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The distribution of *BRAF* gene fusions in solid tumors and response to targeted therapy

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Although the *BRAF* V600E base substitution is an approved target for the BRAF inhibitors in melanoma, *BRAF* gene fusions have not been investigated as anticancer drug targets. In our study, a wide variety of tumors underwent comprehensive genomic profiling for hundreds of known cancer genes using the FoundationOne™ or FoundationOne Heme™ comprehensive genomic profiling assays. *BRAF* fusions involving the intact in-frame BRAF kinase domain were observed in 55 (0.3%) of 20,573 tumors, across 12 distinct tumor types, including 20 novel *BRAF* fusions. These comprised 29 unique 5′ fusion partners, of which 31% (9) were known and 69% (20) were novel. *BRAF* fusions included 3% (14/531) of melanomas; 2% (15/701) of gliomas; 1.0% (3/294) of thyroid cancers; 0.3% (3/1,062) pancreatic carcinomas; 0.2% (8/4,013) nonsmall-cell lung cancers and 0.2% (4/2,154) of colorectal cancers, and were enriched in pilocytic (30%) *vs*. nonpilocytic gliomas (1%; $p < 0.0001$), Spitzoid (75%) *vs*. nonSpitzoid melanomas (1%; $p = 0.0001$), acinar (67%) *vs*. nonacinar pancreatic cancers (<1%; $p < 0.0001$) and papillary (3%) *vs*. nonpapillary thyroid cancers (0%; $p < 0.03$). Clinical responses to trametinib and sorafenib are presented. In conclusion, *BRAF* fusions are rare driver alterations in a wide variety of malignant neoplasms, but enriched in Spitzoid melanoma, pilocytic astrocytomas, pancreatic acinar and papillary thyroid cancers.

BRAF encodes a RAF kinase, which signal downstream of RAS and activate the MAPK pathway, and has emerged as a major oncogenic driver and a potential therapy target in a wide variety of solid tumors and hematological malignancies.[1–4] BRAF signaling is critical for cell division and differentiation and activating BRAF mutations result in uncontrolled growth and tumorigenesis.[2–4] Over 90% of activating *BRAF* mutations in cancer cells occur within the kinase domain at amino acid V600, most commonly resulting in V600E, which is an approved target for the inhibitors dabrafenib and vemurafenib in the treatment of metastatic malignant melanoma.[5–7] Melanomas with other *BRAF* mutations have been shown to respond to BRAF inhibitors, although generally to a lesser extent than tumors harboring V600E.[5–7] Some nonmelanoma malignancies with activating *BRAF* alterations such as V600E have responded to BRAF-targeted therapy,[8–11] whereas others have not.[12]

BRAF gene fusions represent a different mechanism of BRAF activation and have been described in several solid tumor types.[13] However, reports describing the use of anti-BRAF therapies for tumors with *BRAF* fusion alterations have been limited to date. Moreover, first-generation BRAF inhibitors such as sorafenib may not only be ineffective in *BRAF* fusion-driven malignancies, tumor progression may actually be promoted by the use of these agents; thus, drug sensitivity of BRAF fusions remains unclear and controversial.[14] In the following study, genomic profiling of >20,500 malignancies identified *BRAF* gene fusions is in a panorama of tumor types, revealing enrichment in certain histologic subtypes and providing additional examples of response to therapies targeting activated *BRAF* fusions.

Material and Methods

A database of 20,573 consecutive clinical samples of primarily relapsed and refractory solid tumors and hematologic malignancies was evaluated retrospectively to search for *BRAF*

Key words: cancer, solid tumors, *BRAF* fusions, pilocytic astrocytoma, pancreatic acinar carcinoma, Sptizoid melanoma, comprehensive genomic profiling, NGS, targeted therapy
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What's new?

New results may help target a rare genetic alteration that promotes cancer. Activation of the BRAF gene is already known to spur tumor growth, and usually that activation results from a single amino acid substitution. BRAF-inhibiting treatments, then, target that mutation. However, in some cases, BRAF gets revved up by a gene fusion. In our study, the authors tested 20,000 tumors and identified 55 BRAF gene fusions in 12 different tumor types. They found the gene fusions occurred more frequently in certain histologic subtypes, information which will help guide treatment strategies for patients with these tumor subtypes.

gene fusions. Local site permissions to use clinical samples were obtained for our study. Comprehensive genomic profiling (CGP)was performed on all formalin fixed paraffin embedded tissues using a hybrid capture-based next generation sequencing platform (FoundationOne^{TM}) on the Illumina HiSeq2500 instrument.[15] Extracted DNA was adaptor-ligated and capture was performed for all coding exons of 182 cancer-related genes and 37 introns of 14 genes frequently rearranged in cancer (earlier version of the test) or all coding exons from 236 cancer-related and 47 introns of 19 genes frequently rearranged in cancer (current version of the test). Captured libraries were sequenced to a median exon coverage depth of $>600\times$, and resultant sequences were analyzed for base substitutions, insertions, deletions, copy number alterations (focal amplifications and homozygous deletions) and gene fusions, as previously described.[15] The sequence analysis methods and validation of the comprehensive genomic profiling platform used in our study included extensive comparisons to orthogonal methodologies.[14] Base substitution detection is performed using a Bayesian methodology, which allows detection of novel somatic mutations at low mutant allele frequency (MAF) and increased sensitivity for mutations at hotspot sites through the incorporation of tissue-specific prior expectations.[15] Reads with mapping quality <25 are discarded, as are base calls with quality ≤ 2. Final calls are made at MAF $\geq 5\%$ (MAF $\geq 1\%$ at hotspots) after filtering for strand bias (Fisher's test, $p < 1e{-}6$), read location bias (KS test, $p < 1e{-}6$), and presence in two or more normal controls. To detect indels, *de novo* local assembly in each targeted exon is performed using the de-Bruijn approach.[16,17] After read pairs are collected and decomposed, the statistical support for competing haplotypes is evaluated and candidate indels are aligned against the reference genome. Filtering of indel candidates is carried out as described for base substitutions. Gene amplifications and homozygous deletions are detected by comparing complete chromosomal copy number maps to reference process-matched normal control samples. Finally, gene fusions and rearrangements are detected by analysis of chimeric read pairs as follows.[15] Genomic rearrangements are identified by analyzing chimeric read pairs (read pairs for which reads map to separate chromosomes, or at a distance of over 10 kbp). Pairs are clustered by genomic coordinate of the pairs, and clusters containing at least five chimeric pairs (three for known fusions) are identified as rearrangement candidates. Filtering of candidates is performed by mapping quality (MQ >30) and distribution of alignment positions (standard deviation >10). Rearrangements are annotated for predicted function (e.g. creation of fusion gene).

Clinically relevant alterations were defined as those that could be targeted using anticancer therapies currently on the market for any tumor type with known primary site or alterations required for entry in a mechanism-driven registered clinical trial. Local site permissions to utilize clinical samples and approval by the Albany Medical College IRB to analyze and report patient data were obtained for our study. The frequencies of *BRAF* fusions in the various tumor types were evaluated for significance using the Fisher's exact test.

Results

BRAF fusions containing the intact BRAF kinase domain were observed in 55 (0.3%) of the 20,573 tumors (Table 1, Supporting Information Table 1 and Fig. 1), including 20 novel *BRAF* fusions. These comprised 29 unique 5′ fusion partners, of which 31% (9) were known and 69% (20) were novel. The median age of the 55 patients whose tumors harbored *BRAF* fusions was 56 years with a range of 1–83 years, with 29 (53%) of those patients female and 26 (47%) male. The primary tumor was sequenced in 33 (60%) of the cases and a metastasis biopsy was sequenced in 22 (40%). Of the 430 distinct tumor types evaluated in our study (Supporting Information Table 2), *BRAF* fusions were distributed across 12 (3%) tumors including melanoma (3%; 14/531); glioma (2%; 15/701); thyroid cancers (1.0%; 3/294); pancreatic carcinoma (0.3%; 3/1,062); nonsmall-cell lung cancer (0.2%; 8/4,013) and colorectal cancers (0.2%; 4/2,154). Additional tumor types for which multiple samples were found with *BRAF* fusions included breast carcinomas and unknown primary carcinomas. Tumor types featuring only a single case included in data analysis with *BRAF* fusion included esophageal, prostatic carcinoma, head and neck carcinoma and soft tissue sarcoma. Four additional *BRAF* fusions were more recently identified each in a single tumor type as follows: rectal adenocarcinoma, uterine endometrial adenocarcinoma, ovarian serous carcinoma and pleural malignant mesothelioma. These four cases, numbers 56 through 59 are included in Table 1 and Supporting Information Table 2, but are not included in the statistical frequency and data analysis due to the fact that these fusions have not been fully characterized.

Table 1. Fifty-five cases of solid tumors with *BRAF* gene fusions

Case number	Tumor group	Histologic diagnosis	Gender	Age	Sample source	Fusion
1	Breast carcinoma	Breast invasive ductal carcinoma (IDC)	F	62	Metastasis	*KIAA1549-BRAF*
5	Breast carcinoma	Breast carcinoma (NOS)	F	61	Metastasis	*KIAA1549-BRAF*
4	Colorectal carcinoma	Colon adenocarcinoma (CRC)	M	56	Primary	*MKRN1-BRAF*
2	Colorectal carcinoma	Colon adenocarcinoma (CRC)	F	71	Metastasis	*TRIM24-BRAF*
6	Colorectal carcinoma	Colon adenocarcinoma (CRC)	F	52	Metastasis	*TRIM24-BRAF*
3	Colorectal carcinoma	Colon adenocarcinoma (CRC)	F	59	Primary	*AGAP3-BRAF*
7	Esophageal carcinoma	Esophagus adenocarcinoma	M	61	Primary	*ZC3HAV1-BRAF*
18	Glioma	Brain desmoplastic infantile ganglioglioma	F	5	Primary	*KIAA1549-BRAF*
12	Glioma	Brain pilocytic astrocytoma	M	17	Primary	*KIAA1549-BRAF*
19	Glioma	Brain pleomorphic xanthoastrocytoma	F	64	Primary	*KIAA1549-BRAF*
20	Glioma	Spinal cord low-grade glioma (NOS)	M	4	Primary	*KIAA1549-BRAF*
14	Glioma	Brain pilocytic astrocytoma	M	31	Primary	*AKAP9-BRAF*
8	Glioma	Brain pleomorphic xanthoastrocytoma	M	2	Primary	*CCDC6-BRAF*
17	Glioma	Brain pilocytic astrocytoma	F	2	Primary	*KIAA1549-BRAF*
21	Glioma	Spinal cord low-grade glioma (NOS)	M	8	Primary	*KIAA1549-BRAF*
11	Glioma	Brain pilocytic astrocytoma	M	6	Primary	*KIAA1549-BRAF*
15	Glioma	Brain pilocytic astrocytoma	M	8	Primary	*KIAA1549-BRAF*
13	Glioma	Brain pleomorphic xanthoastrocytoma	M	21	Primary	*AGK-BRAF*
9	Glioma	Not pilocytic. Anaplastic oligodendroglioma	M	20	Primary	*AGK-BRAF*
16	Glioma	Brain pilocytic astrocytoma	F	2	Primary	*KIAA1549-BRAF*
43	Glioma	Brain pilocytic astrocytoma	M	1	Primary	*KIAA1549-BRAF*
10	Glioma	Not pilocytic. Anaplastic ganglioglioma	F	47	Primary	*KIAA1549-BRAF*
22	Head & Neck Carcinoma	Head and neck neuroendocrine carcinoma	F	53	Primary	*MKRN1-BRAF*
23	Lung Carcinoma	Lung adenocarcinoma	F	60	Metastasis	*EPS15-BRAF*
29	Lung Carcinoma	Lung nonsmall-cell lung carcinoma (NOS)	M	69	Primary	*NUP214-BRAF*
26	Lung Carcinoma	Lung adenocarcinoma	F	69	Primary	*ARMC10-BRAF*
28	Lung Carcinoma	Lung adenocarcinoma	M	70	Primary	*BTF3L4-BRAF*
27	Lung Carcinoma	Lung adenocarcinoma	F	83	Primary	*AGK-BRAF*
24	Lung Carcinoma	Lung adenocarcinoma	M	68	Metastasis	*GHR-BRAF*
25	Lung Carcinoma	Lung adenocarcinoma	F	66	Primary	*ZC3HAV1-BRAF*
30	Lung Carcinoma	Lung nonsmall-cell lung carcinoma (NOS)	M	73	Primary	*TRIM24-BRAF*
35	Melanoma	Cutaneous melanoma Spitzoid	F	62	Primary	*TRIM24-BRAF*
39	Melanoma	Mucosal melanoma non-Spitzoid	F	56	Metastasis	*ZNF767-BRAF*
49	Melanoma	Cutaneous melanoma non-Spitzoid	M	63	Metastasis	*CCDC91-BRAF*
34	Melanoma	Cutaneous melanoma Spitzoid	F	25	Primary	*DYNC1I2-BRAF*
32	Melanoma	Cutaneous melanoma Spitzoid	F	60	Metastasis	*AKAP9-BRAF*
38	Melanoma	Cutaneous melanoma Spitzoid	F	46	Metastasis	*ZKSCAN1-BRAF*
51	Melanoma	Unknown primary melanoma	M	N/A	Metastasis	*GTF2I-BRAF*
42	Melanoma	Cutaneous melanoma non-Sptizoid	M	54	Metastasis	*AGAP3-BRAF*
37	Melanoma	Cutaneous melanoma Spitzoid	F	44	Metastasis	*AGK-BRAF*
41	Melanoma	Cutaneous melanoma Spitzoid	M	27	Metastasis	*MZT1-BRAF*
31	Melanoma	Cutaneous melanoma Spitzoid	F	52	Metastasis	*AGK-BRAF*
33	Melanoma	Cutaneous melanoma non-Spitzoid	F	1	Primary	*RAD18-BRAF*
40	Melanoma	Cutaneous melanoma Spitzoid	F	60	Metastasis	*CUX1-BRAF*

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Table 1. Fifty-five cases of solid tumors with *BRAF* gene fusions (Continued)

Case number	Tumor group	Histologic diagnosis	Gender	Age	Sample source	Fusion
36	Melanoma	Cutaneous melanoma Spitzoid	F	30	Metastasis	*SLC12A7-BRAF*
47	Pancreatic carcinoma	Pancreas ductal adenocarcinoma	M	63	Primary	*MYRIP-BRAF*
46	Pancreatic carcinoma	Pancreas acinar cell carcinoma	F	75	Primary	*SND1-BRAF*
45	Pancreatic carcinoma	Pancreas acinar cell carcinoma	M	67	Metastasis	*SND1-BRAF*
48	Prostatic carcinoma	Prostate acinar adenocarcinoma	M	57	Metastasis	*NUB1-BRAF*
50	Sarcoma	Malignant solid fibrous tumor	F	56	Primary	*KIAA1549-BRAF*
53	Thyroid carcinoma	Thyroid papillary carcinoma	M	61	Primary	*KLHL7-BRAF*
54	Thyroid carcinoma	Thyroid papillary carcinoma	M	67	Primary	*TANK-BRAF*
52	Thyroid carcinoma	Thyroid papillary carcinoma	F	64	Metastasis	*RBMS3-BRAF*
44	Unknown primary carcinoma	Unknown primary, adenocarcinoma	F	N/A	Metastasis	*STRN3-BRAF*
55	Unknown primary carcinoma	Unknown primary, carcinoma (NOS)	M	65	Metastasis	*SND1-BRAF*
S1	Pleura mesothelioma	Pleura mesothelioma	F	48	Primary	*STK35-BRAF*
S2	Rectum adenocarcinoma	Rectum adenocarcinoma	M	56	Metastasis	*ETFA-BRAF*
S3	Uterus endometrial carcinoma	Uterus endometrial adenocarcinoma (NOS)	F	74	Metastasis	*SVOPL-BRAF*
S4	Ovary serous carcinoma	Ovary serous carcinoma	F	62	Metastasis	*JHDM1D-BRAF*

Cases **S1–S4** are supplemental, have not been fully characterized and were not included in the data analysis.

Melanomas

The 14 melanomas harbored *BRAF* fusions and 9 (64%) featured an epithelioid and spindle cell histology characteristic of the so-called Spitzoid melanoma (Fig. 2*a*). For the 531 melanomas evaluated, the enrichment of *BRAF* fusions in Spitzoid melanomas (9/12, 75%) compared to non-Sptizoid tumors (5/519, 1%) was highly significant ($p = 0.0001$). *BRAF* base substitution alterations were identified in 191/531 (36%) melanomas analyzed.

Gliomas

Of the 15 gliomas with *BRAF* fusions detected in our study, 7 (47%) were pilocytic astrocytomas (Fig. 2*b*). Of the 701 gliomas analyzed, the enrichment of *BRAF* fusion in pilocytic astrocytomas (7/23; 30%) compared to the nonpilocytic gliomas (8/678; 1%) was highly significant ($p < 0.0001$). In addition, 3 (38%) of the 8 nonpilocytic gliomas harboring *BRAF* fusions featured high grade anaplastic astrocytoma histology with large histocytic-like giant cells in the pattern of the pleomorphic xanthoastrocytoma. Of the entire set of gliomas evaluated, 28 (4%) featured base substitution alterations in *BRAF*.

Nonsmall-cell lung carcinomas

BRAF fusions were identified in <1% of NSCLC samples. In contrast, 270/4,013 (7%) NSCLC harbored *BRAF* base substitution alterations. All NSCLC with *BRAF* fusions were adenocarcinomas or NSCLC with adenocarcinoma features. *BRAF* fusions were not seen in squamous or small cell lung cancers.

Colorectal carcinomas

Less than 1% of the 2,154 CRC tumors evaluated harbored *BRAF* fusions, in contrast to the 284 (13%) of the CRC that featured *BRAF* base substitution alterations. There were no distinctive morphologic features in the CRC tumors with *BRAF* fusions.

Pancreatic carcinomas

Of 1,062 pancreatic cancers, 3 featured *BRAF* fusions; this subset comprised 2 (67%) acinar carcinomas (Fig. 2*c*) and 1 (33%) ductal adenocarcinoma. The cohort of pancreatic tumors analyzed featured only three acinar carcinomas, and the enrichment of *BRAF* fusions in acinar carcinomas (2/3; 67%) compared to nonacinar carcinomas (1; <0.1%) was significant ($p < 0.0001$).

Thyroid carcinomas

The three thyroid carcinomas with *BRAF* fusions identified in our study were papillary thyroid carcinomas (3/94; 3%), with no fusions identified in nonpapillary thyroid carcinomas (0/200; 0%) ($p = 0.03$). In contrast, *BRAF* base substitutions were found in 82 (28%) of the total thyroid tumors with 65 (79%) of these mutations identified in papillary thyroid carcinomas and 17 (21%) in nonpapillary thyroid tumors. Information pertaining to radiation exposure in the thyroid cancer patients was not available for our study.

Figure 1 summarizes the exon composition of the *BRAF* fusions identified in our study, all 55 of which preserved an intact BRAF kinase domain, encoded by exons 11–18, and are considered activating. Fusions between *KIAA1549* and *BRAF*

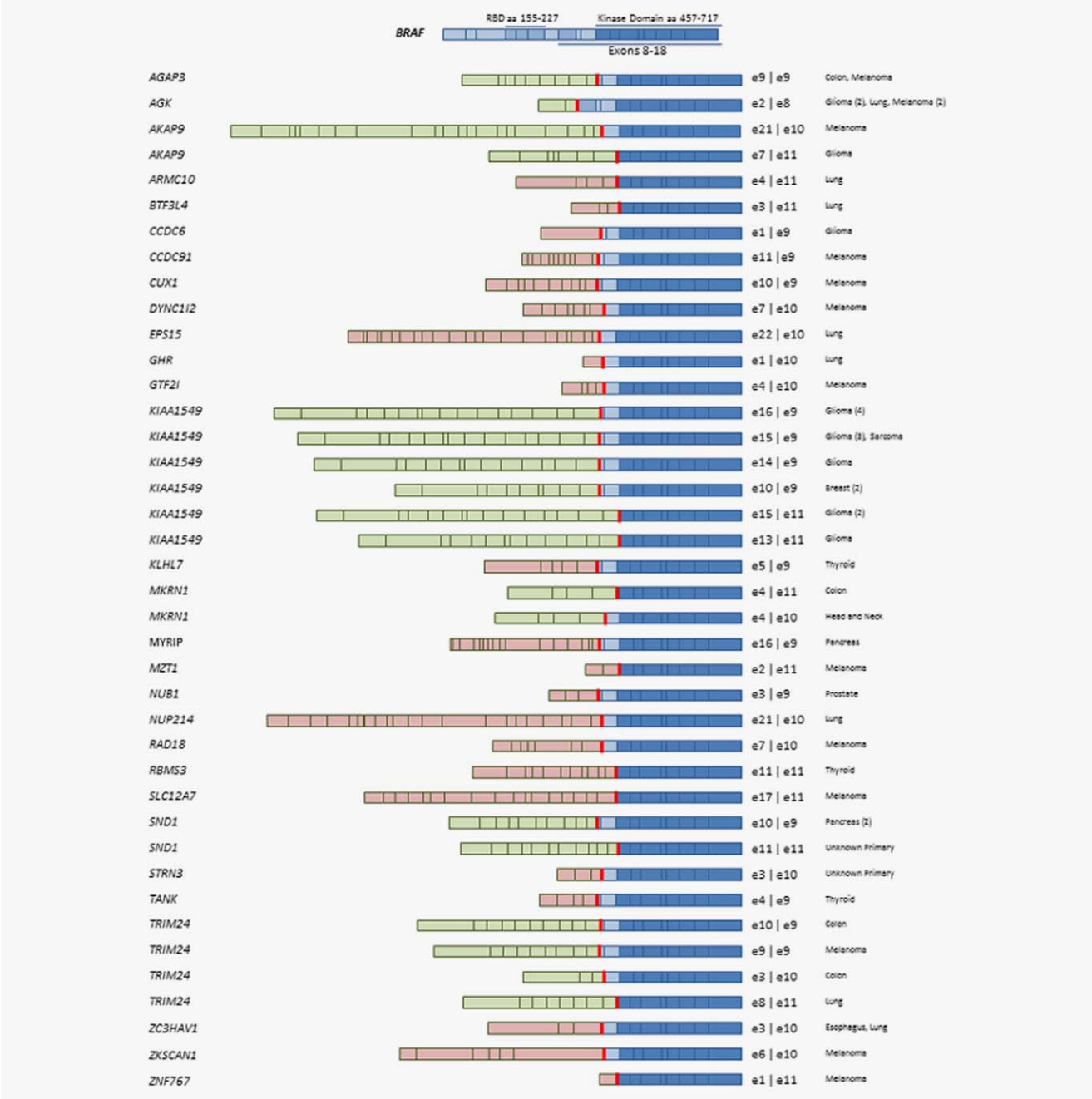


Figure 1. Structure of 55 *BRAF* fusions discovered from 20,573 solid tumors detected by comprehensive genomic profiling. Novel fusions were in pink, and known fusions were in green.

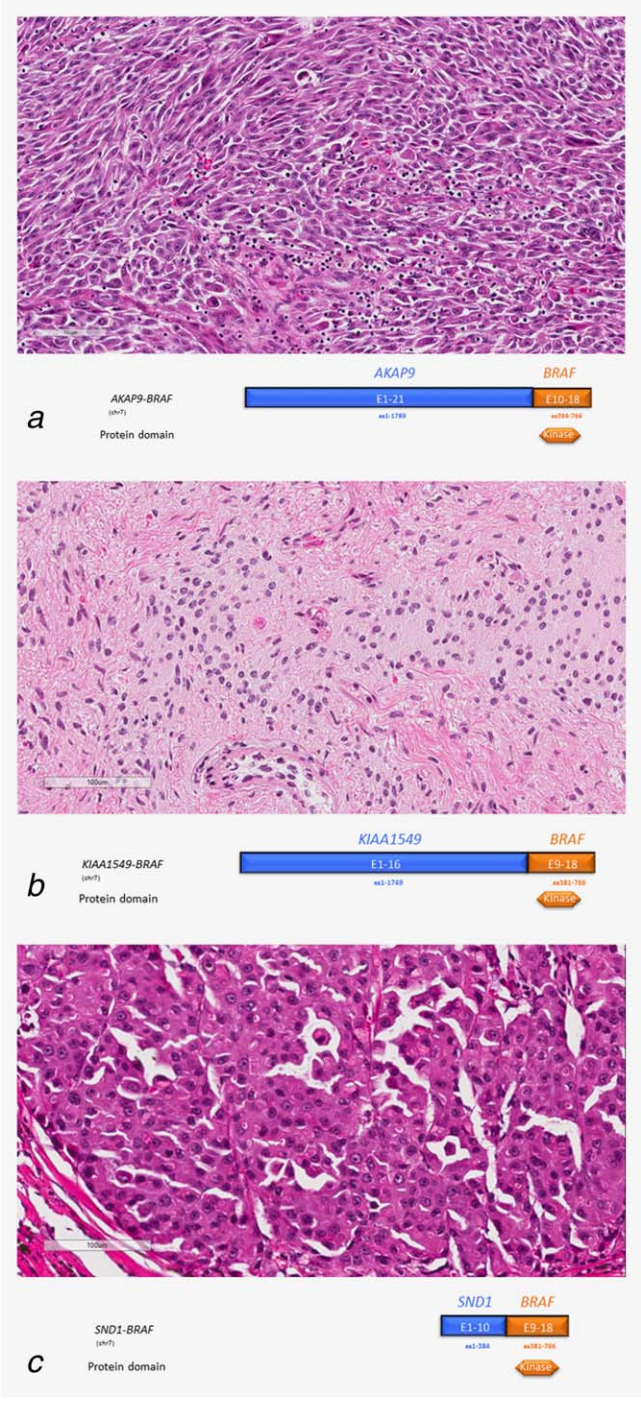
were the most frequent *BRAF* fusions identified in the study and involved 14 (25%) of the 55 *BRAF* fusion positive tumors. Eleven (20%) of the *KIAA1549-BRAF* fusions were identified in brain tumors. The *AGK-BRAF*, *TRIM24-BRAF* and *SND1-BRAF* fusions were the next most frequent, identified in 5, 4 and 3 tumors, respectively. A total of 20 novel fusion partners not previously reported in public databases (COSMIC and TCGA) or the published literature (PubMed) were identified across 20 samples (36%). The remaining 25 fusions have been previously reported (Table 1).[18–26] All 55 *BRAF* fusions were in-frame with breakpoints on the *BRAF* hotspot introns 7, 8, 9 and 10. One fusion *MKRN1-BRAF* (Case 22) was found in a head and neck carcinoma with breakpoint on *MKRN1* Exon 4 and *BRAF* intron 9, which is predicted as in-frame with *MKRN1* exons 1–3, partial exon4 and *BRAF* exons 11-18. *MKRN1-BRAF* was identified in another colorectal carcinoma with a known structure of *MKRN1* (exons 1–4)–*BRAF* (exons 11–18).[24] Most fusions retained *BRAF* exons 9–18 (24/55, 44%).

In the 55 tumors harboring *BRAF* fusions, 207 additional genomic alterations involving the targeted genes of the

sequencing panel were identified in genes such as *CDKN2A/B* (29%), *TP53* (22%), *PTEN* (11%), *PIK3CA* (9%), *PBRM1*, *APC* and *EGFR* (each at 7%). The long tail of additional alterations found in fewer than three tumors included clinically relevant alterations affecting, *MET*, *PDGFRA*, *RET* and *TSC2* (Fig. 3). In 54/55 (98%), tumors the *BRAF* fusion was the only *BRAF* alteration identified, although a single case of metastatic non-Spitzoid melanoma in a 54-year-old man (Case 42) featured both a *BRAF* V600E base substitution and an *AGAP3-BRAF* fusion.

Clinical outcomes are available for only two patients included in our study. A Spitzoid melanoma from a 46-year-old Caucasian woman that harbored a *ZKSCAN1-BRAF* fusion responded to treatment with the MEK inhibitor trametinib given at full dose (2 mg/day orally) (Case 38) (Fig. 4). Subcutaneous tumor nodules exhibited overt clinical responses within 14 days of therapy, and her dominant bulky right lung metastases showed significant response by Day 45 such that she subsequently underwent robotic-assisted lobectomy. This previously unresectable tumor was removed with clean surgical margins, and without any of the 16 recovered lymph nodes involved with melanoma. Similarly, in a recent study, significant clinical activity was demonstrated when trametinib was used in the treatment of a patient with metastatic melanoma harboring a *BRAF* fusion.[27]

A malignant spindle cell tumor of the chest wall treated as a soft tissue sarcoma featured a *KIAA1549-BRAF* fusion (Fig. 5) and responded to treatment with the pan-kinase inhibitor sorafenib in combination with bevacizumab and temsirolimus (Case 50).



Discussion

The above data represent the most diverse series of *BRAF* gene fusions described to date. Although *BRAF* fusions are infrequent in advanced solid tumors, both the present data and the published literature demonstrate enrichment in certain histologic subsets including pilocytic astrocytoma,[14,21,28–30] Spitzoid melanoma,[18,20,31,32] pancreatic acinar carcinoma[33] and papillary thyroid cancer.[2] Other datasets including the COSMIC database accessed in December 2014

Figure 2. *BRAF* fusions in a variety of solid tumors. (*a*) (Case 32) Spitzoid metastatic malignant melanoma in a 60-year-old Caucasian female. Note the diffuse distribution of so-called spindle or elongate cells and mixed with scattered round epithelioid cells with abundant pink cytoplasm and tumor-infiltrating lymphocytes. Lymph node and cutaneous metastases present at the time of sequencing. The tumor features a fusion of *AKAP9* (exons 1–21)–*BRAF* (exons 10–18) (hematoxylin and eosin 100×). (*b*) (Case 15) A pilocytic astrocytoma partially resected from the parietal lobe in an 8-year-old male with a *KIAA1549* (exons 1–16)–*BRAF* (exons 9–18) fusion. Image shows a well-differentiated low-grade astrocytoma with widely separated oval to elongate tumor cell nuclei associated with tangles of eosinophilic fibrils (rosenthal fibers) in the lower right (hematoxylin and eosin 100×). (*c*) (Case 45) Pulmonary metastasis from a primary pancreatic acinar carcinoma in a 67-year-old Caucasian man. Sequencing revealed a rearrangement consistent with an inversion on chromosome 7, juxtaposing the 5′ region of SND1 to the complete kinase domain of BRAF, resulting in the generation of a predicted in-frame *SND1* (exons 1–10)–*BRAF* (exons 9–18) fusion protein (Hematoxylin and eosin X 100). In an expanded study of 44 pancreatic acinar carcinomas, we identified recurrent rearrangements involving *BRAF* and *RAF1* (*CRAF*) in 23% of the tumors. The image shows solid nests of polygonal neoplastic cells with granular eosinophilic cytoplasm (hematoxylin and eosin 100×).

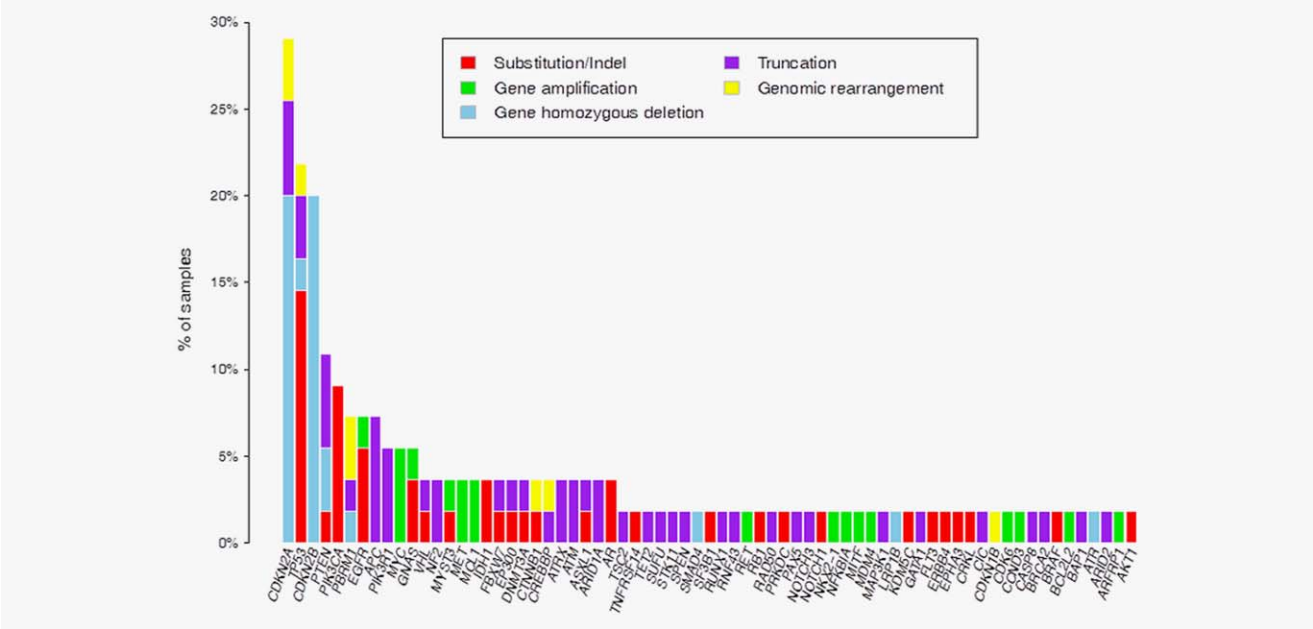


Figure 3. Distribution plot of additional genomic alterations identified in the targeted genes of the sequencing panel in 55 cases of *BRAF* fusion associated solid tumors.

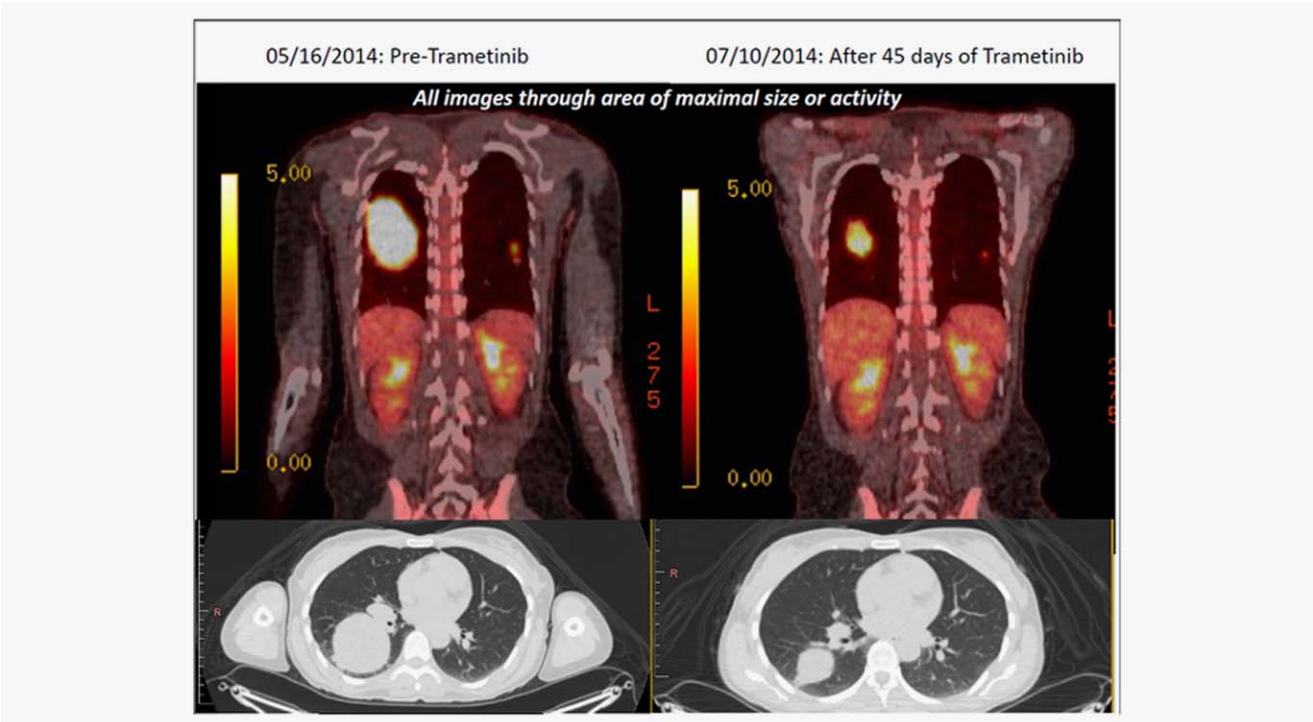


Figure 4. Fused PET/CT imaging results of trametinib therapy in a metastatic Spitzoid melanoma (Case 38) from a 46-year-old Caucasian woman that featured a *ZKSCAN1-BRAF* fusion (*ZKSCAN1* exons 1–5–*BRAF* exons 10–18) and responded to the MEK inhibitor trametinib. Subcutaneous tumor nodules exhibited overt clinical responses within 14 days of therapy, and her dominant bulky right lung metastases showed significant response by Day 45 such that she subsequently underwent robotic-assisted lobectomy. The patient is currently alive with stable disease at 6 months post-thoracic surgery.

for our study report even fewer examples of tumors driven by *BRAF* fusions which are restricted to fewer tumor types.[17] However, it should be noted that the public databases such as COSMIC likely include tumors that were evaluated for *BRAF* base substitutions only and may not have included a sequencing assay capable of detecting gene fusions. Thus,

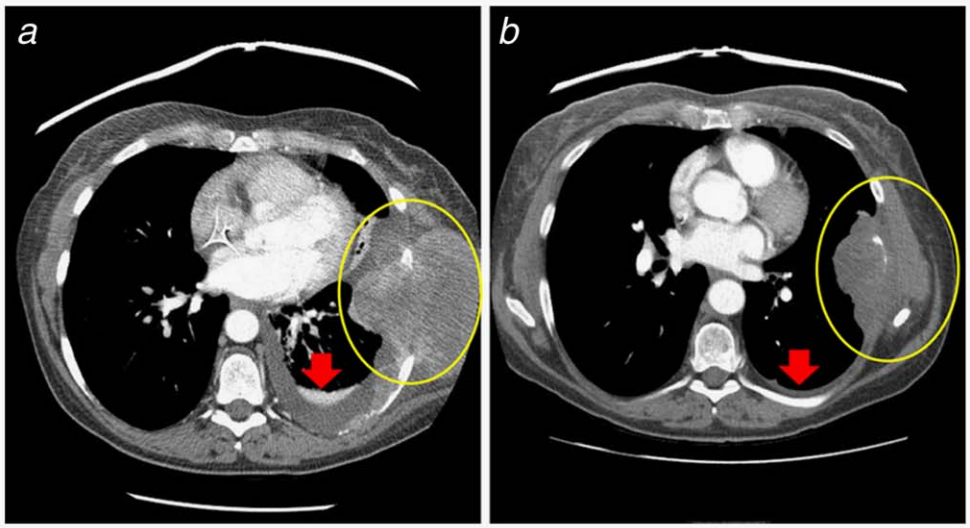


Figure 5. A malignant spindle cell tumor (Case 50) of the chest wall treated as a soft tissue sarcoma that featured a *KIAA1549-BRAF* fusion (*KIAA1549* exons 1–15–*BRAF* exons 9–18) showing pre- and post-treatment CT scan images featuring tumor response to treatment with bevacizumab, temsirolimus and sorafenib.[39] [Permission to re-publish this figure provided by the publisher]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

such discrepancies may be explained by the limitations of analyses not optimized or designed to identify gene fusions. For example, of 4,299 gliomas studied for *BRAF* sequence in COSMIC, 268 (6%) featured an alteration with a 106 (2%) incidence of *BRAF* fusions limited to pilocytic astrocytomas. Similarly, in the melanomas listed in COSMIC, 16,403 tumors included *BRAF* sequencing and 7,110 (43%) had BRAF alterations, but no BRAF fusions were listed in the entire group or in the 53 Spitzoid melanomas in the database. Of 2,533 pancreatic cancers sequenced for *BRAF* at COSMIC, 27 (1%) featured *BRAF* alterations with 0 *BRAF* fusions. Interestingly, in an expanded study of 44 pancreatic acinar carcinomas, we identified recurrent rearrangements involving *BRAF* and *RAF1* (*CRAF*) in 23% of the tumors.[33] Of the 46,463 thyroid tumors sequenced for *BRAF* at COSMIC, 19,297 (42%) had *BRAF* alterations with three (<0.1%) *BRAF* fusions identified all restricted to the papillary carcinoma subtype.

Of interest is the fact that *BRAF* fusions are similar to other kinase fusions in occurring in a mutually exclusive pattern with other activating mutations in the MAP kinase signaling pathway. Only one (2%) *BRAF* V600E base substitution was identified in the 55 cases of *BRAF* fusions which occurred in a case of cutaneous melanoma (Case 42). No *KRAS* mutations were identified in the 55 cases. In contrast, there were the alterations in *GNAS* (3 cases; 5%), *IDH1* (2 cases; 4%) and *EGFR* (4 cases; 7%) in the 55 *BRAF* fusion-positive tumors.

The greater frequency and wider tumor-type distribution of *BRAF* fusions presented in the current study in comparison with COSMIC database is most likely the result of differing techniques used in the tumor analysis. The COSMIC database includes tumors sequenced by nonhybrid capture-based technologies either not optimized to identify or unable to detect gene fusions. The current assay utilized a DNA bait set only.[15] A small series of *BRAF* rearrangements was also uncovered in this cohort of >20,000 clinical tumor samples, but these alterations could not be completely characterized using DNA sequencing alone. It is possible that, with RNA sequencing, these rearrangements could be more precisely characterized as *BRAF* fusions.

Figure 1 shows the exon composition of the *BRAF* fusions identified in this cohort, which includes both a series of previously described fusions and a set of novel fusions described here for the first time. Although direct *in vitro* assays were not conducted as part of our study, based on the published studies for the known *BRAF* fusions and using published models for confirming activation and prediction of the protein amino acid sequences, we expect that the novel fusions identified to be similarly oncogenic. Several *BRAF* fusions, including many identified here, have been previously characterized as activating and oncogenic.[18–22] Modeling and protein domain analysis shows that these fusions, as well as the 20 novel fusions described in Figure 1, all maintain the kinase domain of BRAF, suggesting a universal mechanism of BRAF activation, irrespective of the 5′ fusion partner. Previous studies have shown that loss of the autoinhibitory region upstream of the BRAF kinase domain, which is predicted for all of the fusions described here, leads to activation of BRAF signaling.[34] Although the adverse prognostic significance of *BRAF* base substitution, such as V600E, is widely described for a variety of solid tumors,[35–37] given their rarity, the significance of *BRAF* fusions for clinical outcome is unknown.

Evidence supporting the treatment of solid tumors harboring *BRAF* fusions with therapies targeting this kinase has

started to emerge.[18,38,39] As shown in Figures 4 and 5, tumor responses to kinase inhibitors in combination with nontargeted cytotoxic agents indicate that RAF kinases or downstream signaling pathways can be targeted when activated by *BRAF* fusion. Sorafenib, a multikinase inhibitor that inhibits RAF, has had limited efficacy as an anticancer drug in patients with BRAF activating point mutations.[40] In Figure 5, sorafenib was used to treat the soft tissue sarcoma with a *KIAA1549-BRAF* fusion, but the MTOR inhibitor temsirolimus and the antiangiogenic antibody therapeutic bevacizumab were also given to the patient, and these latter therapies may well have provided the primary tumor response shown in the tumor images.[39] In a study of low-grade astrocytomas, the impact of sorafenib therapy was mixed with both deleterious effects and stabilized disease seen.[14] Studies on melanoma, in contrast, have shown evidence of significant benefit from sorafenib treatment.[41] Thus, the sensitivity of *BRAF* fusion-driven malignancies to sorafenib remains unclear and controversial. In addition, the major tumor response in the patient with the Spitzoid metastatic melanoma featuring a *ZKSCAN1-BRAF* fusion shown in Figure 4 responded to the MEK inhibitor trametinib rather than to a RAF kinase inhibitor. Unfortunately, the extremely low frequency of *BRAF* fusions in solid tumors precludes a prospective randomized clinical trial evaluating the efficacy of treatment with RAF kinase and MEK inhibitors. However, the expanded clinical use of next-generation DNA sequencing and comprehensive genomic profiling in oncology practice may provide data from Phase I trials and published case reports that will validate the use of agents targeting *BRAF* fusions and bring significant clinical improvement for patients with disease driven by this rare but distinctive genomic alteration.

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