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DETECTION OF THE BRAF V600E MUTATION IN COLON CARCINOMA – CRITICAL EVALUATION OF THE IMUNOHISTOCHEMICAL APPROACH

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Abstract

Recently *BRAF* V600E mutant-specific antibody (clone VE1) became available to immunohistochemically pinpoint the occurrence of these *BRAF* mutant proteins in different tumors, such as colon carcinoma. Detection of *BRAF* mutations is important for the accurate application of targeted therapy against *BRAF* serine-threonine kinase activation. In this study we evaluated 113 colon carcinomas including 95 primary and 27 metastatic tumors with the VE1 antibody using Leica Bond-Max automated immunohistochemistry. To ensure comprehensive *BRAF* V600E mutation detection, all cases were evaluated using 4 molecular methods (Sanger sequencing, the cobas® 4800 *BRAF* V600 Mutation Test, *BRAF* V600 allele-specific PCR, and *BRAF* V600 qPCR) with nearly 100% concordance. Molecular and immunohistochemical studies were blinded. Furthermore, all cases were evaluated for *KRAS* and *NRAS* mutations as parameters mutually exclusive with *BRAF* mutations offering parallel evidence for *BRAF* mutation status. Strong to moderate VE1-positivity was seen in 34 tumors. 12 colon carcinomas showed weak VE1 immunohistochemical staining and 67 were entirely negative. An identical c.1799T>A single nucleotide substitution leading to the *BRAF* V600E mutation was identified in 27 of 113 (24%) colon carcinomas. A majority of *BRAF* mutant tumors were located in the right side of colon and had mismatch repair deficiency. V600E mutation negative carcinomas were more often sigmoid tumors and usually showed intact mismatch repair proteins and *KRAS* or *NRAS* mutations. The sensitivity and specificity of positive result (strong to moderate staining) of VE1 immunohistochemistry were 85% and 68%, respectively. If any positivity would be considered, then the specificity declined to 51% with no significant improvement of sensitivity. Therefore, only strong positivity should be considered when using the VE1 antibody and Leica Bond-Max

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automated immunohistochemistry with these parameters. Although VE1 antibody can be useful in the screening of colon carcinomas for BRAF V600E mutants protein, molecular genetic confirmation is always necessary for mutation diagnosis.

Keywords

BRAF V600E; colon cancer; immunohistochemistry; Sanger sequencing; the cobas® 4800 BRAF V600 Mutation Test; BRAF V600 allele-specific PCR; BRAF V600 qPCR

INTRODUCTION

BRAF gene encodes a serine/threonine-protein kinase B-Raf (BRAF), which belongs to the family of growth signal transduction non-receptor protein kinases. Oncogenic activation of BRAF leads to constitutive kinase activity and phosphorylation of downstream targets of the RAS/RAF/MAPK signaling pathway.¹ Gain-of-function *BRAF* mutations have been identified in different types of cancer, such as colon carcinoma, melanoma, papillary thyroid carcinoma, and some lymphomas, among others, as listed by COSMIC, the catalogue of somatic mutations in cancer (<http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/>).

In colon carcinoma, the most common *BRAF* mutation is c.1799T>A point mutation leading to single amino acid substitution V600E. Detection of this *BRAF* mutation in colon carcinoma has potential as a prognostic marker and also a treatment target for new BRAF inhibitors, such as vemurafenib.^{2,3} Moreover, presence of V600E mutations might indicate resistance to anti-epidermal growth factor receptor (EGFR) therapy as seen in *KRAS* mutants, which are unlikely to benefit from EGFR inhibitor treatment.^{4,5} Thus, *KRAS* and *BRAF* testing prior to such treatment would help to target these expensive treatments to appropriate patients.⁶ However, the Evaluation of Genomic Applications in Practice and Prevention Working Group (EGAPP) recently stated that the power of *BRAF* V600E testing to guide anti-EGFR therapy is probably low.⁷

Various molecular genetic assays have been employed to identify *BRAF* V600E mutation.^{8–10} More recently, a *BRAF* V600E mutant-specific monoclonal antibody (clone VE1) was introduced and used to identify this BRAF-mutant protein in archival formalin fixed paraffin embedded tissue specimens from different malignancies including colon carcinoma.^{11,12} Some studies have reported near to complete concordance between immunohistochemically identified BRAF V600E mutant expression and detection of V600E mutation in colon carcinomas.^{9,13–16} However, one study concluded that immunohistochemistry with VE1 antibody is not a useful surrogate for genotyping in colorectal carcinomas.¹⁷

The aim of this study was to further evaluate the sensitivity and specificity of V600E mutant-specific antibody (VE1) to detect *BRAF* V600E mutation in colon cancers thoroughly analyzed for *BRAF* and *RAS* mutations, as the latter are mutually exclusive with *BRAF* and thus offer parallel evidence for *BRAF* gene status.

MATERIAL and METHODS

Study material and design

One hundred and thirteen anonymized and annotated colon carcinoma specimens from Europe and United States were selected for this study based on availability of representative material. Following microdissection, the tumor tissue was processed for DNA extraction and immunohistochemical studies. Immunostainings were performed in the Laboratory of Pathology (LP), while screening for *BRAF* mutations was performed independently in three laboratories employing four different analytical systems: Sanger sequencing (LP), the cobas® 4800 *BRAF* V600 Mutation Test (Department of Biology and Genetics, Medical University of Gdansk, Poland), and *BRAF* allele-specific PCR, and qPCR (Department of Molecular Diagnostics, Holycross Cancer Center, Kielce, Poland). Multiple methods for mutation analysis were used to ensure comprehensive detection of the *BRAF* mutation status. All immunohistochemical and mutation analysis studies were done blindly without knowledge of results from the other studies.

Immunohistochemistry

Multitissue blocks containing 30–50 colon carcinomas were prepared as previously described.¹⁸ Immunostaining was performed with Leica Bond-Max automatic immunostainer (Leica, Bannockburn, IL) following 25 minutes incubation at room temperature in Bond™ Epitope Retrieval Solution 1 (Leica Biosystems, Catalog No. AR9961). Expression of the *BRAF* V600E-mutant protein was evaluated immunohistochemically using a mouse monoclonal VE1 antibody (Spring™ Bioscience, Pleasanton, CA) diluted 1:200, with 30 min. incubation at room temperature). The dilution selected was the lowest dilution yielding a strong signal in a series of positive controls (V600E mutant tumors, including malignant melanomas) while giving no staining or a weak blush in negative controls. The polymer detection was performed for 15 min., based on the observation that a shorter detection time (8 min.) did not significantly reduce false positive staining in colon carcinoma specimens (similar positivity commonly remained in smooth muscle and occasionally in normal mucosa). The immunostaining was scored arbitrarily as negative (no staining), weakly (pale staining significantly differing from background), moderately positive (more than pale, with tinctorial quality intermediate between weak and strong) and strongly positive (deep, golden brown staining).

Expression of DNA-mismatch repair proteins was evaluated using antibodies to MLH1, MSH2, MSH6 proteins from Cell Marque (www.cellmarque.com) and PMS2 from BD Pharmingen (www.bdbiosciences.com). MLH1 was diluted 1:600, MSH 1:500, MSH6 1:80, and PMS2 1:200. Nuclear staining scored as positive or negative, with the presence of positive normal cell components in each case.

Tissue selection and DNA extraction

Formalin fixed paraffin embedded (FFPE) tumor samples were selected for DNA extraction by enriching the tumor content by H&E stained slide-guided microdissection. Ten 5µ thick sections were deparaffinized with xylene, washed twice in ethanol, lyophilized, and incubated with 10ug/ul proteinase K (Roche Diagnostics, Indianapolis, IN) in Hirt-Buffer at

55°C for 24 hours. Subsequently, DNA was recovered using the Maxwell®16 robotic system and DNA IQ™ Casework Pro Kit for Maxwell®16 (Promega, Madison, WI) as previously reported.¹⁹

PCR amplification for Sanger sequencing

BRAF codon 600 flanking sequences were PCR amplified using the following primers: 5' TCTTCATGAAGACCTCACAG 3' and 5'AGCCTCAATTCTTACCATCC 3'. PCR amplifications were performed with AmpliTaq Gold® DNA polymerase (Applied Biosystems, Roche, Branchburg, NJ) following standard three-temperature PCR protocol with denaturing at 94°C, annealing at 50°C, and extension at 72°C. 50µl PCR reactions were evaluated on agarose gels. The 155 bp PCR products were extracted using QIAquick Gel Extraction Kit (www.qiagen.com) and submitted for sequencing with the above mentioned primers. Sanger sequencing of PCR amplification products was performed by MacroGenUSA, Rockville, MD. Obtained sequences were analyzed and aligned with *BRAF* reference sequence, NM_004333.4 (www.ncbi.nlm.nih.gov).

Apparently negative for *BRAF* V600E cases were evaluated for alternative mutations in *KRAS* codons 12, 13, 61 and 146 and *NRAS* codons 12, 13 and 61 by PCR amplification and direct Sanger sequencing of PCR amplification products. Primer sequences and PCR conditions are listed in Table 1.

The cobas® 4800 BRAF V600 Mutation Test

Mutational analysis was performed using the cobas® 4800 BRAF V600 Mutation Test and cobas® 4800 system according to the manufacturer's protocol obtained from Roche Molecular Systems, NJ, USA (www.cobastbrafstest.com).

BRAF V600 allele-specific PCR

BRAF V600 allele-specific PCR was performed using the wild type (WT)-specific BRAFek15f 5' TCATAATGCTTGCTCTGATAGGA 3' and BRAFek15r 5' GGCCAAAATTTAATCAGTGGA 3' primers and V600E mutation-specific BRAFek15mutA 5' GGTGATTTTGGTCTAGCTACAGA 3' primer. PCR conditions consisted of one 10 min cycle in 95°C and thirty-five cycles of 15s 95°C and 1 min 60°C incubation. Appropriate positive and negative controls were included in each experiment. PCR products were analyzed using MultiNA Microchip Electrophoresis System following manufacturer instructions (Shimadzu, Japan). The 224-bp and 126-bp PCR amplification products represented WT and mutant *BRAF* allele respectively.

BRAF V600 qPCR

Quantitative PCR (qPCR) via TaqMan assay targeting 68bp of *BRAF* exon 15 was performed using Rotor-Gene® Q (Qiagen, Synge-Biotech, Poland) with forward 5' AGACCTCACAGTAAAAATAGGTGATTTTGG 3' and reverse 5' GATGGGACCCACTCCATCG 3' primers. The cycling conditions consisted of one 10 min cycle at 95°C and fifty cycles of 10s 95°C and 30s 67 °C incubation. Appropriate positive and negative controls were included in each experiment. *BRAF* V600E mutant-specific probe (6FAM-TAGCTACAGAGAAATC-MG-BNFQ) and *BRAF* WT-allele-specific probe

(VIC-CTAGCTACAGTGAAATC-MGB-NFQ) were used.²⁰ The qPCR data were analyzed using Rotor-Gene® Q software version 2.2.3

RESULTS

Clinicopathologic data

One hundred and thirteen colonic adenocarcinomas diagnosed in 52 men and 61 women were analyzed. The median and mean ages of colon cancer patients were identical, 68 years. The 95 primary tumors were from cecum (n=27), ascending colon (n=7), hepatic flexure (n=3), transverse colon (n=8), splenic flexure (n=3), descending colon (n=3), sigmoid colon (n=32), and unspecified location in colon (n = 12). 18 metastatic tumors were from the liver (n=9), lung (n=3), brain (n=2), and one of each from the ovary, pancreas, pelvis, and small bowel mesentery. Most tumors were gland-forming adenocarcinomas, but 12 were mucinous carcinomas with extensive extracellular mucin production and 3 tumors were composed of solid sheets of epithelial cells and scant fibrous stroma (medullary type of histology).

Comparison of immunohistochemical and molecular genetic studies

Summary of the results on VE1-immunohistochemistry in relation to BRAF V600E mutation and mismatch repair status is shown in Table 2.

Twenty-four of 45 colon carcinomas with any VE1-positivity (strong, moderate or weak) and 3 of 67 VE1- negative cases were V600E mutants. However, 23 of 34 cases with strong to moderate VE1 immunoreactivity were V600E mutants, while only 1 of 12 tumors with weak VE1 immunostaining carried the V600E mutation.

The sensitivity for BRAF mutation detection with the VE1 antibody was 85% (23 of 27) if strong to moderate reaction was scored as positive result and 89% (24 of 27) if any (including weak) immunostaining was interpreted as a positive result. The specificity of this assay was 68% if only strong to moderate staining was considered positive. However, it declined to 51% if any positive staining was considered to indicate BRAF V600E mutant expression.

Most cases (20/26, 77%) with loss of MLH1/PMS2 expression carried *BRAF* V600E mutation and two were *KRAS* exon 12 mutants. Also, one *KRAS* exon 12 mutant had loss of MSH2/MSH6 expression. BRAF immunohistochemistry was concordant with molecular results in 18 of 20 cases with loss of MLH1/PMS2 expression. Two tumors with loss of MLH1/PMS2 expression and *BRAF* V600E mutation lacked VE1 immunoreactivity. Also, two *KRAS* exon 12 mutants with loss of MLH1/PMS2 expression showed false positive BRAF immunostaining.

Immunohistochemical studies

Thirty-four colon carcinomas showed strong (n=2) to moderate positivity with VE1 antibody while 67 were entirely negative. The remaining 12 tumors displayed weak staining. Mismatch repair-deficient status was detected in 28 tumors. There were 26 cases with loss of MLH1/PMS2 and 2 tumors with loss of MSH2/MSH6. In addition, colonic smooth muscle commonly showed variable granular VE1-immunoreactivity, and colonic mucosa was

occasionally positive, usually weakly. Representative examples of immunostainings are shown in Figures 1 and 2.

Molecular studies

V600E *BRAF* mutation—An identical c.1799T>A single nucleotide substitutions leading to V600E mutation was identified in 27 of 113 (24%) tumors. Sanger sequencing identified 26 mutants and this was confirmed independently by the cobas® 4800 *BRAF* V600 Mutation Test, allele-specific PCR, and qPCR targeting *BRAF* V600 in blinded experiments in different laboratories. However, one additional mutant was detected by the cobas® 4800 *BRAF* V600 Mutation Test, allele-specific PCR, and qPCR. In this case, evaluation of microdissected tissue used for DNA extraction revealed high amount of non-tumor cells, mainly tumor infiltrating lymphocytes. Subsequent Sanger sequencing of DNA isolated from tumor cell rich area confirmed *BRAF* V600E mutation. No other mutations than V600E were found in *BRAF* exon 15.

***KRAS* and *NRAS* mutations**—Mutations in *KRAS* codons 12, 13, 61 and 146 were identified in 40 *BRAF* V600 WT tumors. 27 mutations were identified in codon 12 including ten c.35G>A, eight c.35G>T, six c.34G>T, two c.35G>C and one c.34G>A. Six identical c.38G>A mutations were identified in codon 13. One mutation, c.183A>T affected codon 61. Five mutations were found in codon 146 including four c.436G>A, and one c.437C>T. No mutations was found in *NRAS* codon 12 and 13, while in codon 61 c.182A>G and c.183A>C were identified in two cases.

Clinicopathologic and molecular profile of false positive and false negative cases

The clinicopathologic features of discordant, VE1 false-positive and false-negative cases are summarized in Tables 3 and 4. *BRAF*-WT colon carcinomas with strong to moderate VE1 staining consisted of 7 primary and 4 metastatic colon carcinomas. Three of these tumors showed mismatch repair gene deficiency. *KRAS* codon 12 or 13 mutations were identified in 8 cases.

Three VE1- negative and 1 with weakly VE1-positive colon carcinomas carried a *BRAF* V600E mutation. Three of these tumors were from the right colon (cecum=1, ascending colon=2) and one from sigmoid colon. Mismatch repair gene deficiency was documented in three cases including sigmoid tumor.

DISCUSSION

Recently, a *BRAF* V600E mutant-specific antibody VE1 became available for immunohistochemical screening of such *BRAF* mutant tumors.¹¹ Initial studies showed that this antibody could identify *BRAF* V600E mutants in a spectrum of cancers, including colorectal carcinomas.²¹ Because V600E represents a great majority of *BRAF* mutations in colon carcinoma,¹ immunohistochemistry of the V600E mutant protein could be an alternative mutation detection method to molecular genetic assays. However, a recent study challenged the specificity of VE1 antibody and its use for the immunohistochemical evaluation of *BRAF* V600E mutants in colon carcinomas.¹⁷

In the present study, a cohort of 113 colon carcinomas was analyzed immunohistochemically for the expression of BRAF V600E mutant protein and the status of BRAF V600E and other BRAF exon 15 mutations by molecular genetic techniques.

Our results showed that sensitivity and specificity of VE1 immunohistochemistry for V600E mutant were 85% and 68%, respectively, provided that only strong to moderately positive staining was considered positive. Based on our results on a highly sensitive detection system such as Leica Bond-Max under the described conditions, weak staining should not be considered positive, as this lowers the specificity to 51% without significant improvement in the detection sensitivity. Our results differ from those of two previous studies showing 100% sensitivity and 100% specificity of VE-1 immunohistochemistry in the diagnosis of colon carcinoma BRAF V600E mutants.^{14,15} However, our results show almost two times higher sensitivity than those reported in another study.¹⁷

A significant number (11 of 86) of BRAF V600E mutation negative (BRAF-WT) tumors showed strong to moderate VE1 staining. The negative results of mutation analysis were confirmed by multiple tests including additional evaluation of DNA extracted from microdissected VE1 positive tumor cell islands. The clinicopathologic and molecular genetic profiles of these false positive cases were similar to those of VE1-negative BRAF-WT colon carcinomas. 73% of BRAF-WT tumors with strong to moderate VE1 staining, including three mismatch deficient tumors, carried KRAS mutations. Since KRAS and BRAF mutations are mutually exclusive in colon carcinoma,²³ these tumors clearly represent cases with false-positive VE1 immunohistochemical staining. A recent study of 52 colorectal carcinomas reported weak cytoplasmic staining in 17% of analyzed cases including 3 KRAS mutants.¹⁷

At present, the explanation for false-positive VE1-staining remains unknown. However, such a problem seems to occur with different immunohistochemical detection systems and platforms including manual staining, and automated Dako, Leica Bond, and Ventana stainers.^{16,17,24} Granular cytoplasmic VE1-immunostaining seen in normal gastrointestinal smooth muscle and nuclear positivity in normal mucosa cells in our study was also reported by others indicating incomplete specificity of the VE1 antibody to the BRAF V600E mutant protein and existence of cross reactivity with other epitopes apparently present even in normal tissues.^{14,16,24}

In this study, strong false positive VE1 staining was seen in 2 colon carcinomas. Two colon carcinomas with strong VE1 staining and lack of BRAF V600E mutation were also previously reported.^{13,24} Also, one study detailed three thyroid papillary carcinomas with strong VE1-immunoreactivity unassociated with BRAF mutations.²² Moreover, recent studies showed diffuse, strong VE1 immunoreactivity in pituitary adenomas unassociated with the BRAF V600E mutation.^{25,26} Therefore, occasionally encountered strong false positive staining with the VE1-antibody in colon carcinomas could also represent “true immunoreactivity” with unknown homologous epitopes.

In this study, four BRAF V600E mutants revealed either no or weak immunohistochemical staining with VE1 antibody representing false negative results. Clinical characteristics of

these tumors, female gender, the proximal colonic location and mismatch repair gene deficiency were compatible with the characteristics of sporadic microsatellite unstable colon cancer.²⁷ A recent series reported five *BRAF* mutant colorectal carcinomas with negative VE1 immunohistochemistry.¹⁷ Decreased immunoreactivity due to low level of *BRAF* mutant expression or suboptimal antigen preservation during specimen processing may explain these false negative results.

Previous studies showed that VE1 immunohistochemistry coupled with other tests might be useful for Lynch syndrome screening as *BRAF* mutation shows positive correlation with mismatch repair deficiency in sporadic microsatellite-unstable colorectal cancer.^{13,16,24} In this study, 90% of microsatellite-unstable tumors with *BRAF* V600E mutations were identified by positive VE1 staining, although two false negative and two false positive cases were seen as well. Thus, use of *BRAF* V600E mutant immunohistochemistry might be useful for initial screening for Lynch syndrome although nevertheless should be followed by molecular genetic testing.

The detection of small tumor foci with *BRAF* V600E mutation could be assisted by immunohistochemical testing. Furthermore, immunohistochemical approach could also be useful in cases with low numbers of tumor cells allowing selection of material for molecular testing via methods such as laser capture microdissection. Theoretically, evaluation of *BRAF* mutation status in cases with significant DNA degradation might also benefit from the immunohistochemical approach, although molecular assays targeting *BRAF* V600E mutation are often based on short amplicons relatively well preserved even in significantly degraded DNA.

Targeted therapy of colon carcinoma depends on precise tumor genotyping and consists of simultaneous evaluation of numerous genes from RAS/RAF/MAPK and other signaling pathways involved in tumor progression.⁴ Recently developed low cost sequencing platforms allow concurrent sequencing of multiple targets.²⁸ This may diminish the role of *BRAF* V600E immunohistochemistry in tailoring treatment for individual patients, especially considering the potential false-positive and -negative results.

In summary, our study showed that VE1 immunohistochemistry can identify colon cancer *BRAF* V600E mutants with relatively high sensitivity and moderate specificity. This antibody may be helpful in screening for *BRAF* mutant colon carcinomas, but the *BRAF* mutation status should always be verified by molecular genetic studies.

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Figure 1.

Two examples of BRAF V600E mutant colon carcinomas with strong VE1 immunoreactivity. The upper row shows a mucinous carcinoma and lower row a poorly differentiated gland-forming adenocarcinoma. Both tumors are MLH1-negative and mismatch repair-deficient.

Figure 2.

Examples of aberrant results of VE1 immunoreactivity. A. False negative *BRAF* V600E mutant colon carcinoma with a solid/medullary pattern showing no significant immunoreactivity. B. Weakly positive staining in a *BRAF* WT tumor. C. Strongly positive metastatic colon carcinoma representing a false positive case. D. This colon carcinoma is negative, but smooth muscle fibers show VE1 immunoreactivity.

Table 1

Primer sequences and PCR conditions used to amplify targets for Sanger sequencing.

Gene	PCR amp target	Forward primer (5' 3')	Reverse primer (5' 3')	A temp (°C)	Amplicon (bp)
BRAF	Exon 15	TCTTCATGAAGACCTCACAG	AGCCTCAATTCTTACCATCC	50	125
KRAS	Exon 2	GTGTGACATGTTCTTAATATAGTCA	AGAAATGGTCTGCACCAAGAAATTAT	48	215
KRAS	Exon 3	CCAGACTGTGTTTCTCCCTT	TACACAAAGAAAAGCCCTCCC	50	157
KRAS	Exon 4	AGAGTTAAGGACTCTGAAGA	CAGTGTTACTTACCTGTCTT	45	220
NRAS	Exon 2	GACTGAGTACAAAACCTGGTGG	CACCTCTATGTGGGATCAT	48	112
NRAS	Exon 3	CCCCCAGGATTCTTACAGAA	ATACACAGAGAGGAAGCCTTCG	49	140

Table 2

Summary of the results on VE1-immunohistochemistry in relation to confirmed *BRAF* V600E mutation and mismatch repair protein status in 133 colon carcinomas.

VE1 (<i>BRAF</i> V600E) immunostaining category	No. of tumors	No. of <i>BRAF</i> V600E mutants*	No. of mismatch repair deficient <i>BRAF</i> mutants*	No. of mismatch repair deficient non- <i>BRAF</i> mutants*
Strongly to moderately positive	34	23	18	0
Weakly positive	12	1	0	2
Negative	67	3	2	6
Total no. of cases	133	27	20	8

* identified by molecular genetic studies

Table 3

Clinicopathologic features of VE-1 positive *BRAF*-WT colon carcinomas.

Case	Age	Sex	Location	Histology	MLH1	PMS2	MSH2	MSH6	KRAS
1	38	M	Cecum	Adenocarcinoma NOS	(-)	ND	(+)	(+)	c.38G>A
2	75	M	Cecum	Adenocarcinoma NOS	(+)	(+)	(+)	(+)	c.35G>A
3	77	M	Cecum	Adenocarcinoma mucinous type	(+)	(+)	(-)	(-)	WT
4	43	M	Descending colon	Adenocarcinoma NOS	(-)	(-)	(+)	(+)	c.38G>A
5	46	F	Sigmoid colon	Adenocarcinoma NOS	(+)	(+)	(+)	(+)	WT
6	66	F	Sigmoid colon	Adenocarcinoma mucinous type	(+)	(+)	(+)	(+)	c.35G>A
7	52	F	Colon NOS	Adenocarcinoma NOS	(+)	(+)	(+)	(+)	c.34G>T
8	50	M	Brain metastasis	Adenocarcinoma solid/medullary type	(+)	(+)	(+)	(+)	c.34G>T
9	64	M	Cerebellum metastasis	Adenocarcinoma NOS	(+)	(+)	(+)	(+)	WT
10	51	F	Liver metastasis	Adenocarcinoma NOS	(+)	(+)	(+)	(+)	c.35G>T
11	51	F	Lung metastasis	Adenocarcinoma NOS	(+)	(+)	(+)	(+)	c.35G>T

Table 4

Clinicopathologic features of VE1-negative or weakly positive *BRAF* V600E mutant colon carcinomas.

Case	Age	Sex	Location	Histology	MLH1	PMS2	MSH2	MSH6	VE1
1	65	M	Cecum	Adenocarcinoma NOS	(-)	(-)	(+)	(+)	weak (+)
2	65	F	Ascending colon	Adenocarcinoma mucinous type	(+)	(+)	(+)	(+)	(-)
3	77	F	Ascending	Adenocarcinoma NOS	(-)	(-)	(+)	(+)	(-)
4	65	F	Sigmoid colon	Adenocarcinoma solid/medullary type	(-)	(-)	(+)	(+)	(-)