ORIGINAL ARTICLE

Characteristics of and Treatment Strategies for Advanced *EGFR*-Mutant NSCLC With Concomitant *BRAF* Variations

Xue-Wu Wei, MD,[a,b] Jia-Yi Deng, MD,[a,b] Chong-Rui Xu, PhD,[a] Zhi-Hong Chen, MD,[c] Dong-Qin Zhu, PhD,[d] Qian Wu, PhD,[d] Xu-Chao Zhang, PhD,[c] Yi-Long Wu, MD,[a] Qing Zhou, PhD[a,b,*]

[a]*Guangdong Lung Cancer Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, People's Republic of China*
[b]*School of Medicine, South China University of Technology, Guangzhou, People's Republic of China*
[c]*Guangdong Provincial Key Laboratory of Translational Medicine in Lung Cancer, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, People's Republic of China*
[d]*Department of Research and Development, Nanjing Geneseeq Technology Inc., Nanjing, People's Republic of China*

Received 24 February 2022; revised 22 May 2022; accepted 25 May 2022
Available online - 9 June 2022

ABSTRACT

Introduction: *BRAF* variants were reported resistant mechanisms to EGFR tyrosine kinase inhibitors (TKIs) in *EGFR*-mutant NSCLC. Nevertheless, characteristics and subsequent treatment strategies of such patients remain unclear.

Methods: From October 2016 to May 2020, patients with advanced NSCLC for whom next-generation sequencing detected mutations of both *BRAF* and *EGFR* were retrospectively included. From June 2020 to January 2021, patients with *EGFR*-mutant NSCLC who acquired the *BRAF* V600E mutation after progression on osimertinib were prospectively enrolled to explore the efficacy and safety of EGFR plus BARF co-inhibition.

Results: A total of 58 patients were retrospectively identified and five prospectively included. *BRAF* variants were acquired after a median time of 22.7 months from initial diagnosis. The frequency of variations in *TP53, PIK3CA, RB1, MET, LRP1B, APC, CDKN2A, MYC, ERBB2,* and *SMAD4* was all more than 10%; these mutations affected the cell cycle or p53 pathway and the EGFR downstream and bypass pathways. The median progression-free survival was 5.0 months for patients on chemotherapy and 2.1 months for those on TKIs not targeting both of EGFR and BRAF ($p = 0.019$). The median PFS was 7.8 months in five patients who received EGFR plus BRAF co-inhibitory drugs. RAS signaling was activated on disease progression.

Conclusions: Variations in the *EGFR* downstream and bypass pathways were frequent in patients with dual mutations of *EGFR* and *BRAF*. The efficacies of TKIs not targeting both EGFR and BRAF were inferior to chemotherapy. EGFR plus BRAF co-inhibition improved efficacy. Such treatment strategies should be further explored.

Keywords: BRAF; EGFR; NSCLC; Characteristics; Treatment strategy

***Corresponding author.**

Disclosure: Prof. Zhou reports receiving honoraria from AstraZeneca, United Kingdom, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Merck Sharp & Dohme, United Kingdom, Pfizer, Roche, and Sanofi, outside the submitted work. Prof. Wu reports receiving advisory services for AstraZeneca, Boehringer Ingelheim, Novartis, Switzerland, and Takeda; personal fees from AstraZeneca, BeiGene, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Merck Sharp & Dohme, Pfizer, Roche, and Sanofi; and grants from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Hengrui, and Roche outside of the submitted work. The remaining authors declare no conflict of interest.

Address for correspondence: Qing Zhou, PhD, Guangdong Lung Cancer Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, 106 Zhongshan Er Road, Guangzhou 510080, People's Republic of China. E-mail: gzzhouqing@126.com

Cite this article as: Wei XW, Deng JY, Xu CR, et al. Characteristics of and treatment strategies for advanced *EGFR*-mutant NSCLC with concomitant *BRAF* variations. *JTO Clin Res Rep*. 2022;3:100348.

ISSN: 2666-3643

https://doi.org/10.1016/j.jtocrr.2022.100348

Introduction

EGFR tyrosine kinase inhibitors (TKIs) are routinely given to patients with advanced *EGFR*-mutant NSCLC. Nevertheless, acquired resistance to such drugs is inevitable, and treatment remains challenging. Resistance mechanisms include activation of *EGFR*-dependent or -independent pathways and histologic transformation.[1] The mechanisms of *EGFR*-dependent drug resistance have been well studied and include the acquired T790M mutation for first- or second-generation EGFR TKIs and the acquired C797S mutation for the third-generation EGFR TKI.[2,3] *EGFR*-independent resistance is less common, and more complicated, including amplification of *ERBB2* and *MET* and mutation of *PI3KCA*, *KRAS*, and *BRAF*.[4,5]

Acquired *BRAF* mutations or fusions develop in 1% to 3% patients with NSCLC who receive EGFR TKIs.[4,5] The RAS-RAF-MEK-ERK signaling pathway is a key regulator of cell growth; BRAF (a serine/threonine kinase) operates downstream of RAS. Usually, three classes of *BRAF* mutations are recognized based on the different activation mechanisms, which are as follows: RAS-independent active monomers (class 1), constitutively active dimers (class 2), and kinase-dead or -impaired mutations (class 3).[6,7] *BRAF* V600 encodes an active monomer; most other *BRAF* mutants are constitutively active RAS-independent dimers. In clinical trials, vemurafenib or dabrafenib plus trametinib effectively treated patients with NSCLC with *BRAF* V600E mutation but not those with *BRAF* non-V600E mutations.[8,9] Given the increasing accessibility of next-generation sequencing (NGS) in clinical practice, both *BRAF* V600E and non-V600E mutations are now frequently identified in tumors. Nevertheless, the clinical significance of acquired *BRAF* variants remains poorly understood.

In vitro, an acquired *BRAF* V600E mutation rendered some *EGFR*-mutant cell lines insensitive to EGFR TKIs; such insensitivity was eliminated by *BRAF* inhibitors.[10,11] Several case studies have reported that combinations of EGFR TKIs and BRAF inhibitors were effective (mediating EGFR + BRAF co-inhibition).[12,13] Nevertheless, no clinical trial on the safety and efficacy of such co-inhibition has been performed. Aboubakar Nana and Ocak[14] reviewed case reports on the efficacies and toxicities of EGFR plus BRAF co-inhibition and suggested that combination strategies might be appropriate.[14,15] Here, we retrospectively studied the clinical and genetic characteristics of patients with *EGFR* plus *BRAF*-mutant NSCLC and their prognoses. We also prospectively investigated the efficacy and safety of EGFR plus BRAF co-inhibition.

Materials and Methods

Study Patients

From October 2016 to May 2020, patients with advanced NSCLC with *EGFR* and *BRAF* co-mutations were retrospectively included. Clinical information was extracted from the electronic medical records of Guangdong Provincial People's Hospital. From June 2020 to January 2021, patients with acquired *BRAF* V600E mutation after failure of osimertinib were recommended to accept an EGFR plus BRAF co-inhibition therapy. This study was approved by the Ethics Committee of Guangdong Provincial People's Hospital (approval number GDREC2019304H). Written informed consent was provided by all participants. The study adhered to the principles of the Declaration of Helsinki.

Next-Generation Sequencing

At initial diagnosis or disease progression, tumor tissues or body fluids (pleural effusion, plasma, cerebrospinal fluid) were collected from all patients and subjected to panel NGSs exploring the status of 168, 196, 425, or 520 cancer-relevant genes.[16,17] A total of 75 genes evaluated by all four panels were analyzed in the present study. The clinical significance of *BRAF* mutations or fusions was that of the annotations of OncoKB (https://www.oncokb.org/) (a Food and Drug Administration–recognized, public, human genetic variant database).[18] Tier I and II variants are considered clinically significant, but tier III and IV variants are insignificant.[19]

Treatment and Efficacy

Patients initially received oral osimertinib 80 mg once daily plus vemurafenib 480 mg twice daily or osimertinib 80 mg once daily plus dabrafenib 150 mg twice daily plus trametinib 2 mg once daily until disease progression or the development of unacceptable adverse events. Computed tomography and brain magnetic resonance imaging (if needed) were performed 4 weeks after treatment initiation and then every 8 weeks. Responses were evaluated based on the Response Evaluation Criteria in Solid Tumors version 1.1 criteria. Progression-free survival (PFS) was defined as the time from the start of treatment to disease progression or death. Adverse events were recorded. Overall survival (OS) was defined as the time from identification of *BRAF* variants to death from any cause. Global OS was defined as the time from the initial diagnosis to death from any cause.

Statistical Analysis

Categorical variables were compared using the chi-square or Fisher's exact test. The nonparametric test

used was the ranked sum test. In terms of survival analyses, Kaplan-Meier curves were compared using the log-rank test and hazard ratios (HRs) calculated using the Cox's proportional hazards model. A two-sided p value less than 0.05 was considered significant. All statistical analyses were performed by IBM SPSS version 22.0 software.

Results

Patient Characteristics

A total of 58 patients were retrospectively identified and five patients were prospectively included (Supplementary Fig. 1). In total, 63 patients with *EGFR*-mutant NSCLC with concomitant *BRAF* mutations (n = 53) or fusions (n = 10) were included. Clinical and demographic characteristics are listed in Table 1. The *BRAF* variants were V600E (44.4%, 28 of 63), non-V600E (39.7%, 25 of 63), and fusions (15.9%, 10 of 63) (Fig. 1*A*); 52.4% (33 of 63) of *BRAF* mutations occurred in the tyrosine kinase domain (Fig. 1*B*); and 90.5% patients (57 of 63) developed *BRAF* variants on disease progression. A total of 49 patients exhibited clinically significant *BRAF* mutations or fusions (Supplementary Table 1).

Clinical Characteristics of EGFR-Mutant NSCLC With Concomitant BRAF Variations

The median time from initial diagnosis of advanced NSCLC to the detection of acquired *BRAF* variations was 22.7 months (95% confidence interval [CI]: 26.4–38.7 mo); the times were similar for the three *BRAF* subtypes (V600E versus non-V600E versus fusion: 22.6 versus 30.5 versus 26.0 mo, $p = 0.639$; Fig. 2*A*). Most patients (61.4%, 35 of 57) acquired *BRAF* variants after failure of second-line *EGFR* TKI treatment (Fig. 2*B*). Before detection of the *BRAF* variants, gefitinib/icotinib/erlotinib followed by osimertinib was the most common treatment (49.1%, Fig. 2*C*). Of all patients, 61.4% (35 of 57) developed *BRAF* variants after osimertinib, including three who received osimertinib as first-line treatment. The global median OS of all patients was 53.6 months (95% CI: 42.3–85.2 mo). The median OS of the patient cohort was 11.6 months (95% CI 9.7 mo–not applicable) from the time of detection of *BRAF* variants. A somewhat longer OS was achieved by patients with *BRAF* V600E (V600E versus non-V600E versus fusion: 16.8 versus 15.2 versus 10.7 mo, $p = 0.72$).

Molecular Characteristics of EGFR-Mutant NSCLC With Concomitant BRAF Variations

The median mutation count was seven in all patients (Fig. 2*D*) and was significantly higher in patients with non-V600E than V600E mutations (5 versus 8.5, $p = 0.004$). Apart from the *BRAF* variants, variations with frequencies more than 10% were noted in *TP53*, *PIK3CA*, *RB1*, *MET*, *LRP1B*, *APC*, *CDKN2A*, *MYC*, *ERBB2*, and *SMAD4* (Fig. 1*C*), thus in genes involved in the cell cycle/p53 pathway, *EGFR* downstream pathways (the PI3K and RAS pathways), and other receptor tyrosine kinases. On multivariate analysis, *RB1* mutations were significantly enriched in patients with non-V600E mutations (Supplementary Table 2, $p = 0.04$), as were *TP53* mutations (the latter marginally) ($p = 0.09$). Of 11 patients with *RB1* alterations, nine underwent tumor rebiopsy; no histologic transformation was evident.

A total of 33 patients underwent NGS before the development of *BRAF* variants (Supplementary Fig. 2). As *BRAF* variants were acquired, the frequencies of variations in *PIK3CA*, *APC*, *MYC*, *LRP1B*, *CDNK2A*, *KRAS*, and *SMAD4* also increased. *BRAF* V600E and *BRAF* fusions were uncommon in patients with preexisting *PIK3CA* alterations, but common in patients receiving osimertinib (Supplementary Table 3).

Potential Impact of De Novo BRAF Variants on EGFR-Mutant NSCLC

Concomitant *BRAF* variants were detected in six treatment-naive patients with NSCLC and included five *BRAF* non-V600E mutations (P422L, G466R, E533K, D555Y, and E611Q) and one *BRAF* fusion (*BRAF*∼*IGR*) (B8: upstream *MRPS33*). *BRAF* G466R is a tier II variant; the other five variants have not been reported. The impacts of mutations P422L, E533K, D555Y, and E611Q on protein structure were predicted to be benign by PolyPhen-2[20] (Supplementary Table 4). The *BRAF*∼*IGR* (B8: upstream MRPS33) fusion did not include the *BRAF* kinase domain. All patients received first-line *EGFR* TKIs and achieved an objective response rate (ORR) of 83.3% (five of six) with a median PFS of more than 12 months.

Efficacies of Following Treatments in Patients With Clinically Significant BRAF Variants

A total of 35 patients with acquired *BRAF* variants of clinical significance have received further treatment; most underwent chemotherapy (48.6%, 17 of 35: single nab-paclitaxel/docetaxel, n = 2; nab-paclitaxel/pemetrexed + cisplatin/carboplatin, n = 6; taxol/pemetrexed + cisplatin/carboplatin + bevacizumab, n = 9) or received TKIs (45.7%, 16/35) (Fig. 3*A*). Overall, the ORR and disease control rate were 14.3% (5 of 35) and 57.1% (20 of 35); the median PFS was 3.5 months. Of the TKI group, most patients (75.0%, 12 of 16) solely targeted *EGFR* only. The ORR and disease control rates were 6.3% (1 of 16) and 43.8% (7 of 16) in the TKI group but 23.5% (4 of 17) and 64.7% (11 of 17) in the chemotherapy group. The PFS was significantly longer in the chemotherapy than in the TKI group (5.0 versus 2.1

Table 1. Demographic and Clinical Characteristics of Cohort Patients

Characteristics	Total (N = 63)	V600E (n = 28)	Non-V600E (n = 25)	Fusion (n = 10)	*p*
Age (y), median					0.412
≤60	39 (61.9)	17 (60.7)	14 (56.0)	8 (80.0)	
>60	24 (38.1)	11(39.3)	11 (44.0)	2(20.0)	
Sex, n (%)					0.218
Female	39 (61.9)	14 (50.0)	18 (72.0)	7 (70.0)	
Male	24 (38.1)	14 (50.0)	7 (28.0)	3 (30.0)	
Smoking, n (%)					0.215
Yes	10 (15.9)	5 (17.9)	2 (8.0)	3 (30.0)	
No	53 (84.1)	23 (82.1)	23 (92.0)	7 (70.0)	
Pathology, n (%)					1
ADC	62 (98.4)	27 (96.4)	25 (100.0)	10 (100.0)	
ASC	1 (1.6)	1 (3.6)	0 (0.0)	0 (0.0)	
Stage, n (%)					0.691
IIIB-IVA	12 (19.0)	4 (14.3)	2 (8.0)	0 (0.0)	
IVB	51 (81.0)	24 (85.7)	23 (92.0)	10 (100.0)	
Brain metastasis, n (%)					0.59
Yes	33 (52.4)	16 (57.1)	11 (44.0)	6 (60.0)	
No	30 (47.6)	12 (42.9)	14 (56.0)	4 (40.0)	
PS, n (%)					1
0-2	61 (93.7)	27 (96.4)	24 (96.0)	10 (100.0)	
3	2 (6.3)	1 (3.6)	1 (4.0)	0 (0.0)	
EGFR subtype, n (%)					0.374
L858R	30 (47.6)	13 (46.4)	9 (36.0)	3 (30.0)	
19Del	33 (52.4)	15 (53.6)	16 (64.0)	7 (70.0)	
EGFR T790M status, n (%)					0.037
Yes	37 (58.7)	19 (67.9)	10 (40.0)	8 (80.0)	
No	26 (41.3)	9(32.1)	15 (60.0)	2 (20.0)	

ADC, adenocarcinoma; ASC, adenosquamous carcinoma; PS, performance status.

mo, log-rank test: $p = 0.019$, Fig. 3*B*). Among the 35 patients, 18 were positive for T790M before detection of *BRAF* variants, but eight lost T790M when *BRAF* variants were detected; seven patients acquired T790M and *BRAF* variants concurrently. Thus, the T790M positivity rate was 48.6% (17 of 35) when *BRAF* variants were detected. On multivariate Cox regression analysis, both the number of lines of previous treatment (1 versus ≥2: HR = 0.61, 95% CI: 0.38–0.99, $p = 0.044$) and treatment (TKI versus chemotherapy: HR = 3.61, 95% CI: 1.54–8.45, $p = 0.003$) were independently associated with PFS, whereas T790M status and the previous use of osimertinib were not (Supplementary Table 5).

We identified patients who had been naive for osimertinib or chemotherapy after acquiring *BRAF* variants and explored the potential impacts of the *BRAF* variants (Fig. 4). Five patients acquired *BRAF* V600E (n = 4) or G466E (n = 1) and concurrent T790M, after failure of the first- or second-generation *EGFR* TKIs. All received osimertinib as the next treatment. No patient achieved an objective response; the median PFS was 3.4 months. Four patients acquired *BRAF* fusions of the kinase domain-containing 3' region (Fig. 4). Two patients developed progressive disease after icotinib or afatinib and acquired *BRAF* fusions and T790M. One patient achieved a partial response but the PFS was short (4.8 mo); another experienced progressive disease at 1 month. Ten chemo-naive patients received chemotherapies as their next treatments after acquiring *BRAF* mutations (Fig. 4). Four achieved a partial response (40.0%) with a disease control rate of 80%. The median PFS was 6.0 months (range: 0.7–11.6 mo).

Efficacy and Safety of Combinations of Osimertinib and BRAF Inhibitors

Osimertinib plus vemurafenib (n = 4) or osimertinib plus dabrafenib plus trametinib (n = 1) was prospectively given to five patients. The median PFS was 7.8 months (range: 2.2–12.3 mo) (Fig. 5*A*). The disease control rate was 100%. Two patients achieved an objective response (Fig. 5*B*). Grade 3 rash was observed in one patient; other grade 1 or 2 adverse events included fatigue, arthralgia, fever, diarrhea, and anorexia (Supplementary Table 6).

Resistance Profiling of Patients on Combinations of Osimertinib and BRAF Inhibitors

Two patients (P20 and P21) underwent plasma or tissue-based NGS testing on failure of EGFR plus BRAF

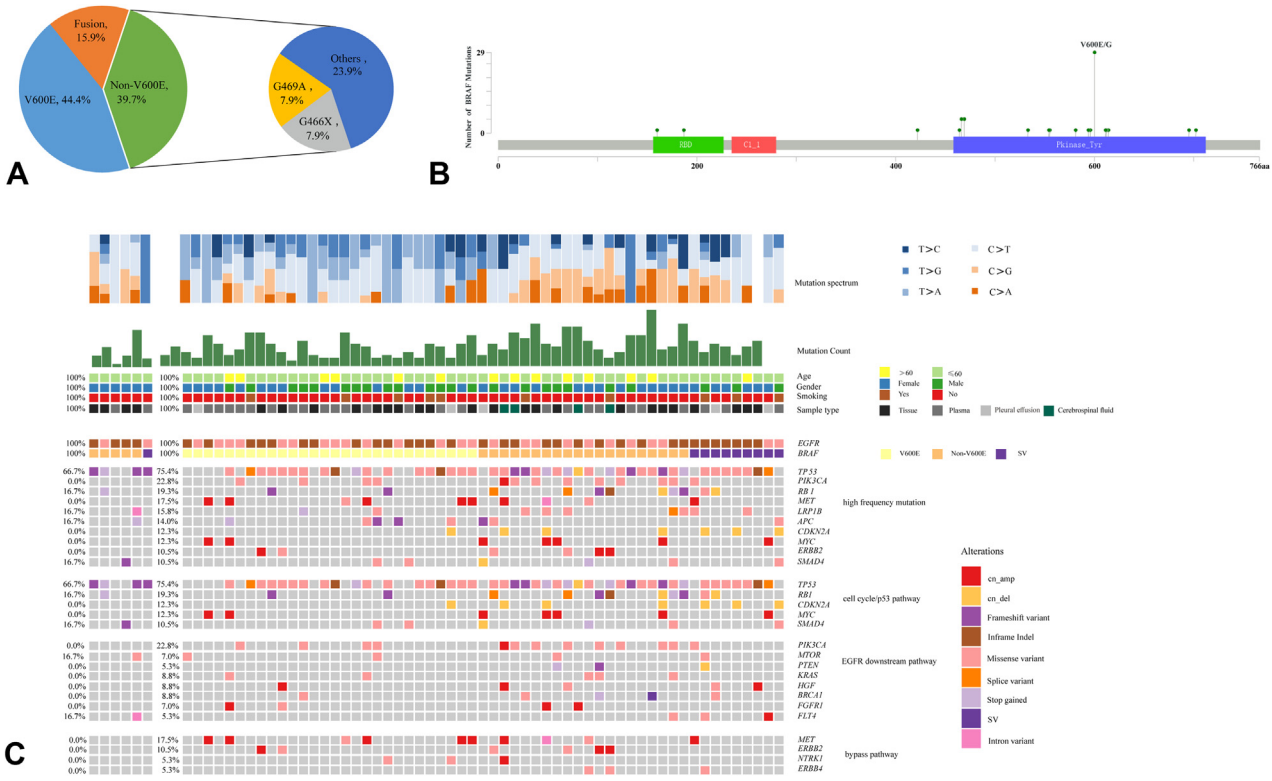

Figure 1. (*A*) The distribution of *BRAF* variants; (*B*) the distribution of *BRAF* mutations; and (*C*) the oncoprints of the clinical information and the gene profiles of 63 patients; genes that were altered at rates greater than 5% are illustrated. amp, amplification; del, deletion; SV, structural variation.

co-inhibition (Supplementary Table 7). P20 exhibited stable disease (with a PFS of 7.8 mo) but developed progressive disease. Given the difficulty of rebiopsy, plasma NGS was performed instead. Multiple mutations including *KRAS* G12R, *HRAS* Q61K, and *PIK3CA* E542K had been acquired. P21 seemed to be primarily resistant

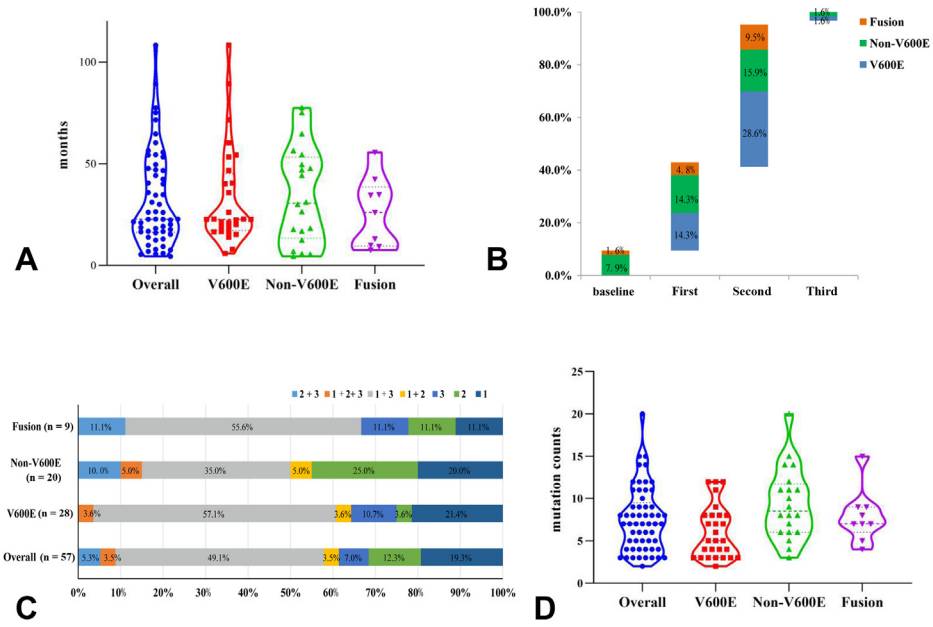

Figure 2. (*A*) Time from initial diagnosis to acquisition of *BRAF* variants. (*B*) The lines of TKI treatments to the times of acquisition of *BRAF* variants. (*C*) The TKI sequences before acquisition of *BRAF* variants: 1, 2, and 3 indicate the first-, second-, and third-generation *EGFR* TKIs, respectively. (*D*) The mutation counts in the overall population and patients of different *BRAF* subtypes. TKI, tyrosine kinase inhibitor.

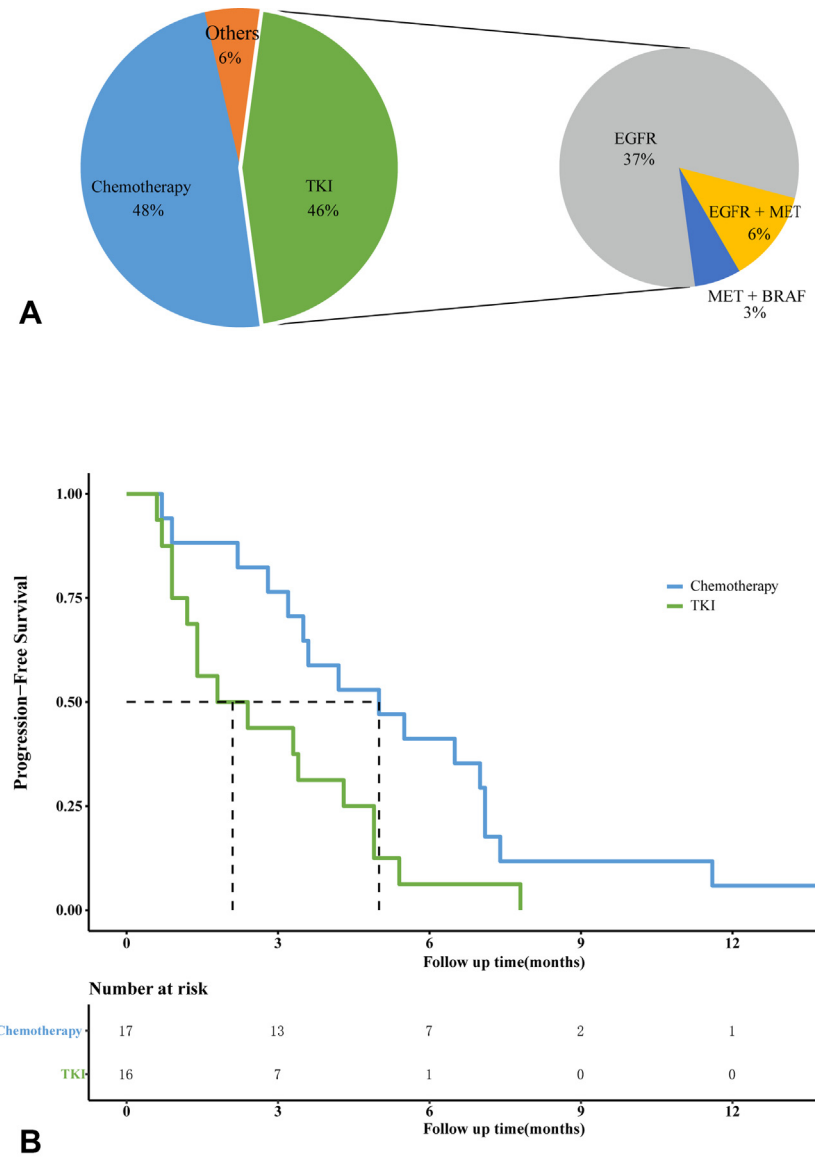

Figure 3. (*A*) Treatments after acquisition of clinically significant *BRAF* variants and the targets of TKIs; others indicated that one patient received chemotherapy plus PD-L1 inhibitors, another one received anlotinib. (*B*) Kaplan-Meier curves of patients who received chemotherapies and TKIs. PD-L1, programmed death-ligand 1; TKI, tyrosine kinase inhibitor.

to treatment; she experienced pleural effusion and enlargement of the left supraclavicular lymph node at a short PFS of 2.2 months. Compared with previous pleural effusion-based NGS, *NARS* Q61K and an *MYC* amplification were newly identified in the enlarged lymph node.

Discussion

To the best of our knowledge, this is the first study to explore patient characteristics and treatment strategies in those with both *EGFR*-mutant NSCLC and concomitant *BRAF* variants. The *BRAF* variants developed in subclones and acquired late during disease progression of *EGFR*-mutant NSCLC. Acquired *BRAF* variants may weaken the efficacies of subsequent EGFR TKIs; chemotherapy was superior to TKIs that did not target both EGFR and BRAF. Our pilot study revealed that EGFR plus BRAF co-inhibition might afford clinical benefits (with manageable toxicity) for such patients.

In a preclinical study, class 1 *BRAF* variants (*BRAF* V600) were sensitive to Food and Drug Administration–approved BRAF inhibitors (dabrafenib or vemurafenib) but class 2 or 3 *BRAF* variants were not.[7] This was replicated in a clinical trial of vemurafenib.[9] *BRAF* V600E was the most common acquired *BRAF* variant in our present patient cohort. Schrock et al. reported that *BRAF* kinase fusions were associated with acquired resistance

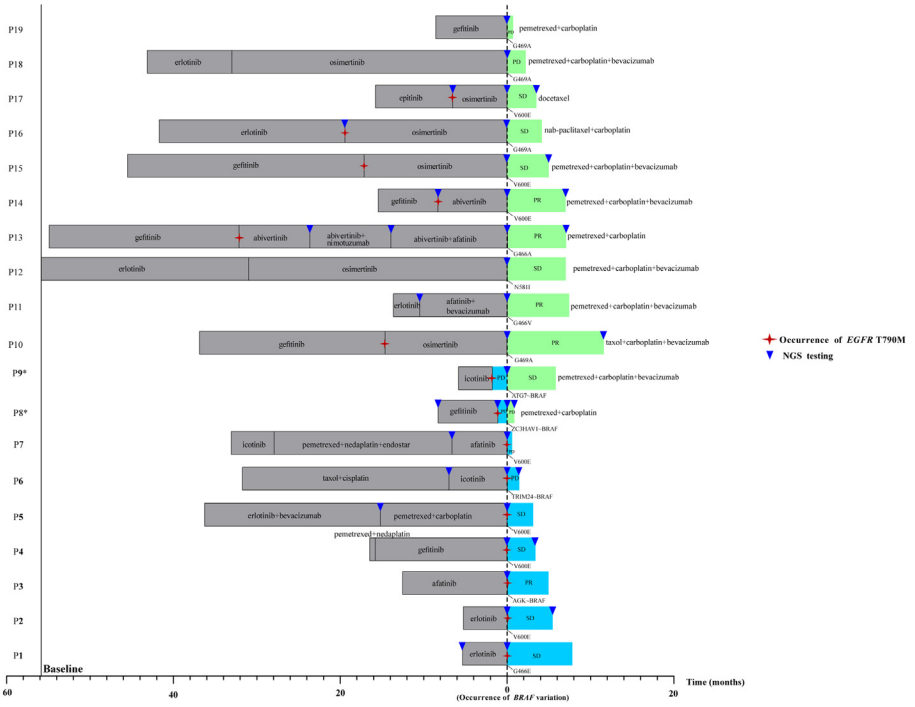

Figure 4. The efficacies of chemotherapy for chemo-naive patients and osimertinib for patients with both *BRAF* variants and T790M after failure of previous EGFR TKIs. Chemo is shown by the green bar; the chemo-regimens are indicated. Osimertinib is indicated by the blue bar. *These two patients experienced disease progression after 1 month and *BRAF* fusions were subsequently detected. Chemo, chemotherapy; NGS, next-generation sequencing; P, patient; PD, progressive disease; PR, partial response; SD, stable disease; TKI, tyrosine kinase inhibitor.

to EGFR TKIs.[21] In our present study, we identified a new *BRAF* kinase fusion partner (*RELCH*),[5] also known as *KIAA1468*. The encoded protein is involved in intracellular cholesterol transport and was a fusion partner of *RET*.[22] Vojnic et al.[23] introduced an *AGK~BRAF* fusion into the H1975 cell line (L858R+ and T790M+); growth inhibition by osimertinib was impaired. One of our patients acquired an *AKG~BRAF* fusion and T790M after progression on afatinib. Although this patient evidenced a partial response to single-agent osimertinib, the PFS was short (4.9 mo). Combined inhibition using osimertinib and trametinib, or a pan-RAF inhibitor, may be optimal for such patients. Apart from *BRAF* variants, a high frequency of tumor-promoting variants was observed in our cohort, especially *PI3KCA* mutation and *MET* amplification. The genetic profiles of those who acquired *BRAF* V600E and non-V600 mutations differed. Although statistical significance was not attained, the genetic background seemed to be "cleaner" in the *BRAF* V600E group. Thus, *BRAF* V600E may confer high-level resistance to EGFR TKIs, emphasizing that EGFR plus BRAF co-inhibition may be useful.

Peng et al.[24] comprehensively reported co-mutations of different *BRAF* subtypes with *EGFR*, including *BRAF* V600E and non-V600E mutations and fusions/rearrangements. Nevertheless, the impacts of those variants on later treatments were unclear.[24] Although we found concomitant *BRAF* variants in treatment-naive patients with *EGFR*-mutant NSCLC, these seemed to have minimal impact on subsequent EGFR TKI treatments (Supplementary Table 4). The variants may not have affected protein structure. In other words, de novo *BRAF* variants may be benign in *EGFR*-mutant patients, in line with the observation that an *EGFR*-activating mutation is seldom co-mutated with other driver genes in treatment-naive patients.[25] *BRAF* V600E and *BRAF* fusions conferred resistance to osimertinib in first-line settings.[26] Given the fact that most of the patients in our study developed *BRAF* variants after osimertinib indicates the importance of NGS to find resistant mechanisms for patients after failure of osimertinib. In addition, osimertinib is now the standard first-line treatment for advanced *EGFR*-mutant NSCLC, whether acquired *BRAF* variants more frequently occurred in patients who received osimertinib than other EGFR TKIs remained unknown. In our cohort, TKI treatment was of limited efficacy in patients with acquired *BRAF* variants. Nevertheless, chemotherapy remains an option and rather effective as in previous reports.[27,28] Notably, *BRAF* V600E was concurrently acquired with T790M after first- or second-generation EGFR TKIs. Moreover, subsequent osimertinib was not very

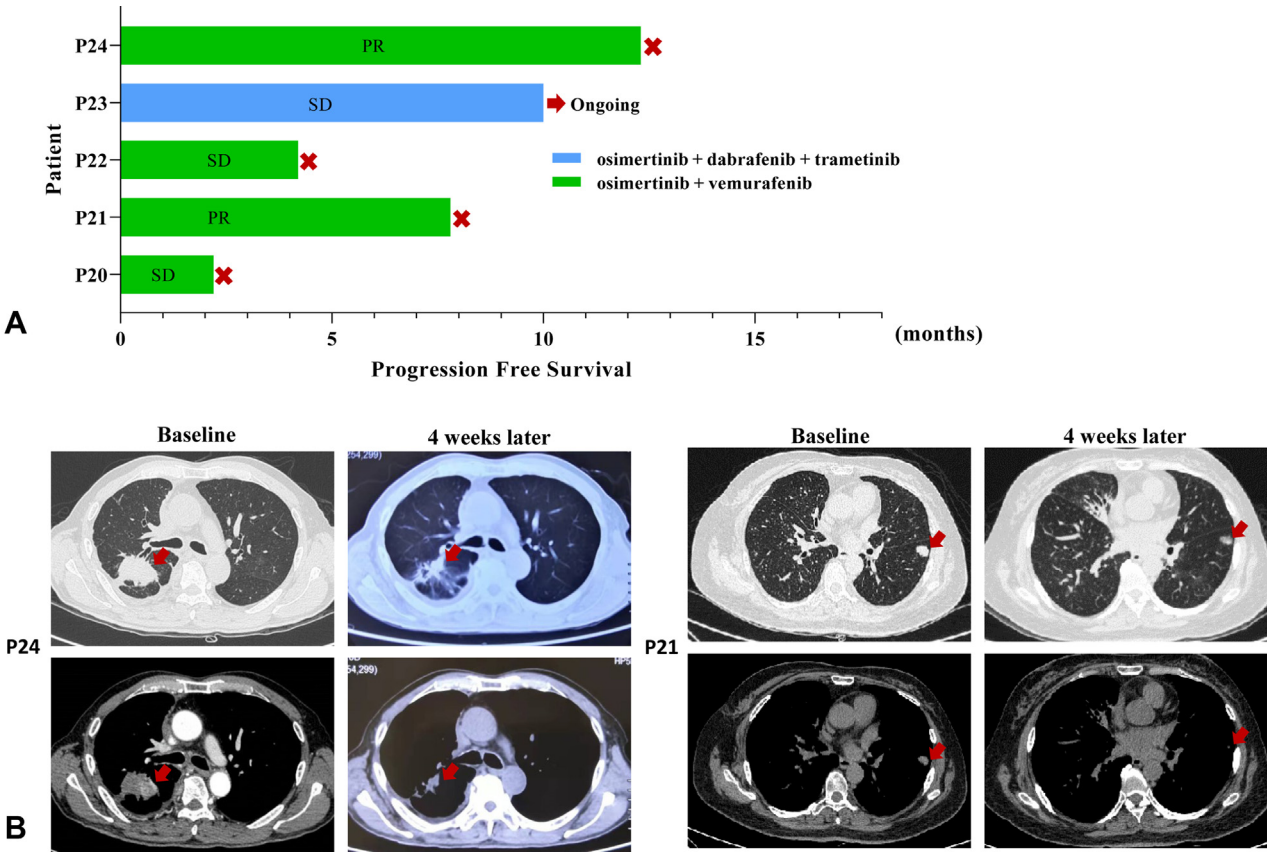

Figure 5. (*A*) Efficacy of EGFR plus BRAF co-inhibition in five patients with *EGFR*-mutant NSCLC who acquired *BRAF* V600E after progression on osimertinib. (*B*) Computed tomographic images of P21 and P24 before and 4 weeks after EGFR plus BRAF co-inhibition. P, patient; PR, partial response; SD, stable disease.

effective. This may part explain why some patients with acquired T790M do not respond to osimertinib.

In a clinical trial of patients with NSCLC with *BRAF* mutations, the discontinuation rate of vemurafenib at 960 mg twice daily attained 24%, and 60% of patients experienced dose reductions/treatment delays.[9] Although no phase 1 trial has explored co-inhibition by EGFR and BRAF, case reports and a literature review support the efficacy of a combined strategy but also indicate that full doses of vemurafenib and osimertinib are usually followed by dose reductions or treatment cessation.[15,29] In the present study, although the vemurafenib dose was only 480 mg twice daily, two of four patients required dose reductions. In a clinical trial, the ORR and PFS of vemurafenib were 44.8% and 5.2 months for patients with NSCLC with *BRAF* V600, respectively.[9] The efficacy of osimertinib plus vemurafenib in our study was similar. Our pilot study also indicated that clinical benefits improved by co-inhibition of EGFR and BRAF, although vemurafenib was delivered at half the regular dose. The optimal doses, safety, and efficacy of EGFR plus BRAF co-inhibition should be further investigated in a clinical trial. The genetic profiles of two patients were explored after both developed progressive disease on a combination of osimertinib and BRAF inhibitors. Both had acquired functional mutations in *RAS* genes. *RAS* mutations reportedly conferred resistance to osimertinib.[4] Activation of RAS signaling in patients with melanoma also conferred resistance to BRAF inhibitors.[30] On activation of RAS signaling, RAS-driven heterodimerization of BRAF and CRAF increases, enhancing drug resistance.[31] Together, the data suggest that *RAS* mutations may mediate resistance to EGFR plus BRAF co-inhibition.

Our work had several limitations. First, given the difficulties to rebiopsy, NGS was performed on tumor tissue, plasma, cerebral fluid, and pleural effusions. Second, approximately 30% of patients had *BRAF* variants after first-line EGFR TKI treatments, but some lacked baseline NGS panel data. Thus, such patients may have had preexisting *BRAF* variants. Third, the sample size of the pilot study on EGFR plus BRAF co-inhibition was small.

In conclusion, we found that acquired *BRAF* variants may reduce EGFR TKI efficacies. A combination of osimertinib with BRAF inhibitors improved efficacy in

patients acquiring *BRAF* V600E mutations after failure of osimertinib. EGFR plus BRAF co-inhibition should be investigated in a clinical trial.

CRediT Authorship Contribution Statement

Xue-Wu Wei: Investigation, Formal analysis, Data curation, Writing—original draft, Visualization.

Jia-Yi Deng, Chong-Rui Xu: Resources, Data curation.

Zhi-Hong Chen: Project administration.

Dong-Qin Zhu, Qian Wu: Visualization.

Xu-Chao Zhang: Resources.

Yi-Long Wu: Supervision.

Qing Zhou: Conceptualization, Methodology, Writing—review and editing, Funding acquisition.

Acknowledgments

The study was supported by the National Natural Science Foundation of China (grant number: 82072562 to Dr. Zhou), the High-level Hospital Construction Project (grant number: DFJH201810 to Dr. Zhou), and the GDPH Scientific Research Funds for Leading Medical Talents in Guangdong Province (grant number: KJ012019428 to Dr. Zhou). The authors thank the patients and their families for participating in this study.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2022.100348.

References

1. Lim SM, Syn NL, Cho BC, Soo RA. Acquired resistance to EGFR targeted therapy in non-small cell lung cancer: mechanisms and therapeutic strategies. *Cancer Treat Rev*. 2018;65:1–10.
2. Nguyen KS, Kobayashi S, Costa DB. Acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancers dependent on the epidermal growth factor receptor pathway. *Clin Lung Cancer*. 2009;10:281–289.
3. Jia Y, Yun CH, Park E, et al. Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. *Nature*. 2016;534:129–132.
4. Westover D, Zugazagoitia J, Cho BC, Lovly CM, Paz-Ares L. Mechanisms of acquired resistance to first- and second-generation EGFR tyrosine kinase inhibitors. *Ann Oncol*. 2018;29:i10–i19.
5. Ricordel C, Friboulet L, Facchinetti F, Soria JC. Molecular mechanisms of acquired resistance to third-generation EGFR-TKIs in EGFR T790M-mutant lung cancer. *Ann Oncol*. 2018;29:i28–i37.
6. Dankner M, Rose AAN, Rajkumar S, Siegel PM, Watson IR. Classifying BRAF alterations in cancer: new rational therapeutic strategies for actionable mutations. *Oncogene*. 2018;37:3183–3199.
7. Yao Z, Yaeger R, Rodrik-Outmezguine VS, et al. Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS. *Nature*. 2017;548:234–238.
8. Planchard D, Smit EF, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously untreated BRAFV600E-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. *Lancet Oncol*. 2017;18:1307–1316.
9. Mazieres J, Cropet C, Montane L, et al. Vemurafenib in non-small-cell lung cancer patients with BRAF(V600) and BRAF(nonV600) mutations. *Ann Oncol*. 2020;31:289–294.
10. Ho CC, Liao WY, Lin CA, Shih JY, Yu CJ, Yang JC. Acquired BRAF V600E mutation as resistant mechanism after treatment with osimertinib. *J Thorac Oncol*. 2017;12:567–572.
11. La Monica S, Minari R, Cretella D, et al. Acquired BRAF G469A mutation as a resistance mechanism to first-line osimertinib treatment in NSCLC cell lines harboring an EGFR exon 19 deletion. *Target Oncol*. 2019;14:619–626.
12. Ribeiro M, Knebel FH, Bettoni F, et al. Impressive response to dabrafenib, trametinib, and osimertinib in a metastatic EGFR-mutant/BRAF V600E lung adenocarcinoma patient. *NPJ Precis Oncol*. 2021;5:5.
13. Schaufler D, Ast DF, Tumbrink HL, et al. Clonal dynamics of BRAF-driven drug resistance in EGFR-mutant lung cancer. *NPJ Precis Oncol*. 2021;5:102.
14. Aboubakar Nana F, Ocak S. Targeting BRAF activation as acquired resistance mechanism to EGFR tyrosine kinase inhibitors in EGFR-mutant non-small-cell lung cancer. *Pharmaceutics*. 2021;13:1478.
15. Mauclet C, Collard P, Ghaye B, Hoton D, Nana FA. Tumor response to EGFR/BRAF/MEK co-inhibition in a patient with EGFR mutated lung adenocarcinoma developing a BRAF(V600) mutation as an acquired resistance mechanism. *Lung Cancer*. 2021;159:42–44.
16. Zhang YC, Chen ZH, Zhang XC, et al. Analysis of resistance mechanisms to abivertinib, a third-generation EGFR tyrosine kinase inhibitor, in patients with EGFR T790M-positive non-small cell lung cancer from a phase I trial. *EBiomedicine*. 2019;43:180–187.
17. Li YS, Jiang BY, Yang JJ, et al. Unique genetic profiles from cerebrospinal fluid cell-free DNA in leptomeningeal metastases of EGFR-mutant non-small-cell lung cancer: a new medium of liquid biopsy. *Ann Oncol*. 2018;29:945–952.
18. Chakravarty D, Gao J, Phillips SM, et al. OncoKB: a precision oncology knowledge base. *JCO Precis Oncol*. 2017;2017:PO.17.00011.
19. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn*. 2017;19:4–23.
20. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248–249.

21. Schrock AB, Zhu VW, Hsieh WS, et al. Receptor tyrosine kinase fusions and BRAF kinase fusions are rare but actionable resistance mechanisms to EGFR tyrosine kinase inhibitors. *J Thorac Oncol*. 2018;13:1312–1323.
22. Nakaoku T, Tsuta K, Ichikawa H, et al. Druggable oncogene fusions in invasive mucinous lung adenocarcinoma. *Clin Cancer Res*. 2014;20:3087–3093.
23. Vojnic M, Kubota D, Kurzatkowski C, et al. Acquired BRAF rearrangements induce secondary resistance to EGFR therapy in EGFR-mutated lung cancers. *J Thorac Oncol*. 2019;14:802–815.
24. Peng P, Lv G, Hu J, Wang K, Lv J, Guo G. Co-mutations of epidermal growth factor receptor and BRAF in Chinese non-small cell lung cancer patients. *Ann Transl Med*. 2021;9:1321.
25. Guo Y, Song J, Wang Y, et al. Concurrent genetic alterations and other biomarkers predict treatment efficacy of EGFR-TKIs in EGFR-mutant non-small cell lung cancer: a review. *Front Oncol*. 2020;10:610923.
26. Ríos-Hoyo A, Moliner L, Arriola E. Acquired mechanisms of resistance to osimertinib—the next challenge. *Cancers (Basel)*. 2022;14:1931.
27. Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol*. 2011;12:735–742.
28. Wu YL, Zhou C, Hu CP, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol*. 2014;15:213–222.
29. Sun M, Wang X, Xu Y, et al. Combined targeting of EGFR and BRAF triggers regression of osimertinib resistance by using osimertinib and vemurafenib concurrently in a patient with heterogeneity between different lesions. *Thorac Cancer*. 2022;13:514–516.
30. Rizos H, Menzies AM, Pupo GM, et al. BRAF inhibitor resistance mechanisms in metastatic melanoma: spectrum and clinical impact. *Clin Cancer Res*. 2014;20:1965–1977.
31. Yao Z, Torres NM, Tao A, et al. BRAF mutants evade ERK-dependent feedback by different mechanisms that determine their sensitivity to pharmacologic inhibition. *Cancer Cell*. 2015;28:370–383.