

Genomically Driven Tumors and Actionability across Histologies: *BRAF*-Mutant Cancers as a Paradigm

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Abstract

The diagnosis, classification, and management of cancer are traditionally dictated by the site of tumor origin, for example, breast or lung, and by specific histologic subtypes of site-of-origin cancers (e.g., non-small cell versus small cell lung cancer). However, with the advent of sequencing technologies allowing for rapid, low cost, and accurate sequencing of clinical samples, new observations suggest an expanded or different approach to the diagnosis and treatment of cancer—one driven by the unique molecular features of the tumor. We discuss a genomically driven strategy for cancer treatment using *BRAF* as an example. Several key points are highlighted: (i) molecular aberrations can be shared across cancers; (ii) approximately

15% of all cancers harbor *BRAF* mutations; and (iii) *BRAF* inhibitors, while approved only for melanoma, have reported activity across numerous cancers and related disease types bearing *BRAF* aberrations. However, *BRAF*-mutated colorectal cancer has shown poor response rate to *BRAF* inhibitor monotherapy, striking a cautionary note. Yet, even in this case, emerging data suggest *BRAF*-mutated colorectal cancers can respond well to *BRAF* inhibitors, albeit when administered in combination with other agents that impact resistance pathways. Taken together, these data suggest that molecular aberrations may be the basis for a new nosology for cancer. *Mol Cancer Ther*; 15(4); 533–47. ©2016 AACR.

Introduction

A wealth of data now suggests that molecular aberrations may be shared across multiple histologies (1). As an example, *BRAF* mutations can be detected in melanoma, colorectal tumors, lung and ovarian cancers, hairy cell leukemia, histiocytosis and many other related disease types (2; Fig. 1; Table 1). Indeed, a small subset of almost all types of malignancies may harbor a *BRAF* mutation (3, 4). Of special importance in this regard is the fact that several drugs that effectively target the *BRAF*-mutant protein product have been developed (Table 2). For instance, the *BRAF* inhibitors, vemurafenib and dabrafenib, have both been approved for *BRAF*-mutant melanoma based on results from the phase III BRIM-3 study (5) and the phase III BREAK-3 study (6), respectively.

A key conundrum now debated in the cancer community is whether or not targeted drugs approved for one type of histology should be administered to other histologies harboring the cognate aberration. For instance, should a *BRAF* inhibitor

approved for *BRAF*-mutant melanoma be given to a patient with a *BRAF*-mutant tumor other than melanoma? A corollary to this question is the precise criteria needed in order to extrapolate predictive data on a biomarker for a given targeted therapy in one cancer to another cancer. These questions are of tremendous importance for the following reasons: (i) molecular aberrations, in particular amplifications, loss, and mutations, do not appear to segregate well by histology (1, 2, 4); (ii) numerous targeted drugs are becoming clinically available and they have been developed to inhibit a specific cancer signal that may be found in multiple tumor types, hence their rational application would be in tumors bearing the cognate target (3); and (iii) molecular anomalies are found in a very small percentage of diverse cancers (7), and the rarity in each histologic type presents a near-impossible challenge for classic randomized or even nonrandomized trials to determine efficacy histology by histology.

Newer study designs are beginning to accommodate these challenges. For instance, histology-agnostic trials (so-called bucket or basket trials) might include patients with a wide variety of histologies as long as they all harbor the cognate aberration. As an example, a histology-agnostic trial of the *BRAF* inhibitor vemurafenib can include diverse types of cancers, providing that they carry *BRAF* mutation (e.g., VE BASKET study; 8). However, these types of trials are still often perceived as signal finding. If a variety of histologies respond, what should be the next steps to approval and/or pay or coverage? To what extent can we be certain or do we need to be certain that each histology bearing the mutation will respond before it is acceptable to administer drugs across cancers based on their molecular, rather than histologic, classification? Does molecular classification actually represent a biology-based nosology?

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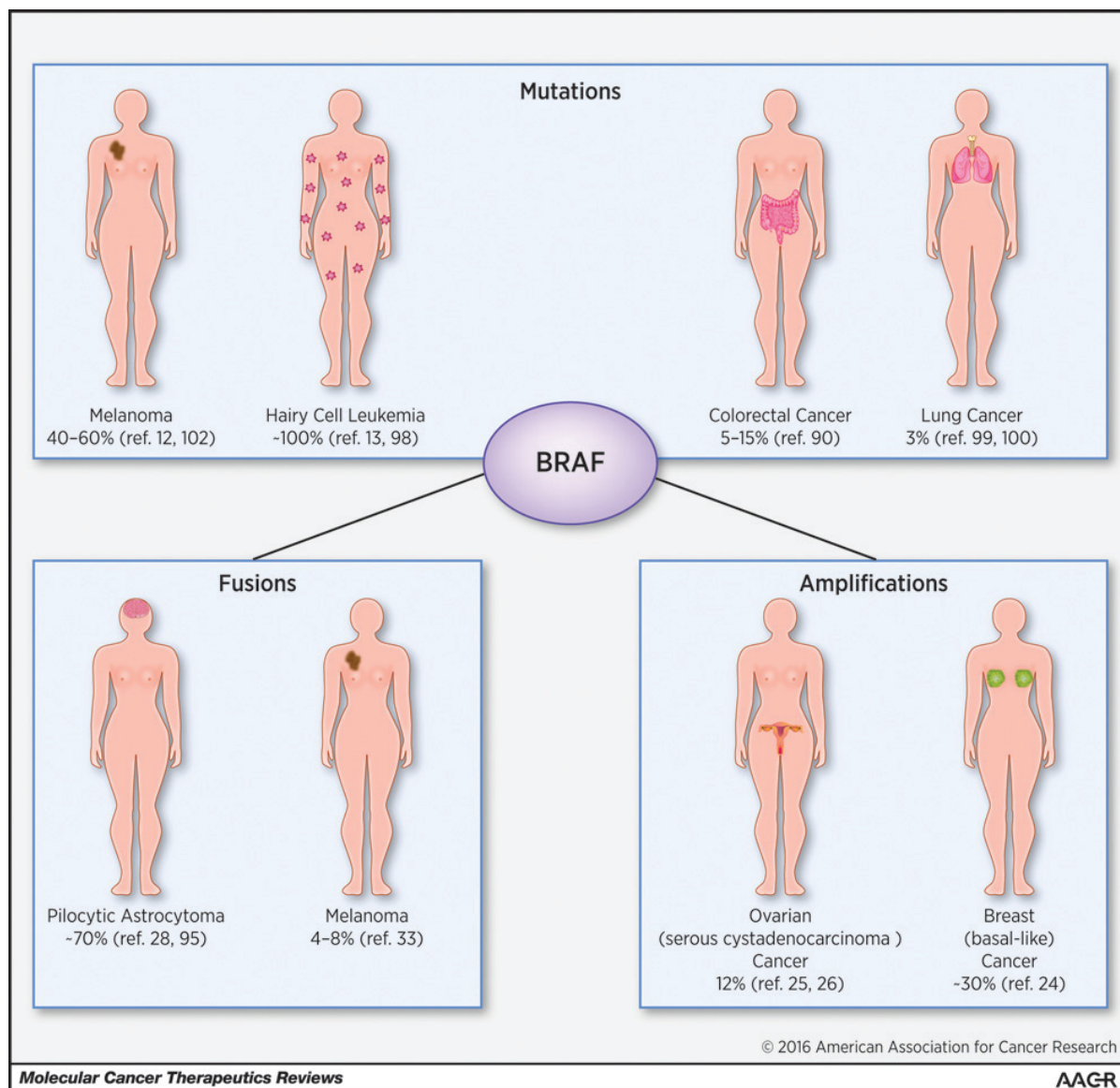


Figure 1.

Examples of organ of origin tumors that have different types of *BRAF* aberrations. For a comprehensive list of tumor types having *BRAF* aberrations, please refer to Table 1.

Herein we review this topic, using *BRAF*-mutant malignancies as a paradigm. The choice of *BRAF* was considered apt for the following reasons: (i) *BRAF* mutations as well as other *BRAF* anomalies (amplifications, fusions) have been described in a wide variety of tumors; (ii) two *BRAF* inhibitors and a MEK inhibitor have already been approved for *BRAF*-mutant melanoma; and (iii) there is a rich literature demonstrating responses, albeit at times in small numbers of patients, with the use of *BRAF* inhibitors in a variety of *BRAF*-mutation bearing cancers (9, 10). On the other hand, *BRAF*-mutant colorectal cancers have proved more resistant to *BRAF* inhibitor monotherapy, hence striking a cautionary note. The observations in *BRAF*-mutant tumors may

therefore inform future conceptualization of genomically driven treatment.

***BRAF* Mutations in Diverse Cancers**

BRAF is mutated in about 15% of all cancers (3, 11) and *BRAF* mutations can be found in solid tumors, hematologic malignancies, and related disease types (Table 1). For some cancers, *BRAF* mutations are very frequently detected: melanoma [40%–60% of patients (12)] and hairy cell leukemia [~100% (13)].

The predominant mutation detected in *BRAF*-mutated cancers is the V600E mutation, representing approximately 70% to 90%

Table 1. *BRAF* mutations in diverse cancers^a

Cancer	<i>BRAF</i> mutation frequency	Source	Comments
Cholangiocarcinoma	3%–22%	Goeppert et al (93) Tannapfel et al (94)	<i>BRAF</i> V600E (60%) <i>BRAF</i> V600D (13%) Other codons (27%)
Chronic lymphocytic leukemia	2.8%	Jebaraj et al (95)	
Colorectal cancer	5%–15%	Pakneshan et al (96)	<i>BRAF</i> V600E
MSI unstable	27.8%–51.8%	Domingo et al (97)	
MSI stable	5%–7.5%	Samowitz et al (98)	
Erdheim-Chester disease	54%	Benlloch et al (99)	
Ganglioglioma	43%	Haroche et al (100)	<i>BRAF</i> V600E
GIST	2%–13%	Gupta et al (101)	<i>BRAF</i> V600E
		Hostein et al (102)	<i>BRAF</i> V600E
		Miranda et al (103)	
Glioblastoma	1.7%	cBioPortal (25,26)	<i>BRAF</i> V600E
Hairy cell leukemia	~100%	Sakata-Yanagimoto (104)	<i>BRAF</i> V600E
		Tiacci et al (13)	
Kidney cancer	3%	COSMIC (23)	<i>BRAF</i> V600E (85%) Other codons (5%)
Lung cancer adenocarcinoma	3%	Cooper et al (105) Paik et al (106)	<i>BRAF</i> V600E (50%) <i>BRAF</i> G469A (39%) <i>BRAF</i> D594G (11%) <i>BRAF</i> V600E
Langerhans cell histiocytosis	25%–38%	Go et al (107) Haroche et al (100)	
Melanoma	~60%	Davies et al (12) Hodis et al (108)	<i>BRAF</i> V600E (80%) <i>BRAF</i> V600K (8%) <i>BRAF</i> V600R (1%) Other codons (10%) <i>BRAF</i> V600E (38%) Other codons (62%) <i>BRAF</i> V600E
Multiple myeloma	~6%	Lohr et al (109)	
Ovarian cancer	35%–60%	Grisham et al (110)	
Serous borderline	44.6%–71%	Bosmuller et al (111)	
Low-grade serous	5.3%–14%		
Pancreatic cancer	1%–16%	Schultz et al (112) COSMIC (23)	Schultz et al reported all mutations detected were non- <i>BRAF</i> V600E (112). COSMIC reported ~55% of <i>BRAF</i> mutations were <i>BRAF</i> V600E. <i>BRAF</i> - <i>KIAA1549</i> fusion
Pilocytic astrocytoma	70%–80%	Korshunov et al (28) Gupta et al (101)	
Pleomorphic xanthoastrocytoma	66%	Schindler et al (113)	<i>BRAF</i> V600E
Prostate cancer	1.6%	COSMIC (23)	<i>BRAF</i> V600E (<1%) <i>BRAF</i> V600X (84%) <i>BRAF</i> V600E
Papillary thyroid cancer	30%–80%	Xing (114)	

^aMultiple other tumors may have a small incidence of *BRAF* mutations not described here. Additionally some tumors may have *BRAF* amplification or fusions as noted in the comments column or as discussed in the section entitled "Abnormalities in the *BRAF* gene other than Mutations".

of all mutations in *BRAF* (12, 14–16). Substitution of glutamic acid (E) for valine (V) at codon 600 of the *BRAF* protein affects the activation segment of the protein by mimicking the phosphorylation of the kinase domain, causing a change in structure that favors the active conformation (14, 17). Experimental studies have confirmed that the *BRAF* V600E mutations are activating, resulting in increased *BRAF* kinase activity in *in vitro* studies, as well as activation of downstream effectors and oncogenic transformation in cell-based studies (12, 18, 19).

Other activating mutations in *BRAF* include additional mutations affecting codon 600 that result in substitutions other than glutamic acid. In *BRAF*-mutated melanoma, the *BRAF* V600K mutation is found at a frequency of approximately 7% to 19% (16, 20). Other rare mutations affecting codon 600 include *BRAF* V600D (0.1%), *BRAF* V600R (1%), and *BRAF* V600M (0.3%; 20). Furthermore, activating mutations in *BRAF* that affect codons other than 600 include L597 substitutions (0.5%), and K601E (0.7%; 20). Table 1 lists several other non-V600 mutations in *BRAF* and their frequencies in detected cancers (for responsiveness

of non-V600E mutations to *BRAF* inhibitors, see section entitled "*BRAF* mutations other than V600E").

In addition, inactivating or "low-activity" mutations in *BRAF* have been identified and characterized; they typically involve substitutions at codon 594 (19, 21), although missense mutations at other codons (including codon 466) have also been shown to result in *BRAF* kinase inactivation or reduced activation (18).

Abnormalities in the *BRAF* Gene Other Than Mutations

In addition to mutations, other types of *BRAF* aberrations are found in cancer, including amplification and *BRAF* fusions. *BRAF* amplification involving either the wild-type gene or mutant versions of the gene is predicted to result in increased *BRAF* activity in tumor cells (22). In some cases where *BRAF* mutations are rare, *BRAF* amplifications dominate. For example, while mutations in *BRAF* are found in only 1% of breast cancers (23), *BRAF* amplification has been reported in 30% of basal-like

Table 2. Examples of clinically available BRAF inhibitors and their applications

Drug name	Target(s)	Approximate IC ₅₀ for BRAF	Development status	Indications/Stage of development ^a	Comments	Refs
Vemurafenib	BRAF V600E, BRAF, RAF1, ARAF, SRMS, TNK2, FGR, MAP3K5	31 nmol/L (BRAF V600E) 100 nm (BRAF)	Approved for BRAF V600E	Unresectable or metastatic melanoma with BRAF V600E mutation		FDA label (115) Bollag et al (116)
Dabrafenib	BRAF V600E, BRAF V600D, BRAF V600K, BRAF, RAF1	1.84 nm (BRAF V600E) 3.2 nmol/L (BRAF)	Approved for BRAF V600E	-Single agent for unresectable or metastatic melanoma with BRAF V600E mutation -In combination with trametinib for unresectable or metastatic melanoma with BRAF V600E/K mutation		FDA label (117)
Trametinib	MAP2K1, MAP2K2	N/A	Approved for BRAF V600E/K	Single agent or in combination with dabrafenib for unresectable or metastatic melanoma with BRAF V600E/K mutation		FDA label (62,118)
Sorafenib	BRAF, KDR, PDGFRA, PDGFRB, KIT, FLT4, FLT3, RET, RAF1, FLT1	38 nmol/L (BRAF V600E) 25 nmol/L (BRAF)	Approved but not related to BRAF aberrations	-Unresectable hepatocellular carcinoma -Advanced renal cell carcinoma - Locally recurrent, or metastatic, progressive, differentiated thyroid carcinoma	-Also in phase II trial for BRAF-mutant (excluding BRAF V600 mutations) solid tumors (NCT02029001) -Not validated clinically as an effective BRAF inhibitor	Wilhelm et al (119)
Regorafenib	BRAF, FLT1, KDR, FLT4, KIT, TEK, PDGFRA, PDGFRB, FGFR1, FGFR2, NTRK1, MAPK1, ABL1	19 nmol/L (BRAF V600E) 28 nmol/L (BRAF)	Approved but not related to BRAF aberrations	-Metastatic colorectal cancer -Locally advanced, unresectable, or metastatic GIST	-Also in phase II trial for BRAF- or RAS-mutant colorectal cancer (NCT02175654) -Not validated clinically as an effective BRAF inhibitor	Wilhelm et al (120)
Pazopanib	BRAF, FLT1, KDR, FLT4, PDGFRA, PDGFRB, KIT, FGFR1, FGFR3, CSF1R, LCK, ITK	410 nmol/L (BRAF)	Approved but not related to BRAF aberrations	-Advanced renal cell carcinoma -Advanced soft tissue sarcoma	-Also in phase I trial in combination with dabrafenib for BRAF-mutant advanced malignant tumors (NCT01713972) -Less effective at inhibition of BRAF V600E; at 1 μmol/L can achieve ~80% inhibition of wild-type BRAF versus only ~40% inhibition of BRAF V600E -Not validated clinically as an effective BRAF inhibitor	Kitagawa et al (121)
ARQ 736	BRAF, RAF1	2.7 nmol/L (BRAF V600E) 2.6 nmol/L (BRAF) ^b	Investigational	Phase I	In a phase I trial (now completed) for solid tumors with BRAF and/or NRAS mutations (NCT01225536)	Chen et al (122)
CEP-32496	BRAF V600E, BRAF, ABL1, BCR-ABL1, RET, EPHA2	60 nmol/L (BRAF V600E) >2,000 nmol/L (BRAF) ^c	Investigational	Phase I/II	Phase II portion of trial selecting for melanoma or colorectal cancer with either BRAF V600E or BRAF V600K mutation (NCT01817781)	James et al (123)

(Continued on the following page)

Table 2. Examples of clinically available BRAF inhibitors and their applications (Cont'd)

Drug name	Target(s)	Approximate IC ₅₀ for BRAF	Development status	Indications/Stage of development ^a	Comments	Refs
LGX818	BRAF V600E, BRAF	4 nmol/L (BRAF V600E) ^c	Investigational	Phase III	-In phase III trial for BRAF V600E-mutated melanoma (NCT01909453) -In a phase II trial for solid tumors (excluding melanoma and colorectal cancer) and hematologic malignancies with BRAF V600 mutation (NCT01981187)	Stuart et al (124)
MLN2480	BRAF, ARAF, RAFI	Information not available	Investigational	Phase I	-Clinical testing in solid tumors and melanoma (NCT01425008) -Not yet featured in trials with BRAF mutation requirements	
PLX8394	BRAF V600E, BRAF, RAFI	Information not available	Investigational	Phase I/II	Phase II portion of trial selecting for BRAF-mutated solid tumors and hairy cell leukemia (NCT02012231)	
PLX3603	BRAF V600E, BRAF	Information not available	Investigational	Phase I	In phase I trial for solid tumors with BRAF V600E mutation (NCT01143753)	
RAF265	BRAF, RAFI, KDR	<100 nmol/L (BRAF V600E, BRAF) 140 nmol/L (BRAF V600E) ^c	Investigational	Phase II	In a phase I trial (now completed) in combination with MEK162 for patients with solid tumors containing BRAF V600E or NRAS or KRAS mutations (NCT01352273)	Stuart et al (125)
RO5126766	BRAF, RAFI, MAP2K1, MAP2K2	8.2 nmol/L (BRAF V600E) 160 nmol/L (BRAF)	Investigational	Phase I	-In phase I trial (now completed) for patients with solid tumors (NCT00773526) -Not yet featured in trials with BRAF mutation requirements	Martinez-Garcia (126)
XL281	BRAF, RAFI	Information not available	Investigational	Phase I/II	In phase I/II trial as monotherapy or in combination with cetuximab for colorectal cancer with BRAF V600E mutation or with KRAS codon 12 or 13 mutations (NCT01086267)	

^aRelevant examples of development are given.

^bIC₅₀ values presented are for ARQ 680, which is the active moiety of the prodrug ARQ 736.

^cCellular IC₅₀ value.

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