



Triple-targeted therapy of dabrafenib, trametinib, and osimertinib for the treatment of the acquired *BRAF* V600E mutation after progression on EGFR-tyrosine kinase inhibitors in advanced *EGFR*-mutated non-small cell lung cancer patients

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Background: The B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) V600E mutation is responsible for approximately 3% of acquired resistance mechanisms to epidermal growth factor receptor (*EGFR*)-tyrosine kinase inhibitors (TKIs) in advanced *EGFR*-mutant non-small cell lung cancer (NSCLC). This study investigated the efficacy and safety of a triple-targeted therapy combining *EGFR*/*BRAF*/mitogen-activated protein kinase kinase (MEK) inhibitors of dabrafenib, trametinib, and osimertinib in NSCLC patients with acquired *BRAF* V600E mutation after *EGFR*-TKI treatment.

Methods: A multi-center retrospective review of medical records was performed to analyze *EGFR*-mutated advanced Chinese NSCLC patients who acquired the *BRAF* V600E mutation following *EGFR*-TKI treatment. All patients subsequently received dabrafenib, trametinib, and osimertinib. The clinical characteristics, progression-free survival (PFS), and adverse events (AEs) were documented. The *in-vivo* drug response of patient-derived organoids (PDOs) was observed. Next-generation sequencing (NGS) was performed upon progression to triple-targeted therapy.

Results: Thirteen patients with *BRAF* V600E mutations were included. Following triple-targeted therapy,

the corresponding objective response rate and disease control rate were 61.5% and 92.3%, respectively. The median PFS was 13.5 months (95% confidence interval: 6.6–20.4). PDOs derived from one patient's tumor sample were established, revealing that the triple-targeted therapy had a significantly lower half-maximal inhibitory concentration (IC_{50}) value compared to other regimens. The tumor growth inhibitory rate was 99.36% for dabrafenib, trametinib, and osimertinib; 99.25% for osimertinib plus vemurafenib; 98.92% for osimertinib, encorafenib, and cetuximab; and 62.83% for pemetrexed plus carboplatin. NGS analysis identified major resistance mechanisms following the triple-targeted therapy, including the *EGFR*-dependent pathway, *EGFR* and *BRAF* V600E-dependent pathway, and an off-target mechanism.

Conclusions: *EGFR*/*BRAF*/*MEK* triple-targeted therapy is an effective and safe approach for treating *EGFR*-mutated NSCLC patients resistant to *EGFR*-TKIs with acquired *BRAF* V600E mutations.

Keywords: Non-small cell lung cancer (NSCLC); epidermal growth factor receptor (EGFR); resistance; B-Raf proto-oncogene; serine/threonine kinase V600E (*BRAF* V600E); treatment strategy

Submitted Apr 24, 2024. Accepted for publication Sep 02, 2024. Published online Oct 28, 2024.

doi: 10.21037/tlcr-24-358

View this article at: <https://dx.doi.org/10.21037/tlcr-24-358>

Introduction

Lung cancer remains a leading cause of cancer-related mortality globally, with non-small cell lung cancer (NSCLC) accounting for 85% of cases (1). The epidermal growth factor receptor (*EGFR*) gene mutation is a predominant driver mutation, particularly in lung adenocarcinoma, the most common NSCLC subtype. Notably prevalent in East Asian populations, *EGFR* mutations occur in approximately 50% of lung adenocarcinoma cases, prompting the adoption of *EGFR*-tyrosine kinase inhibitor (*EGFR*-TKI) as the primary first-line treatment for *EGFR*-mutated NSCLC patients (2,3). Despite initial success of *EGFR*-TKIs, acquired resistance (AR) inevitably develops in most advanced NSCLC patients.

Overcoming resistance to *EGFR*-TKI treatment poses an unmet challenge. While various on-target and off-target AR mechanisms have been identified, 30–50% of resistance mechanisms remain unknown, and chemotherapy is currently a practical and standard therapeutic strategy (4,5). One notable off-target downstream pathway contributing to AR in *EGFR*-mutated lung cancer is the presence of the B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) V600E mutation (6,7). The *BRAF* V600E mutation acts as an oncogenic driver via mitogen-activated protein kinase (MAPK), affecting approximately 3% of patients treated with *EGFR*-TKIs (8). Preclinical models suggest potential success in overcoming AR mediated by the *BRAF* V600E mutation through the combination of targeted therapies

inhibiting *EGFR* and *BRAF* or mitogen-activated protein kinase kinase (*MEK*) simultaneously (7,9). Sporadic cases also have demonstrated clinical improvement and good tolerance with the triple combination of osimertinib, dabrafenib, and trametinib (10–12).

There have been no large-scale multicenter trials to compare the efficacy of the triple-targeted therapy (combination of *EGFR*/*BRAF*/*MEK* inhibitors) with dual-targeted therapy (combination of *BRAF*/*MEK* inhibitors) and clarify the safety of triple-targeted therapy. In this multicenter retrospective study, we investigated the real-world efficacy and safety of a triple-targeted therapy involving dabrafenib, trametinib, and osimertinib for NSCLC patients with an acquired *BRAF* V600E mutation after *EGFR*-TKI treatment (13). Additionally, *in-vitro* experiments using patient-derived organoids (PDOs) were conducted to gain deeper insights into the drug response dynamics potential resistance mechanisms associated with this triple-targeted therapy (13). We present this article in accordance with the STROBE reporting checklist (available at <https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-358/rc>).

Methods

Study design and patients

In this real-world, multicenter study, we retrospectively reviewed the medical records of advanced Chinese NSCLC

patients who acquired *BRAF* V600E mutations following EGFR-TKI treatment. These patients were admitted to seven participating hospitals (Guangdong Provincial People's Hospital, Dongguan People's Hospital, Peking University Shenzhen Hospital, Shenzhen People's Hospital, The First Affiliated Hospital of Guangzhou Medical University, West China Hospital of Sichuan University, The First Affiliated Hospital of Sun Yat-sen University) between December 15, 2018, and October 30, 2023. The inclusion criteria were as follows: (I) pathologically confirmed stage IIIB/IIIC/IV NSCLC according to the 8th edition of the American Joint Committee on Cancer staging manual; (II) presence of *EGFR* mutations and receipt of EGFR-TKIs as a first- or later-line therapy; (III) development of an acquired *BRAF* V600E mutation upon progression with EGFR-TKI treatment and subsequent treatment with osimertinib, dabrafenib, and trametinib. Patients without complete medical records or follow-up information were excluded. The study was conducted in accordance with the

Declaration of Helsinki (as revised in 2013). Research Ethics Committee of Guangdong Provincial People's Hospital approved the study (approval No. GDREC2019217H). Informed consent was waived due to the retrospective nature of the present study. All participating hospitals/institutions were informed and agreed to the study.

Data collection and treatment procedures

The baseline demographic and clinicopathological data were extracted from the medical record system. *EGFR* and *BRAF* genotyping were performed using polymerase chain reaction or next-generation sequencing (NGS).

Patients initially received osimertinib at a daily oral dose of 80 mg, dabrafenib at 150 mg twice daily orally, and trametinib at 2 mg once daily orally. The treatment efficacy was assessed according to the Response Evaluation Criteria in Solid Tumors (version 1.1). Progression-free survival (PFS) was measured from the start of triple-therapy to the occurrence of progressive disease or the last follow-up. Adverse events (AEs) were graded using the Common Terminology Criteria for Adverse Events, version 5.0.

Upon progression during triple-targeted therapy, NGS analysis and fluorescence in situ hybridization (FISH) testing were performed to detect resistance mechanisms (14).

PDO cultures and drug sensitivity tests

Malignant effusion samples were collected at the Guangdong Provincial People's Hospital for the PDO experiments. The PDO cultures and sensitivity tests involving anticancer drugs were performed according to established procedures (15), the detailed information and concentrations of drug compounds are presented in Table S1. Cell viability was assessed using a CellTiter-Glo 3D assay (Promega, Madison, WI, USA). The half-maximal inhibitory concentration (IC₅₀) values were calculated using GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA).

Statistical analysis

Continuous variables were expressed as the mean \pm standard deviation or median and interquartile range. Categorical variables were reported as counts and percentages. PFS was analyzed by Kaplan-Meier survival curves. Statistical analyses were performed using SPSS 25.0 software.

Highlight box

Key findings

- Triple-targeted therapy [epidermal growth factor receptor (EGFR)/B-Raf proto-oncogene, serine/threonine kinase (BRAF)/mitogen-activated protein kinase (MEK)] exhibited significant efficacy and safety in treating non-small-cell lung cancer (NSCLC) patients with acquired *BRAF* V600E mutations post-EGFR-tyrosine kinase inhibitor (TKI) treatment. Notably, it achieved an objective response rate of 61.5%, a disease control rate of 92.3%, and a median progression-free survival (PFS) of 13.5 months.

What is known and what is new?

- The *BRAF* V600E mutation arises in approximately 3% of EGFR-TKI-treated patients. Prior research has indicated the potential of targeted therapies against EGFR and BRAF or MEK mutations to enhance outcomes in resistant cases.
- This study with real-world evidence affirmed the efficacy of triple-targeted therapy for this specific patient subgroup. Additionally, it validated the therapy's superior efficacy over standard chemotherapy through patient-derived organoids. Furthermore, it identified major resistance mechanisms.

What is the implication, and what should change now?

- Triple-targeted therapy is a treatment option for EGFR-mutated NSCLC patients with acquired *BRAF* V600E mutations post-EGFR-TKI resistance.
- Further research into resistance mechanisms is warranted to refine treatment strategies. Updating clinical guidelines and practices to acknowledge the efficacy of triple-targeted therapy may lead to improved patient outcomes.

Table 1 Baseline demographic information and characteristics of patients with an acquired *BRAF* V600E mutation after EGFR-TKI resistance

| Clinical characteristics | Value (n=13) |
|----------------------------------|------------------|
| Age (years) | 53.0 (39.5–66.5) |
| ≥60 | 4 (30.8) |
| <60 | 9 (69.2) |
| Gender | |
| Female | 11 (84.6) |
| Male | 2 (15.4) |
| Smoking history | |
| Non-smoker | 11 (84.6) |
| Smoker | 2 (15.4) |
| Clinical stage | |
| IVA | 3 (23.1) |
| IVB | 10 (76.9) |
| ECOG PS | |
| 0 | 2 (15.4) |
| 1 | 9 (69.2) |
| ≥2 | 2 (15.4) |
| Metastatic site | |
| Bone | 7 (53.8) |
| Anterior mediastinal lymph nodes | 5 (38.4) |
| Liver | 2 (15.4) |
| Lung | 5 (38.4) |
| Brain | 4 (30.8) |
| Others | 6 (46.2) |
| Histology | |
| Adenocarcinoma | 13 (100.0) |
| EGFR mutation | |
| Exon 19 del | 9 (69.2) |
| Exon 21 L858 | 4 (30.8) |
| Prior treatment line | |
| 1 | 2 (15.4) |
| 2 | 5 (38.5) |
| 3 | 3 (23.1) |
| ≥4 | 3 (23.1) |
| Genetic test specimen | |
| Tissue | 7 (53.8) |
| Plasma | 4 (30.8) |
| Pleural effusion | 2 (15.4) |

Data are presented as n (%) or median (interquartile range). Stages IVA and IVB are classified according to the eighth edition of the TNM staging system for lung cancer. ECOG PS, Eastern Cooperative Oncology Group Performance Status; *BRAF*, B-Raf proto-oncogene, serine/threonine kinase; *EGFR*, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

Results

Baseline characteristics

Thirteen patients with an acquired *BRAF* V600E mutation receiving the triple-targeted therapy were included in this study. The median age of the patients was 53.0 years old, and 84.6% were female. The triple-targeted therapy was initiated as the second-line treatment for 2 (15.4%), as the third-line treatment for 5 (38.5%) patients and as treatment beyond the third line for 6 (46.2%) patients (*Table 1*). The median follow-up time was 19.8 months [95% confidence interval (CI): 10.2–29.5].

Clinical outcomes

As of October 30, 2023, twelve patients were evaluated for having the best response. Eight patients (61.5%) achieved a partial response (PR) and 4 (30.8%) achieved stable disease, resulting in an objective response rate (ORR) of 61.5% and a disease control rate of 92.3%. The median PFS was 13.5 months (95% CI: 6.6–20.4) (*Figure 1A,1B*).

Patient No. 5 achieved PR of triple-targeted therapy. Thoracic lesion exhibited significant reduction (75%) following the therapy. The triple-targeted therapy maintained its efficacy for a duration of 15 months (*Figure 1C*).

Patient No. 13 initially received dabrafenib and trametinib after experiencing progression of EGFR-TKI therapy upon the emergence of *BRAF* V600E. Although a PR was initially achieved with the dual therapy, the disease progressed after 3 months. Subsequently, triple-targeted therapy was started, resulting a PR after 2 months (*Figure 1D*).

PDOs for drug response

PDOs were successfully established using a malignant effusion sample from patient No. 10 who had received osimertinib as first-line therapy and osimertinib plus bevacizumab as second-line therapy. The drug sensitivity test showed that the triple-targeted therapy had a significantly lower IC₅₀ value compared to the other treatment regimens. The tumor growth inhibitory rate was 99.36% for dabrafenib, trametinib, and osimertinib; 99.25% for osimertinib plus vemurafenib; 98.92% for osimertinib, encorafenib, and cetuximab; and 62.83% for pemetrexed plus carboplatin (*Figure 2A-2D*). The thoracic lesions of the corresponding patient exhibited a significant reduction in size after the triple-targeted therapy (*Figure 2E*).

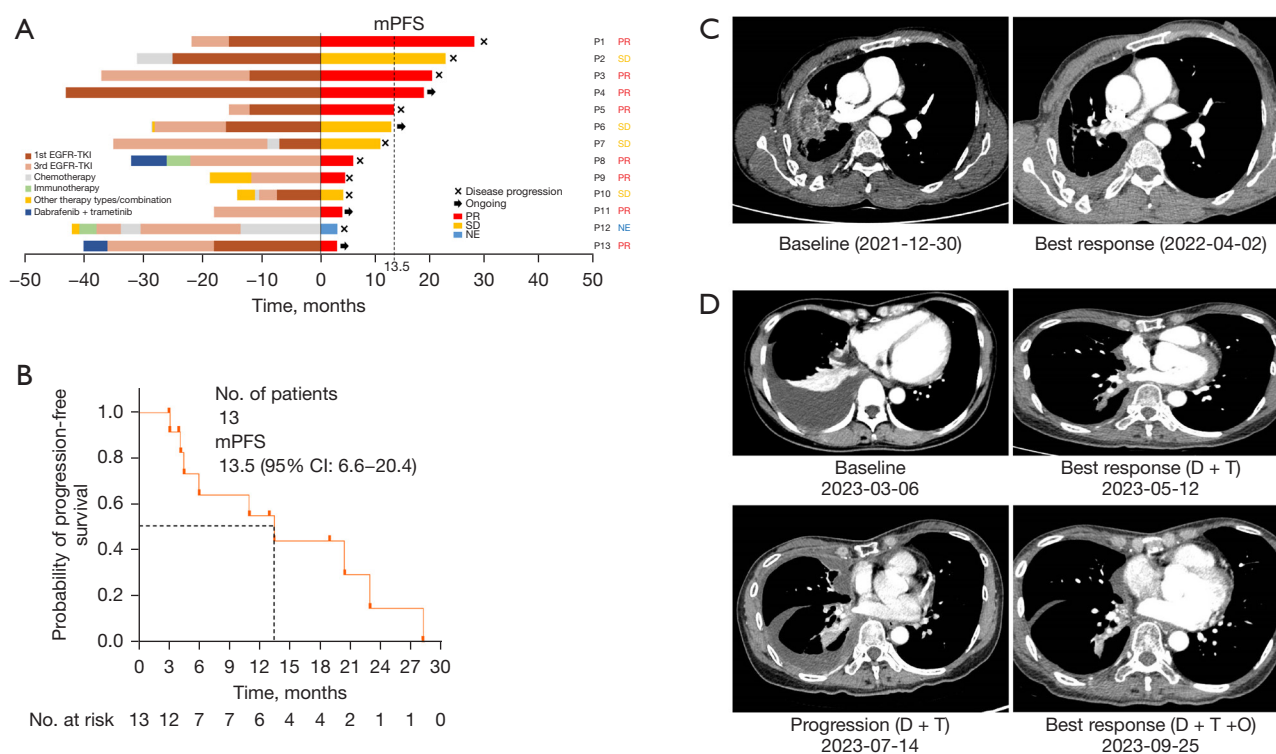


Figure 1 Efficacy and patient cases of triple-targeted therapy. (A) Swimmer plot. The x-axis at 0 represents the start of triple-targeted therapy. The left side of the y-axis indicates the treatment timelines before triple-targeted therapy, and the right side of the y-axis represents the treatment assessment after triple-targeted therapy. (B) Kaplan-Meier survival curve for progression-free survival. (C) Patient case—thoracic lesion changes before and after triple-targeted therapy. Patient No. 5 achieved a partial response after triple-targeted therapy. The thoracic lesions exhibited a significant reduction (75%) following the therapy. The triple-targeted therapy maintained its efficacy for a duration of 15 months. (D) Patient case—changes of thoracic lesions at baseline, after dual therapy, at progression, and after triple-targeted therapy. Patient No. 13 initially received dabrafenib and trametinib after experiencing progression on EGFR-TKI therapy upon the emergence of a *BRAF* V600E mutation. Although a partial response was initially achieved with the dual therapy, the disease progressed after 3 months. Subsequently, triple-targeted therapy was started, resulting in a PR after 2 months. mPFS, median progression-free survival; PR, partial response; SD, stable disease; NE, non-evaluable; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; 1st EGFR-TKI, first generation EGFR-TKI; 3rd EGFR-TKI, third generation EGFR-TKI; *BRAF*, B-Raf proto-oncogene, serine/threonine kinase; D + T, dabrafenib + trametinib; D + T + O, dabrafenib, trametinib and osimertinib.

Safety profiles

AE related to triple-targeted therapy included diarrhea (n=6, 46.2%), pyrexia (n=3, 23.1%), anemia (n=3, 23.1%), nausea (16.7%), mouth ulcer (15.4%), decreased appetite (7.7%), rash (7.7%) and edema (7.7%), among which, \geq Grade 3 anemia were observed in two cases (15.4%) (Table 2).

Patient No. 5 developed Grade 4 anemia after the triple-targeted therapy. This situation was alleviated after drug withdrawal, red blood cell transfusions and erythropoietin treatment. Dosing is resumed at a half dose (osimertinib at 80 mg Qd, dabrafenib at 75 mg bid, and trametinib at

1 mg qd) after hemoglobin rose to 80 g/L and patient status improved (Figure 3), and the patient did not experience any AEs after resuming dosing. Patient No. 5 experienced PFS for 1 month after resuming dosing.

Exploration of AR mechanisms following triple-targeted therapy

Seven patients underwent NGS analysis to identify the primary resistance mechanism after receiving the triple-targeted therapy. The heatmap of NGS-detected genetic alterations exhibited significant differences between before

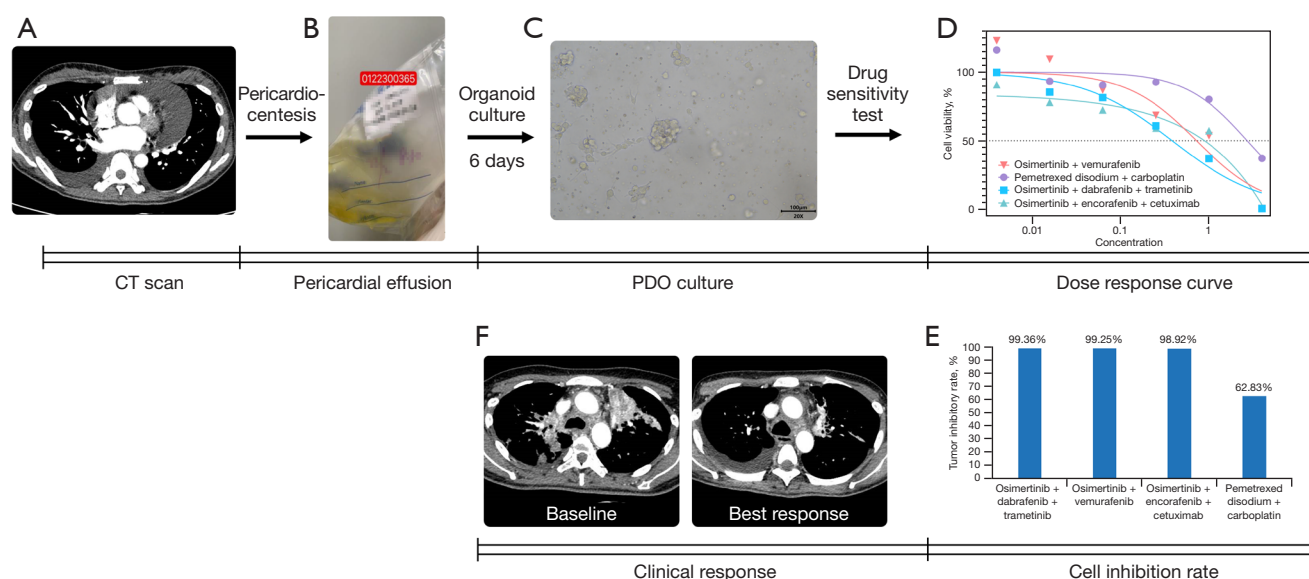


Figure 2 The procedure of the PDO experiment. (A) Chest CT image depicting malignant epicardial effusion sampled for the PDO model. (B) Pericardial effusion for PDO sampling. (C) PDO culture: on the 6th day of cultivation, the organoids displayed an average diameter of 63.67 μm (with a range of approximately 21.03–126.22 μm), and a total count of around 3,000 organoids, as illustrated in the light microscope image. (D) Cell proliferation inhibitory rates. (E) IC_{50} values. (F) Efficacy of the triple-targeted therapy shown in the chest CT images from patient No. 9. PDO, patient-derived organoid; CT, computed tomography; IC_{50} , half-maximal inhibitory concentration.

Table 2 Triple-targeted TRAEs

| TRAEs | All grades, n (%) | Grade 3–4, n (%) |
|--------------------|-------------------|------------------|
| Diarrhea | 6 (46.2) | 1 (7.7) |
| Pyrexia | 3 (23.1) | 0 |
| Anemia | 3 (23.1) | 2 (15.4) |
| Mouth ulcer | 2 (15.4) | 0 |
| Nausea | 1 (7.7) | 0 |
| Decreased appetite | 1 (7.7) | 0 |
| Rash | 1 (7.7) | 0 |
| Edema | 1 (7.7) | 0 |
| Unknown | 4 (33.3) | 0 |

TRAE, treatment-related adverse event.

and after triple-targeted therapy resistance (Figure 4A). The gene profiles became more intricate post-resistance. The predominant changes post-resistance included BRAF loss, reactivation of the EGFR pathway, and the emergence of bypass activation events involving MET proto-oncogene, receptor tyrosine kinase (MET)/neurotrophic tyrosine receptor kinase (NTRK). In most cases of post-

triple-targeted therapy resistance, *TP53* mutations (5 out of 7) appeared, often accompanied by multiple gene amplifications. These resistance mechanisms can be categorized into three types, namely the *EGFR*-dependent pathway (n=2), the *EGFR* and *BRAF* V600E-dependent pathway (n=4), and an off-target mechanism (n=1). Among the patients categorized into the *EGFR*-dependent pathway, neither exhibited *BRAF* mutations, yet in one patient, *EGFR* C797S emerged upon progression (Figure 4B). Furthermore, one patient demonstrated *MET* amplification as further confirmed by NGS (CN = 7.0), FISH (c-Met/CSP7 ratio: 8.6), and immunohistochemical (IHC) staining (70%+++, 30%++), indicating an off-target resistance mechanism to the triple-targeted therapy (Figure 4C).

Discussion

This study evaluated the efficacy of a novel triple-targeted therapy involving the EGFR/BRAF/MEK inhibitors dabrafenib, trametinib, and osimertinib in a real-world setting. We specifically examined its potential in NSCLC patients who acquired *BRAF* V600E mutations after prior EGFR-TKI treatment. Our findings highlight a robust clinical response to this triple regimen, along with manageable AEs and a

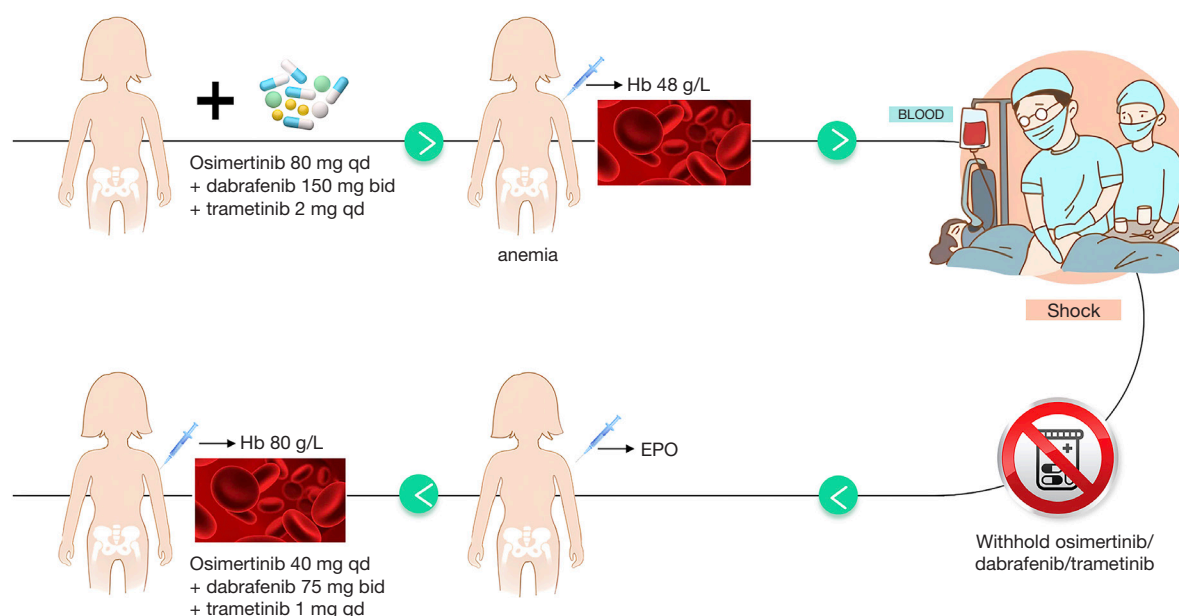


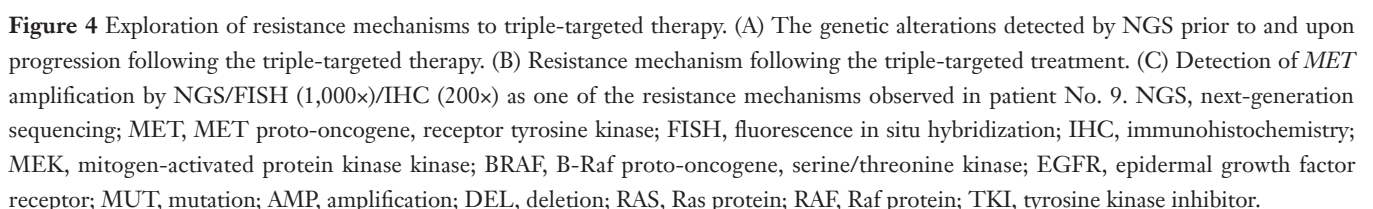
Figure 3 The clinical management of grade 4 anemia. EPO, erythropoietin; Hb, hemoglobin.

promising PFS of 13.5 months. Utilizing PDOs, we not only validated the clinical effectiveness and but also identified the molecular resistance mechanisms, primarily categorized into the *EGFR*-dependent pathway, *EGFR* and *BRAF* V600E-dependent pathway, and off-target mechanisms, thereby providing valuable directions for subsequent therapeutic strategies.

BRAF, a serine-threonine kinase within the RAS/RAF/MEK/ERK signaling cascade, plays a pivotal role in regulating cell proliferation, differentiation, and survival by activating downstream targets MEK1 and MEK2 (16). The *BRAF* V600E mutation, a prominent *BRAF* mutation subtype in NSCLC, presents a therapeutic challenge. Immunotherapy has demonstrated limited clinical efficacy in this cohort. The IMMUNOTARGET study reported an ORR of 24% and a PFS of 3.1 months with immune checkpoint inhibitors monotherapy in advanced NSCLC patients with *BRAF* alterations (17). The AcSé vemurafenib monotherapy for *BRAF* V600E-mutated NSCLC displayed an ORR of 37.1–44.9% and a PFS of 5.2–6.5 months (5,18). Notably, trials have validated the efficacy of *BRAF*/MEK inhibitors, specifically dabrafenib and trametinib, in managing advanced NSCLC patients with *BRAF* V600E mutations (19). The BRF113928 trial reported an ORR of 64%, a median PFS of 14.6 months and a median OS of 24.6 months (20). As a second-line treatment, dabrafenib plus trametinib yielded encouraging outcomes, with a median PFS of 10.2 months and

a median OS of 18.2 months (21). For Chinese patients with *BRAF* V600E-mutated NSCLC, the combined regimen of dabrafenib plus trametinib achieved an impressive ORR of 75% (22). The National Comprehensive Cancer Network, European Society of Medical Oncology, and Chinese Society of Clinical Oncology guidelines all recommend dabrafenib plus trametinib as a preferred treatment of *BRAF* V600E-mutated NSCLC.

EGFR downstream signaling pathways include PI3K/AKT, RAS/MAPK/ERK, and JAK/STAT pathways (5). *BRAF* V600E mutation observed in 3% of patients post-*EGFR*-TKI treatment is a primary alteration in the MAPK pathway triggering *EGFR*-TKI resistance (8,9,16,23). Dual inhibition of *EGFR* and MEK has emerged as a potential approach against RAS mutation-mediated osimertinib resistance in *EGFR*-mutated NSCLC (24,25). Preliminary studies support combining osimertinib with an MEK inhibitor to overcome resistance (26–28). For NSCLC patients developing *BRAF* V600E mutations following *EGFR*-TKI treatment, case reports have highlighted the feasibility of *EGFR*/*BRAF*/MEK co-inhibitors, yielding an ORR of 50–71.4%, and a median PFS of up to 11 months (12,29,30). In this study, the majority received triple-targeted therapy as a third- or subsequent-line treatment. Interestingly, *EGFR* mutations persisted alongside *BRAF* V600E mutations upon *EGFR*-TKI resistance, emphasizing the need for combination therapies (16,30). Patient No.



9, progressing after dabrafenib and trametinib treatment, exhibited improvement with the triple-targeted therapy.

Standard chemotherapy post-EGFR-TKI failure in *EGFR*-mutated NSCLC results in a median PFS of approximately five months (4). Trials exploring combinations of immunotherapy with chemotherapy post-EGFR-TKI progression (ORIENT-31, Keynote 789), reported inconsistent findings (14,31). Preclinical studies revealed increased tumor sensitivity when co-treating tumor cells expressing both *EGFR* T790M and *BRAF* V600E mutations with osimertinib and a *BRAF* V600E inhibitor (9). Our study, through the use of PDOs (32), observed superior efficacy of dabrafenib, trametinib, and osimertinib compared to current standard chemotherapy.

Adverse reactions to triple-targeted therapy typically include fever, rash, nausea, fatigue, reduced appetite, diarrhea, and pain, manageable through symptomatic treatment or by dosage adjustment (30). Consistent with previously reported safety profiles, our study found most AEs, including gastrointestinal symptoms, fever, oral mucositis, and rash, to be mild and controllable. Grade 3–4 anemia in two patients, one progressing to shock, was swiftly resolved with transfusions and erythropoietin treatment (33). Temporary dosage reduction, later resumed at half the initial, maintained efficacy.

Molecular profiling is a prerequisite for precision medicine in NSCLC. Liquid biopsy has emerged as a valuable alternative to nucleic acid testing (16). The loss of sensitivity to EGFR-TKIs can primarily be categorized into three groups: mutations acquired within the target gene (*EGFR*), such as the T790M or C797S mutation; activation of bypass signaling pathways, represented by *RAS* mutations or *MET* amplification; and histologic transformation into small cell lung cancer (34). *BRAF*/MEK inhibition resistance in *BRAF*-mutated NSCLC is mainly due to the recurrent MAPK pathway activation (35). The resistance mechanism with triple-targeted therapy involves EGFR-dependent pathways, or *EGFR* and *BRAF* V600E-dependent pathway. The mechanisms of resistance to dual-targeted and triple-targeted therapies differ notably, particularly with the emergence of *BRAF* loss, reoccurrence of the EGFR pathway with the C797S mutation appearing post-resistance, and in some cases *MET* amplification.

This retrospective study, albeit with a small sample size, highlights the potential efficacy and safety of triple-targeted therapy involving dabrafenib, trametinib, and osimertinib in treating acquired *BRAF* V600E mutation after progression on EGFR-TKIs. However, further confirmation is necessary through large-sample, multi-center randomized controlled

trials. Additionally, the different sample sources for NGS detection after triple-targeted therapy resistance may impact the determination of resistance mechanisms.

Conclusions

This is the largest cohort study to date demonstrating that EGFR/*BRAF*/MEK triple-targeted therapy may be an effective and safe approach for acquired *BRAF* V600E mutations in *EGFR*-mutated NSCLC post-EGFR-TKIs resistance. The PDO model confirms its superior efficacy over chemotherapy, serving as a promising predictive tool. A multifaceted resistance mechanism involves alterations in EGFR/*BRAF*-dependent pathways and off-target mechanisms.

Acknowledgments

A preliminary version of this work was presented at ESMO ASIA as poster presentation in 2023. We thank all patients who participated in this study as well as their families. We also thank Burning Rock Biotech, Berry Oncology, and Nanjing Geneseeq Technology Inc. for their help with NGS data analysis.

Funding: This study was supported by the National Natural Science Foundation of China (No. 81972164 to J.J.Y.) and Guangdong Provincial Key Laboratory of Lung Cancer Translational Medicine (No. 2017B030314120).

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-24-358/rc>

Data Sharing Statement: Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-24-358/dss>

Peer Review File: Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-24-358/prf>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-24-358/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related

to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Research Ethics Committee of Guangdong Provincial People's Hospital approved the study (approval No. GDREC2019217H). Informed consent was waived due to the retrospective nature of the present study. All participating hospitals/institutions were informed and agreed the study.

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Cite this article as: Weng CD, Liu KJ, Jin S, Su JW, Yao YH, Zhou CZ, Li YF, Chen ZX, Chen HJ, Li YY, Tang KJ, Yang JJ. Triple-targeted therapy of dabrafenib, trametinib, and osimertinib for the treatment of the acquired *BRAF* V600E mutation after progression on EGFR-tyrosine kinase inhibitors in advanced *EGFR*-mutated non-small cell lung cancer patients. *Transl Lung Cancer Res* 2024;13(10):2538-2548. doi: 10.21037/tlcr-24-358

Table S1 Details of drug compounds used in this study and their normalized concentrations

| Compound name | Supplier | Cat. No. | Actual concentration when the normalized concentration is 1 (μM) | Maximum concentration for drug test (μM) |
|---------------|----------|----------|---|---|
| Osimertinib | MCE | HY-15772 | 2 | 8 |
| Dabrafenib | MCE | HY-14660 | 2 | 8 |
| Trametinib | MCE | HY-10999 | 0.2 | 0.8 |
| Vemurafenib | MCE | HY-12057 | 2 | 8 |
| Encorafenib | MCE | HY-15605 | 2 | 8 |
| Pemetrexed | MCE | HY-10820 | 75 | 300 |
| Carboplatin | MCE | HY-17393 | 12.5 | 50 |