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The Utility of BRAF Testing in the Management of Papillary Thyroid Cancer

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ABSTRACT

Over the last decade, investigators have developed a clearer understanding of the genetic alterations underlying thyroid carcinogenesis. A number of biomarkers involved in the pathogenesis of differentiated thyroid cancer have undergone intensive study, not only for their role in tumorigenesis, but also for their potential utility as diagnostic and prognostic indicators and therapeutic targets. This review summarizes the current literature surrounding *BRAF* and its significance in thyroid cancer. Further, we discuss how molecular analysis can be integrated into management algorithms for thyroid nodules and papillary thyroid cancer. We also review what is known, to date, about the association of *BRAF* and papillary microcarcinoma as well as using targeted therapies for *BRAF* as adjuvant treatment for metastatic papillary thyroid cancer. *The Oncologist* 2010;15:1285–1293

INTRODUCTION

Papillary thyroid carcinoma (PTC) accounts for the majority of thyroid cancers (~85%) and generally carries an excellent prognosis, with a 10-year survival rate >90% [1]. However, a small cohort of PTC patients goes on to develop recurrent and/or metastatic cancer and ultimately succumb to the disease. Better methods of identifying and treating these patients are very much needed. Over the last decade, an improved understanding of the genetic basis underlying the development of thyroid cancer has evolved that will undoubtedly lead to necessary improvements in the management of PTC patients.

It has now been well-established that the development of PTC involves activation of the mitogen-activated protein kinase (MAPK) signaling pathway, which mediates cellular response to various growth signals. Derangements of this pathway play a central role in uncontrolled cell proliferation and faulty apoptosis. The *BRAF* oncogene is a strong activator of that pathway, and has been implicated in a number of human cancers, including malignant melanoma, colorectal carcinomas, and sarcomas [2]. *BRAF* is located on chromosome 7q24 and encodes a serine–threonine kinase. After activation by RAS, BRAF phosphorylation triggers a series of activation events along the MAPK cas-

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cade [3]. A *BRAF* point mutation at codon 600 results in a valine to glutamate (V600E) alteration, leading to constitutive MAPK pathway stimulation. The *BRAF* V600E mutation is the most common genetic alteration in PTC and has been reported to occur in up to 80% of papillary thyroid cancers [4], although most experts quote a prevalence of ~45% in PTCs [5]. Nikiforov nicely summarized the role of *BRAF* and other key genetic mutations and rearrangements in the pathogenesis of thyroid cancer [3].

Among the various histologic subtypes of PTC, *BRAF* V600E mutation is most commonly found in the conventional and tall-cell histologic variants (67%–68% and 80%–83%, respectively), and less commonly found in the follicular variant (12%–18%) of PTC [6, 7]. *BRAF* mutations may also occur in thyroid lymphomas and anaplastic and poorly differentiated thyroid cancers, but have not been identified in follicular or medullary carcinomas and have only very rarely been identified in benign hyperplastic nodules [8]. Approximately 95% of *BRAF* mutations involve V600E [5]; other *BRAF* mutations have also been identified in PTC, although they are much less common and are not associated with the same tumor phenotype. Chiosea and colleagues provide an excellent review of other rare *BRAF* mutations that have been reported in the literature [9]. For the remainder of this article, reference to *BRAF* is to the V600E mutation.

BRAF IN THE DIAGNOSIS OF PTC

Fine-needle aspiration biopsy (FNAB) is the gold standard for the evaluation of thyroid nodules because it is safe, quick, cost-effective, and accurate [10]. When malignant cells are seen on cytology, the decision to proceed to surgery is simple, and most experts agree on the extent of initial thyroidectomy (i.e., near-total or total thyroidectomy) [11]. However 10%–15% of thyroid nodule FNABs fall into the indeterminate category, which includes follicular and oncocytic neoplasms, follicular lesions of undetermined significance, and suspicious nodules, according to the most current categorization of thyroid nodules established by the Bethesda Criteria of the National Cancer Institute [12]. At present, diagnostic thyroidectomy is recommended to definitively exclude malignancy in patients with indeterminate lesions. Although the risk is extremely low in expert hands, thyroid surgery is not without a risk for complications and carries health care costs. Although FNAB is highly accurate and specific, another complementary diagnostic adjunct is needed to help reduce the need for diagnostic thyroidectomy and/or better define the extent of initial surgery. Molecular testing has risen to the forefront as an exciting focus of research in this area over the last decade.

A number of centers have recently evaluated *BRAF* mutation analysis in the preoperative setting. In a prospectively evaluated Italian cohort of patients with nodules deemed suspicious sonographically, 48 $BRAF^+$ nodules were identified after ultrasound-guided FNAB followed by direct DNA sequencing and restriction fragment length polymorphism analysis for the *BRAF* V600E mutation. Seven patients with benign cytology underwent thyroidectomy because their nodules harbored the mutation, and all seven had conventional PTC on final histology. The investigators reported that *BRAF* mutational analysis increased the sensitivity of cytology for PTC from 77% to 87% [13]. Jo and colleagues prospectively evaluated 101 thyroid nodules with ultrasound-guided FNAB (43 benign, 30 malignant, 24 indeterminate or suspicious, four nondiagnostic) and *BRAF* V600E mutational analysis using pyrosequencing. Thyroidectomy was performed in 54 patients with malignant/indeterminate nodules (22 malignant and seven indeterminate nodules were $BRAF^+$) and one patient with a nondiagnostic nodule that was $BRAF^+$. All $BRAF^+$ nodules, including the one nondiagnostic and seven indeterminate nodules, were PTC on final histopathology, yielding a sensitivity of 75% [14]. Xing et al. [15] prospectively evaluated 45 patients who had FNA either in the outpatient setting or in the operating room immediately prior to thyroidectomy; both direct DNA sequencing and a colorimetric gene detection method were used to carry out *BRAF* mutation analysis on the cytology specimens. The sensitivity, specificity, and negative predictive value (NPV) of FNAB *BRAF* testing in that study were 50%, 100%, and 78%, respectively [15]. The same group subsequently evaluated FNAB *BRAF* mutation status as a potential risk stratification tool by correlating mutation status with final histopathology and clinical outcomes in 190 PTC (134 conventional, 41 follicular variant, 15 tall-cell variant) patients undergoing total or near-total thyroidectomy. In some cases the DNA isolation was from fresh FNAB specimens, whereas in other cases it was retrospectively obtained from archived samples. The mutation was identified in 38% of the PTCs and was a strong predictor of capsular invasion ($p = .05$), extrathyroidal extension ($p = .04$), lymph node metastasis ($p = .002$), and tumor persistence/recurrence ($p = .002$). In that study, the sensitivity, specificity, positive predictive value (PPV), and NPV of a $BRAF^+$ FNAB specimen to predict PTC persistence/recurrence were 68%, 66%, 36%, and 88%, respectively.

At the University of Pittsburgh, since 2006 we have routinely used *BRAF* testing as part of a panel of molecular markers used as a diagnostic tool to improve the accuracy of FNAB. Nikiforov and his colleagues at the Universities of Cincinnati and Colorado prospectively evaluated 470

Table 1. Summary of papers evaluating *BRAF* testing of FNAB specimens

Study	Country	Study design	*n* of PTCs on final histopathology	Cytology of confirmed PTCs	Mutation(s) tested	Sensitivity for PTC	Specificity for PTC	PPV for PTC	NPV for PTC
Bentz et al. (2009) [51]	USA	Retrospective	40	22 PTC, 17 indeterminate, 1 benign	*BRAF*	42.5%	100%	100%	17.9%
Cantara et al. (2010) [52]	Italy	Prospective	74	46 PTC, 7 indeterminate, 8 benign, 13 nondiagnostic	*BRAF*[a], *RAS*, *RET*, *TRK*, *PPRγ*	44.6%	100%	100%	79.7%
Cohen et al. (2004) [17]	USA	Retrospective	54	23 PTC, 29 indeterminate, 2 benign	*BRAF*	32%	97.3%	95.7%	52.9%
Domingues et al. (2005) [53]	Portugal	Prospective	11	9 PTC, 1 indeterminate, 1 benign	*BRAF*[a], *RET/PTC*	27.3%	100%	100%	61.9%
Jin et al. (2006) [25]	USA	Retrospective	58	57 PTC, 1 indeterminate	*BRAF*	53.5%	100%	100%	32.5%
Jo et al. (2009) [14]	Korea	Prospective	40	30 PTC, 9 indeterminate, 1 nondiagnostic	*BRAF*	75%	100%	100%	85.9%
Kim et al. (2008) [54]	Korea	Retrospective	75	57 PTC, 18 indeterminate	*BRAF*	84%	100%	100%	70%
Marchetti et al. (2009) [55]	Italy	Retrospective	90	56 PTC, 33 indeterminate, 1 benign	*BRAF*	65.6%	100%	100%	40.4%
Nam et al. (2010) [56]	Korea	Prospective	85	68 PTC, 16 indeterminate or nondiagnostic, 1 benign	*BRAF*	71.8%	100%	100%	86.9%
Nikiforov et al. (2009) [16]	USA	Prospective	38	18 PTC, 17 indeterminate, 3 benign	*BRAF*[a], *RAS*, *RET/PTC*, *PAX8/PPARγ*	47%	100%	100%	70.6%
Ohori et al. (2010) [24]	USA	Prospective	20	20 indeterminate[b]	*BRAF*[a], *RAS*, *RET/PTC*, *PAX8/PPARγ*	15%	100%	100%	85.1%
Pizzolanti et al. (2007) [57]	Italy	Prospective	16	13 PTC, 3 indeterminate	*BRAF*[a], *RET/PTC*	68.8%	100%	100%	86.8%
Rowe et al. (2006) [58]	USA	Retrospective	19	19 indeterminate[b]	*BRAF*	15.8%	100%	100%	23.8%
Salvatore et al. (2004) [59]	Italy	Retrospective	69	54 PTC, 11 indeterminate, 4 nondiagnostic	*BRAF*[a], *RET/PTC*	37.7%	100%	100%	38.6%
Sapio et al. (2007) [60]	Italy	Prospective	21	21 indeterminate[b]	*BRAF*[a], *Galectin-3*	47.6%	100%	100%	91.8%
Xing et al. (2004) [15]	USA	Prospective	16	10 PTC, 6 indeterminate	*BRAF*	50%	100%	100%	78.4%
Zatelli et al. (2009) [13]	Italy	Prospective	74	45 PTC, 23 indeterminate, 6 benign	*BRAF*	63.5%	99.7%	97.9%	93.6%
17 Total			800		Average	49.5%	99.8%	99.6%	64.5%

[a]Results here reflect only *BRAF*$^+$ specimens, though other mutations were tested in the study.
[b]These studies excluded patients with definite cancer on FNAB.
Abbreviations: FNAB, fine-needle aspiration biopsy; NPV, negative predictive value; PPV, positive predictive value; PTC, papillary thyroid cancer.

FNAB samples from 328 consecutive patients and tested them for *BRAF*, *RAS*, *PAX8-PPARγ*, and *RET/PTC* mutations, correlating the mutation status results with cytology, surgical pathology, or clinical follow-up results. The *BRAF* and *RAS* point mutations were detected using real-time polymerase chain reaction (PCR) and fluorescence melting curve analysis, whereas reverse transcription PCR was used to detect *RET/PTC* and *PAX8-PPARγ* rearrangements. In the study, 97% of mutation-positive nodules were papillary or follicular carcinoma on final histology. Molecular testing alone had a sensitivity of 62%, but when combined with cytology, the sensitivity rose to 80% with a PPV of 98%. In particular, molecular testing was helpful for indeterminate nodules, with a PPV of 100% for these lesions; 15 of 52 nodules with indeterminate cytology harbored a mutation and all 15 were malignant on final histology (13 PTC and two follicular carcinoma) [16]. We recently published a retrospective analysis of 44 *BRAF*$^+$ PTC thyroidectomy patients who had undergone preoperative *BRAF* testing on their FNAB specimens; 31 of the FNAB specimens were positive (29 PTCs and two inadequate cytological specimens), translating into a sensitivity of 70% [7]. These and other studies evaluating *BRAF* testing on FNAB specimens are summarized in Table 1. It is important to note in Table 1 that in both studies that did not report a 100% specificity and 100% PPV, it was because there was one *BRAF*$^+$ case of anaplastic carcinoma [13, 17]. Furthermore, reports without data to calculate sensitivity, specificity, PPV, and NPV were excluded from the table [7, 18, 19].

To the best of our knowledge, there have only been six

cases documented in the literature of false-positive *BRAF* testing. The first report was from Korea, where the *BRAF* mutation is highly prevalent, and describes a patient with an indeterminate $BRAF^{+}$ nodule by FNAB who, on final histopathology, had atypical hyperplasia in a background of Hashimoto's thyroiditis. The authors speculated that the atypical hyperplasia could have been a precursor lesion for PTC [20].

More recently, using dual-priming oligonucleotide (DPO)-based multiplex PCR analysis, which can detect *BRAF* V600E in only 2% of cells within a population of wild-type cells, false-positive BRAF testing occurred in five of 226 (2%) FNABs. Upon repeat testing of DNA extracted both from the stored preoperative FNA specimen and after microdissection of the biopsied nodule, *BRAF* mutation was unable to be detected in any of the five nodules, suggesting that the false-positive testing was a result of the overly sensitive assay. In that study, the overall sensitivity and specificity of BRAF testing alone to predict malignancy were 83% and 99%, respectively. In agreement with other published studies, the authors concluded that BRAF testing improved the sensitivity of FNAB in predicting malignancy, although limitations of the testing technique should also be considered in determining malignancy risk [21].

In addition to DPO-based PCR analysis, a number of other techniques to detect the *BRAF* mutation have been described. These include direct sequencing, pyrosequencing, PCR-based single-strand conformation polymorphism, and restriction fragment length polymorphism. Depending on the technique, the tests can detect *BRAF* V600E if it is present in 2%–20% of the cells within an otherwise wild-type background, and the sensitivity, specificity, and PPV are similar [22, 23]. Regardless of which method for *BRAF* mutation detection is used, patients should be appropriately counseled accordingly. In addition, negative molecular testing does not eliminate the need for diagnostic thyroidectomy in patients with cytology results in the indeterminate category.

Molecular testing is also proving to be particularly helpful among thyroid lesions now classified under the new Bethesda Criteria category of "follicular lesion of undetermined significance" (FLUS) [12]. Ohori et al. [24] recently reviewed 100 patients treated at our institution with 117 FNAB diagnoses of either FLUS or atypia of undetermined significance, and compared the results for *BRAF*, *RAS*, *RET/PTC1*, and *PAX8-PPARγ* testing with those of surgical histopathology. Of the 12 FNAB samples with positive molecular results, three of 12 were $BRAF^{+}$ and all were PTC on final histopathology. The other nine were also PTCs, but were positive for other mutations (seven *NRAS61*, one *HRAS61*, one *PAX8-PPARγ*) [24]. Although the current practice for FLUS FNAB results at our institution is to repeat the FNAB in short-interval follow-up, molecular testing may soon direct clinicians when to move directly to diagnostic surgery, as well as the extent of surgery (below). Based on our experience, and after thorough preoperative discussion with consenting patients, we currently recommend upfront total thyroidectomy for FLUS with positive mutational analysis results.

The 2009 Revised American Thyroid Association (ATA) Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer provide a level C recommendation in support of the use of molecular markers to help guide the management of patients with indeterminate cytology [11]. Testing for *BRAF* and other mutations can be done reliably and reproducibly on already collected FNAB specimens [25]; however, it is routinely used in only a handful of centers nationally. The current data on *BRAF* testing support its use as a complementary adjunct to routine cytologic analysis, and when positive it is helpful in determining the extent of thyroidectomy for patients with otherwise indeterminate results. Future cost-efficacy analyses may provide the driving force for widespread implementation of preoperative *BRAF* testing on thyroid FNA samples, but such analyses must necessarily consider clinical criteria in assessing which groups of cytology specimens should be tested.

The Prognostic Utility of *BRAF*

In general, risk stratification plays a key role in the management of patients with PTC, because patients with high-risk tumors are treated more aggressively with adjuvant therapies and patients with high-risk tumors undergo more frequent follow-up [11]. A number of risk stratification tools have been developed, but none have gained universal acceptance; some risk factors, such as completeness of resection, can only be assigned postoperatively after histopathological characteristics become available. The ability to stratify PTC patients into different risk categories *pre*operatively could allow for optimization of the initial surgical procedure, such as the extent of thyroidectomy, the addition of central compartment lymph node dissection (CCND), and the extent of lymph node dissection. To date, no risk stratification classification system is completely accurate, and additional methods to predict aggressive disease are needed.

Beyond its strong correlation with PTC, the *BRAF* V600E mutation is well described to associate with poor prognosis [6, 7, 26–28]. Clinicopathologic parameters established to represent aggressive behavior and poor

prognosis include extrathyroidal extension, multicentricity, local recurrence, lymph node metastasis, and distant metastases [29, 30]. Lupi et al. [6] evaluated 500 PTCs, of which 43% were $BRAF^+$, and found that patients with $BRAF^+$ PTC, compared with those with $BRAF^-$ PTC, had a higher incidence of extrathyroidal extension, nodal metastasis, multicentricity, and advanced stage. Kebebew and colleagues followed 314 thyroid cancer patients for a median of 6 years and found that the *BRAF* V600E mutation was independently associated with recurrent and persistent PTC by multivariate analysis [26]. An association between the *BRAF* V600E mutation and disease-specific survival has also been demonstrated. Elisei and colleagues retrospectively evaluated a small cohort of PTC patients with a median follow-up of 15 years and observed shorter survival in the group with *BRAF* V600E mutation by multivariate analysis [27]. We recently demonstrated that patients with $BRAF^+$ PTCs were more likely to require cervical reoperation than those with $BRAF^-$ PTCs (10% versus 3%; $p = .04$), even in short-interval follow-up [7]. It is most likely that a complex "molecular signature" will one day more precisely identify aggressive PTC, but at present *BRAF* is the marker with the greatest prognostic utility.

Though the body of literature associating the *BRAF* mutation with poor prognostic features is impressive, some of the data are retrospective, and *BRAF* status has not been incorporated into standard PTC management algorithms as of yet. It remains unclear whether identification of this mutation in isolation, regardless of the presence or absence of other clinicopathologic characteristics, should prompt clinicians to treat PTC patients with more aggressive adjuvant therapies and/or closer long-term surveillance. More study in this regard is required.

BRAF and Papillary Thyroid Microcarcinoma

Papillary thyroid microcarcinoma (PTMC) is defined as papillary carcinoma ≤1.0 cm [31]. Although the vast majority of PTMCs are low risk, up to two thirds are associated with cervical lymph node metastasis. The disease-specific mortality rate from PTMC is reportedly up to 2% in some series [32], and recurrence rates have varied in the range of 3%–17%, depending on the length of follow-up and whether recurrence was defined biochemically (by thyroglobulin elevation) or by local recurrence with histopathologic confirmation [33–36]. Because the best management of patients with PTMC remains very unclear, clinical tools are urgently required to help identify the small subgroup of patients with an aggressive tumor and thereby place such patients into high-risk management algorithms postoperatively.

Given what is already known about the significance of the *BRAF* V600E mutation in PTC, it is not surprising that a number of groups have evaluated its prognostic utility for PTMC. Unlike conventional PTC, for which the prevalence of the mutation is ~45%, the prevalence of the *BRAF* mutation in PTMC is somewhat lower, at ~30% [28]. A notable exception was seen in a recently published review of 1,150 Korean patients with PTC by Park et al. [36]. The frequency of the *BRAF* V600E mutation was 67.2% in macroPTC, with a comparable frequency of 65.6% among PTMC cases. In addition, they reported a comparable prevalence between PTC and PTMC for both extrathyroidal extension (72.4% versus 52.2%) and lymph node metastasis (51.8% versus 34.9%), with mean follow-up times of 53 months and 84 months, respectively [36]. Conversely, Lee et al. [37] found that 24 of 64 (38%) PTMC patients who underwent total thyroidectomy with CCND carried the *BRAF* V600E mutation in their tumor; no patient had distant metastasis, but the $BRAF^+$ cohort had a significantly higher incidence of extrathyroidal extension (50% versus 10%; $p = .001$) and nodal metastasis (50% versus 15%; $p = .003$). Similarly, Kwak and colleagues evaluated the association of the *BRAF* V600E mutation with not only known prognostic factors but also with ultrasound characteristics in 339 PTMC patients. On multivariate analysis, they found that *BRAF* mutation was associated with tumor size, extracapsular invasion, and a higher tumor–node–metastasis stage. There was no significant association with any ultrasound characteristic, but there was a trend toward an association of *BRAF* and marked hypoechogenicity ($p = .06$) [38]. A study of 214 consecutive Italian patients with classic PTMC found that 41% of tumors harbored the *BRAF* V600E mutation and noted that size, gender, vascular invasion, extrathyroidal extension, and multifocality showed no significant correlation with *BRAF* status. However, patients with $BRAF^+$ tumors were significantly older than those who did not harbor the mutation (52.7 years versus 33.4 years; $p < .001$) [39].

To summarize, although these results are not entirely consistent, we know that a $BRAF^+$ status in PTMC is also associated with poor clinicopathologic features in most reports, and that may translate into a higher risk for disease recurrence and shorter survival. Whether patients with $BRAF^+$ PTMC would benefit from completion thyroidectomy, lymphadenectomy, or radioactive iodine (RAI) ablation remains to be seen. This is a particularly exciting area of thyroid cancer research because the management of mi-

crocarcinoma is so controversial, with a notable dearth of clinical practice recommendations based on high-level evidence.

Incorporating *BRAF* Status into Management Algorithms

The initial management of PTC is surgical, but in this area there is much room for improvement. Current ATA guidelines definitively recommend near-total or total thyroidectomy for all tumors >1 cm as well as therapeutic neck dissection for patients with clinically positive central or lateral neck lymphadenopathy [11]. However, only 20%–30% of indeterminate thyroid nodules harbor cancer after diagnostic thyroid lobectomy, resulting, even in the modern era, in many thyroidectomies for what proves to be benign thyroid tissue. Furthermore, patients are frequently required to submit to the distress and risks of reoperative completion total thyroidectomy once the final pathology confirms a carcinoma, and/or must later undergo reoperative cervical lymphadenectomy. The ability to identify which PTC patients have aggressive PTC could very usefully allow the surgeon to tailor the operative approach at initial surgery and also could potentially provide durable cure.

Because *BRAF* positivity has clear diagnostic utility and is an established poor prognostic factor in PTC patients, we recently evaluated its use for optimizing initial surgical management, comparing 106 $BRAF^+$ PTC patients with 100 $BRAF^-$ control patients, all of whom underwent thyroidectomy [7]. *BRAF* testing occurred either preoperatively on FNAB specimens or on the surgical specimen. Compared with controls, a higher proportion of the $BRAF^+$ patients: had suspicious sonographic features on preoperative ultrasound (50% versus 33%; $p = .03$); underwent total thyroidectomy as the initial surgical procedure (87% versus 67%; $p < .001$); had tall-cell morphology (30% versus 6%; $p < .001$), extrathyroidal extension (57% versus 15%; $p < .001$), lymphovascular invasion (40% versus 21%; $p = .003$), and level VI lymph node metastases (36% versus 12%; $p = .003$); and have since required cervical reoperation for persistent or recurrent disease (10% versus 3%; $p = .04$). Of the 44 $BRAF^+$ PTC patients who were able to receive preoperative *BRAF* testing of FNAB specimens, 31 of 44 were $BRAF^+$, yielding an FNAB detection rate of 70%. FNAB *BRAF* testing was negative in 13 of 44 cases (30%) in this study, largely resulting from either extraction of inadequate DNA or FNAB of a nodule that was not the $BRAF^+$ nodule. On final histopathology, all 31 patients had PTC, including two patients whose FNAB specimens had inadequate yield but were $BRAF^+$, yielding an FNAB PPV of 100%. We further were able to show that, altogether, preoperative knowledge of a $BRAF^+$ status could have beneficially altered the initial surgical management in 24% of the 75 PTC patients whose preoperative *BRAF* status was either unknown or was falsely negative [7]. Based on these findings, to informed and consenting patients with $BRAF^+$ cytology we now routinely offer total thyroidectomy as initial surgery. Similarly, Xing et al. [19] recently observed a greater than fourfold higher risk for tumor persistence/recurrence among $BRAF^+$ PTC patients than among patients with mutation-negative tumors ($p = .002$), and concluded that *BRAF* status may be a useful decision-making tool regarding whether or not to do a prophylactic central compartment neck dissection at initial surgery. Our current management algorithm for *BRAF* mutation detected on FNAB is illustrated in Figure 1.

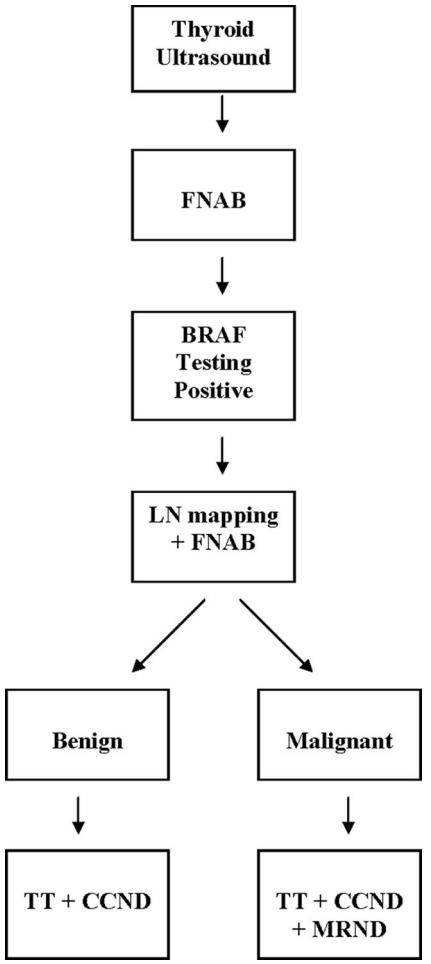

Figure 1. Management algorithm of papillary thyroid cancer with a *BRAF* mutation detected on FNA cytology.

Abbreviations: CCND, central compartment node dissection; FNAB, fine-needle aspiration biopsy; LN, lymph node; MRND, modified radical neck dissection; TT, total thyroidectomy.

BRAF status may also guide the need for and extent of initial lymphadenectomy. Proponents of CCND argue that, although this approach does not improve survival, it may decrease the risk for locoregional recurrence and improve staging accuracy, allowing for a more precise assessment of the need for postoperative adjuvant therapy [40]. However, these claims have yet to be demonstrated in the literature, and the benefits of CCND must very much be weighed against the often-described higher risks for recurrent laryngeal nerve injury and hypoparathyroidism [41]. Because the presence of the *BRAF* mutation was associated with central lymph node metastasis in the multiple studies already discussed, *BRAF* testing may help to delineate which PTC patients should receive prophylactic CCND at the time of thyroidectomy, especially because ultrasonography has been shown to be inaccurate at identifying central compartment lymphadenopathy preoperatively [42]. A prospective, randomized trial examining this issue would be reasonable and ethical because true equipoise remains.

BRAF as a Therapeutic Target for PTC

The overall 10-year survival rate for patients with differentiated thyroid cancer (DTC) is generally excellent; however, there remains a small proportion of patients who recur and/or develop distant metastases and succumb. Treatment options for this subgroup are limited, and new therapies are necessary, especially because the mortality from DTC has not improved in past two to four decades [43]. Given the prevalence and prognostic significance of the *BRAF* mutation in PTC, it is an obvious choice for the study of targeted molecular therapeutic options.

It is hypothesized that one reason why patients with $BRAF^+$ PTC have a poorer prognosis is because the mutation confers resistance to the conventional adjuvant therapies used to treat PTC. The effectiveness of RAI ablation for DTC depends on the ability of thyrocytes to take up iodide via the sodium-iodide symporter (NIS) located on the basolateral aspect of the follicular cell membrane. In vitro studies have documented significantly lower expression levels of NIS in *BRAF* $V600E^+$ PTCs [44]. In 2009, Riesco-Eizaguirre et al. [45] described a novel mechanism whereby *BRAF*-induced activation of transforming growth factor β and subsequent activation of the Smad signaling pathway led to NIS repression in thyroid cancer, a process that their group had already described in normal thyroid cells. Liu and colleagues were able to demonstrate, in a human PTC cell line, the restoration of the expression of several important genes involved in iodide metabolism that had previously been silenced by the *BRAF* V600E mutation [46]. In a recent study by Ricarte-Filho et al. [47], *BRAF* mutations were present in a high proportion of RAI-resistant PTC tumors and metastases. $BRAF^+$ PTC may therefore not respond to RAI ablation or thyroid-stimulating hormone suppression.

A number of *BRAF* inhibitors have been investigated as potential new agents for targeted therapies. Perhaps the agent most familiar to clinical and surgical oncologists is BAY43–9006, or sorafenib. This potent kinase inhibitor has activity against a number of tyrosine protein kinases, including RAF, c-KIT, platelet-derived growth factor, vascular endothelial growth factor receptor (VEGFR)-2, and VEGFR-3, and has been evaluated in the setting of metastatic thyroid cancer. Salvatore et al. [48] studied the effects of sorafenib on six thyroid cancer cell lines expressing the *BRAF* V600E mutation and found lower proliferation in cancer cells but not in normal thyroid cells. The mechanism for sorafenib's effects is not yet understood, but it does not appear to be through reinduction of RAI avidity [49]. A recent phase II trial evaluated patients with differentiated ($n = 27$), medullary ($n = 1$), and poorly differentiated ($n = 2$) thyroid cancers treated with sorafenib (400 mg twice daily). Twenty-three percent had a partial response and 53% had stable disease, with an overall median progression-free survival duration of 79 weeks [50]. A phase III trial evaluating its utility in refractory thyroid cancer is currently in recruitment and the results are highly anticipated.

Conclusions

The *BRAF* V600E mutation plays a central role in the pathogenesis of PTC, prompting further investigation of *BRAF* as a diagnostic and prognostic tool. The presence of a *BRAF* mutation in an FNAB specimen has a >95% PPV for PTC, further increasing the sensitivity of an already accurate test. When available, *BRAF* FNAB testing facilitates optimal oncologic surgery performed at the initial operation. Furthermore, $BRAF^+$ PTC tends to be more aggressive, and the mutation should alert clinicians to categorize patients accordingly as high risk and consider postoperative adjuvant therapies and more frequent cancer surveillance. The presence of *BRAF* is less frequent in papillary microcarcinoma and may also be associated with a poorer prognosis. Finally, *BRAF* as a target for new therapies to treat high-risk patients with recurrent or metastatic disease who have exhausted conventional therapies holds exciting promise.

Author Contributions

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