



Precision medicine: BRAF mutations in thyroid cancer

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Abstract: Mutations in BRAF are commonly seen in thyroid carcinoma and can have therapeutic and prognostic implications. The BRAF V600E mutation is seen in approximately 40% of cases of papillary thyroid carcinoma (PTC), and is associated with aggressive clinicopathological features, higher recurrence rate, and disease related mortality. Significant advances have occurred in the development of new therapeutic targets of the MAP kinase pathway, which is activated in the setting of BRAF mutations, including BRAF and mitogen extracellular kinase (MEK) inhibitors. BRAF alterations other than V600E are less common in PTC, and their clinical significance remains to be established. Here we present a case of a male with poorly differentiated thyroid carcinoma, with papillary and follicular features, who was noted to have a BRAF K601E mutation. The patient was subsequently treated with a BRAF inhibitor, dabrafenib, and MEK inhibitor, trametinib.

Keywords: Thyroid cancer; BRAF mutation; RET mutation; V600; K601E

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Introduction

Thyroid cancer is a highly prevalent malignancy with a low overall mortality. As per the SEER database, in 2019, there are an estimated 52,070 new cases of thyroid cancer (14,260 in men and 37,810 in women) and about 2,170 deaths in the United States (1). While the overall cure rate is high with standard therapy, including surgery and radioiodine ablation, there are still patients who die of the disease when standard therapy fails or is not an option. Identifying treatments for these select patients is key.

The distinct histologic subtypes of thyroid cancer including papillary, follicular, medullary, and anaplastic carcinoma, have their own unique pathologic and clinical manifestations, and rates of disease related mortality. There are diverse genetic alterations within each subtype with varying clinical implications. Common alterations include RET/PTC rearrangements, BRAF point mutations, RAS mutations, and TP53 mutations (2). In papillary thyroid

carcinoma (PTC), BRAF mutations, and specifically BRAF V600E mutation, are the most common alterations. BRAF V600E mutation is associated with aggressive clinicopathological features and increased disease related mortality. Non-V600 BRAF mutations are also seen, but have less clinically relevant data available. BRAF mutations may provide therapeutic targets for patients with more aggressive thyroid carcinoma. Understanding the mechanism of these mutations and the role of drugs that target the aberrant pathway leading to tumor cell proliferation, is essential to further decreasing thyroid cancer related mortality.

Case presentation

A 77-year-old male was initially diagnosed with thyroid carcinoma in November 2013, after MRI lumbar spine showed a 5 cm L4 expansile mass. A staging CT scan of the chest, abdomen, and pelvis at the time showed an enlarged

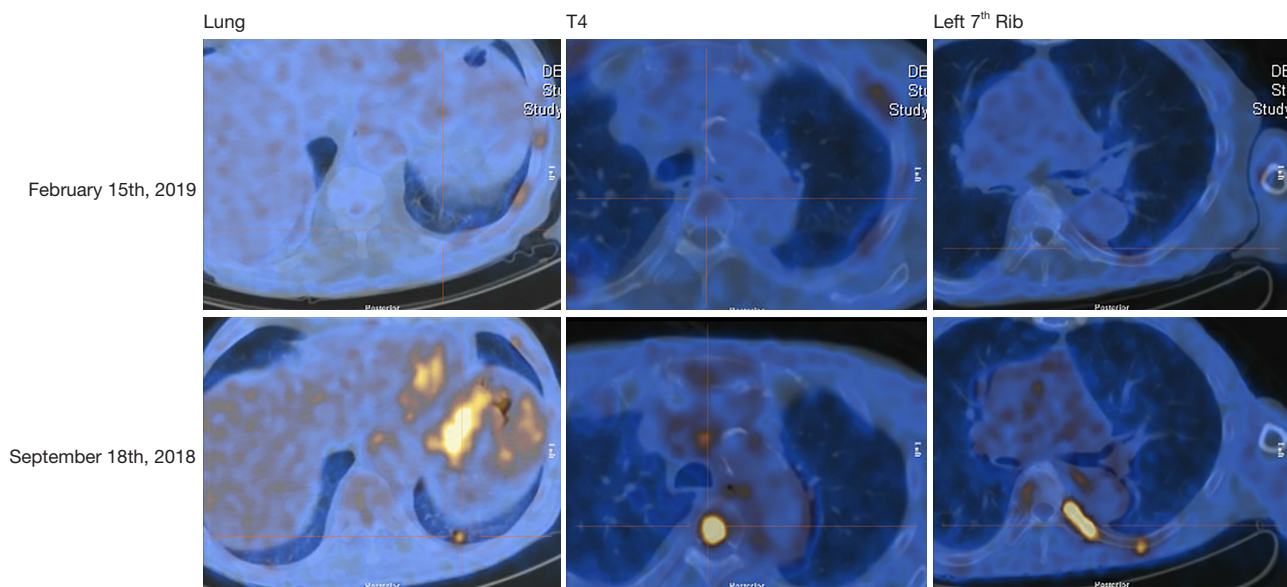


Figure 1 PET/CT scan findings before and after initiating treatment with dabrafenib and trametinib. Top images are post treatment and show resolution of previously seen left lower lobe nodule and marked improvement in osseous metastases.

right thyroid. Bone scan showed bilateral rib cage and left sternoclavicular joint activity. The patient was treated with radiation to the L3–L5 vertebral bodies in January 2014, followed by a thyroidectomy. Pathology from the surgery revealed a 3.6 cm poorly differentiated thyroid carcinoma with follicular and papillary features, high-grade regions, and lymph node metastasis. In March 2014, the patient had a repeat MRI which showed a persistent large expansile L4 mass. He was treated with 200 mci of radioactive iodide and thyroid suppression. In August 2014, a repeat PET scan showed mildly FDG avid lytic sternal and L4 lesions, for which the patient was started on denosumab. He then had multiple imaging studies consistent with metastases to the lung and progression in numerous bony sites. Lenvatinib, 24 mg daily, was started in June 2017 with multiple toxicities, including fatigue, weight loss, and diarrhea, requiring dose reduction to 14 mg daily by March of 2018.

In September 2018, PET-CT scan showed progression of pulmonary and bony metastases, as well as an increase in thyroglobulin from 195 to 434 over two months, suggesting resistance to lenvatinib. Given progression of disease on various lines of treatment, ctDNA via Foundation One was ordered for evaluation of actionable mutations. The ctDNA testing showed a BRAF K601E actionable mutation, as well as a CHEK2 splice site 592+1G > A and TP53 Q136H.

Given this finding and review of the literature, the

decision was made to treat the patient with a BRAF inhibitor (dabrafenib) in combination with a mitogen extracellular kinase (MEK) inhibitor (trametinib). Repeat imaging showed an excellent response to treatment (*Figure 1*), but given adverse effects, including severe lower extremity edema, the medication was discontinued after approximately six weeks (with the last 3 weeks of treatment only consisting of single agent trametinib). Despite initial impressive response on imaging, the toxicity and subsequent rapid physical decline, associated with a rise in thyroglobulin and clear radiologic progression of disease (MRI spine with new large lumbosacral lesion), the patient was transitioned to hospice.

Molecular tumor board

Molecular alterations in thyroid cancer

There are various forms of thyroid cancer, each with their own distinct pathologic, molecular, and clinical findings. The primary subtypes include papillary (most common and accounts for ~80% of cases), follicular (including Hurthle cell), medullary, and anaplastic. Several highly prevalent genetic alterations are uniquely seen in the various subtypes (*Table 1*) and include RET/PTC rearrangements, point mutations of BRAF, RAS mutations, and PAX8-peroxisome proliferator-activated receptor γ (PPAR γ) fusion oncogene

Table 1 Molecular alterations in thyroid cancer

Thyroid cancer subtype	Molecular alteration	Frequency
Papillary	BRAF mutations (primarily V600E)	60–70%
	TERT promoter mutations (exclusively in adults)	10–20%
	RET fusion (CCDC6 and NCOA4 are most common)	10–20%
	RTK fusions (RET > NTRK)	15%
	RAS (NRAS > HRAS > KRAS)	13%
Follicular	RAS (NRAS > HRAS > KRAS)	60%
Hurthle cell	TERT promoter mutations	50–60%
	TP53 mutations	20%
Medullary	RET mutation (M918T is most common)	Sporadic >60%, hereditary >90%
Anaplastic	TP53 mutations	65%
	TERT promoter mutations	65%
	BRAF mutations	40%
	RAS mutations	20–30%

(3,4). These mutations seen are typically mutually exclusive of other major driver mutations (5). As such, BRAF mutations are more common in adults and are the major somatic genetic alterations driving the formation of PTC in the adult population (5). On the other hand, RET/PTC rearrangements tend to occur in children and are the major somatic genetic alterations that drive the formation of PTC in that population. It is important to understand the various molecular alterations, as they have therapeutic impacts.

RET mutations

RET is a proto-oncogene that encodes a transmembrane protein belonging to the receptor tyrosine kinase family and is primarily expressed in tissues of neuroectodermal origin. When mutated, it can drive the development of various malignancies, including papillary thyroid cancer (RET fusions) and medullary thyroid cancer (RET mutations). While the majority of medullary thyroid cancers are sporadic, approximately 25% are inherited, and can be secondary to MEN2A, MEN2B, or other non-MEN familial conditions. Classically, those with RET changes are treated with multi-kinase inhibitor

drugs, including cabozantinib and vandetanib; however, due to less selective targeting, these agents are associated with significant toxicity. LOXO-292 is a potent and highly selective RET inhibitor (fusion and mutation independent) that was granted a breakthrough therapy designation for the treatment of those with advanced RET fusion-positive thyroid cancer who require systemic therapy, have had disease progression following prior treatment, and have no acceptable alternative treatment options. This designation was based on the results of the LIBRETTO-001 trial, a phase 1/2 study in patients with advanced RET fusion-positive solid tumors and medullary thyroid cancer. The preliminary results were presented at the American Thyroid Association in 2018 (6). The study included 29 patients with RET-mutant medullary thyroid cancer and nine patients with RET fusion-positive thyroid cancer. Patients were heavily pretreated and an overall response rate of 56% (95% CI; 35–75%) was seen among those with RET-mutant medullary thyroid cancer and 78% (95% CI; 40–97%) in those with RET fusion-positive thyroid cancer. In the ARROW trial, BLU-667, another selective RET inhibitor, demonstrated clinical activity against thyroid cancer with RET gene alterations. This trial included 60 patients with RET-mutated medullary thyroid carcinoma and 5 patients with RET-fusion + PTC. Overall response rate was 47% (95% CI: 33–62%) and responses occurred regardless of MKI resistance or RET genotype (7).

BRAF mutations

BRAF mutations in thyroid cancer occur almost exclusively in PTC and PTC-derived anaplastic thyroid carcinoma (ATC). They can be subdivided into classical V600E mutations, other RAF non-V600 mutations, RAF fusions, and RAF deletions. Mutations in BRAF can cause hyperactivation of the BRAF protein, leading to increased activity in the MAP kinase pathway and subsequent tumorigenesis. As the MEK protein is located downstream in the MAP kinase pathway, it is also hyperactivated by these mutations (8) (Figure 2).

Amongst the BRAF mutations, BRAF V600E mutation is most common and accounts for more than 90% of these mutations (9,10). It is a point mutation, substituting thymine with adenine at position 1799 on exon 15, resulting in glutamine for a valine at residue 600. It is an early and common driver mutation in well-differentiated thyroid tumors, with subsequent late-event mutations that promote

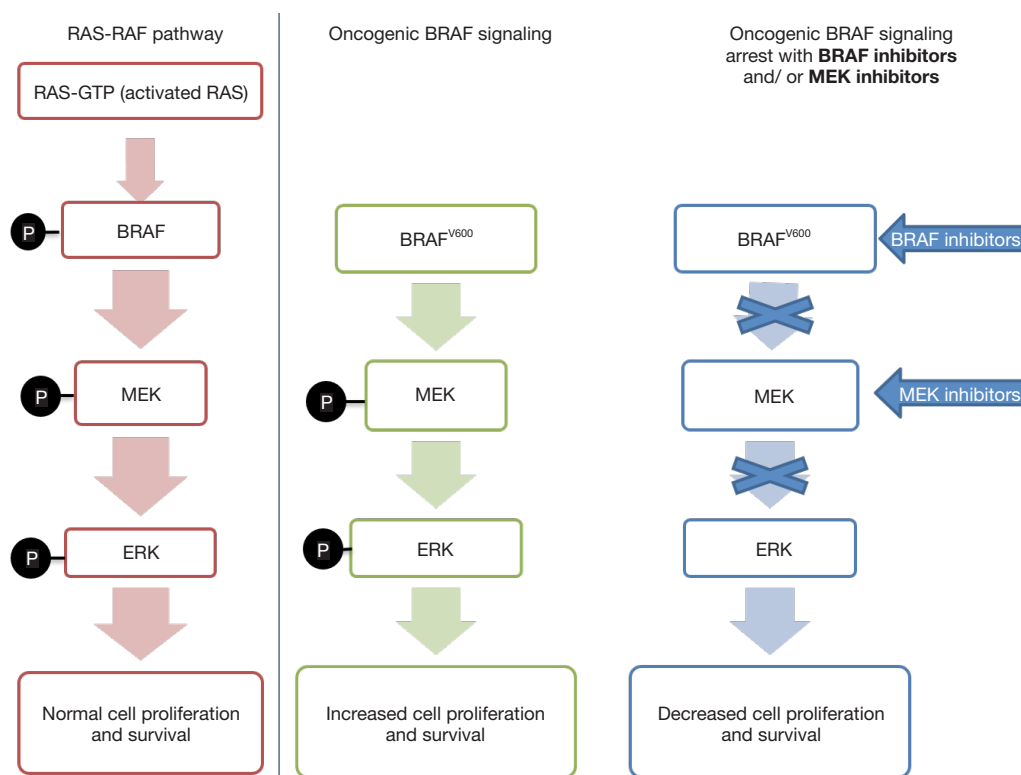


Figure 2 RAS-RAF-MEK-ERK signaling pathway. Ras directly interacts with and activates Raf. Raf phosphorylates and activates MEK, which in turn phosphorylates and activates ERKs. A BRAF V600 mutation leads to constitutive activation, independent of external factors. Selective binding by BRAF inhibitors or MEK inhibitors blocks the constitutively activated pathway and downstream activity. MEK, mitogen extracellular kinase.

progressive dedifferentiation to ATC.

Non-V600 mutations (as in our case) can either increase or decrease kinase activity relative to wild type BRAF. Oncogenesis can occur via dysregulated kinase activity in a similar manner to V600E mutations, or via increased dimerization/transactivation of the partner protomer, CRAF, leading to hyperactivation. These mutations can lead to constitutive activation of BRAF and evade ERK-mediated feedback.

The role of BRAF inhibitors has been primarily studied in those with V600E mutations, with a large portion of the literature stemming from melanoma patients. First generation BRAF inhibitors include Vemurafenib and Dabrafenib. These agents only block the monomeric mutant BRAF (not dimers), and are associated with an increased risk of development of resistance, as well as pro-oncogenic adverse effects, including new skin cancers. The skin toxicities are due to paradoxical activation of MAP kinase pathway in epithelial cells that harbor the

RAS mutation and bypass the inhibition of BRAF. Second-generation BRAF inhibitors, including sorafenib and LYS3009120 (a pan-RAF inhibitor) inhibit BRAF in active (dimer) formation, and thereby minimize the paradoxical pathway activation in Ras mutant cells. MEK inhibitors, including trametinib and selumetinib, block MEK kinase activity within the MAP kinase signaling pathway, and can decrease the risk of adverse events related to paradoxical ERK activation compared to monotherapy with BRAF inhibitors.

Data supporting treatment with BRAF inhibitors, alone, or in combination with MEK inhibitors, is primarily based on studies in those with V600 mutations. Patients with melanoma with BRAF V600E or V600K mutations were noted to have improved response rates and response duration when treated with combination therapy versus dabrafenib alone (11). A recent study in metastatic melanoma with V600E or V600K mutations looked at the 5 year outcomes of first line treatment with combination

dabrafenib and trametinib and showed long-term benefit in approximately one third of the patients (12). A phase II trial of vemurafenib in patients with BRAFV600E positive metastatic or unresectable papillary thyroid cancer, refractory to radioactive iodine, showed a partial response in 38.5% of those never treated with a multi-kinase inhibitor targeting VEGFR (13), although a high rate of grade 3 and 4 adverse events were noted. More recently, a phase II, open-label trial looked at the efficacy and safety of dabrafenib and trametinib combination in BRAF V600E-mutated anaplastic thyroid cancer (14). There were 16 patients enrolled in the study and the overall response rate was 69% (95% CI: 41–89%). One patient had a complete response and 10 patients had a partial response. Seven subjects had ongoing responses at the time of the analysis. This led to the FDA approval of combination dabrafenib and trametinib in BRAF V600E mutated anaplastic thyroid cancer.

BRAF K601E

While BRAF V600E is the most widely identified BRAF alteration, our patient was noted to have a BRAF K601E mutation. This is a specific mutation at codon K601 in exon 15 of the BRAF genome which substitutes lysine (K) to a glutamic acid (E) at position 601 in BRAF, and leads to increased kinase activity. The mutation is in the activation segment of the kinase domain and is adjacent to V600. It occurs in about 5% of patients with melanoma, and is the third most common BRAF mutation (15,16).

Pre-clinical studies suggest that downstream signaling induced by the K601E mutant may be blocked by trametinib. In a study in melanoma, vemurafenib treatment of all of the BRAF mutant-expressing cells led to a decrease in phospho-MEK and ERK protein levels, whereas treatment with the MEK inhibitor led to a more dramatic decrease in phospho-ERK signaling, suggesting treatment response to both agents (15). In a phase II study of trametinib in patients with metastatic BRAF mutant cutaneous melanoma, one patient with a BRAF K601E mutation experienced a partial response (17). A case report of a patient with melanoma and a BRAF K601E mutation noted complete response to single agent trametinib over 36 months (18). Based on this case report and the data for treatment with combination BRAF inhibitor/MEK inhibitors in BRAF V600 mutated ATC, the consensus at our molecular tumor board was to treat our patient with the combination.

Conclusions

This case highlights the importance of testing for molecular targets in the treatment of thyroid cancer that has progressed on standard therapy. Currently, the approval for BRAF and MEK inhibitors in thyroid cancer is primarily limited to ATC. There is an active clinical trial investigating the use of dabrafenib with or without trametinib in patients with recurrent thyroid cancer (NCT01723202) with documented BRAF mutations. Additionally, the MATCH screening trial (NCT02465060) is assessing dabrafenib and trametinib in those with BRAF V600 mutations, and an arm has been completed with the use of single agent trametinib in BRAF non-V600 mutations, while a current active arm is investigating the single agent MEK inhibitor, ulixertinib for tumors with BRAF non-V600E/K mutations or BRAF fusions. The results of these studies and others will help elucidate the role of these medications in BRAF-mutated papillary thyroid cancer and other BRAF driven malignancies.

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Footnote

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