterized by a variable combination of complex architecture comprised of papillary, follicular, solid, squamoid, morular, and cribriform growth (DeLellis 2004, Tomoda, Miyauchi et al. 2004, Jung, Choi et al. 2009).

2.2.2 Other Variants

2.2.2.1 Tall Cell Variant

It has been widely recognized that the tall cell variant of PTC is associated with a particularly aggressive clinical behavior characterized by a higher propensity for locally invasive disease, metastatic spread (regional and distant), higher recurrence rate, and increased mortality when compared to classical PTC or FVPTC (Ostrowski and Merino 1996, Ito, Hirokawa et al. 2008, Jalisi, Ainsworth et al. 2010, Morris, Shaha et al. 2010, Kazaure, Roman et al. 2012, Axelsson, Hrafnikelsson et al. 2015, Shi, Liu et al. 2016). Histologically, this tumor with papillary growth pattern is characterized by tumor cells that are at least two to three times as tall as they are wide (Johnson, Lloyd et al. 1988, DeLellis 2004). Although, the 2004 WHO classification of thyroid carcinomas and some expert endocrine pathologists require that these tumors have predominant (≥50%) tall cell cytomorphology (DeLellis 2004, LiVolsi 2010), others
use a lower threshold. Specifically, a 30% cutoff has been adopted by some based on data that shows that patients with PTCs that harbor at least 30% tall cell cytomorphology have more distant metastasis, recurrences, and deaths than patients with classical PTC (Johnson, Lloyd et al. 1988). Furthermore, disease-specific survival and recurrence-free survival in patients with PTCs composed of 30-49% tall cells is similar to patients with PTCs composed of $\geq 50\%$ tall cells (Ganly, Ibrahimpasic et al. 2014).

2.2.2.2 Oncocytic Variant

Oncocytic (Hurthle cell, oxyphilic) change is defined as cellular enlargement characterized by the accumulation of altered mitochondria, which results in an ample eosinophilic granular cytoplasm (Asa 2004). PTCs with oncocytic cytology may harbor different architectures and as such should be further classified as per their pattern of growth (e.g. “oncocytic classical PTC”) (Seethala 2014, Mete and Asa 2016).

Herrera et. al. (Herrera, Hay et al. 1992) reported that oncocytic PTCs (defined as tumors with exclusive or predominant oncocytic cytology) are associated with a higher 10-year recurrence rate and disease-specific mortality than classical PTCs (Herrera, Hay et al. 1992). However, other studies of oncocytic PTC suggest that the behavior of this tumor may not be more aggressive than classical PTC (Berho and Suster 1997), particularly if the tumor is encapsulated (Woodford, Nikiforov et al. 2010). These studies utilized inconsistent definitions and perhaps this partly explains the different results.
2.2.2.3 Clear Cell Variant

Clear cells, which in some occasions also have a partially oncocytic cytoplasm, may be found in classical or FVPTCs (DeLellis 2004). The outcome of patients with thyroid tumors containing clear cells appears to be more dependent on the underlying tumor architecture than on the presence or amount of clear cells (Carcangiu, Zampi et al. 1985) but studies formally evaluating the prognostic significance of this cytological change are lacking.

2.2.2.4 Warthin-like Variant

Apel, Asa, and LiVolsi (Apel, Asa et al. 1995) originally described this variant of PTC after examination of 13 thyroid carcinomas that resembled Warthin’s tumor of the salivary gland. These relatively rare tumors (Yeo, Bae et al. 2015, Kim, Shin et al. 2016) with classical architecture are composed of oncocytic cells and arise in the background of a lymphocytic infiltrate (Apel, Asa et al. 1995, Baloch and LiVolsi 2000). The Warthin-like variant of PTC appears to have a similar clinical behavior to classical PTC (Apel, Asa et al. 1995, Yeo, Bae et al. 2015).

2.3 Selected Patterns of Invasion

As per the definition utilized by Mete and Asa (Mete and Asa 2016) invasive growth in thyroid carcinoma refers to the presence of any one of the following features: angioinvasion, lymphatic invasion, capsular invasion, invasion of the surrounding normal thyroid parenchyma, perineural invasion, and ETE. For FVPTCs, which are frequently encapsulated, an adequate assessment of invasive features requires that the neoplasm be submitted as a whole for microscopic evaluation (Mete and Asa 2016).
2.3.1 Extrathyroidal Extension

Approximately 14% of PTCs present with ETE (Hay, Johnson et al. 2016), which is defined as tumor invasion outside the thyroid gland and into the surrounding tissues. Some authors describe ETE on the basis of whether it is grossly visible during surgery (macroscopic ETE) or exclusively detected on histopathology (microscopic ETE) (Andersen, Kinsella et al. 1995, Arora, Turbendian et al. 2008, Rivera, Ricarte-Filho et al. 2010). According to the 7th edition of the American Joint Committee on Cancer (AJCC) TNM thyroid cancer staging system, ETE is subclassified as minimal (pT3) if the thyroid carcinoma extends to the sternothyroid muscle or perithyroidal soft tissues or moderate (pT4a) to very advanced (pT4b) if the tumor extends beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, recurrent laryngeal nerve, carotid artery, or mediastinal vessels (Edge 2010). Significant ETE (pT4) is usually appreciated at the macroscopic level while pT3 is determined by histologic evaluation of the tumor. The pathologic diagnosis of pT3 is subjective, and diagnostic concordance amongst expert pathologists is poor (Su, Wenig et al. 2016). One of the potential explanations may be related to Mete et al.’s (Mete, Rotstein et al. 2010) finding that the thyroid gland lacks a defined anatomical fibrous capsule and instead harbors a thin, incomplete pseudocapsule composed of fibroadipose tissue. This implies that tumor cells in perithyroidal fat may not denote invasion beyond the thyroid. An additional important aspect is that although skeletal muscle invasion can be reliably used as a criterion for ETE, this is not applicable at the isthmus where muscle fibers of Soemmering’s muscle may normally be seen within the gland of certain individuals (Mete, Rotstein et al. 2010).

Extrathyroidal extension is a marker of aggressiveness in PTC, and it is one of the elements that is consistently found in the different PTC staging systems utilized around the world.

2.3.2 Angioinvasion (Vascular Invasion)

The diagnostic criteria used to identify angioinvasion are controversial; nevertheless, how criteria are applied appear to be the factor that determines its usefulness as a marker of aggressive behavior (Mete and Asa 2012). With angioinvasion rigidly defined as tumor invasion through a vessel wall or a thrombus adherent to the intravascular tumor Mete and Asa (Mete and Asa 2011) found a prevalence of this feature in only 3% of follicular cell-derived carcinomas while Nishida et al. (Nishida, Katayama et al. 2002) reported a remarkably high prevalence of
46.9%. Interestingly, while Mete and Asa (Mete and Asa 2011) found that approximately 33% of the angioinvasive WDTC gave rise to distant metastatic disease (mean follow-up of 5.3 years), Nishida et al. (Nishida, Katayama et al. 2002), found that only 11% of patients with angioinvasive thyroid carcinomas developed distant metastasis (median follow-up of 4.9 years). Although, Nishida et al. (Nishida, Katayama et al. 2002) may have applied the definition of angioinvasion differently or described a cohort of tumors that for some reason had a high prevalence of vascular invasion, these investigators were able to demonstrate the association of this feature with tumor recurrence.

2.3.3 Capsular Invasion and Thyroid Parenchyma Invasion

Thyroid carcinomas may be encapsulated or unencapsulated. Encapsulated tumors are considered invasive when there is invasion of the tumor capsule, surrounding parenchyma, angioinvasion (Heffess and Thompson 2001), lymphatic invasion, or perineural invasion (Mete and Asa 2016). In the absence of a tumor capsule, neoplastic infiltration of the adjacent parenchyma is regarded as a malignant feature (Heffess and Thompson 2001). Concordance in the diagnosis of capsular invasion, however, is low even amongst expert pathologists (Elsheikh, Asa et al. 2008). While some pathologists diagnose capsular invasion when there is penetration of the entire capsule by the tumor [as per 2004 WHO criteria (DeLellis 2004)] or when there is a satellite tumor nodule identical to the main tumor just outside the thyroid capsule, others make the diagnosis based on the presence of incomplete capsular transgression (Ghossein 2009, Baloch and LiVolsi 2014). Also, the term “minimally invasive” is not uniformly used among practicing pathologists. For example, the 2004 WHO classification of thyroid carcinomas and some experts recommend this term to describe tumors with limited capsular or vascular invasion (Ruegemer, Hay et al. 1988, Heffess and Thompson 2001). Others, however, prefer to reserve it
to describe tumors with exclusive capsular invasion (D'Avanzo, Treseler et al. 2004, Baloch and LiVoltsi 2007). This distinction is of clinical significance as patients with minimally invasive tumors defined by the latter criteria have a better 5-year survival rate (98%) compared to those with tumors defined by the former criteria (80%) (D'Avanzo, Treseler et al. 2004).

2.4 Oncogenesis and Predominant Molecular Signatures of Papillary Thyroid Carcinoma

2.4.1 Mitogen-Activated Protein Kinase and Phosphatidylinositol-3 Kinase Signaling Pathways

Papillary thyroid carcinoma initiation and progression occur through the accumulation of genetic and epigenetic alterations, including somatic mutations, chromosomal rearrangements, microRNA deregulation, and aberrant gene methylation (Nikiforov and Nikiforova 2011). Of these, virtually mutually exclusive activating somatic alterations of genes encoding effectors in the mitogen-activated protein kinase (MAPK) signaling pathway constitute the main driver events (Figure 4) (Cancer Genome Atlas Research 2014). Indeed, the \( \text{BRAF}^{V600E} \) mutation is found in approximately 45% of PTCs (Xing 2005) and mutations in \( \text{RAS} \), which also deregulate the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT) pathway, have a prevalence of about 30-45% (Yip, Nikiforova et al. 2015, Xing 2016).

2.4.1.1 The \( \text{BRAF}^{V600E} \) Mutation and the \( \text{BRAF}^{V600E} \)-like Signature

\( \text{BRAF} \) can be activated by different mechanisms including point mutations, small in-frame deletions or insertions, or chromosomal rearrangement (Nikiforov and Nikiforova 2011). Of these, the most common mechanism is the point mutation \( \text{BRAF}^{V600E} \) accounting for >90% of cases (Xing 2007). Activation of the MAPK signaling pathway via the \( \text{BRAF}^{V600E} \) oncogene
results in deregulated cell proliferation/growth as well as dedifferentiation (Cancer Genome Atlas Research 2014). As shown in Figure 4, when compared to PTCs with RAS mutations, \( \text{BRAF}^{V600E} \)-harboring tumors show a higher output of the ERK (extracellular regulated mitogen-activated protein kinase) transcriptional program, likely due to monomeric RAF signaling leading to insensitivity of the \( \text{BRAF}^{V600E} \) oncoprotein to ERK-mediated inhibitory feedback (Cancer Genome Atlas Research 2014).

It is crucial to consider how the \( \text{BRAF}^{V600E} \) mutation is connected with PTC morphology and clinicopathological features. A correlation between genotype, gene expression and PTC morphology was recognized more than a decade ago (Nikiforova, Kimura et al. 2003, Giordano, Kuick et al. 2005). Most recently, The Thyroid Cancer Genome (TCGA) Research Network’s (Cancer Genome Atlas Research 2014) genomic characterization of PTC corroborated this relationship in a cohort of 402 PTCs submitted to WES. This multicenter study showed that classical PTCs were the predominant histologic variant within the \( \text{BRAF}^{V600E} \)-mutated group (also enriched in tall cell variant PTCs) while FVPTCs predominated within the RAS-mutated group (Cancer Genome Atlas Research 2014) (Figure 4). Also, in the large multicenter study by Xing et al. (Xing, Alzahrani et al. 2015) published last year, 56% (813/1,448) of classical PTCs harbored a \( \text{BRAF}^{V600E} \) mutation. On the other hand, the \( \text{BRAF}^{V600E} \) signature is tightly linked to high-risk clinicopathological features including ETE, lymph nodal metastasis, and advanced TNM stages (Nikiforova, Kimura et al. 2003, Xing, Westra et al. 2005, Riesco-Eizaguirre, Gutierrez-Martinez et al. 2006, Xing 2007, Elisei, Ugolini et al. 2008, Chakraborty, Narkar et al. 2012, Elisei, Viola et al. 2012, Lee, Jang et al. 2013, Daliri, Abbaszadegan et al. 2014, Liu, Zhang et al. 2014, Yip, Nikiforova et al. 2015, Kim, Myong et al. 2016). Accordingly, this mutation has been shown to be associated with less favorable outcomes such as disease persistence/recurrence and disease-related mortality (Riesco-Eizaguirre, Gutierrez-Martinez et al.)
Given these established associations it is not surprising that some (Kim, Kim et al. 2005, Xing, Westra et al. 2005), albeit not all (Xing, Alzahrani et al. 2015) studies have revealed that the $BRAF^{V600E}$ mutation loses its prognostic value when PTC architecture is accounted for. Therefore, the independent prognostic value of detecting this genotype, particularly in classical PTC remains uncertain.

Although some have shown that FVPTCs lack the $BRAF^{V600E}$ mutation (Trovisco, Soares et al. 2005, Howitt, Jia et al. 2013, Onder, Ozturk Sari et al. 2016), others have found that 7.7%-62.5% FVPTCs harbor this alteration (Di Cristofaro, Marcy et al. 2006, Fugazzola, Puxeddu et al. 2006, Lee, Lee et al. 2007, Eloy, Santos et al. 2011, Chakraborty, Narkar et al. 2012, Jung, Im et al. 2012, Nam, Jung et al. 2012, Fernandez, Piccin et al. 2013, Lee, Jung et al. 2013, Min, Lee et al. 2013, Park, Kim et al. 2013, Rossi, Martini et al. 2015, Walts, Mirocha et al. 2015, Xing, Alzahrani et al. 2015), with the largest of these studies showing a prevalence of 20.6% (Xing, Alzahrani et al. 2015). Such variation is not entirely surprising given the controversies surrounding the pathological diagnosis of FVPTC. Based on the work by Jakubowski and Hunt (Jakubowski and Hunt 2009), the existence of even a small percentage of papillary architecture in a predominantly follicular-patterned PTC is associated with $BRAF^{V600E}$ mutations. Consequently, pathologists that allow for the presence of scant papillae in “FVPTC” may find the $BRAF^{V600E}$ mutation within this group. Following this same rationale, the complete absence of papillae would likely be associated with less or no $BRAF^{V600E}$ mutations in the FVPTC category. For example, in the study by Order et al. (Onder, Ozturk Sari et al. 2016), where FVPTCs lacked the $BRAF^{V600E}$ mutation, tumors with any papillae were classified within the classical architecture category.
The TCGA Research Network quantified the extent to which the gene expression profile of PTCs resembled either $BRAF^{V600E}$-mutated tumors ($BRAF^{V600E}$-like molecular signature) or RAS mutated tumors ($RAS$-like molecular signature) (Cancer Genome Atlas Research 2014). For instance, tumors that exhibit *rearranged during transfection* (RET) fusions, which are known to be frequent oncogenic drivers in pediatric PTC and radiation-induced PTC (Romei, Ciampi et al. 2016), are $BRAF^{V600E}$-like (Figure 4). Conversely, neurotrophic tyrosine kinase receptor ($NTRK$) fusions are neutral with respect to the $BRAF^{V600E}$-like or $RAS$-like signature (Cancer Genome Atlas Research 2014). Interestingly, a recent prospective study showed that PTCs with RET fusions are associated with advanced TNM stages and disease recurrence (Yip, Nikiforova et al. 2015). Also, Prasad et al. (Prasad, Vyas et al. 2016) found that pediatric PTCs that harbor either RET fusions or $NTRK$ fusions are larger, more frequently associated with lymphovascular invasion, and more commonly solid and diffuse sclerosing variants when compared to $BRAF^{V600E}$-mutated tumors.

### 2.4.1.2 Mutations in RAS and the RAS-like Signature

In contrast to the $BRAF^{V600E}$ mutation, RAS mutations ($H-RAS$, $K-RAS$, $N-RAS$) have been associated with less aggressive clinical behavior (Yip, Nikiforova et al. 2015, Xing 2016) and a follicular architecture (FVPTC) (Cancer Genome Atlas Research 2014, Yip, Nikiforova et al. 2015). Signaling in RAS-mutated tumors also differs from tumors with the $BRAF^{V600E}$ mutation because the former is characterized by activation of the PI3K signaling pathway and a less robust output from the ERK transcriptional program (Cancer Genome Atlas Research 2014) (Figure 4). Indeed, tumors with activating RAS mutations and those with the RAS-like signature (e.g. most tumors with $EIF1AX$ mutations and $BRAF^{K601E}$ mutations) are more differentiated than their $BRAF^{V600E}$-like counterparts (Cancer Genome Atlas Research 2014).
Figure 4. Downstream signaling of \( \text{BRAF}^{V600E} \)-like and \( \text{RAS} \)-like papillary thyroid carcinomas. The majority of PTCs are driven by mutually exclusive somatic activating mutations in either \( \text{RAS} \) or \( \text{BRAF}^{V600E} \), which deregulate the MAPK pathway. The \( \text{BRAF}^{V600E} \) oncoprotein (left), commonly expressed in classical PTCs, signals as a monomer and is insensitive to ERK inhibitory feedback. This results in a robust activation of the MAPK pathway with a higher output of the ERK transcriptional program, represented in particular by DUSP mRNAs. With mutations in \( \text{RAS} \) (right), which predominantly occur in FVPTCs, there is activation of PI3K/AKT signaling and a rather modest activation of the MAPK pathway due to negative feedback from ERK to RAF dimers. Paradoxically, \( \text{RAS} \)-like PTCs show higher phosphorylation of the ERK substrate p90, which is associated with mTOR activation (probably through inhibition of TSC2) and BAD phosphorylation with concurrent BCL2 overexpression, leading to antiapoptosis. PTCs with \( \text{RET} \) fusions have a similar molecular signature to those with \( \text{BRAF}^{V600E} \) mutations, while those with the \( \text{BRAF}^{K601E} \) mutation are \( \text{RAS} \)-like. \( \text{NTKR1/3} \) fusions harboring-PTCs are largely neutral. Abbreviations: BAD, BCL2 associated agonist of death; BCL2, apoptosis regulator BCL2; DUSPs, dual specific phosphatases; ERK, extracellular regulated mitogen-activated protein kinase; \( \text{NTKR1/3} \), neurotrophic tyrosine kinase receptor type 1 and 3; p90, p90 ribosomal s6 kinase; RET, rearranged during transfection; TSC2, tuberous sclerosis 2 protein. Reprinted from Cell, volume 159, The Cancer Genome Atlas Research Network, Integrated Genomic Characterization of Papillary Thyroid Carcinoma, pages 676-690, Copyright 2014, with permission from Elsevier.
2.5 Risk Stratification

The extent of surgical treatment for suspected PTC is principally guided by preoperative thyroid nodule cytology and radiological findings including thyroid nodule size, evidence of ETE or the presence of regional lymph nodal metastasis. Subsequently, postoperative staging systems centered on clinicopathological features that are available shortly after initial therapy are utilized to predict the risk of mortality and disease persistence/recurrence. This information is used to direct additional therapy such as the need for more extensive surgery or radioactive iodine treatment (Figure 5). The 2016 American Thyroid Association (ATA) guidelines for thyroid cancer management recommend the AJCC TNM staging system (7th edition) and the 2009 ATA initial risk stratification for such purpose (Haugen, Alexander et al. 2016). In contrast, these guidelines do not recommend the routine evaluation of the BRAF mutational status because the incremental benefit of adding this information to the current risk stratification scheme has not been established (Haugen, Alexander et al. 2016).

After the initial treatment, response to therapy is assessed with imaging studies (neck ultrasound or other imaging modalities when indicated) and tumor markers (thyroglobulin and thyroglobulin antibodies) to provide a better estimate of a patient’s risk of recurrence over time (Momesso and Tuttle 2014, Haugen, Alexander et al. 2016). This dynamic risk assessment strategy results in individualized thyroid stimulating hormone (TSH) suppression targets and surveillance strategies (Figure 4) (Momesso and Tuttle 2014, Haugen, Alexander et al. 2016).
Figure 5. General outline of papillary thyroid carcinoma management. Currently, risk stratification is the cornerstone of individualized papillary thyroid carcinoma management. The process of risk assessment is dynamic and continues after initial treatment.

2.5.1 The TNM Staging System and the MACIS System

The TNM staging system includes tumor size, regional nodal status, ETE, distant metastasis status, and age (<45 or ≥45 years) (Edge 2010). It has been used for decades both because of its simplicity and the fact that its various editions have consistently predicted cancer-specific survival (Brierley, Panzarella et al. 1997, Wu, Young et al. 2000, Lang, Lo et al. 2007, Lang, Lo et al. 2007, Verburg, Mader et al. 2010, Vrachimis, Gerss et al. 2014, Tanase, Thies et al. 2015). Similarly, the MACIS (Metastases, Age, Completeness of Resection, Invasion, Size)
system is a validated tool to predict cancer-specific mortality (Hay, Bergstralh et al. 1993, Brierley, Panzarella et al. 1997, Wu, Young et al. 2000, Passler, Prager et al. 2003, Powers, Dinauer et al. 2004, Lang, Lo et al. 2007, Lang, Lo et al. 2007). In fact, these systems are the highest ranked by proportion of variance explained, which is a statistical measure of how well they predict the outcome of interest (Lang, Lo et al. 2007). Nevertheless, they are less than perfect instruments accounting for no more than 30% of the observed variance in disease-specific mortality (Momesso and Tuttle 2014).

The MACIS system was derived from a cox model analysis, and stepwise variable selection based on 1779 patients with PTC with >26,000 patient years of follow-up (Hay, Bergstralh et al. 1993). In contrast with the TNM staging system, regional lymph nodal status was not included because the presence of metastatic lymphadenopathy lacked influence on cumulative mortality in both patients with intrathyroidal tumors and those with locally invasive disease (Hay 1990). This difference implies that some patients who are assigned to an advanced TNM stage (predictive of lower cancer-specific survival) would be allocated to a MACIS category that is associated with a negligible risk of mortality.

2.5.2 2009 American Thyroid Association Risk Stratification System

Because the AJCC TNM stages do not correlate with the risk of persistent/recurrent disease (Tuttle, Tala et al. 2010), the 2009 ATA guidelines for the management of patients with WDTC proposed a novel three-tier risk stratification system to assess this risk (American Thyroid Association Guidelines Taskforce on Thyroid, Differentiated Thyroid et al. 2009). This system includes histopathological features that are not part of the TNM or MACIS systems (e.g. angioinvasion and aggressive histologic variants) as well imaging and biochemical features that highly suggest disease persistence (post-radioactive iodine scan positivity outside the neck and
elevated thyroglobulin levels) (American Thyroid Association Guidelines Taskforce on Thyroid, Differentiated Thyroid et al. 2009). Several studies have retrospectively validated the 2009 ATA risk stratification system, which also predicts the likelihood of achieving remission (Tuttle, Tala et al. 2010, Castagna, Maino et al. 2011, Vaisman, Shaha et al. 2011, Fernandez, Piccin et al. 2013, Pitoia, Bueno et al. 2013). Unfortunately, the system shows a proportion of variance explained of <35% (Tuttle, Tala et al. 2010, Castagna, Maino et al. 2011).

2.5.3 Revised 2009 American Thyroid Association Risk Stratification System and Clinical lymph node Metastasis

The 2016 ATA WDTC guidelines revised the 2009 ATA risk stratification system to reflect the risk of recurrence that is associated with certain features including the presence of bulky metastatic lymphadenopathy (Haugen, Alexander et al. 2016). Indeed, clinical lymph node metastases (defined as metastatic lymph nodes identified by physical examination, imaging or intraoperative examination) are associated with a median recurrence rate of 22% (10-42%) (Randolph, Duh et al. 2012). Moreover, metastatic lymph nodes ≥3 cm in patients ≥50 years old independently predict disease-specific survival (Sugitani, Kasai et al. 2004). Metastatic lymphadenopathy of this size also predicts disease-free survival in patients of all ages without distant metastasis at presentation (Sugitani, Kasai et al. 2004). In contrast, patients who lack lymph nodal involvement (clinical or pathological) have a median risk of recurrence of 2% (0-9%) (Randolph, Duh et al. 2012).

2.6 Summary

The variability in the diagnosis of classical PTC and FVPTC is probably due to multiple factors including the criteria used to make the diagnosis and how these criteria are applied.
Ultimately, this variability may have resulted in an apparent overlap between morphology and molecular signatures of PTC, namely, the presence of the $BRAF^{V600E}$ mutation in FVPTCs. This hypothesis is supported by a previous study that showed that in PTCs with mixed follicular and papillary architecture the $BRAF^{V600E}$ signature was present in both the areas of follicular and papillary growth (Jakubowski and Hunt 2009). Perhaps, as suggested by the TCGA Research Network (Cancer Genome Atlas 2014) the classification of thyroid cancer needs to be revised to separate follicular-patterned WDTCs from those with classical architecture, and more accurately reflect the differences in genotype, morphology, and clinical features between and within $RAS$-like PTCs and $BRAF^{V600E}$-like PTCs.
CHAPTER 3
MATERIALS AND METHODS

3 Study Design

In a retrospective cohort study design, we reviewed 183 PTC cases submitted to the UHN pathology department between 2002-2004 and 2006-2012 respectively. We identified age, sex, histologic variant, status of invasiveness including angioinvasion, AJCC TNM cancer staging 7th edition, MACIS score, 2009 ATA risk categories, clinical lymph node metastasis, and presence of metastatic disease at time of surgical pathology diagnosis for each surgical specimen and corresponding patient. In addition, for patients followed at the UHN after surgical treatment of their PTC, follow-up time and structural disease recurrences were noted. All variables were correlated to \( BRAF^{V600E} \) and \( RAS \) mutations. The tissue samples had been previously sequenced for these and other mutations as part of unrelated research protocols (Cheng, Serra et al. 2011) including the TCGA Research Network’s genomic characterization of PTC (Cancer Genome Atlas Research 2014). The 2002-2004 group served as a discovery cohort while the 2006-2012 cohort was used to validate initial findings. The cohorts were not pooled given different methods for mutational analysis, different mechanisms to allocate tumors into the classical and FVPTC categories, and heterogeneous follow-up strategies.

3.1 Study Population

3.1.1 Selection of Cases

We had a total of 239 samples that had been submitted to the UHN pathology department in two separate cohorts: 2002-2004 and 2006-2012. These were cases where patients had consented and primary tumor tissue was available at the UHN biobank; therefore, they were only
a proportion of the approximately 400 cases of thyroid cancer diagnosed yearly at this institution. Also, the tumor samples had all been submitted in toto for microscopic examination (except for a small tissue sample for the biobank in grossly identified dominant tumors exceeding 1 cm) and reviewed by at least one of the expert thyroid pathologists. The data on the surgical pathology report were used to record the histologic variant of each tumor included in the discovery and validation cohorts. Tumors with any amount of true papillae in the background of follicular growth were defined as classical PTCs, whereas those of FVPTCs displayed exclusive follicular (microfollicular and/or macrofollicular) architecture. The discovery cohort included 131 consecutive cases of PTC of all sizes (Figure 6) while the validation cohort included 108 cases of PTC $\geq$ 1 cm selected for the TCGA project (Figure 7). From each case, the dominant (largest) tumor was selected for analysis. The study populations included local cases of PTC (treated and followed locally) and cases referred from outside the UHN (pathology reviewed at UHN after surgical treatment but followed subsequently at outside institutions). In the validation cohort, the tumor sections were required to contain an average of 60% tumor cell nuclei with less than 20% necrosis. Both cohorts included patients who had not received external beam radiation or chemotherapy for the treatment of their PTC. A research ethics board approval was obtained for the retrospective study of both cohorts.

3.1.2 Exclusion of Cases

Of the original 239 samples, fifteen were excluded from the discovery cohort and forty-one were excluded from the validation cohort (Figures 6 and 7). All samples with an undifferentiated thyroid carcinoma component were excluded from both cohorts. Also, all tumors other than classical or FVPTCs with or without oncocyic cytological changes, clear cell cytology or focal areas of solid growth were excluded (Figures 6 and 7). PTCs with more than one somatic mutation were excluded as well. Specifically, from the discovery cohort eight PTCs
were excluded because of their histologic variant (1 tall cell variant, 3 Warthin-like variants, 1 cribriform-morular variant, 2 diffuse sclerosing variants, and 1 solid variant) and three additional tumors because of the presence of double somatic mutations ($KRAS^{G12C}$ and $BRAF^{V600E}$, $NRAS^{Q61R}$ and $STK11^{F354L}$, $NRAS^{Q61R}$ and $EGFR^{S768I}$). From the validation cohort, two cribriform-morular variants of PTC and a PTC with multiple mutations ($EIF1AX^{A113_splice}$, $KRAS^{Q61K}$, $BRAF^{V600E}$) were excluded. The reason for exclusion of these cases was to ensure a homogenous pool. One case of multifocal PTC that contributed two separate specimens with two different architectures and different mutations was excluded from the discovery cohort (Figure 6). This case was exceptional in that the two dominant tumors were submitted for molecular analysis (as opposed to the rest of the cohort where only one tumor was generally analyzed). From that same cohort, the smaller of two FVPTCs specimens from the same patient was excluded to maintain a 1:1 relationship between tissue sample and corresponding patient (Figure 6).

For analysis involving MACIS scores/categories one classical PTC case from the discovery cohort was excluded. The exception was a patient in whom a biopsy proven microscopic left sided PTC was not resected due to paralysis of the recurrent laryngeal nerve during a right hemithyroidectomy. This case was also excluded from analysis involving ATA risk stratification.

In four cases belonging to the discovery cohort and five cases from the validation cohort, it was not possible to determine whether there were clinical lymph node metastases (cN1) because of a combination of lack of preoperative imaging information, non-informative operative notes and lack of lymph nodes in the surgical pathology specimen. These cases were excluded from analysis involving the cN1 variable.
Figure 6. Study population discovery cohort. One hundred and sixteen cases were selected from the initial one hundred and thirty one cases with patient consent and sufficient research material at the institutional biobank. ATA, American Thyroid Association; cN1, clinical lymph node metastasis; FVPTC, follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma; UHN, University Health Network.
Figure 7. Study population validation cohort. This cohort consisted of sixty-seven cases that had been selected for the TCGA Research Network’s characterization of papillary thyroid carcinoma (Cancer Genome Atlas 2014). ATA, American Thyroid Association; cN1, clinical lymph node metastasis; cN0, no clinical lymph node metastasis; FVPTC, follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma; TCGA, The Cancer Genome Atlas; UHN, University Health Network; WES, whole-exome sequencing.
Angioinvasion status was indeterminate in three cases from the discovery cohort and ten cases from the validation cohort; therefore, these cases were excluded from analysis involving this variable.

For analysis involving the outcome variable “structural recurrence” sixteen cases from the discovery cohort and five cases from the validation cohort were excluded due to a follow-up of <6 months (n=8 and n=1, respectively) or no imaging studies (including functional imaging with diagnostic whole body scans) done at any point during follow-up (n=8 and n=4, respectively).

3.2 Measurement

Patient medical record number was used to identify electronic charts and abstract the data. Molecular analysis results for the discovery cohort were provided by the UHN molecular pathology laboratory (Suzanne Kamel-Reid, PhD), while the TCGA Research Network provided the results for the validation cohort (Cancer Genome Atlas Research 2014). I performed the final pooling of all the information collected from both cohorts and although I was unblinded, the data was gathered based on factual chart content. Crucially, UHN endocrine pathologists (Doctors Sylvia Asa, Ozgur Mete, and Daniel Winer) were blinded to the genetic status of the individual patients at the time of initial histopathological examination and reporting of these tumors.

Somatic mutations for the 2002-2004 cohort were determined by the molecular pathology team from the Pathology Department at UHN. These individuals were blinded to the clinical status of the patient. Molecular analysis of the 2006-2012 cohort was performed by members of the TCGA Research Network (Cancer Genome Atlas Research 2014) who were also blinded to clinicopathological data.
3.2.1 Definitions

Papillary thyroid carcinoma and its morphologic variants were defined as per 2004 WHO criteria (DeLellis 2004) with a few exceptions. Most importantly, a FVPTC diagnosis was rendered in tumors with an exclusive follicular architecture and complete absence of true papillae (as opposed to “virtually” no papillae). Of note, seven PTCs with follicular architecture (three from the discovery cohort and four from the validation cohort) contained a focal (<10%) component of solid growth. Also, 3 classical PTCs in the discovery cohort had focal solid growth. In line with international expert recommendations, these tumors with focal solid growth were classified as FVPTCs or classical PTCs with focal solid growth. Secondly, tall cell variant of PTC was defined as a tumor with classical architecture containing at least 30% tall cell cytomorphology. Finally, solid variant PTC was defined as a tumor containing >30% solid architecture.

The PTC with classical architecture category included the following tumors: classical with focal solid, oncocyic classical with focal solid, oncocyic classical, and classical with focal dedifferentiation. PTCs with follicular architecture included: oncocyic follicular variant, clear cell follicular variant, follicular variant with focal solid, and follicular variant with focal dedifferentiation. Therefore, in this thesis PTC with follicular architecture and FVPTC are used interchangeably. The same principle applies to the terms PTC with classical architecture and classical PTC.

Follicular variant PTCs were classified as encapsulated if they were completely surrounded by a tumor capsule, partially encapsulated if the capsule was incomplete, or unencapsulated if a tumor capsule was lacking. Additionally, FVPTCs were classified as
invasive if they invaded the tumor capsule, the surrounding normal thyroid parenchyma, nerves, vessels, or extrathyroidal tissues.

As per UHN guidelines with the exception of solid tumors exceeding 1 cm in which a small tumor fragment obtained from the tumor center was submitted for biobanking purposes, all tumors were submitted entirely for microscopic examination.

Cancer-specific survival was assessed with the 7th version of the AJCC TNM pathological (p) staging system (Edge 2010) as follows:

1. **Primary tumor (T)**

   T1  Tumor size \( \leq 2 \text{ cm} \) in greatest dimension, noninvasive
   
   T2  Tumor > 2 cm but \( \leq 4 \text{ cm} \), noninvasive
   
   T3  Tumor >4 cm, limited to the thyroid or any tumor with minimal ETE (extends to the sternothyroid muscle or perithyroidal soft tissues). Of note, since the thyroid gland is surrounded by a fibroadipose tissue pseudocapsule (Mete, Rotstein et al. 2010), at the UHN, PTC presence in fibroadipose surrounding the thyroid gland is not considered as pT3.
   
   T4  Moderately to very advanced disease: tumor extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve, prevertebral fascia or encases carotid artery or mediastinal vessels

2. **Regional lymph nodes (N)**

   NX  Regional nodes cannot be assessed
   
   N0  No regional lymph node metastasis
   
   N1  Regional lymph node metastasis
N1a  Metastases to level VI

N1b  Metastases to retropharyngeal lymph nodes, superior mediastinal nodes or any of the following cervical levels: I, II, III, IV, V, VIII

3. Distant metastases (M)

M0  No distant metastasis is found
M1  Distant metastasis present

TNM stages were divided in the four categories shown below. However, for statistical analysis, TNM stages were in some instances grouped in different categories: a. stages I/II vs. stages III/IV or b. stages I/II/III vs. stage IV.

<table>
<thead>
<tr>
<th>TNM stages</th>
<th>5-Year cancer-specific survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: &lt;45 years without M1; ≥45 years with T1N0M0 or T1NXM0</td>
<td>97.1%</td>
</tr>
<tr>
<td>II: &lt;45 with M1; ≥45 years with T2N0M0 or T2NXM0</td>
<td>92.8%</td>
</tr>
<tr>
<td>III: ≥45 years with T3 or N1a</td>
<td>82.0%</td>
</tr>
<tr>
<td>IV: ≥45 years with T4 or N1b or M1 (combined IVA, IVB, and IVC)</td>
<td>41.4%</td>
</tr>
</tbody>
</table>

Mortality risk was assessed using MACIS scores (Hay, Bergstralh et al. 1993) which were based on the following criteria:

1. **Distant metastasis**: clinical or pathological M0 or MX=no distant metastases; clinical or pathological M1= distant metastases present.
2. **Age** in years.

3. **Completeness of resection**: resection margins negative or unable to determine margin status but complete gross resection = completely resected; positive margins with either microscopic or macroscopic residual carcinoma = incompletely resected.

4. **Size of tumor** in cm: maximum tumor diameter.

Using these criteria, the scores were calculated with the ATA’s thyroid cancer staging calculator ([http://www.thyroid.org/thyroid-cancer-staging-calculator](http://www.thyroid.org/thyroid-cancer-staging-calculator)). Although, MACIS score is typically grouped in four categories that reflect different 20-year mortality rates (Hay, Bergstralh et al. 1993), in some circumstances we combined it into two categories (“grouped or combined MACIS categories”) for the purpose of data analysis:

<table>
<thead>
<tr>
<th>MACIS score (risk category)</th>
<th>20-year mortality</th>
<th>20-year mortality for grouped risk categories (I/II and III/IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;6 (I)</td>
<td>0.9%</td>
<td></td>
</tr>
<tr>
<td>6-6.9 (II)</td>
<td>11.3%</td>
<td>0.9-11.3%</td>
</tr>
<tr>
<td>7 to 7.9 (III)</td>
<td>44.4%</td>
<td>44.4%-76.5%</td>
</tr>
<tr>
<td>&gt;8 (IV)</td>
<td>76.5%</td>
<td></td>
</tr>
</tbody>
</table>

Papillary thyroid carcinoma recurrence risk was estimated based on the 2009 ATA guidelines ([American Thyroid Association Guidelines Taskforce on Thyroid, Differentiated Thyroid et al. 2009](http://www.thyroid.org)) as follows:
1. **Low-risk** (must meet all criteria): pT1/T2, lymph node status = N0 or NX, intrathyroidal pT3, complete macroscopic resection.

2. **Intermediate-risk**: lymph node status N1, pT3 due to extrathyroidal extension, pT4, M1, high-risk variants (tall cell or columnar cell variants), definitive angioinvasion.


The revised 2009 ATA risk stratification system (included in the 2016 ATA guidelines) was not utilized because it provides similar risk estimates to the original system (Pitoia, Jerkovich et al. 2015) and it has not been validated in as many patients as the older system.

The term ETE was used to describe tumors with both minimal (pT3) and moderately to advanced (pT4) carcinoma extension outside the thyroid gland given the strict definition of pT3 used at this institution.

Angioinvasion was diagnosed when tumor cells invaded through a vessel wall or a thrombus adherent to the intravascular tumor was present (Mete and Asa 2011). Cases were called indeterminate for angioinvasion when there was a focus suspicious but not diagnostic for angioinvasion and this focus could not be further clarified due to tissue related limitations or artifacts.

Clinically apparent lymph nodal metastasis (cN1) was defined as metastatic lymph nodes identified by physical examination, imaging or intraoperative examination (Randolph, Duh et al. 2012).

Follow-up was defined as the time in years between surgical pathology diagnosis and the last clinical or radiological assessment.
Structural recurrence was defined as the presence of biopsy proven PTC or imaging evidence highly suggestive of PTC (discovered by US, CT, MRI or a radioactive iodine scan) occurring after a patient was considered free of disease (negative imaging studies, thyroglobulin, and thyroglobulin antibodies) or in addition to persistent disease.

3.3 Analytic Methods

3.3.1 Discovery Cohort

A hundred and thirty one tissue specimens collected from 2002 through 2004 had been submitted for mutational analysis prior to the initiation of this study. DNA from formalin-fixed paraffin-embedded sections of these PTCs was subjected to a panel of twenty-three multiplexed assays interrogating 286 mutations in twenty-three genes on a MassARRAY platform (Sequenom). Mutation status was determined using TyperAnalyzer software and manual analysis.

3.3.2 Validation Cohort

A hundred and eight samples collected during 2006-2012 were submitted to the TCGA Research Network for analysis. Mostly for technical reasons only 70 tumors were analyzed on all major platforms (SNP arrays, exomes, RNA-seq, miRNA-seq, and DNA methylation). The strategy and methods used to analyze these samples has been previously described in detail in the supplementary material of the article entitled “Integrated Genomic Characterization of Papillary Thyroid Carcinoma” (Cancer Genome Atlas Research 2014). Briefly, for mutational analysis, WES was performed on the Illumina HiSeq 2000 platform using the V3 Sequencing Kits and the Illumina 1.3.4 pipeline to produce paired-end sequenced data. Basic alignment and sequence
quality control was done on the sequence data-processing pipeline “Picard” (http://picard.sourceforge.net/) and the Cancer genome analysis pipeline “Firehouse” (http://www.broadinstitute.org/cancer/cga/) at the Broad Institute. The latter pipeline, which is a series of tools for analyzing massively parallel sequencing data for tumor samples and their patient-matched normal DNA samples, performed mutation calling, rearrangement detection, small deletion and insertion identification, and detection of significantly recurring mutated genes. Following Firehouse processing, false somatic mutations were filtered out with the Panel of Normals filter. Somatic single nucleotide variants from the WES data were identified with Strelka (v1.0.6) and RADIA (RNA AND DNA Integrated Analysis). Significantly mutated genes were identified with MutSig 1.5.

3.4 Statistical Analysis

The statistical software SPSS 20 for Mac was used for analysis except for cases in which cross-tabulations were more than 2x2 and an exact test was required. For these calculations a free online resource that performs the Freeman-Halton extension of Fisher’s exact test (Freeman and Halton 1951) for 2x3 or 2x4 contingency tables was utilized. Also, in two instances where exact tests for >2x4 tables were required, SPSS 23 for Windows was used. Odds ratios (OR) and their corresponding 95% confidence intervals were calculated using the following online tool:


---

1 The SPSS 20 software used for this thesis does not perform exact tests for cross-tabulations that are more than 2x2, therefore, these free online resources were utilized: http://vassarstats.net/fisher2x3.html and http://vassarstats.net/fisher2x4.html.
Descriptors were reported as percentages and proportions as well as with medians and range or means and standard deviation where appropriate. Percentages and proportions were rounded to the tenths. According to each variable distribution, differences in means or between groups were analyzed with Student’s t or Mann-Whitney U and differences in proportions using $\chi^2$ or Fisher’s exact test. Fisher's exact test was used in instances where more than 20% of the cells had expected cell frequencies of less than five. ORs were used to compare the relative odds of the occurrence of the outcome of interest, given exposure to the variable of interest. The 95% confidence interval (CI) was used to estimate the precision of the OR. $p$ values were reported as exact values rounded to the thousandths except when the $p$ value was $<0.001$ in which case it was reported as $p < 0.001$. Statistical significance was considered reached at $p < 0.05$. 
CHAPTER 4
RESULTS

4 Patient Characteristics

General characteristics of the discovery and validation cohort populations are shown in Table 3. A total of 183 patients, 116 from the discovery cohort and 67 from the validation cohort, were included for analysis. Most patients were female (74.9%), had FVPTCs (54.6%) and low risk disease [TNM Stage I PTC (63.4%), MACIS category I (80.8%), low ATA risk category (57.1%)]. Also, most subjects were treated with a total thyroidectomy without lymph nodal dissection (70.5%), and radioactive iodine (85.4%). Mean age at the time of surgical pathology diagnosis was 44.0 years ($SD = 14.1$) for the combined cohorts. Although the average age in the discovery cohort was lower (43.7 years, $SD = 12.4$) than in the validation cohort [48.9 years, $SD= 16.3$; $t (182) = -2.188, p = 0.031$], the proportion of patients $\geq 45$ years (Table 3) was comparable [$\chi^2 (1) = 3.042, p = 0.081$]. Median follow-up for the combined cohorts was 4.6 years (range = 0-13.8) with individuals in the discovery cohort having longer follow-up than those in the validation cohort ($Mdn = 7.5$ years and 4.0 years respectively; $U= 2,276, p <0.001$).

Follicular variant PTCs and classical PTCs were similarly represented in the discovery and validation cohorts [$\chi^2 (1) = 0.183, p = 0.669$] (Table 3). Surgical treatment was comparable between groups as well (Freeman-Halton extension of Fisher’s exact test, $p = 0.165$) (Table 3). Both cohorts were also similar with respect to gender [$\chi^2 (1) = 3.30, p = 0.068$], ATA risk categories (Freeman-Halton extension of Fisher’s exact test, $p = 0.556$), and TNM stages [$\chi^2 (3) = 7.036, p = 0.071$] (Table 3).
Table 3. General characteristics of cohorts

<table>
<thead>
<tr>
<th>Variables</th>
<th>Discovery cohort N=116 (%)</th>
<th>Validation cohort N=67 (%)</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total N=183 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>92 (79.3)</td>
<td>45 (67.2)</td>
<td>0.068</td>
<td>137 (74.9)</td>
</tr>
<tr>
<td>Male</td>
<td>24 (20.7)</td>
<td>22 (32.8)</td>
<td></td>
<td>46 (25.1)</td>
</tr>
<tr>
<td><strong>Age in years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>64 (55.2)</td>
<td>28 (41.8)</td>
<td>0.081</td>
<td>93 (50.5)</td>
</tr>
<tr>
<td>≥45</td>
<td>52 (44.8)</td>
<td>39 (58.2)</td>
<td></td>
<td>91 (49.5)</td>
</tr>
<tr>
<td><strong>TNM Stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>77 (66.4)</td>
<td>39 (58.2)</td>
<td>0.071</td>
<td>116 (63.4)</td>
</tr>
<tr>
<td>II</td>
<td>10 (8.6)</td>
<td>13 (19.4)</td>
<td></td>
<td>23 (12.6)</td>
</tr>
<tr>
<td>III</td>
<td>19 (16.4)</td>
<td>6 (9.0)</td>
<td></td>
<td>25 (13.7)</td>
</tr>
<tr>
<td>IV</td>
<td>10 (8.6)</td>
<td>9 (13.4)</td>
<td></td>
<td>19 (10.4)</td>
</tr>
<tr>
<td><strong>MACIS categories&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>96 (83.5)</td>
<td>51 (76.1)</td>
<td></td>
<td>147 (80.8)</td>
</tr>
<tr>
<td>II</td>
<td>17 (14.8)</td>
<td>7 (10.4)</td>
<td>0.002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24 (13.2)</td>
</tr>
<tr>
<td>III</td>
<td>2 (1.7)</td>
<td>7 (10.4)</td>
<td></td>
<td>9 (4.9)</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>2 (3.0)</td>
<td></td>
<td>2 (1.1)</td>
</tr>
<tr>
<td><strong>ATA risk categories&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>69 (60.0)</td>
<td>35 (52.2)</td>
<td>0.556</td>
<td>104 (57.1)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>42 (36.5)</td>
<td>30 (44.8)</td>
<td></td>
<td>72 (39.6)</td>
</tr>
<tr>
<td>High</td>
<td>4 (3.5)</td>
<td>2 (3.0)</td>
<td></td>
<td>6 (3.3)</td>
</tr>
<tr>
<td>Table 3. Continuation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Variables</strong></td>
<td><strong>Discovery cohort N=116 (%)</strong></td>
<td><strong>Validation cohort N=67 (%)</strong></td>
<td><strong>p value</strong></td>
<td><strong>Total N=183 (%)</strong></td>
</tr>
<tr>
<td><strong>Surgical treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobectomy</td>
<td>4 (3.4)</td>
<td>2 (3.0)</td>
<td>0.165</td>
<td>6 (3.3)</td>
</tr>
<tr>
<td>Total thyroidectomy</td>
<td>87 (75.0)</td>
<td>42 (62.7)</td>
<td></td>
<td>129 (70.5)</td>
</tr>
<tr>
<td>Total thyroidectomy with lymph nodal dissection</td>
<td>25 (21.6)</td>
<td>23 (34.3)</td>
<td></td>
<td>48 (26.2)</td>
</tr>
<tr>
<td><strong>Radioactive iodine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>91 (97.8%)</td>
<td>44 (67.7%)</td>
<td>&lt;0.001</td>
<td>135 (85.4%)</td>
</tr>
<tr>
<td>No</td>
<td>2 (2.2%)</td>
<td>21 (32.35)</td>
<td></td>
<td>23 (14.6%)</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td>62 (53.4)</td>
<td>38 (56.7%)</td>
<td>0.669</td>
<td>100 (54.6)</td>
</tr>
<tr>
<td>FVPTC</td>
<td>54 (46.6)</td>
<td>29 (43.3%)</td>
<td></td>
<td>83 (45.4)</td>
</tr>
<tr>
<td>Classical PTC</td>
<td>54 (46.6)</td>
<td>29 (43.3%)</td>
<td></td>
<td>83 (45.4)</td>
</tr>
<tr>
<td><strong>Multifocality</strong></td>
<td>66 (56.9%)</td>
<td>48 (71.6%)</td>
<td>0.047</td>
<td>114 (62.3%)</td>
</tr>
<tr>
<td>Yes</td>
<td>50 (43.1%)</td>
<td>19 (28.4%)</td>
<td></td>
<td>69 (37.7%)</td>
</tr>
<tr>
<td>No</td>
<td>50 (43.1%)</td>
<td>19 (28.4%)</td>
<td></td>
<td>69 (37.7%)</td>
</tr>
</tbody>
</table>

ATA, American Thyroid Association; FVPTC, follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma.

aχ² or Fisher’s exact test.
bMACIS score and ATA risk could not be calculated for one of the patients in the discovery cohort, therefore, n=115.
cFisher’s exact test for grouped MACIS categories (I/II and III/IV).
dIn the first cohort information was available for 93 cases and in the second cohort for 65 cases.

Although the mean MACIS score was the same in both cohorts ($Mdn = 4.7; U = 4,215.0 \ p = 0.290$), higher MACIS categories (III/IV) were more prevalent in the validation cohort (13.5%) than the discovery cohort (1.7%) (Fisher’s exact test; $p = 0.002$). In fact, the odds of a MACIS score of ≥7 were 8.8 times higher if patients belonged to the validation cohort than if they
belonged to the discovery cohort (95% CI: 1.83-41.91). As expected due to clinical practice changes patients in the discovery cohort were more frequently treated with radioactive iodine than those in the validation cohort \( \chi^2 (1) = 27.975, p < 0.001 \) (Table 3). Another difference between the groups is that tumors in the validation cohort were more frequently multifocal \( \chi^2 (1) = 3.931, p = 0.047 \) (Table 3).

4.1 **Authentic Identification of Classical Architecture Selects \( \text{BRAF}^{V600E} \) Mutants**

4.1.1 Discovery Cohort

Genotype-architecture correlations are delineated in Figure 8 and Table 4. Sixty-two (53.4%) of 116 PTCs had follicular architecture. Of these FVPTCs, twenty harbored mutations, the majority of which were in \textit{RAS} (n=18, 90.0%) (Figure 8). Remarkably, there were no \( \text{BRAF}^{V600E} \) mutations within the follicular architecture subgroup. One FVPTC harbored a novel mutation in serine/threonine kinase 11 (\textit{STK11}): \textit{STK11}W332*.

Capsular invasion status was available in 61/62 FVPTCs and a description of the tumor capsule was complete in 59/62 cases. Eighteen of forty-four (40.9%) invasive FVPTCs were encapsulated, twenty-two (50.0%) had a partial capsule, two were unencapsulated (4.5%), and in the remaining two the information was not available (4.5%). Seventeen of sixty-one (27.9%) FVPTCs were noninvasive; of these tumors nine were completely encapsulated. Eighteen (66.7%) of the twenty-seven completely encapsulated FVPTCs, were invasive.
Thirty-six (66.7%) of the fifty-four tumors with classical architecture harbored mutations. The vast majority of these classical PTCs (n=34, 63.0%) were BRAF-V600E-mutated while only one carcinoma (1.9%) harbored a RAS mutation. Not surprisingly, the BRAF-V600E signature was strongly associated with classical architecture \(\chi^2(1) = 55.223, p < 0.001, \text{Cramer's } V = 0.690\) while the RAS signature was related to follicular architecture \(\chi^2(1) = 15.568, p < 0.001, \text{Cramer's } V = 0.366\).

![Diagram](image)

**Figure 8.** Distribution of somatic mutations in classical and follicular variant papillary thyroid carcinomas within the discovery cohort. The BRAF-V600E mutation was exclusive to classical papillary thyroid carcinomas while RAS mutations were overrepresented in follicular variant papillary thyroid carcinomas. Not surprisingly, the BRAF-K601E mutation was only found in a papillary thyroid carcinoma with follicular architecture. Interestingly, a novel mutation in STK11 was found in a follicular variant papillary thyroid carcinoma while a mutation in EGFR was present in a classical PTC. Please also refer to Table 4 and Footnote 3.
Table 4. Genotype-architecture correlations

<table>
<thead>
<tr>
<th>Mutation drivers - n, architecture</th>
<th>Discovery cohort n=56</th>
<th>Validation cohort n=42</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRAF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$BRAF^{V600E}$</td>
<td>34, classical 1, follicular 0</td>
<td>22, classical 1 follicular 1, follicular 1, follicular</td>
</tr>
<tr>
<td>$BRAF^{K601E}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$BRAF^{TAPTP488del}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RAS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$NRAS^{Q61R}$</td>
<td>5, follicular 2, follicular; 3, follicular; 1 classical 1, follicular 0</td>
<td>5 follicular; 1 classical 5, follicular none 0 1, follicular 0 1, classical 1, follicular</td>
</tr>
<tr>
<td>$NRAS^{Q61K}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$KRAS^{G12C}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$KRAS^{G12D}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$KRAS^{G12V}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$KRAS^{Q61R}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$HRAS^{Q61K}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$HRAS^{Q61R}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EGFR$^{S768I}$</strong></td>
<td>1, classical</td>
<td>0</td>
</tr>
<tr>
<td><em><em>STK11$^{W332</em>}$</em>*</td>
<td>1, follicular</td>
<td>0</td>
</tr>
<tr>
<td><strong>EIF1AX</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$EIF1AX^{A113V}$</td>
<td>0</td>
<td>1, follicular</td>
</tr>
<tr>
<td>$EIF1AX^{G9R}$</td>
<td>0</td>
<td>1, follicular</td>
</tr>
<tr>
<td>$EIF1AX^{G8R}$</td>
<td>0</td>
<td>1, follicular</td>
</tr>
</tbody>
</table>

---

2 Since we had not observed any $BRAF^{V600E}$ mutants in follicular variant papillary thyroid carcinomas (FVPTCs) belonging to the discovery cohort, after completion of this study we proceeded to review the surgical pathology specimen submitted for the TCGA project and learned that this was a classical PTC that originated from a patient with multifocal classical (second dominant tumor) and FVPTC (dominant tumor). That is, the second dominant classical PTC was incorrectly analyzed as FVPTC due to a clerical error.

3 Given the rarity of $RAS$ mutations in classical papillary thyroid carcinoma (classical PTC), after completion of this study classical PTCs with a $RAS$ mutation were reviewed. In this particular case, a clerical error was committed and a similarly sized FVPTC belonging to the same case was submitted.
4.1.2 Validation Cohort

Genotype-architecture correlations are outlined in Table 4 and Figure 9. Thirty-eight (56.7%) of 67 tumors were FVPTCs. Of these, 18 had mutations, most of which were in RAS (n=12, 66.7%). Three FVPTCs harbored alterations in BRAF; namely, \textit{BRAF^{V600E}}, \textit{BRAF^{K601E}}, and \textit{BRAF^{TAPTP488del}} (Table 4 and Footnote 2). The three \textit{EIF1AX} (eukaryotic translation initiation factor 1A, X-linked) mutations found in the cohort were exclusive to the FVPTC category.

In one encapsulated FVPTC capsular invasion was indeterminate. Twenty-two of the remaining thirty-seven FVPTCs (59.5%) were invasive. Fifteen of these tumors were encapsulated and seven had a partial capsule. The fifteen (15/37, 39.5%) noninvasive FVPTCs were classified as follows: five completely encapsulated tumors, six partially encapsulated tumors, and four tumors without a capsule.

Twenty-four (82.8%) of twenty-nine tumors with classical architecture had mutations. The vast majority (n=22, 91.7%) of classical PTCs were \textit{BRAF^{V600E}}-mutated while only two were reported to harbor RAS mutations (Table 4 and Footnote 3). Accordingly, the \textit{BRAF^{V600E}} signature was strongly associated with classical PTCs [$\chi^2 (1) = 39.125, p < 0.001, \text{Cramer’s V= 0.764}$] while the RAS signature was associated with FVPTCs [$\chi^2 (1) = 6.062, p = 0.014, \text{Cramer’s V= 0.301}$].
Figure 9. Distribution of somatic mutations in classical and follicular variant papillary thyroid carcinomas within the validation cohort. The \(BRAF^{V600E}\) mutation was commonly found in classical papillary thyroid carcinomas while other \(BRAF\) variants were exclusive to the follicular variant papillary thyroid carcinoma category. \(RAS\) mutations were overrepresented in follicular variant papillary thyroid carcinomas and \(EIF1AX\) mutations were only detected in this category. Please also refer to Table 4 and Footnotes 2-3.

4.2 Classical Papillary Thyroid Carcinomas Harbor Distinct Clinicopathological Characteristics

4.2.1 Classical Architecture is Associated with Clinical Lymph Node Metastasis

4.2.1.1 Discovery Cohort

Ascertainment of cN was possible in 112 cases of which twenty-two (19.6%) had metastatic lymphadenopathy. In twenty-one cases (19 classical PTCs, 2 FVPTCs) the size of the affected lymph nodes was available and it ranged from 0.7 cm to 4.8 cm (\(M = 2.4, SD = 1.2\)).
Subjects harboring classical PTCs more frequently presented with cN1 (20/54, 37.0%) compared to their FVPTC counterparts (2/58, 3.4%) \[\chi^2(1) = 18.687; p < 0.001\] (Figure 10). In fact, the odds of cN1 were 16.5 times higher if patients had classical PTCs than if they had FVPTCs (95% CI: 3.62-74.9).

![Figure 10](image)

**Figure 10.** Classical architecture is associated with clinical lymph node metastasis in the discovery cohort. Patients with classical papillary thyroid carcinomas more frequently harbored clinically evident metastatic lymph nodes compared to those with follicular variant papillary thyroid carcinomas.

### 4.2.1.2 Validation Cohort

Determination of cN1 status was possible in sixty-two cases. In nineteen of these cases the size of metastatic lymphadenopathy was available and it ranged from 0.9 to 3.8 cm (\(M=2.1, SD=0.9\)). Clinical lymph node metastases were overrepresented in patients with classical PTCs (16/27, 55.6% vs. 4/35, 11.4% patients with FVPTCs) \[\chi^2(1) = 13.964; p < 0.001\] (Figure 11)
with the odds of cN1 being 9.7 times higher if patients had classical PTCs than if they had FVPTCs (95% CI: 2.67-35.14).

![Bar chart showing percentage of cases with clinical lymph node metastasis (cN1) present or absent, with classical architecture and follicular architecture](image)

**Figure 11.** Classical architecture is associated with clinical lymph node metastasis in the validation cohort. Clinical lymph node metastases were more prevalent in classical papillary thyroid carcinomas than their follicular variant counterparts.

### 4.2.2 Tumor Architecture is Associated with Extrathyroidal Extension in the Discovery Cohort

#### 4.2.2.1 Discovery Cohort

Extrathyroidal extension was present in nine of 116 cases (7.8%). Classical architecture was associated with this specific type of invasive growth with all nine tumors belonging to this category (9/54 vs. 0/62 FVPTCs; Fisher’s exact test, *p* = 0.001; OR 26.1, 95% CI: 1.48-460.03).
4.2.2.2 Validation Cohort

Three of sixty-seven (4.5%) PTCs presented with ETE and although this pattern of invasive growth was only found in classical PTCs (3/29, 10.3%) the proportion of cases was not significantly higher compared to FVPTCs (0/30; Fisher’s exact test, $p = 0.076$).

4.2.3 Tumor Architecture is not Associated with Angioinvasion

4.2.3.1 Discovery Cohort

Angioinvasion was present in twelve (10.6%) of 113 tumors. Classical PTCs were more frequently angioinvasive (8/44, 15.4%) than FVPTCs (4/57, 6.6%), but the difference was not significant [$\chi^2 (1) = 2.304; p = 0.129$].

4.2.3.2 Validation Cohort

Seven percent (4/57) of PTCs showed vascular invasion. Although, angioinvasion was overrepresented within classical PTCs (2/20, 10.0% vs. 2/37, 5.4% of FVPTCs) this was not statistically significant (Fisher’s exact test, $p = 0.607$).

4.2.4 Classical Architecture is Associated with More Advanced TNM Stages

4.2.4.1 Discovery Cohort

Papillary thyroid carcinoma architecture was significantly associated with TNM stages (Freeman-Halton extension of Fisher’s exact test, $p <0.001$) (Figure 12). Indeed, a higher proportion of patients with classical PTCs (10/54, 18.5%) than those with FVPTCs (0/62)
presented with stage IV disease (Fisher’s exact test, $p < 0.001$; OR 29.5, 95% CI: 1.68-515.57). Similarly, after combining TNM stages into two categories (I/II, III/IV) patients with classical PTC presented with more advanced stages (19/ 54, 35.2%) than patients with FVPTC (10/62, 16.1%) [$\chi^2(1) = 5.590; p = 0.018$].

![Bar chart showing percentage of patients within architecture by TNM stage](chart.png)

**Figure 12.** Classical architecture is associated with more advanced Tumor, Node, Metastasis (TNM) stages in the discovery cohort. Patients with classical papillary thyroid carcinoma more frequently presented with stage IV disease compared to those with follicular variant papillary thyroid carcinomas. ***$p < 0.001$ vs. stages I/II/III.

### 4.2.4.2 Validation Cohort

There was a significant association between PTC architecture and TNM stages (Freeman-Halton extension of Fisher’s exact test, $p < 0.001$) (Figure 13). Patients with classical PTCs
presented more frequently with stage IV disease (8/29, 27.6%) than patients with FVPTCs (1/38, 2.6%) (Fisher’s exact test, $p = 0.008$; OR 14.1, 95% CI: 1.65-120.61). The prevalence of combined TNM stages III/IV was also higher in patients with classical tumors (19/56, 33.9% vs.10/61, 16.4% patients with FVPTCs) [$\chi^2 (1) = 9.607; p = 0.002$].

![Graph showing the proportion of patients with classical and follicular architecture across different TNM stages](image)

**Figure 13.** Classical architecture is associated with more advanced Tumor, None, Metastasis (TNM) stages in the validation cohort. Patients with classical papillary thyroid carcinoma more frequently presented with stage IV disease compared to those with follicular variant papillary thyroid carcinomas. ***$p < 0.008$ vs. stages I/II/III.***
4.2.5 Classical Architecture is not Associated with MACIS Categories

4.2.5.1 Discovery Cohort

MACIS scores could be calculated for 115 patients and they were not significantly different between those with classical PTCs ($Mdn = 4.7$) and FVPTCs ($Mdn = 4.6$; $U = 1,368.0$, $p = 0.123$). There was also no association between PTC architecture and whether individuals fell into a higher or lower grouped MACIS score category (Fisher’s exact test, $p = 0.210$).

4.2.5.2 Validation Cohort

MACIS scores for all 67 cases were calculated. Although scores were higher in patients with classical PTCs ($Mdn = 5.0$) than patients with FVPTCs ($Mdn = 4.6$), this difference was not significant ($U = 503.0$, $p = 0.544$). Moreover, there was no association between PTC architecture and whether subjects fell into higher (III,IV) or lower (I,II) grouped MACIS categories (Fisher’s exact test, $p = 0.485$).

4.2.6 Classical Architecture is Associated with Higher ATA Risk Categories

4.2.6.1 Discovery Cohort

As shown in Figure 14 PTC architecture and ATA risk categories were significantly associated (Freeman-Halton extension of the Fisher’s exact test, $p < 0.001$).
**Figure 14.** Papillary thyroid carcinoma architecture is associated with American Thyroid Association risk categories in the discovery cohort. Patients with classical papillary thyroid carcinomas were overrepresented in the American Thyroid Association intermediate/high-risk categories.

Patients with classical PTCs were more frequently stratified into the intermediate/high-risk categories (37/53, 69.8%) compared to their FVPTC counterparts (9/62; 14.5%) \[\chi^2(1) = 36.403; p < 0.001\] (OR 13.6, 95% CI: 5.44-34.11).

### 4.2.6.2 Validation Cohort

Tumor architecture was significantly related to ATA risk categories (Freeman-Halton extension of Fisher’s exact test, \(p = 0.004\)) (Figure 15) with a higher proportion of patients with classical PTCs in the intermediate/high-risk categories (20/29, 69.0%) than patients with FVPTCs (12/38; 31.6%) \[\chi^2(1) = 9.214; p = 0.002\] (OR 4.8, 95% CI: 1.70-13.70).
Figure 15. Papillary thyroid carcinoma architecture is associated with American Thyroid Association risk categories in the validation cohort. Patients with classical papillary thyroid carcinomas were overrepresented in the American Thyroid Association intermediate/high-risk categories.

4.2.7 Tumor Architecture is not Associated with Structural Recurrences

4.2.7.1 Discovery Cohort

A hundred patients (100/116, 86.2%) in whom structural recurrence analysis was possible had a median follow-up of 7.8 years (1.3-13.8). There were only three structural recurrences, all arising from classical PTCs. However, recurrent disease was not significantly overrepresented in patients with classical PTCs (3/49, 6.1%) compared to those with FVPTCs (0/51; Fisher’s exact test, \( p = 0.114 \)).
All three recurrences occurred in regional lymph nodes. Two of the patients with recurrent disease had presented with cN1 (1.7 cm and 3 cm in size) while the other patient had been diagnosed with central lymph node metastasis as well as a small noninvasive primary tumor at the time histologic examination (T1bN1a).

4.2.7.2 Validation Cohort

Sixty-two cases (62/66, 93.9%), with a median follow-up of 4.1 years (1.5-8.2) were included in the analysis of structural recurrences. Five patients (8.1%) developed structural disease. In three cases, these recurrences arose from classical PTCs (3/27, 11.1%) while the other two originated from FVPTCs (2/35, 5.7%). Two of the classical PTC recurrences occurred in regional lymph nodes and one in the thyroid bed. All three patients with recurrent classical PTCs initially presented with cN1 whereas only one patient with recurrent FVPTC (regional node recurrence) had that type of clinical presentation. The remaining patient with recurrent FVPTC harbored an angioinvasive primary tumor that contained an area of focal dedifferentiation. This patient had originally presented with bony metastasis and subsequently developed new distant metastases. There was no association between classical PTC architecture and structural recurrence (Fisher’s exact test, $p = 0.645$). Lack of an association persisted after excluding the case of recurrent FVPTC in which a focal poorly differentiated component was present (Fisher’s exact test, $p = 0.313$).
4.3 The \textit{BRAF}^{V600E} Signature is Associated with Clinical Lymph Node Metastasis and Extrathyroidal Extension

4.3.1 Discovery Cohort

Clinical lymph node metastases were present in twenty-two of 112 (19.6\%) subjects. Patients with \textit{BRAF}^{V600E}-mutated PTCs more frequently presented with cN1 (14/34, 41.2\%) than those with tumors with a wild-type (WT) status for this mutation (8/78, 10.3\%) [$\chi^2 (1) = 14.342$; $p < 0.001$]. The odds of a patient having cN1 were 6.1 times higher if they had a \textit{BRAF}^{V600E}-mutated tumor than if they had a carcinoma lacking the mutation (95\% CI: 2.25-16.66).

Nine (7.7\%) of 116 tumors showed ETE. This pattern of invasive growth was overrepresented in \textit{BRAF}^{V600E}-harboring PTCs (7/34, 20.6\%) compared to FVPTCs (2/82, 2.4\%; Fisher’s exact test, $p = 0.003$) with the odds of ETE being 10.4 times higher if the tumor had a \textit{BRAF}^{V600E} mutation than if it did not (95\% CI: 2.03-52.98).

4.3.2 Validation Cohort

Nineteen (30.6\%) of sixty-two patients had cN1 at the time of surgical pathology diagnosis. A higher proportion of patients with \textit{BRAF}^{V600E}-mutated tumors (13/21, 61.9\%) than patients with tumors lacking the mutation (6/41, 14.6\%) presented with cN1 [$\chi^2 (1) = 14.600$; $p < 0.001$]. Indeed, the odds of a patient having cN1 were 9.5 times higher if they had a \textit{BRAF}^{V600E}-harboring carcinoma than if they had a tumor lacking this genotype (95\% CI: 2.76-32.60).

Three of sixty-seven (4.5\%) PTCs presented with ETE. Tumors with the \textit{BRAF}^{V600E} mutation more frequently harbored this pattern of invasion (3/23; 13.0\% vs. 0/44 \textit{BRAF}^{V600E}-WT PTCs; Fisher’s exact test, $p = 0.037$).
4.4 The $\text{BRAF}^{V600E}$ Signature is not Associated with Angioinvasion in Papillary Thyroid Carcinomas within the Discovery and Validation Cohorts

Twelve (10.6%) of 113 tumors in the discovery cohort were angioinvasive. The proportion of angioinvasive $\text{BRAF}^{V600E}$-mutated tumors (3/33, 9.1%) and angioinvasive PTCs lacking the mutation (9/80, 11.2%) was comparable (Fisher’s exact test, $p = 1.000$).

Four (7.0%) of fifty-seven PTCs in the validation cohort showed vascular invasion. Although angioinvasion was overrepresented in $\text{BRAF}^{V600E}$-mutated tumors (2/20, 10.0% vs. 2/37, 5.4% PTCs lacking the mutation) this was not significant (Fisher’s exact test, $p = 0.281$).

4.5 The $\text{BRAF}^{V600E}$ Signature is Associated with More Advanced TNM Stages in Papillary Thyroid Carcinoma

4.5.1 Discovery Cohort

As shown in Figure 16, there was a significant association between TNM stages and the $\text{BRAF}^{V600E}$ mutational status (Freeman-Halton extension of Fisher’s exact test, $p < 0.001$). Patients with $\text{BRAF}^{V600E}$-mutated PTCs (9/34, 26.5% vs. 1/82, 1.2% $\text{BRAF}^{V600E}$-WT status) more frequently presented with stage IV disease (Fisher’s exact test, $p < 0.001$; OR 29.2, CI: 3.52-241.51). Moreover, subjects with $\text{BRAF}^{V600E}$-mutated tumors were more likely to be assigned to the TNM stage III/IV disease category (14/82, 17.1%) compared to patients harboring tumors that lacked this genotype (15/34, 44.1%) [$\chi^2 (1) = 9.375$; $p = 0.002$]. Indeed, the odds of having stage III/IV disease were 3.8 times if patients had $\text{BRAF}^{V600E}$-mutated tumors than if they did not (95% CI: 1.60-9.32).
Figure 16. The \textit{BRAF}^{V600E} signature is associated with more advanced Tumor, None, Metastasis (TNM) stages in the discovery cohort. Patients with \textit{BRAF}^{V600E}-mutated tumors had a higher prevalence of stage IV disease compared to those without the mutation. ***$p < 0.001$ vs. stages I/II/III.

4.5.2 Validation Cohort

As shown in Figure 17, TNM stages and \textit{BRAF}^{V600E} mutational status were significantly associated (Freeman-Halton extension of Fisher's exact test, $p = 0.003$). Patients with \textit{BRAF}^{V600E}-harboring tumors more frequently presented with stage III/IV disease (9/23, 39.1%) than patients with PTCs lacking the mutation (6/44, 13.6%) [$\chi^2 (1) = 5.650; p = 0.029$]. The odds of stage III/IV disease were 4.1 times higher if patients had a tumor with a \textit{BRAF}^{V600E} mutation than if their tumor lacked this genotype (95% CI: 1.23-13.53). Of note, although patients who had \textit{BRAF}^{V600E}-mutated carcinomas more frequently presented with stage IV disease (6/23, 26.1% vs. 3/44, 6.8% patients with PTCs lacking the mutation) this was not statically significant (Fisher's exact test, $p = 0.54$).
Figure 17. The $BRAF^{V600E}$ signature is associated with more advanced Tumor, None, Metastasis (TNM) stages in the validation cohort. Patients with $BRAF^{V600E}$-mutated tumors had a higher prevalence of stage III/IV disease compared to those without the mutation. ***$p < 0.029$ stages III/IV vs. stages I/II.

4.6 The $BRAF^{V600E}$ Signature is not Associated with MACIS Categories in Papillary Thyroid Carcinoma

4.6.1 Discovery Cohort

The median MACIS score for patients with $BRAF^{V600E}$-mutated carcinomas and those with $BRAF^{V600E}$-WT PTCs was the same [$Mdn=4.7$; $n=115$, $U = 1,557.50$, $p = 0.206$]. Furthermore, the proportion of patients with $BRAF^{V600E}$-mutant PTCs ($1/33$, $3.0\%$) with a
MACIS score ≥7 (MACIS categories III/IV), was similar to their $BRAF^{V600E}$ WT counterparts (1/82, 1.2%; Fisher’s exact test, $p = 0.493$).

4.6.2 Validation Cohort

MACIS scores were comparable between patients with $BRAF^{V600E}$-harboring PTCs ($Mdn = 5.1$) and those with tumors that lacked this genotype [$Mdn= 4.7; n=67, U = 534.0, p = 0.712$]. Also, the proportion of patients with $BRAF^{V600E}$-mutated carcinomas with a MACIS score ≥7 (4/23, 17.4%) did not differ significantly from those with PTCs that lacked this genotype (5/44, 11.4%; Fisher’s exact test, $p = 0.481$).

4.7 The $BRAF^{V600E}$ Signature is Associated with Higher ATA Risk Categories in Papillary Thyroid Carcinoma

4.7.1 Discovery Cohort

As shown in Figure 18, $BRAF^{V600E}$ mutational status was significantly associated with ATA risk categories (Freeman-Halton extension Fisher’s exact test, $p < 0.001$). Patients with $BRAF^{V600E}$-mutated PTCs were more frequently stratified into the combined intermediate/high-risk category (23/33, 69.7%) than patients with PTCs with WT status for the mutation (23/82, 28.0%) [$\chi^2(1) = 17.006; p < 0.001$] (OR 5.9, 95% CI: 2.43-14.30).
**Figure 18.** The \( BRAF^{V600E} \) signature is associated with the American Thyroid Association risk categories in the discovery cohort. Patients with \( BRAF^{V600E} \)-mutated tumors were more frequently stratified into the intermediate/high-risk American Thyroid Association risk categories.

### 4.7.2 Validation Cohort

\( BRAF^{V600E} \) mutational status was significantly associated with ATA risk categories (Figure 19) (Freeman-Halton extension of Fisher’s exact test, \( p = 0.003 \)). Patients with \( BRAF^{V600E} \)-mutated PTCs were more frequently stratified into the combined intermediate/high-risk category (17/23, 73.9%) than patients with tumors that lacked the mutation (15/44, 34.1%) \( \chi^2 (1) = 9.600; p = 0.002 \) (OR 5.5, 95% CI: 1.79-16.79).
Figure 19. The \( \text{BRAF}^{V600E} \) signature is associated with the American Thyroid Association risk categories in the validation cohort. Patients with \( \text{BRAF}^{V600E} \)-mutated tumors were more frequently stratified into the intermediate/high-risk American Thyroid Association risk categories.

4.8 The \( \text{BRAF}^{V600E} \) Signature is not Associated with Structural Recurrences in Papillary Thyroid Carcinoma

4.8.1 Discovery Cohort

Three (3.0\%) of 100 patients developed structural recurrences. The presence of the \( \text{BRAF}^{V600E} \) mutation (2/69, 2.9\%) was not associated with this outcome (vs. 1/31, 3.2\% \( \text{BRAF}^{V600E} \) WT PTCs; Fisher’s exact test, \( p =1.000 \)).

4.8.2 Validation Cohort

Five (8.1\%) of sixty-two patients were diagnosed with structural recurrences. Although recurrent disease was overrepresented in the \( \text{BRAF}^{V600E} \)-mutated carcinoma category (3/21,
14.3% vs. 2/41, 4.9% PTCs lacking the mutation), this was not statistically significant (Fisher’s exact test, \( p = 0.325 \)).

### 4.9 The BRAF\textsuperscript{V600E} Signature is not Associated with Clinical Lymph Node Metastasis, Extrathyroidal Extension, or Angioinvasion in Classical Architecture Papillary Thyroid Carcinoma

#### 4.9.1 Discovery Cohort

Thirty-four (62.3%) of fifty-four patients with classical PTCs presented with cN1. Patients with \( \text{BRAF}^{\text{V600E}} \)-mutated PTCs more frequently harbored cN1 (14/34, 41.2%) than those with \( \text{BRAF}^{\text{V600E}} \) WT PTCs (6/20, 30.0%). This difference, however, was not significant \([\chi^2(1) = 0.675; p = 0.411]\).

Clinical lymph node metastasis size was available for nineteen cases, thirteen of which presented with PTCs harboring a \( \text{BRAF}^{\text{V600E}} \) mutation. Mean cN1 size was comparable between \( \text{BRAF}^{\text{V600E}} \)-mutated carcinomas (2.1 cm, \( SD = 1.27 \)) and \( \text{BRAF}^{\text{V600E}} \)-WT PTCs (2.9 cm, \( SD = 0.91 \); \( t(17) = 1.458, p = 0.163 \)).

Nine (16.7%) of fifty-four classical PTCs showed ETE. Albeit a higher proportion of \( \text{BRAF}^{\text{V600E}} \)-mutated tumors with ETE (7/34, 20.6%) than \( \text{BRAF}^{\text{V600E}} \) WT PTCs (2/20, 10.0%), this difference was not significant (Fisher’s exact test, \( p = 0.458 \)).

Eight (15.4%) of fifty-two tumors with classical PTCs were angioinvasive. This pattern of invasive growth was underrepresented within the \( \text{BRAF}^{\text{V600E}} \)-mutated PTC category (3/33,
9.1% vs. 5/19; 26.3% in PTCs lacking the mutation) but this was not statistically significant (Fisher’s exact test, \( p = 0.124 \)).

4.9.2 Validation Cohort

Fifteen of twenty-seven patients (55.6%) with classical PTCs presented with cN1. While 80% of these cN1 originated from \( \text{BRAF}^{V600E} \)-mutated tumors, the proportion of \( \text{BRAF}^{V600E} \)-harboring PTCs with cN1 (12/20, 60.0%) was not significantly different than PTCs lacking this genotype (3/7, 42.9%; Fisher’s exact test, \( p = 0.662 \)).

Three (10.3%) of twenty-nine classical PTCs presented with ETE. This pattern of invasion was more frequent in \( \text{BRAF}^{V600E} \)-mutated PTCs (3/22, 13.6% vs. 0/7 PTCs lacking this mutation) but the difference was not significant (Fisher’s exact test, \( p = 0.557 \)).

Only two of twenty angioinvasive classical PTCs harbored the \( \text{BRAF}^{V600E} \) mutation (2/14 \( \text{BRAF}^{V600E} \) mutants vs. 0/6 tumors without the mutation); this yielded no association between the \( \text{BRAF}^{V600E} \) mutational status and angioinvasion (Fisher’s exact test, \( p = 1.000 \)).

4.10 The \( \text{BRAF}^{V600E} \) Signature is not Associated with TNM Stages in Classical Architecture Papillary Thyroid Carcinoma

4.10.1 Discovery Cohort

The \( \text{BRAF}^{V600E} \) signature was not associated with TNM stages in patients with classical PTCs (Freeman-Halton extension of Fisher’s exact probability test, \( p = 0.168 \)), albeit a higher proportion of individuals with \( \text{BRAF}^{V600E} \)-harboring tumors (9/34, 26.5% vs.1/20, 5.0% patients with \( \text{BRAF}^{V600E} \) WT PTCs) who presented with stage IV disease.
4.10.2 Validation Cohort

A slightly lower proportion of patients with $BRAF^{V600E}$-mutated PTCs presented with stage IV disease (6/22, 27.3%) than those with tumors lacking the mutation (2/7, 28.6%). In contrast, proportionally more patients with $BRAF^{V600E}$-harboring tumors (3/22, 13.6%) were assigned to stage III disease (vs. 0/7 PTCs lacking the mutation). These differences in proportions were not significant (Freeman-Halton extension of Fisher’s exact probability test, $p = 0.835$).

4.11 The $BRAF^{V600E}$ Signature is not Associated with MACIS Categories in Classical Architecture Papillary Thyroid Carcinoma

4.11.1 Discovery Cohort

The distribution of MACIS scores was not statistically different between patients with $BRAF^{V600E}$ mutated tumors and those with carcinomas harboring a WT status for this mutation [$n =55$, $U = 393.50$, $p= 0.524$]. Furthermore, the proportion of cases with a combined higher MACIS category (III/IV) was comparable between the $BRAF^{V600E}$-mutated group (1/33, 3.0%) and their WT counterparts (1/20, 5.0%; Fisher’s exact test, $p =1.000$).

4.11.2 Validation Cohort

The distribution of MACIS scores was similar in patients with $BRAF^{V600E}$-mutated tumors and those with carcinomas lacking this genotype [$n= 29$, $U = 335.0$, $p= 0.799$]. In addition, although the proportion of patients with $BRAF^{V600E}$-mutated tumors who presented with
a MACIS score $\geq 7$ (7/22, 31.8%) was higher than those patients with tumors that lacked this genotype (1/7, 14.3%) this difference was not significant (Fisher’s exact test, $p = 0.635$).

4.12 The $BRAF^{V600E}$ Signature is not Associated with ATA Risk Categories in Classical Architecture Papillary Thyroid Carcinoma

4.12.1 Discovery Cohort

A lower proportion of patients with $BRAF^{V600E}$-harboring classical PTCs (1/33, 3.0%) than those with $BRAF^{V600E}$ WT carcinomas (2/20, 10.0%) were clustered into the high-risk category. In contrast proportionally more patients with $BRAF^{V600E}$-mutant PTCs (22/33, 66.7% vs.12/20, 60.0% patients with PTCs lacking the mutation) were represented in the intermediate-risk category. These differences in proportions, however, were not statistically significant (Freeman-Halton extension Fisher’s exact test, $p = 0.707$).

4.12.2 Validation Cohort

Twenty-nine patients with classical PTCs were allocated into the different ATA risk categories: nine (31.0%) presented with low-risk for disease recurrence, nineteen (65.5%) with intermediate-risk, and one (3.4%) with high-risk; the latter was a $BRAF^{V600E}$ mutant. Because only one patient was allocated to the high-risk category, the analysis was performed combining the intermediate and high-risk ATA categories. Proportionally more patients with $BRAF^{V600E}$-mutated tumors (6/22, 72.7% vs. 4/7, 57.1% patients with PTCs lacking the mutation) were stratified into the intermediate/high-risk category but this was not significant (Fisher’s exact test, $p = 0.642$).
4.13 The \( \text{BRAF}^{V600E} \) Signature is not Associated with Structural Recurrences in Classical Architecture Papillary Thyroid Carcinoma

4.13.1 Discovery Cohort

Three of forty-nine (6.1\%) patients with classical PTCs went on to develop structural recurrences. The proportion of \( \text{BRAF}^{V600E} \)-mutated tumors (1/31, 3.2\%) that gave rise to structural disease was comparable with the proportion of recurrent PTCs lacking this genotype (2/18, 10.0\%; Fisher’s exact test, \( p = 0.546 \)).

4.13.2 Validation Cohort

Three of twenty-seven (11.1\%) patients with classical PTCs developed structural recurrences. While \( \text{BRAF}^{V600E} \)-harboring PTCs recurred more frequently (2/30, 15\%) that those with a WT genotype for this mutation (0/7), this was not statistically significant (Fisher’s exact test, \( p = 0.545 \)).

4.14 The \( \text{RAS} \) Signature is not Associated with High-Risk Clinicopathological Features

4.14.1 Discovery Cohort

There was no association between \( \text{RAS} \) mutational status and angioinvasion (Fisher’s exact test = 0.687), ETE (Fisher’s exact test = 0.352), grouped TNM stages (Fisher’s exact test = 0.395), grouped MACIS categories (Fisher’s exact test = 1.000), or grouped ATA risk categories (Fisher \%) \( [\chi^2 (1) = 3.405; p = 0.077] \) (Table 5). Conversely, \( \text{RAS} \)-mutated carcinomas less
frequently harbored cN1 (0/16) compared to their *RAS* WT counterparts (22/96, 22.9%; Fisher’s exact test, *p* = 0.038).

<table>
<thead>
<tr>
<th>Table 5. Association of <em>RAS</em> mutational status and clinicopathological characteristics in the discovery cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Angioinvasion&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Extrathyroidal extension</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>TNM stages</td>
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<tr>
<td>I/II</td>
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<tr>
<td>III/IV</td>
</tr>
<tr>
<td>MACIS categories&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>I/II</td>
</tr>
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<td>III/IV</td>
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<tr>
<td>ATA risk categories&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Intermediate/High</td>
</tr>
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</table>

WT; wild-type  
<sup>a</sup> *χ²* or Fisher’s exact test  
<sup>b</sup> Three cases excluded  
<sup>c</sup> One case excluded
4.14.2 Validation Cohort

As shown in Table 6, there was no association between RAS mutational status and angioinvasion (Fisher’s exact test, \( p = 1.000 \)), ETE (Fisher’s exact test, \( p = 1.000 \)), cN1 (Fisher’s exact test, \( p = 0.192 \)) grouped TNM stages (Fisher’s exact test = 0.719), grouped MACIS categories (Fisher’s exact test, \( p = 0.672 \)), or grouped ATA risk categories [\( \chi^2 (1) = 1.029; p = 0.310 \)].

<table>
<thead>
<tr>
<th>Table 6. Association of RAS mutational status and clinicopathological characteristics in the validation cohort</th>
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<tr>
<td><strong>RAS</strong> <strong>mutant (%)</strong></td>
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</tr>
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</tr>
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</tr>
<tr>
<td><strong>Clinical lymph node metastasis</strong></td>
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<td><strong>TNM stages</strong></td>
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<tr>
<td>I/II</td>
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<tr>
<td>III/IV</td>
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Table 6. Continuation

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<thead>
<tr>
<th>MACIS categories</th>
<th>$RAS$ mutant (%) n=14</th>
<th>$RAS$ WT (%) n=53</th>
<th>$p$ value$^a$</th>
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<td>I/II</td>
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<td>III/IV</td>
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<td>8 (15.1)</td>
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<table>
<thead>
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<th>ATA risk categories</th>
<th>$RAS$ mutant (%) n=14</th>
<th>$RAS$ WT (%) n=53</th>
<th>$p$ value$^a$</th>
</tr>
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<tr>
<td>Low</td>
<td>9 (64.3)</td>
<td>26 (49.1)</td>
<td>0.310</td>
</tr>
<tr>
<td>Intermediate/High</td>
<td>5 (35.7)</td>
<td>27 (50.9)</td>
<td></td>
</tr>
</tbody>
</table>

WT; wild-type  
$^a$ $\chi^2$ or Fisher’s exact test  
b. Ten cases excluded  
c. Five cases excluded

4.15 Characteristics of Papillary Thyroid Carcinomas that Gave Rise to Distant Metastasis

4.15.1 Discovery Cohort

Two patients in the discovery cohort had distant metastatic disease. One of these metastatic PTCs arose from a $BRAF$ WT classical PTC while the other one originated from a FVPTC that lacked mutations. Both of these were angioinvasive, showed ETE, and were associated with bulky cN1 (2.5-4 cm) at the time of diagnosis.

4.15.2 Validation Cohort

In this cohort, two patients with FVPTCs developed distant metastases. One of them had an angioinvasive FVPTC with focal dedifferentiation while the other patient had large (4.2 and 3.9 cm) multifocal noninvasive tumors. The patient with an angioinvasive FVPTC presented
with symptomatic bony metastasis and had a primary tumor with an $NRAS^{Q61R}$ mutation. The patient with large primaries was diagnosed with a sacral metastasis after initial radioactive iodine treatment; his tumor harbored no driver mutation.
4.16 Patient Characteristics

4.17 Discussion

This study was aimed to determine whether the application of strict morphologic criteria to classify classical and FVPTCs is associated with distinct molecular signatures and whether these two approaches to the categorization of PTC correlate with clinical behavior. To achieve these objectives we relied on well-established morphologic and molecular bodies of data. This allowed us to quickly focus on whether the detection of any true fibrovascular core containing papillae in a PTC would select BRAF\textsuperscript{V600E}-mutated carcinomas while an exclusive follicular architecture would segregate more closely with RAS mutations. In addition, I aimed to determine whether there was an association between tumor architecture, molecular signature, and selected clinicopathological features (extrathyroidal extension, angioinvasion, clinical lymph node metastasis, TNM stage, MACIS score, ATA risk categories, and structural recurrences). In order to answer this question we retrospectively reviewed two separate cohorts of patients with PTC diagnosed by expert thyroid pathologists who require an exclusive follicular architecture to render a FVPTC diagnosis, i.e., they classify WDTCs with any papillae as classical PTCs in the absence of special cytomorphology other than oncocytic change or solid growth exceeding 30% of the tumor volume. We initially tested our hypothesis in a discovery cohort that was comprised of 116 patients with tumor samples analyzed on a MassARRAY platform. Subsequently, I proceeded to support our findings with another 67 patients (validation cohort) whose tumor
samples had been submitted to WES through the TCGA Research Network (Cancer Genome Atlas Research 2014).

**The $BRAF^{V600E}$ Signature and Papillary Thyroid Carcinoma Architecture**

The foremost finding in the current study was that the $BRAF^{V600E}$ signature, considered by many groups to be associated with more aggressive clinical behavior, segregated almost exclusively with PTCs displaying any amount of classical architecture. In fact, as shown in Table 7 only 1/100 (0.01%) FVPTCs (0/62 in the discovery cohort and 1/38 in the validation cohort) harbored a $BRAF^{V600E}$ mutation. In contrast, 56/83 (67.4%) classical PTCs (34/54, discovery cohort; 22/29, validation cohort) were $BRAF^{V600E}$-mutated. Because no $BRAF^{V600E}$ mutations were noted within FVPTCs that belonged to the discovery cohort, one single $BRAF^{V600E}$-harboring FVPTC in the validation cohort was re-examined after completion of the study. We discovered that due to a clerical error the submitted tissue for molecular analysis was actually a classical PTC originating from a thyroidectomy specimen containing two distinct foci of PTC with a dominant FVPTC and second dominant classical PTC. Whether or not this case is taken into consideration, our results are in line with the report by Jakubowski and Hunt (Jakubowski and Hunt 2009) who found that PTCs with mixed follicular and papillary architecture that carry a $BRAF^{V600E}$ mutation harbor this molecular signature in both the areas of classical and follicular growth. This is also consistent with the report by Trovisco *et al.* (Trovisco, Soares *et al.* 2005) who noted that none of their 71 FVPTCs with or without oncocytoic cytological changes were $BRAF^{V600E}$ mutants (Table 7) while 39/97 (46.4%) classical PTCs with or without oncocytoic changes harbored the mutation. Furthermore, it is in keeping with a recent report by Onder *et al.* where all fifteen pediatric FVPTC cases with 100% follicular architecture lacked the $BRAF^{V600E}$ mutation (Table 7), while 13/24 (54.2%) classical PTCs harbored the $BRAF^{V600E}$ mutation.
Conversely, our finding contrasts with the 17.4% to 62.5% $BRAF^{V600E}$ mutant frequency reported in FVPTCs in other studies (Table 7). Of note, nearly all of these latter studies did not clearly define the percentage of follicular architecture required to make a FVPTC diagnosis or adhered to the WHO classification, which requires virtually no papillary structures, i.e., an almost exclusive follicular architecture (Fugazzola, Puxeddu et al. 2006, Eloy, Santos et al. 2011, Jung, Im et al. 2012, Nam, Jung et al. 2012, Park, Kim et al. 2013, Rossi, Martini et al. 2015). Additionally, some of these studies used percentages of follicular architecture that ranged from >50% to approximately >95% (Lee, Jung et al. 2013, Walts, Mirocha et al. 2015), or in one case the authors claimed to have required an exclusive follicular architecture (Min, Lee et al. 2013) (Table 7). Our results also differ from the outcomes obtained by pathologists outside of the UHN who, as part of the TCGA Research Network, were asked to classify tumors into FVPTCs if they contained 99% follicular architecture (Cancer Genome Atlas Research 2014). Specifically, in this large PTC cohort, the proportion of $BRAF^{V600E}$-mutated tumors within the FVPTC category was significantly higher when the diagnosis was made at other institutions (11/45, 24.4%) compared to the UHN (1/38, 2.6%) [$\chi^2 (1) = 7.926; p = 0.005$] (Cancer Genome Atlas Research 2014) (Supplemental Table 2, 402 WES cases). Indeed, the odds of discovering a FVPTC with a $BRAF^{V600E}$ mutation were twelve times higher if the tumor samples were reviewed by other pathologists than if they were examined by UHN pathologists who used exclusion of any true papillae for the diagnosis of FVPTC (95% CI: 1.47-97). It is important to mention that in the TCGA Research Network’s study PTC samples from both the UHN and other institutions were uniformly submitted to WES, which implicates that the method used for mutational analysis does not account for the different results. Furthermore, we observed similar results in our discovery and validation cohorts albeit the different methods used for mutational analysis (Sequenom mass array and WES respectively).
Together, these data suggest that the method used for \(BRADF^{V600E}\) mutation ascertainment does not account for the discordance between our results and what other groups have published.

It is of noteworthy that the vast majority of FVPTCs in our study were invasive or lacked a complete capsule (52/59 discovery cohort; 31/37, validation cohort). Therefore, the absence of \(BRADF^{V600E}\)-mutated FVPTCs cannot be explained by a biased inclusion of less histologically aggressive tumors. Instead, it is most likely attributed to the morphologic criteria used to diagnose FVPTC. Specifically, while other groups that permit less than exclusive follicular architecture to render a FVPTC diagnosis have reported that 25.0-46.2% of infiltrative FVPTCs harbor \(BRADF^{V600E}\) mutations (Rivera, Ricarte-Filho et al. 2010, Eloy, Santos et al. 2011, Lee, Jung et al. 2013, Walts, Mirocha et al. 2015), all sixty-four invasive FVPTCs in our study (43 in the discovery cohort and 21 in the validation cohort) lacked this genotype. One exception is the study by Min et al. (Min, Lee et al. 2013) who found that 47.6% of 58 predominantly invasive FVPTCs with exclusive follicular architecture harbored \(BRADF^{V600E}\) mutations (Table 7). It is unclear why this Korean study yielded remarkably different results from our findings. Although a particularly high prevalence of \(BRADF^{V600E}\) mutations in this ethnic group has been reported (Ahn, Park et al. 2012, Jung, Im et al. 2012, Nam, Jung et al. 2012), other Korean studies (Lee, Jung et al. 2013, Park, Kim et al. 2013) have not shown such high percentages of \(BRADF^{V600E}\) mutations within the FVPTC category (Table 7).

With respect to completely encapsulated FVPTCs, we found that all noninvasive tumors also lacked \(BRADF^{V600E}\) mutations (0/9 discovery cohort; 0/5 validation cohort). These findings are concordant with those of Rivera et al. (Rivera, Ricarte-Filho et al. 2010), Howitt et al. (Howitt, Jia et al. 2013), and Nikiforov et al. (Nikiforov, Seethala et al. 2016) who found no
**BRAF**\(^{V600E}\) mutations in encapsulated or well-demarcated noninvasive FVPTCs with >99% follicular architecture.

<table>
<thead>
<tr>
<th>First author, year, reference</th>
<th>Country</th>
<th>Component of follicular architecture required to render a FVPTC diagnosis</th>
<th>Method used for mutational analysis of the <strong>BRAF</strong> gene</th>
<th>Number of FVPTCs (% <strong>BRAF</strong>(^{V600E}) mutants)</th>
<th><strong>BRAF</strong>(^{V600E}) mutants within the infiltrative or nonencapsulated FVPTC category</th>
<th><strong>BRAF</strong>(^{V600E}) mutants within the encapsulated or well-demarcated FVPTC category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trovisco et al., 2005 (Trovisco, Soares et al. 2005)</td>
<td>Portugal, Spain, Brazil, Russia</td>
<td>Not specified(^d)</td>
<td>SSCP; PCR and direct sequencing when a case presented aberrant band in SSCP analysis</td>
<td>0/71 (0%)(^e)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Di Cristofaro et al., 2006 (Di Cristofaro, Marcy et al. 2006)</td>
<td>France</td>
<td>&gt;99%</td>
<td>PCR and direct sequencing</td>
<td>13 (7.7%)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Fugazzola et al., 2006 (Fugazzola, Puxeddu et al. 2006)</td>
<td>Italy</td>
<td>Not specified(^d)</td>
<td>SSCP or by PCR and direct sequencing</td>
<td>51 (17.6%)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Rivera et al., 2010 (Rivera, Ricarte-Filho et al. 2010)</td>
<td>USA</td>
<td>Up to 99%(^e)</td>
<td>Sequenom Mass array</td>
<td>47(^f) (10.6%)</td>
<td>5/19 (26.3%)</td>
<td>0/28 (0%)</td>
</tr>
<tr>
<td>Eloy et al., 2011 (Eloy, Santos et al. 2011)</td>
<td>Portugal</td>
<td>Not specified(^d)</td>
<td>PCR and direct sequencing</td>
<td>29(^h) (17.2%)</td>
<td>4/16 (25.0%)</td>
<td>1/12 (8.3%)</td>
</tr>
<tr>
<td>Nam et al., 2012, (Nam, Jung et al. 2012)</td>
<td>Korea</td>
<td>Not specified(^d)</td>
<td>PCR and direct sequencing</td>
<td>32 (62.5%)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Jung et al., 2013 (Jung, Im et al. 2012)</td>
<td>Korea</td>
<td>Not specified(^d)</td>
<td>PCR and direct sequencing</td>
<td>24 (45.8%)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>First author, year, reference</td>
<td>Country</td>
<td>Component of follicular architecture required to render a FVPTC diagnosis</td>
<td>Method used for mutational analysis of the BRAF gene</td>
<td>Number of FVPTCs (% BRAF&lt;sup&gt;V600E&lt;/sup&gt; mutants)</td>
<td>BRAF&lt;sup&gt;V600E&lt;/sup&gt; mutants within the infiltrative or nonencapsulated FVPTC category</td>
<td>BRAF&lt;sup&gt;V600E&lt;/sup&gt; mutants within the encapsulated or well-demarcated FVPTC category&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Howitt et al., 2013 (Howitt, Jia et al. 2013)</td>
<td>USA</td>
<td>Exclusive or 99%</td>
<td>Sequenom Mass array</td>
<td>28 (0%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>None</td>
<td>0/28&lt;sup&gt;b&lt;/sup&gt;(0%)</td>
</tr>
<tr>
<td>Park et al., 2013 (Park, Kim et al. 2013)</td>
<td>Korea</td>
<td>Not specified&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Pyrosequencing</td>
<td>132 (32.6%)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Min et al., 2013 (Min, Lee et al. 2013)</td>
<td>Korea</td>
<td>100%</td>
<td>PCR and direct sequencing</td>
<td>58 (47.6%)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Not reported</td>
<td>2/7 (28.5%)</td>
</tr>
<tr>
<td>Lee et al., 2013 (Lee, Jung et al. 2013)</td>
<td>Korea</td>
<td>&gt;50%&lt;sup&gt;j&lt;/sup&gt;</td>
<td>PCR and direct sequencing</td>
<td>58 (24.1%)</td>
<td>7/28 (25.0%)</td>
<td>7/30 (23.3%)</td>
</tr>
<tr>
<td>Fernandez et al., 2013 (Fernandez, Piccin et al. 2013)</td>
<td>Italy</td>
<td>Not defined</td>
<td>PCR and direct sequencing</td>
<td>94 (31.9%)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Rossi et al., 2015 (Rossi, Martini et al. 2015)</td>
<td>Italy</td>
<td>Not defined</td>
<td>PCR and direct sequencing</td>
<td>46 (17.4%)</td>
<td>6/15 (40.0%)</td>
<td>2/31 (6.5%)</td>
</tr>
<tr>
<td>Xing et al., 2015 (Xing, Alzahrani et al. 2015)</td>
<td>USA, Korea, Italy, Japan, Poland, Australia, Spain, Czech Republic</td>
<td>Varied amongst the medical centers that participated in this multicenter study</td>
<td>PCR and direct sequencing, SSCP, fluorescence melting curve analysis</td>
<td>431 (20.6%)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>First author, year, reference</td>
<td>Country</td>
<td>Component of follicular architecture required to render a FVPTC diagnosis</td>
<td>Method used for mutational analysis of the (BRAF) gene</td>
<td>Number of FVPTCs (% (BRAF^{V600E}) mutants)</td>
<td>(BRAF^{V600E}) mutants within the infiltrative or nonencapsulated FVPTC category</td>
<td>(BRAF^{V600E}) mutants within the encapsulated or well-demarcated FVPTC category</td>
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<tr>
<td>Walts et al., 2015 (Walts, Mirocha et al. 2015)</td>
<td>USA</td>
<td>&gt;95%</td>
<td>PCR and direct sequencing(^k)</td>
<td>47 (34.0%)</td>
<td>12/26 (46.2%)</td>
<td>4/22 (19.0%) (^1)</td>
</tr>
<tr>
<td>Onder et al. 2016 (Onder, Ozturk Sari et al. 2016)</td>
<td>Turkey</td>
<td>100%(^m)</td>
<td>Probe based quantitative PCR</td>
<td>15 (0%)</td>
<td>0/6 (0%)</td>
<td>0/9 (0%)</td>
</tr>
<tr>
<td>Nikiforov et al., 2016 (Nikiforov, Seethala et al. 2016)</td>
<td>USA, Italy</td>
<td>&gt;99%</td>
<td>ThyroSeq v.2 next generation sequencing</td>
<td>37(^n)</td>
<td>Not done</td>
<td>0/37(^o) (0%)</td>
</tr>
<tr>
<td>Current study—both cohorts (not published)</td>
<td>Canada</td>
<td>100%(^a)</td>
<td>Sequenom Mass array and whole-exome sequencing on the Illumina HiSeq 2000</td>
<td>100(^p) (0.01%)</td>
<td>1/52 (discovery)</td>
<td>0/9 (discovery)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/32 (validation)</td>
<td>0/5 (validation)</td>
</tr>
</tbody>
</table>

FVPTC, follicular variant papillary thyroid carcinoma; SSCP, Single-strand conformational polymorphism; USA, United States of America

\(^a\)This column represents encapsulated tumors unless specified otherwise

\(^b\)Authors used criteria proposed by LiVolsi (Livolsi 1990) and Rosai (Rosai, Carcangiu et al 1992)

\(^c\)Including oncocytic papillary thyroid carcinomas with follicular architecture

\(^d\)Authors used 2004 World Health Organization Classification of papillary carcinoma (DeLellis 2004) which describes FVPTCs as tumors composed of follicles with “virtually no papillary structures”

\(^e\)All FVPTCs in this study had \(\leq 1\%\) papillary formations

\(^f\)Encapsulated FVPTCs were totally surrounded by a fibrous capsule and infiltrative tumors were partially encapsulated or totally unencapsulated with invasion of normal thyroid tissue

\(^g\)Encapsulated FVPTCs were completely surrounded by a tumor capsule with our without signs of focal invasion whereas infiltrative tumors where classified as such if they displayed an infiltrative/poorly circumscribed growth pattern

\(^h\)These tumors entirely or partially lacked a tumor capsule and had a well-circumscribed edge with no invasion of the adjacent thyroid parenchyma

\(^i\)The total number of cases with \(BRAF^{V600E}\) mutations was not reported, instead a percentage was provided. Therefore, the exact number of infiltrative FVPTCs could not be calculated

\(^j\)In addition to \(>50\%\) follicular architecture, the authors required that these tumors completely lack well-formed papillae

\(^k\)In this study sequencing was performed on grossly tissue as opposed to the other studies where microdissected tissue was utilized

\(^l\)The authors used the term “well circumscribed” to define tumors that showed a sharply demarcated border with or without an identifiable capsule

\(^m\)This information was obtained through a personal communication with the senior author (O M) These tumors completely lacked true papillae

\(^n\)Only a subset of encapsulated FVPTCs (37/138) were submitted to molecular analysis; these tumors were completely encapsulated and noninvasive

\(^o\)Seven PTCs with follicular architecture (three from the discovery cohort and four from the validation cohort) had less than exclusive follicular architecture with a focal component of solid growth. All tumor completely lacked true papillae

\(^p\)These 100 samples include one case belonging to the discovery cohort where the pathology report did not indicate whether the tumor was invasive or whether it contained a capsule as well as one case in the validation cohort where the invasion status was reported as indeterminate

\(^q\)After completion of the study we discovered that this classical PTC was incorrectly labeled as FVPTC

\(^r\)These are invasive encapsulated tumors or partially encapsulated tumors

\(^s\)These are completely encapsulated noninvasive FVPTCs
Additionally, in the present study, none of the invasive encapsulated PTCs (17 in the discovery cohort and 15 in the validation cohort) harbored $BRAF^{V600E}$ mutations. In contrast, in other bodies of work where the encapsulated FVPTC category has included both noninvasive and invasive tumors, $BRAF^{V600E}$-mutated carcinomas have been found (Eloy, Santos et al. 2011, Min, Lee et al. 2013) (Table 7).

**Epidermal Growth Factor Receptor (EGFR) Mutation in Papillary Thyroid Carcinoma**

We found one $EGFR$ mutation ($EGFR^{S768I}$) in a classical PTC belonging to the discovery cohort. Although this pathogenic $EGFR^{S768I}$ variant has been previously described in non-small cell lung cancer (Improtta, Pettinato et al. 2016) it has not been reported in thyroid carcinoma (ClinVar 2016). Other mutations in $EGFR$ have been found in poorly differentiated and anaplastic thyroid carcinoma (Lee, Lee et al. 2007, Masago, Asato et al. 2009) as well as a few cases of classical and FVPTC (Masago, Asato et al. 2009). There is evidence suggesting that upregulation of EGFR may be a marker of aggressive PTC, and play a role in thyroid cancer dedifferentiation (Landriscina, Pannone et al. 2011, Fisher, Jani et al. 2013).

**RAS and Other Molecular Signatures of Follicular Variant Papillary Thyroid Carcinoma**

Consistent with their prevalence in PTCs with follicular architecture (Zhu, Gandhi et al. 2003, Castro, Rebocho et al. 2006, Di Cristofaro, Marcy et al. 2006, Eloy, Santos et al. 2011), we found that 30.0% of FVPTCs (30/100 total; 18/62, discovery cohort and 12/38, validation cohort) were $RAS$-mutated. This yielded a significant association between the $RAS$ signature and follicular architecture ($p < 0.001$, Cramer’s $V = 0.366$, discovery cohort; $p = 0.014$, Cramer’s $V = 0.301$, validation cohort). In contrast, only 3.6% of classical PTCs harbored a $RAS$ mutation (3/83 total; 1/54, discovery cohort and 2/29, validation cohort). The latter is not surprising as a
small percentage of classical PTCs with predominant follicular architecture harbor a RAS signature. Moreover, two cases with double RAS and $BRAF^{V600E}$ mutations that were excluded from this series displayed both follicular and classical growth.

Follicular variant PTCs also harbored relatively infrequent genetic alterations including the $BRAF^{K601E}$ mutation (1/62, discovery cohort; 1/38, validation cohort), mutations in $EIF1AX$ (three tumors in the validation cohort: $EIF1AX^{A113V}$, $EIF1AX^{X69R}$, $EIF1AX^{X86R}$) and the novel $STK11^{W332*}$ mutation (1/62, discovery cohort). In keeping with these results, the $BRAF^{K601E}$ mutation has been only described in FVPTCs (Trovisco, Soares et al. 2005, Jung, Im et al. 2012, Rossi, Martini et al. 2015). Further, $EIF1AX$ mutations were recently recognized as oncogenic drivers in follicular-patterned carcinomas through the TCGA Research Network’s molecular characterization of PTC (Cancer Genome Atlas Research 2014). Regarding $STK11$, also known as liver kinase B1 ($LKB1$), it is a widely expressed tumor suppressor kinase that when mutated in the germline gives rise to Peutz-Jeghers syndrome (Lizcano, Goransson et al. 2004) with eight cases in the English literature of individuals with this syndrome harboring PTC (Wei, LiVolsi et al. 2016). Additionally, this year, Wei et al. (Wei, LiVolsi et al. 2016) found two different $STK11$ mutations ($STK11^{R342G}$ and $STK11^{c.862+1_862+16delGTGGAGCCTCATCCC}$) in thyroid carcinomas from adults over 70 years old without Peutz-Jeghers syndrome. Although these tumors had coexisting somatic mutations, namely $TP53^{R342G}$ and $BRAF^{V600E}$, based on the known oncogenic role of the STK11 protein it was proposed that it may play a role in PTC oncogenesis (Wei, LiVolsi et al. 2016). In the present study, an $STK11^{W332*}$ mutation was found in a minimally invasive 4.5 cm oncocytic FVPTC of a 58-year-old female patient with no history of Peutz-Jeghers syndrome or radiation exposure and no other driver mutations.
Architecture and Clinicopathological Features

Classical PTCs were associated with higher risk clinicopathological features compared to their FVPTC counterparts. Specifically, patients with classical PTCs more frequently harbored cN1 (OR 16.5, 95% CI: 3.62-74.91, discovery cohort; OR 9.7, 95% CI: 2.67-35.14, validation cohort), tumors with ETE (OR 26.1, 95% CI: 1.48-460.03, discovery cohort), TNM stage IV disease (18.5% vs. 0%, p < 0.001, discovery cohort; 27.6% vs. 2.6%, p = 0.008, validation cohort), and an intermediate/high-risk ATA category (OR 13.6, 95% CI: 5.44-34.11, discovery cohort; OR 4.8, 95% CI: 1.70-13.70, validation cohort). These results are concordant with the large multinational study by Shi et al. (Shi, Liu et al. 2016) where patients with classical PTCs were shown to have a higher prevalence of carcinomas with ETE, lymph nodal metastasis (pN1), and TNM stage IV than those with FVPTCs. They are also in keeping with smaller studies where patients with classical PTCs more frequently harbored pN1 (Lam, Lo et al. 2005, Adeniran, Zhu et al. 2006) and tumors with ETE (Lang, Lo et al. 2006). Interestingly, in one study, although patients with classical PTCs harbored more pN1 than those with FVPTCs, the proportion of cN1 was comparable (Lang, Lo et al. 2006).

MACIS scores were similar between patients with classical PTCs and FVPTCs (p = 0.123, discovery cohort, p = 0.544, validation cohort). This may be partly explained by comparable average ages between patients with FVPTCs and classical PTCs in both the discovery (M=43.2 years vs. M=44.6; p = 0.555) and validation (M=47.8 years vs. M=50.3; p = 0.549) cohorts as well as a similar proportion of M1 (1.6% vs 1.9%, p=1.000, discovery cohort; 2.6% vs. 0%, p = 1.000, validation cohort) at the time of surgical pathology diagnosis (Appendix tables 1 & 2). Additionally, although incomplete resections (R1) were more common in patients with classical PTCs than in patients with FVPTCs (22.6% vs. 8.1%, p =0.028, discovery cohort;
24.1% vs. 2.7%, \( p = 0.18 \), validation cohort), FVPTCs were larger than classical PTCs in the discovery \( (Mdn = 2.2 \text{ cm vs. } Mdn = 2.0 \text{ cm;} \ p = 0.037) \) and validation cohorts \( (Mdn = 2.6 \text{ cm vs. } Mdn = 1.7 \text{ cm;} \ p = 0.018) \) (Appendix table 1, 2). These results differ from Lang et al.’s (Lang, Lo et al. 2006) study where patients with classical PTCs had higher MACIS scores than those with FVPTC.

The proportion of angioinvasive classical PTCs and angioinvasive FVPTCs was not significantly different in both the discovery \( (15.4\% \text{ vs. } 6.6\%,, \ p = 0.129) \) and validation cohorts \( (10.0\% \text{ vs. } 5.4\% \ p = 0.607) \). Concordantly, in the study by Mete et al. (Mete and Asa 2011) classical PTCs \( (n = 26) \) and FVPTCs \( (n = 20) \) were similarly represented in a group of angioinvasive PTCs \( [\chi^2 \text{ goodness of fit } (2) = 0.783, \ p = 0.376] \). Patients with classical PTCs and FVPTCs also had a statistically comparable proportion of structural recurrences in the discovery \( (6.1\% \text{ vs. } 0, \ p = 0.114) \) and validation cohorts \( (11.1\% \text{ vs. } 5.7\%, \ p = 0.645) \). This result differs from the study by Shi et al. (Shi, Liu et al. 2016) where patients with classical PTCs had a higher tumor recurrence rate \( (16.1\%) \) than those with FVPTCs \( (9.1\%) \). Shi et al.’s study, however, used a broad definition for tumor recurrence as they included both persistent disease and tumor marker positivity (Shi, Liu et al. 2016). It is noteworthy, that cN1 have been shown to be an independent risk factor for recurrence (Sugitani, Kasai et al. 2004, Bardet, Malville et al. 2008, Ito, Fukushima et al. 2009, Lee, Sung et al. 2016) and based on the fact that patients with classical PTCs in our study had a higher prevalence of cN1 one could hypothesize that a larger sample size might have allowed for the detection of a difference in recurrence rates between patients with classical and FVPTCs.
Predominant Molecular Signatures and Clinicopathological Features

Given that the \( Braf^{V600E} \) signature is the most common driver mutation in PTC (Cancer Genome Atlas Research 2014), a lot of research has been done aiming to identify its clinical implications. Although, there is discordance in the literature, meta-analyses support both the role of the \( Braf^{V600E} \) mutation as a marker of high-risk clinicopathological features including ETE, advanced TNM stages and pN1 as well as its association with classical architecture (Li, Lee et al. 2012, Tufano, Teixeira et al. 2012, Zhang, Liu et al. 2016). The \( Braf^{V600E} \) mutation has also been found to be associated with ATA risk categories (Nam, Jung et al. 2012, Fernandez, Piccin et al. 2013). Concordant with these findings, in the present body of work the \( Braf^{V600E} \) signature was associated with ETE (OR 10.4, 95% CI: 2.03-52.98, discovery cohort; 13.0% vs .0%, \( p = 0.037 \), validation cohort), cN1 (OR 6.1, 95% CI: 2.25-16.66, discovery cohort; OR 9.5, 95% CI: 2.76-32.60 validation cohort), TNM stage IV disease (OR 29.2, 95% CI: 3.52-241.51, discovery cohort), and the intermediate/high-risk ATA category (OR 5.9, 95% CI: 2.43-14.30, discovery cohort; OR 5.5, 95% CI: 1.79-16.79, validation cohort). In contrast, the \( Braf^{V600E} \) signature was not associated with higher MACIS scores in either cohort in agreement with previous reports (Nam, Jung et al. 2012, Niederer-Wust, Jochum et al. 2015). Tumors with \( Braf^{V600E} \) mutations were also not significantly overrepresented in cases of structural recurrence in the discovery (2.9% vs. 3.2%, \( p =1.000 \)) and validation (14.3% vs. 4.9%, \( p = 0.325 \)) cohorts. The latter finding contrasts data from the considerably larger study (n=2,099) by Xing et. al. (Xing, Alzahrani et al. 2015) which showed that the recurrence rate in \( Braf^{V600E} \)-positive tumors was higher compared to \( Braf^{V600E} \)-negative PTCs. Finally, while Elisei et al. (Elisei, Viola et al. 2012) showed that this mutation was an independent predictor of the risk of persistent/recurrent disease, Fernandez et al. found that this molecular signature was not associated with recurrence-free survival (Fernandez, Piccin et al. 2013).
In contrast with the \(BRAF^{V600E}\) signature, \(RAS\) mutations have been shown to not be associated with high-risk clinicopathological features (Eloy, Santos et al. 2011, Eloy, Santos et al. 2011, Medici, Kwong et al. 2015, Yip, Nikiforova et al. 2015). In fact, \(RAS\)-harboring PTCs are recognized as particularly indolent tumors, especially when they are noninvasive (Xing 2016). Concordantly, in this study, patients with \(RAS\)-mutated tumors and \(RAS\) WT PTCs did not differ significantly in terms of the prevalence of TNM stages III/IV (15.8\% vs. 26.8\%, \(p = 0.395\), discovery cohort; 14.3\% vs. 24.5\%, \(p = 0.395\), validation cohort), higher grouped ATA risk categories (21.1\% vs. 43.8\%, \(p = 0.077\). discovery cohort), presence of angioinvasion in the tumor (5.3\% vs. 11.7\%, \(p = 0.687\), discovery cohort; 7\% vs 7.1\%, \(p =1.000\), validation cohort), or PTCs with ETE (0\% vs 9.3\%, \(p = 0.352\), discovery cohort; 0\% vs. 5.7\%, \(p =1.000\), validation cohort). Moreover, in the discovery cohort, cN1 were less frequent in patients with \(RAS\)-mutated tumors (0\% vs. 22.9\%, \(p = 0.038\)).

**The \(BRAF^{V600E}\) Signature in Classical Papillary Thyroid Carcinomas and Clinicopathological Features**

In this study the \(BRAF^{V600E}\) signature was strongly associated with classical architecture in both the discovery \([\chi^2 (1) = 52.223, p < 0.001, \text{Cramer’s }V = 0.690]\) and validation \([\chi^2 (1) = 39.125, p <0.001, \text{Cramer’s }V = 0.764]\) cohorts. This finding is concordant with the other institutions involved in the TCGA Research Network’s “Integrated Genomic Characterization of Papillary Thyroid Carcinoma” \([\chi^2 (1) = 30.856, p < 0.001, \text{Cramer’s }V = 0.336]\) (Cancer Genome Atlas Research 2014) (Appendix table 3) and the large multicenter study by Xing et al. (Xing, Alzahrani et al. 2015) \([\chi^2 (1) = 167.667, p < 0.001, \text{Cramer’s }V = 0.299]\). This tight relationship between classical tumor architecture and its predominant molecular signature may partly explain why other investigators (Xing, Westra et al. 2005, Rivera, Ricarte-Filho et al. 2010, Cheng, Serra
et al. 2011) have found that although the $BRAF^{V600E}$ mutation correlates with high-risk clinicopathological features in PTC in general, this relationship is lost when limiting the analysis to classical PTC cases. In a recent Korean study restricted to classical PTCs ($BRAF^{V600E}$ prevalence = 79.4%, 85/107) this molecular signature did not correlate with high-risk clinicopathological features (Ahn, Park et al. 2012). In this same ethnic group, classical papillary microcarcinomas with $BRAF^{V600E}$ mutations also did not show higher risk clinicopathological features than classical papillary microcarcinomas lacking this genotype (Kim, Kim et al. 2005). Furthermore, Lin et al. reported that in a Taiwanese cohort of 78 patients with classical PTCs, the $BRAF^{V600E}$ mutation (present in 73% of cases) was not associated with pN1, ETE, or advanced TNM stage (Lin, Fu et al. 2016). In keeping with the previous data, I found that the $BRAF^{V600E}$ mutation within the classical PTC subgroup was not associated with cN1 (41.2% vs 30.0%, $p = 0.411$, discovery cohort; 60.0% vs 42.9%, $p = 0.662$ validation cohort), ETE (20.6% vs 10.0%, $p = 0.458$, discovery cohort; 13.6% vs 0%, $p = 0.557$, validation cohort) or angioinvasion (9.1% vs 26.3%, $p = 0.124$ discovery cohort, 14.3% vs 0%, $p = 1.000$, validation cohort). The $BRAF^{V600E}$ signature also lacked association with TNM stages ($p = 0.168$, discovery cohort; $p = 0.835$, validation cohort) or ATA risk categories ($p = 0.707$, discovery cohort; $p = 0.642$, validation cohort). Furthermore, this mutation was not associated with structural recurrences in classical PTCs belonging to the discovery (3.2% vs. 10% $BRAF^{V600E}$-negative tumors, $p = 0.546$) or validation cohort (15.0% vs 0% $BRAF^{V600E}$-negative tumors, $p = 0.545$). This result is consistent with Kim et. al.'s (Kim, Kim et al. 2005) study where the $BRAF^{V600E}$ mutation failed to independently predict the risk of recurrence in classical PTCs. It is also in keeping with the report by Onder et. al. (Onder, Ozturk Sari et al. 2016) who found that the $BRAF^{V600E}$ signature was not associated with a lower disease-free survival in pediatric classical PTC cases. However, it differs from the large multicenter study by Xing et al. where the
BRAF\textsuperscript{V600E} mutation was significantly associated with recurrences in classical PTC (Xing, Alzahrani et al. 2015).

Papillary Thyroid Carcinomas that Gave Rise to Distant Metastasis

Although only three (1.6%) patients had distant metastasis at the time of surgical pathology diagnosis, it is important to recognize that all tumors that gave rise to distant metastasis shared histopathological characteristics that are known to be associated with a more aggressive clinical course. Namely, these PTCs were angioinvasive, two of them invaded extrathyroidal tissues, and two were associated with bulky lymph nodal metastasis of up to 4 cm. It is noteworthy, that in our series these high-risk clinicopathological features had a relatively low prevalence, which implies that in this study they were of particular clinical relevance. For example, only 10.6% of PTCs (12/113) in the discovery cohort and 7.0% of PTCs (4/57) in the validation cohort were angioinvasive. This means that 16.7% (2/12) of patients with angioinvasive PTCs in the discovery cohort and 25% (1/4) of patients with angioinvasive PTCs in the validation cohort presented with distant metastatic disease. Similarly, ETE was present in only 7.8% (9/116) of cases in the discovery cohort but 22.2% (2/9) of carcinomas that invaded extrathyroidal tissues gave rise to distant metastasis.

Limitations

One of this study’s major limitations is inherent to its design as a retrospective analysis. Additionally, the retrospective review of the samples did not involve quantification of the papillary architecture component of each PTC. Another model to address my hypothesis would now be a prospective study. Here at the UHN the main advantage of this type of study design would be the unbiased inclusion of all sequential cases of PTC. Notwithstanding the above
mentioned limitation, the sharp contrast between the prevalence of the \(BRAF^{V600E}\) mutation within the FVPTC category in this study (1/100, 0.01%) and previous reports including the large multicenter study by Xing et al. (Xing, Alzahrani et al. 2015) (89/431, 20.6%) supports the concept that the authentic identification of any amount of true papillae in PTC is a strong morphologic predictor of the \(BRAF^{V600E}\) mutation. One might argue that these data reflect the expertise of pathologists from the study centre. However, it appears that it is the lack of true papillae that is paramount for an accurate and clinically relevant variant designation in PTCs since another center using the same criteria reached identical results (Onder, Oztürk Sari et al. 2016).

Secondly, this study was based on the morphology and molecular signature of each patient’s largest tumor and did not account for multifocality, which was present in 56.9% (66/116) of cases in the validation cohort and 71.6% (48/67) in the validation cohort. Therefore, it is possible that in cases where the patient had coexistent FVPTC and classical PTC, the measured outcomes of interest might have been attributed to the incorrect tumor architecture. One might hypothesize, based on previous literature, that the more likely error would have been to attribute lymph nodal metastasis (and hence potentially also more advanced TNM stages and ATA risk categories) to a dominant FVPTC when in fact coexisting classical PTCs could have been the culprit.

Thirdly, the prevalence of FVPTC in this study was approximately 50%, which is higher than the commonly reported prevalence of <30% (Mao and Xing 2016). This is interesting given one would have predicted that our rigid definition of FVPTC (complete lack of papillae and exclusive follicular architecture) would have resulted in a higher prevalence of classical PTC. The potential explanations for this unusually high proportion of FVPTCs include: selection bias,
referral bias, a different epidemiology of PTC in the studied population, or a lower threshold to diagnose FVPTC. For instance, although approximately 400 thyroid carcinoma cases are diagnosed each year at the UHN only samples where patient consent and sufficient tissue available at the UHN biobank were included in the discovery and validation cohorts. This implies that the high prevalence of FVPTCs in this study may not represent the true prevalence of these tumors at the UHN. However, it should be mentioned that although rare, the high proportion of FVTCPs observed in the present body of work is not unique. For example, in the University of Pittsburg, the proportion of FVPTC has significantly increased over the last four decades; while 25.5% of PTC cases harbored follicular architecture in the year 2000, nine years later 56.6% of all carcinomas had a follicular pattern of growth (Jung, Little et al. 2014).

Fourthly, the author was unblinded to both the morphology and molecular results permitting the biased re-examination of surgical pathology samples once the study was finalized. Namely, to better understand why a minority of cases showed a molecular signature that contrasted the predominant findings (i.e. classical PTCs with a RAS signature and a FVPTC with a BrafV600E signature), these few cases were re-examined by pathologists to look for potential distinctive morphologic features. Instead, we found two clerical errors where the wrong tumor from multifocal PTC cases was sent for analysis. In the validation cohort a “FVPTC” shown to have a BrafV600E mutation was actually a classical PTC that originated from a thyroidectomy specimen with multifocal disease consisting of a dominant FVPTC and a second dominant classical PTC. In addition, from this same cohort, we identified that a classical PTC sample was sent for analysis as a FVPTC; this tumor harbored a RAS mutation. Since only a few tumors were re-reviewed, one cannot exclude the possibility that other clerical errors were made. However, since our study’s conclusions are concordant with previously published reports (Table 7), it is unlikely that other potential clerical errors would have significantly changed our findings.
An additional limitation of this study is the potential for a type 2 error. This body of work aimed to identify associations between PTC architecture or mutational profile and clinicopathological features of a limited group of cases. However, it was not designed to detect a statistically significant difference in the proportion of recurrences or grouped MACIS categories in patients with PTCs harboring different architecture or mutational profile. It was also not devised to detect a statistically significant difference in the proportion of adverse clinicopathological features between classical PTCs with or without the \(BRAF^{V600E}\) mutation. Therefore, the lack of statistical significance that was obtained in the above-mentioned scenarios may potentially be attributable to a small sample size. Although it is tempting to perform post-hoc power analysis, this practice has been discouraged and instead when considered appropriate one could use confidence intervals to assess whether “clinically significant” values exist in the range of statistically plausible values (Goodman and Berlin 1994, Levine and Ensom 2001).

Future studies would be required to determine whether the utilization of strict criteria to classify PTC identifies classical tumors as a group at higher risk of structural recurrences.

Lastly, since large studies have shown that classical PTCs and those harboring the \(BRAF^{V600E}\) signature have a higher risk of persistent/recurrent disease, the low prevalence of structural recurrences in this study might be partly responsible for our negative findings. Although we cannot determine the reasons for a low recurrence rate potential explanations include a follow-up time of <10 years (when the majority of recurrences generally occur) in the discovery and validation cohorts as well as different surveillance and treatment strategies. Namely, patients in the discovery cohort frequently lacked periodic imaging of the neck and periodic measurements of both thyroglobulin and thyroglobulin antibodies while those in the validation cohort received proportionally less radioactive iodine treatment. Albeit this limitation, one important aspect to consider is that the large studies showing an association between the
BRAFV600E signature and persistent/recurrent disease did not evaluate structural recurrences only (Elisei, Viola et al. 2012, Xing, Alzahrani et al. 2015). This latter type of recurrence is generally of more clinical significance than the exclusive elevation in tumor markers. Also, regarding the BRAFV600E signature and its relationship with the risk of PTC recurrence, it is crucial to recognize that this mutation is tightly linked with high-risk clinicopathological features; therefore, it is difficult to determine the contribution to this risk that can be independently attributable to it. This point is illustrated by the fact that the BRAFV600E mutation has not consistently been shown to independently predict the risk of recurrence in multivariate analysis (Fernandez, Piccin et al. 2013), particularly when the analysis is restricted to classical PTCs (Kim, Kim et al. 2005).

Another important point is that BRAFV600E-harboring PTCs comprise at least four biologically relevant distinct molecular subtypes (Cancer Genome Atlas Research 2014). This heterogeneity provides a biological basis for the discordant results in clinical studies exploring the predictive value of the BRAFV600E mutation. It has been suggested that the BRAFV600E mutation, together with other molecular changes including the micro-RNA signature of the tumor (i.e. increased miR21 and decreased miR204 linked to more aggressive variants), TERT promoter mutations (associated with less differentiated more aggressive tumors), and somatic copy number alterations in chromosome 1qis are better predictors of clinical behavior than the BRAFV600E mutation alone (Cancer Genome Atlas Research 2014).

Strengths

One of the strengths of this study is that a thorough review of tumor samples was possible given that complete sampling is standard at the UHN. This potentially reduces the probability of not being able to detect the presence of scant papillae or invasive features. In that regard, it is interesting that other retrospective studies evaluating PTC architecture and its correlation with
molecular signatures often do not clearly state whether the slides that were reviewed were the product of complete sampling (Trovisco, Soares et al. 2005, Rivera, Ricarte-Filho et al. 2010, Howitt, Jia et al. 2013, Min, Lee et al. 2013, Rossi, Martini et al. 2015, Walts, Mirocha et al. 2015, Xing, Alzahrani et al. 2015).

Another strength of this study is that the expert endocrine pathologists who examined the PTC samples were blinded to the molecular profile of PTCs at the time of diagnosis. Furthermore, these pathologists use homogenous criteria to classify PTC and invasive growth (Mete and Asa 2016) reducing the probability of interobserver variability and presumably resulting in concordant results across the discovery and validation cohorts.

4.18 Conclusions

There has been much debate about the clinical implications of detecting the presence of the highly prevalent *BRAF*<sup>V600E</sup> mutation in PTC. While the *BRAF*<sup>V600E</sup> molecular signature is associated with more aggressive clinical behavior including a higher risk of recurrence (usually regional and that can be detected with ultrasound) in a subset of tumors, knowledge of the mutational status does not routinely change the initial management of this disease. Moreover, although PTC incidence rates have been increasing steadily, this has not been accompanied by a concomitant proportional increase in mortality rates. Since mutational analysis is not performed in the vast majority of countries around the world and pathologists are more likely to be available in some areas, more rigorous criteria to ensure appropriate PTC classification might be of more clinical value to a broader population. Conversely, in places where pathologists are not available, the molecular classification of PTC could provide clinically relevant information. The present body of work supports the TCGA Research Network’s proposal regarding a pathologic
classification of PTC that would more accurately reflect the tight relationship between PTC morphology and its molecular signatures. That is, a classification that distinguishes WDTC with a follicular growth pattern (RAS-like tumors) from classical PTC (BRAFV600E-like tumors) (Figure 20). This work also highlights the fact that the arbitrary <1% papillary growth that is allowed in the commonly used definition of FVPTC is unsubstantiated by molecular evidence. A definition of FVPTC that demands exclusive follicular architecture appears to be more biologically accurate. Furthermore, this study supports the concept that the authentic identification of classical PTC selects a more clinically aggressive group of tumors compared to FVPTCs (Figure 20). This latter point is of utmost importance because along with recent studies it suggests that the treatment of some follicular-patterned lesions should be different than classical PTCs.

**Figure 20.** Schema illustrating the distinction between follicular and classical papillary thyroid carcinomas based on the current study and “The Integrated Genomic Characterization of Papillary
Thyroid Carcinoma”. Our work (left) and The Cancer Genome Atlas Research Network’s study (right) (Cancer Genome Atlas Research 2014), which included all 67 tumor specimens from the University Health Network belonging to the validation cohort, showed that follicular variant papillary thyroid carcinomas (FVPTCs) are molecularly distinct from classical papillary thyroid carcinomas (PTCs).

4.19 Future Directions

The results presented in this thesis provide further support for a classification of PTC where tumors of an exclusive follicular pattern are separated from WDTCs with true papillae. It also substantiates the current approach to PTC classification at the UHN. As such, this work may serve as the basis for future studies as described below.

1. To assess whether the quantity of papillary growth in classical PTC impacts its clinicopathological features.

The percentage of papillary growth in a PTC is usually estimated on the basis of visual inspection, as such, it is likely to vary in between and within observers. Jakubowski and Hunt (Jakubowski and Hunt 2009) found that PTCs with at least 50% of follicular growth and a variable amount of papillary growth ranging from 10% to 50% often harbored the $BRAF^{V600E}$ mutation. However, there are no studies looking at the impact (clinical and molecular) of different percentages of papillary growth within a predominantly follicular-patterned tumor. Digital quantification of papillary growth in classical PTCs included in the present study would allow us to accurately determine the proportion of these tumors that harbor different degrees of papillary architecture including the subgroup with scant papillae (<1% papillary growth) (Figure 21). The data obtained from this assessment could further support the observation that PTCs classified with strict criteria are molecularly distinct. It would also expand our knowledge on the
features of $BRAF^{V600E}$-harboring PTCs with predominant follicular architecture. If indeed the percentage of papillary growth in PTC is clinically relevant and other groups validate our findings, this could potentially have an impact on how pathologists report classical PTC cases and also how tumors with different degrees of follicular architecture are treated.

**Digital quantification of papillary growth in classical papillary thyroid carcinomas from the discovery and validation cohorts**

- >50% papillary growth
- >1% to <50% papillary growth
- <1% papillary growth

**Comparison of clinicopathological features and molecular signatures between the groups**

**Figure 21.** Schema illustrating a potential study of the impact of papillary growth in classical papillary thyroid carcinomas with different degrees of follicular growth. Digital quantification of papillary growth in classical papillary thyroid carcinomas included in the present body of work would potentially allow us to better understand whether classical papillary thyroid carcinomas with predominant follicular architecture have different clinicopathological features.
2. To retrospectively analyze the clinical outcomes and molecular signatures of noninvasive FVPTCs (NIFTPs) and compare them to classical PTCs with scattered true papillae.

The recent proposal to change the nomenclature of encapsulated or well-demarcated noninvasive FVPTCs to NIFPT was prompted by physician’s overzealous treatment of this particularly indolent tumor (Nikiforov, Seethala et al. 2016). The authors of this proposal, which was based on the retrospective review of invasive and noninvasive FVPTCs, felt that a different name that excluded the word carcinoma would decrease overtreatment. Interestingly, one of the recommendations on the diagnostic criteria for this entity was that tumors could have up to 1% of papillae (Nikiforov, Seethala et al. 2016). However, the present body of work suggests that using this arbitrary cutoff could potentially result in classical PTCs with a \(BRAF^{V600E}\) mutation to be included into the NIFPT category. A retrospective study comparing the molecular signatures and clinicopathological features of NIFPTs with complete lack of papillae with noninvasive well-demarcated classical PTCs with up to 1% of papillae in the background of follicular architecture (these tumors would be called NIFPTs at other institutions) would allow us to understand whether the proposed morphologic criteria for NIFPT need to be revised. This study could follow proposed study number one, where we would digitally quantify papillary growth in classical PTC.

3. To compare the clinical outcomes and molecular signatures of noninvasive FVPTCs (NIFTPs) vs. invasive FVPTCs and classical PTCs by using rigid criteria to define each category.

In the present study FVPTCs were analyzed as a group and compared to classical PTCs. However, recent data has shown that NIFTPs are associated with different outcomes and molecular signatures compared to their invasive counterparts (invasive FVPTCs) (Xu and
Ghossein 2015). We could utilize the cases in the current study to compare the molecular signatures and clinicopathological features of invasive FVPTCs, NIFPTPs and classical PTCs. This retrospective analysis would allow us to determine the impact of excluding NIFPTs from the FVPTC category. We could also define whether \( RAS \) mutations and other less common variations are more frequently found in invasive vs. noninvasive FVPTCs. This proposed study could serve as a platform for a prospective clinical study examining whether more extensive surgery (e.g., extent of nodal dissection) may be advantageous for individuals with classical variant PTC as opposed to invasive FVPTCs.

4. To assess the impact of complete tumor sampling on the detection of scattered papillary structures in order to provide justification for the development of universal pathology practice guidelines.

At the UHN PTCs are submitted virtually entirely for microscopic examination (please refer to methods section). We hypothesize that the lack of recognition of true papillae as well as incomplete tumor sampling could be partly responsible for the misclassification of some PTC cases and hence the reported overlap between FVPTC and the \( BRAF^{V600E} \) mutation. Specifically, we propose that classical PTCs with predominant follicular growth may harbor the diagnostic papillary structures in a part of the tumor; i.e. in a few but not all tissue blocks. A retrospective review of classical PTC cases with predominant follicular architecture included in this study would allow us to determine whether the identification of scant papillae truly requires complete tumor sampling. That is, we would review all slides from tumors that were entirely examined and map the extent of papillae in each slide. If the diagnostic papillary growth is only identified in a few blocks but is absent in most sections this would provide support for complete tumor sampling. Subsequently, if we are able to show that complete sampling impacts the detection of
papillary growth, we would propose that other centers in Ontario adopt the practice and
determine whether it changes the prevalence of FVPTC and classical PTC diagnoses in their
institution. The outcome of this study could help in the establishment of evidence-based
recommendations related to tumor handling in pathology departments.

5. To retrospectively re-examine all multifocal PTCs in both cohorts.

In this study we did not account for tumor multifocality, which was present in more than
50% of cases. Re-examination of all multifocal PTCs would allow us to identify the authentic
tumor of origin giving rise to metastatic lymphadenopathy in cN1 or pN1 cases. We would also
be able to determine whether there were any additional clerical errors.
References


Canadian Cancer Society’s Advisory Committee on Cancer Statistics (2015). Canadian Cancer Statistics. [Toronto], [Canadian Cancer Society]: volumes.


Castagna, M. G., F. Maino, C. Cipri, V. Belardini, A. Theodoropoulou, G. Cevenini and F. Pacini (2011). "Delayed risk stratification, to include the response to initial treatment (surgery and radioiodine ablation), has better outcome predictivity in differentiated thyroid cancer patients." Eur J Endocrinol 165(3): 441-446.

PPARgamma rearrangement is frequently detected in the follicular variant of papillary thyroid carcinoma. J Clin Endocrinol Metab 91(1): 213-220.


ClinVar (2016) "NM_005228.3 (EGFR):c.2303G>T (p.Ser768Ile)."


Koo, J. S., S. Hong and C. S. Park (2009). "Diffuse sclerosing variant is a major subtype of papillary thyroid carcinoma in the young." Thyroid 19(11): 1225-1231.


"NTRK fusion oncogenes in pediatric papillary thyroid carcinoma in northeast United States." Cancer 122(7): 1097-1107.


Bethesda, Md., Armed Forces Institute of Pathology ; under the auspices of Universities Associated for Research and Education in Pathology.


SEER Program (1975-2013). Surveillance, Epidemiology, and End Results Program Stat Fact Sheets 1975-2013: Thyroid Cancer

Seethala, R. R. A., S.L.; Carty, S. E.; Hodak, S.P.; McHugh, J.B.; Shah, J.; Thompson, L.D.R; Nikiforov, Y.E. (2014) "Protocol for the Examination of Specimens From Patients With Carcinomas of the Thyroid Gland.".


Tanase, K., E. D. Thies, U. Mader, C. Reiners and F. A. Verburg (2015). "The TNM system (version 7) is the most accurate staging system for the prediction of loss of life expectancy in differentiated thyroid cancer." Clin Endocrinol (Oxf).


Vaisman, F., A. Shaha, S. Fish and R. Michael Tuttle (2011). "Initial therapy with either thyroid lobectomy or total thyroidectomy without radioactive iodine remnant ablation is associated with very low rates of structural disease recurrence in properly selected patients with differentiated thyroid cancer." Clin Endocrinol (Oxf) 75(1): 112-119.


## Appendices

### Appendix table 1. Papillary thyroid carcinoma architecture and its relationship with clinicopathological features

<table>
<thead>
<tr>
<th>Variables</th>
<th>Discovery cohort N=116</th>
<th>Validation cohort N=67</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follicular (n= 62)</td>
<td>Classical (n = 54)</td>
</tr>
<tr>
<td></td>
<td>*p value</td>
<td>*p value</td>
</tr>
<tr>
<td>Clinical lymph nodes&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
</tr>
<tr>
<td>Absent</td>
<td>96.6%</td>
<td>63.0%</td>
</tr>
<tr>
<td>Present</td>
<td>3.4%</td>
<td>37.0%</td>
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<tr>
<td>Extrathyroidal extension</td>
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<td></td>
</tr>
<tr>
<td>Absent</td>
<td>100%</td>
<td>83.3%</td>
</tr>
<tr>
<td>Present</td>
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<td>16.7%</td>
</tr>
<tr>
<td>Angioinvasion&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>93.4%</td>
<td>84.6%</td>
</tr>
<tr>
<td>Present</td>
<td>6.6%</td>
<td>15.4%</td>
</tr>
<tr>
<td>TNM Stages</td>
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<tr>
<td>I</td>
<td>71.0%</td>
<td>61.1%</td>
</tr>
<tr>
<td>II</td>
<td>12.9%</td>
<td>3.7%</td>
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<td>III</td>
<td>16.1%</td>
<td>16.7</td>
</tr>
<tr>
<td>IV</td>
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<td>18.5%</td>
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<tr>
<td>MACIS categories&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>96.2%</td>
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<tr>
<td>III and IV</td>
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<tr>
<td>ATA Risk Category (2009)&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Low</td>
<td>85.5%</td>
<td>31.2%</td>
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<tr>
<td>Intermediate and High</td>
<td>14.5%</td>
<td>69.8%</td>
</tr>
</tbody>
</table>

*Chi Square or Fisher’s exact test  
<sup>a</sup>n=112 in discovery cohort (58 FVPTCs); n=62 (35 FVPTCs) in validation cohort  
<sup>b</sup>n=113 in discovery cohort (57 FVPTCs); n=57 (37 FVPTCs) in validation cohort  
<sup>c</sup>n=115 in discovery cohort (53 classical PTCs)  
<sup>d</sup>n=113 in discovery cohort (51 classical PTCs)
## Appendix table 2. BRAF status and clinicopathological features of papillary thyroid carcinoma

<table>
<thead>
<tr>
<th>Variables</th>
<th>Discovery cohort N=116</th>
<th>Validation cohort N=67</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No BRAF&lt;sup&gt;V600E&lt;/sup&gt; (n= 82)</td>
<td>BRAF&lt;sup&gt;V600E&lt;/sup&gt; (n = 34)</td>
</tr>
<tr>
<td>Clinical lymph nodes&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>89.7%</td>
<td>58.8%</td>
</tr>
<tr>
<td>Present</td>
<td>10.3%</td>
<td>41.2%</td>
</tr>
<tr>
<td>Extrathyroidal extension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>97.6%</td>
<td>79.4%</td>
</tr>
<tr>
<td>Present</td>
<td>2.4%</td>
<td>20.6%</td>
</tr>
<tr>
<td>Angioinvasion&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>88.8%</td>
<td>90.9%</td>
</tr>
<tr>
<td>Present</td>
<td>11.2%</td>
<td>9.1%</td>
</tr>
<tr>
<td>TNM Stages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I and II</td>
<td>82.9%</td>
<td>55.9%</td>
</tr>
<tr>
<td>III and IV</td>
<td>17.1%</td>
<td>44.1%</td>
</tr>
<tr>
<td>MACIS categories&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I and II</td>
<td>98.8%</td>
<td>97.0%</td>
</tr>
<tr>
<td>III and IV</td>
<td>1.2%</td>
<td>3.0%</td>
</tr>
<tr>
<td>ATA Risk Category (2009)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>72.0%</td>
<td>30.3%</td>
</tr>
<tr>
<td>Intermediate and High</td>
<td>28.0%</td>
<td>69.7%</td>
</tr>
</tbody>
</table>

*Chi Square or Fisher’s exact test

<sup>a</sup>n=112 in discovery cohort (78 PTCs without the BRAF<sup>V600E</sup> mutation); n=62 in validation cohort (41 PTCs without the BRAF<sup>V600E</sup> mutation)

<sup>b</sup>n=113 in discovery cohort (80 PTCs without the BRAF<sup>V600E</sup> mutation); n=57 in validation cohort (37 PTCs without the BRAF<sup>V600E</sup> mutation)

<sup>c</sup>n=115 in discovery cohort (33 PTCs with the BRAF<sup>V600E</sup> mutation)

<sup>d</sup>n=113 in discovery cohort (31 PTCs with the BRAF<sup>V600E</sup> mutation)
Appendix table 3. *BRAF* status and clinicopathological features of classical papillary thyroid carcinoma

<table>
<thead>
<tr>
<th>Variables</th>
<th>Discovery cohort</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=54</td>
<td>N=29</td>
</tr>
<tr>
<td><strong>BRAF</strong> status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V600E (n=34)</td>
<td>*p value</td>
<td>V600E (n=22)</td>
</tr>
<tr>
<td>No<strong>BRAF</strong> V600E (n=20)</td>
<td></td>
<td>No<strong>BRAF</strong> V600E</td>
</tr>
<tr>
<td>Clinical lymph nodes(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>70.0%</td>
<td>58.8%</td>
</tr>
<tr>
<td>Present</td>
<td>30.0%</td>
<td>41.2%</td>
</tr>
<tr>
<td>Extrathyroidal extension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>90.0%</td>
<td>79.4%</td>
</tr>
<tr>
<td>Present</td>
<td>10.0%</td>
<td>20.6%</td>
</tr>
<tr>
<td>Angioinvasion(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>73.7%</td>
<td>90.9%</td>
</tr>
<tr>
<td>Present</td>
<td>26.3%</td>
<td>9.1%</td>
</tr>
<tr>
<td>TNM Stages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I and II</td>
<td>80.0%</td>
<td>55.9%</td>
</tr>
<tr>
<td>III and IV</td>
<td>20.0%</td>
<td>44.1%</td>
</tr>
<tr>
<td>MACIS categories(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I and II</td>
<td>95.0%</td>
<td>97.0%</td>
</tr>
<tr>
<td>III and IV</td>
<td>5.0%</td>
<td>3.0%</td>
</tr>
<tr>
<td>ATA Risk Category (2009)(^d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>30.0%</td>
<td>30.3%</td>
</tr>
<tr>
<td>Intermediate and High</td>
<td>70.0%</td>
<td>69.7%</td>
</tr>
</tbody>
</table>

\(^*\)Chi Square or Fisher’s exact test

\(^a\)n=27 in validation cohort (20 PTCs with the **BRAF**\(^V600E\) mutation)

\(^b\)n=52 in discovery cohort (33 PTCs with the **BRAF**\(^V600E\) mutation); n=20 in validation cohort (14 PTCs with the **BRAF**\(^V600E\) mutation)

\(^c\)n=53 in discovery cohort (33 PTCs with the **BRAF**\(^V600E\) mutation)

\(^d\)n=53 in discovery cohort (33 PTCs with the **BRAF**\(^V600E\) mutation)
Appendix table 4. Comparison of selected clinicopathological features between follicular variant papillary thyroid carcinoma cases and classical papillary thyroid carcinoma cases in the discovery cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>FVPTC (n=62)</th>
<th>Classical PTC (n=54)</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.2 (11.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.6 (13.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.555</td>
</tr>
<tr>
<td>Age ≥45</td>
<td>26 (41.9%)</td>
<td>26 (48.1%)</td>
<td>0.576</td>
</tr>
<tr>
<td>Male</td>
<td>7 (11.3%)</td>
<td>17 (31.5%)</td>
<td>0.007</td>
</tr>
<tr>
<td>Tumor size in cm</td>
<td>2.2 (0.8-7.0)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.0 (0.4-6.0)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.037</td>
</tr>
<tr>
<td>R1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5 (8.1%)</td>
<td>12 (22.6%)</td>
<td>0.028</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>1 (1.6%)</td>
<td>1 (1.9%)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

FVPTC, follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma; R1, complete macroscopic resection with positive microscopic margins

<sup>a</sup>χ², Fisher’s exact test, Student’s t, or Mann-Whitney U

<sup>b</sup>Expressed as mean with standard deviation in parenthesis

<sup>c</sup>Expressed as median with the range in parenthesis

<sup>d</sup>Margins could not be determined in one classical PTC; n=116
### Appendix table 5. Comparison of selected clinicopathological features between follicular thyroid carcinoma cases and classical papillary thyroid carcinoma cases in the validation cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>FVPTC</th>
<th>Classical PTC</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=38)</td>
<td>(n=29)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.8 (15.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.3 (17.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.549</td>
</tr>
<tr>
<td>Age ≥45</td>
<td>21 (55.3%)</td>
<td>18 (62.1%)</td>
<td>0.576</td>
</tr>
<tr>
<td>Male</td>
<td>7 (18.4%)</td>
<td>15 (51.7%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Tumor size in cm</td>
<td>2.6 (1.1-6.1)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.7 (1.0-6.6)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.014</td>
</tr>
<tr>
<td>R1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1 (2.7%)</td>
<td>7 (24.1%)</td>
<td>0.018</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>1 (2.6%)</td>
<td>0</td>
<td>1.000</td>
</tr>
</tbody>
</table>

FVPTC, follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma; R1, complete macroscopic resection with positive microscopic margins  
<sup>a</sup> χ², Fisher’s exact test, Student’s t, or Mann-Whitney U  
<sup>b</sup>Expressed as mean with standard deviation in parenthesis  
<sup>c</sup>Expressed as median with the range in parenthesis  
<sup>d</sup>Margin status could not be determined in one FVPTC case; n=66

### Appendix table 6. Association between the \(BRAF^{V600E}\) signature and tumor architecture in whole-exome sequencing cases from “The Integrated Genomic Characterization of Papillary Thyroid Carcinoma”<sup>a</sup>

<table>
<thead>
<tr>
<th>Variable</th>
<th>FVPTC</th>
<th>Classical PTC</th>
<th>p value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=45)</td>
<td>(n=229)</td>
<td></td>
</tr>
<tr>
<td>(BRAF^{V600E})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11 (24.4%)</td>
<td>157 (68.6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>34 (75.6%)</td>
<td>72 (31.45)</td>
<td></td>
</tr>
</tbody>
</table>

FVPTC, follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma  
<sup>a</sup>This information was obtained from The Thyroid Cancer Genome Atlas Research Network’s “Integrated Genomic Characterization of Papillary Thyroid Carcinoma” Supplemental table 2; University Health Network cases were excluded (Cancer Genome Atlas Research 2014)  
<sup>b</sup>χ²
Appendix table 7. Association between the \textit{BRAF}^{V600E} signature and tumor architecture in the large multicenter study by Xing \textit{et al.}^{a}

<table>
<thead>
<tr>
<th>Variable</th>
<th>FVPTC (n=431)</th>
<th>Classical PTC (n=1,448)</th>
<th>( p ) value\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAFV600E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>89 (20.6%)</td>
<td>813 (56.1%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>342 (79.4%)</td>
<td>635 (43.9%)</td>
<td></td>
</tr>
</tbody>
</table>

FVPTC, follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma

\textsuperscript{a}Xing \textit{et al.} (Xing, Alzahrani \textit{et al.} 2015) in “Association Between BRAFV600E Mutation and Recurrence of Papillary Thyroid Cancer”

\textsuperscript{b}\( \chi^2 \)
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