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Molecular features and clinical outcomes of EGFR-mutated, MET-amplified non-small-cell lung cancer after resistance to dual-targeted therapy

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Abstract

Background: Some studies of dual-targeted therapy (DTT) targeting epidermal growth factor receptor (EGFR) and mesenchymal-epithelial transition (MET) have shown promising efficacy in non-small-cell lung cancer (NSCLC). Consequently, patient management following DTT resistance has gained significance. However, the underlying resistance mechanisms and clinical outcomes in these patients remain unclear.

Objectives: This study aimed to delineate the molecular characteristics and survival outcomes of patients with NSCLC harboring *EGFR* mutations and acquired *MET* amplification after developing resistance to DTT.

Design: We conducted a retrospective analysis of patients with NSCLC with EGFR mutations and acquired MET amplification who exhibited resistance to EGFR/MET DTT.

Methods: Next-generation sequencing (NGS) was performed on patients with available tissue samples before and/or after the development of resistance to DTT. Stratified analyses were carried out based on data sources and subsequent salvage treatments. Univariate/multivariate Cox regression models and survival analyses were employed to explore potential independent prognostic factors.

Results: The study included 77 NSCLC patients, with NGS conducted on 19 patients. We observed many resistance mechanisms, including EGFR-dependent pathways (4/19, 21.1%), MET-dependent pathways (2/19, 10.5%), EGFR/MET co-dependent pathways (2/19, 10.5%), and EGFR/MET-independent resistance mechanisms (11/19, 57.9%). Post-progression progression-free survival (pPFS) and post-progression overall survival (pOS) significantly varied among patients who received the best supportive care (BSC), targeted therapy, or chemotherapy (CT), with median pPFS of 1.5, 3.9, and 4.9 months, respectively ($p = 0.003$). Median pOS were 2.3, 7.7, and 9.2 months, respectively ($p < 0.001$). The number of treatment lines following DTT resistance and the Eastern Cooperative Oncology Group performance status emerged as the independent prognostic factors.

Conclusion: This study revealed a heterogeneous landscape of resistance mechanisms to EGFR/MET DTT, with a similar prevalence of on- and off-target mechanisms. Targeted therapy or CT, as compared to BSC, exhibited the potential to improve survival outcomes for patients with advanced NSCLC following resistance to DTT.

Keywords: *EGFR* mutation, *MET* amplification, NSCLC, tyrosine kinase inhibitors, resistance

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Introduction

The identification of oncogenic drivers has revolutionized the management of advanced non-small-cell lung cancer (NSCLC). Targeted therapies, particularly tyrosine kinase inhibitors (TKIs) tailored to specific driver genes, such as epidermal growth factor receptor (*EGFR*) mutations, *ALK* rearrangements, *ROS1* rearrangements, *NTRK* fusions, mesenchymal–epithelial transition (*MET*) amplifications, or mutations, have yielded remarkable responses in select patients, marking a pivotal advancement in the treatment of metastatic NSCLC. Among these driver genes, *EGFR* mutations have been prominently observed, with an incidence of approximately 15% in Western populations and up to 40% in Asian NSCLC cohorts.[1] Notably, Chinese patients with NSCLC exhibit an even higher incidence, reaching 60%.[2] For individuals with advanced NSCLC, first-line therapy utilizing different generations of EGFR-TKIs has consistently demonstrated superior clinical efficacy when compared to traditional chemotherapy (CT). Nevertheless, resistance to EGFR-TKIs invariably emerges, driven by various factors, including the acquisition of new genetic aberrations. Among these, *MET* amplification accounts for approximately 5–15% of patients with NSCLC who develop acquired resistance to first- or second-generation EGFR-TKIs.[3] Moreover, it is worth noting that nearly 60% of *MET* amplifications do not co-occur with the T790M mutation. Importantly, *MET* amplification has emerged as the primary mechanism of resistance to third-generation EGFR-TKIs, with a prevalence ranging from 19% to 25%.[4–6] The primary consequence of *MET* amplification is the activation of the HER3-PI3K signaling axis, resulting in sustained downstream pathway activation and resistance to EGFR-TKIs.[7,8] Several MET TKIs, including crizotinib, savolitinib, tepotinib, and capmatinib, have been developed and have exhibited promising efficacy in patients with *MET* amplification. For individuals who acquire *MET* amplification or overexpression following EGFR-TKI treatment, the prevailing view is that dual-target inhibition concurrently targeting the EGFR and MET pathways may offer synergistic therapeutic advantages to overcome acquired drug resistance. Three multicenter clinical trials,[9–11] led by the Guangdong Lung Cancer Institute (GLCI), have initially demonstrated the potential effectiveness of EGFR/MET dual-targeted therapy (DTT) as a treatment option for *EGFR*-mutated NSCLC with acquired *MET* amplification. However, following the failure of DTT, there is a dearth of established treatment modes for these patients, highlighting the growing significance of salvage therapy in the era of increasing DTT utilization.

Despite the burgeoning adoption of DTT, the molecular characteristics and clinical outcomes of *EGFR*-mutant, *MET*-amplified NSCLC following resistance to dual-target EGFR/MET regimens remain obscure. Current investigations into this matter rely predominantly on case reports and genetic analyses with limited sample sizes. Resistance mechanisms to dual-target therapy include a spectrum of possibilities, including EGFR-dependent, MET-dependent, and other mechanisms. Lim *et al.*[12] conducted a comprehensive exome analysis of tissues from three patients who developed acquired resistance to osimertinib and volitinib through tissue analysis both before and after dual-target treatment. Their findings unveiled secondary *MET* abnormalities, including acquired *MET* p.D1246H mutations, *MET* p.Y1230C mutations, augmented *MET* copy numbers, as well as other alterations, including ERBB2 mutations, increased amplification and copy numbers of *CCNE*, *CCND1*, *CDK6*, and *EGFR*. Mutations of *MET* Y1248H and D1246N were found to be a possible way for cancer cells to stop responding to dual-target therapy. These mutations could be overcome *in vitro* by Type II MET inhibitors such as cabozantinib, BMX 777607, and AMG 458.[13,14] Consequently, conducting comprehensive studies with larger sample sizes focusing on molecular characteristics, prognostic factors, and salvage therapy strategies post-DTT resistance would be instrumental in optimizing treatment approaches for DTT-resistant patients, ultimately enhancing their survival prospects.

In our study, we retrospectively investigated the clinical outcomes of patients with NSCLC harboring *EGFR* mutations and acquired *MET* amplification after developing resistance to EGFR/MET DTT. We delved into the molecular characteristics both before and after resistance to EGFR/MET DTT, and we compared the clinical outcomes associated with different salvage therapies for these patients.

Materials and methods

Patients and data extraction

This study comprised two cohorts of patients with NSCLC with *EGFR* mutations and acquired *MET* amplification who developed resistance to EGFR/MET DTT. The first cohort encompassed

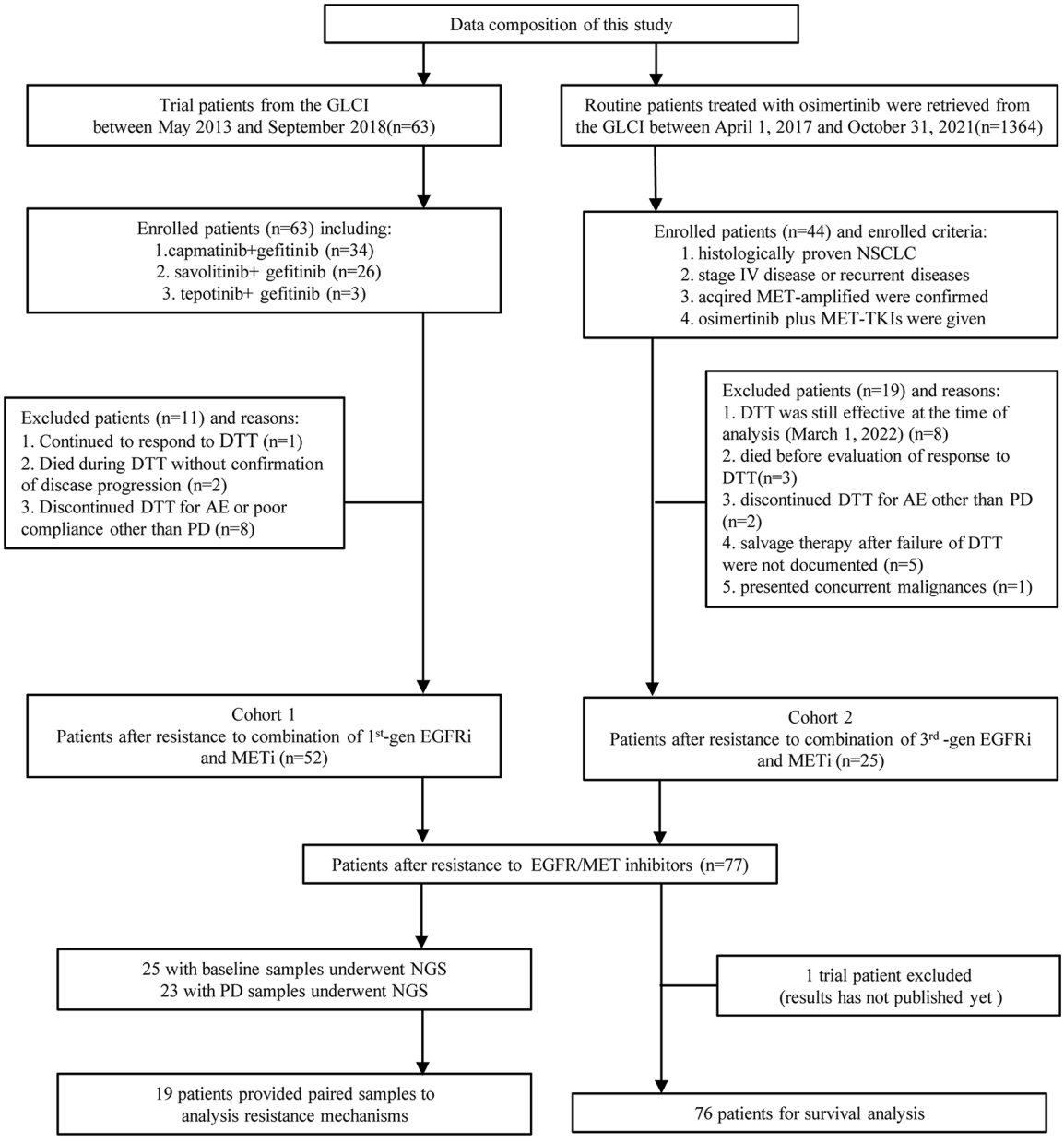

Figure 1. Flow chart of this study.
AE, adverse events; 1st-gen EGFRi, first-generation EGFR-TKI; 3rd-gen EGFRi, third-generation EGFR-TKI; DTT, dual-targeted therapy; METi, MET-TKI; NGS, next-generation sequencing; PD, progressive disease.

patients from three clinical trials, while the second cohort was a real-world investigation (Figure 1).

Between May 2013 and September 2018, a total of 63 patients with NSCLC at the GLCI, experiencing acquired resistance to first-generation EGFR-TKIs due to *MET* amplification, underwent EGFR/MET dual-target therapy in the context of three clinical trials: the phase Ib/II study of capmatinib (INC280) plus gefitinib, the phase Ib study of MET-TKI savolitinib plus gefitinib, and the INSIGHT study.[9–11] Patient eligibility criteria encompassed successful enrollment in the mentioned trials and receipt of dual-target therapy. The assessment of disease progression during DTT adhered to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[15]

Patients continuously treated with osimertinib were identified from outpatient and inpatient medical records spanning from 1 April 2017 to 31 October 2021. Enrollment criteria included

histologically confirmed NSCLC, stage IV or recurrent disease, acquired MET amplification, MET copy number variation, administration of osimertinib plus MET-TKI (based on the available evidence-based medicine and with informed consent, we administered DTT to a specific group of patients with MET amplification after developing resistance to Osimertinib), and confirmed disease progression during DTT in accordance with RECIST version 1.1. Exclusions were made for patients in whom DTT remained effective as of 1 March 2022, patients who passed away during dual-target therapy without confirmed disease progression, and patients who discontinued dual-target therapy for reasons other than the progressive disease (PD) and lacked documented salvage therapy after DTT failure or presented concurrent malignancies.

Definitions of MET amplifications

MET amplifications were defined based on the criteria established in the TATTON study[16]: local tissue fluorescent *in situ* hybridization (FISH; *MET* gene copy number ≥ 5 or *MET*-CEP7 ratio ≥ 2), local tissue immunohistochemistry (MET 3+ expression in $\geq 50\%$ of tumor cells), or next-generation sequencing (NGS; ≥ 5 copies of *MET* over tumor ploidy).

NGS-based analysis of genetic characteristics

NGS analysis was conducted on 25 patients before initiating EGFR/MET DTT and in 23 patients after resistance to EGFR/MET DTT had developed. Paired analysis of NGS results was performed for a total of 19 patients. Tumor tissues or body fluids, including pleural effusion, plasma, and cerebrospinal fluid, were collected from patients and subjected to panel NGS analysis to assess the status of 168, 196, 425, or 520 cancer-relevant genes.

Biological samples for NGS-based analysis were processed by two commercial laboratories, Burning Rock Biotech and Geneseeq Technology Inc. (Nanjing, China), using optimized protocols as previously described.[17] These samples were maintained under the custody of GLCI.

Patients' follow-up and treatment response evaluation

Patient follow-up continued until 1 June 2022. The duration of follow-up was calculated from the date of dual-target therapy progression to the date of death or the last examination. Treatment responses were evaluated according to RECIST version 1.1.

Patient follow-up responses to treatment included the following categories: complete response (CR), partial response (PR), stable disease (SD), and PD. All responses are indicated using a percentage (%). Furthermore, the objective response rate (ORR) was defined as CR + PR, and the disease control rate (DCR) was defined as CR + PR + SD. Post-progression progression-free survival (pPFS) was defined as the period from radiologically confirmed EGFR/MET DTT progression until radiologically confirmed progression with further-line treatment, death, or the last follow-up. Conversely, post-progression overall survival (pOS) was defined as the period from the radiologically confirmed progression of EGFR/MET dual-targeting therapy until death or the last follow-up. Progression-free survival (PFS) was the period from the initiation of EGFR/MET DTT to radiologically confirmed progression, while overall survival (OS) was the period from the initiation of EGFR/MET DTT until death or the last follow-up.

Statistical analysis

Categorical variables were presented as numbers (N) and percentages (%) and compared using the chi-square or Fisher's exact test (if an expected value ≤ 5 was encountered). The continuous variable 'age' was reported as mean (SD) and compared using Student's independent *t*-test or one-way ANOVA between groups. Other continuous variables were expressed as median (IQR) and compared using the Mann–Whitney *U* or Kruskal–Wallis test between groups. Univariate and multivariate Cox proportional-hazard regression models were utilized to explore potential factors associated with patient pPFS and pOS. If an independent variable exhibited a p-value of <0.20 in the univariate results, it was entered into the subsequent multivariate model. Independent variables in the multivariate regression model were identified as factors associated with pPFS or pOS. Factor performance was further assessed using the Kaplan–Meier method and compared using a log-rank test among groups. Hazard ratios (HR) and 95% confidence intervals (CI) were reported in the Cox modeling results. An HR >1 indicated a worse prognosis, while an HR <1 indicated a better prognosis compared to the reference

category. All analyses were conducted using IBM SPSS Version 26 (SPSS Statistics V26, IBM Corporation, Somers, NY, USA). A *p*-value of <0.05 was considered statistically significant. The reporting of this study adheres to the STROBE Statement – a checklist of items that should be included in reports of observational studies.

Results

Clinical and pathological characteristics of patients

This study comprised a total of 77 patients, with 52 having received first-generation EGFR/MET TKIs and 25 receiving third-generation EGFR/MET TKIs (Table 1). Patients receiving first- or third-generation EGFR-TKIs and MET inhibitors in DTT exhibited similar characteristics. However, most patients (59.6%) received DTT as second-line therapy in cohort 1, while the majority (84.0%) underwent DTT as third-line or later therapy in cohort 2. T790M mutation testing was performed on the majority of patients before DTT, and it was negative in most cases (75.3%). However, post-DTT progression, T790M mutation testing was conducted in only 37 (48.1%) patients. Resistance to DTT predominantly resulted from extracranial progression (81.8%), with a smaller proportion experiencing intracranial progression (11.7%). Only 6.5% of patients exhibited both extracranial and intracranial progression after DTT. Extensive progression was the primary cause of DTT failure (68.8%).

The distribution of EGFR mutation subtypes among patients using first- and third-generation EGFR-TKIs in DTT was similar. *MET* amplifications were redefined using the criteria from the TATTON study, with positive results in 64 patients (83.1%), evenly distributed across different patient subgroups.

Distinct genetic mutation profile after resistance to DTT

Among the 77 patients in this study, NGS tests were conducted on 25 patients [Figure 2(a)] before initiating dual-target therapy and on 23 patients [Figure 2(b)] after resistance had developed. Paired NGS analysis was performed on a total of 19 patients. Genetic abnormalities were analyzed in 19 pre-dual-target and post-resistant tumor tissue or body fluid samples available from patients [Figure 2(c)].

Classic *EGFR* mutations were present in the 19 patients at baseline before dual-target therapy (*EGFR* mutations were not detected in the NGS of P58 tissue, but *EGFR* L858R was detected in his previous CSF and peripheral blood NGS), among which *EGFR* L858R was present in 10 patients (52.6%), *EGFR*-19del in nine patients (47.4%), coexisting *EGFR* T790M in six patients (31.6%), and coexisting *EGFR* amplification in 10 patients (52.6%). After DTT, most patients retained these classical *EGFR* mutations, with only one patient (P62) losing the *EGFR* L858R mutation, and five patients losing the *EGFR* amplification. Acquired EGFR pathway-dependent resistance mechanisms were detected in five patients: *EGFR* T790M ($n=1$), *EGFR* C797S/Y ($n=3$), *EGFR* L718Q/V ($n=1$), *EGFR* G796S ($n=2$), and *EGFR* copy number amplification (cn amp) ($n=2$).

Prior to DTT, *MET* amplification was found in 15 patients (78.9%). After DTT, loss of *MET* amplification was observed in nine patients (47.4%). Secondary variants of *MET* were detected in four patients, including D1228H/N/Y, Y1230C/H, D1231Y, R1004G, V1237I, and *MET* fusion. In addition, other potential resistance mechanisms were identified, including small-cell lung cancer (SCLC) transformation, *BRAF* V600E, *KRAS* G60D, *NRAS* Q61R, *PIK3CA* E545K, *PIK3CA* L339R, *ERBB2* amp, and *MYC* amp.

Multiple resistance mechanisms could coexist in patients, such as P66, who harbored both EGFR and MET-dependent pathway mechanisms and *ERBB2* amplification, and P77, who exhibited SCLC transformation and *PIK3CA* E545K. One mechanism may predominate in EGFR/MET DTT resistance, leading to the grouping of 19 patients into four categories based on the dominant resistance mechanism [Figure 3(a)]. Drug resistance mechanisms after EGFR/MET DTT were classified into on-targeted and off-targeted bypass mechanisms. On-targeted mechanisms encompassed the EGFR-dependent pathway (Group a), the MET-dependent pathway (Group b), and the EGFR/MET-co-dependent pathway (Group c). Off-targeted bypass mechanisms (Group d) included various drug resistance mechanisms that were not reliant on the EGFR or MET pathways. A summary of the regimens and efficacy

Table 1. Baseline patient demographics and disease characteristics in two cohorts after resistance to DTT.

Group	First EGFR/MET TKI (*n* = 52)	Third EGFR/MET TKI (*n* = 25)	Overall (*n* = 77)	*p* Value
Sex				0.515
Male	25 (48.1%)	14 (56.0%)	39 (50.6%)	
Female	27 (51.9%)	11 (44.0%)	38 (49.4%)	
Age (years)				0.922
Mean (SD)	59.15 (10.47)	58.91 (9.96)	59.07 (10.24)	
Age groups (years)				0.505
<60	27 (51.9%)	15 (60.0%)	42 (54.5%)	
⩾60	25 (48.1%)	10 (40.0%)	35 (45.5%)	
ECOG performance status				0.205
0–1	45 (86.5%)	18 (72.0%)	63 (81.8%)	
>1	7 (13.5%)	7 (28.0%)	14 (18.2%)	
Histological subtype				0.009
Adenocarcinoma	52 (100.0%)	21 (84.0%)	73 (94.8%)	
Squamous cell carcinoma	0 (0.0%)	2 (8.0%)	2 (2.6%)	
Small cell carcinoma	0 (0.0%)	2 (8.0%)	2 (2.6%)	
Smoking history				0.907
Non-smoker	41 (78.8%)	20 (80.0%)	61 (79.2%)	
Ever smoker	11 (21.2%)	5 (20.0%)	16 (20.8%)	
Lines of DTT				<0.001
2	31 (59.6%)	4 (16.0%)	35 (45.5%)	
⩾3	21 (40.4%)	21 (84.0%)	42 (54.5%)	
DTT_BOR				0.197
PR	20 (38.5%)	12 (48.0%)	32 (41.6%)	
SD	21 (40.4%)	5 (20.0%)	26 (33.8%)	
PD	11 (21.2%)	8 (32.0%)	19 (24.7%)	
Pre_DTT_MET				0.747
Positive	44 (84.6%)	20 (80.0%)	64 (83.1%)	
Negative	8 (15.4%)	5 (20.0%)	13 (16.9%)	
EGFR subtype				0.750
Exon 19 deletion	25 (48.1%)	14 (56.0%)	39 (50.6%)	
Leu858RArg	26 (50.0%)	11 (44.0%)	37 (48.1%)	

(Continued)

Table 1. (Continued)

Group	First EGFR/MET TKI ($n=52$)	Third EGFR/MET TKI ($n=25$)	Overall ($n=77$)	*p* Value
Unknown	1 (1.9%)	0 (0.0%)	1 (1.3%)	
Pre_DTT_T790M				0.066
Positive	6 (11.5%)	7 (28.0%)	13 (16.9%)	
Negative	40 (76.9%)	18 (72.0%)	58 (75.3%)	
Unknown	6 (11.5%)	0 (0.0%)	6 (7.8%)	
Post_DTT_T790M				0.006
Positive	10 (19.2%)	6 (24.0%)	16 (20.8%)	
Negative	9 (17.3%)	12 (48.0%)	21 (27.3%)	
Unknown	33 (63.5%)	7 (28.0%)	40 (51.9%)	
Brain metastases				0.334
No	35 (67.3%)	14 (56.0%)	49 (63.6%)	
Yes	17 (32.7%)	11 (44.0%)	28 (36.4%)	
PD_mode				0.092
Local PD	13 (25.0%)	11 (44.0%)	24 (31.2%)	
Extensive PD	39 (75.0%)	14 (56.0%)	53 (68.8%)	
Progression intra/extracranial				0.900
Intracranial	6 (11.5%)	3 (12.0%)	9 (11.7%)	
Extracranial	43 (82.7%)	20 (80.0%)	63 (81.8%)	
Both	3 (5.8%)	2 (8.0%)	5 (6.5%)	

BOR, best of response; DTT, dual-targeted therapy; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; MET, mesenchymal–epithelial transition; PD, progressive disease; TKI, tyrosine kinase inhibitors.

of DTT and the salvage therapy chosen after DTT resistance in these four groups of patients is presented in Supplemental Table S1. Typical cases from each dominant resistance mechanism group are illustrated in Figure 3(b)–(e).

Salvage treatments after resistance to DTT

Salvage treatments for patients following progression during EGFR/MET DTT included best supportive care (BSC; $n=17$), TKIs ($n=24$), CT ($n=35$), and one patient received an anti-EGFR/MET dual antibody (EMB01 clinical trial). As the results of this study have not been published, we excluded this patient (P73) from the clinical outcomes analysis. Treatment decisions were made through discussions between patients and their oncologists, considering financial conditions, physical status, rebiopsy results (pathological and genetic tests), and patient preferences.

The clinical characteristics, including gender, age, smoking history, Eastern Cooperative Oncology Group (ECOG) performance status (PS), histology, and line of DTT, were well-balanced among the three treatment groups mentioned above (Table 2). We assessed the best response of salvage therapies, pPFS, and pOS (Supplemental Figures S1A–D).

In the BSC group, five patients received local treatment, while the remaining 12 patients received only symptomatic supportive treatment. Among the 24 patients in the TKI group, 16 received

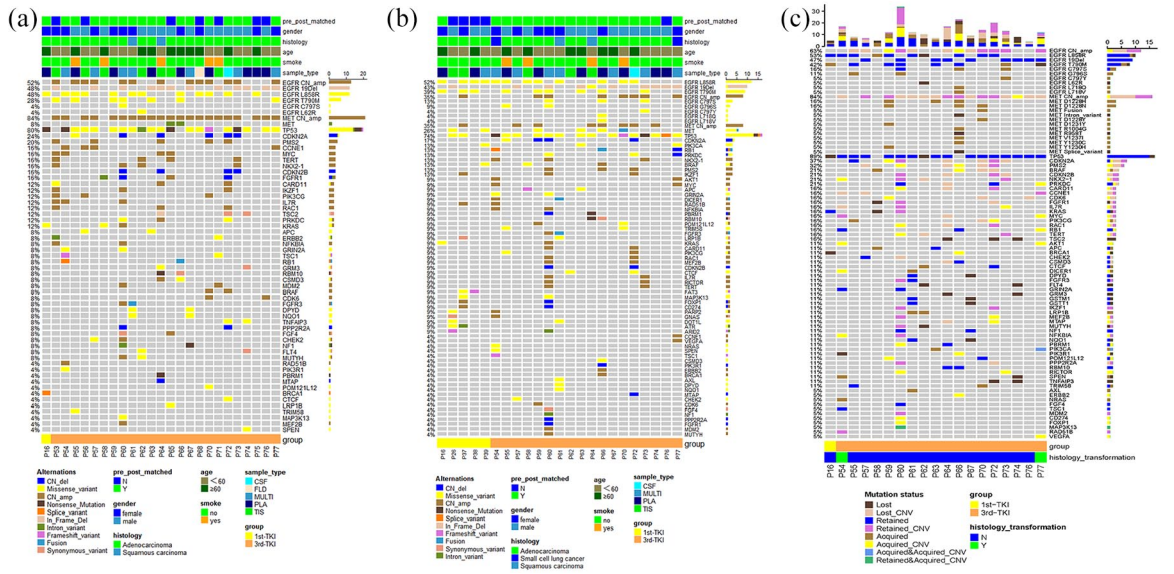

Figure 2. Genetic global profile before and after disease progression of DTT. Each column represents one patient. All detected alterations in EGFR and MET are captured. First-TKI is for patients who received first-generation EGFR-TKI plus MET-TKI as DTT and third-TKI is for patients who received third-generation EGFR-TKI plus MET-TKI as DTT. (a) Gene characteristics before DTT ($n = 25$). (b) Gene characteristics after disease progression of DTT ($n = 23$). (c) Comparison of the genetic changes of the 19 patients based on NGS results before DTT and after disease progression of DTT.
CN_amp, copy number amplification; CN_del, copy number deletion; CSF, cerebrospinal fluid; DTT, dual-targeted therapy; EGFR, epidermal growth factor receptor; FLD, fluid; MET, mesenchymal–epithelial transition; MULTI, multiple, indicating that both matched tissue and plasma samples were available for analysis; NGS, next-generation sequencing; PLA, plasma; pre-/post-matched, patients with paired samples before DTT and after disease progression; TIS, tissue; TKI, tyrosine kinase inhibitors.

different TKIs from pre-treatment, and 11 of them were selected based on the resistance mechanism, while five did not. Six patients, five of whom had local progression, received local treatment in combination with the original dual-targeted plan, and two patients continued the previous regimen. In the CT group, 12 patients received CT combined with bevacizumab, four received CT combined with EGFR-TKIs, and 19 received CT alone.

Responses and pPFS, pOS in different salvage treatments of patients after resistance to DTT

The ORR of the BSC, TKI, and CT groups were 5.9%, 16.7%, and 20.0%, respectively ($p = 0.474$). The DCR of the salvage treatment groups was 5.9%, 45.8%, and 77.1%, respectively ($p < 0.001$). Notably, the CT group had a significantly higher DCR compared to the TKI group [Figure 4(a) and (b)]. We analyzed the survival outcomes of patients after DTT progression in the three salvage treatment groups. pPFS differed significantly among patients who received BSC, targeted therapy (TKI), or CT [log-rank $p = 0.003$; Figure 4(c)], with median pPFS durations of 1.5 months (95% CIs: 0.3–2.7 months), 3.9 months (95% CI: 1.9–5.9 months), and 4.9 months (95% CI: 3.7–6.0 months), respectively. Pairwise analysis revealed significantly longer pPFS in the TKI group compared to the BSC group ($p = 0.026$). Similarly, the CT group exhibited significantly longer pPFS than the BSC group ($p = 0.001$). However, pPFS did not significantly differ between the TKI and CT groups ($p = 0.258$). pOS also varied significantly among the three treatment groups [log-rank $p < 0.001$; Figure 4(d)], with median pOS of 2.3 months (95% CI: 0.0–4.9 months) for the BSC group, 7.7 months (95% CI: 3.8–11.5 months) for the TKI group, and 9.2 months (95% CI: 6.5–12.0 months) for the CT group. Pairwise analysis showed significantly longer pOS in the TKI group ($p = 0.006$) and the CT group ($p < 0.001$) compared to the BSC group. pOS was similar between the TKI and CT groups ($p = 0.338$).

Based on the inclusion or exclusion of anti-vascular therapy, we divided the CT group into two subgroups: CT combined with bevacizumab and CT without bevacizumab. The baseline characteristics of the two groups were well-balanced (Supplemental

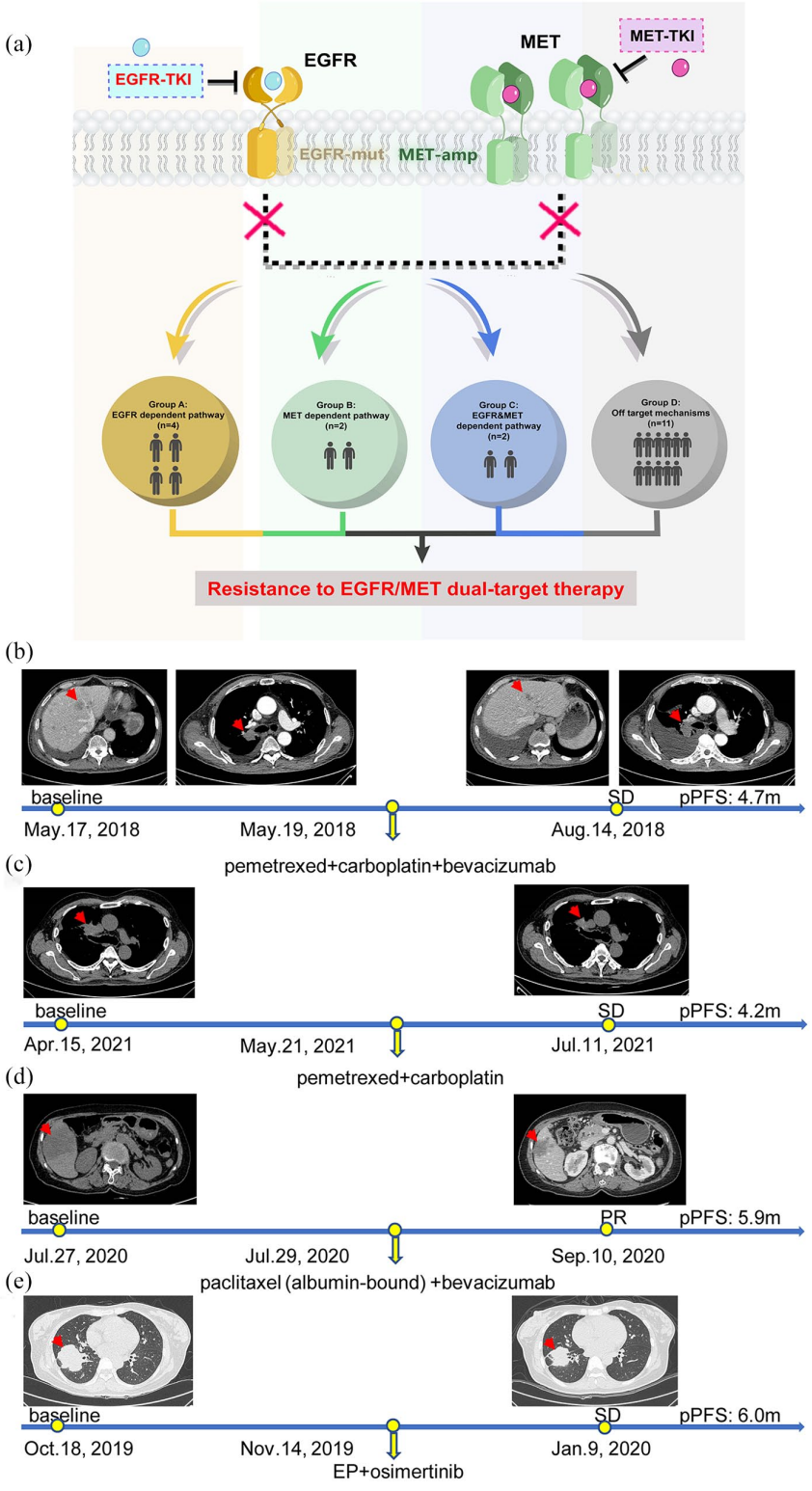

Figure 3. The landscape of potential resistance mechanisms of DTT in 19 patients and typical cases. (a) The landscape of potential resistance mechanisms of DTT is summed up through data from 19 patients, and divided into four groups according to on-targeted and off-targeted bypass mechanisms. The on-targeted mechanisms included the EGFR-dependent pathway (Group a), the MET-dependent pathway (Group b), and the EGFR/MET-co-dependent pathway (Group c). The off-targeted bypass mechanisms (Group d) included various drug resistance mechanisms that were not dependent on the EGFR or MET pathways. Unknown refers to patients in whom putative resistance mechanisms were not identified. (b)–(e) Typical cases of four groups.
DTT, dual-targeted treatment; EGFR, epidermal growth factor receptor; MET, mesenchymal–epithelial transition; SCLC, small-cell lung cancer.

Table 2. Baseline patient demographics and disease characteristics in three treatment groups after resistance to DTT.

Group	BSC (*n* = 17)	TKI (*n* = 24)	CT (*n* = 35)	Overall (*n* = 76*)	*p* Value
Generation of EGFR-TKI					0.114
First generation	15 (88.2%)	14 (58.3%)	23 (65.7%)	52 (68.4%)	
Third generation	2 (11.8%)	10 (41.7%)	12 (34.3%)	24 (31.6%)	
Sex					0.881
Male	8 (47.1%)	13 (54.2%)	17 (48.6%)	38 (50.0%)	
Female	9 (52.9%)	11 (45.8%)	18 (51.4%)	38 (50.0%)	
Age (years)					0.805
Mean (SD)	60 (10)	58 (10)	60 (11)	59 (10)	
Age groups (years)					0.317
<60	8 (47.1%)	16 (66.7%)	17 (48.6%)	41 (53.9%)	
⩾60	9 (52.9%)	8 (33.3%)	18 (51.4%)	25 (46.1%)	
ECOG performance status					0.695
0–1	13 (76.5%)	19 (79.2%)	30 (85.7%)	62 (81.6%)	
>1	4 (23.5%)	5 (20.8%)	5 (14.3%)	14 (18.4%)	
Histological subtype					0.234
Adenocarcinoma	17 (100.0%)	22 (91.7%)	33 (94.3%)	72 (94.7%)	
Squamous cell carcinoma	0 (0.0%)	2 (8.3%)	0 (0.0%)	2 (2.6%)	
Small-cell carcinoma	0 (0.0%)	0 (0.0%)	2 (5.7%)	2 (2.6%)	
Smoking history					0.652
Non-smoker	14 (82.4%)	20 (83.3%)	26 (74.3%)	60 (78.9%)	
Ever smoker	3 (17.6%)	4 (16.7%)	9 (25.7%)	16 (21.1%)	
Lines of DTT					0.774
2	9 (52.9%)	10 (41.7%)	16 (45.7%)	35 (46.1%)	
⩾3	8 (47.1%)	14 (58.3%)	19 (54.3%)	41 (53.9%)	
DTT_BOR					0.593
PR	9 (52.9%)	10 (41.7%)	12 (34.3%)	31 (40.8%)	
SD	6 (35.3%)	7 (29.2%)	13 (37.1%)	26 (34.2%)	
PD	2 (11.8%)	7 (29.2%)	10 (28.6%)	19 (25.0%)	
Pre_DTT_MET					0.571
Positive	15 (88.2%)	21 (87.5%)	27 (77.1%)	63 (82.9%)	
Negative	2 (11.8%)	3(12.5%)	8(22.9%)	13 (17.1%)	

(Continued)

Table 2. (Continued)

Group	BSC ($n=17$)	TKI ($n=24$)	CT ($n=35$)	Overall ($n=76$*)	p Value
EGFR subtype					0.960
Exon 19 deletion	8 (47.1%)	13 (54.2%)	17 (48.6%)	38 (50.0%)	
Leu858RArg	9 (52.9%)	11 (45.8%)	17 (48.6%)	37 (48.7%)	
Unknown	0 (0.0%)	0 (0%)	1 (2.9%)	1 (1.3%)	
Pre_DTT_T790M					0.439
Positive	2 (11.8%)	4 (16.7%)	7 (20.0%)	13 (17.1%)	
Negative	13(76.5%)	20 (83.3%)	24 (68.6%)	57 (75.0%)	
Unknown	2 (11.8%)	0 (0.0%)	4 (11.4%)	6 (7.9%)	
Post_DTT_T790M					0.083
Positive	1 (5.9%)	7 (29.2%)	7 (20.0%)	15 (19.7%)	
Negative	2 (11.8%)	7 (29.2%)	12 (34.3%)	21 (27.6%)	
Unknown	14 (82.4%)	10 (41.7%)	16 (45.7%)	40 (52.6%)	
Brain metastases					0.837
No	11 (64.7%)	14 (58.3%)	23 (65.7%)	48 (63.2%)	
Yes	6 (35.3%)	10 (41.7%)	12 (34.3%)	28 (36.8%)	
PD_mode					0.092
Local PD	7 (41.2%)	9 (37.5%)	8 (22.9%)	24 (31.6%)	
Extensive PD	10 (58.8%)	15 (62.5%)	27 (77.1%)	52 (68.4%)	
Progression intra/extracranial					0.005
Intracranial	5 (29.4%)	4 (16.7%)	0 (0.0%)	9 (11.8%)	
Extracranial	12 (70.6%)	19 (79.2%)	31 (88.6%)	62 (81.6%)	
Both	0 (0.0%)	1 (4.2%)	4 (11.4%)	5 (6.6%)	

*Since the results of this study have not been published, we excluded this patient (P73) from the clinical outcomes analysis.
BOR, best of response; BSC, best supportive care; CT, chemotherapy; DTT, dual-targeted therapy; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; MET, mesenchymal–epithelial transition; PD, progressive disease.

Table S2A). pPFS showed marginal significance between the two groups (log-rank $p=0.079$, Supplemental Figure S2A), with median pPFS of 6.1 and 4.7 months, respectively. Although pOS did not significantly differ between the two groups (log-rank $p=0.419$), median pOS were 12.3 and 7.8 months, respectively (Supplemental Figure S2B).

Immune checkpoint inhibitors (ICIs) were not administered as salvage therapies at the time following DTT failure, and only a small fraction of patients ($n=9$) received ICIs after salvage treatments. Patients receiving ICIs as post-line treatment following salvage treatments exhibited longer pOS than those who did not (15.8 months *versus* 6.5 months, $p=0.027$) (Figure 4E, Supplemental Table S2B). However, since few patients received ICIs (only nine patients), a larger sample size is required to confirm the impact on survival.

Identification of independent prognostic factors

Multivariate Cox regression analysis revealed that the generation of EGFR-TKIs in DTT, the PFS

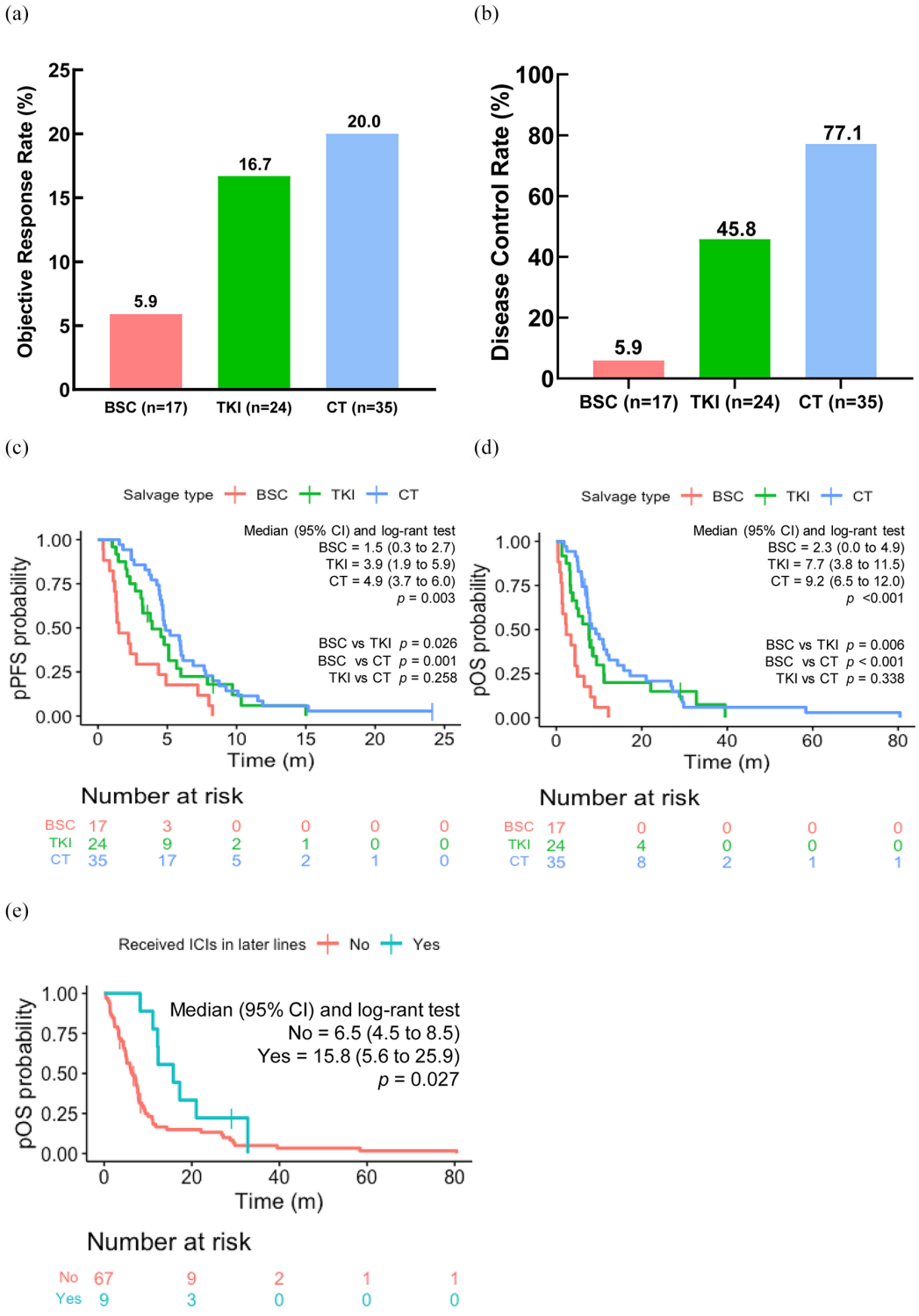

Figure 4. Subgroup survival analysis and response to salvage therapies after resistance to EGFR/MET DTT. (a) ORR of patients receiving BSC, TKI, and chemotherapy treatments. (b) DCR of patients receiving BSC, TKI, and chemotherapy treatments. (c and d) Kaplan–Meier curves illustrating pPFS (a) and pOS (b) of patients who received BSC, TKI, and chemotherapy. The risk table below indicates the number of patients in each group at the specified time. (e) Kaplan–Meier curve illustrating the pOS of patients who received and did not receive ICIs in later lines. The risk table below indicates the number of patients in each group at the specified time. P15 received surgery after DTT progression but did not receive systematic treatment; so, he was included in the BSC group. The efficacy of his salvage treatment was CR.

BSC, best supportive care; CT, chemotherapy; DCR, disease control rate; DTT, dual-targeted treatment; ICIs, Immune checkpoint inhibitors; ORR, objective response rate; pPFS, post-progression progression-free survival; pOS, post-progression overall survival; TKI, tyrosine kinase inhibitor.

of DTT, and salvage therapies were independent factors affecting pPFS among all clinical characteristics (Supplemental Table S3A). Furthermore, the number of treatment lines after resistance to DTT ($p < 0.001$) and ECOG PS ($p = 0.060$, marginally significant) were independent prognostic factors for pOS (Supplemental Table S3B).

The pPFS was 4.6 months (95% CI 4.0–5.2 months) for patients who received the first generation of EGFR-TKI in DTT and 4.2 months (95% CI 3.1–5.3 months) for those who received the third generation of EGFR-TKI ($p = 0.050$, Figure S3A). The pOS was 6.5 months (95% CI 4.8–8.2 months) for patients receiving the first generation and 7.8 months (95% CI 7.0–8.6 months) for those receiving the third generation of EGFR-TKI in DTT ($p = 0.648$, Supplemental Figure S3B). Patients who received the first generation of EGFR-TKI in DTT showed significantly better pPFS than those who received the third generation, while the pOS of these patients in the two cohorts were similar.

Multivariate Cox regression analysis also indicated that a longer PFS of DTT might be associated with a longer pPFS. Patients were divided into two groups according to the median PFS (mPFS) of DTT, which was 4.2 months. The pPFS was significantly different between patients with a PFS of DTT less than 4.2 months and those with a PFS of DTT equal to or greater than 4.2 months, with median pPFS of 3.9 months (95% CI: 1.6–6.2 months) and 4.8 months (95% CI: 4.1–5.4 months), respectively (log-rank $p = 0.041$, Figure S3C). The pOS was not significantly different between the two groups (log-rank $p = 0.899$, Supplemental Figure S3D).

A better ECOG PS was also associated with pOS. Patients were categorized based on the ECOG PS score as score 0–1 group and score > 1 group. The Kaplan–Meier curve illustrated that the pPFS was significantly different between the two groups (log-rank $p = 0.014$, Figure S3E), with median pPFS durations of 4.7 months (95% CI: 4.2–5.1 months) and 2.8 months (95% CI: 1.3–4.2 months), respectively. Although pOS was also significantly different between the two groups (log-rank $p = 0.026$, Figure S3F), the median pOS was 7.7 months (95% CI: 6.8–8.5 months) and 4.4 months (95% CI: 0.1–8.7 months), respectively.

The number of treatment lines patients received after resistance to DTT was positively correlated with longer pOS. The Cox proportional-hazards model results showed that the status of T790M before DTT did not significantly influence pPFS and pOS. Among the first EGFR/MET TKI patients, 6 (11.5%) were T790M positive, while 40 (76.9%) were T790M negative. The ORRs of DTT in these two groups were 0% and 47.5%, respectively ($p = 0.066$). The PFS of DTT significantly differed between these two groups, with mPFS of 1.9 months (95% CI: 0.9–2.9 months) and 4.5 months (95% CI: 2.8–6.1 months), respectively (log-rank $p = 0.049$). However, the OS rates of DTT, as well as pPFS and pOS, were not significantly different between the two groups (log-rank $p = 0.586$, $p = 0.513$, $p = 0.323$, respectively; Supplemental Table S4).

Discussion

This study represents the largest clinical research effort to investigate the molecular characteristics, salvage treatment options, and efficacy following resistance to EGFR/MET DTT. We meticulously examined paired pre- and post-DTT samples from 19 patients for molecular characteristic analysis through parallel NGS analysis, and we assessed the clinical outcomes of 76 patients after developing resistance to DTT. We categorized potential resistance mechanisms into four distinct groups: the EGFR-dependent pathway group, the MET-dependent pathway group, the EGFR/MET-co-dependent pathway group, and the off-target bypass group, including various drug resistance mechanisms not reliant on the EGFR or MET pathways. Furthermore, we explored optimal salvage therapies and identified potential independent prognostic factors based on real-world evidence.

While the mechanisms of acquired resistance to EGFR-TKI or MET-TKI monotherapy have been partially elucidated, the resistance mechanism to EGFR/MET DTT remains elusive. In theory, these mechanisms encompass alterations of the EGFR-dependent pathway, MET-dependent pathway abnormalities, and off-target bypass alterations. In our study, we evaluated the potential molecular mechanisms of resistance to EGFR/MET DTT in 19 patients. Notably, we observed instances of the loss of *EGFR* mutations (*EGFR* T790M) and *MET* amplification, which were not uncommon. The acquired mutations we detected included previously reported mechanisms of on-target and off-target resistance to EGFR-TKI and MET-TKI monotherapies.

Regarding the MET pathway, the acquisition of missense mutations in *MET* D1228 and Y1230 was considered one of the drug resistance mechanisms for Type I MET-TKI, which may be reversible with Type II MET-TKI treatment.[13,18] MET inhibitors can be categorized into two distinct classes based on their functional properties. Type I inhibitors specifically target and bind to the active conformation of MET, including examples like crizotinib (Type Ia), capmatinib, tepotinib, and savolitinib (Type Ib). Conversely, Type II inhibitors preferentially bind to the inactive conformation of MET, as exemplified by cabozantinib. It is worth noting that Type II inhibitors often exhibit a broader spectrum of inhibition toward various kinase targets, potentially leading to off-target side effects.[19,20] These two mutations (MET D1228 and Y1230) were also detected in our cohort. In the EGFR pathway, acquired *EGFR* amplification was detected in two patients, with one patient showing a significant increase in copy number after developing drug resistance. This suggests that *EGFR* amplification could contribute to DTT resistance, which has been previously reported in patients treated with INC280 plus gefitinib, indicating its potential role in mediating resistance to EGFR-TKI/MET-TKI.[13,18]

In terms of the EGFR-dependent pathway, *EGFR* C797S/Y is a common drug resistance mechanism for third-generation TKIs, second only to *MET* amplification. While trans-mutations may be overcome by combining first- and third-generation EGFR-TKIs,[21] cis-mutations may be addressed with cetuximab combined with brigatinib.[22] However, in our study, patients did not receive these combination therapies.

Rare mutations, such as *EGFR* L718V/Q, were detected in our study, which has demonstrated sensitivity to afatinib *in vitro*.[23] In addition, other rare mutations, such as *EGFR* G796S (a solvent-front mutation in exon 20 of EGFR), were observed, which pose a steric hindrance to osimertinib.[4]

Independent drug resistance mechanisms of EGFR/MET DTT, such as *BRAF* and *PIK3CA* mutations involved in MAPK/PI3K signal transduction, have been reported and were also observed in our study. Furthermore, *MYC* amplification, found in our study, may drive resistance in patients with *MET* amplification and resistance to capmatinib for NSCLC, suggesting MYC inhibitors as an alternative treatment strategy.[24]

Some uncommon resistance mechanisms are consistent with previous literature reports. For instance, the functional deficiency of *RBM10* (nonsense mutation) is a biomarker of a poor response to EGFR inhibitor treatment.[25] *AXL*-mediated resistance to EGFR-TKI in lung cancer, which we observed in our study, has been previously reported as well.[26] AXL overexpression has been shown to enhance the survival of drug-tolerant resistant cells and expedite the emergence of the T790M mutation.

Toshimitsu Yamaoka *et al.*[27] suggested in an *in vitro* cell line study that IGF-1R abnormalities mediate resistance to EGFR/MET-TKI. In addition to this, our study uncovered two patients who underwent a transformation to small-cell lung cancer (SCLC). One of these patients received a PCB (Carboplatin + Pemetrexed + Bevacizumab) regimen and achieved a PR, while the other received an EP (Cisplatin+Etoposide) regimen plus osimertinib and achieved SD with a shorter pPFS. Patients who undergo transformation to SCLC exhibit considerable heterogeneity, making the choice of treatment following this transformation an area that warrants further exploration.

A deeper understanding of genetic changes, rebiopsy practices, and NGS analysis following resistance to pre-line targeted therapy could offer more precision therapy opportunities, potentially improving the survival rates of patients with advanced NSCLC.[28] Such approaches have become routine for patients with *EGFR*-mutated NSCLC who have previously received EGFR-TKIs.

MET amplification is a common mechanism of acquired resistance to EGFR-TKIs. Dual-targeted EGFR/MET inhibition strategies, like osimertinib and savolitinib, have demonstrated effectiveness in studies like the TATTON[16] and SAVANNAH[29] trials. Consequently, the role of DTT is expected to grow in importance in the future, leading to its broader approval and use. However, determining optimal post-treatment strategies after resistance to EGFR/MET DTT remains challenging due to the limited sample sizes in previous studies.

Historically, the standard second-line treatment for NSCLC involved single-agent CT, with response rates ranging from 6.7% to 9.1% and mPFS of only 2.9–3 months.[30–32] Nevertheless, salvage treatments often extend to the third or even fourth line of therapy, involving targeted therapy, CT, or a combination of CT with anti-angiogenesis treatments after resistance to DTT. In our study, we observed that some patients treated with CT plus EGFR-TKIs after DTT progression achieved an efficacy rate of around 20% and a mPFS of 4–7 months. This suggests that such treatment regimens may offer more benefit to patients compared to single-agent CT.

In our study, even among patients in the BSC group, 76.5% of patients had 0–1 ECOG score, but without continued systematical antitumor therapy or further biopsy to clarify the drug resistance mechanism after DDT progression. That may be due to factors such as the third-generation EGFR-TKI not yet available at that time, the low patients' willingness to chemotherapy, and so on. Moreover, our study suggested that even with a good ECOG score, BSC also resulted in significantly poorer survival outcomes compared to the TKI and CT groups. This underscores the importance of encouraging patients with good physical performance after progression on targeted therapy to undergo re-biopsy and continue antitumor treatment.

Numerous salvage therapies are available for patients with acquired resistance to DTT. Precision-targeted therapy based on specific genetic alterations is a reasonable option, especially for patients with NSCLC with *EGFR* T790M mutations acquired after using a first-generation EGFR-TKI. In our study, some patients resistant to first-generation EGFR-TKIs were T790M positive before DTT, and while combination treatment with capmatinib/tepotinib and gefitinib did not yield clinical benefit, these patients achieved good efficacy with third-generation EGFR-TKIs post-DTT. This suggests that T790M and *MET* amplification may represent independent mechanisms of EGFR-TKI resistance. Patients with both T790M positivity and MET amplification may not benefit from the combination of first-generation EGFR-TKIs and MET inhibitors. However, these patients, having previously been treated with this combination, may still benefit from third-generation EGFR-TKIs.

In cases where acquired *MET* secondary mutations or other resistance mechanisms are present, combination therapy with a Type II MET inhibitor, such as cabozantinib,[13,18] or other targeted agents may be considered. Some retrospective studies have reported that continuing systemic treatment can still yield benefits after local relapse or progression.[33] This explains why certain patients in our study who experienced local progression opted for local treatment in combination with previous DTT.

However, it is worth noting that in our study, the median pPFS for the 24 patients who received TKIs as salvage therapy did not exceed 4 months, with an ORR and DCR of only 16.7% and 45.8%, respectively. This indicates that transitioning or continuing TKI treatment requires a favorable therapeutic effect based on matching the resistance mechanism. Currently, many potential resistance mechanisms have not yet been approved for clinical use, posing challenges such as the lack of drugs to overcome these resistance mechanisms and issues related to drug accessibility. By contrast, CT combined with EGFR-TKIs and CT combined with bevacizumab showed superior efficacy compared to TKIs alone. Therefore, for heavily treated TKI patients, CT-based treatments should be considered the optimal strategy.

While only a small number of patients in our study received ICIs for post-line treatment, findings from other trials are noteworthy. For instance, the IMpower150 study[34] demonstrated that atezolizumab plus bevacizumab plus carboplatin plus paclitaxel treatment provided better PFS in EGFR-TKI-resistant NSCLC patients compared to bevacizumab plus carboplatin plus paclitaxel. In the ORIENT-31 trial,[35] sintilimab plus biosimilar bevacizumab (IBI305) plus CT significantly improved PFS in patients with *EGFR*-mutated NSCLC who had progressed on EGFR-TKI treatment. The mPFS was 7.2 months in the sintilimab plus IBI305 plus CT group, 5.5 months in the sintilimab plus CT group, and 4.3 months in the CT alone group. Nine of the patients in our study used ICIs in back-line treatment, and pOS was significantly improved compared to patients without immunotherapy. These findings suggest that immunotherapy could play a role in the future treatment of patients with dual-target resistance.

It is important to note that for advanced tumors, OS remains the gold standard for evaluating treatment benefits, as PFS does not necessarily translate into OS. In the case of patients with

advanced NSCLC harboring the T790M mutation, while osimertinib improved PFS compared to platinum-pemetrexed CT,[36] it did not show significant benefits in OS[37] because a significant number of patients in the control arm crossed over to receive osimertinib. Our multivariate analysis results suggest that the number of treatment lines and PS scores are independent prognostic factors. PS is a well-recognized prognostic factor for lung cancer, with better PS indicating a more favorable prognosis.[38] However, the potential positive correlation between the number of treatment lines and prognosis in patients with advanced NSCLC has not been extensively explored in the literature. It is important to recognize that, as a retrospective study, the observed correlation between the number of treatment lines and prognosis does not imply causation and may involve confounding factors. Patients with better PS scores may be more capable of receiving and tolerating multiple lines of antitumor treatment, resulting in longer survival. Therefore, further research is needed to investigate the causal relationship between the number of treatment lines and prognosis. Patient management and care that enable patients to tolerate and be eligible for additional anticancer therapies upon treatment failure could be critical for the OS of patients with resistance to dual-target therapy.

However, we must acknowledge the limitations of this study, including its retrospective design and small sample size. The inherent limitations of conducting research on a small sample size at a single center emphasize the need for multicenter clinical studies to determine the most effective salvage therapy for NSCLC patients who have developed resistance to EGFR/MET DTT. Our study, although limited in scale, has provided valuable insights into the various resistance mechanisms and treatment approaches for these patients. To validate these findings, more extensive studies involving larger patient populations are essential. In addition, it is worth noting that our study did not comprehensively assess the efficacy of ICIs, as only a small subset of patients received this treatment following salvage therapy. Given the potential significance of ICIs in this context, further analysis is warranted to explore their role and contribution to patient outcomes.

In summary, this study reveals a heterogeneous landscape of resistance to EGFR/MET dual-target therapy. TKIs or CT appear to improve the survival outcomes for patients with NSCLC who have developed resistance to EGFR/MET TKIs and may represent the optimal salvage therapy for these patients.

Declarations

Ethics approval and consent to participate

Ethics approval was obtained from the Research Ethics Committee of the Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences (approval no. GDREC2019217H). Written informed consent was provided by all patients for the use of their clinical data before receiving treatment.

Consent for publication

Not applicable.

Author contributions

Mei-Mei Fang: Conceptualization; Data curation; Formal analysis; Writing – original draft; Writing – review & editing.

Jiang-Tao Cheng: Data curation; Validation; Writing – review & editing.

Yu-Qing Chen: Data curation; Formal analysis; Writing – original draft; Writing – review & editing.

Xiao-Cheng Lin: Data curation; Visualization; Writing – review & editing.

Jun-Wei Su: Data curation; Visualization; Writing – review & editing.

Yi-Long Wu: Conceptualization; Supervision; Writing – review & editing.

Hua-Jun Chen: Conceptualization; Writing – review & editing.

Jin-Ji Yang: Conceptualization; Methodology; Project administration; Writing – review & editing.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The anonymized datasets used in this study can be available upon reasonable request to the corresponding author.

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Supplemental material

Supplemental material for this article is available online.

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