REVIEW

Agents to treat BRAF-mutant lung cancer

Jean G Bustamante Alvarez MD, MS, Gregory A Otterson MD
Division of Medical Oncology, Department of Internal Medicine, The James Cancer Center and Solove Research Institute, The Ohio State University, Columbus, OH 43210, USA

Abstract
BRAF mutations are seen in up to 3.5–4% of the non-small cell lung cancer (NSCLC) patients. BRAF V600E mutations account for 50% of these cases, and the remaining BRAF mutations are non-V600E. The biologic behavior of BRAF-mutated lung tumors tends to be more aggressive and resistant to chemotherapy, but responses to tyrosine kinase inhibitors such as BRAF inhibitors with or without MEK inhibitors have provided another effective tool to attain better response rates when compared to cytotoxic chemotherapy. New strategies such as immunotherapy are becoming as well another option to treat in the second-line setting patients with BRAF-mutated NSCLC.

Keywords: dabrafenib, immunotherapy, lung neoplasm, proto-oncogene protein B-raf, trametinib.

Citation
Bustamante Alvarez JG, Otterson GA. Agents to treat BRAF-mutant lung cancer. Drugs in Context 2019; 8: 212566. DOI: 10.7573/dic.212566

Introduction
The BRAF gene encodes for a serine/threonine kinase that belongs to the RAS-RAF-MEK-ERK axis that regulates cellular growth. The prevalence of BRAF-mutated lung cancer is between 1.5 and 3.5% without any ethnic predilection. BRAF mutations are seen in 3–5% of the nonsquamous non-small cell lung cancer (NSCLC) population according to several reports. BRAF V600E point mutation accounts for half of those mutations. Histologically, BRAF V600E-mutated adenocarcinomas are mucinous with a micropapillary growth pattern and intense thyroid transcription factor-1 (TTF-1) expression. Besides adenocarcinoma, BRAF mutations have been reported in sarcomatoid carcinomas in 9 out of 125 patients (7%) in one series. Large-cell neuroendocrine carcinomas of the lung-harbored BRAF mutations in 9 out of 300 cases (3%) in another series. The Cancer Genome Atlas (TCGA) project revealed a 3% mutation rate in squamous cell lung cancer. BRAF mutations are more frequent in female patients; however, non-V600E mutations seem to occur with a higher frequency in males. Most patients harboring BRAF mutations are current or former smokers. Retrospective analyses of patients with BRAF V600E mutations have shown inferior responses to platinum-based chemotherapy when compared to BRAF non-V600E-mutated patients or wild-type patients; however, these differences have not been statistically significant and are of questionable clinical relevance. Most BRAF V600E-mutated tumors have an aggressive micropapillary pattern that is associated with shorter progression-free survival (PFS) and overall survival (OS) in univariate analysis (hazard ratio [HR] 2.67; p<0.001 and HR 2.97; p<0.001, respectively) and multivariate analysis (HR, 2.19; p<0.011 and HR, 2.18; p<0.14, respectively). BRAF non-V600E tumors were found to have micropapillary histology in only 12% of the cases. In addition, when comparing V600E-mutated subjects with individuals without BRAF mutations, disease-free survival (DFS) was 15.2 versus 52.1 months (p<0.001), respectively, and OS was 29.3 versus 72.4 months (p<0.001), respectively. BRAF-mutated tumors were negative for KRAS, but in two subjects a concomitant deletion 19 epidermal growth factor receptor (EGFR) mutation was reported.

BRAF mutations have also been classified into three classes: (1) Class I mutations are BRAF V600 and signal as monomers, (2) Class II are BRAF non-V600 that function as dimers and they are kinase-activating, and (3) Class III are BRAF non-V600 and kinase impaired but able to amplify ERK signaling when there is upstream tyrosine kinase activation or other alterations increasing the Rat sarcoma (RAS) activity. Clinicopathological characteristics showed that class I patients less frequently harbor brain metastasis upon diagnosis (9 versus 29% and 31% for classes II and III, respectively). PFS of patients with class I mutations was superior to classes II and III (n=14, n=5, and n=4, respectively) when treated with...
carboplatin and pemetrexed (5.1 versus 1.4 months and 4.9 months, respectively). OS was also superior for class I when treated without targeted therapy compared to patients with classes II and III mutations (median OS of 40, 14, and 15.6 months, respectively).6

**Molecular pathways**

*BRAF* gene encodes a serine/threonine-protein kinase that regulates normal cell growth and proliferation. The amino acid residues that specifically encode the kinase domain of *BRAF* are 457–717. The activation loop of the kinase is located within the residues 596–600, which interact with the phosphate-binding loop keeping the kinase locked. Once the activation loop is phosphorylated, *BRAF* can also phosphorylate and thus activate the mitogen-activated 2 kinase 1 and 2 (MAP2K 1/2) signaling pathway (also known as MEK1/2), which will phosphorylate the tyrosine and threonine residues of the MAPK ERK1/2 proteins. ERK1/2 will activate by phosphorylation proteins of the MAPKAPKK family and cytoskeletal proteins such as vimentin and keratin-8. ERK 1 and 2 will also translocate to the nucleus activating transcription factors such as FOS, TP53, and ELK1.7

**Evidence for BRAF and MEK inhibitors combination for NSCLC**

A basket trial with vemurafenib showed an overall response rate (ORR) of 42% for patients with *BRAF V600*-mutated NSCLC and a PFS of 7.3 months. A total of 20 patients were enrolled in this trial. Moreover, 18 of 20 NSCLC patients had *BRAF V600E* mutation,1 patient had *BRAF V600G* mutation, and another had a *BRAF V600* unknown type of mutation. At 12 months, the PFS rate was 23% (95% CI: 6–46) and the median overall survival (mOS) had not been reached but the preliminary rate was 66% (95% CI: 36–85).8 The final report of the expanded NSCLC cohort showed 3 and 20 confirmed responses of 8 previously untreated patients and 54 previously treated patients, respectively. A total of 27 patients had stable disease, including previously treated and untreated patients, and the median duration of response was 7.2 months (95% CI: 5.5–18.4). The untreated cohort had a median PFS of 12.9 months (95% CI: 4.0–Not Evaluable [NE]) and a NE median OS (95% CI: 6.0–NE). The previously treated cohort had a median PFS of 6.1 months (95% CI: 5.1–8.3) and a median OS of 15.4 (95% CI: 8.2–22.6).9 Mazieres and colleagues reported their experience with vemurafenib in 100 patients harboring *BRAF V600E* mutation. This cohort of patients had progressed to one or more lines of standard treatment. In total, 43 patients had a partial response (PR), 21 had stable disease (SD), 16 had progressive disease (PD), and 12 had deaths before assessment. Moreover, 8 patients were not evaluable. The mean ORR was 44.9% (95% CI: 35.2–54.8). Responses lasted a median of 6.5 months (5.1–7.3). Median PFS was 5.2 months (3.8–6.9), and median OS was 9.3 months. Reasons for stopping therapy were PD (55 patients), adverse events (n=23), death (n=3), unclear (n=1), and patient’s preference (n=9).10 A phase II, multicenter, nonrandomized, open-label clinical trial studied the efficacy of the BRAF inhibitor, dabrafenib, on patients with *BRAF V600E* stage IV NSCLC. In total, 78 patients received dabrafenib after one or more prior chemotherapy regimens for metastatic disease and 6 patients received dabrafenib as first-line treatment. The median follow-up was 10.7 months. A confirmed ORR was evidenced in 33% (95% CI: 23–43) of the pretreated patients (26 of 78). The disease control rate (DCR) that included SD, PR, and complete response (CR) was 58% (45 of 78 patients [95% CI: 46–67] [Table 1]. In this trial, ORR and DCR were higher in patients with only one prior line of therapy compared to those with two or more prior lines of therapy (ORR of 38% and DCR of 65% versus ORR of 29% and DCR of 50%, respectively).11

Cohort B of this same study comprised 57 patients previously treated who received the combination of dabrafenib and trametinib. The investigator-assessed confirmed overall response was 63.2% (36 of 57; 95% CI: 49.3–75.6). In total, 2 subjects (4%) had a CR according to investigator assessment, and 34 subjects (60%) had a PR. The investigator-assessed PFS was 9.7 months (95% CI: 6.9–19.6); and the median duration of response was 9.0 months (95% CI: 6.9–18.3). Notably, at data cut-off, 50% (18 of 36) of confirmed responses were ongoing. Median time to response was 6 weeks from starting treatment. The OS at 6 months was 82% and at data cut-off (11.6 months of follow-up) 23 (40%) of 57 patients had died.12

Cohort C of this clinical trial included 36 previously untreated patients. They were given first-line treatment with dabrafenib and trametinib. The investigator and independent review committee reported an ORR of 64%, DCR of 72% and 75% when independently assessed and investigator assessed, respectively. There were two complete responses. PFS was 10.9 months, and OS was 24.6 months. The investigator-assessed median duration of response was 10.4 months.13

The European EURAF cohort also reported their experience with BRAF inhibitors. The sample evaluated by this group included 35 patients of whom 29 had *BRAF V600E* mutations and 6 had *BRAF non-V600E* mutations. The BRAF inhibitors included in this study were vemurafenib, dabrafenib, and sorafenib. Five patients received targeted therapy in the first line and 29 as subsequent therapy. Four patients were treated with a sequential BRAF inhibitor strategy. In total, 34 patients had advanced stage NSCLC (stage III or IV). Moreover, 2 patients with *BRAF V600E* mutations had a CR (none in the *BRAF non-V600E* cohort). A total of 16 patients had a PR (11 had *BRAF V600E* mutation). Stable disease was seen in 11 patients of whom 10 had *BRAF V600E* mutation. These results translated to a 54% ORR (95% CI: 32.8–74.4) and DCR of 96% (95% CI: 78.9–99.9) in the 25 patients with *BRAF V600E* mutation exposed to vemurafenib. In this study, one patient with *BRAF G596V* was reported to have a partial response to vemurafenib.14

A case report of a patient with metastatic NSCLC and BRAF Y412C inactivating mutation achieved a complete response for more than 4 years with dasatinib. *BRAF G466V* inactivating
mutations have been reported to confer sensitivity to dasatinib in preclinical studies.\textsuperscript{15} K\textit{RAS} mutations occur in 20–30\% of NSCLC, and it has been proposed as a potential resistance mechanism after patients with \textit{BRAF} V600E mutations are exposed to \textit{BRAF} inhibitors. Preclinical models have shown that \textit{BRAF}- and \textit{KRAS}-mutated murine lung cancers share the MAP kinase pathway (MEK) as a downstream effector; moreover, when exposed to MEK inhibitors, there is a regression of these tumors.\textsuperscript{16} In a case report of a patient with NSCLC and concomitant \textit{BRAF} V600E and \textit{KRAS} mutation, resistance developed after attaining a partial response for 8 months with dabrafenib monotherapy. In this case, the proposed mechanism of resistance was considered to be due to feedback activation of the RAS pathway with increased signaling to the nuclei by way of the unblocked MAP kinase axis.\textsuperscript{17} Other proposed resistance mechanisms described in the literature include the following: (1) the switch from full-length \textit{BRAF} V600E to an aberrant type in NSCLC cell lines with \textit{BRAF} V600E mutation exposed to \textit{BRAF} inhibitors. The product of the aberrant \textit{BRAF} V600E is the protein p61VE. The expression of this protein was absent when parental cells were treated with \textit{BRAF} and MEK inhibitors indicating that this combination could prevent the emergence of resistance through this mechanism. (2) Relief of \textit{BRAF} V600E dependence by engagement of the EGFR signaling via c-Jun-mediated upregulation of EGFR ligands (i.e. HB-EGF, EREG, AREG, TGF-\alpha) and increasing protein kinase B (AKT) activation.\textsuperscript{18} There are two ongoing phase I clinical trials with ERK1/2 inhibitor to treat different \textit{BRAF}-mutated cancers such as NSCLC and to address the emergence of resistance to \textit{BRAF} and MEK inhibitors (NCT02857270 and NCT02711345).

### Immunotherapy in patients with \textit{BRAF} mutations

A retrospective study of 30 patients evaluated the response to immunotherapy in patients with \textit{BRAF} mutations. Moreover, 21 of the patients had \textit{BRAF} V600E mutations (Cohort A) and 18 patients had \textit{BRAF} non-V600E mutations (Cohort B); 57\% of cohort A and 55\% of cohort B subjects received immunotherapy, showing an ORR of 25 and 33, respectively. No correlation between the level of PD-L1 expression and response was noted. The anti-PD-1 agents used included...
pembrolizumab and nivolumab in different lines of treatment. In the past, high levels of PD-L1 expression have been reported in patients with BRAF-mutant NSCLC (up to 50% of cases) compared to a 28% of high PD-L1 expression seen in the overall population of NSCLC. High tumor mutation burden was seen in approximately 18% of BRAF-mutated NSCLC population, which is consistent with what is seen in the overall population as well. In this retrospective analysis, two ‘hyperprogressors’ had high levels of PD-L1. No statistical significance was seen when comparing outcomes in terms of PFS and OS within cohort A and cohort B subjects.19

Li and colleagues reported a case of metastatic NSCLC with BRAF V600E mutation and PD-L1 positivity with a tumor proportion score (TPS) score of 90% treated with pemetrexed and sorafenib as part of a clinical trial. The patient had a durable response lasting 2 years with this combination. Upon progression, she was treated with dabrafenib that provided her with 19 months of clinical and radiological response. Subsequently, she was treated with pembrolizumab for only two cycles due to immune-related side effects (colitis and pneumonitis) but surprisingly, 12 weeks after stopping pembrolizumab, a significant response was seen and maintained for at least seven more months (PFS to pembrolizumab was not reached when this case was reported).5

Another retrospective analysis reported the experience with immunotherapy in patients harboring BRAF mutations. This study had 48 patients with mutated BRAF. In this cohort, most patients received immune checkpoint inhibitors as a second-line treatment. The median age of patients was 61 years old, 24 patients were male, and one-third of patients were smokers. The ORR was 24%, OS: 13.6 months, PFS 3.1 months. These results signal some activity of immunotherapy in BRAF-mutated NSCLC; however, due to a small sample, definite conclusions cannot be drawn.20

Conclusion

Targeting BRAF V600E mutations with a combination of BRAF and MEK inhibitors appear to be the best frontline option for patients with this oncogenic driver. There are insufficient data to determine the responses for patients with BRAF non-V600E mutations. According to phase II trials, the responses to combined BRAF/MEK inhibitors in the first-line setting is higher than if given in the second line or beyond. BRAF V600E appears to confer aggressive biology, and cytotoxic chemotherapy is inferior when used in the first-line setting. The second-line treatment is unclear at this moment. Immune checkpoint inhibitors appear to have some activity in retrospective analyses, but further prospective trials are needed to establish their efficacy in this subset of patients.
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