BRAF Inhibition in BRAFV600E-Positive Anaplastic Thyroid Carcinoma

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Abstract

The efficacy of targeted monotherapy for BRAFV600E-positive anaplastic thyroid carcinomas (ATC) is not established. We report 2 cases of BRAFV600E-positive ATC treated with a BRAF inhibitor. A 49-year-old woman with a T4bN1bM0 ATC manifested symptomatic metastatic disease 8 weeks after radical chemoradiotherapy. Within 1 month of BRAF inhibitor monotherapy, a complete symptomatic response was observed, with FDG-PET scan confirming metabolic and radiologic response. Treatment was terminated after 3 months because of disease progression. The patient died 11 months after primary diagnosis. A 67-year-old man received first-line BRAF inhibitor for a T4aN1bM0 ATC. Within 10 days of treatment his pain had stabilized and his tumor had clinically halved in size. Stable disease was achieved for 11 weeks but the patient died 11 months after diagnosis because of disease progression. BRAF inhibitor monotherapy in ATC may obtain clinical benefit of short duration. Upfront combination therapy should be investigated in this patient subgroup.


Background

The ability to rapidly identify therapeutically targetable mutations is impacting clinical practice in oncology, and has led to the use of potentially novel treatment avenues for patients with tumors that have traditionally had no effective treatments available. For tumors that harbor known targetable mutations, treatments directed at those mutations can be highly effective and well tolerated.1,2

Anaplastic thyroid carcinoma (ATC) is an uncommon, highly aggressive tumor with a dismal prognosis.3,4 The worldwide incidence of the disease varies geographically and ranges from 0.8% to 9.8%.3,5 Although metastatic differentiated thyroid cancer occurs more commonly and can usually be treated readily with iodine-131 therapy, ATC always lacks iodine-avidity. No effective systemic therapies exist for ATC, with conventional chemotherapy regimens, typically anthracyclines, demonstrating low response rates of short duration.6 Therefore, the median survival for ATC is less than 6 months. From a molecular perspective, TP53 mutations have been commonly reported in ATC, but given the lack of effective systemic treatment options for the disease, it has been of particular interest to note reports of the identification of targetable mutations, including BRAFV600E, NRAS, HRAS, and PIK3CA mutations.3,7,8

The identification of the BRAFV600E mutation in a subgroup of ATC creates the opportunity to investigate the efficacy of BRAF inhibition for this disease,9 with effective targeted agents already in clinical use for other malignancies. The BRAFV600E mutation results in the constitutive activation of the serine-threonine kinase, which drives cellular proliferation through the mitogen-activated protein kinase (MAPK) pathway.10

The identification of “druggable oncogenes,” such as the BRAFV600E mutation, has led to the use of a num-

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ber of promising monotherapeutic regimens. However, the development of acquired resistance to monotherapy with such agents is well-known, and is a relatively early phenomenon occurring through a variety of different mechanisms that can be histotype-specific. For this reason, the efficacy of upfront combination therapy using both a BRAF inhibitor and an MEK/MAPK inhibitor was investigated in metastatic melanoma and was shown to be more effective than monotherapy with a BRAF inhibitor alone. However, the efficacy of monotherapy or combination tyrosine kinase inhibitor therapy for other tumors that harbor the BRAFV600E mutation, including ATC, is not yet established, and because of the rarity of ATC, cannot be readily examined within the ideal setting of prospective clinical trials. Indeed, the use of monotherapy may not be the most effective first-line strategy. Therefore, clinical experience with these approaches can help guide treatment decisions for individual patients in the absence of robust trial data.

 Accordingly, here we report 2 cases of patients diagnosed with ATC who received BRAF inhibitor monotherapy for BRAFV600E-positive disease.

**Case #1**

A 49-year-old woman presented with a 5-week history of a rapidly enlarging neck mass. Beyond a history of smoking 10 to 15 cigarettes per day for 30 years, the patient had no other significant medical comorbidities and was taking no regular medications. On clinical examination she was found to have a left-sided thyroid lesion with ipsilateral palpable nodes. CT imaging of the neck confirmed a 4.3-cm lesion arising from the left lobe of the thyroid, with extensive necrotic ipsilateral nodal metastases, and marked displacement and encasement of surrounding structures. FDG-PET revealed high uptake in the known disease sites but did not demonstrate evidence of metastatic disease. An ultrasound-guided core biopsy of the thyroid mass demonstrated necrotic tumor that was AE1/AE3-positive, with features consistent with ATC. The tumor was initially staged as T4bN1bM0 disease. After discussion at a multidisciplinary meeting, the tumor was not considered suitable for primary resection because of the radiologic presence of tracheal involvement and also as a result of the lack of a distinct prevertebral resection plane. Therefore, the patient was urgently commenced on treatment with 70 Gy of radiotherapy administered with weekly paclitaxel. Treatment was well tolerated.

At a routine posttreatment review appointment 8 weeks after the completion of chemoradiotherapy, the patient reported a 3-day history consistent with a unilateral brachial plexopathy. CT scans demonstrated the enlargement of the primary thyroid lesion, which had increased to 6 cm, but was accompanied with a reduction in the lymphadenopathy. Mass effect and displacement of the scalene muscles were also detected, with new areas of linear enhancement of soft tissue along the course of the brachial plexus, which was thought to account for the patient's symptoms. In addition, new mediastinal lymphadenopathy and 4 parenchymal lung lesions were detected, of which the largest measured 2.7 cm. After the patient's pain responded to intermittent low-dose opioids, she was referred for consideration of palliative systemic treatment options.

Image-guided core biopsy of the largest lung lesion obtained tissue that demonstrated a poorly differentiated epithelioid malignancy with no features of a differentiated thyroid carcinoma, and no glandular or squamous differentiation. Some strongly positive AE1/AE3 cells were observed, consistent with a carcinoma. The tumor did not demonstrate any TTF-1, thyroglobulin, CK5, RCCAb, S100, or Melan-A staining. The pathologic findings in the clinical context were consistent with metastatic ATC, and a second primary lung carcinoma was considered less likely. DNA extracted from the lung biopsy demonstrated the presence of the BRAFV600E mutation on Sanger sequencing.

During a hospital admission for management of a pain crisis related to a recurrence of the brachial plexopathy and for commencement of anticoagulation to treat an incidentally discovered thrombosis of the subclavian vein, off-label, compassionately accessed dabrafenib at 150 mg twice daily was commenced. For this reason, the efficacy of upfront combination therapy using both a BRAF inhibitor and an MEK/MAPK inhibitor was investigated in metastatic melanoma and was shown to be more effective than monotherapy with a BRAF inhibitor alone. However, the efficacy of monotherapy or combination tyrosine kinase inhibitor therapy for other tumors that harbor the BRAFV600E mutation, including ATC, is not yet established, and because of the rarity of ATC, cannot be readily examined within the ideal setting of prospective clinical trials. Indeed, the use of monotherapy may not be the most effective first-line strategy. Therefore, clinical experience with these approaches can help guide treatment decisions for individual patients in the absence of robust trial data.

Accordingly, here we report 2 cases of patients diagnosed with ATC who received BRAF inhibitor monotherapy for BRAFV600E-positive disease.
A 67-year-old man presented with a 2-month history of an enlarging tender left thyroid mass and odynophagia. CT imaging revealed a 3.7-cm thyroid mass with tracheal deviation and adherence, with unilateral lymphadenopathy. Fine-needle aspiration demonstrated features consistent with ATC. FDG-PET demonstrated a 4.7-cm thyroid lesion but did not reveal distant disease. The patient’s tumor was initially staged as T4aN1bMO. Apart from being a current smoker with a 25 pack-year smoking history, the patient had no comorbidities. The

uptake value (SUV$_{\text{max}}$) of the mediastinal nodal mass reduced from 12.8 to 5.9 (−54%), the lung metastasis SUV$_{\text{max}}$ reduced from 45.8 to 5.3 (−88%), and the SUV$_{\text{max}}$ of a left cervical node reduced from 20 to 4.6 (−73%). Similarly, the mediastinal nodal mass reduced in maximal dimension from 26 to 16 mm, the largest lung metastasis reduced from 52 to 32 mm, and the left cervical nontarget node reduced from 13 to 10 mm. At this time, treatment was well tolerated, with localized grade 1 skin rash as the only toxicity.

In the second month of therapy at routine review, FDG-PET scan demonstrated asymptomatic isolated disease progression in the mediastinal nodal mass (Figure 1C). Resection of the progressive mediastinal disease was performed by mediastinoscopy for the purpose of obtaining more tissue to determine mechanisms of resistance and other molecular aberrations through whole exome sequencing via enrollment in a translational research study. However, the resected tissue was extensively necrotic and no viable tissue suitable for further sequencing was obtained. By the third month of therapy, the patient developed symptoms consistent with superior vena caval obstruction (SVCO), with FDG-PET CT scan demonstrating widespread disease progression and an intravascular FDG-avid lesion extending from the known subclavian thrombosis (Figure 1D). Dabrafenib therapy was ceased.

The patient received palliative radiotherapy for the possibility of intravascular disease within the right subclavian vein, given the symptomatic SVCO. However, the patient’s symptoms of SVCO continued to progress off dabrafenib therapy. She died 11 months after her initial diagnosis and 7 months after detection of metastatic disease.
patient declined both radical surgery and chemoradiotherapy.

A core biopsy was performed to confirm the tumor histology and to determine BRAF mutation status. The thyroid biopsy showed a malignancy consisting of pleomorphic ovoid to spindle-shaped cells and some normal thyroid tissue. The tumor cells were negative for AE1/AE3, thyroglobulin, TTF-1, S100, Melan-A, smooth muscle actin, and desmin. Features of follicular, papillary, or poorly differentiated thyroid carcinoma were not identified. In the context of the clinical and radiologic findings, the histopathologic features were consistent with ATC. A BRAF V600E mutation was demonstrated on high-resolution melting polymerase chain reaction and confirmed by Sanger sequencing.

Off-label, compassionately accessed dabrafenib at 150 mg twice daily was commenced in the setting of increasing opiate requirements for neck pain secondary to the rapidly enlarging tumor, which measured 7.5 cm maximally at this time. At a scheduled review 10 days after the commencement of therapy, tumor-related symptoms had significantly reduced and the palpable thyroid mass had clinically halved in size. After a month of therapy, grade 2 palmar-plantar erythema and the development of keratoacanthomas were noted, but neither of these required intervention. Clinical benefit on BRAF inhibition continued for 11 weeks, until a dramatic relapse of pain recurred with a rapid increase in tumor size from 3 to 7.9 cm measured clinically. At this time, dabrafenib was ceased. CT imaging did not demonstrate other sites of disease.

Repeat core biopsy was taken at the time of progression to examine the molecular basis of treatment resistance and to determine alternative treatment options. Exome sequencing of more than 6,000 genes accessed through participation in a translational research study confirmed the presence of the BRAF V600E mutation (allelic frequency 34%), in addition to a TP53 R273C mutation (allelic frequency 41%), and 2 germline mutations of uncertain significance. An ERCC2 C259Y mutation was observed with a variant frequency of 48% in whole blood, but this mutation was not enriched in the tumor, which demonstrated a 38% variant frequency. A VHL P192S was also detected with a variant frequency of 46% in whole blood, but this again was not enriched in the tumor DNA (48%). There was also evidence of extensive copy number variation in the tumor sample. Molecular profiling of the pretreatment tumor sample has been subsequently performed, which demonstrated the BRAF V600E mutation with an allelic frequency of 32%. However, the baseline allelic frequency of the TP53 R273C mutation was 33%.

Given the symptomatic disease progression, the patient received 50 Gy in 20 fractions of palliative radiotherapy to the thyroid, with clinical improvement but no regression in the size of the tumor. Two months after completion of radiotherapy, pain increased and the patient deteriorated. This patient also died 11 months after diagnosis.

Discussion

The molecular profiling of tumors may lead to therapeutic opportunities for diseases with limited treatment options. Since the discovery of oncogene addiction and development of effective therapies combatting molecular dependencies, such as the BRAF V600E mutation in melanoma, it has been a matter of considerable clinical interest as to whether such therapies can be used in other diseases demonstrating the same molecular phenotype.

Given the aggressiveness of the disease and the limited treatment options, the clinical need exists for the development of effective systemic therapies for ATC. Guidelines from both the American Thyroid Association (ATA) and NCCN suggest an approach to the management of patients with ATC, highlighting the need for multidisciplinary input to communicate to patients the dismal natural history of the disease and to assist in the determination of appropriate treatment goals. However, in the advanced setting where surgery is not feasible, only moderate-level evidence at best exists for the use of systemic therapy. In the metastatic setting, only nonrandomized data from small trials exist for the use of both chemotherapy and targeted therapies that generally do not obtain any significant tumor response.

This highlights the clinical need for more relevant data for these patient subgroups, and therefore it is suggested that their participation in clinical trials is paramount. However, because of the rarity of disease, the rapid demise of patients with ATC, and the smaller subgroup of patients with BRAF V600E mutated tumors, it would be a considerable feat to perform robust clinical trials to investigate genotype-specific therapeutic options. Therefore, in the light
of these barriers, clinical experience with treatment can provide insight and direction for further approaches, as reported here.

The discovery of the BRAFV600E mutation, and other targetable mutations, in a subset of these tumors has led to the hope that targeted therapies may have potential benefit for patients with the disease. For ATC, the V600E point mutation is the most commonly reported mutation in BRAF, although the reported frequency is variable between studies and ranges between 6% and 50%.7,8,21–25 In general, the sample sizes from studies are small and the number of ATCs examined is usually fewer than 20 samples in each study. ATCs can arise both de novo and as areas of dedifferentiated tumor arising in differentiated tumors, such as differentiated papillary thyroid carcinoma. The reported presence of BRAFV600E mutations in only a portion of differentiated papillary thyroid carcinomas suggests the possibility that BRAF mutations are a seminal molecular event that triggers thyroid carcinogenesis or dedifferentiation or confers aggressiveness.8,23,25–30 As such, detailed studies using highly sensitive sequencing techniques or BRAFV600E immunohistochemistry to examine possible heterogeneity of BRAF mutations in differentiated thyroid cancers are warranted.

Early studies using BRAF inhibitors in differentiated thyroid tumors demonstrate some benefit.31,32 However, although studies are underway examining the efficacy of BRAF inhibition and combination targeted therapy in thyroid tumors, these only focus on differentiated thyroid malignancies, where BRAF mutations occur more commonly.33 Our 2 cases of patients with BRAFV600E-positive ATC demonstrate that the presence of the mutation may be successfully targeted by dabrafenib. Both our patients experienced rapid clinical improvement associated with disease response, confirming dependency on BRAF signaling. In addition, as both patients lived longer than the median survival for the disease, it is possible that the natural history may have been altered by the targeted therapy. The marked early metabolic response on FDG-PET observed in the first patient is consistent with a direct effect on glycolytic metabolism through abrogation of oncogenic signaling, as observed in BRAFV600E malignant melanoma treated with BRAF inhibitor therapy.34 However, the development of treatment resistance was also noted to occur early in both patients.

For ATC, the rapid development of resistant disease on BRAF inhibitor monotherapy suggests that this may not be the best approach. Treatment resistance was observed in our 2 patients’ tumors at 1 to 3 months. In contrast, resistance to monotherapy with BRAF inhibitors in metastatic melanoma develops with a median of more than 5 months. Based on known mechanism of resistance to BRAF monotherapy, the efficacy of upfront combination therapy using both a BRAF inhibitor and an MEK inhibitor was investigated in metastatic melanoma.17,18 In phase III studies that investigated the efficacy of upfront combination therapy compared with monotherapy with a BRAF inhibitor, the primary end point of improved progression-free survival was met (hazard ratio for progression or death in the combination arm, 0.75; 95% CI, 0.57–0.99; \( P = .03 \)).18 This recent demonstration of superior but modest survival gained with upfront combination therapy for metastatic melanoma argues that the treatment algorithms for other tumors should also be reconsidered.18 In addition, an understanding of the mechanisms for resistance to BRAF inhibition will be essential for establishing the optimal treatment approach. Thyroid cancer cell line studies have highlighted distinct mechanisms of resistance to BRAF inhibitors compared with those that occur commonly in metastatic melanoma, such as the feedback-mediated/ligand-dependent upregulation of the HER3 signaling pathway.16 Exome sequencing of the second patient’s tumor after the development of clonal resistance did not identify an obvious molecular cause, although it is impossible to determine the significance of detected mutations given the absence of comprehensive molecular profiling of a pretreatment tumor specimen. A known pathogenic mutation in TP53 R273C was identified, with TP53 mutations recognized as a common event in poorly differentiated and anaplastic thyroid malignancies.28,35–41 It is possible that these mutations may be a key event contributing to dedifferentiation in anaplastic tumors in the multistep process of carcinogenesis. Although a germline mutation was detected in VHL, the specific mutation has not been associated with the Von-Hippel Lindau syndrome and is associated with hereditary polycythemia with an unknown predisposition to malignancy.42

Thus, for ATC, given the rapid clinical response and equally rapid development of resistant disease described in the patient cases presented here, consid-
eration of upfront combination therapy may provide a more effective treatment strategy. Murine models of BRAF\(^{V600E}\)-positive ATC have demonstrated significantly prolonged survival in mice treated with combination therapy with a BRAF inhibitor and an MEK inhibitor compared with those treated with a BRAF inhibitor alone, suggesting that this may indeed be a favorable clinical approach.

**References**


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