Combined osimertinib, dabrafenib and trametinib treatment for advanced non-small cell lung cancer patients with an osimertinib-induced \textit{BRAF} V600E mutation

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ABSTRACT

Introduction: Previous studies have reported an acquired **BRAF** V600E mutation as a potential resistance mechanism to osimertinib treatment in advanced NSCLC patients with an activating mutation in **EGFR**. However, the therapeutic effect of combining dabrafenib and trametinib with osimertinib remains unclear. Here we report treatment efficacy in two cases with acquired **BRAF** V600E mutations.

Methods: Two patients with an **EGFR** exon 19 deletion and a T790M mutation, both treated with osimertinib, acquired a **BRAF** V600E mutation at disease progression. Following the recommendation of the molecular tumor board, a concurrent combination of dabrafenib and trametinib plus osimertinib was administered.

Results: Because of toxicity, one patient ultimately received a reduced dose of dabrafenib and trametinib combined with a normal dose of osimertinib. Clinical response in this patient lasted for 13.4 months. Re-biopsy upon tumor progression revealed loss of **BRAF** V600E and emergence of **EGFR** C797S. The other patient, treated with full doses of the combined therapy, had progression with metastases in lung and brain one month after starting therapy.

Conclusion: **BRAF** V600E may be a resistance mechanism induced by osimertinib in **EGFR**-mutated advanced NSCLC. Combined treatment using dabrafenib/trametinib concurrently with osimertinib needs to be explored for osimertinib-induced **BRAF** V600E mutation.
INTRODUCTION

Osimertinib was approved by the Food and Drug Administration in 2018 for the treatment of epidermal growth factor receptor (EGFR)-mutant non-small cell lung cancer (NSCLC) patients. Initially, osimertinib was used only for patients with the resistance-inducing T790M mutation, acquired after 1st and/or 2nd generation EGFR tyrosine kinase inhibitor (TKI) treatment. Recently, osimertinib has been approved for the first-line treatment of EGFR-mutant patients. Despite impressive initial responses, patients with advanced NSCLC inevitably develop resistance to osimertinib, with a median time to progression of 10.1 months. Several actionable mechanisms of resistance have been identified, including an acquired v-raf murine sarcoma viral oncogene homolog B1 (BRAF) V600E mutation. Treatment options for osimertinib-resistant patients with an acquired BRAF V600E mutation have not been established and are still in an early phase of development.

Here we report on the treatment outcomes of dual EGFR and BRAF inhibition for EGFR-mutant NSCLC patients who acquired the BRAF V600E mutations after progressing on osimertinib. In addition, we provide an overview of the treatment outcomes for similar cases reported in literature.

MATERIALS AND METHODS

A retrospective analysis of 80 NSCLC patients with actionable EGFR mutations treated with osimertinib between January 1, 2015 and March 1, 2020 revealed an acquired BRAF V600E mutation in two patients. Re-analysis of the pre- and post-osimertinib samples was performed for BRAFV600E using the highly sensitive droplet digital polymerase chain reaction (ddPCR) assay (dHsaMDV2010027; Bio-Rad, Hercules, CA) following standard procedures, as described previously. The two patients were anonymized for the investigators. The study protocol complies with the Research Code of the University Medical Center Groningen (UMCG) and national ethical and professional guidelines.

CASES

Patient 1 was a 56-year-old never smoking Caucasian female diagnosed with stage IV adenocarcinoma of the lung. An EGFR exon 19 deletion p.(L747_A750delinsP) was identified by next generation sequencing (NGS) using an amplicon-based Ion Torrent platform on plasma DNA. The patient received afatinib as first-line treatment. After 11 months, she had progressive disease with an EGFR p.(T790M) in addition to the previously observed EGFR exon 19 deletion. Osimertinib was started as a second-line treatment. After more than
eight months, the patient presented with progressive disease in the pulmonary lesion and brain metastases. Re-biopsy of the pulmonary lesion and NGS showed loss of the \(EGFR\) p.(T790M) and gain of a \(BRAF\) p.(V600E) in addition to the \(EGFR\) exon 19 deletion. To establish whether the \(BRAF\) p.(V600E) was pre-existing or acquired, ddPCR was performed on the pre-treatment axillary lymph node biopsy. No \(BRAF\) p.(V600E) mutant droplets were detected, while there were 6740 wild-type \(BRAF\) droplets, equaling a limit of detection of 0.04 %. As both biopsies originated from the same site, the mutation likely developed during osimertinib treatment. After discussion in the UMCG Molecular Tumor Board (MTB), the patient started combination treatment with osimertinib, dabrafenib and trametinib. The patient responded clinically within two weeks, although brain metastases progressed at magnetic resonance imaging (MRI) after six weeks. Treatment had to be stopped because of a pneumonitis (Figure 1A). The clinical condition of the patient was too poor to start new treatment, and she died five months later.

Patient 2 was a 66-year-old never smoking Caucasian male diagnosed with stage IV adenocarcinoma of the lung. Diagnostic tests revealed an \(EGFR\) exon 19 deletion p.(E746-A750del) in the baseline tumor biopsy. The patient received gefitinib treatment for 11.2 months. At disease progression, an \(EGFR\) p.(T790M) mutation was detected in addition to the previously detected \(EGFR\) exon 19 deletion, and treatment was changed to osimertinib. A partial tumor response was observed that was sustained for 20 months. Analysis of a biopsy taken at progression indicated presence of a \(BRAF\) p.(V600E) in addition to the previously observed \(EGFR\) exon 19 deletion and p.(T790M). Re-analysis by ddPCR of the pre- and post-osimertinib biopsies, both taken from the subcarinal lymph node, confirmed \(BRAF\) p.(V600E) in the post-osimertinib sample and no detectable \(BRAF\) p.(V600E) mutation in the pre-osimertinib sample. The limit of detection was 0.06 %, with a total 5212 \(BRAF\) wild-type droplets in the pre-osimertinib sample. Based on the observed mutations, the patient was discussed in the UMCG-MTB, and subsequently treated with a combination of osimertinib, dabrafenib and trametinib. The patient showed a partial tumor response for all tumor sites, but required dose reduction because of repeated grade 2 pyrexia, a common toxicity criterion (V 4.0), grade 2 nausea and grade 2 vomiting. The dosages were reduced to 50 mg dabrafenib twice per day, 0.5 mg trametinib once per day and 80 mg osimertinib once per day. Tumor mass showed a remarkable reduction after three months according to the positron emission tomography–computed tomography (PET-CT) image (Figure 1B). After 13.4 months, the patient had progressive disease with loss of the \(BRAF\) V600E and an acquired \(EGFR\) p.(C797S) that coexisted with the previously observed \(EGFR\) exon 19 deletion at similar variant allele frequencies (VAF) (77 % and 83 %, respectively), while the p.(T790M) mutation was observed only at a very low frequency (VAF of 2%). The absence of the \(BRAF\) p.(V600E) mutation was confirmed using ddPCR, with 36,119 wild-type droplets and no mutant droplets, reaching a limit of detection of 0.008 %. Based on these results, and after
evaluation the UMCG-MTB, gefitinib treatment was proposed and started. Three weeks after start of this treatment, the clinical condition of the patient worsened rapidly as a result of pneumonitis without tumor progression, and the patient died a few days later.

**Figure 1. Overview of treatment history and observed mutations before and during treatment of two patients with an EGFR exon 19 deletion.** Overviews of patient 1 (A) and patient 2 (B). The blue bar represents the treatment periods of the different TKI regimens. The CT and MRI images are ordered according to the time point relative to the treatment. *EGFR, epidermal growth factor receptor gene; m, months.*
DISCUSSION

*BRAF* V600E is a known oncogenic driver occurring in approximately 2% of NSCLC.\(^7\) Clinical studies have shown effectivity of the *BRAF* inhibitor dabrafenib in combination with the MEK inhibitor trametinib in *BRAF* V600E-mutated NSCLC patients.\(^8\) However, the effectiveness of dabrafenib and trametinib plus osimertinib as a treatment strategy for NSCLC patients who acquired *BRAF* V600E as a resistance mechanism to EGFR-TKI treatment is largely unknown. One out of two patients in this study responded to the treatment for 14 months until loss of the *BRAF* V600E mutation and induction of a new resistant mutation (*EGFR C797S*). The other patient had brain metastases, and the main clinical deterioration under treatment came from progression of brain metastases. This patient did not respond to the treatment, possibly due to limited drug penetration across the blood-brain barrier,\(^9\) or to a different (non-*BRAF* V600E-mediated) resistance mechanism in the brain. In both patients, a pneumonitis developed on TKI after exposure to the combined treatment. It is unknown know whether this was a result of toxicity.

To date, treatment outcome has been published for five similar cases (Table 1). Abdulla et al. reported two patients, the first of which demonstrated a reduction of tumor size after two weeks of dabrafenib/trametinib and a slight increase four weeks thereafter.\(^10\) A second patient presented with clinical progression after four weeks of dabrafenib and trametinib treatment. After switching treatment to a combination of both osimertinib and dabrafenib, an impressive metabolic response was observed by 18F-2-fluoro-2-deoxy-d-glucose (FDG) PET/CT (-33 %) within two weeks.\(^10\) Solassol et al. reported a patient who was treated with dabrafenib and trametinib alternated with osimertinib because of hepatic progression in which a *BRAF* V600E was detected.\(^11\) This patient responded to treatment for six months. The final two cases, reported by Huang et al. and Zhou et al (with an *EGFR* exon 19 deletion and with L858R, respectively) who were treated with a combination of dabrafenib, trametinib and osimertinib concurrently, after acquiring a *BRAF* V600E mutation upon treatment with osimertinib, which had been administrated based on an acquired T790M.\(^12,13\) Both patients demonstrated tumor shrinkage during reported treatment times of two and seven months, respectively. Treatment was ongoing at the time of the case reports, and as such, the PFS was not yet known. Despite the limited number of patients treated thus far, promising results have been obtained with the combined treatment.
Table 1. Overview of patients with an osimertinib-induced \( \text{BRAF} \) V600E mutation and their treatment details from literature and this study

<table>
<thead>
<tr>
<th>No.</th>
<th>Reference</th>
<th>Baseline ( \text{EGFR} ) mutation</th>
<th>Line*</th>
<th>Mutations at resistance to osimertinib</th>
<th>Treatment</th>
<th>PFS (months)</th>
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<tr>
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<tr>
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<td>( \text{BRAF} ) V600E Loss of T790M</td>
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* Line of treatment; b Treatment ongoing; c Dabrafenib/trametinib and osimertinib were alternated every month.

\( \text{EGFR} \), epidermal growth factor receptor gene; No., patient identifier; PFS, progression-free survival.

**CONCLUSION**

Our results indicate that the TKI-induced \( \text{BRAF} \) V600E mutation is an acquired resistance mechanism to osimertinib. Out of five patients receiving combined dabrafenib, trametinib, and osimertinib, the four patients without brain metastasis showed a clinical response, while the fifth patient with brain metastasis did not respond. Combined treatment with dabrafenib/trametinib and osimertinib thus seems to be effective, especially in patients without brain metastasis. To define the most optimal treatment strategy for patients with \( \text{EGFR} \) activating mutations who develop \( \text{BRAF} \) V600E mutations after initial response, further studies with similar treatment regimens and data on PFS and OS are required.
Chapter 9

Acknowledgements
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Conflicts of interest
The authors declare no conflicts of interest related to this study.
REFERENCES


