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Clinical outcomes of *EGFR*+/*MET*amp+ vs. *EGFR*+/*MET*amp- untreated patients with advanced non-small cell lung cancer

Kai-Cheng Peng[1,2] | Jun-Wei Su[2] | Zhi Xie[2] | Han-Min Wang[2] | Mei-Mei Fang[2] | Wen-Feng Li[2] | Yu-Qing Chen[1,2] | Xu-Hui Guan[1,2] | Jian Su[2] | Hong-Hong Yan[2] | Xu-Chao Zhang[2] | Hai-Yan Tu[2] | Qing Zhou[2] | Hua-Jun Chen[2] | Yi-Long Wu[2] | Jin-Ji Yang[1,2]

[1]School of Medicine, South China University of Technology, Guangzhou, China

[2]Guangdong Lung Cancer Institute, Guangdong Provincial Key Laboratory of Translational Medicine in Lung Cancer, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China

Correspondence
Jin-Ji Yang, Guangdong Lung Cancer Institute, Guangdong Provincial Key Laboratory of Translational Medicine in Lung Cancer, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, 510080, China. School of Medicine, South China University of Technology, Guangzhou, 510006 China.
Email: yangjinji@gdph.org.cn

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Abstract

Background: *MET* dysregulation has been implicated in the development of primary and secondary resistance to EGFR tyrosine kinase inhibitor (TKI) therapy. However, the clinicopathological characteristics and outcomes of patients harboring *EGFR*-sensitive mutations and de novo *MET* amplifications still need to be explored.

Methods: A total of 54 patients from our hospital with non-small cell lung cancer harboring *EGFR*-sensitive mutations and/or de novo *MET* amplifications were included in this study. Survival rates were estimated by the Kaplan–Meier method with log-rank statistics. Lung cancer organoids (LCOs) were generated from patient-derived malignant pleural effusion to perform drug sensitivity assays.

Results: Fifty-four patients with the appropriate clinicopathological characteristics were enrolled. *MET* FISH was performed in 40 patients who were stratified accordingly into two groups: *EGFR*+/*MET*amp- ($n = 22$) and *EGFR*+/*MET*amp + ($n = 18$). Survival rates for *EGFR*+/*MET*amp- and *EGFR*+/*MET*amp + patients respectively, were as follows: the median progression-free survival (PFS) was 12.1 and 1.9 months ($p<0.001$); the median post-progression overall survival (pOS) was 25.6 and 11.6 months ($p = 0.023$); the median overall survival (OS) was 33.2 and 12.7 months ($p = 0.013$). Drug testing conducted in LCOs derived from malignant pleural effusion from *EGFR*+/*MET*amp + patients showed that dual targeted therapy was more effective than TKI monotherapy.

Conclusion: *EGFR*+/*MET*amp + patients treated with first-line TKI monotherapy had poor clinical outcomes. Dual targeted therapy showed potent anticancer activity in the LCO drug testing assay, suggesting that it is a promising first-line treatment for *EGFR*+/*MET*amp + patients. Randomized controlled trials are needed to further validate these results.

KEYWORDS
de novo *MET* amplification, *EGFR*-sensitive mutation, non-small cell lung cancer, patient-derived organoid, targeted therapy

Kai-Cheng Peng and Jun-Wei Su contributed equally.

INTRODUCTION

Lung cancer is the second most common cancer type and is the leading cause of cancer-related deaths worldwide. According to the Global Cancer Statistics 2020, lung cancer in China is ranked first among all cancers in terms of incidence and number of deaths.[1] In the last decade, targeted therapies for driver mutations, such as epidermal growth factor receptor (*EGFR*) mutations and anaplastic lymphoma kinase (*ALK*) rearrangement, have revolutionized the treatment of advanced non-small cell lung cancer (NSCLC).[2] However, approximately 20%–30% of patients harboring *EGFR* mutations develop primary resistance to EGFR-tyrosine kinase inhibitor (EGFR-TKI) therapy.[3] In these patients, the development of drug resistance poses a major obstacle for the long-term clinical remission of the disease. Several preclinical and clinical studies have found that the c-*MET* proto-oncogene (*MET*) amplification underlies the mechanism of acquired resistance in 5%–20% of patients with NSCLC harboring *EGFR* mutations and treated with EGFR-TKIs. This resistance develops particularly after the administration of first-line third-generation EGFR inhibitors, such as osimertinib.[4–9] Parallel activation of downstream signaling proteins can lead to EGFR-TKI resistance, with *MET* amplification leading to PI3K pathway activation and tumor resistance, suggesting that dual inhibition of two activating proteins upstream the PI3K pathway (EGFR and MET) may have a synergistic therapeutic effect.[10,11] The TATTON study (NCT02143466) indicated a median progression-free survival (PFS) of 5.4 months, suggesting that osimertinib in combination with savolitinib showed acceptable safety and antitumor efficacy in NSCLC patients with *MET* amplification who adopted the third-generation EGFR-TKI therapy.[12] Three multicenter clinical trials led by the Guangdong Lung Cancer Institute showed activation of the HGF/MET signaling pathway to be an oncogenic driver in *EGFR*-mutant NSCLC, mediating primary and secondary resistance to EGFR-TKI therapies.[13–15]

De novo *MET* amplification has previously been reported in approximately 2%–26% of patients with *EGFR*-mutant NSCLC.[16–19] However, the incidence of *EGFR*-mutant NSCLC coexisting with de novo *MET* amplification varies among studies because of different detection methods and definitions of *MET* amplification. Fluorescence in situ hybridization (FISH) is considered the standard method to confirm *MET* amplification. *MET* to centromere of chromosome 7 (*MET* / CEP7) ratio can be used to distinguish polyploid amplification from focal amplification.[20] Currently, the criteria for positive *MET* amplification in FISH are *MET* signals per cell ≥5 (Cappuzzo scoring system) and *MET*/CEP7 ratio ≥2.[20] Studies have shown that there is a significant difference in PFS between patients with *EGFR* mutations and MET immunohistochemistry (IHC) strong staining of more than 75% tumor cells compared to patients carrying only *EGFR* mutations, suggesting that MET overexpression at the protein level may cause primary resistance to EGFR-TKI therapies in patients with *EGFR*-mutant advanced NSCLC. Higher expression levels correlate with a greater chance for the development of primary resistance and with a worse outcome and survival rates.[21] Another study concluded that the overall survival (OS) of patients with de novo *MET* amplification who did not receive *MET* inhibitors was shorter than that of patients treated with *MET* inhibitors. However, there was no difference in OS between patients treated with or without crizotinib based on *MET* amplification levels.[22] This study suggests that *MET* amplification may not be a prognostic factor for survival, but rather a predictor of response to MET-inhibitor therapy.

First-line dual-targeted regimens are not routinely recommended for NSCLC patients harboring *EGFR* mutations and de novo *MET* amplifications, and there are no large clinical trials for this group of patients. For this reason, exploring further treatment options for this population is necessary. Wang et al. also found that de novo *MET* amplification detected by FISH was an independent predictor of PFS in EGFR-TKI–treated patients.[18] More effective treatments are required for patients with advanced NSCLC with de novo *MET* amplifications and *EGFR* mutations.

Patient-derived organoids (PDOs) are 3-dimensional (3D) organotypic structures, which can perfectly recapitulate the heterogeneity and diversity of tumors. They also show a high degree of consistency with clinical specimens in genotype and phenotype, exhibit a response to antitumor drugs, and are available in a short period of time.[23–25] The establishment of tumor PDOs for the screening of antitumor drugs provides a powerful reference for clinical treatment.

Thus, in our study, we retrospectively investigated 54 advanced lung adenocarcinoma patients harboring *EGFR* mutations and de novo MET overexpression or *MET* amplification at the Guangdong Provincial People's Hospital in China from January 2014 to December 2020. We evaluated the tumor inhibition rate and drug sensitivity in two different patient-derived lung cancer organoids (LCOs) models derived from malignant pleural effusion.

METHODS

Patients

We conducted a retrospective study on 54 advanced lung adenocarcinoma patients harboring *EGFR*-sensitive mutations and/or de novo MET overexpression or *MET* amplification at the Guangdong Provincial People's Hospital in China from January 2014 to December 2020. Ethical approval was obtained from the Research Ethics Committee of Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences (approval no. GDREC2019397H). When available, tumor samples from these patients were subjected to a *MET* FISH assay to determine *MET* baseline levels, and according to the results, the 54 patients were divided into three groups: *EGFR*+/*MET*amp-, *EGFR*+/*MET*amp+, and unknown. The medical records of all patients were examined, and the information regarding clinicopathological characteristics and

medical history was collected. The clinical response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1). Survival outcomes were evaluated based on digital medical records or telephone follow-up appointments. The PFS interval was calculated from the start of first-line EGFR-TKI or MET-TKI therapies until disease progression or death. The post-progression overall survival (pOS) interval was calculated from the start of the second progression to EGFR-TKI, MET-TKI, or dual targeted therapy with best response treatment until death from any cause. The OS interval was calculated from the start of the TKI treatment until either death from any cause or the last follow-up appointment (October 1, 2021).

Statistical analysis

Patient clinicopathological characteristics and outcomes are reported as absolute values, percentages, mean ± SD, or median (95% CI) values, as appropriate. Kaplan–Meier estimates and the log-rank test were applied to evaluate PFS, pOS, and OS. All survival data were analyzed using the SPSS 26.0 (IBM Corp.) and GraphPad Prism 8.0.1 (GraphPad Software) software. The chi-square test was used to compare differences between two groups, and two-sided *p*-values less than 0.05 were considered to denote statistical significance. Half-maximal inhibitory concentration (IC50) values were calculated using the GraphPad Prism 7.0 software (GraphPad Software).

EGFR-sensitive mutation analysis

Tissue samples were first collected from primary tumors or metastatic sites before any treatment (i.e., untreated specimens). Upon patient approval to test for *EGFR* mutations, tumor tissues were subjected to next-generation sequencing (NGS), an amplification refractory mutation system (ARMS), or polymerase chain reaction (PCR). Subsequently, the *EGFR*-mutant protein expression levels were assessed in the available untreated tumor tissues by using antibodies specifically recognizing EGFR variants with the exon 19 E746-A750 del deletion and exon 21 L858R mutation (Cell Signaling Technologies). The criterion for evaluating protein expression by IHC was staining intensity, ranging from negative (−: complete absence of staining or faint staining in less than 10% of cells) to triple positive (+++: strong staining of tumor cells).

MET IHC and *MET* FISH

MET IHC (clone SP44, Roche Tissue Diagnostics) was performed at the pathological sections. MET overexpression was defined as positive if more than 50% of tumor cells showed strong staining intensity. IHC scoring was strictly performed by a trained pathologist subjected to inter-laboratory proficiency testing.

MET amplification was determined by *MET* FISH (Vysis SA) and was considered to be positive when the following criteria were met: *MET* gene copy number ≥5, *MET* to centromere of chromosome 7 (*MET* / CEP7) ratio ≥2, and focal amplification present in more than 10% of tumor cells.

Human malignant effusion collection and processing

In this retrospective study, malignant pleural effusion was collected from two advanced lung adenocarcinoma patients at the Guangdong Provincial People's Hospital and was used to generate LCO cultures. The obtained samples were diagnosed based on pathological assessment. The research protocol was approved by the Research Ethics Committee of Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences (Guangzhou, China).

Tissue preparation and LCO culturing

The culture of patient-derived lung cancer organoids (LCOs) in our study was performed following a similar protocol as previously described.[26] Malignant pleural effusion (200–800 ml) was obtained by thoracentesis, stored aseptically in heparinized (10 U/ml) sterile bottles, and transferred to the laboratory on ice for further processing within 4 hours of collection. The effusion samples were centrifuged for 3 min at 112 rcf and lysed with Red Blood Cell Lysis Buffer. The cells were precipitated in a second centrifugation step and were washed once with HEPES buffer (Thermo Fisher Scientific). Subsequently, the cell pellet was resuspended in Accuroid lung cancer medium (ALCM; Accurate International Biotech Co. Ltd) and the cells were counted. The organoid cultures were established by mixing 100 µl of cell suspension with 200 µl of Matrigel (Corning Inc.) and allowing 30 µl of the mixture to solidify upside down on prewarmed 6-well culture plates (Corning Inc.) at 37°C for 30 min. Finally, 3 ml of ALCM was added to each well. The medium was changed every 2–3 days.

Drug sensitivity assay

Organoids cultured more than 2 weeks were harvested and dissociated using 1× TrypLe reagents (Thermo Fisher Scientific). The digested cells were counted using a cell counter (Countstar). The harvested cells and cell clusters were mixed in a membrane-bottomed microwell (MBM) + Matrigel (1:1 ratio) and seeded onto 384-well white plates on ice, 2000–3000 cells were seeded in each well, and then 50 µl of

MBM was added to each well after gelation. Following 72 h of culture to allow the formation of organ-like structures, a liquid-handling robotic system was used to dispense a dilution series (50, 10, 2, 0.4, 0.08, and 0.016 μM) of drugs on the organoids. After 3–4 days of incubation with the drugs, cell viability was determined using the CellTiter-Glo assay (Promega). The plates were agitated for 30 min at room temperature prior to the luminescence measurements. Half-maximal inhibitory concentration (IC50) values were calculated using the GraphPad Prism 7.0 (GraphPad Software) software.

TABLE 1 Baseline characteristics of enrolled patients with *EGFR* sensitive mutation and de novo *MET* alteration in *EGFR*+/*MET*amp- and *EGFR*+/*MET*amp + groups

Group	*EGFR*+/*MET*amp-($n = 22$)	*EGFR*+/*MET*amp + ($n = 18$)	*p*-value
Ages (years)			
Mean ± standard deviation	52.5 ± 10.3	58.3 ± 8.8	
Age group			$p = 0.106$
<60	16 (72.7%)	8 (44.4%)	
≥60	6 (27.3%)	10 (55.6%)	
Sex			$p = 0.526$
Male	12 (54.5%)	12 (66.7%)	
Female	10 (45.5%)	6 (33.3%)	
ECOG PS score			$p = 0.704$
0 ∼ 1	21 (95.5%)	17 (94.4%)	
2	0 (0.0%)	1 (5.6%)	
3	1 (4.5%)	0 (0.0%)	
Smoking status			$p = 1.000$
Never smoker	14 (63.6%)	12 (66.7%)	
Smoker	8 (36.4%)	6 (33.3%)	
Histology			
Adenocarcinoma	22 (100.0%)	18 (100.0%)	
Stage			
IV	22 (100.0%)	18 (100.0%)	
Brain metastases			$p = 0.498$
Yes	5 (22.7%)	6 (33.3%)	
No	17 (77.3%)	12 (66.7%)	
Type of *EGFR* mutation			$p = 0.341$
19 deletion	13 (59.1%)	7 (38.9%)	
21 L858R	9 (40.9%)	11 (61.1%)	
MET IHC (H-score)			$p = 0.005$
NA	0 (0.0%)	4 (22.2%)	
0 < 150	0 (0.0%)	2 (11.1%)	
150 < 200	2 (9.1%)	0 (0.0%)	
200 ≤ 300	20 (90.9%)	12 (66.7%)	
Treatment in first-line			$p = 0.008$
First generation EGFR TKI	21 (95.5%)	11 (61.1%)	
Second generation EGFR TKI	1 (4.5%)	2 (11.1%)	
Third generation EGFR TKI	0 (0.0%)	4 (22.2%)	
MET TKI	0 (0.0%)	1 (5.6%)	
Subsequent treatment			$p < 0.001$
EGFR TKI	9 (41.0%)	1 (5.6%)	
MET TKI	0 (0.0%)	2 (11.1%)	
Unknown	12 (54.5%)	6 (33.3%)	
Dual targeted therapy	1 (4.5%)	9 (50.0%)	

Abbreviations: IHC, immunohistochemistry; TKI, tyrosine kinase inhibitor.

RESULTS

Baseline patient characteristics

We enrolled 54 patients harboring *EGFR*-sensitive mutations and MET overexpression or *MET* amplification; these included 48 with de novo MET overexpression and six with de novo *MET* amplification (without MET IHC at the original baseline level). In most patients ($n = 48$), treatment-naive tumor samples were available that were subsequently tested by *MET* FISH. Based on the assay results of the treatment-naïve tumor samples, 40 of the patients were divided into two groups: an *EGFR*+/*MET*amp- group ($n = 22$) and an *EGFR*+/*MET*amp + group ($n = 18$) (Figure S1). The age, gender, Eastern Cooperative Oncology Group Performance Status (ECOG PS) score, presence of brain metastases, and type of *EGFR* mutation were well balanced between the two groups (Table 1). In the *EGFR*+/*MET*amp- group, 90.9% of the patients (20/22) had a MET IHC H-score between 200 and 300, compared to only 66.7% of the patients in the *EGFR*+/*MET*amp + group (12/18). Interestingly, there were statistically significant differences in the MET IHC results following first- and subsequent-line TKI regimens. First-generation EGFR-TKIs were commonly chosen as a first-line treatment in 95.5% (21/22) of the *EGFR*+/*MET*amp- patients and in 61.1% (11/18) of the *EGFR*+/*MET*amp + patients. However, for the subsequent-line treatment, only 41% (9/22) of the *EGFR*+/*MET*amp- patients selected EGFR-TKIs, while half (9/18) of the *EGFR*+/*MET*amp + patients chose dual targeted therapy. Overall, we found 35.1% (12/34) of treatment-naive available tumor samples to be positive for *MET* amplification (Figure S1). For the remaining patients (n = 14), there was not enough material for a *MET* FISH assay, and they were subsequently assigned to the unknown group.

Presentation of typical cases from the *EGFR*+/*MET*amp- and *EGFR*+/*MET*amp + groups

One case from each group was selected as a typical example of the pattern of *EGFR*-mutant protein expression, MET overexpression, and *MET* amplification. The results were accompanied by a treatment timeline and included computed tomography (CT) scanning images at some important time points. Case 1 was a middle-aged female patient from

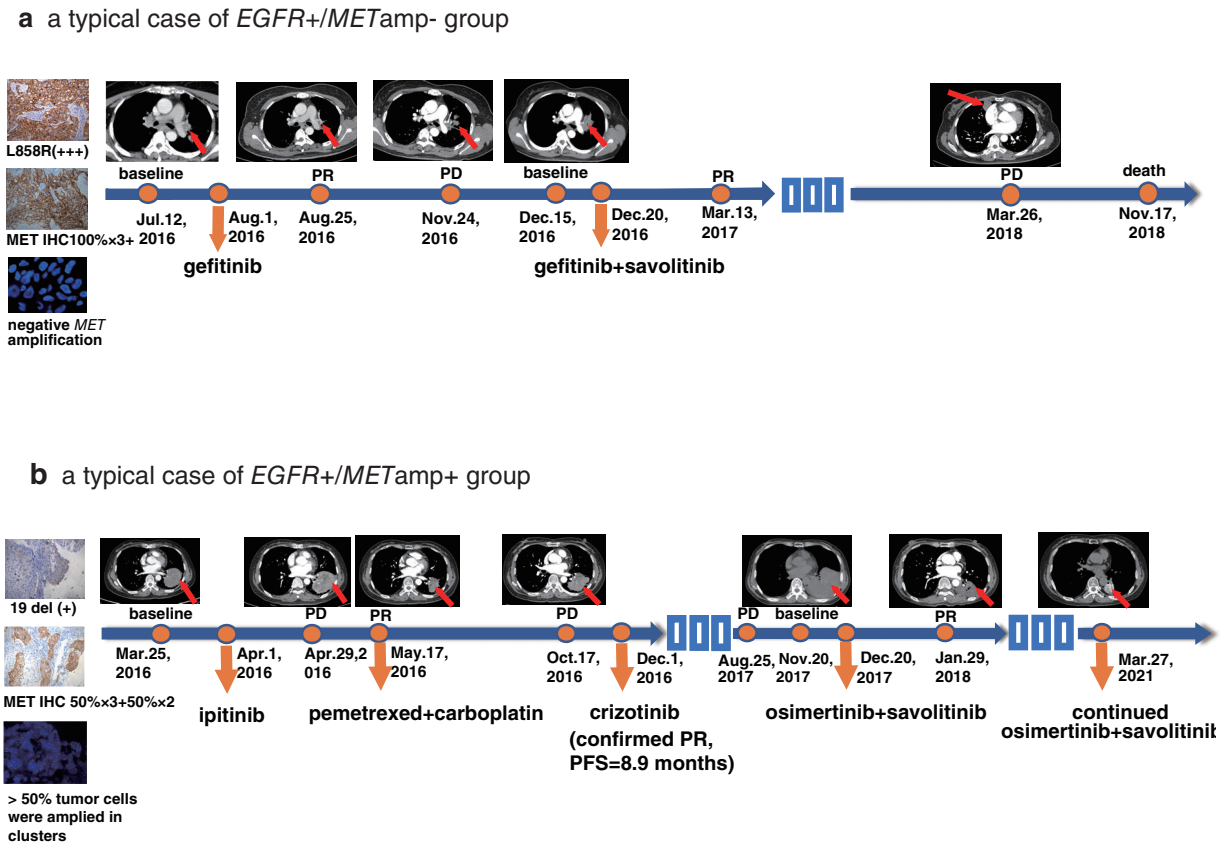

FIGURE 1 Typical case presentation of *EGFR*+/*MET*amp- and *EGFR*+/*MET*amp + patients. (a) An *EGFR*+/*MET*amp- patient responded to gefitinib and savolitinib for 15.4 months after developing resistance to gefitinib. (b) An *EGFR*+/*MET*amp + patient who was resistant to TKI monotherapy (ipitinib or crizotinib) in prior-line treatments benefited from osimertinib and savolitinib dual therapy until the last follow-up date. *EGFR*, *epidermal growth factor receptor*; PR, partial response; PD, progressive disease; IHC, immunohistochemistry

the *EGFR*+/*MET*amp- group and a nonsmoker diagnosed with advanced lung adenocarcinoma without brain metastases. She was found to be harboring the *EGFR* L858R mutation and had de novo MET overexpression (Figure 1a). Baseline measurements in her supraclavicular lymph nodes showed negative *MET* amplification by FISH. Gefitinib was used as a first-line treatment for 3.8 months, and the best response was partial response (PR). Subsequently, the supraclavicular lymph nodes tested positive for both the *EGFR* mutation (confirmed by NGS) and *MET* amplification

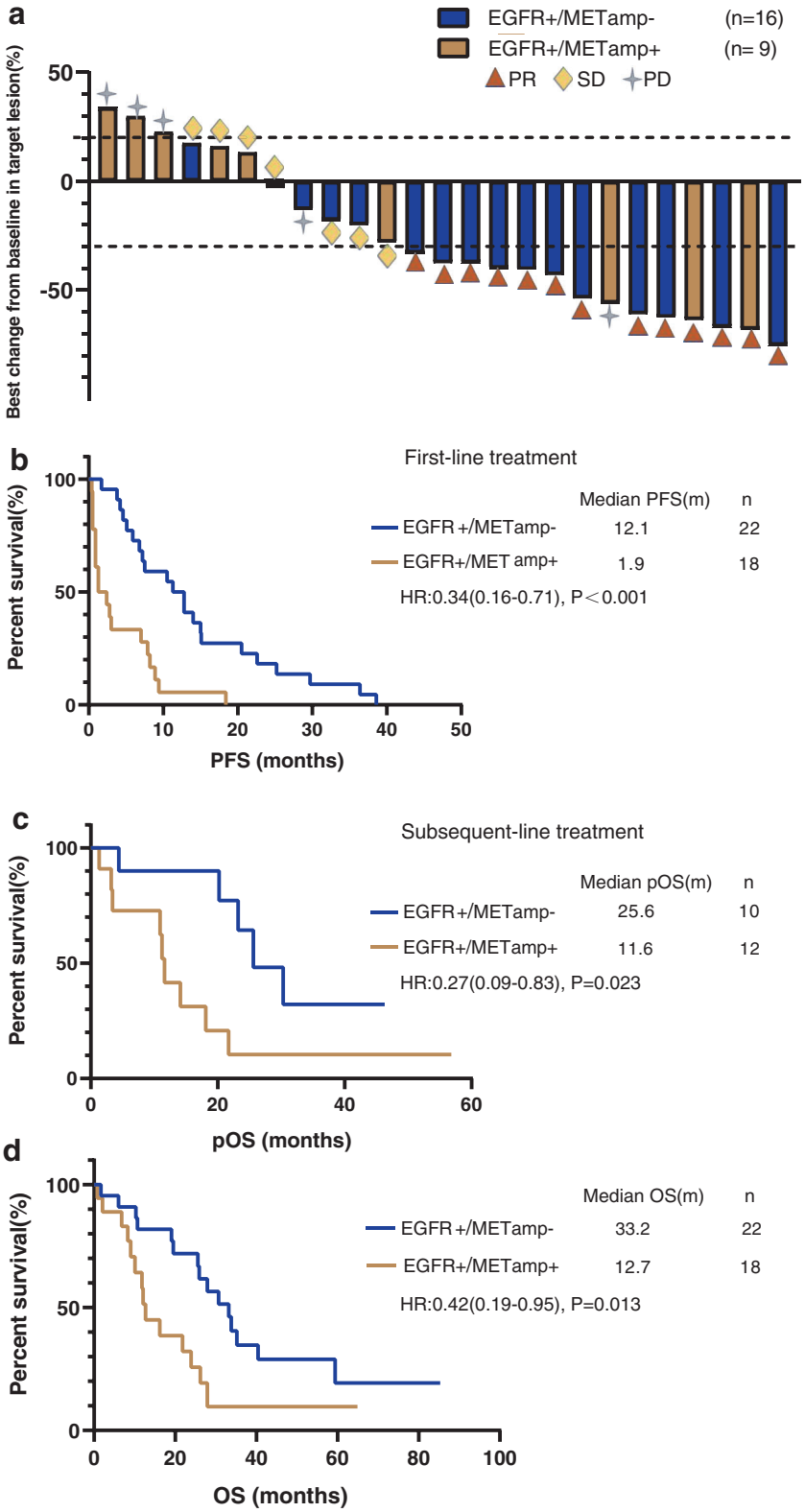

FIGURE 2 Treatment outcomes for patients belonging to the *EGFR*+/*MET*amp- and *EGFR*+/*MET*amp + groups. (a) Best percent change in target lesion size and response to first-line treatment for *EGFR*+/*MET*amp- and *EGFR*+/*MET*amp + patients. (b) Progression-free survival of first line treatment for patients in the two groups ($n = 40$). (c) Post-progression overall survival of subsequent line treatment for patients in the two groups ($n = 22$). (d) Kaplan–Meier estimates of OS for patients in the two groups ($n = 40$). *EGFR*, *epidermal growth factor receptor*; PR, partial response; PD, progressive disease; SD, stable disease; PFS, progression-free survival; pOS, post-progression overall survival; OS, overall survival; HR, hazard ratio

(confirmed by FISH), for which the patient was enrolled in a clinical trial (NCT02374645) and accepted gefitinib plus savolitinib as a subsequent-line treatment, but the best response was still PR. After a response of 15.4 months, the patient developed resistance to the dual targeted therapy, and NGS identified the *EGFR* T790M mutation and *EGFR* amplification. The patient died before the last follow-up appointment.

Case 2 was a middle-aged female patient from the *EGFR*+/*MET*amp + group, who was a nonsmoker and was diagnosed with advanced lung adenocarcinoma with brain metastases. She was harboring an *EGFR* exon 19 deletion and de novo MET overexpression (Figure 1b). Baseline *MET*

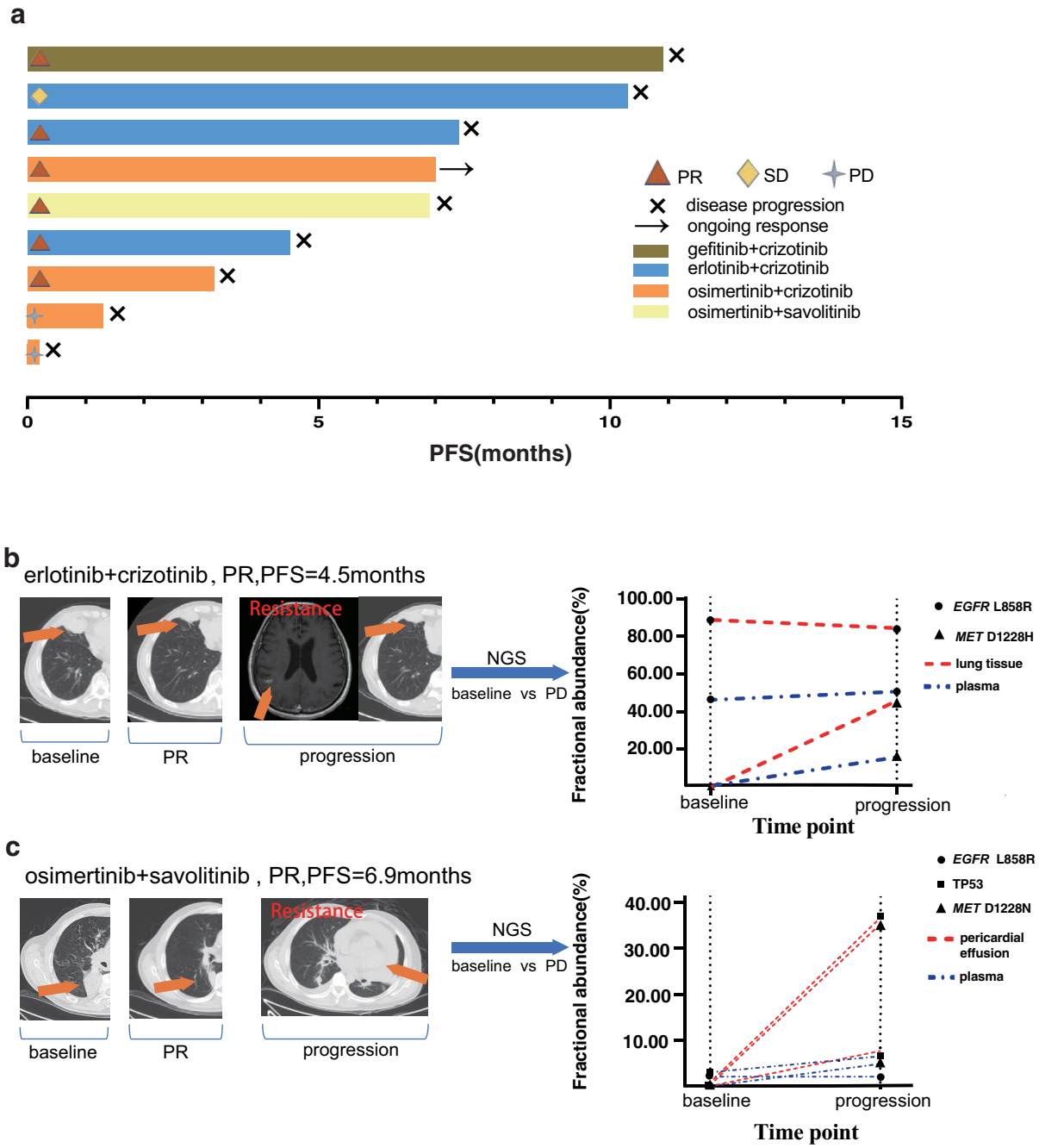

FIGURE 3 Efficacy and resistance mechanisms of dual targeted therapy for *EGFR*+/*MET*amp + patients. (a) Swimmer plot showing treatment duration, outcome events, and response to both EGFR and MET TKIs in *EGFR*+/*MET*amp + patients ($n = 9$). Eight progression events were reported before the end of data collection. (b) In one of nine patients treated with erlotinib and crizotinib for 4.5 months, *MET* D1228H mutation was identified by NGS in both plasma and the lung tumor. (c) One of nine patients was treated with osimertinib and savolitinib for 6.9 months and suffered from pericardial effusion, in which *MET* D1228N mutation was found, and which was also identified in the plasma. *EGFR*, *epidermal growth factor receptor*; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival; NGS, next-generation sequencing

FISH measurements in primary lung tissue identified amplified *MET* signal clusters in more than 50% of the tumor cells. Ipitinib was used as first-line treatment for less than 1 month. Chemotherapy was administered as a second-line treatment for 5 months. Because *MET* FISH was still positive for *MET* amplification, the patient received crizotinib for 8.9 months as a third-line treatment. Considering that the patient was still harboring the *EGFR* exon 19 deletion and *MET* amplification, a combination therapy of osimertinib and savolitinb was selected for the next follow-up regimen. A chest CT scan revealed considerable reduction of the primary tumor and malignant pleural effusion. The patient had PR for nearly 4 years until the follow-up date, and her ECOG PS was 1.

Response and survival of *EGFR*+/*MET*amp- and *EGFR*+/*MET*amp + patients

When collectively considering the patients belonging to the *EGFR*+/*MET*amp- and *EGFR*+/*MET*amp + groups, we found that 97.5% (39/40) received EGFR-TKIs and 2.5% (1/40) received MET TKI as a first-line treatment. The treatment efficacy was evaluated for 16 patients in the *EGFR*+/*MET*amp- group and nine patients in the *EGFR*+/*MET*amp + group (Figure 2a), and their objective response rate was 68.8% and 22.2%, respectively. It is worth noting that 44.4% (4/9) of patients in the *EGFR*+/*MET*amp +- group did not benefit from the first-line TKI treatment. The median PFS was 12.1 months in the *EGFR*+/*MET*amp- group and 1.9 months in the *EGFR*+/*MET*amp + group ($p < 0.001$; Figure 2b). In addition, based on measurements performed on clinical material wherever available, 45.5% (10/22) of patients in the *EGFR*+/*MET*amp- group and 66.7% (12/18) in the *EGFR*+/*MET*amp + group were eligible for subsequent-line TKI treatment. In the two groups, the median pOS was 25.6 and 11.6 months, respectively ($p = 0.023$; Figure 2c), while the median OS was 33.2 and 12.7 months, respectively ($p = 0.013$; Figure 2d). The median PFS, pOS, and OS for the unknown group were 10.4 ($p < 0.001$), 14.8 ($p = 0.100$), and 22.6 ($p = 0.047$) months, respectively (Figure S2 A, B, and C).

Resistance mechanisms of subsequent-line dual targeted therapy in *EGFR*+/*MET*amp + patients

A total of 75% (9/12) *EGFR*+/*MET*amp + patients were treated with both EGFR- and MET-TKIs. At the time of the last follow-up appointment, only one patient maintained PR but the others developed progressive disease (PD) and

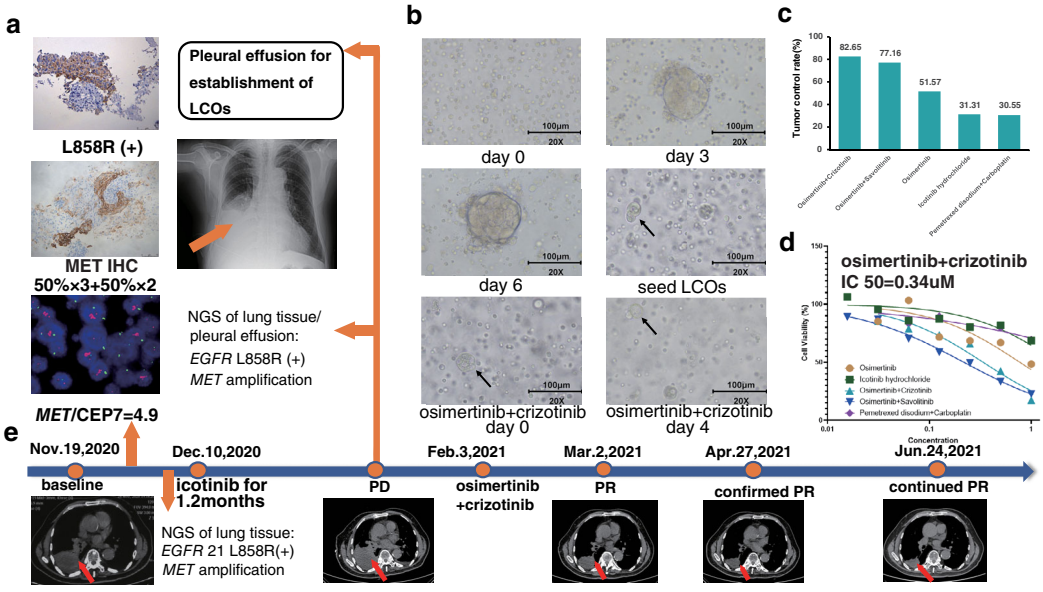

FIGURE 4 Drug screening in LCO cultures recapitulates the clinical response of dual targeted therapy in *EGFR*+/*MET*amp + patients. (a) Positive *EGFR*-mutant protein expression, MET protein expression, and *MET* amplification were detected in the lung tumor tissue. (b) Summary of the experimental design, from the establishment of LCO cultures to the drug sensitivity assay. (c) Tumor inhibition rates in five LCO cultures, each treated with a different drug. (d) Dose–response curves of the five LCO cultures treated with the indicated drug concentrations. Representative viability curves generated from the luminescence signal intensities are shown. (e) Pleural effusion-derived LCO cultures were established and used to predict partial responses to combined osimertinib and crizotinib therapy after resistance development to icotinib first-line treatment. IHC, immunohistochemistry; *MET*/CEP7, *MET* relative to chromosome 7 centromere; LCO, lung cancer organoid; *EGFR*, *epidermal growth factor receptor*; NGS, next-generation sequencing; IC50, 50% inhibitory concentration; PD, progressive disease; PR, partial response

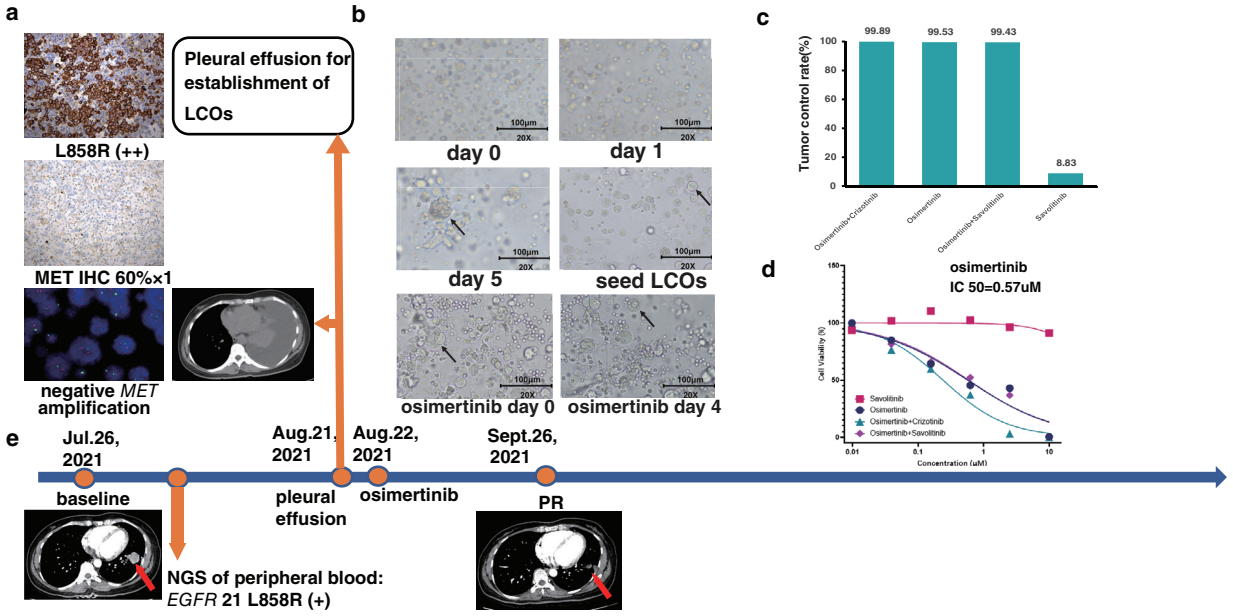

FIGURE 5 Treatment outcome and LCO drug sensitivity in a patient harboring only the *EGFR* exon 21 L858R mutation. (a) Positive *EGFR*-mutant protein expression, negative MET protein expression, and negative *MET* amplification were detected in pleural effusion. (b) Summary of the experimental design, from the establishment of LCO cultures to the drug sensitivity assay. (c) Tumor inhibition rates in four LCO cultures, each treated with a different drug. (d) Dose–response curves of the four LCO cultures treated with the indicated drug concentrations. Representative viability curves generated from the luminescence signal intensities are shown. (e) Pleural effusion-derived LCO cultures were established and used to predict partial responses to osimertinib first-line treatment. IHC, immunohistochemistry; LCO, lung cancer organoid; IC50, 50% inhibitory concentration; *EGFR*, *epidermal growth factor receptor*; NGS, next-generation sequencing; PR, partial response

stopped the dual targeted therapy. The objective response rate for dual targeted treatment was 66.7% (6/9; Figure 3a). NGS analysis was performed in only two of the patients who developed resistance to the dual targeted treatment. Patient 1 responded to erlotinib and crizotinib for 4.5 months, and the best response was PR. NGS analysis identified the *MET* D1228H mutation in both plasma and lung tumor tissue (Figure 3b). Patient 2 was treated with osimertinib and savolitinib and benefited for nearly 7 months. Subsequently, *MET* D1228N mutation was detected in his pericardial effusion and plasma (Figure 3c).

LCO **drug testing recapitulates the clinical response to** EGFR/MET **dual targeted therapy**

The malignant pleural effusion of a patient diagnosed with advanced lung adenocarcinoma and harboring both *EGFR* exon 21 L858R mutation and de novo MET overexpression was collected after he developed primary resistance to first-line icotinib treatment for 1.2 months. *MET* FISH was retrospectively performed on a sample from the lung needle biopsy that was performed at the local hospital where the patient was treated with first-line icotinib. *MET* amplification (*MET*/CEP7 = 4.9) was identified, indicating an *EGFR*+/*MET*amp + lung adenocarcinoma. Determination of half-maximal inhibitory concentration (IC50) values in an in vitro drug sensitivity assay performed on LCOs derived from the patient malignant pleural effusion suggested the potential efficacy of combinatorial osimertinib and crizotinib treatment (IC50 = 0.34 uM). Accordingly, he received dual TKI therapy as the second-line treatment from February 2021. A dramatic response was achieved 1 month later. The best response was PR, and the patient was still benefiting from this regimen at the end of the data collection period, demonstrating the concordance between the drug sensitivity of malignant effusion-derived LCOs and the objective tumor response (Figure 4).

A similar organoid model was established from malignant pleural effusion collected from a treatment-naive *EGFR*-mutant patient. The results of the in vitro drug sensitivity testing showed the potential efficacy of osimertinib (IC50 = 0.57 uM). It was also demonstrated that dual targeted therapy with MET-TKIs (savolitinib or crizotinib) and an EGFR-TKI (osimertinib) did not enhance the anticancer activity of osimertinib in the patient-derived *EGFR*-mutant organoid model. Thus, she received first-line osimertinib monotherapy and achieved PR (Figure 5).

Hematoxylin and eosin (HE) staining was also performed in both LCO models to test for the presence of cancer cells in the LCOs (Figure S3 A and B).

DISCUSSION

An increasing number of studies show *MET* amplification after the development of resistance to EGFR-TKI therapies.[12–14,27,28] However, there are few studies concerning de novo *MET* amplification in patients with *EGFR*-mutant NSCLC. De novo *MET* amplification has been reported in approximately 2%–26% of patients with NSCLC positive for *EGFR* mutations.[16–19] This motivated us to retrospectively investigate the clinical outcomes of *EGFR*+/*MET*amp- and *EGFR*+/*MET*amp + patients with advanced NSCLC. Our study revealed that, compared to *EGFR*+/*MET*amp- patients, *EGFR*+/*MET*amp + patients had a worse response to first-line EGFR-TKI monotherapy and a poorer survival rate (median PFS: 1.9 vs. 12.1 months, $p<0.001$; median pOS: 11.6 vs. 25.6 months, $p = 0.023$; median OS: 12.7 vs. 33.2 months, $p = 0.013$). Meanwhile, drug sensitivity assays performed on LCOs derived from malignant pleural effusion were able to recapitulate the objective tumor response to EGFR/MET dual targeted therapy for *EGFR*+/*MET*amp + patients. To the best of our knowledge, this is the first retrospective study with a relatively large sample size to reveal a worse response/survival of *EGFR*+/*MET*amp + patients receiving first-line TKI monotherapy. Furthermore, the drug sensitivity data from the *EGFR*+/*MET*amp + patient-derived LCO models might provide supporting evidence for the design of future randomized controlled trials of EGFR/MET dual targeted therapy as a first-line treatment.

Our study highly emphasized the importance of *EGFR*+/*MET*amp + NSCLC. Peng et al. has suggested that *MET* amplification identified by NGS may not be sufficiently robust to serve as an effective predictive biomarker.[29] On the contrary, *MET* amplification detected by FISH has been gradually recognized as an oncogenic driver in NSCLC although MET overexpression identified by IHC is a poor prognostic factor for advanced NSCLC. In previous studies, targeting MET overexpression in advanced NSCLC with MET inhibitors failed.[30–33] In our study, the median PFS for *EGFR*+/*MET*amp + patients with advanced NSCLC receiving first-line EGFR-TKI monotherapy was only 1.9 months, indicating primary resistance to TKI monotherapy. However, for *EGFR*+/*MET*amp + patients treated with second- or further-line EGFR/MET dual targeted therapy, the response rate was 66.7% (6/9). Furthermore, in two *EGFR*+/*MET*amp + patients who developed resistance to the EGFR/MET dual targeted therapy, *MET* D1228H and *MET* D1228N mutations were detected by NGS after the resistance development. Acquired second-site mutations, such as *MET* D1228N/H, are considered to underlie the resistance mechanisms against type I MET inhibitors, such as crizotinib.[34–36] In summary, de novo *MET* amplification may be an oncogenic driver in *EGFR*-mutant NSCLC and treatable by drugs. Since *EGFR*+/*MET*amp + patients are a small subset of *EGFR*-mutant NSCLC patients, they should receive more attention in clinical trials and practice in the future.

Tremendous efforts have been made to establish reliable preclinical models to predict responses to anticancer therapy, which include among others the development of cancer cell lines and patient-derived xenograft models (PDXs). More recently, LCOs, 3D organotypic structures that maintain the original tissue heterogeneity, have attracted increased attention. Chen et al. showed that in vitro drug response in PDOs had a high correlation with the predictions based on the mutation profiles of the primary tumors.[26] Kim et al. also suggested that the in vitro drug screening in patient-derived LCO systems may prove useful for predicting patient-specific drug responses.[37] In our study, we established LCOs from the malignant pleural effusion of *EGFR*-mutant and *EGFR*+/*MET*amp +- adenocarcinoma patients. Interestingly, LCOs derived from *EGFR*+/*MET*amp + adenocarcinoma were more sensitive to combination targeted therapy. On the contrary, LCOs derived from *EGFR*-mutant adenocarcinoma showed similar sensitivity to osimertinib and osimertinib/savolitinib or crizotinib. The drug responses of the two different LCO models were clinically consistent with the tumor drug response in the corresponding patients, suggesting that randomized controlled trials of first-line EGFR/MET dual targeted therapy for *EGFR*+/*MET*amp + patients with advanced NSCLC are warranted. Thus, we intend to conduct a prospective, pilot study comparing osimertinib and savolitinib combination therapy with osimertinib monotherapy as first-line treatment for patients with de novo *MET*-amplified, *EGFR*-mutant advanced NSCLCs (NCT05163249).

Nevertheless, there were some limitations in the present study. Considering the single-center retrospective design of the study and small sample size, we would encourage larger prospective studies to validate and expand on our findings. In addition, in our study, the patients for whom de novo *MET* amplification could not be verified by FISH were classified simply as one unknown group, and their baseline clinicopathologic characteristics were collectively compared with those of the *EGFR*+/*MET*amp- and *EGFR*+/*MET*amp +- groups (Table S1). Due to lack of tissues, we could not subdivide the unknown group into more specific group types, although the treatment outcomes for this group lay in between those for the other 2 groups (Figure S2).

In summary, our findings showed that *EGFR*+/*MET*amp + patients with advanced NSCLC had a significantly worse response to first-line EGFR-TKI monotherapy and poorer survival. LCOs drug testing in vitro demonstrated better anticancer activity of dual targeted therapies, suggesting a promising first-line treatment for *EGFR*+/*MET*amp +- patients. A future large prospective clinical study and further in vitro experiments are needed to validate these findings.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

ORCID

Kai-Cheng Peng https://orcid.org/0000-0002-8094-9720
Xu-Chao Zhang https://orcid.org/0000-0002-4138-8115
Qing Zhou https://orcid.org/0000-0002-0478-176X

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SUPPORTING INFORMATION

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