Original Article

Co-mutations of epidermal growth factor receptor and BRAF in Chinese non-small cell lung cancer patients

Panli Peng[1#], Guoli Lv[2#], Jinwei Hu[3]^, Kai Wang[3], Junhong Lv[4], Gang Guo[5]

[1]Oncology No. 2 Department, Guangdong Second Provincial General Hospital, Guangzhou, China; [2]Department of Thoracic Surgery in the Aged, the First Affiliated Hospital of Kunming Medical University, Kunming, China; [3]OrigiMed Co., Ltd., Shanghai, China; [4]Thoracic Surgeons Department, Guangdong Second Provincial General Hospital, Guangzhou, China; [5]Department of Thoracic Surgery, Yunnan Cancer Center, Kunming, China

Contributions: (I) Conception and design: P Peng, G Lv; (II) Administrative support: J Lv; (III) Provision of study materials or patients: P Peng, G Lv; (IV) Collection and assembly of data: J Hu, K Wang; (V) Data analysis and interpretation: J Hu, K Wang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Prof. Junhong Lv. Thoracic Surgeons Department, Guangdong Second Provincial General Hospital, Guangzhou, China. Email: Junhonglv@163.com; Gang Guo. Department of Thoracic Surgery, Yunnan Cancer Center, No.519, Kun Zhou Road, Xi-shan District, Kunming 650000, China. Email: 13888480101@139.com.

Background: Epidermal growth factor receptor (EGFR) and BRAF are 2 driver genes in non-small cell lung cancer (NSCLC) which are normally mutually exclusive. It has been previously reported that the existence of BRAF V600E in EGFR-mutated NSCLC patients could cause resistance to EGFR tyrosine kinase inhibitors (TKIs), but the influence of other BRAF actionable mutations on resistance to EGFR-TKIs has not yet been investigated. Understanding the coexistence of EGFR and BRAF actionable mutations in Chinese NSCLC patients may be essential for further treatment and prognostic prediction.

Methods: A total of 127 Chinese NSCLC patients harboring EGFR and BRAF co-mutations were enrolled in this study. We analyzed the mutation profiles of these patients through next-generation sequencing (NGS). We explored the associations between somatic mutations and patient characteristics, including tumor stage and age, among others.

Results: The frequency of EGFR and BRAF co-mutation was 0.91% in Chinese NSCLC patients, compared with 0.97% in Western NSCLC patients (cBioPortal). Among the 127 patients with both EGFR and BRAF mutations, 93 of them harbored clinically significant mutations. The remaining 34 patients were found to have mutations of uncertain significance of either EGFR or BRAF. TP53 was the most frequently mutated gene in BRAF and EGFR co-mutation patients, accounting for around 58% (N=54/93). MET active mutations (amplification and exon 14 skipping) accounted for 12% (N=11/93). Approximately 18% of patients (N=17/93) with significant EGFR mutations were detected to have fusions/rearrangements of the BRAF gene. BRAF fusion was more likely detected in EGFR exon19del patients compared with non-exon19del patients (P value =0.015). In addition, EGFR T790M, the most TKI-resistant mutation, was not found in any patient with BRAF fusion/rearrangement.

Conclusions: This study is the first to show different subtypes of EGFR and BRAF co-mutations in Chinese NSCLC patients. The prognosis of EGFR-TKI treatment may vary according to different BRAF actionable mutations. Aside from BRAF V600E, class II/III and BRAF fusions were found, which provides clues for investigating the resistance mechanisms of EGFR-TKIs in the future.

Keywords: BRAF; fusion; epidermal growth factor receptor (EGFR); non-small cell lung cancer (NSCLC); co-mutation

^ ORCID: 0000-0003-2399-2586.

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Introduction

The incidence and mortality of lung cancer rank first among all types of cancers worldwide (1). Non-small cell lung cancer (NSCLC) is the major histological subtype of lung cancer, accounting for approximately 75–80% of all cases (2). Epidermal growth factor receptor (*EGFR*) is the most frequent somatic mutation driver gene in NSCLC, detected in ~30–40% of Asian patients (3). With the development of precision medicine, tyrosine kinase inhibitors (TKIs) have become the standard drug treatment for NSCLC patients harboring *EGFR* somatic mutations and have greatly improved overall survival. However, acquired resistance (AR) to *EGFR*-TKIs always occurs after targeted therapy. Different mechanisms have been discovered to be responsible for AR, including on-target (EGFR-dependent) and off-target (EGFR-independent) (4). Such as *EGFR* T790M mutation (~50–60%) after first- or second-generation EGFR TKIs, *MET* amplification (~20%), and transformation to small-cell lung cancer (SCLC) (~5–10%), among others (5). *BRAF* is another driver gene found in NSCLC. The frequency of *BRAF* mutations is relatively low (~2–5%) (6-8). *BRAF* alterations were found in 4.4% of Chinese NSCLC patients (N=1,200) (9). *BRAF* mutations include V600E, promoting several fold kinase hyperactivation; non-V600E activating mutations, rearrangements, N-terminal deletions (NTDs), kinase domain duplications (KDDs), and fusions, resulting in constitutive activation of BRAF and downstream ERK signalling (10-12). Dabrafenib and trametinib are approved for the management of advanced NSCLCs that harbor *BRAF* V600E mutations.

EGFR and *BRAF* mutations are normally mutually exclusive, as the coexistence of *EGFR* and *BRAF* somatic mutations are uncommon in NSCLC patients. The frequency of *EGFR* and *BRAF* co-mutation in the western population is around 0.97% [cBioPortal database (http://www.cbioportal.org)]. With the accumulation of NSCLC patients, some studies have reported the existence of actionable *BRAF* mutations in *EGFR*-mutated NSCLC patients. A study on 5,125 Chinese NSCLC patients found that only 2 of them harbored both *EGFR* and *BRAF* mutations (13). Another study on patients with AR to EGFR-TKIs detected *BRAF* mutations in 2 patients (G469A and V600E), and cell line experiments demonstrated that *BRAF* V600E could cause resistance to erlotinib (14). These studies highlight the possibility that *BRAF* mutations are likely to be another emerging mechanism of AR to EGFR-TKIs. In a study of 326 non-squamous NSCLC patients, 240 (73.6%) had *EGFR* mutations, and of these 240 patients with *EGFR* determination, 2.9% had *BRAF* mutations (15). *BRAF* was shown to be altered in 4.5% of western NSCLC patients, and 37.4% (n=397) had *BRAF* V600E, 38% had *BRAF* non-V600E activating mutations, and 18% had *BRAF* inactivating mutations. Rearrangements were observed at a frequency of 4.3% (10).

Limited to the number of patients carrying both *EGFR* and *BRAF* mutations, the influence of different *BRAF* mutations on EGFR-TKIs is not yet clear. This study aims to determine the incidence of various *EGFR* and *BRAF* co-mutations in Chinese NSCLC patients and the influence of different types of *BRAF* mutations (including short variants, copy number changes, and rearrangements) on EGFR-TKI-treated Chinese NSCLC patients. We present the following article in accordance with the STROBE reporting checklist (available at https://dx.doi.org/10.21037/atm-21-3570).

Methods

Sample collection

We retrospective analysed from 13,976 Chinese NSCLC patient samples that formalin-fixed, paraffin-embedded (FFPE) tumor samples and matched blood samples were collected and prepared according to standard procedures. All cases were diagnosed with lung cancer according to the World Health Organization criteria based on hematoxylin and eosin staining reviewed by experienced pathologists, including lung adenocarcinoma, squamous cell lung carcinoma, and adenosquamous carcinoma of the lung, among other types. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of Guangdong Second Provincial General Hospital (No.: GZR-2020-KT-39-01) and informed consent was taken from all the patients.

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Next-generation sequencing (NGS)

Genomic alterations of patients were detected by the tissue-based 450 genes panel assay for FFPE samples with paired blood as normal control, and circulating tumor DNA (ctDNA) based for 329 or 18 genes panel. All samples were sequenced in a College of American Pathologists (CAP) accredited and Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory (OrigiMed, Shanghai, China).

At least 50 ng of cancer tissue DNA was extracted from each 40-mm FFPE tumor sample using a DNA Extraction Kit (QIAamp DNA FFPE Tissue Kit) according to the manufacturer's protocol. All coding exons of 450 key cancer-related genes and selected introns of 36 genes commonly rearranged in solid tumors were incorporated into the custom hybridization capture panel (Yuansu^{TM}, OrigiMed) (16). Libraries were constructed and each diluted to 1.05 nM and then sequenced with a mean coverage of 900× for tissue samples (minimum 700×) and 300× for matched blood samples on an Illumina NextSeq-500 Platform (Illumina Incorporated, San Diego, CA, USA).

Bioinformatics analysis, variant identification, and annotation

Genomic alterations including single nucleotide variants (SNVs), short and long insertions/deletions (Indels), copy number variations (CNVs), gene rearrangements, and fusions were subjected to advanced analysis. First, reads were aligned to a human genome reference sequence (hg19) by Burrows-Wheeler-Alignment (BWA) (17), and polymerase chain reaction (PCR) duplicates were removed using Picard (available online: https://broadinstitute.github.io/picard/). Second, SNVs and short Indels were identified by MuTect (18) after quality recalibration and realignment using GATK (Broad Institute, Cambridge, MA, USA) and an in-house pipeline. Short Indels were then calibrated using the results from Pindel (19). The log-ratio per region of each gene was calculated, and customized algorithms were used to detect copy number changes. Tumor cellularity was estimated by allele frequencies of sequenced SNPs. A customized algorithm was developed to detect gene rearrangements and long Indels. Reliable somatic alterations were detected in the raw data by comparing tumor tissues with matched blood control samples. At minimum, 5 reads and a minimum variant allele frequency of 1% were required to support alternative calling. For the calling of gene rearrangements and fusions, aligned reads with an abnormal insert size of over 2,000 or 0 bp were collected and used as discordant reads. Next, the discordant reads with a distance less than 500 bp formed clusters that were further assembled to identify potential rearrangement breakpoints. The breakpoints were confirmed by the BLAST-like alignment tool and the resulting chimeric gene candidates were annotated.

Statistical analysis

The variants were divided into 4 tiers after identification (20): Tier I, variants with strong clinical significance; Tier II, variants with potential clinical significance; Tier III, variants of unknown clinical significance; and Tier IV, variants deemed benign or likely benign. Tier I and Tier II were considered clinically significant mutations, which is the focus of future analysis. IBM SPSS Statistics (Version 20.0; IBM Corp, Armonk, NY) was used for statistical analysis. For all test, $P<0.05$ was defined as statistically significant.

Results

A total of 127 patients harboring both *EGFR* and *BRAF* mutations were included in this study. Of these patients, 93 harbored clinically significant mutations of both *EGFR* and *BRAF*. The remaining 34 patients had mutations of uncertain significance of either *EGFR* or *BRAF*. We aimed to explore the association between *BRAF* mutations and EGFR-TKI AR, and focused on the analysis of 93 patients harboring both *EGFR* and *BRAF* clinically significant mutations.

Demographic and clinicopathological data of the patients

The demographics and clinicopathological data of patients in the cohort are summarized in *Table 1*. The median age of patients at the time of sampling was approximately 60 years (range, 33–82 years), and females were moderately overrepresented compared with males (56% of patients were female). There were approximately equal numbers of male and female patients older than 60. However, female patients were overrepresented in the ≤60 age group (66% *vs.* 34%), which is slightly different to Chinese lung cancer patients (9). Regarding histological subtypes, most patients had lung adenocarcinoma (95%), while all other patients had squamous cell lung carcinoma. Patients were classified into main clinical stages (I–IV) according to both pathology and medical history following the American Journal of

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Critical Care Cancer Staging Manual (version 8; *Table 1*). More than half of the patients were late stage (III–IV).

Profiling of 18 actionable genes of EGFR and BRAF co-mutation NSCLC patients

We analyzed 18 actionable genes of the 93 NSCLC patients, including *AKT1* , *ALK*, *BRAF*, *CDKN2A*, *DDR2*, *EGFR*, *ERBB2*, *KRAS*, *MAP2K1*, *MET*, *NRAS*, *NTRK1*, *PIK3CA*, *PTEN*, *RB1*, *RET*, *ROS1*, and *TP53*. Profiling of the 18 actionable genes of the 93 NSCLC patients was conducted as shown in *Figure 1*. *TP53* was the most frequently mutated gene in *BRAF* and *EGFR* co-mutated patients, accounting for approximately 58% (N=54/93). *MET* active mutations (amplification and exon 14 skipping) accounted for 12% (N=11/93). *CDKN2A* mutations accounted for 8.6% (N=8/93) and *PIK3CA* mutations accounted for 7.5% (N=7/93). *CDKN2A* and *PIK3CA* mutations were more frequently observed in late-stage (III–IV) *EGFR* and *BRAF* co-mutated patients.

Table 1 Clinical characteristics of 127 *EGFR* and *BRAF* co-mutation NSCLC patients

Characteristics	Subtypes	No. of patients (%)
Age	Mean (SD)	59.7 (8.3)
	Median age [range]	59 [33–82]
Gender	Male	56 (44.1)
	Female	71 (55.9)
Histology	Lung adenocarcinoma	121 (95.3)
	Squamous cell lung carcinoma	6 (4.7)
Stage	I	25 (19.7)
	II	10 (7.9)
	III	13 (10.2)
	IV	51 (40.2)
	Unknown*	28 (22)

*, patients with unknown clinical stage indicated that the clinical stages were not clarified according to the information from physicians. NSCLC, non-small cell lung cancer; *EGFR*, epidermal growth factor receptor.

Distribution of EGFR mutations in NSCLC patients

In regards to *EGFR* mutations, which excluded amplification of *EGFR*, 86% of *EGFR*-mutant patients harbored hotspots, including L858R (37%), exon 19 deletion (32%), T790M (13%), and exon 20 insertions (4%). *Figure 2* shows the profile of *EGFR* genomic alterations. Moreover, uncommon mutations accounted for 14% of *EGFR* mutations. Uncommon *EGFR* mutations were defined as mutations other than L858R, exon 19del, and exon 20ins, including KDD (exon18_exon25dup) and G719/S768 mutation.

Distribution of EGFR and BRAF subtypes of EGFR and BRAF co-mutation NSCLC patients

BRAF mutations included short variants and fusions.

Figure 1 Profiling of 18 actionable genes of NSCLC. Substitution, a sequence change where, compared to a reference sequence, one nucleotide is replaced by one other nucleotide. Indel, a sequence change where, compared to a reference sequence, one or more nucleotides are inserted or deleted. Truncation, a stop gain of substitution or frameshift indel mutation. NSCLC, non-small cell lung cancer.

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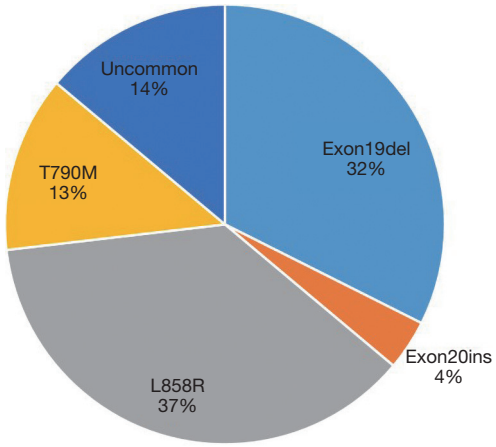

Figure 2 Distribution of *EGFR* mutations of NSCLC patients. NSCLC, non-small cell lung cancer; *EGFR*, epidermal growth factor receptor.

Short variants of *BRAF* were divided into 3 classes: RAS-independent and signal as active monomers (class 1), constitutively active dimers (class 2), and impaired kinase activity or are kinase-dead (class 3) (21). *BRAF* fusions/rearrangements were divided into 3 types depending on different partner genes and breakpoints. including: (I) likely fusion, refer to the 5' region of a novel partner gene with the kinase domain-containing 3' region of *BRAF*; (II) known fusion, refer to the 5' region of a known partner gene with the kinase domain-containing 3' region of *BRAF*; (III) Rearrangements, any other forms were classified as rearrangements. Approximately 18% of patients (N=17/93) with clinically significant EGFR mutations were found to have combined BRAF fusions/rearrangements (*Table 2*).

The number of short variants of *BRAF* was 12 for class 1, 14 for class 2, and 13 for class 3. There were 9 uncommon

Table 2 The 17 *BRAF* fusions/rearrangements in *EGFR*-mutated NSCLC patients

Patient ID	BRAF fusion/rearrangement	Kinase domain	Fusion/rearrangement classification
P89	AGK-BRAF	Kinase domain included	Known fusion
P90	AGK-BRAF	Kinase domain included	Known fusion
P83	CUX1-BRAF	Kinase domain included	Known fusion
P77	NRF1-BRAF	Kinase domain included	Known fusion
P12	NRF1-BRAF	Kinase domain included	Known fusion
P65	MKRN1-BRAF	Kinase domain included	Known fusion
P45	MGAM-BRAF	Kinase domain included	Likely fusion
P36	CNTNAP2-BRAF	Kinase domain included	Likely fusion
P78	TERF1-BRAF	Kinase domain included	Likely fusion
P51*	WDR91-BRAF	Kinase domain included	Likely fusion
P68	ADCK2-BRAF	Kinase domain included	Rearrangement
P5	BRAF-CUL1	Kinase domain included	Rearrangement
P29	BRAF-CALD1	Kinase domain not included, hot breakpoint region	Rearrangement
P2	BRAF-CHRM2	Kinase domain not included, hot breakpoint region	Rearrangement
P81	BRAF-MYO5B	Kinase domain not included, hot breakpoint region	Rearrangement
P31	7q34-BRAF	Kinase domain included	Rearrangement
P67	7q22.1-BRAF	Kinase domain included	Rearrangement

*, this patient also harbored a BRAF V600E hotspot mutation. Additionally, 7q34 and 7p22.1 represent the locations of the intergenic regions in the rearrangement. Partner gene reserved region refers to the regions of the partner gene in the rearrangement. NSCLC, non-small cell lung cancer; *EGFR*, epidermal growth factor receptor.

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Table 3 Classification of *BRAF* mutations in *EGFR*-mutated NSCLC patients

BRAF mutation	BRAF short variant	Number of patients
Class 1	V600E	12
Class 2	G469A	2
	G469R	2
	G469V	2
	G469S	1
	K601E	3
	L597Q	1
	L597R	1
	V600_K601delinsE	1
	V600_K601insPATV	1
Class 3	D594N	1
	D594G	2
	G466A	1
	G466E	2
	G466R	1
	G466V	1
	G596A	1
	G596R	2
	N581S	2
Uncommon	Q257R	2
	E275K	1
	V471F	1
	K499E	1
	T599I	1
	N486_P490del	3
Amplification		28
Fusion/ rearrangement		17

NSCLC, non-small cell lung cancer; *EGFR*, epidermal growth factor receptor.

active mutations. Also, there were 28 patients harboring *BRAF* amplification, and 17 patients had *BRAF* fusion and rearrangement (*Table 3*). Of these 17 patients, *EGFR* mutations were mainly exon19del (N=12/17) (*Table 4*). *BRAF* fusion was more likely detected in *EGFR* exon19del patients compared with non-exon19del patients (P value =0.015). There have been 6 known fusions reported in the literature, including *AGK-BRAF* in sporadic pediatric papillary thyroid carcinoma (22), *CUX1-BRAF* in metanephric adenoma (23), *NRF1-BRAF* in urothelial carcinoma (24), and *MKRN1-BRAF* in thyroid carcinomas (25). Additionally, there were 4 *BRAF* likely fusions and 7 *BRAF* rearrangements, and 9 of them were with a novel partner. NTDs and KDDs were not detected in our cohort. The breakpoints of *BRAF* fusion/rearrangement were located at the known hot regions (intron7/8/10) (*Figure 3*).

EGFR mutations found in patients with *BRAF* rearrangements were mainly exon19del (N=12/17). Two patients carried *EGFR* L858R and another 2 had *EGFR* amplification. *EGFR* KDD was also found in 1 patient. *EGFR* T790M was not found in any patient with BRAF fusion/rearrangement. Furthermore, 28 of 93 patients (about 30%) with significant *EGFR* mutations harbored *BRAF* amplification. Of these patients, *EGFR* amplification, L858R, exon19del, and T790M accounted for around 71% (N=20/28), 43% (N=12/28), 46% (N=13/28), and 21% (N=6/28), respectively. *EGFR* amplification was the most frequent mutation type with *BRAF* amplification.

There were 2 patients with emerging *BRAF* fusions following progression on TKI therapy (*Figure 4*): (I) *EGFR* exon 19del was identified in patient A. The patient was started on gefitinib in January 2017 and disease progression occurred 1 year later. A repeat lung biopsy detected *EGFR* T790M, and osimertinib treatment was used for about year and a half. Unfortunately, the disease progressed again after osimertinib treatment. A lung biopsy revealed that this patient harbored *EGFR* exon19del, *EGFR* amplification, and *CUX1-BRAF* fusion, and T790M disappeared. (II) Patient B was similar to patient A. The patient experienced rapid disease progression after osimertinib treatment and harbored *NRF1-BRAF* fusion.

Discussion

In this study, we present, to our knowledge, the largest cohort of *BRAF* and *EGFR* co-mutation Chinese NSCLC patients. A total of 127 Chinese NSCLC patients harboring co-mutations of *EGFR* and *BRAF* mutations were enrolled in this study. *BRAF* fusion was more likely detected in *EGFR* exon19del patients compared with non-exon19del patients (P value =0.015). Aside from *BRAF* V600E, class II/III and *BRAF* fusions were found, which provides clues for investigating the resistance mechanisms of EGFR-TKIs in the future.

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Table 4 Different patterns of BRAF and EGFR actionable mutations

EGFR classification	BRAF classification					
	Amp	Class 1	Class 2	Class 3	Fusion/rearrangement	Uncommon
Amp	20	2	0	1	5	0
exon19del	11	6	1	3	12	2
exon20ins	1	0	2	0	0	1
L858R	13	6	9	6	2	5
T790M	6	3	0	2	0	3
Uncommon	1	1	3	6	1	0

EGFR, epidermal growth factor receptor.

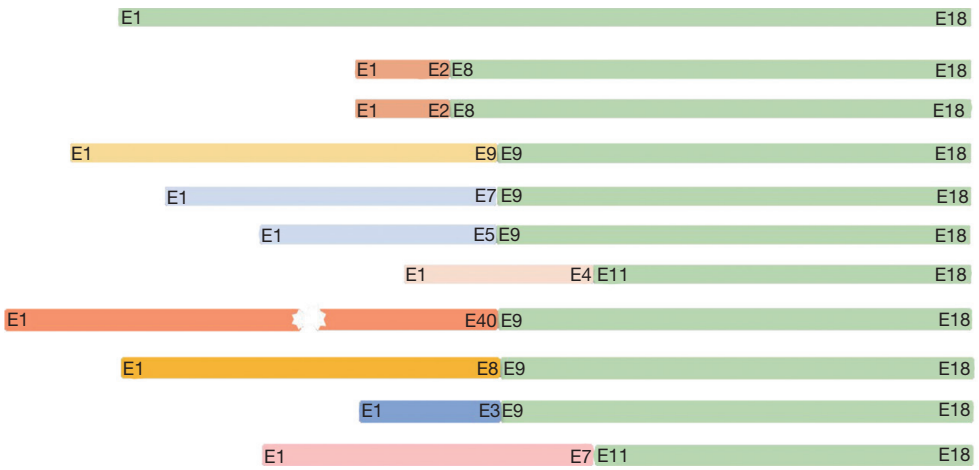

Figure 3 BRAF fusions detected in *EGFR*-mutated NSCLC patients. The *BRAF* gene consists of 18 exons. Green square represents the reserved regions of *BRAF* and squares with other colors refer to the reserved regions of different partner genes. As MGAM-*BRAF* fusion contains 40 exons of MGAM, a blank was used for representation. NSCLC, non-small cell lung cancer; *EGFR*, epidermal growth factor receptor.

Pathological activation of the RAS/RAF/MEK/ERK (MAPK) pathway is observed across multiple tumor types, and *BRAF* alterations in lung cancer can be targeted by MEK inhibitors or pan-RAF inhibitors. *BRAF* mutations were found in 4–5% of NSCLC patients. The US Food and Drug Administration (FDA) has approved combined dabrafenib and trametinib therapy for metastatic NSCLC with *BRAF* V600E mutation. *EGFR* mutations, the most common alterations in lung cancer, account for the majority of druggable targets in lung adenocarcinoma. Over the past decades, the optimization of *EGFR* inhibitors has revolutionized the treatment options for patients suffering from this disease (26). For lung cancer patients with *EGFR* exon 19 deletions or an exon 21 Leu858Arg mutation, the standard first-line treatments are first-generation (gefitinib, erlotinib) or second-generation (afatinib) TKIs. EGFR-TKIs improve response rates, time to progression, and overall survival. Unfortunately, patients with *EGFR*-mutant lung cancer develop disease progression after a median of 10 to 14 months on EGFR-TKIs. Different mechanisms of AR to first-generation and second-generation EGFR-TKIs have been reported. Optimal treatments for the various mechanisms of AR have not yet been clearly defined, except for the T790M mutation. Osimertinib has been approved for patients with T790M-positive NSCLC with AR to EGFR-TKIs. For other TKI resistance mechanisms, combination therapy may be considered (27).

Usually, combined *BRAF* and *EGFR* mutations are

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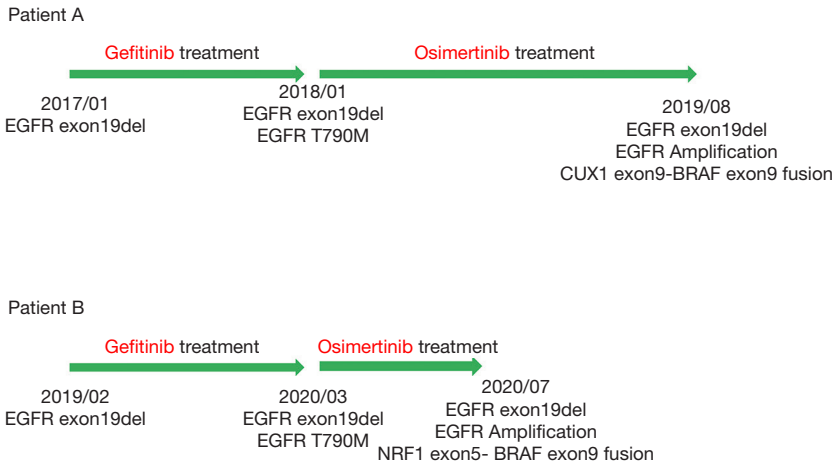

Figure 4 Time of therapy of 2 lung adenocarcinoma patients. *EGFR*, epidermal growth factor receptor.

rare in NSCLC. The frequency of *EGFR* and *BRAF* co-mutation was 0.91% in Chinese NSCLC patients, similar to Western patients. Among the 127 patients with both *EGFR* and *BRAF* mutations, 93 of them harbored clinically significant mutations. The remaining 34 patients had mutations of uncertain significance of either *EGFR* or *BRAF*. Approximately 18% of patients (N=17/93) with significant *EGFR* mutations were detected to have fusions/rearrangements of the *BRAF* gene. Of these 17 patients, *EGFR* mutations were mainly exon19del. Two patients carried *EGFR* L858R and another 2 had *EGFR* amplification. *EGFR* KDD (exon18_exon25dup) was also found in 1 patient. In addition, *EGFR* T790M, as the most TKI-resistant mutation, was not found in any patient with *BRAF* fusion/rearrangement. This may indicate that *BRAF* fusion is an AR mechanism against osimertinib, similar to *EGFR* C797S after osimertinib treatment in T790M patients (28).

The prognosis of EGFR-TKI treatment can vary according to different *BRAF* actionable mutations. Multiple genetic mechanisms have been identified in EGFR-mutant lung cancers as mediators of AR to EGFR-TKIs. The most common mechanisms of AR include secondary *EGFR* mutation, *MET* amplification, and histologic transformation (29,30). We also found that *BRAF* fusion was more likely detected in patients with *EGFR* exon19del. Additionally, several novel *BRAF* fusion partners were detected. Aside from *BRAF* V600E, class II/III were found, which provides clues for investigating the resistance mechanisms of EGFR-TKIs in the future. Although *BRAF* fusion may seem to be an obvious therapeutic target, the US FDA-approved *BRAF* inhibitors have not been effective against *BRAF* fusions. Regarding off-target (EGFR independent) resistance mechanisms, combinations of EGFR TKIs with different drugs (including other TKIs, monoclonal antibodies, chemotherapy and vaccines) are currently under investigation (4). Several clinical trials was ongoing including Biomarker-driven approaches, such as combination of osimertinib and the MET TKI savolitinib for MET amplification and the objective response rate (ORR) was 30% in patients previously treated with third-generation EGFR TKIs, with a median PFS of 5.4 months (31). Also the combination therapy has been tried for fusion caused resistance of EGFR TKI, there was 2 NSCLC patients with EGFR-mutant and RET-fusion was treated with osimertinib and BLU-667 and was well tolerated with rapid radiographic response (32). Among melanomas that harbor *BRAF* fusions, response to trametinib has been described, which indicates that NSCLC tumors that harbor *BRAF* fusions may also benefit from monotherapy with MEK inhibitors (33,34). These findings revealed that combined inhibition of *EGFR* and *MEK* (with osimertinib and trametinib) or *BRAF* (with a pan-RAF inhibitor) are potential therapeutic strategies that should be explored. Due to the limitations of the number of samples, more follow up data about therapy response information is needed for further research.

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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. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of Guangdong Second Provincial General Hospital (No.: GZR-2020-KT-39-01) and informed consent was taken from all the patients.

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