BRAF V600E VE1 antibody

Abstracts summary sheet

Instructional Statement for BRAF abstracts summary sheet

The BRAF V600E mutation is a common mutation among malignancies of widely diverse type, including melanoma, colorectal carcinoma, papillary thyroid carcinoma, hairy cell Leukaemia, lung adenocarcinoma and low-grade ovarian serous tumours.

The VENTANA anti-BRAF V600E (VE1) antibody detects the protein product of the BRAF V600E gene mutation. This antibody has been demonstrated to be both highly sensitive and specific when compared to genomic methods such as DNA sequencing or PCR-based assays.

The following document includes available abstracts that demonstrate performance of the VE1 antibody in several disease areas.

Colorectal – Lynch Syndrome


In colorectal carcinoma BRAF V600E mutation is associated with presence of microsatellite instability (MSI-H), but not with hereditary non-polyposis colorectal cancer (HNPCC)/Lynch syndrome. Therefore, the incorporation of BRAF V600E mutation analysis into the laboratory testing can differentiate sporadic colorectal cancer from Lynch syndrome in patients with MSI-H.

The immunohistochemistry (IHC) with anti-BRAF V600E (VE1) mouse monoclonal antibody and DNA sequencing for BRAF V600E mutation was performed on 91 MSI-H colorectal specimens from patients tested for Lynch syndrome. Out of 91 cases 11 cases were positive for BRAF V600E mutation by Sanger sequencing and also by IHC. Seventy nine cases out of the remaining 80 cases classified as BRAF wild type showed negative staining with anti-BRAF V600E (VE1) antibody. There was one apparent false-positive case by BRAF IHC. All tumours positive for BRAF V600E by IHC were also negative for MLH1 and PMS2.

These data indicate that there is a high concordance between immunohistochemistry and DNA sequencing [90 of 91 cases (98.9%)]. Overall, immunohistochemistry was able to detect BRAF V600E mutation in MSI-H colorectal cancers with a sensitivity of 100% and specificity of 98.8%.

These data suggest that immunohistochemical staining with anti–BRAF V600E (VE1) antibody should be included in the diagnostic panel for patients with Lynch syndrome.

Colorectal


In this study, anti-BRAF V600E (VE1) mutation–specific antibody was used to evaluate the BRAF V600E protein expression in the colon cancer samples with known BRAF V600E mutation status that was determined by a multiplex allele specific PCR-based assay. The samples included 75 cases of primary colon carcinomas (stage III), 50 cases were BRAF V600E positive and 25 cases did not carry BRAF V600E mutation (BRAF wild-type). The slides were scored by two pathologists who were blinded to clinical and mutation data. Anti-BRAF V600E (VE1) antibody identified mutant BRAF V600E proteins in 49 of 74 colon cancers.
A PCR-based assay confirmed the presence of BRAF V600E mutation in all 49 cancers. In contrast, the 25 tumours that were wild-type for BRAF did not show any staining with anti-BRAF V600E (VE1) antibody. One case was not suitable for evaluation.

**In summary, this study showed that the IHC using mutation-specific anti-BRAF V600E (VE1) antibody identified all tumours with BRAF V600E mutation and exhibited complete concordance with a PCR-based method. These results support the use of IHC in clinical practice as a strategy to screen colorectal cancers for BRAF V600E mutation.**

**Thyroid**


The V600E mutation of the BRAF gene appears to be a driving mutation in the development and progression of papillary thyroid carcinoma. The major goal of this study was to evaluate the expression of the mutated BRAF V600E protein in papillary thyroid carcinoma using anti-BRAF V600E (VE1) mutation-specific antibody.

One hundred and forty four patients were evaluated. Seventy-six patients with papillary thyroid carcinomas (52.8%) showed diffuse cytoplasmic expression of the mutated BRAF protein. The presence of the T1799A point mutation was confirmed by sequencing. Interestingly, the expression of the mutated BRAF V600 protein is not a marker of disease aggressiveness but is seen in the early, clinically favorable microcarcinomas as well as in invasive tumours.

**In summary the data show that IHC detection of the mutated BRAF V600E protein is a reliable and specific method for detection of the BRAF V600E mutation in papillary thyroid carcinoma.**

**Hairy Cell Leukaemia**


Hairy cell Leukaemia (HCL) is an uncommon hematological malignancy characterised by infiltration of bone marrow and spleen with abnormal B-lymphocytes. Recent studies showed that BRAF V600E mutation is present in virtually all cases of HCL, however this mutation is extremely rare in other B-cell neoplasms.

The major goal of this study was to evaluate whether the anti-BRAF V600E (VE1) antibody can distinguish HCL from HCL mimics, such as HCL variant and splenic marginal-zone lymphoma. A total of 52 FFPE tissue specimens (46 bone marrow, 6 spleen) were evaluated for the expression of BRAF protein mutated at V600E. The presence of BRAF V600E mutation was confirmed in all 32 HCL cases. Importantly, all non-HCL cases were scored negative for BRAF V600E mutation. In addition, 30 HCL cases and 20 cases of HCL mimics were also evaluated for the presence of the BRAF V600E mutation by direct sequencing. In this study the BRAF V600E mutation was found in 28 out of 30 HCL cases whereas no BRAF mutation was found among the 20 HCL mimics. In a follow-up study 228 mature B-cell neoplasms were screened with anti-BRAF V600E (VE1) antibody. One case of chronic lymphocytic Leukaemia was found to be positive for BRAF and the presence of a BRAF V600E mutation was confirmed by sequencing.

**Overall, these studies show that IHC with anti-BRAF V600E (VE1) antibody can be used to distinguish HCL from other low grade B-cell neoplasms which can mimic HCL.**
**Lung**


BRAF V600E mutation is considered to be an uncommon mutation in non-small cell lung carcinoma (NSCLC), however the NSCLC patients with this mutation may benefit from targeted therapy. Therefore, it is important to develop the reliable detection methods for the identification of the NSCLC patients carrying BRAF V600E mutation.

This study compared IHC with other methods for the detection of BRAF V600E in primary lung adenocarcinoma. BRAF mutations were analyzed by DNA sequencing and IHC with anti-BRAF V600E (VE1) antibody in 450 patients selected out of 1509 NSCLC patients that were wild-type for EGFR, KRAS, PI3KA, Her2 and EML4-ALK. A BRAF mutation was detected in 40 out of 450 tumour biopsies (9%) by DNA sequencing. Out of these 40 cases 21 cases (5%) had BRAF V600E mutation, while 19 cases (4%) had other mutation than BRAF V600E. These 40 cases were subsequently evaluated by IHC with anti-BRAF V600E (VE1) antibody. The positive staining was detected in 19 of 21 (90%) BRAF V600E-mutated tumours and no staining was found in all cases with mutations other than V600E in BRAF gene.

It was concluded that IHC with the mutation specific antibody against BRAF V600E is a specific and sensitive method for the detection of BRAF V600E and may be an alternative to molecular biology for the detection of this type of mutation in NSCLC.

**Ovarian**


Mutations in BRAF gene are common in serous ovarian borderline tumours, whereas high-grade serous ovarian carcinomas only rarely show this feature. In this study IHC with anti-BRAF V600E (VE1) antibody was compared with allele-specific PCR followed by sequencing for the detection of the BRAF V600E mutation in a large series of serous ovarian carcinoma and serous borderline tumours. Negative or weak, diffuse background staining of BRAF V600E was detected in all 141 cases of high-grade serous ovarian cancer. The absence of BRAF V600E mutation was confirmed by molecular analysis in all these cases. By contrast, one (14%) of 7 low-grade serous carcinomas and 22 (71%) of 31 serous borderline tumours showed moderate to strong BRAF VE1 positive staining. The BRAF V600E mutation was confirmed by allele-specific PCR and sequencing in all the cases positive for BRAF V600E staining by IHC. However, two BRAF V600E positive cases with low tumour cell content required microdissection to confirm the presence of the mutation.

In summary, these data suggest that IHC with the anti-BRAF V600E (VE1) antibody is a specific and sensitive tool for detection of the BRAF V600E mutation in serous ovarian tumours and may provide a practical screening test, especially in samples with sparse tumour cell content.
Brain metastases


The prognosis of patients with brain metastases is poor. In this study a total series of 1,120 tumour specimens (855 brain metastasis, 157 primary tumours, 78 extra-cranial metastases) from 874 patients were evaluated for the BRAF V600E mutation using a mutation-specific anti-BRAF V600E (VE1) antibody. In addition, DNA sequencing was performed to validate immunohistochemical results in 85 cases.

BRAF V600E protein was found by IHC in brain metastases in 42/76 (55.3%) melanomas, 1/15 (6.7%) ovarian cancers, 4/72 (5.5%) colorectal cancers, 1/355 (0.3%) lung cancers, 2/6 thyroid cancers and 1/2 choriocarcinomas. Gene sequencing was conclusive in 70/85 (82.3%) cases and inconclusive in 15/85 (17.3%) cases. There was high concordance between IHC and DNA sequencing (68/70 or 97.1% cases with conclusive gene sequencing).

The results of this study indicate that BRAF V600E mutation is found in a broad range of tumour types and that immunohistochemical detection of BRAF V600E mutant protein is a promising tool for patient therapeutic stratification.