

Tissue-agnostic Activity of BRAF plus MEK inhibitor in BRAF V600 mutant tumors

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Abstract

BRAF plus MEK inhibitor combinations are currently FDA approved for melanoma, non-small cell lung cancer, and anaplastic thyroid cancer. The lack of clinical benefit with BRAF inhibition in BRAF V600 mutated colorectal cancer has prevented its tissue-agnostic drug development. We reviewed the AACR GENIE database for the prevalence of BRAF V600 mutations across tumor types. We reviewed the literature for case reports of clinical responses, outcomes in patients with BRAF V600 mutation-positive non-melanoma malignancies who received BRAF inhibitor therapy, and data from published adult and pediatric trials. BRAF V600 mutations are prevalent across multiple non-melanoma malignancies (> 40 different tumor types), lead to oncogene addiction, and are clinically actionable in a broad range of adult and pediatric non-melanoma rare malignancies. Continued tissue-agnostic drug development is warranted beyond the current BRAF plus MEK approved cancers.

Introduction

The MAP kinase pathway was first implicated in the pathogenesis of melanoma, where mutations in the *BRAF* gene, specifically the V600E site, lead to constitutively active kinase leading to downstream cancer cell proliferation(1, 2). After discovery of the mutant *BRAF* V600E, efforts to inhibit this kinase were focused on developing drugs to block the active form and induce cell death of cells with overactivation of BRAF. Although tumors harboring BRAF V600 alterations respond to BRAF inhibitors, acquired resistance develops quickly. In order to avoid and/or delay resistance, a combination strategy of MEK inhibition plus BRAF inhibition was evaluated and showed synergistic benefit(3).

Beyond melanoma, mutations in the *BRAF* gene have also been implicated in hairy cell leukemia, colon cancer, non-small-cell lung cancer (NSCLC), anaplastic thyroid cancer, and ovarian cancer among others (4). The first of the drugs to show clinical activity inhibit BRAF was vemurafenib(5) followed by dabrafenib and later by encorafenib(6). Vemurafenib, dabrafenib and encorafenib serve as potent inhibitors of the active mutant BRAF V600E kinase. Further, inhibition of the MEK kinase, a member of the MAP kinase pathway, has also been shown to improve outcomes for patients with advanced melanoma (7-9). The success of this MEK kinase inhibition strategy has been shown in other malignancies as well(10, 11). Although BRAF plus MEK inhibitor combinations have shown responses in multiple tumors, the reason that BRAF plus MEK inhibitors were not viewed or pursued as tissue-agnostic drugs like NTRK inhibitors for *NTRK* fusion positive tumors is that in colon cancer there was a lack of benefit derived from single-agent use of vemurafenib (12). This unresponsiveness in

colorectal cancer stalled tissue-agnostic drug development and hence tumor specific drug development pathways were pursued and approval was sought in a tumor specific manner. However, it is important to note that the addition of an EGFR inhibition to BRAF and MEK inhibitors for colon cancer provided a significantly longer survival for patients with *BRAF* V600E-mutated colon cancer(13). This example shows that perhaps in some circumstances, BRAF plus MEK inhibitors may need supplemental inhibition to block an additional driver, EGFR, to overcome innate drug resistance and failure(13, 14).

Currently, the FDA approvals for BRAF plus MEK inhibitors include vemurafenib plus cobimetinib and encorafenib plus binimetinib for melanoma, dabrafenib plus trametinib for melanoma, non-small cell lung cancer, and anaplastic thyroid cancer, and vemurafenib for Erdheim Chester disease. In addition, encorafenib plus cetuximab is FDA-approved for metastatic colorectal cancer with a BRAF V600E mutation alteration. Since, mutations in the *BRAF* V600E are found across a breadth of tumor histologies that include a wide variety of rare and orphan cancers, perhaps the drugs that inhibit *BRAF* V600E and MEK should be considered as tissue agnostic targeted drugs and pursued further for drug development (with the exception of colorectal cancer where additional EGFR inhibition is needed). Results from the vemurafenib-basket study (15) and the NCI-match trial (16) reveal that BRAF pathway inhibition is active in more than 20 unique cancer types. In this article, we review the evidence from available literature, real-world data from published case studies and clinical trials on the role of BRAF plus MEK inhibition in multiple BRAF V600 positive adult and pediatric malignancies beyond melanoma.

Prevalence of BRAF V 600 alterations

We queried the AACR GENIE database to assess the prevalence of BRAF V600E mutations across various tumor types. Among the 96,324 samples queried from the AACR GENIE database, the BRAF V600E mutation was reported in 43 different tumor types across 2,963 samples (3.07%) (**Figure 1A and B**). BRAF V600E was most commonly present in thyroid cancer (40.9%), parathyroid cancer (31.8%), melanoma (26.1%), Langerhans cell histiocytosis (25.7%), and head and neck NOS (14.3%). These results highlight the prevalence of BRAF V600E across various tumor types and unveil the possible opportunities for targeted therapy.

Real world evidence of tissue-agnostic efficacy of BRAF inhibitors

We conducted a literature search using NCBI PubMed from 2012-2019 for case reports, series, and clinical trials using the search terms: “dabrafenib,” “trametinib,” “vemurafenib,” “encorafenib,” “binimetinib,” and “cobimetinib.” Patients with BRAF V600 mutation-positive non-melanoma cancers were included in the analysis. The information of interest (e.g., age, previous treatments, progression-free survival [PFS] and overall survival [OS]) was manually extracted from the text and supplementary material in the manuscripts. This data was used to calculate median PFS and OS across a diverse set of non-melanoma cancers.

The review of literature revealed 178 cases across 69 tumor types that were identified and categorized in accordance with the NIH Cancer Classification (**Table 1A**).

The most common cases identified with the BRAF V600E mutation included Erdheim-Chester disease (n = 30, 16.9%), papillary thyroid carcinoma (n=16, 8.9%), anaplastic thyroid carcinoma (n = 13, 7.3%), and hairy cell leukemia (n = 13, 7.3%). The mean of the patients' ages was 43.9 years old, with a range from 5 weeks to 90 years old. Most patients were between 54 to 72 years of age (n = 35 patients or 20.4%) closely followed by patients between 1-18 years of age (n = 32 patients or 18.6%) being the next most common. Regarding therapy, dabrafenib (BRAf), dabrafenib plus trametinib (BRAf plus MEK), trametinib (MEK), vemurafenib (BRAf), vemurafenib plus trametinib (BRAf plus MEK), vemurafenib plus cobimetinib (BRAf plus MEK) were used in 34% (n =56), 16% (n = 27), 4% (n = 6), 44% (n = 72), 1% (n = 1), 2% (n =3) of cases, respectively (**Table 1B**).

For the patients with BRAF V600E-mutated Erdheim-Chester Disease (ECD) the median PFS was 3.0 months, median OS was 33.0 months, and median duration of response (DOR) was 9.0 months, which included treatment with dabrafenib or vemurafenib.(17-26) For the patients with BRAF V600E-mutated thyroid cancer, the median PFS was 11.3 months, median OS was 14.0 months, and median DOR was 9.3 months, which included patients treated with dabrafenib, dabrafenib plus trametinib, and vemurafenib.(27-34) Overall, across a cohort of tumor histologies, median PFS was 6.5 months, and median OS was 28.5 months with a median duration of response of 8.0 months (**Supplemental Figure 1**).

Activity of BRAF inhibition in BRAF positive non-melanoma malignancies from published studies

We reviewed 16 adult studies and 6 pediatric studies conducted in non-melanoma malignancies. Among the adult studies, responses were reported in multiple malignancies harboring a BRAF V600 alteration like hairy-cell leukemia, anaplastic and papillary thyroid cancer, non-small-cell lung cancer, multiple myeloma, biliary tract cancer, pancreatic cancer, and Langerhans cell histiocytosis(**Table 2**) (10, 11, 15, 16, 35-46). Dabrafenib in combination with trametinib or vemurafenib was most commonly studied. In all studies which compared single agent drugs to combination therapy, the objective response rate (ORR) in combination treatment was superior. The most dramatic responses of these studied treatments were seen in hairy cell leukemia with ORR of 87% in vemurafenib plus rituximab and 78% in dabrafenib plus trametinib (44, 46). The ORR was 100% in vemurafenib alone in the U.S. study and 96-100% in dabrafenib alone (42, 43). Notable responses were also observed in thyroid cancer. In one of the most aggressive forms of thyroid cancer, anaplastic thyroid cancer the ORR was 69% with dabrafenib plus trametinib in an interim analysis of the ROAR study (10). Recently, definite benefit of this combination was confirmed in an updated analysis that included the full enrollment of 36 patients and more than 4 years of additional study follow-up(47). ORR was 56%, with 50% of responders still in response at 12 months(47). In papillary thyroid cancers, ORR's were 35% in dabrafenib plus trametinib and 38.5% in vemurafenib alone (39, 40). Given these positive results, BRAF-targeted therapies may prove to be effective in various cancer types. In the vemurafenib basket study of 172 patients with 26 unique cancer types, an overall response rate of 33% was reported, and responses were observed in 13 unique cancer types (15). Interestingly, the NCI-Match study studying the combination of dabrafenib and trametinib across

diverse tumor types also showed an ORR of 38% and responses in 7 distinct tumor types(16).

There is also evidence that BRAF V600 mutations can be targeted successfully in pediatric patients (**Table 3**) (48-53). Among the pediatric studies, the breadth of current literature is in pediatric neuro-oncology. Pediatric gliomas are the primary tumor type studied. In three studies, favorable response were seen with ORR's of 80%, 44%, and 100% (49, 50, 52).

Totality of evidence of BRAF inhibition in adult and pediatric pan-cancers

BRAF V600 mutations are prevalent across a breadth of tumor histologies, and there are multiple non-melanoma FDA-approvals of drugs that inhibit the BRAF/MEK pathway (**Table 4**). A basket study of non-melanoma BRAF V600 alterations included cohorts which derived benefit from vemurafenib(54). Similarly, in the collected case studies thyroid cancers were one of the most abundant types of cancer, and these patients derived meaningful benefits from BRAF inhibition. Patients with hairy cell leukemia have BRAF V600 alterations in 100% of cases with a 96% response rate (41, 42). These examples suggest that BRAF V600 may be a tumor agnostic biomarker. Critics are quick to cite findings of a study which used single-agent vemurafenib to treat BRAF V600-mutated colorectal cancer with ORR of ~5% and PFS of 2.1 months (12). However, it may be that some cancer types, such as colorectal cancer may require additional inhibition of co-occurring alterations/pathways. By targeting additional pathways, such as EGFR, ORRs increase to 26% and PFS increases to 4.3 months(13). It is important to note that beyond colorectal cancer there is no other

cancer that has a tissue specific innate mechanism of resistance to BRAF inhibition that is prevalent widely.

NSCLC is yet another example of BRAF V600 inhibition leading to improved outcomes with 42% ORR and PFS of 7.3 months(54). Like the findings with a median duration of response of 8.0 months for all patients, 43% of patients had a response and the median treatment duration was 5.9 months with no patients progressing on vemurafenib(54). Another basket study also presented a median PFS of 5.8 months and OS of 17.6 months across an array of histologies which appears to be congruent with our findings of median PFS of 6.5 months and median OS of 28.5 months across pan-cancers(15). Brain tumors appear to be the 7th most abundant BRAF V600-mutated cancers, and in those patients vemurafenib has been shown to have an ORR of 25% and median PFS of 5.5 months (55).

To date, there are nine FDA-approved indications for BRAF plus MEK inhibitors in various malignancies including melanoma (**Table 4**). Single-agent vemurafenib was first approved in 2011 for unresectable or metastatic melanoma with BRAF V600E mutation followed by the combination of dabrafenib plus trametinib for the same indication in 2013, and encorafenib plus binimetinib in 2018. The combination of dabrafenib plus trametinib has also been approved for use in metastatic NSCLC with BRAF V600E mutation, as adjuvant therapy for BRAF V600E- or V600K-melanoma, and locally advanced or metastatic BRAF V600E-mutated anaplastic thyroid cancer. Vemurafenib combined with cobimetinib is approved for both BRAF V600E- or V600K-mutated metastatic melanoma. Vemurafenib as a single agent is approved for patients with BRAF V600-mutated Erdheim-Chester Disease. Encorafenib is approved when

used in combination with cetuximab for patients with metastatic BRAF V600E-mutated colorectal cancer.

Conclusion

The current and growing number of indications for BRAF plus MEK inhibitors in various malignancies along with the presented case studies serve as compelling evidence that BRAF may be a tumor agnostic target. Our literature review of cases and multiple studies are congruent with the reported non-melanoma basket studies and show that there may be a wide variety of malignancies that could benefit from access to these drugs. Since BRAF plus MEK inhibition is standard of care in multiple tumor types, a combination approach should be used for tissue agnostic studies as well (56-58). Furthermore, an agnostic drug indication will increase patient access to medications and potentially offer an additional line of treatment option to these rare cancer patients. The issue here is access to a potentially lifesaving therapy or a therapy conferring clinical benefit in a rare disease patient harboring a BRAF V600 alteration. If it is not approved for a particular indication, it is quite challenging to access the drug. Approval provides more efficient access to patients. The increased use of next-generation sequencing in the treatment arsenal of cancer enables potential target identification and easier approval of clinically meaningful drugs (58-60). Comprehensive review of the BRAF V600 landscape reveals the prevalence in multiple rare non-melanoma malignancies and identifies BRAF V600 as a tissue agnostic target.

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Table 1 A. List of unique malignancies harboring BRAF V 600E alteration with activity on BRAF plus or minus MEK inhibitors reported in case studies

Adult Wilms tumor	Lung adenocarcinoma
ameloblastoma	malignant peripheral nerve sheath tumor
anaplastic astrocytoma	malignant pleural mesothelioma
anaplastic ganglioma	metastatic ameloblastoma
anaplastic pleomorphic xanthoastrocytoma	metastatic colorectal carcinoma
anaplastic thyroid carcinoma	metastatic papillary thyroid carcinoma
appendiceal adenocarcinoma	metastatic upper tract urothelial carcinoma
brainstem ganglioglioma	multiple myeloma
cholangiocarcinoma	mutated ganglioma
clear cell carcinoma	mutated high-grade glioma
colorectal adenocarcinoma	non-small cell lung adenocarcinoma
dendritic cell sarcoma	neurofibromatosis type 1-associated glioblastoma
desmoplastic infantile astrocytoma	ovarian carcinoma
encephalocraniocutaneous lipomatosis	papillary craniopharyngioma
epithelioid glioblastoma	papillary thyroid carcinoma
Erdheim-Chester disease	pediatric invasive gliofibroma
ganglioma	peduncular anaplastic ganglioma
ganglioneurocytoma	pilocytic astrocytoma
gastrointestinal stromal tumor	pilomyxoid astrocytoma
glioblastoma	pleomorphic xanthoastrocytoma
glioblastoma without epithelioid cells	pulmonary Langerhans cell histiocytosis
gnathic ameloblastoma	pilocytic astrocytoma
hairy cell leukemia	renal cell carcinoma
High-grade glioma	right colon adenocarcinoma
histiocytic sarcoma	salivary duct carcinoma
infiltrative pleomorphic glioma	serous ovarian cancer
Langerhans cell histiocytosis	spinal ganglioma
low-grade serous ovarian adenocarcinoma	urothelial carcinoma

Table 1B. Review of literature of individual case reports of BRAF V600 tumors treated with BRAF plus or minus MEK inhibitor. Table shows percentage of Cases Using Each Drug(s)

dabrafenib (BRAf)	34% (n =56)
dabrafenib plus trametinib (BRAf plus MEK)	16% (n = 27)
trametinib (MEK)	4% (n = 6)
vemurafenib (BRAf)	44% (n = 72)
vemurafenib plus trametinib (BRAf plus MEK)	1% (n = 1)
vemurafenib plus cobimetinib (BRAf plus MEK)	2% (n =3)

Table 2. Previously published studies in adult patients with non-melanoma BRAF-altered cancers

Drug	Tumor Type	Number of Patients	Objective Response Rate or Overall Response Rate	Other Comments	Reference
dabrafenib plus trametinib	BRAF V600E mutant biliary tract cancer	43	ORR = 51 %	626 patients with biliary tract cancer were locally prescreened for the BRAFV600E mutation. Based on local BRAF testing, 57 patients with BRAFV600E-mutated biliary tract cancer were identified, of whom 43 were enrolled Included in NCCN guidelines	(35)
vemurafenib	BRAF V600 mutant non-melanoma malignancies	172	33%	In total, 172 patients with 26 unique cancer types were treated, achieving an overall response rate of 33% and median duration of response of 13 months. Responses were observed in 13 unique cancer types, including historically treatment-refractory tumor types such as cholangiocarcinoma, sarcoma, glioma, neuroendocrine carcinoma, and salivary gland carcinomas.	(15)
dabrafenib plus trametinib	BRAF V600E mutant solid tumors, lymphomas, or multiple myeloma	29	38%	The median overall survival was 28.6 months.	(16)
dabrafenib plus trametinib	BRAF V600E mutant metastatic NSCLC	93	68.4%		(36)
dabrafenib plus trametinib	BRAF V600E mutant metastatic NSCLC	36	64%		(11)
dabrafenib plus trametinib	BRAF V600E mutant metastatic NSCLC	59	63.2%		(37)
dabrafenib	BRAF V600E mutant advanced non-small-cell lung cancer	84	33%		(38)
vemurafenib	BRAF V600E mutant metastatic or unresectable papillary thyroid cancer refractory to radioactive iodine	51	38.5%		(39)

dabrafenib plus trametinib	Locally Advanced or Metastatic BRAF V600 mutant Anaplastic Thyroid Cancer	16 (interim data) 36 (Final data)	69% (interim data) 56 % (Final data)	First FDA approved therapy of Anaplastic thyroid cancer. This updated analysis confirms the definitive benefit of dabrafenib plus trametinib in ATC with long-term follow-up	(10, 47)
dabrafenib plus trametinib	BRAF V600 mutant papillary thyroid carcinoma	53	35%		(40)
	BRAF mutations in hairy cell leukemia			This study was only identifying BRAF mutations in HCL	(41)
vemurafenib	hairy-cell leukemia that had relapsed after treatment with a purine analogue or who had disease that was refractory to purine analogues	26 Italian study 24 U.S. study	96% Italian study 100% U.S. study		(42)
dabrafenib	relapsed or refractory hairy cell leukemia	10	96-100%		(43)
vemurafenib plus rituximab	Refractory or relapsed hairy cell leukemia	30	87%		(44)
dabrafenib plus trametinib	BRAF V600E mutant high-grade (HGG) and low-grade glioma (LGG)	45	33% in HGG and 69 % in LGG	Included in NCCN guidelines	(45, 61)
dabrafenib plus trametinib	Recurrent/refractory BRAF V600E mutated hairy cell leukemia	43	78%		(46)

Table 3. Previously published studies in pediatric patients with non-melanoma BRAF-altered cancers

Drug	Tumor Type	Number of Patients	Objective Response Rate or Overall Response Rate	Other Comments	Reference
dabrafenib plus trametinib	BRAF V600E high-grade gliomas	3	N/A	Patient 1 remained disease free for 20 months at which time he presented with disseminated disease recurrence and died 2 months later. Patient 2 has remained on therapy with a small amount of stable disease for 32 months. Patient 3 remained on therapy with stable disease for 23 months.	(48)
dabrafenib or vemurafenib	BRAF V600E Pediatric Gliomas	67	80%	Poor prognostic factors in conventional therapies, such as concomitant homozygous deletion of <i>CDKN2A</i> , were not associated with lack of response to BRAF inhibition.	(49)
dabrafenib	BRAF V600E Pediatric low-grade glioma	32	44%		(50)
dabrafenib plus trametinib	BRAF V600E Wilms tumor	1	N/A	The patient remains in a complete radiographic response 12 months after starting therapy and continues to receive dabrafenib and trametinib with minimal treatment-emergent toxicities.	(51)

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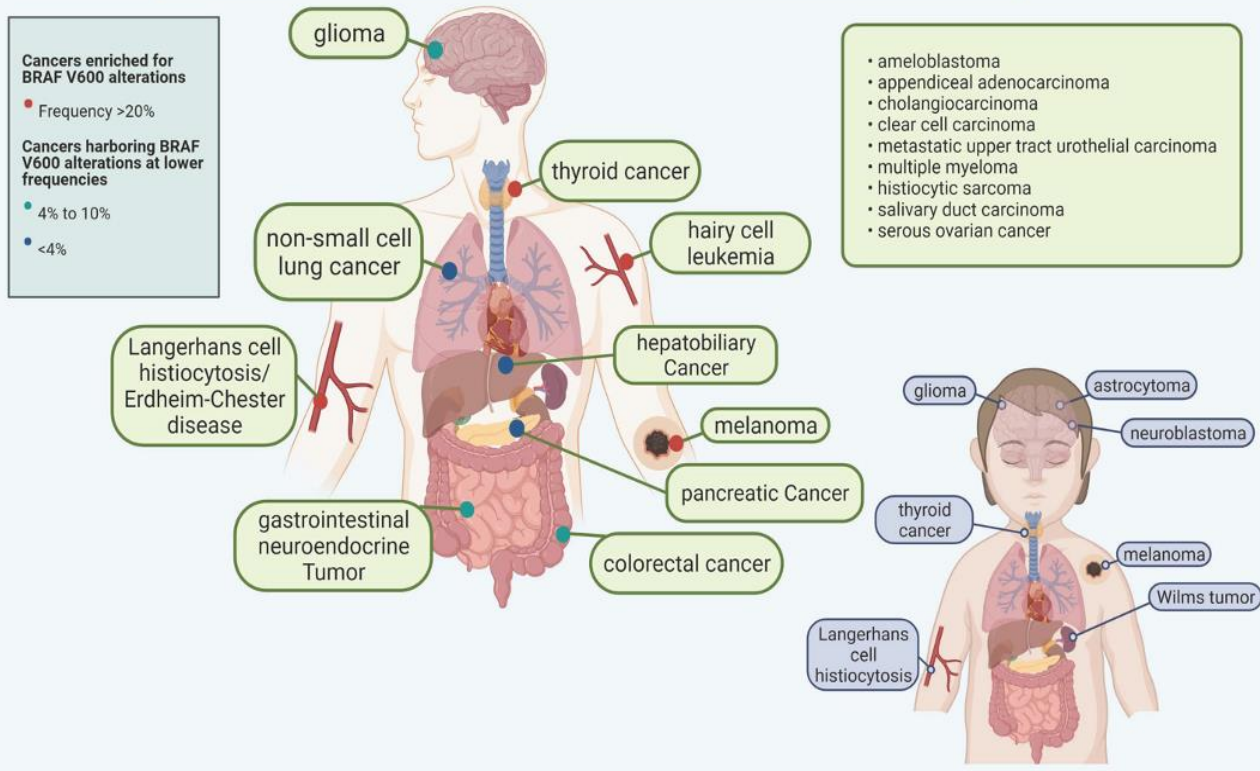
vemurafenib	recurrent or progressive BRAF V600E mutant brain tumors	19	32%		(52)
Dabrafenib	BRAF V600 mutation-positive tumors	27	Not reported	In this first clinical trial in pediatric patients with pretreated <i>BRAF</i> V600-mutant tumors, dabrafenib was well tolerated while achieving target exposure levels; the average treatment duration was >1 year with many patients still on treatment.	(53)

Table 4. FDA-approvals of BRAF plus MEK inhibitors in non-melanoma cancers

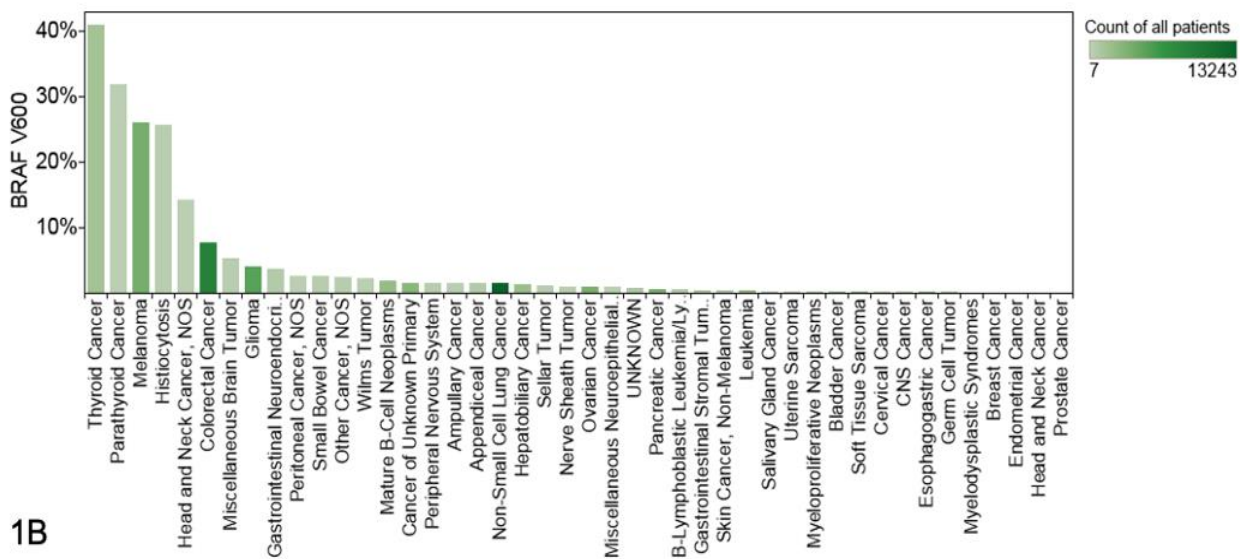
Drug/Combination	Non-Melanoma Indications (Date of FDA Approval)
dabrafenib plus trametinib	Metastatic non-small cell lung cancer (NSCLC) with BRAF V600E mutation (6/22/2017)
	Locally advanced or metastatic anaplastic thyroid cancer (ATC) with BRAF V600E mutation and with no satisfactory locoregional treatment options (5/4/2018)
vemurafenib alone	Treatment of patients with Erdheim-Chester Disease (ECD) with BRAF V600 mutation (11/6/2017)
vemurafenib plus cobimetinib	No non-melanoma indication
encorafenib	In combination with cetuximab for the treatment of adult patients with metastatic colorectal cancer (CRC) with a BRAF V600E mutation (4/9/2020)
encorafenib plus binimetinib	No non-melanoma indication

Figure Legend: Figure 1A. Distribution of BRAF V600 mutations in adult and pediatric tumors
Figure 1B. Frequency of BRAF V600 mutations by tumor histology Figure Panel A is a cartoon schematic with examples of various BRAF-mutated nonmelanoma cancers and the distribution in adult and pediatric tumors. Figure Panel B shows the frequency of BRAF V600 mutations in 43 different tumor types across 2,963 samples in the AACR GENIE database.

Distribution of BRAF V600 alterations in adult and pediatric tumors



1A



1B