BRAF mutations are potentially targetable alterations in a wide variety of solid cancers

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Abstract (updated)

Background: The BRAF gene mutations are potentially targetable with several treatment modalities approved in a limited number of cancer types. The present study explored the spectrum and frequencies of BRAF mutations in a wide variety of solid tumors.
Materials and Methods: 36,312 solid tumors were profiled using different gene sequencing assays (Sanger and Next-generation sequencing, Caris Life Sciences, Phoenix, AZ) and immunohistochemistry.
Results: Overall, 5% of all solid tumors harbored BRAF mutations (77% V600E and 23% non-V600E). As expected, BRAF mutations were detected in 38% of thyroid cancers (4% non-V600E), 35% malignant melanomas (13% non-V600E), 9% colorectal (10% non-V600E), 6% small intestinal cancers (92% non-V600E) and 4% NSCLC (65% non-V600E). Non-V600E mutations with functional impact on kinase activity included (% variant/non-V600E): V600K (24%), D594G (7%), G469A (9%), G466V (6%), and G469V (5%). Some other cancer types also harbored BRAF mutations (GBM; 75% V600E and 25% non-V600E, biliary tract cancers; 43% V600E, 57% non-V600E), while esophageal/GEJ, gastric, liver, pancreatic and head and neck cancers were mostly devoid of BRAF mutations. The most common concurrent alterations associated with potential resistance to BRAF inhibitors, and with implications for dual-targeting approaches, included mutations of PIK3CA (7%), PTEN (6%), KRAS (5%), NRAS (2%), AKT1 and NRAS (1%, respectively), and over-expression of EGFR (53%). Of interest, overexpression of EGFR in BRAF-mutated tumors was highest in the tumor type with poor clinical response to BRAF inhibitors (colorectal cancer with 80%), and lowest in melanoma (6%) where monotherapy has been more successful.
Conclusions: Activating BRAF mutations (both V600E and non-V600E) are potentially targetable in a substantial proportion of various solid malignancies. A subset of BRAF-mutated cancers harbors additional genetic alterations and/or over-express EGFR which may require expanded/dual targeting treatment to overcome resistance.

Background

The *BRAF* gene, located on chromosome 7q34, is a constitutive part of the MAPK/ERK signaling pathway involved in cancer initiation and progression. *BRAF* is one of the most frequently mutated genes in human cancer (~7%) (1). Since 2002 when Davies et al. described *BRAF* mutations in a subset of human neoplasms (2) there have been numerous studies exploring *BRAF* status in human neoplasms (e.g. melanoma, thyroid, colorectal, lung, ovarian cancer, hairy cell leukemia, multiple myeloma, histiocytoses) (3-4).

The *BRAF* gene (V600E and non-V600E) mutations are clinically relevant due to the targeted treatment modalities (e.g. vemurafenib, dabrafenib, tramatenib) that have been approved in a limited number of cancers so far (3-4).

In the present study we explored the spectrum and frequencies of BRAF mutations in a large cohort of solid tumors and analyzed other potentially targetable biomarkers co-existing with BRAF mutations.

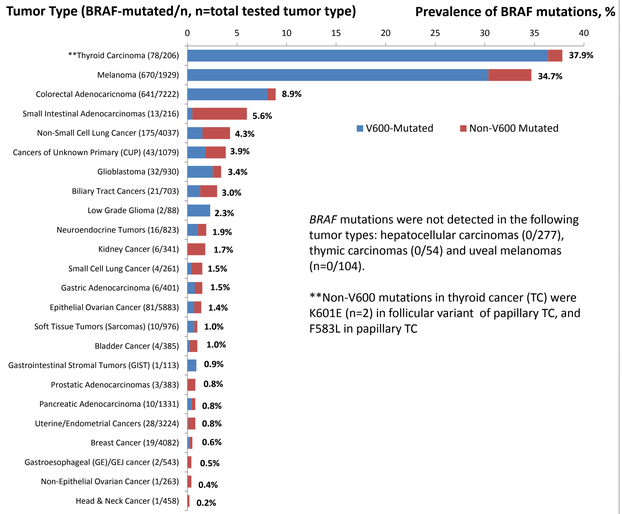
Methods

Tissue samples

A cohort of 36,312 solid tumors were profiled at Caris Life Sciences, (Phoenix, AZ) using Sanger and NGS assays. ***Next-generation sequencing (NGS) and Sanger sequencing:*** NGS was performed on enriched tumor genomic DNA isolated from formalin-fixed paraffin embedded samples using the Illumina MiSeq platform. Specific regions of the genome were amplified using the Illumina TruSeq Amplicon - Cancer Panel. The NGS panel included 45 different genes listed here: http://www.carismolecularintelligence.com/next-generation-sequencing-profile). All variants were detected with >99% confidence based on the frequency of the mutation present and the amplicon coverage using a mutation frequency threshold of 10% (4, 5). All regions that were sequenced achieved a minimum of 100x coverage and overall samples had an average coverage of >500x. **Sanger Sequencing** was used to explore selected regions of *BRAF, KRAS, c-KIT, EGFR,* and *PIK3CA*. Testing also included one or more of the following: protein expression (IHC), gene amplification (C/FISH), microsatellite instability testing by fragment analysis (FA).

Results

**Full analysis involved an additional 11293 patients profiled since abstract submission



BRAF mutations were not detected in the following tumor types: hepatocellular carcinomas (0/277), thymic carcinomas (0/54) and uveal melanomas (n=0/104).

**Non-V600 mutations in thyroid cancer (TC) were K601E (n=2) in follicular variant of papillary TC, and F583L in papillary TC

Figure 1. *BRAF* mutation spectrum across solid tumors. Of 36,312 solid tumors tested, 1867 harbor BRAF mutation, for a mutation frequency of 5%. Frequency displayed is % of *BRAF* mutations among total tested, across tumor types. Blue and Red bars indicate the distribution of V600- and non-V600 mutations across tumor types, respectively.

Results, contd.

Table 1. Frequency of V600 Mutations across Tumor Types

Histoytpe	V600E	V600K	V600R	V600E(2)	V600D	V600M	V600_ K601delinsE	V600_ W604delinsR
CRC	578	-	-	-	-	-	1	-
Melanoma	462	82	12	3	1	1	3	-
TC	75	-	-	-	-	-	-	-
NSCLC	58	2	-	-	-	-	2	1
CUP	20	1	-	-	-	-	-	-
EOC	40	-	-	-	-	-	-	-
GBM	24	-	-	-	-	-	-	-
NET	9	-	-	-	-	-	-	-
Biliary Tract	9	-	-	-	-	-	-	-
Sarcomas	7	-	-	-	-	-	-	-
Breast	6	-	-	-	-	1	-	-
ECD*	4	-	-	-	-	-	-	-
Uterine	3	-	-	-	-	-	-	-
Gastric	3	-	-	-	-	-	-	-
Pancreatic	3	-	1	-	-	-	1	-
Low Grade Glioma	2	-	-	-	-	-	-	-
GIST	1	-	-	-	-	-	-	-
SBA	1	-	-	-	-	-	-	-

*Erdheim-Chester Disease 4/4 V600E-mutated

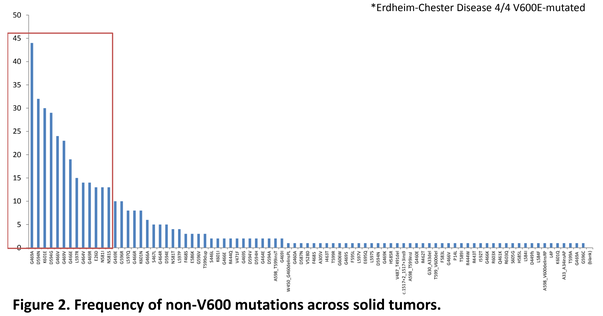


Figure 2. Frequency of non-V600 mutations across solid tumors.

Table 2. Most frequent non-V600 mutations are described with preclinical and clinical implications

Variant	Most-observed tumor type	Clinical (C) or Pre-clinical (P) Data Regarding Treatment Implications	Citations
G469A	NSCLC	C/P : Likely resistant to vemurafenib C: Potentially responsive to sorafenib	G469A: Porcelli et al. (2015) Journal of Translational Medicine, 13(Suppl 1):P3
G469V	NSCLC		
G469R	Melanoma, CRC		G469L: Gautschi, et al. (2013) Lung Cancer, 82(2):365-7. G469R: Sereno, M, et al. (2015) Anticancer Drugs, 26(9):1004-7.
D594N	NSCLC	P: kinase dead mutation, utility to BRAF inhibitors are not supported, however, if found in the presence of activated RAS, then MEK inhibitors may be of potential benefit.	Heidorn, et al. (2010) Cell 140(2):209-21.
D594G	CRC		
L597R/S	CRC, Melanoma, Ovarian	C: Likely responsive to vemurafenib C: Likely responsive to MEK inhibition (TAK-733, trametinib)	L597R: Bahadoran, et al. (2013) J Clin Oncol, 31(19) L597S: Dahlman, et al. (2012) Cancer Discovery, 2:791-797. L597Q: Bowyer, et al. (2014). Melanoma Res, 24(5):504-8.
G464V	CRC, NSCLC	P: Slightly higher levels of BRAF signaling compared with wildtype BRAF, however vemurafenib lacks activity against this alteration.	Yang, et al. (2010) Cancer Res 70, 5518.
G466E	Melanoma, Ovarian	P: low kinase activity compared to V600E, however still activates ERK through CRAF, sorafenib is a better inhibitor of CRAF than mutant BRAF	Wan, et al. (2004) Cell, 116(6):855-867.
G466V	NSCLC		
N581I	NSCLC	P: low kinase activity compared to V600E, however still activates ERK through CRAF, sorafenib is a better inhibitor of CRAF than mutant BRAF	Wan, et al. (2004) Cell, 116(6):855-867.
N581S	Uterine, Ovarian		
K601E	Melanoma, NSCLC	C: Likely responsive to MEK inhibition (trametinib)	K601E: Bowyer, et al. (2014). Melanoma Res, 24(5):504-8.

Results, contd.

Figure 3. Potential therapeutically relevant alterations that co-occur with BRAF

BRAF Status	EGFR pos.	PIK3CA pathway: PTEN loss	PIK3CA pathway: AKT	PIK3CA pathway: PIK3CA	PIK3CA pathway: PTEN	PIK3CA pathway: STK11	RAS pathway: KRAS	RAS pathway: HRAS	RAS pathway: NRAS
V600E	58% (148/255)	49% (536/1046)	2% (8/532)	8% (50/653)	6% (31/509)	2% (9/521)	0.2% (2/927)	0.4% (2/465)	1% (4/705)
other V600	17% (5/30)	44% (55/98)	1% (1/76)	1% (1/90)	6% (4/68)	1% (1/71)	0% (0/89)	0% (0/65)	0% (0/90)
non-V600	42% (84/201)	35% (246/378)	1% (5/337)	8% (28/367)	7% (21/322)	5% (15/323)	19% (73/393)	2% (6/303)	5% (20/369)
V600E vs. other V600 (K,R,D)	*0.0001*	*ns*	*ns*	*0.0142*	*ns*	*ns*	*ns*	*ns*	*ns*
V600E vs. non-V600	*0.0007*	*0.0001*	*ns*	*ns*	*ns*	*0.018*	*0.0001*	*ns*	*0.0001*

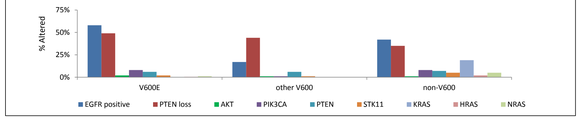


Figure 3. Co-occurring alterations in BRAF-mutated patients, according to specific BRAF alterations. EGFR positivity and PTEN loss are detected by IHC, whereas AKT, PIK3CA, PTEN, STK11, KRAS, HRAS and NRAS are detected by sequencing methods. Positive EGFR status was significantly higher in V600E solid tumors than other V600 mutations (V600K/R/D, etc.) and non-V600 mutations, as well. PIK3CA was found at higher frequency in V600E and non-V600, than in V600K/R/D, etc. KRAS occurred at significantly higher frequency in non-V600 BRAF-mutated tumors than V600E.

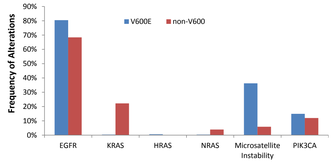


Figure 4. Comparison of co-occurring alterations in BRAF-mutated colorectal cancers. RAS mutations are more frequent in non-V600, however 4 BRAF V600E mutated CRC tumors harbored concurrent activating RAS mutations: (KRAS: Q61L, G13D; NRAS:V45I, HRAS:c.290+2T>C). MSI was more frequent in V600E mutated CRC compared to non-V600-mutated CRC (36% vs. 6%).

Conclusions

- Activating *BRAF* mutations (both V600E and non-V600E) are potentially targetable in a substantial proportion of various solid malignancies.
- The V600E mutations were more prevalent (77%) although a substantial proportion of non-V600E mutations with functional impact on kinase activity was also detected: V600K (24%), D594G (7%), G469A (9%) G466V (6%) and G469V (5%).
- A subset of BRAF-mutated cancers harbors additional genetic alterations (*KRAS, PIK3CA, PTEN, AKT1, NRAS*) and/or over-express EGFR, which may require expanded/dual targeting treatment to overcome a potential resistance mechanism.

References

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