Clinical Benefit From BRAF/MEK Inhibition in a Double Non-V600E BRAF Mutant Lung Adenocarcinoma: A Case Report

Roxana Reyes,[1] Clara Mayo-de-las-Casas,[2] Cristina Teixidó,[3,4] Carlos Cabrera,[1] Elba Marín,[4] Ivan Vollmer,[5] Pedro Jares,[3] Mónica Garzón,[2] Miguel Ángel Molina-Vila,[2] Noemí Reguart[1,4]

Clinical Practice Points

- Dual BRAF-MEK inhibition is the standard-of-care treatment in patients with advanced non–small-cell lung cancer (NSCLC) with V600E B-Raf proto-oncogene, serine-threonine protein kinase (*BRAF*) mutations.
- Non-V600 *BRAF* mutations constitute a heterogeneous group of genetic alterations that comprise distinct functional classes with different clinical features, biological characteristics, and for which optimal management has not yet been defined.
- The unprecedented long-lasting benefit to BRAF/MEK targeted inhibition in our case of a dual G469A and W604C mutation highlights the urgent need of prospective clinical trials to further evaluate the role of class-specific therapies in this subset of patients with non-common *BRAF*-mutated NSCLC.

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Introduction

On June 2017, a 69-year-old man, who was a heavy smoker, with a history of non-resolving pneumonia, was referred to our hospital. Thorax computerized tomography (CT) and a positron emission tomography-CT revealed 2 consolidations with air bronchogram and multiple bilateral lung micronodules with a ground-glass opacity pattern (Figure 1A). A lung CT-guided biopsy yielded the diagnose of lung adenocarcinoma with a lepidic pattern positive for thyroid transcription factor-1 (cT4 cN0 cM1a, stage IVA).[1] Next-generation sequencing (NGS) with Oncomine Solid Tumor panel (Life Technologies, Carlsbad, CA) revealed 2 concomitant *BRAF* mutations in exons 11 and 15; pGly469Ala, c.1406G>C and pTrp604Cys, c.1812G>T; with variant allele frequency (VAF) of 34.3% and 37.4%, respectively. The sample was negative for other genetic alterations, and the programmed death-ligand 1 expression was negative by immunohistochemistry (clone 22C3). Further testing with another NGS panel (GeneReader Qiagen, Hilden, Germany) confirmed the presence of both mutations with similar VAF (38.5% and 37.7%) (Figure 2). In light of the results, although double BRAF/MEK inhibition is only approved for *BRAF*-V600 mutations, in August 2017, a decision was made to initiate first-line targeted therapy with oral dabrafenib (RAF inhibitor) 150 mg twice daily and trametinib (MEK inhibitor) 2 mg once daily, under compassionate use and with prior patient's consent.

A first CT evaluation performed after 2 months of treatment showed disappearance of some metastatic lesions and a remarkable widespread decrease in density of the 2 major consolidations, changing from part solid to an extended ground-glass opacity component (Figure 1B). In the last follow-up imaging assessment, performed 15 months after treatment initiation, disease remains stable with no signs of progression according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 (Figure 1C). Overall, treatment with dabrafenib and trametinib was well-tolerated, with mild (grade 1) fatigue and dry skin the only treatment-related adverse events reported.

[1]Department of Medical Oncology, Hospital Clínic, Barcelona, Spain
[2]Pangaea Oncology, Hospital Universitari Quirón Dexeus, Barcelona, Spain
[3]Department of Pathology, Hospital Clínic, Barcelona, Spain
[4]Translational Genomics and Targeted Therapeutics in Solid Tumors, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[5]Department of Thoracic Radiology, Hospital Clínic, Barcelona, Spain

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Address for correspondence: Noemí Reguart, MD, PhD, Department of Medical Oncology, Hospital Clínic, Villarroel 170, CP: 08036, Barcelona, Spain
E-mail contact: nreguart@clinic.cat

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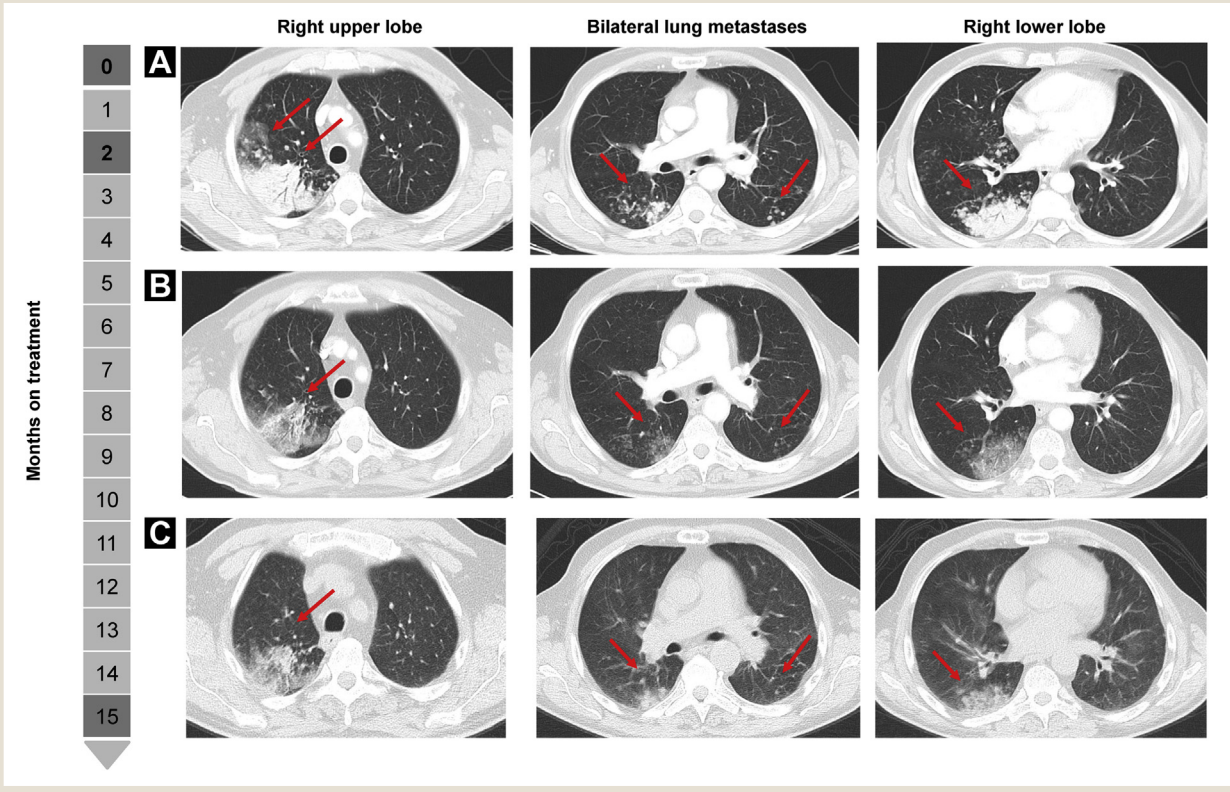
Figure 1 Thoracic Assessment by Chest Computed Tomography on *BRAF/MEK* Inhibition. A, Different Sections of Chest Computed Tomography Taken at Diagnosis Showing an Extensive Consolidation With Air Bronchogram and Peripheral Ground-Glass Opacity in the Right Upper Lobe, Presence of Bilateral Micronodules Corresponding to Lung Metastases, and a Second Confluent Consolidation in the Right Lower Lobe (Red Arrows). B and C, CT Scans Obtained at 2 (B) and 15 (C) Months of Treatment, Displaying a Progressive Decrease In Tumor Size as Well as in Tumor Density With an Almost Resolution of the Bilateral Micronodules (Red Arrows). Tumor Response was Assessed as Stable Disease according to Response Evaluation Criteria In Solid Tumors, v 1.1



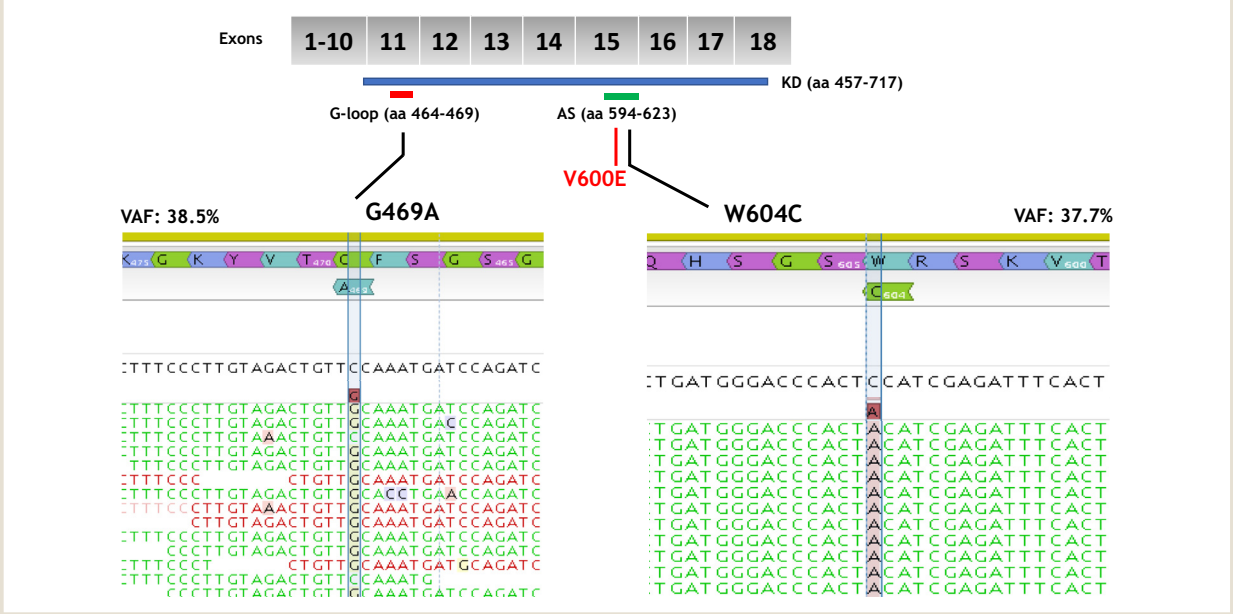
Discussion

The identification of distinct molecular driver alterations with potential for target inhibition has dramatically improved the outcomes of patients with advanced NSCLC.[2] Currently, several targeted therapies, such as those inhibiting the epidermal growth factor receptor (*EGFR),* anaplastic lymphoma kinase *(ALK),* c-Ros oncogene 1 (*ROS1)*, and *BRAF*-V600E mutations have been approved for the treatment of oncogenic NSCLC tumors. Therefore, mutational testing by NGS has been progressively incorporated into the clinical practice, enabling identification of a wider spectrum of genetic alterations, among them the non-V600E *BRAF* mutations.[2]

BRAF mutations have been reported in the range of 1% to 4% in large series of patients with NSCLC.[3-6] Nonetheless, in NSCLC, unlike melanoma, one-half of all *BRAF* mutations are located outside codon 600 (non-V600)[5,7] (Figure 3A). Combined treatment with the BRAF/MEK inhibitors, dabrafenib and trametinib, is the first targeted therapy approved for the treatment of metastatic NSCLC with *BRAF* V600E mutations, based on their substantial antitumor activity with an overall response rate of 64% and a median progression-free survival (PFS) of 10.9 months in *BRAF* V600E-naive patients.[8] Meanwhile, treatment with platinum-based chemotherapy remains the first-line standard-of-care in patients with advanced NSCLC with non-V600 *BRAF* mutations. The role of immunotherapy has also been recently explored in patients with BRAF mutations,[9,10] reporting overall response rates ranging between 24% and 30%, and PFS and median overall survival (OS) of approximately 3 and 13 months, respectively. Preliminary data of these studies do not provide any evidence of differential outcomes based on *BRAF*-mutation subtypes or programmed death-ligand 1 expression levels, but certainly indicate a similar activity of immunotherapy in *BRAF* V600E and non-V600E mutant NSCLC.

BRAF activating mutations are often located clustered within the conserved glycine-rich loop (G-Loop), activation segments (AS), and in residues that stabilize the interactions between these regions.[11] The G469A mutation, located at the G-Loop in exon 11 (Figure 2), is the most frequent non-V600 mutation described in NSCLC (Figure 3B) and seems to be moderately sensitive to BRAF/MEK inhibition in vitro.[12] A new preclinical work has re-classified *BRAF* mutations into 3 functional classes based not only on their kinase activity[13] but also on their signaling mechanism; although V600 BRAF mutants signal as RAS-independent active monomers with a constitutively strong kinase activity (class I), non-V600 BRAF mutants signal as RAS-independent activated dimers with either high/intermediate kinase activity (class II) or as RAS-

Figure 2 Diagram of BRAF Gene With Exons Represented in Boxes and Respective Location of Both Mutations Herein Reported (G469A in Exon 11 and W604C in Exon 15) With Regard to the Most Common Hotspot V600E Activation Mutation in Red. The Conserved Glycine Motif (G-Loop) in Exon 11 is Indicated With a Red Bar and the AS in Exon 15 With a Green bar, Both Contained in the KD Depicted in a Blue Bar. VAFs for Both Mutations Reported Are Included (GeneReader, Qiagen (Hilden, Germany)



Abbreviations: AS = activation segment; KD = kinase domain; VAFs = variant allelic frequencies.

dependent heterodimers with either lack/low kinase activity (class III).[7,14]

Despite biochemical differences, there are no clear clinicopathologic phenotypes for each one, although tumors with V600E mutations are almost exclusive to infiltrating adenocarcinoma and display a great micropapillary component, whereas tumors with non-V600 mutations can also be present in squamous cell carcinoma and display a more heterogeneous histologic pattern with less presence of a micropapillary component.[5] Recently, a retrospective study has explored the impact of *BRAF* mutation class on disease characteristics and clinical outcomes in *BRAF*-mutant lung cancer. Classes II and III had more aggressive clinical features (including higher incidence of brain metastases) and significantly shorter PFS on chemotherapy compared with class I V600-mutants.[14]

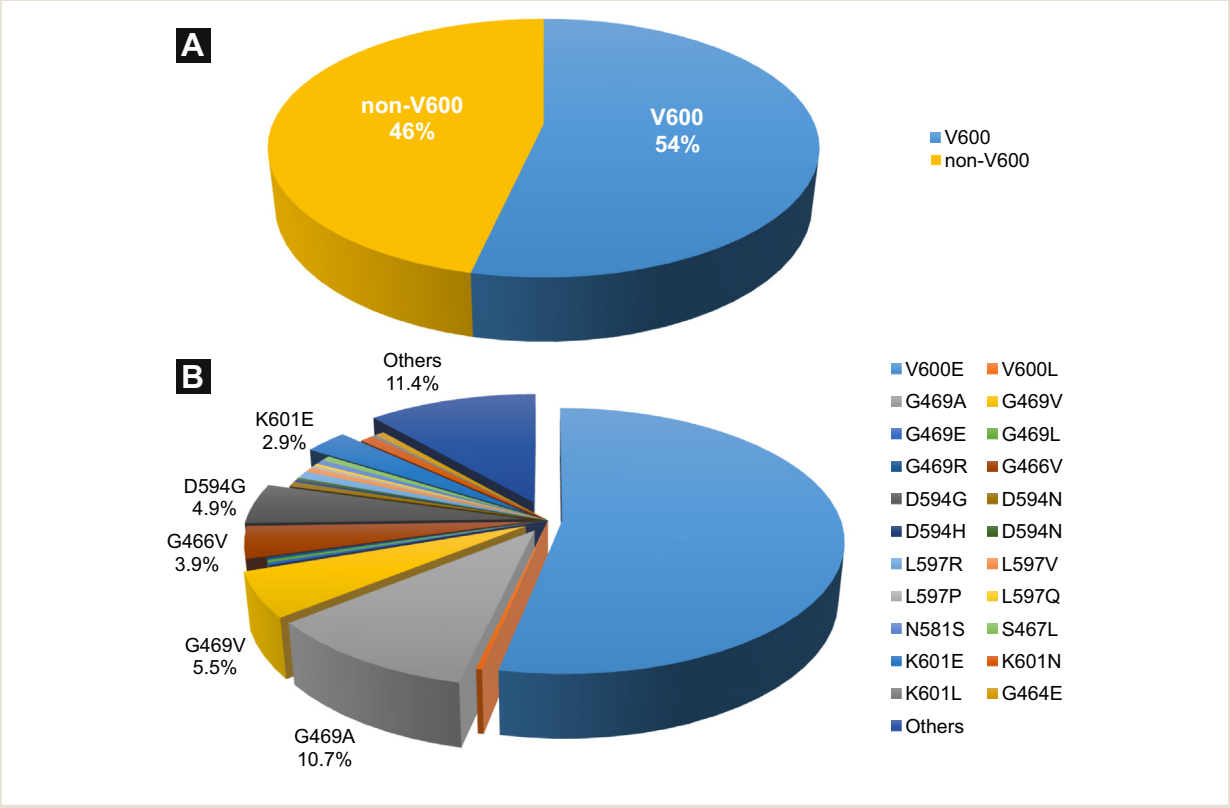
Data about response to BRAF and MEK inhibitors in patients harboring non-V600 *BRAF* mutations is still insufficient and open to discussion. This is owing to the fact that inclusion in most pivotal clinical trials was restricted to V600E mutations, and on the other hand, many genetic tests currently used in clinical practice test for individual targetable hotspot mutations such as *BRAF*-V600E, resulting in underreported non-V600 alterations.

In the European multicenter (EURAF) cohort study, which included 29 patients with V600E and 6 patients with non-V600 (one G469A) *BRAF* mutations, only 1 patient with a non-V600E mutation (G596V) obtained benefit from the single BRAF inhibitor vemurafenib compared with 54% of V600E patients.[15] Of note, a detrimental outcome was observed for non-V600E patients compared with the V600E subgroup (PFS, 1.5 vs. 9.3 months; and OS 11.8 vs. 25.3 months, respectively).[15] Thereby, 17 patients with *BRAF* non-V600 mutations (4 with G469) were enrolled in the NSCLC cohort of the French AcSé trial, which explored the efficacy of vemurafenib as a single agent across pretreated diverse types of tumors harboring *BRAF* mutations. The study has recently updated outcomes reporting median PFS and OS of 1.8 and 5.2 months, respectively, concluding that vemurafenib in monotherapy is not effective in patients harboring non-V600 *BRAF* mutations.[16]

The fact that our patient responded to dabrafenib and trametinib is of particular importance, as this is the first report indicating the potential sensitivity of double BRAF/MEK inhibition in a patient with advanced NSCLC with a G469A *BRAF* mutation. Our patient achieved a long-term benefit (15 months) with BRAF/MEK inhibition, outperforming the median PFS of 10.9 months reported in untreated patients with BRAF V600E-mutant NSCLC[8] and the dismal 1.5 and 1.8 months reported in the retrospective EURAF cohort and AcSé trial for non-V600 patients.[15,16]

Concurrent *BRAF* mutations and other genetic alterations is not rare in NSCLC (approximately 16%-17%).[3] However, the coexistence of 2 *BRAF* mutations has not been previously described in NSCLC; a literature search has only revealed a double *BRAF* mutation V600E/V600M and tandem *BRAF* mutations at exon 15 in primary melanomas.[17,18] As such, we confirmed the presence of both mutations by 2 independent

Figure 3 A, Frequency of V600 and Non-V600 *BRAF* Mutations in Lung Adenocarcinoma. B, Most Frequent Mutations Found Within the *BRAF* kinase Domain (aa 457-717)



Data obtained from the Catalogue of Somatic Mutations in Cancer (COSMIC) database.

NGS platforms, which resulted in comparable VAFs (34.3% and 37.4% with Oncomine; 38.5% and 37.7% with GeneReader, for G469A and W604C, respectively). Together, these results led us to hypothesize that both mutations were concomitantly present in the same tumor cell population.

Unlike G469A, the novel W604C mutation in BRAF can be found in databases such as the Catalogue of Somatic Mutations in Cancer (COSMIC), but there are no reports in the literature about its functional or clinicopathologic relevance. However, the mutation is located in a "hypermutated segment" within the kinase domain at exon 15 of BRAF (Figure 2). Activating mutations in kinases have been reported to cluster within those segments, suggesting that W604C might be actionable.[19]

Conclusion

BRAF mutations constitute a heterogeneous group of genetic alterations that comprise distinct functional classes with different clinical features and biological characteristics. Although we cannot establish whether the antitumor activity observed in our case responded to the likely actionability of class II G469A or the unclear significance of the novel W604C mutation, our case endorses the need of further evaluate the role of class-specific therapies to effectively target these specific molecular subsets of *BRAF*-mutant NSCLC.

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Disclosure

The authors have stated that they have no conflicts of interest.

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