Original Research *Highlight article*

Prevalence of class I–III BRAF mutations among 114,662 cancer patients in a large genomic database

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Impact statement

These data represent the largest aggregation of BRAF mutations within a single clinical database to our knowledge. The relative proportions of both BRAF V600 mutations and non-V600 mutations are informative in all cancers and by malignancy, and can serve as a definitive gold-standard for BRAF mutation cancer incidence by malignancy. The rate of BRAF mutation in human cancer in a real-world large database is lower than previously reported likely representing testing more broadly across tumor types. The relative percentages of Class II and Class III BRAF mutations are higher than previously reported, representing almost 35% of BRAF mutations in cancer. These findings provide support for the development of effective treatments for non-V600 BRAF mutations in cancer.

Abstract

BRAF mutations are relatively common in many cancers, particularly melanoma, colorectal cancer, and thyroid cancer and to a lesser extent in lung cancer. These mutations can be targeted by BRAF and MEK inhibitors, which exhibit good clinical activity. There are conflicting reports of the various relative rates of BRAF Class I mutations (V600 locus), defined as those that exhibit extremely strong kinase activity by stimulating monomeric activation of BRAF, Class II, define as non-V600 mutations that activate BRAF to signal as a RAS-independent dimer, and Class III mutations, defined as "kinase-dead" with low kinase activity as compared to wild type BRAF. Prospective studies have largely focused on patients with tumors harboring Class I BRAF mutations (limited to the V600 locus) where response rates up to 70% with BRAF plus MEK inhibition have been demonstrated. We report on the relative prevalence of various types of BRAF mutations across human cancers in a cohort of 114,662 patients that received comprehensive genomic profiling using next-generation sequencing. Of these patients, 4517 (3.9%) a pathogenic or presumed pathogenic BRAF mutation (3.9%). Of these, 1271 were seen in melanoma, representing 39.7% of all melanomas sequenced, representing the highest rate in all tumors. Class I (V600) mutations were seen overall in 2841 patients (62.1% of BRAF mutations, 2.4% of total cancers). Class II mutations were seen in 746 tumors (16.5% of BRAF mutant, 0.7% of total), and Class III mutations were seen in 801 tumors (17.7% of BRAF, 0.7% of total). Knowledge of the relative prevalence of these types of mutations can aid in the development of agents that might better address non-V600 mutations in cancer.

Keywords: Cancer, biomarkers, kinase, melanoma, BRAF, colon

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Introduction

BRAF is a member of the RAS/RAF/MEK/ERK pathway, an intracellular signaling pathway that drives cell growth and division. When mutated, BRAF can serve as an oncogene leading to the development of cancer. BRAF mutations have been identified in multiple tumor types including melanoma, colorectal cancer, thyroid carcinoma, gliomas, histiocytic neoplasms, lung cancer, ovarian, breast, liver, hairy cell leukemia, multiple myeloma, and sarcoma.[1–7] When harboring certain mutations, BRAF can signal as a RAS-independent monomer or dimer, resulting in constitutive downstream activation and uncontrolled cell division and tumor growth. The most common activating mutation in BRAF-associated cancer is a single

nucleotide substitution mutation at position 1799 T > A, resulting in replacement of the native valine (V) with glutamic acid (E) at codon 600, which leads to BRAF activation as a RAS-independent monomer. The presence of a V600E mutation has significant prognostic and therapeutic implications for a variety of cancers, including melanoma, thyroid, lung, and colorectal cancer.

Approximately 40% of cutaneous melanomas show constitutive BRAF activation through a mutation at the V600 locus. The canonical V600E accounts for the majority, but V600K or other mutations can also occur. Patients with BRAF-V600-mutant melanoma exhibit dramatic responses to BRAF-directed small molecule inhibitors, such as vemurafenib and dabrafenib.[8,9] However, almost all tumors will ultimately acquire resistance to single-agent therapy. The addition of a MEK inhibitor, such as trametinib to dabrafenib, cobimetinib to vemurafenib, and, most recently, binimetinib to encorafenib, increases response rates, duration of response, and survival times in BRAF V600-mutated melanoma[10–12] by preventing reactivation of the pathway through various resistance mechanisms. As a result, combination therapy is now the preferred treatment over single-agent BRAF inhibition in BRAF V600E mutations. Consistent with what is seen in melanoma, single target inhibition of the MAPK signaling cascade in NSCLC is inferior to a doublet strategy in the 2% of NSCLC cases with BRAF V600E mutations. The use of dabrafenib in combination with trametinib is now FDA-approved for treatment in advanced NSCLC with a V600E BRAF mutation.[13]

While approximately 12% of colorectal cancers exhibit the BRAF V600E mutation, treatment with single-agent BRAF inhibitors has not demonstrated therapeutic efficacy.[14] However, recent studies investigating the use of a BRAF inhibitor in combination with other inhibitors of the MAPK pathway have demonstrated increased efficacy. The BEACON clinical trial regimen combines the BRAF inhibitor encorafenib, the MEK inhibitor binimetinib, and the EGFR antibody cetuximab. Results from the Phase III portion of the study demonstrated an overall survival benefit in BRAF-mutated colorectal cancer with the triplet therapy as compared to standard second-line chemotherapy.[15] Further analyses showed that the doublet of encorafenib with cetuximab had equivalent benefit to the triplet regimen while reducing toxicities. As a result, current guidelines recommend the doublet therapy for the treatment of these patients.[16]

The adoption of large-panel next-generation sequencing by oncologists has increased the identification of non-V600E BRAF mutations in cancer patients. These mutations have no standard therapeutic options; however, they have been shown to possess characteristics[17] that could allow the prediction of response to various agents. Delineation of pathogenic BRAF mutations to three separate classes been discussed previously and may serve as a template to guide therapeutic strategies.[18–20] These studies propose to separate the mutations based on three biochemical and signal aspects: (a) kinase activity, (b) RAS-dependency, and (c) dimerization status. Class I mutations are at the V600 codon and exhibit extremely strong kinase activity by stimulating monomeric activation of BRAF. Class II are non-V600 mutations that activate BRAF to signal as a RAS-independent dimer, with a weaker kinase activity and downstream phosphorylation effects than seen in Class I. Finally, Class III mutations are deemed "kinase-dead" or low activity as compared to wild type. Class III mutations cannot directly phosphorylate MEK, but retain activity to bind CRAF in a RAS-dependent manner and may still contribute to oncogenesis through activation of CRAF.[21]

In addition to a review of the literature, here we describe a large international clinical genomics database of 4517 canonical and non-canonical BRAF mutations seen among 114,662 cases of cancers. We correlate these findings with the current literature surrounding the classes of BRAF mutations and describe current treatment recommendations for each type of BRAF mutation. This large dataset provides robust real-world incidence rates of BRAF mutations by both type of malignancy and mutation classification, adding to the understanding of these mutations and how they affect treatment selection.

Materials and methods

Case selection

A total of 114,662 tumors with next-generation sequencing results performed by a commercial CLIA-certified laboratory (Caris Life Sciences, Phoenix, AZ) from December 2012 to July 2019 were retrospectively analyzed for molecular alterations in the BRAF gene. Formalin-fixed paraffin-embedded (FFPE) samples were sent for analysis as part of standard care from treating physicians around the world. The tissue diagnoses were submitted on the basis of pathologic assessment of physicians who requested the assays and were further verified by a board-certified oncological pathologist at the central laboratory.

Next-generation sequencing

NGS was performed on genomic DNA isolated from FFPE tumor samples using the NextSeq or MiSeq platform (Illumina, Inc., San Diego, CA). For NextSeq sequenced tumors ($n = 77{,}828$), a custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, CA). For MiSeq sequenced tumors ($n = 37{,}122$), specific regions of 47 genes were amplified using the customized Illumina TruSeq Amplicon Cancer Hotspot panel. All variants were detected with > 99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of > 500 and an analytic sensitivity of 5%. Prior to molecular testing, tumor enrichment was achieved by harvesting targeted tissue using manual microdissection techniques. Genetic variants identified were interpreted by board-certified molecular geneticists and categorized as "pathogenic," "presumed pathogenic," "variant of unknown significance," "presumed benign," or "benign," according to the American College of Medical Genetics and Genomics (ACMG) standards.

Classification of the BRAF variants was based on review of clinical and preclinical studies: class 1 mutations are

those that affect BRAF V600 position and have shown to activate downstream pathway as monomers; class 2 mutations are those that function as RAS-independent dimers with reported increased kinase activity in clinical or preclinical studies; class 3 mutations show impaired or diminished kinase activity and activate the MAPK pathway through enhanced RAS binding.

Results

A total of 4517 out of 114,662 tumors had a pathogenic or presumed pathogenic BRAF mutation (3.9%). The majority of mutations were at the V600 codon (class 1), with 2841 class 1 mutations seen (62.1% of BRAF mutations, 2.4% of total cancers). Class 2 mutations were seen in 746 tumors (16.5% of BRAF mutations, 0.7% of total cancers), and class 3 mutations were seen in 801 tumors (17.7% of BRAF mutations, 0.7% of total cancers). Other pathogenic or presumed pathogenic mutations, that have not been classified into any of the 3 major classes, were seen in 129 cases (2.9% of BRAF mutations).

Table 1 shows the frequency of specific BRAF mutations, grouped by category, seen in the cohort. The most common BRAF mutation was V600E, seen in 2544 tumors (90% of class 1 mutations, 56.3% of all BRAF mutations). V600K was found in 251 patients (8.8% of class 1 mutations), while other V600 mutations (R, L, and D) were seen in a total of 46 patients. Commonly observed class 2 mutations included G469A (160 patients) and K601E (132 patients), making up 21.4% and 17.7% of class 2 patients, respectively. Commonly observed class 3 mutations included D594G (159 patients), D594N (139 patients), and G466V (94 patients), making up 19.9%, 17.4%, and 11.7% of class 3 mutations.

The greatest number of BRAF mutations was found in colorectal cancer ($n = 1280$), melanoma ($n = 1271$), and non-small cell lung cancer ($n = 772$) (Table 2). However, as a fraction of the total number of cases profiled, the highest incidence rates were seen in melanoma (39.7%), thyroid cancer (33.3%), and small intestinal malignancies (8.9%) (Figure 1). While class 1 mutations were the most common mutation class in most cancer types (79.1% of BRAF mutated colorectal cancer, 77.5% of BRAF mutated melanoma, 97% of BRAF mutated thyroid cancer), other cancers had a much lower percentage of class 1 mutations (30.7% of BRAF-mutated lung cancer, 39.7% of BRAF-mutated pancreatic cancer) (Figure 2). The distribution of BRAF mutations seen in the cohort across the transcribed gene can be seen in the lollipop plot in Figure 3.

Discussion

These data represent the largest aggregation of BRAF mutations within a single clinical database to our knowledge. The relative proportions of both BRAF V600 mutations and non-V600 mutations are informative in all cancers and by malignancy, and can serve as a definitive gold-standard for BRAF mutation cancer incidence by malignancy.

Table 1. Class 1, 2, and 3 BRAF mutations seen in full cohort of 114,662 sequenced patients.

Mutation	Class	N occurences	% of cases
CLASS 1 TOTAL		*2841*	*2.5*
V600E/V600E(2)	1	2544	2.2
V600K	1	251	0.2
V600R	1	42	0
V600L	1	3	0
V600D	1	1	0
CLASS 2 TOTAL		*746*	*0.7*
G469A	2	160	0.1
K601E	2	132	0.1
G469V	2	84	0.1
G469R	2	63	0.1
G464V	2	38	0
L597R	2	34	0
K601N	2	30	0
E586K	2	20	0
T599dup	2	19	0
L597Q	2	19	0
V600_K601delinsE	2	18	0
L485F	2	16	0
N486_P490del	2	14	0
G464R	2	13	0
V471F	2	10	0
K601Q	2	3	0
Q257R	2	3	0
T599R	2	3	0
V487_P492delinsA	2	3	0
L597S	2	3	0
G464A	2	2	0
K499E	2	2	0
L505H	2	2	0
K601T	2	2	0
V600_K601delinsEN	2	1	0
V600_S605delinsEISRWR	2	1	0
L505F	2	1	0
L485_P490delinsY	2	1	0
I463S	2	1	0
CLASS 3 TOTAL		*801*	0.7
D594G	3	159	0.1
D594N	3	139	0.1
G466V	3	94	0.1
N581S	3	81	0.1
G466E	3	67	0.1
G596R	3	46	0
N581I	3	43	0
G466R	3	33	0
G466A	3	28	0
S467L	3	18	0
G469E	3	17	0
N581Y	3	16	0
K483E	3	14	0
D594A	3	10	0
D594E	3	10	0
D594H	3	8	0
F595L	3	5	0
D287H	3	4	0
D594V	3	3	0
G596C	3	3	0
T599A	3	2	0
N581K	3	1	0
Other Pathogenic/ Presumed Pathogenic		*129*	*0.1*
Total		4517	3.9

Table 2. Relative prevalence of BRAF mutation by type and by cancer type.

Cancer types	N of tumors sequenced	BRAF (ALL)	CLASS 1	CLASS 2	CLASS 3	Other pathogenic
Melanoma	3203	1271	985	160	101	25
Thyroid carcinoma	496	165	161	4	0	0
Multiple myeloma	39	6	3	1	2	0
Small intestinal malignancies	742	66	11	18	33	4
Colorectal adenocarcinoma	14,680	1280	1012	80	171	17
Cancer of unknown primary	2894	120	68	22	25	5
Non-small cell lung cancer	18,944	772	237	264	237	34
Cholangiocarcinoma	2068	79	38	12	27	2
Low grade glioma	478	15	12	2	0	1
Others	1551	40	23	10	6	1
High grade glioma	3186	66	55	4	7	0
Lymphoma	295	6	2	4	0	0
Gastric adenocarcinoma	1791	34	10	11	10	3
Neuroendocrine tumors	1956	35	22	7	5	1
Pancreatic adenocarcinoma	4565	68	27	27	8	6
Bladder cancer	1959	28	1	14	9	4
Ovarian surface epithelial carcinomas	16,583	235	122	33	69	11
Prostatic adenocarcinoma	2009	27	0	19	3	5
Uveal melanoma	268	3	2	1	0	0
Non-melanoma, non-Merkel skin cancers	460	5	1	2	1	1
Small cell lung cancer	1015	8	2	2	4	0
Uterine cancers	10,889	82	7	23	43	9
Cervical cancer	1862	13	1	5	7	0
Soft tissue tumors	2439	15	10	3	2	0
Esophageal and esophagogastric junction	2399	13	3	2	8	0
Breast carcinoma	10,478	42	18	8	16	0
Kidney cancer	1312	4	1	2	1	0
Head and neck cancers	1487	4	1	1	2	0
All cancer types	114,662	4517	2841	746	801	129

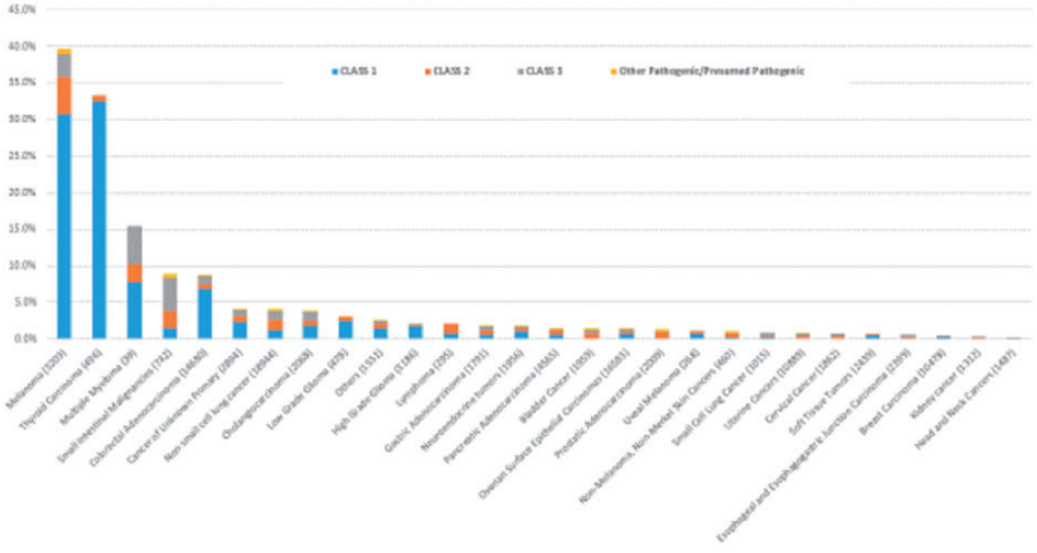

Figure 1. Prevalence of class 1, 2, and 3 BRAF mutations in various cancer types.

It has previously been reported that pathogenic BRAF mutations are present in about 8% of all cancers.[4,22] Our dataset shows a lower rate of mutation in all cancers, with only 3.9% demonstrating pathogenic mutations. It has also been reported that V600E mutations account for 80–90% of all BRAF mutations,[4,23] where our data show that non-V600E mutation (including other Class I mutations as well as Class II and III) can be more frequent. The reported frequencies of non-V600E mutations vary extensively, with ranges between 1.7 and 30%.[24–28] Our data suggest that V600E mutations represent only 56% of all BRAF mutations in cancer, though the relative percentage was higher in those malignancies known to have large numbers of BRAF mutations (melanoma,

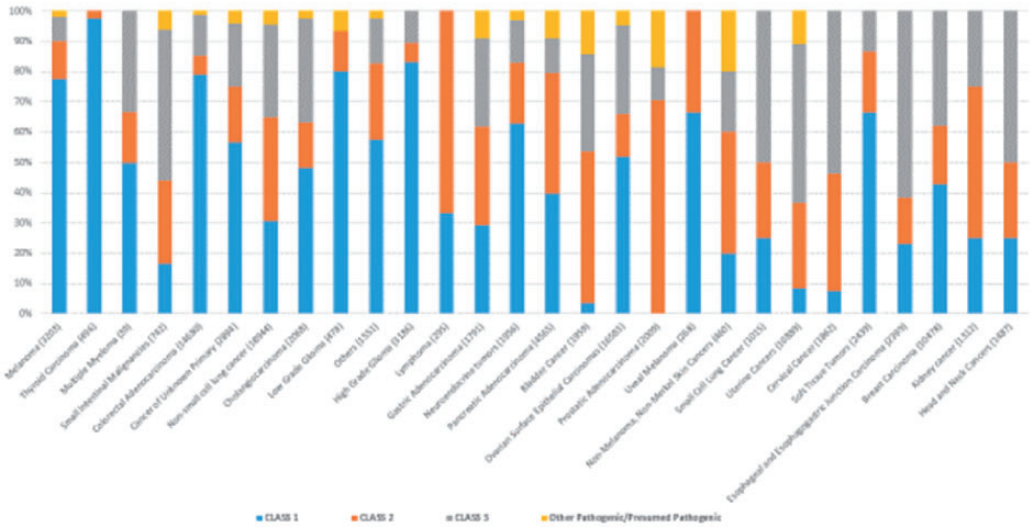

Figure 2. Relative proportion of classes of BRAF mutation by tumor type.

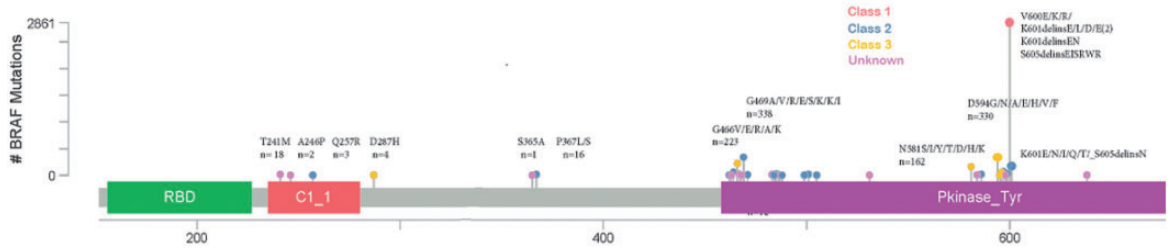

Figure 3. Type and frequency of mutations seen in the database in the BRAF protein. Mutations are plotted along the transcript of the protein by location in the transcript. Individual points ("lollipops") represent the number of mutations at the specific amino acid locus seen in the database. Lollipop color is by class of mutation. Of note, Class 1 mutations are limited to the V600 locus, while class 2 and 3 mutations occur at various other points along the transcript. RBD: Raf-like RAS binding domain; C1_1: phorbol esters/diacylglycerol binding domain (binds the secondary messengers that can stimulate PKC); Pkinase_Tyr: protein tyrosine kinase domain. Figure adapted from cBioportal Mutation Mapper (https://www.cbioportal.org/mutation_mapper).

colorectal and thyroid cancers), and in these malignancies both the overall and relative percentages are similar to previous reports.[4,29–31]

These differences may be due to variations in and specific limitations of assays historically utilized to detect mutations and to the increased clinical use of testing with NGS assays resulting in testing of tumor types not historically tested for BRAF. The previous most common testing method was via the FDA-approved COBAS assay. This assay was designed specifically to detect the V600E alteration and was found to be insufficient in testing for other mutations such as V600K.[17,32,33] It is possible that the current database was slightly biased against BRAF mutations, especially in melanoma, colorectal cancer, and thyroid cancer as some tumors may have been tested using the FDA-approved COBAS test, or a similar assay limited to V600, and sent for NGS testing only when negative. However, the similar rates of BRAF mutation in the FDA-approved indications speak to a minimal effect of selection bias prior to NGS. We believe, therefore, that rather than our dataset underestimating the rate of BRAF pathogenic mutations and overestimating non-V600 mutations, prior reports may have overestimated BRAF mutation rate and underestimated non-V600 rates due to selection bias. These other reports likely overrepresented melanoma, colorectal cancer, and thyroid cancer in their global database, while our real-world database is likely a better representation of the true representation of metastatic cancer in the general population.

The relative percentages and types of mutations reported here are particularly relevant for potential treatment strategies, as the different classes of BRAF mutations can dictate which targeted inhibitors may have therapeutic utility. Recurrent class 1 mutations cause the kinase domain of BRAF to be locked in the active confirmation, leading to strong RAS-independent constitutive phosphorylation and monomeric signaling.[34–38]

Class I mutations including BRAF V600E and V600D have shown an increase in kinase activity by 500–700 fold in *in vitro* studies.[34] V600E mutation increases BRAF activity by forming a salt bridge with K507 at the C-terminal portion of the αC helix,[39] which reproduces the changes that would occur in dimerization providing the ability to signal as a monomer. Importantly, currently approved BRAF therapies function effectively only by inhibiting active monomers.[40–42] This is ideal in limiting off-target effects, but limits their utility in cancers with class II BRAF mutations that require dimerization.[18]

Currently, there are three FDA-approved BRAF inhibitors for patients with class I BRAF V600 mutations in metastatic melanoma: vemurafenib, dabrafenib, and encorafenib.[8,9,12] The mechanism of action for these drugs is as a reversible ATP-competitive inhibitor of BRAF kinase domain. Vemurafenib, dabrafenib, and encorafenib all report about a 50–60% objective response rate in melanoma patients with BRAF V600E/K mutations as well as a significant overall survival benefit.[8,9,40] A small percentage of patients achieved a complete response; however, despite all patients in the study being V600 mutated, intrinsic resistance was seen in about half of the patients.[11,12,43] Even those who did initially respond, the median duration of response to single agent BRAF inhibitors was 5.1 months with dabrafenib, 7.3 months with vemurafenib, and 9.6 months with encorafenib.[9,12,44] There are multiple molecular mechanisms that can lead to inherent or acquired resistance and have been reviewed elsewhere.

Due to high resistance rates, various combination therapies have been explored. Combining BRAF inhibitors with MEK inhibitors, such as cobimetinib, trametinib, or binimetinib, have demonstrated increased therapeutic activity in melanoma and lung cancer,[45–47] while combining with the EGFR inhibitor cetuximab has shown benefit in colorectal cancer.[15] Targeting the MEK protein, which is immediately downstream of BRAF in the MAPK pathway, serves as an effective mechanism to overcome and prevent one of the most common means of resistance. The first MEK inhibitor approved to treat BRAF-mutant patients was trametinib, with the initial indication for single agent use in patients with V600 mutant metastatic melanoma.[11] While the overall response rate and the progression free survival (PFS) for single agent trametinib in BRAF V600 mutant patients was lower than that seen in BRAF inhibitors,[8,11] the combination of trametinib with dabrafenib resulted in an overall response rate of 67%.[10] In addition to an increased response rate, combination therapy also resulted in reduction in select adverse events, particularly cutaneous proliferative events commonly seen with single agent BRAF therapy but uncommonly seen with combination BRAF/MEK therapy.[10,11] The median duration of response with combination therapy was 11.4 months.[48] Pooled overall survival in two studies testing the combination (COMBI-V and COMBI-D) showed a five-year overall survival rate of 34% in the combination arms.[49]

Additional combinations were subsequently approved for BRAF-V600-mutated patients. Vemurafenib plus cobimetinib combination is approved for metastatic melanoma and lung cancer. The approval in melanoma is based on the coBRIM study that demonstrated initial PFS of 12.3 months,[50] with a subsequent study showing an even more dramatic PFS and a median overall survival of 17.4 months.[51] A new combination of encorafenib plus binimetinib has recently been approved for treatment in patients with melanoma (FDA.gov). Median PFS in this study was 12.9 months, with median overall survival not yet reached.[12]

Although the majority of BRAF V600 mutations are V600E, multiple other mutations have been described. These mutations are oncogenic; however, the clinical implications of the different mutations are still largely unclear. The second most common BRAF V600 mutation is the V600K, which substitutes the native valine for lysine.[4,24,52] The prevalence of this mutation has been reported to vary in different populations, being rare in most groups, but more frequent in others such as Australian Caucasians.[17,26,53] This is likely due to environmental and social factors such as length and types of sun exposure. Menzies *et al*.[54] compared patients with V600E to V600K and noted an association with distinct clinicopathological features including age at diagnosis, decreased metastases free survival, and primary location of the melanoma. These melanomas were more frequently seen in sun-exposed areas, whereas V600E mutations develop in areas with intermittent sun exposure.

Other rare mutations at V600 including V600D, V600R, and V600M have also been described.[55] The BRAF V600E2 mutation (GAA gene variant)[17] is less frequent than V600E. In our dataset, non-V600E Class 1 mutations account for about 10% of all codon 600 mutations reported in melanoma. In an initial evaluation of BRAF inhibitor use in patients with BRAF V600E compared to V600M, V600R, V600K, and V600E2, no statistically significant difference was seen in the duration of response or PFS.[55] However, a recent retrospective study in 58 patients with melanoma harboring non-E/K V600 mutations showed a response rate of 56% to combination BRAF+MEK inhibition, which is modestly lower than the response rate in BRAF V600E/K-mutated melanoma.[56]

We reported 16.5% of all BRAF mutations in our dataset were class II. These are less activating compared to BRAF V600 mutations and function as RAS-independent dimers.[57] Class II mutants have subsequently been identified as having either intermediate or high kinase activity[18,19] and subdivided to class IIa and class IIb. Class IIa mutations, such as K601E and L597Q, occur within the activation segment of BRAF have higher intrinsic kinase activity due to removal of interactions with the P-loop, leading to a moderate level of MAPK pathway activation. Class IIb (G464, G469) occur within the glycine-rich p-loop.[18,19,34] Comparatively, class IIb mutants activate the MAPK pathway to a lower extent, but still increased when compared to wild-type BRAF. It has been hypothesized that cancers with these types of mutations may be sensitive to MEK inhibition, with or without BRAF inhibitors, and early studies have shown some responses in patients.[56]

A study by Hallmeyer *et al*. enrolled 31 patients with non-V600E BRAF mutations with 42% with a V600K mutations. The response rate of 23% with vemurafenib alone was similar in patients with and without a V600K mutation, including a small number of responses in Class II mutations (L597S and D594G).[58] On the other hand, in a small retrospective study of non-V600 BRAF mutant melanomas, most of which were class II, there were no responses to BRAF inhibition alone (0/22), while responses were seen with either MEK inhibition alone (2/5) or BRAF + MEK inhibition (5/18).[56] These data suggest that while current BRAF inhibitors have little utility against class II mutations, MEK inhibition may be potentially beneficial, which has been observed in other case series.[59–61] Currently, RAF dimer

inhibitors are being developed that have shown activity in Class II mutations *in vitro*.[62,63]

Class III mutations, which represent 17% of our dataset, are kinase-dead (D594G/N) or low activity (G466V/E) when compared to wild-type BRAF.[34,63] These mutations increase MAPK signaling via wild-type RAF by forming RAF heterodimers between mutant and wild-type proteins. When compared to activating BRAF mutants, low activity BRAF mutant cell signaling occurs in a RAS-dependent manner. Increased binding of class III BRAF mutants to activated RAS is associated with formation of heterodimers of mutant BRAF and wild-type CRAF.[19] Subsequently, ERK activation by these mutants is also CRAF-dependent. Pharmacokinetic inhibition of RAF led to paradoxically active wild-type dimers due to class III BRAF mutants having enhanced RAS dimerization. Because ERK activation requires upstream RAS activation, low-activity and kinase-dead BRAF mutations often co-occur with mutations that dysregulate MAPK signaling such as NF1 deletion/loss-of-function mutation or activating RAS mutation.[19] However, in-human activity in directly inhibiting kinase-dead mutations is limited. Various MAPK inhibition combination strategies may be promising in some tumor specific settings.

The rate of BRAF mutation in human cancer in a real-world large database is lower than previously reported, likely representing testing more broadly across tumor types. The relative percentages of Class II and Class III BRAF mutations are higher than previously reported, representing almost 35% of BRAF mutations in cancer. These findings provide support for the development of effective treatments for non-V600 mutations in cancer.

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DECLARATION OF CONFLICTING INTERESTS

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