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BRAF^{V600} mutations in solid tumors, other than metastatic melanoma and papillary thyroid cancer, or multiple myeloma: a screening study

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Background: Mutations in the *BRAF* gene have been implicated in several human cancers. The objective of this screening study was to identify patients with solid tumors (other than metastatic melanoma or papillary thyroid cancer) or multiple myeloma harboring activating *BRAF*^{V600} mutations for enrollment in a vemurafenib clinical study.

Methods: Formalin-fixed, paraffin-embedded tumor samples were collected and sent to a central laboratory to identify activating *BRAF*^{V600} mutations by bidirectional direct Sanger sequencing.

Results: Overall incidence of *BRAF*^{V600E} mutation in evaluable patients (n=548) was 3% (95% confidence interval [CI], 1.7–4.7): 11% in colorectal tumors (n=75), 6% in biliary tract tumors (n=16), 3% in non-small cell lung cancers (n=71), 2% in other types of solid tumors (n=180), and 3% in multiple myeloma (n=31). There were no *BRAF*^{V600} mutations in this cohort of patients with ovarian tumors (n=68), breast cancer (n=86), or prostate cancer (n=21).

Conclusion: This multicenter, national screening study confirms previously reported incidences of *BRAF*^{V600} mutations from single-center studies. Patients identified with *BRAF*^{V600} mutations were potentially eligible for enrollment in the VE-BASKET study.

Keywords: genetic testing, proto-oncogene proteins B-raf, PLX4032

Introduction

Mutations of the *BRAF* gene were first identified and implicated in human cancers by Davies et al in 2002.[1] *BRAF*, which has been implicated in human cancer, is one of three highly conserved serine–threonine protein kinase genes (*ARAF*, *BRAF*, and *CRAF*) in the RAS–RAF–MEK–ERK cascade.[2] Mutations in the *BRAF* gene have been reported in 7%–15% of all human cancers, with melanoma having one of the highest incidences (40%–70%).[1,2] The most common locus of mutation is at position V600, causing constitutive hyperactivation, proliferation, survival, and oncogenic transformation.[2]

Other tumor types with a marked prevalence of *BRAF*^{V600} mutations include hairy cell leukemia (79%–100%),[3,4] histiocytic conditions, including Erdheim–Chester disease and Langerhans cell histiocytosis (55%),[5] papillary thyroid carcinoma (45%),[6] serous ovarian cancer (35%),[7] colorectal cancer (12%),[1] non-small cell lung cancer (NSCLC; up to 5%),[8] and multiple myeloma (4%).[9] Oncogenic *BRAF*^{V600} mutations are often associated with an aggressive phenotype and shorter disease-free and overall survival than the wild type.[4,6,8,10]

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Development and approval of BRAF inhibitors such as vemurafenib[11] and dabrafenib[12] for the treatment of patients with metastatic melanoma have increased the interest in evaluation of these treatments in other solid tumors with *BRAF* mutations. Previous studies regarding the incidence of *BRAF* mutations in other malignancies were primarily conducted in single centers or with databases that extract mutation data from published reports. Herein, results from a large, multicenter, prospective, national screening study conducted in patients with solid tumors (other than metastatic melanoma or papillary thyroid carcinoma) and multiple myeloma are presented.

Methods

Study design

Twenty oncology specialty centers (19 community centers and one academic center [Vanderbilt University]) in the US were the sites in this study. Patients ≥18 years of age with histologically confirmed solid tumors (other than metastatic melanoma or papillary thyroid cancer) or multiple myeloma were eligible for enrollment. Patients with Eastern Cooperative Oncology Group (ECOG) performance status >2, uncontrolled concurrent malignancy, active/untreated central nervous system metastases, history of or known carcinomatous meningitis, or those who had received prior treatment with a selective BRAF or MEK inhibitor or had an uncontrolled or severe medical illness were excluded from the study. Prior treatment with sorafenib (a multitargeted tyrosine kinase inhibitor) was allowed. Formalin-fixed, paraffin-embedded tumor samples (at least five serially cut, unstained, 5-µm sections) were collected from eligible patients and sent to a central laboratory (Clarient, Inc., Aliso Viejo, CA, USA) to identify *BRAF*^{V600} mutations. Tumor samples were obtained from archival tissue (eg, from the initial diagnosis of cancer) or from fresh biopsy specimens according to institutional standards. Mutations were identified by bidirectional direct Sanger sequencing. Patient characteristics were collected, and included age, cancer diagnosis, and prior cancer treatment. All patients signed written informed consent before enrollment. This study was conducted in compliance with the International Conference on Harmonisation E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki or the laws and regulations of the country in which the research was conducted, whichever afforded the greater protection to the individual. The protocol and the study were approved by the US Oncology Inc. institutional review board and the Vanderbilt University Medical Centre institutional review board. This study was registered with ClinicalTrials.gov (ID, NCT01804140).

Study objectives

The primary objective of this study was to identify patients who had solid tumors (other than metastatic melanoma or papillary thyroid cancer) or multiple myeloma harboring activating *BRAF*^{V600} mutations for enrollment in a phase II clinical study (VE-BASKET; ClinicalTrials.gov ID, NCT01524978).[13] The secondary objective was to provide additional data regarding the incidence and subtype of *BRAF*^{V600} mutations in these tumor types.

Efficacy and safety outcomes

This was a screening study to identify patients with activating *BRAF*^{V600} mutations; therefore, no study treatment was administered. Consequently, there was no formal safety analysis of the study drug. Nonetheless, during the screening/study period, all adverse events (AEs) associated with the sampling or biopsy procedures were monitored and assessed to determine their relationship to study procedures for a maximum of 28 days after an intervention/invasive procedure (biopsy). During this period, any serious AE (SAE) or AE that the investigator considered as having a causal relationship to a protocol-mandated intervention/invasive procedure, regardless of the elapsed time, was reported to the sponsor. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Results

Patients

Between December 2012 and August 2013, 668 patients from 20 US-based institutions provided informed consent: 662 patients were enrolled and 548 patients were evaluable, with mutation results from sampling/biopsy procedures. One hundred fourteen patients were not evaluable because their samples were not available for testing. This includes six patients who died before sampling/biopsy procedures (Figure S1).

Of the 662 patients enrolled, 100 patients (15%) had breast cancer, 100 patients (15%) had NSCLC, 84 patients (13%) had colorectal cancer, 75 patients (11%) had ovarian cancer, 23 patients (3%) had prostate cancer, 37 patients (6%) had multiple myeloma, 17 patients (3%) had a biliary tract tumor (including cholangiocarcinoma), and 226 patients (34%) had other types of solid tumors (Table S1).

Five hundred forty-eight patients were evaluable and were enrolled in the study with mutation results from the sampling/biopsy procedures. Characteristics of evaluable patients at enrollment are summarized in Table 1. Of the 548 evaluable

Table 1 Patient characteristics at enrollment

Characteristic	Tumor type								
	Overall (N=548)	Breast cancer (n=86)	NSCLC (n=71)	Colorectal cancer (n=75)	Ovarian cancer (n=68)	Prostate cancer (n=21)	Multiple myeloma (n=31)	Biliary tract cancer[a] (n=16)	Other cancers (n=180)
Age, median (min–max), years	64 (21–91)	59 (39–89)	65 (41–88)	64 (30–90)	65 (43–89)	70 (59–80)	65 (40–86)	59 (38–87)	66 (21–91)
Sex, n (%)									
Male	221 (40)	2 (2)	38 (54)	36 (48)	0	21 (100)	18 (58)	7 (44)	99 (55)
Female	327 (60)	84 (98)	33 (46)	39 (52)	68 (100)	0	13 (42)	9 (56)	81 (45)
Race, n (%)									
White	485 (89)	70 (81)	59 (83)	68 (91)	62 (91)	19 (90)	28 (90)	15 (94)	164 (91)
Black	47 (9)	13 (15)	10 (14)	4 (5)	5 (7)	2 (10)	2 (6)	1 (6)	10 (6)
Asian	9 (2)	3 (3)	2 (3)	0	0	0	0	0	4 (2)
Other	7 (1)	0	0	3 (4)	1 (1)	0	1 (3)	0	2 (1)
Number of previous lines of therapy, median (min–max)	2 (1–15)	3 (1–14)	2 (1–6)	2 (1–8)	4 (1–15)	4 (1–8)	3 (1–10)	2 (1–3)	2 (1–14)
Number of previous lines of therapy, n (%)									
1	131 (24)	18 (21)	22 (31)	15 (20)	7 (10)	2 (10)	5 (16)	4 (25)	58 (32)
2	154 (28)	18 (21)	27 (38)	27 (36)	13 (19)	4 (19)	10 (32)	6 (38)	49 (27)
3	89 (16)	11 (13)	12 (17)	16 (21)	13 (19)	4 (19)	3 (10)	4 (25)	26 (14)
4	55 (10)	7 (8)	5 (7)	11 (15)	8 (12)	4 (19)	2 (6)	0	18 (10)
≥5	102 (19)	32 (37)	5 (7)	5 (7)	27 (40)	7 (33)	11 (35)	0	15 (8)
Prior cancer treatment, n (%)									
Surgical resection	355 (65)	72 (84)	26 (37)	66 (88)	64 (94)	14 (67)	2 (6)	9 (56)	102 (57)
Systemic therapy	531 (97)	86 (100)	71 (100)	74 (99)	68 (100)	21 (100)	31 (100)	14 (88)	166 (92)
Radiation therapy	256 (47)	65 (76)	43 (61)	28 (37)	10 (15)	16 (76)	15 (48)	5 (31)	74 (41)

Notes: [a]Includes cholangiocarcinoma/cancers of biliary tract; tumors of the gallbladder were classified as "other".
Abbreviations: min, minimum; max, maximum; NSCLC, non-small cell lung cancer.

patients, 3% (95% confidence interval, 1.7–4.7) had tumors with a *BRAF*^{V600} mutation, all of which were of the V600E subtype. *BRAF*^{V600E} mutations were identified in 11% (8/75) of patients with colorectal tumors, 6% (1/16) of patients with biliary tract tumors, 3% (2/71) of patients with NSCLC, 2% (4/180) of patients with other solid tumor types, and 3% (1/31) of patients with multiple myeloma (Table 2). No *BRAF*^{V600E} mutations were identified in patients with ovarian, breast, or prostate cancer. Other subtypes investigated included V600K, V600D, V600R, and other V600 mutations. None of these other *BRAF*^{V600} mutations were identified in the screening population of the study.

Efficacy and safety

This was a screening study. No treatments were administered. Efficacy was not evaluated. No AEs or SAEs resulting from sampling were observed. The study ended early because patient recruitment to the VE-BASKET clinical trial exceeded initial expectations,[13] and it was no longer necessary to screen additional patients.

Discussion

To our knowledge, this is the first national, multicenter screening study to be conducted for *BRAF*^{V600} in multiple tumor types. Of 548 evaluable patients, 16 (3%) had tumors

Table 2 *BRAF*^{V600} mutations overall and by tumor type

BRAF mutation[a]	Tumor type								
	Overall (N=548)	Breast cancer (n=86)	NSCLC (n=71)	Colorectal cancer (n=75)	Ovarian cancer (n=68)	Prostate cancer (n=21)	Multiple myeloma (n=31)	Biliary tract cancer[b] (n=16)	Other cancers (n=180)
BRAF^{V600E}, n (%) [95% CI]	16 (3) [1.7–4.7]	0	2 (3) [0.3–9.8]	8 (11) [4.7–19.9]	0	0	1 (3) [0.1–16.7]	1 (6) [0.2–30.2]	4 (2) [0.6–5.6]

Notes: [a]No other *BRAF*^{V600} mutations were identified; V600K, V600R, V600D, and other V600 mutations were screened for. [b]Includes cholangiocarcinoma/cancers of the biliary tract; tumors of the gallbladder were classified as "other".
Abbreviations: CI, confidence interval (based on Clopper–Pearson estimate); NSCLC, non-small cell lung cancer.

with a *BRAF*^{V600E} mutation. No other *BRAF*^{V600} mutations were identified in this population; however, V600K, V600D, V600R, and V600E(2) have also been identified in patients with metastatic melanoma.[14] *BRAF*^{V600E} mutations were distributed among a range of tumor types, including colorectal cancer (11%), biliary tract cancer (including cholangiocarcinoma, 6%), NSCLC (3%), and multiple myeloma (3%). There were no *BRAF*^{V600} mutations in patients with ovarian tumors, breast cancer, or prostate cancer.

The proportions of patients with *BRAF*^{V600} mutations identified in the screening study were similar to those seen in previously published reports,[1,8–10,15] with the exception of ovarian cancer. It has previously been reported that up to 14% of all ovarian cancers have activating *BRAF* mutations[1] and that *BRAF*^{V600E} mutations are common in low-grade serous ovarian cancer (35%)[7]; however, none of 68 patients of this study were identified with *BRAF*^{V600} mutations. *BRAF*^{V600E} mutations are strongly positively correlated with stage I or II ovarian cancer;[7] the stage, subtype, or grade of patients screened in this study is not available. Additionally, no mutations were found in the 86 patients with breast cancer screened in this study, suggesting that *BRAF* mutations are rare in breast cancer. This finding is supported by data from the Catalogue of Somatic Mutations in Cancer (COSMIC) database (http://cancer.sanger.ac.uk/cosmic), showing documented *BRAF* mutations in ≈1% of almost 5,000 breast cancer samples sequenced to date. *BRAF* mutations have been identified in up to 10% of Asian patients with prostate cancer, but appear to be rare among Caucasian patients.[16–20] The finding of no mutations among 21 patients with prostate cancer is also consistent with data from the COSMIC database, showing documented *BRAF* mutations in ≈1% of almost 2,500 sequenced samples. Limitations of this study include having targeted only mutations at the *BRAF*^{V600} location, which might have excluded patients with mutations at other locations in exon 15 of the *BRAF* allele, and the lack of disease characteristics available for those patients with positive *BRAF*^{V600} mutations.

Patients screened for this study and identified with *BRAF*^{V600} mutations were potentially eligible to be enrolled in the VE-BASKET study, a groundbreaking phase II study. Unlike more traditional study designs, which typically enroll patients with one type of malignancy into cohorts, "basket" design studies can be designed based on the expression of specific cellular mutations, regardless of cancer type.[13]

In conclusion, the results of this screening study suggest that histology-independent trials of rare, molecularly defined populations are feasible and confirm previously reported incidences of *BRAF* mutations in several solid tumors and multiple myeloma, further refining the incidence for ovarian and breast cancers.

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Author contributions

B-MD and IP designed the study; ALC, B-MD, and IP collected the data; B-MD, SA, EM, TR, and IP analyzed the data; AC, B-MD, SA, EM, TR, and IP interpreted the data; SA conducted a literature search; B-MD and SA drafted the figures; and TR provided medical/clinical oversight during the course of the study. All authors contributed toward data analysis, drafting, and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

B-MD and EM are employees of and own stock in Genentech, Inc. SA and TR are employees of Genentech, Inc. IP is a consultant for Roche. ALC reports no conflict of interest in this work.

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Supplementary materials

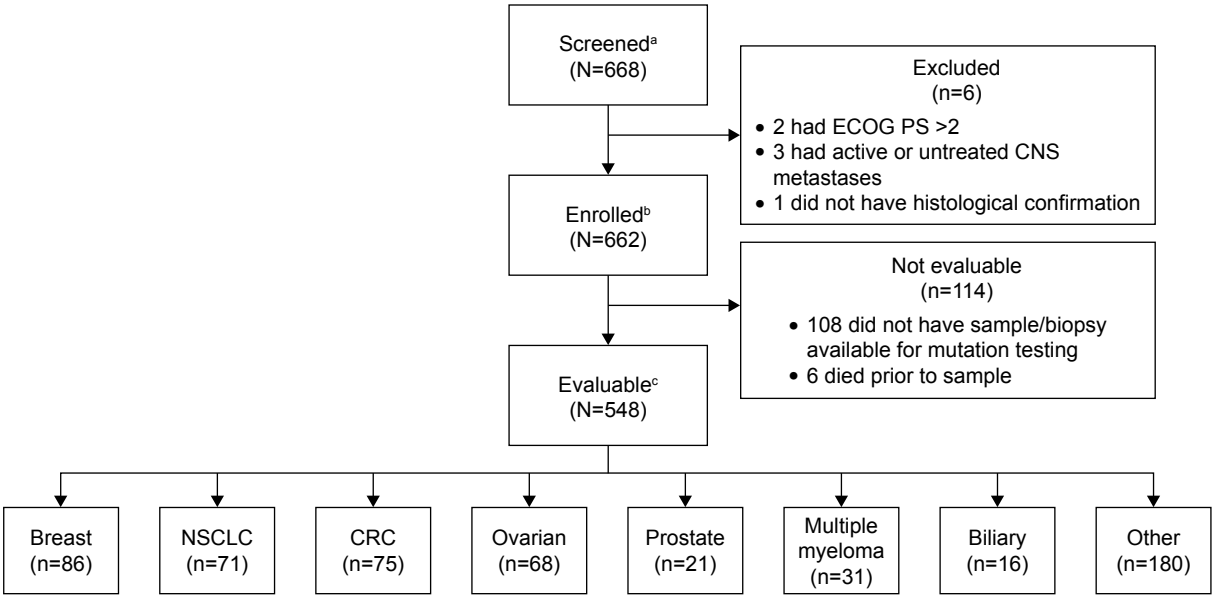

Figure S1 CONSORT diagram.

Notes: [a]Screening population is defined as patients who had signed the informed consent. [b]Enrolled population is defined as patients who met the inclusion/exclusion criteria or provided the tumor samples. [c]Evaluable population is defined as enrolled patients with mutation results from the sampling/biopsy procedures.

Abbreviations: CNS, central nervous system; CRC, colorectal cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status; NSCLC, non-small cell lung cancer.

Table S1 List of other tumor types screened

Tumor type	Patients (n)
Pancreatic cancer	31
Renal cancer	17
SCLC	15
Unknown primary	14
Gastric cancer	13
Bladder cancer	12
Esophageal cancer[a]	12
Soft tissue sarcoma	12
Liver cancer	9
Head and neck cancer	8
Peritoneal cancer	8
Endometrial cancer	6
Sarcoma NOS	6
Chondrosarcoma	4
Gallbladder cancer	4
Glioma	4
Thymic cancer	4
Thyroid cancer	4
Neuroendocrine cancer	4
Small intestine	3
Ampullary cancer	3
Brain cancer	3
Cervical cancer	3
CLL	3
Mesothelioma	3
Vulvovaginal cancer	3
Appendiceal cancer	2
Lung cancer NOS	2
Merkel cell carcinoma	2
Penile cancer	2
Abdominal cancer	1
Adrenocortical carcinoma	1
Anal cancer	1
Ewing's sarcoma	1
Fallopian tube cancer	1
Giant cell sarcoma of bone	1
Langerhans cell histiocytosis	1
Osteosarcoma	1
SCC of the skin	1
Testicular cancer	1

Note: [a]Includes cancer of the gastroesophageal junction.
Abbreviations: CLL, chronic lymphocytic leukemia; NOS, not otherwise specified; SCC, squamous cell carcinoma; SCLC, small cell lung cancer.