Optimal Treatments for NSCLC Patients Harboring Primary or Acquired *MET* Amplification

Dantong Sun, MD[1], Junyan Tao, MD[2], Weihua Yan, MD[3],
Jingjuan Zhu, MD[2], Hai Zhou, MD[2], Yingying Sheng, MD[4],
Chaofan Xue, MD[5], Hong Li, PhD[6], and Helei Hou, MD, PhD[2] [iD]

Abstract

Background: In non-small cell lung cancer (NSCLC) patients harboring *MET* mutations, MET-tyrosine kinase inhibitors (TKIs) have been proven to achieve a good response. However, the relative efficacy of different therapeutics in primary NSCLC patients with *MET* amplification and the treatment options for patients harboring acquired *MET* amplification after the failure of epidermal growth factor receptor (EGFR)-TKIs remain unclear. **Methods:** In total, 33 patients harboring primary *MET* amplification and 9 patients harboring acquired *MET* alterations identified by next-generation sequencing were enrolled. A retrospective analysis was conducted to compare the efficacy of different therapeutics. In addition, studies reporting various treatments for patients harboring *MET* alterations were included in the meta-analysis. **Results:** In our cohort of patients harboring primary *MET* amplification, crizotinib displayed better efficacy than immunotherapy and chemotherapy, as demonstrated both in first-line ($P = .0378$) and second-line treatment regimens ($P = .0181$). The disease control rates for crizotinib, immunotherapy, and chemotherapy were 81.8%, 72.7%, and 63.6%, respectively. In particular, the median progression-free survival (PFS) time after immunotherapy in patients harboring *MET* amplification and high programed death ligand 1 (PD-L1) expression (>50%) was only 77.5 days. The meta-analysis revealed that the median PFS times after crizotinib and immunotherapy were 4.57 and 2.94 months, respectively. In patients harboring acquired *MET* amplification, chemotherapy plus bevacizumab had superior efficacy (310.0 days vs 73.5 days, $P = .0360$) compared with MET-TKIs $\pm$ EGFR-TKIs. **Conclusions:** Immunotherapy showed a low response in patients harboring *MET* alterations, even those with concurrent high PD-L1 expression. MET-TKIs might be an optional treatment with worth-expecting efficacy. However, chemotherapy plus bevacizumab could benefit the subpopulation of patients harboring acquired *MET* amplification after the failure of EGFR-TKIs.

Keywords

NSCLC, *MET* amplification, prognosis, MET-TKIs, immunotherapy

[1] National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
[2] Precision Medicine Center of Oncology, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, China
[3] Department of Pathology, The Affiliated Hospital of Qingdao University, Qingdao, China
[4] Medical College of Qingdao University, Qingdao, Shandong, China
[5] Department of Oncology, The People's Hospital of Huangdao District, Qingdao, China
[6] State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

Corresponding Authors:
Helei Hou, Precision Medicine Center of Oncology, The Affiliated Hospital of Qingdao University, Qingdao 266000, No. 59 Haier Road, Shandong, China.
Email: houhelei@qdu.edu.cn

Hong Li, State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200032, China.
Email: hongli@shsci.org

Abbreviations

AJCC, American Joint Committee on Cancer; ALK, anaplastic lymphoma kinase; CI, confidence interval; DCR, disease control rate; DOR, duration of response; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; EGFR-TKIs, EGFR tyrosine kinase inhibitors; ICIs, immune checkpoint inhibitors; LUSC, lung squamous cell carcinoma; NSCLC, non-small cell lung cancer; NGS, next-generation sequencing; ORR, objective response rate; OS, overall survival; PD, progressive disease; PD-1, programed death 1; PFS, progression-free survival; PR, partial response; RTK, receptor tyrosine kinase; SD, stable disease; TIME, tumor immune microenvironment

Introduction

The management of advanced non-small cell lung cancer (NSCLC) has developed rapidly and relies on the wide usage of novel therapeutics, including targeted therapy, immunotherapy, and so on. Patients harboring actionable genomic alterations, such as *epidermal growth factor receptor (EGFR)*, *anaplastic lymphoma kinase (ALK)* and *ROS1 alterations* can benefit from the corresponding targeted therapy.[1–3] EGFR tyrosine kinase inhibitors (EGFR-TKIs) are currently the standard first-line treatment for advanced NSCLC patients harboring sensitive *EGFR* mutations and have improved outcomes when used as an adjuvant treatment after complete resection, according to the ADAURA study.[4] Currently, systemic administration of monoclonal antibodies against programed death 1 (PD-1) and its ligand PD-L1 has been established as new standard of care for patients with advanced NSCLC.[5,6] Compared with the standard regimen of chemotherapy, treatment with immune checkpoint inhibitors (ICIs) alone or in combination with chemotherapy has brought significant survival benefits for selected NSCLC patients.[5,6] However, patients harboring sensitive *EGFR* mutations or *ALK* rearrangement are believed to have a poor response to immunotherapy. Therefore, actionable genomic alterations are considered to play different roles in the response of NSCLC to different therapeutics.

Alterations in the *MET* gene are identified as actionable oncogenic alterations,[7] and *MET* gene amplification plays an important role in the growth and survival of NSCLC[8] via enhanced binding to receptor tyrosine kinases (RTKs), which can then initiate a series of cellular signaling pathways associated with proliferation and metastasis.[9] In addition to amplification of the *MET* gene, mutations in *MET*, especially exon 14 skipping alterations, are other gain-of-function alterations in *MET*. In NSCLC patients harboring *MET* mutations, the administration of MET-TKIs, such as crizotinib, has been proven in previous studies to achieve a satisfactory response.[10–12] Immunotherapy is another treatment option for NSCLC patients with *MET* alterations.[13–15] However, the efficacy of different therapeutics in NSCLC patients with *MET* amplification remains to be further elucidated.

In this study, we included NSCLC patients with *MET* amplification to estimate the efficacy of various therapeutics in different lines of treatment and further performed a meta-analysis of current and previous studies to determine the therapeutic outcomes based on a larger sample size. Acquired *MET* amplification is an important mechanism of resistance to EGFR-TKI treatment among NSCLC patients harboring concomitant sensitive *EGFR* mutations.[16] However, the therapeutic outcomes after EGFR-TKI treatment failure remain unclear. Therefore, we included another group of patients who received first-line EGFR-TKI therapy and acquired resistance via *MET* amplification to observe the efficacy of different therapeutics.

Methods

Study Patients

In this retrospective study, a total of 33 NSCLC patients (n = 33) harboring primary *MET* amplification, who admitted in our hospital since January 2015, were included in this study. The inclusion criteria were as follows: (a) Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; (b) stage IV disease with pathological tissues available for next-generation sequencing (NGS); (c) primary *MET* amplification; and (d) available follow-up information. The exclusion criteria were as follows: (a) NGS findings—wild-type or *MET* mutation alone or in combination with other actionable genomic alterations (*EGFR*, *ALK*, *ROS1*, *BRAF V600E*, *KRAS*, etc); (b) ECOG performance status > 2; (c) loss to follow-up or refusal to participate.

Another 9 NSCLC patients (n = 9) in the Affiliated Hospital of Qingdao University harboring acquired *MET* amplification were included. The inclusion criteria were as follows: (a) ECOG performance status 0 to 2; (b) stage IV disease with pathological tissues available for NGS; (c) primary sensitive *EGFR* mutations (*19 DEL* or *21 L858R*) and failure of first-line EGFR-TKI treatment; (d) acquired *MET* amplification; and (d) available follow-up information. The exclusion criteria were as follows: (a) treatment with other modalities except EGFR-TKIs; (b) ECOG performance status > 2; and (c) loss to follow-up or refusal to participate.

Basic clinical and demographic information, including age, sex, diagnosis, treatment information, etc was collected from all enrolled patients. All tumors were staged according to the 2019 American Joint Committee on Cancer (AJCC) TNM staging system for lung cancer. Disease progression was diagnosed by 2 professional physicians experienced in clinical medical oncology. The current study was performed according to the instructions of STROBE Statement.[17] All patients were de-identified in this study. This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University, and the investigations were carried out following the guidelines established in the Declaration of Helsinki. Written informed consent was obtained from all patients included in the study, and all experiments were carried out in

accordance with the National Health and Family Planning Commission of the PRC's guidelines.

Targeted Sequencing and Bioinformatic Analysis

In this study, alterations in *MET* and *EGFR* and other actionable genomic alterations were confirmed by NGS. The NGS details have been reported in our previous study.[18] Here, tissue samples from 42 NSCLC patients were analyzed by NGS using 3 capture-based targeted sequencing panels. In addition, data for 143 NSCLC patients (n = 143, 12 patients harboring *MET* alterations) obtained from the cBioPortal for Cancer Genomics[19,20] were included to perform survival analysis of patients stratified by immunotherapy. Data from TIMER[21,22] and TISIDB[23] were used to examine the abundance of infiltrating immune cells in NSCLC patients. In addition, Kaplan–Meier Plotter[24] was used to perform survival analysis.

Meta-Analysis

We searched 4 databases independently—PubMed, ScienceDirect, ClinicalTrials.gov and the Cochrane Library—for literature selection. All MeSH terms for the keywords (MET; NSCLC) were used in the search. The following data were extracted: study ID, a number of patients, *MET* alterations, treatment methods, progression-free survival (PFS) and overall survival (OS). All statistical analyses in the meta-analysis were performed using Stata 14.0 software (Stata Corp.). The results are expressed as incidence rates and 95% confidence intervals (CIs). In this study, we used a random-effects model to perform statistical analyses, and the chi-squared test and I^2 statistic were used to assess inter-study heterogeneity. A *P*-value > .1 and an I^2 < 50% indicated that the heterogeneity was not statistically significant.[25]

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 8.0 software (GraphPad), and Student's *t* test was used to determine statistical significance. *P* values were determined by 2-tailed tests, and *P* < .05 was used to define statistical significance. The log-rank test was used for survival analysis.

Results

the Prevalence of *MET* Alterations and Their Role in the Immunotherapy Response of NSCLC Patients

Two studies of immunotherapy for NSCLC patients from the cBioPortal were selected for the analysis, as shown in Figure 1A. Alterations in common driver genes of NSCLC were detected based on these 2 studies, and the findings suggested that alterations in *KRAS* are the most common genomic signature in NSCLC, with an incidence of 35%, followed by alterations in *EGFR* (14%), *PIK3CA* (8%), *ALK* (7%), *MET* (7%), *ROS1* (6%), *ERBB2* (4%), *BRAF* (4%),

NTRK1 (4%), *RET* (4%), and *NRAS* (2.2%). The PFS times of patients harboring actionable genomic alterations described above, were significantly shorter than those of the wild-type (WT) groups (*P* = .0322), as shown in Figure 1B. In these 2 studies, 12 patients harbored *MET* alterations without any other genomic alterations. Compared with the WT group, *MET* was an independent risk factor for immunotherapy failure in NSCLC (4.2 months vs 6.2 months, *P* = .0149), and patients harboring *MET* alterations had shorter PFS times, as shown in Figure 1C.

Efficacy of Various Therapeutics in NSCLC Patients Harboring Primary *MET* Amplification

To further elucidate the response to different therapeutics among NSCLC patients harboring primary *MET* amplification, 33 NSCLC patients harboring primary *MET* amplification were included in this study. The selected patients were treated with immunotherapy, targeted therapy or chemotherapy. The basic information and clinical features of these 33 patients are summarized in Table 1. Generally, the median age of these patients was 64.0 years and ranged from 33.0 to 78.0 years, and most of the patients were diagnosed with stage IV (97.0%) lung adenocarcinoma (LUAD, 63.6%). A total of 87.9% of NSCLC patients in this study harbored *MET* amplification, and others harbored concomitant alterations of *MET* amplification and concomitant exon14 skipping (12.1%). The median follow-up time was 1308 days, ranged from 273 days to 2343 days.

Immunotherapy

For the 11 patients receiving immunotherapy, only 1 patient was treated with single ICIs and 10 patients were treated with ICIs (anti-PD-1 or anti-PD-L1 antibodies) in combination chemotherapy. As shown in Figure 1D, the median PFS time of patients who received immunotherapy was 75.0 days (95% CI: 34.0-96.0 days). Figure 1E shows the CT scans of 2 typical patients who received immunotherapy; the best response of patient 1 was partial response, but patient 2 did not benefit from the treatment. As concluded in Table 2, the objective response rate (ORR) for the best therapeutic response was 9.1%, and the disease control rate (DCR) was 72.7%.

Crizotinib

In this study, 11 stage IV NSCLC patients (n = 11) included in this study were treated with crizotinib. The median PFS time for this group of patients was 120.0 days (95% CI: 62.0-148.0 days), as shown in Figure 2A. The CT scans shown in Figure 2B demonstrate the treatment process, including the best response (stable disease, SD), of a patient who received crizotinib. The DCR and ORR of crizotinib treatment was 81.8% and 0%, respectively, in this study according to Table 2.
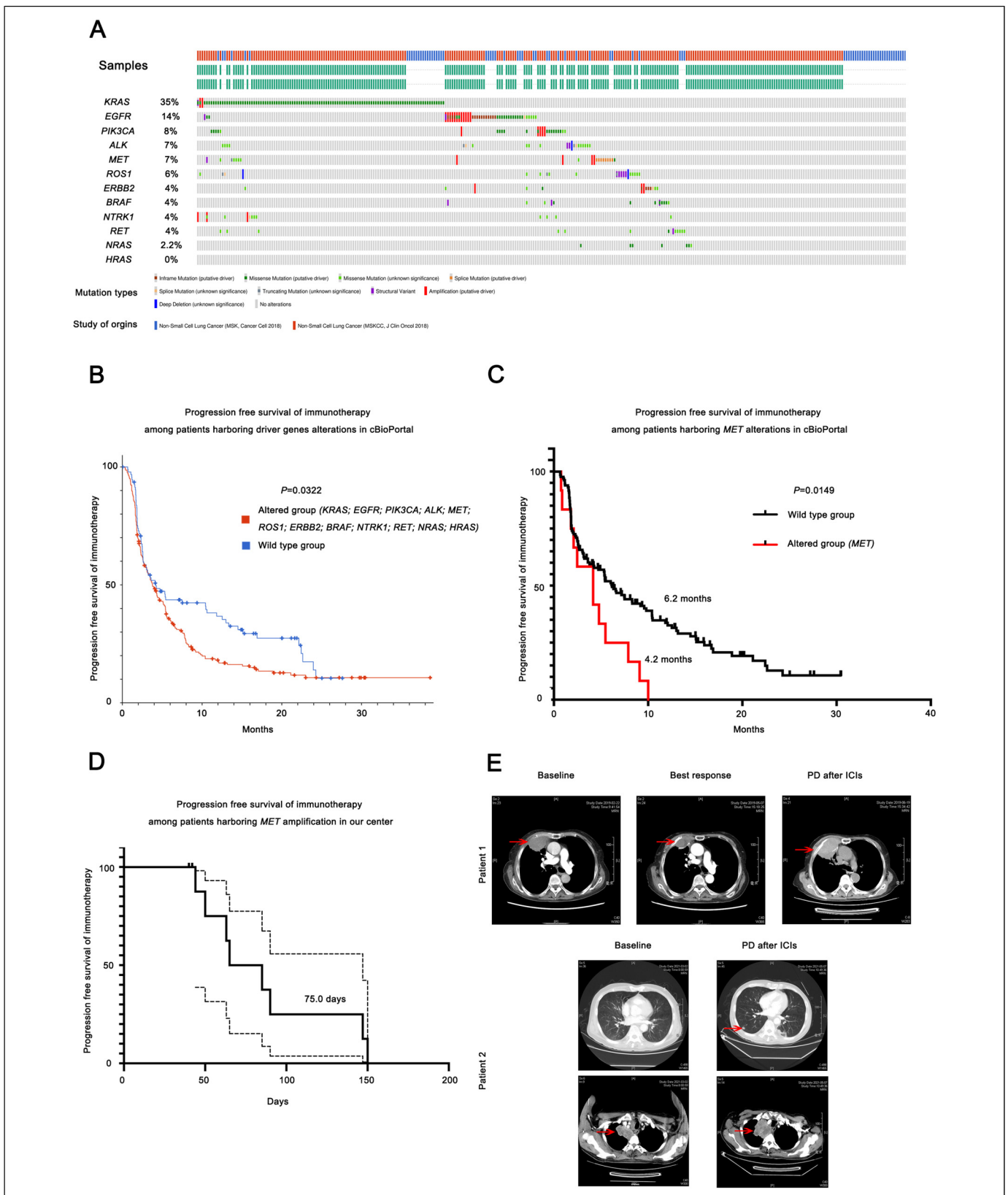
Figure 1. The prevalence of actionable genomic alterations among NSCLC patients and the relationship between MET alterations and immunotherapy. (A) The prevalence of driver genes in cBioPortal; (B) alteration of driver genes is associated with a poor response to immunotherapy; (C) *MET* alterations are associated with a poor response to immunotherapy; (D) the median PFS time of patients harboring primary *MET* amplification who received immunotherapy; (E) representative cases of patients harboring primary *MET* amplification who received immunotherapy. Abbreviations: NSCLC, non-small cell lung cancer; PFS, progression-free survival.

Table 1. The Characteristics of Patients Harboring *MET* Amplification in our Center.

	Total (n = 33)	Immunotherapy (n = 11)	Crizotinib (n = 11)	Chemotherapy (n = 11)
Age				
Medial age	64.0	55.3	62.9	67.4
Range	33.0-78.0	55.0-75.0	33.0-78.0	56.0-78.0
Sex, n (%)				
Male	26 (78.8%)	8 (72.7%)	8 (72.7%)	10 (90.9%)
Female	7 (21.2%)	3 (27.3%)	3 (27.3%)	1 (9.1%)
ECOG performance status, n (%)				
0-2	33 (100%)	11 (100%)	11 (100%)	11 (100%)
> 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tumor histology, n (%)				
LUAD	21 (63.6%)	7 (63.6%)	8 (72.7%)	6 (54.5%)
LUSC	7 (21.2%)	3 (27.3%)	1 (9.1%)	3 (27.3%)
Other	5 (15.2%)	1 (9.1%)	2 (18.2%)	2 (18.2%)
Disease stage, n (%)				
Stage III	1 (3.0%)	1 (9.1%)	0 (0%)	0 (0%)
Stage IV	32 (97.0%)	10 (90.9%)	11 (100%)	11 (100%)
Treatment lines, n (%)				
First line	18 (54.6%)	5 (45.4%)	5 (45.4%)	8 (72.7%)
Second line	14 (42.4%)	5 (45.4%)	6 (54.5%)	3 (27.3%)
Other	1 (3.0%)	1 (9.1%)	0 (0%)	0 (0%)
MET alterations, n (%)				
MET amplification	29 (87.9%)	9 (81.8%)	9 (81.8%)	11 (100%)
MET amplification and exon14 skipping	4 (12.1%)	2 (18.2%)	2 (18.2%)	0 (0%)
PD-L1 expression, n (%)				
≥ 50%	7 (21.2%)	5 (45.4%)	1 (9.1%)	1 (9.1%)
< 50%	16 (48.5%)	3 (27.3%)	4 (36.4%)	9 (91.8%)
Unknown	10 (30.3%)	3 (27.3%)	6 (54.5%)	1 (9.1%)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PD-L1, programed death-ligand 1.

Table 2. The Summary for the Efficacy and Endpoint of Evaluation for Patients in our Center.

	Immunotherapy (n = 11)	Crizotinib (n = 11)	Chemotherapy (n = 11)
Best overall response, n (%)			
Complete response	0 (0%)	0 (0%)	0 (0%)
Partial response	1 (9.1%)	0 (0%)	0 (0%)
Stable disease	7 (63.6%)	9 (81.8%)	7 (63.6%)
Disease progression	3 (27.3%)	2 (18.2%)	4 (36.4%)
Median PFS, days (95% CI)	75.0 (34.0-96.0)	120.0 (62.0-148.0)	60.0 (18.0-72.0)

Abbreviations: DCR, disease control rate; PFS, progression-free survival.

Chemotherapy

Another 11 stage IV NSCLC patients (n = 11) were treated with a standard regimen of chemotherapy in this study. As shown in Figure 2C, the median PFS time of chemotherapy was only 60.0 days (95% CI: 18.0-72.0 days). Figure 2D displays the CT scans of the treatment process of 1 typical patient who had progressive disease (PD) after chemotherapy. The DCR of chemotherapy was 63.6% and the ORR was 0%.

The Comparison of the Different Groups of Patients

The comparison of the PFS times of the patients from the 3 groups shown in Figure 2E indicates that crizotinib treatment seems to be a better choice for NSCLC patients with *MET* amplification than chemotherapy or immunotherapy. In addition, ICIs did not exhibit the expected efficacy, and the prognostic effect of the PD-L1 expression status for ICI treatment did not demonstrate its important role ($P = .7295$) in NSCLC patients with *MET* alterations, as shown in Figure 2F. For those patients with concurrent high PD-L1 expression (≥50%), which accounted for 45.4% of the patients who received immunotherapy in this study, the median PFS time was only 77.5 days, similar to that of patients with concurrent low expression of PD-L1 (50%, median PFS time: 85.0 days).

To further explore the efficacy of different therapeutics in NSCLC patients with *MET* amplification in different lines of treatment, we performed subgroup analysis based on the treatment line. As shown in Figure 2G, crizotinib (first-line: n = 5; second line: n = 6) displayed the best efficacy, followed by ICIs (first-line: n = 5; second line: n = 5) and chemotherapy (first-line: n = 8; second line: n = 3), both in first-line (120.0
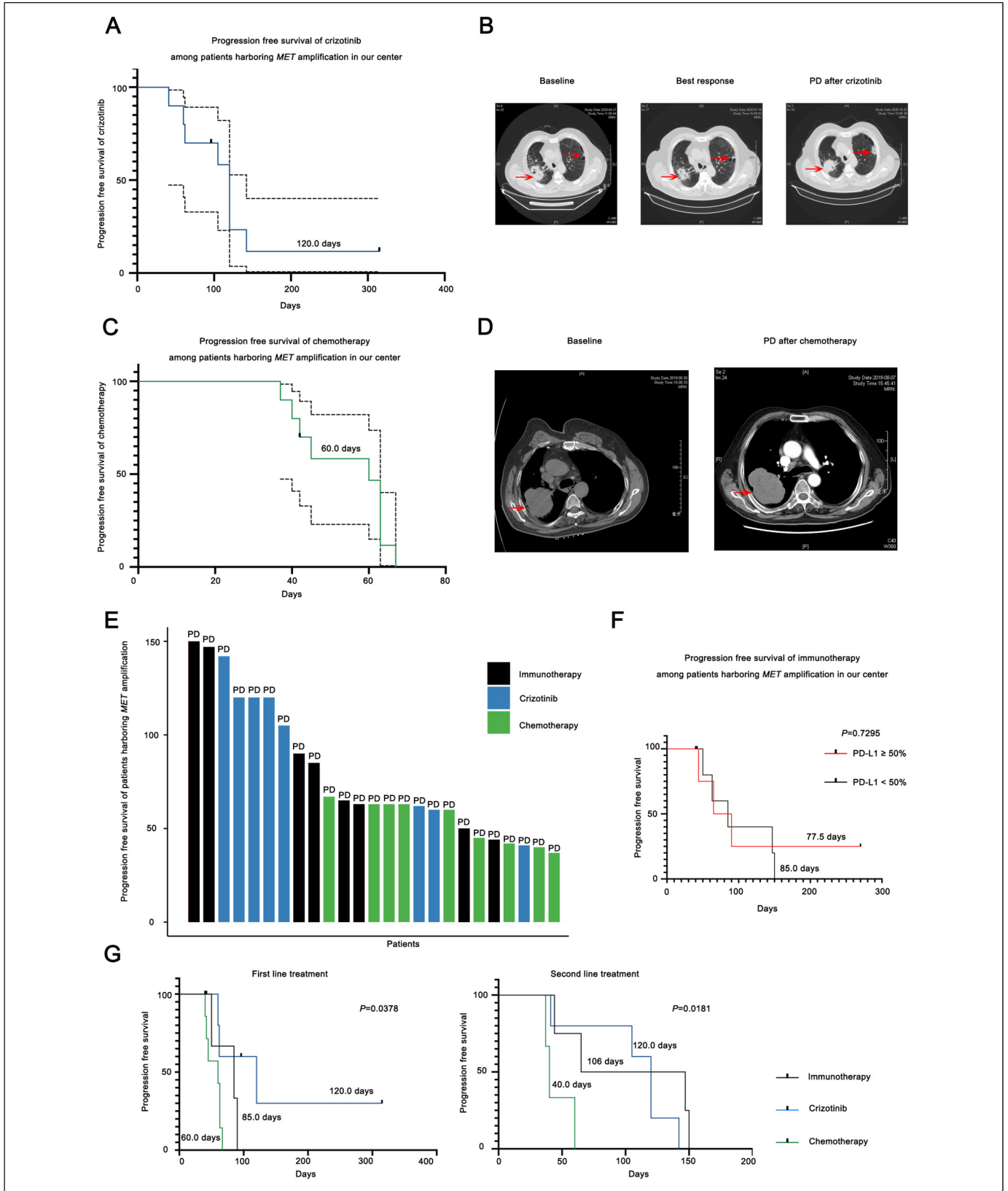
Figure 2. Comparison of different therapeutics among NSCLC patients harboring primary *MET* amplification. (A) The median PFS time of crizotinib in patients harboring primary *MET* amplification; (B) representative case for patients harboring primary *MET* amplification who received crizotinib; (C) the median PFS time of chemotherapy among patients harboring primary *MET* amplification; (D) representative case of patients harboring primary *MET* amplification who received chemotherapy; (E) the comparison of the PFS between patients treated with different therapeutics; (F) the comparison of the response to immunotherapy based on the expression level of PD-L1; (G) the comparison of the efficacy of different therapeutics in different treatment lines among patients harboring primary *MET* amplification. Abbreviations: NSCLC, non-small cell lung cancer; PFS, progression-free survival; PD-L1, programed death ligand 1.

days vs 85.0 days vs 60.0 days, $P = .0378$) and second-line treatment regimens (120.0 days vs 106.0 days vs 40.0 days, $P = .0181$).

Meta-Analysis of Studies on the Treatment of Patients With Primary NSCLC Harboring *MET* Alterations

After literature selection, a total of 6 studies on the treatment of patients with primary NSCLC harboring *MET* alterations were included in the meta-analysis. The basic information of the involved studies is summarized in Table 3. Ninety patients who received crizotinib treatment (n = 90) and 111 patients who received ICI treatment (n = 111) were included in the meta-analysis. As the results of the meta-analysis show (Figure 3A-B), the median PFS time of patients treated with crizotinib (4.57 months, ranging from 3.0 months to 7.4 months) was longer than that of patients treated with ICIs (2.94 months, ranging from 1.9 months to 4.9 months). Assessment of inter-study heterogeneity demonstrated no significant differences between studies and indicated that the results of the meta-analysis were robust.

Efficacy of various Therapeutics in NSCLC Patients Harboring Acquired *MET* Amplification After Resistance to First-Line EGFR-TKIs

As shown in Table 4, 9 stage IV LUAD patients (n = 9) harboring sensitive *EGFR* mutations (7 patients harboring *19 DEL*, 2 patients harboring *21 L858R*) were enrolled and treated with standard first- to third-generation EGFR-TKIs (gefitinib, erlotinib or osimertinib). After treatment with EGFR-TKIs, all 9 LUAD patients developed resistance to EGFR-TKIs, and NGS demonstrated that all of the patients harbored acquired *MET* amplification after first-line treatment with EGFR-TKIs, which constituted the mechanism of resistance. The median PFS time in the second-line treatment group after the failure of EGFR-TKIs was 90.0 days, as shown in Figure 4A. We further explored the differences between the efficacy of different therapeutics as second-line treatments in these patients. As shown in Figure 4B-C, chemotherapy plus bevacizumab (n = 3)

may have superior efficacy (310.0 days versus 73.5 days, $P = .0360$) compared with MET-TKIs $\pm$ EGFR-TKIs (n = 6).

MET is Associated With the Immune Microenvironment and Predicts Poor Prognosis in Patients With NSCLC

In this study, we found that NSCLC patients harboring *MET* amplification did not benefit from the therapeutics and had poor prognoses; therefore, we further explored the potential mechanism related to *MET*-induced insensitivity to the treatment. As shown in Figure 5A, the protein expression of MET was positively associated with that of a series of molecules related to inhibition of the tumor immune microenvironment (TIME), including CD274 (PD-L1, $P < .0001$), PDCD1 (PD-1, $P = .0017$), PDCD1LG2 (PD-L2, $P < .0001$) and TIGIT ($P < .0001$), which suppress the tumor immune response via the PD1/PD-L1 pathway or suppress T-cell activation by promoting the generation of mature immunoregulatory dendritic cells, and that of molecules related to poor prognosis or even hyperprogressive disease with ICI treatment, including MDM2 ($P < .0001$) and CCND1 ($P < .0001$). As shown in Figure 5B, amplification of the *MET* gene was found to be associated with a trend of low infiltration of the TIME, especially in lung squamous cell carcinoma. Furthermore, the MET protein serves as a biomarker for the poor prognosis of NSCLC patients, as shown in Figure 5C. NSCLC patients with higher MET protein expression had poor disease-free survival ($P < .0001$) and OS ($P < .0001$).

Discussion

The standard treatment for NSCLC patients has recently been considerably transformed with the development of targeted therapy and immunotherapy. NSCLC patients harboring actionable genomic alterations, such as sensitive *EGFR* mutations and *ALK* rearrangement, benefit from standard treatment with the corresponding TKIs.[26,27] However, previous studies proved that *EGFR* and *ALK* alterations are associated with a poor response to ICI treatment.[28] Therefore, patients harboring actionable genomic alterations respond differently to therapeutics. *MET* alteration is a relatively rare genomic alteration in

Table 3. The Summary for Studies on the Treatment of NSCLC Patients Harboring *MET* Alterations.

Study ID	Patients number	*MET* alterations	Therapeutics	PFS, months (95%CI)	OS, months (95%CI)
Camidge, et al[10]	38	*Amplification*	Crizotinib	5.1 (1.9-7.0)	11.0 (7.1-15.9)
Wong, et al[11]	19	*Exon 14 skipping mutation*	Crizotinib	3.0 (NA)	NA
Awad, et al[12]	22	*Amplification/Exon 14 skipping mutation*	Crizotinib	7.4 (3.3-NR)	NA
Current study	11	*Amplification/Exon 14 skipping mutation*	Crizotinib	3.6 (2.1-4.9)	NA
Guisier, et al[13]	30	*Exon 14 skipping mutation*	ICIs	4.9 (2.0-11.4)	13.4 (9.4-NR)
Mazieres, et al[14]	36	*Amplification/Exon 14 skipping mutation*	ICIs	3.4 (1.7-6.2)	NA
Sabari, et al[15]	24	*Exon 14 skipping mutation*	ICIs	1.9 (1.7-2.7)	18.2 (12.9-NR)
Wong, et al[11]	10	*Exon 14 skipping mutation*	ICIs	2.4 (NA)	NA
Current study	11	*Amplification/Exon 14 skipping mutation*	ICIs	2.5 (1.1-3.2)	NA

Abbreviations: PFS, progression-free survival; OS, overall survival; ICIs, immune checkpoint inhibitors; NA, not available; NR, not reached.
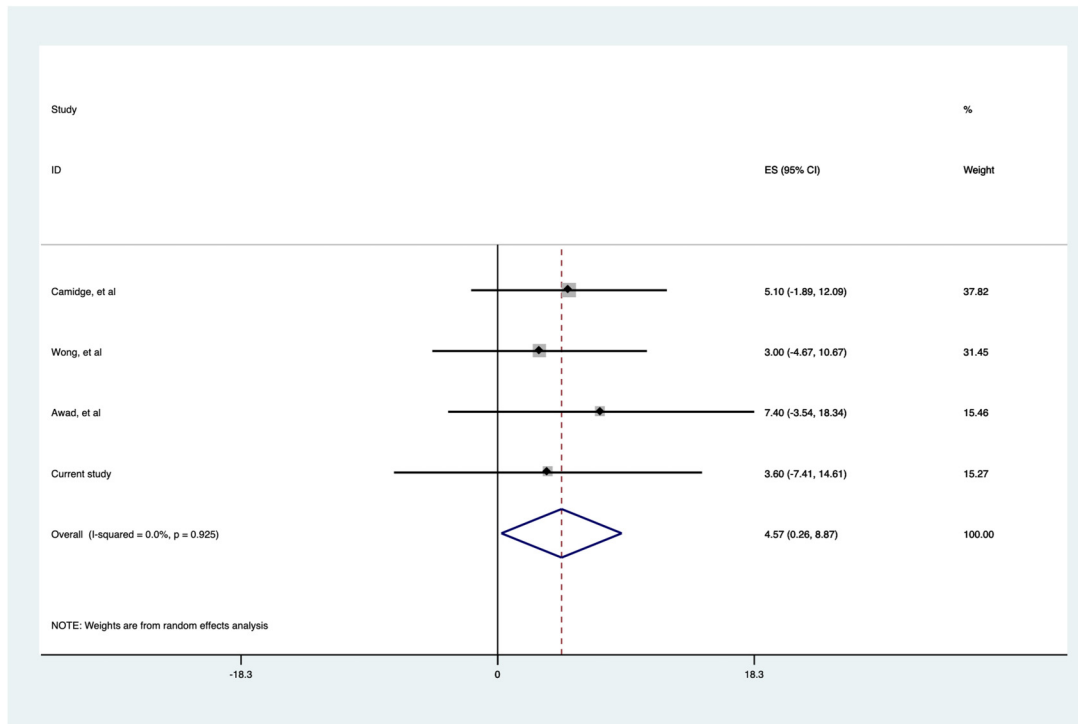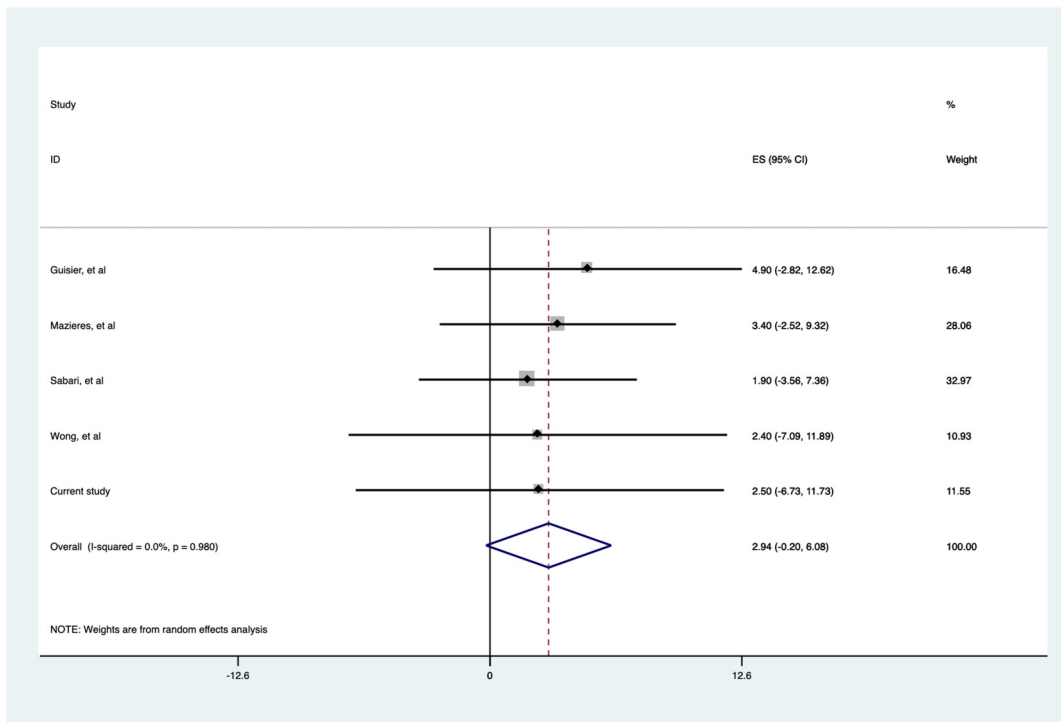
Figure 3. Meta-analysis of studies on the treatment of non-small cell lung cancer (NSCLC) patients harboring primary *MET* alterations. (A) Meta-analysis of patients who received crizotinib in the included studies; (B) meta-analysis for patients who received immunotherapy in the included studies.

NSCLC patients,[29] and in this study, approximately only 7% of patients harbored *MET* amplification and/or mutation. Although multiple studies[10–15] have explored the response of NSCLC patients harboring *MET* alterations to different therapeutics, the sample sizes were small, and no study has made a horizontal comparison of different therapeutics in NSCLC patients harboring *MET* amplification. In this study, we enrolled a group of patients who received 3 types of treatment—immunotherapy,

Table 4. Basic Information for Patients Harboring Acquired *MET* Amplification After the Failure of EGFR-TKIs Treatment in our Center.

Patient ID	Age	Sex	Tumor histology	EGFR mutation	Previous TKIs treatment	Treatment after TKIs resistance	PFS, days (status)
1	62	Male	LUAD	*21 L858R*	Osimertinib	Chemotherapy + Bevacizumab	121 (PD)
2	55	Male	LUAD	*19 Del*	Gefitinib	Chemotherapy + Bevacizumab	310 (PD)
3	60	Male	LUAD	*19 Del*	Gefitinib	Chemotherapy + Bevacizumab	161 (SD)
4	53	Male	LUAD	*21 L858R*	Osimertinib	Crizotinib + Icotinib	16 (Deceased)
5	61	Male	LUAD	*19 Del*	Erlotinib	Crizotinib	1 (Deceased)
6	49	Female	LUAD	*19 Del*	Gefitinib	Crizotinib	87 (PD)
7	62	Female	LUAD	*19 Del*	Gefitinib	Crizotinib + Gefitinib	60 (PD)
8	69	Female	LUAD	*19 Del*	Gefitinib	INC280 + Osimertinib	127 (PD)
9	53	Male	LUAD	*19 Del*	Osimertinib	INC280 + Gefitinib	90 (PD)

Abbreviations: EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors; PFS, progression-free survival; LUAD, lung adenocarcinoma; PD, progressive disease.
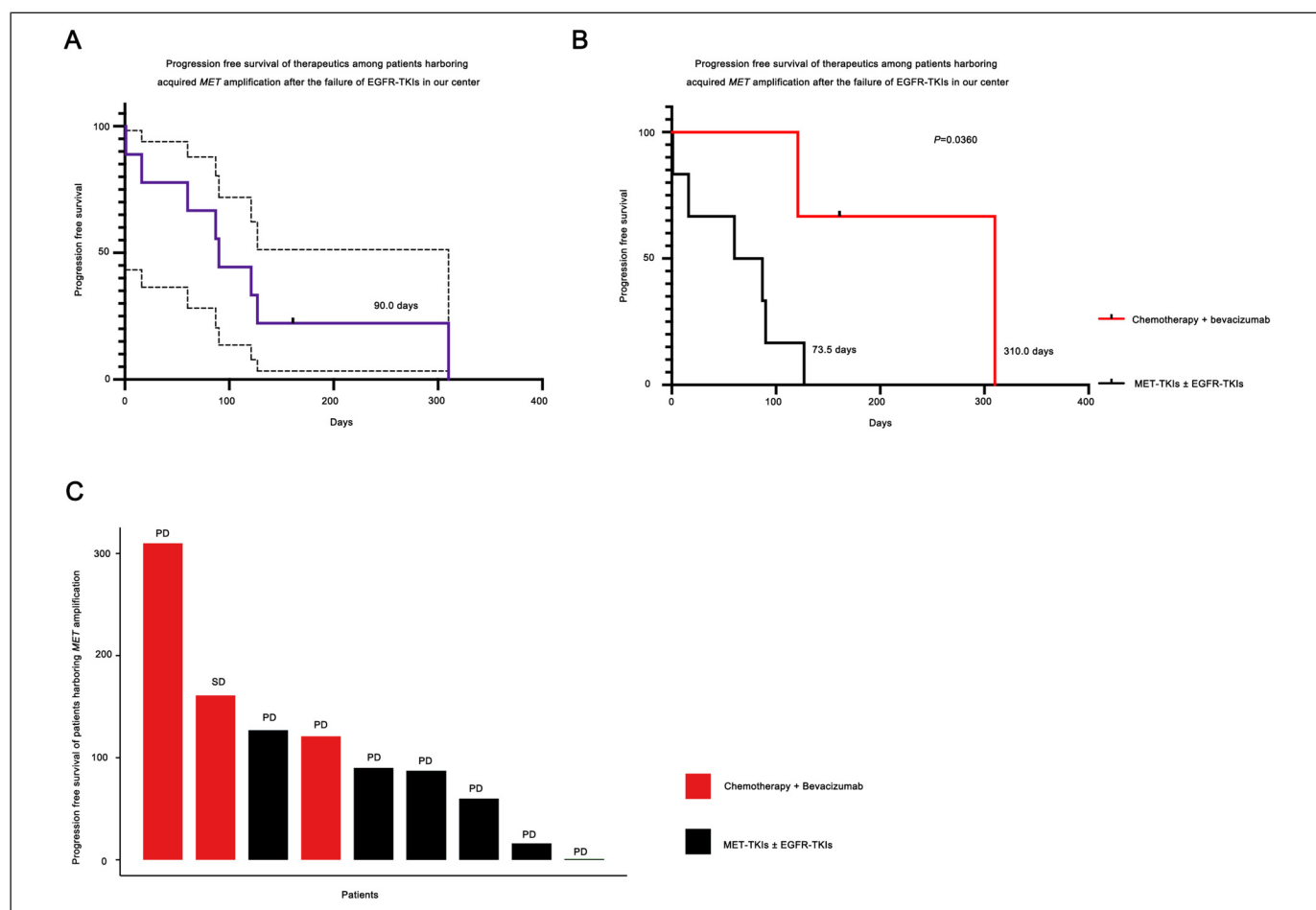

Figure 4. Treatment of patients harboring acquired *MET* amplification after the failure of first-line treatment with EGFR-TKIs. (A) The median PFS time after second-line treatment among patients harboring acquired *MET* amplification; (B) survival analysis of the PFS time between patients treated with different therapeutics; (C) the comparison of the PFS time between patients treated with different therapeutics. Abbreviations: PFS, progression-free survival; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.
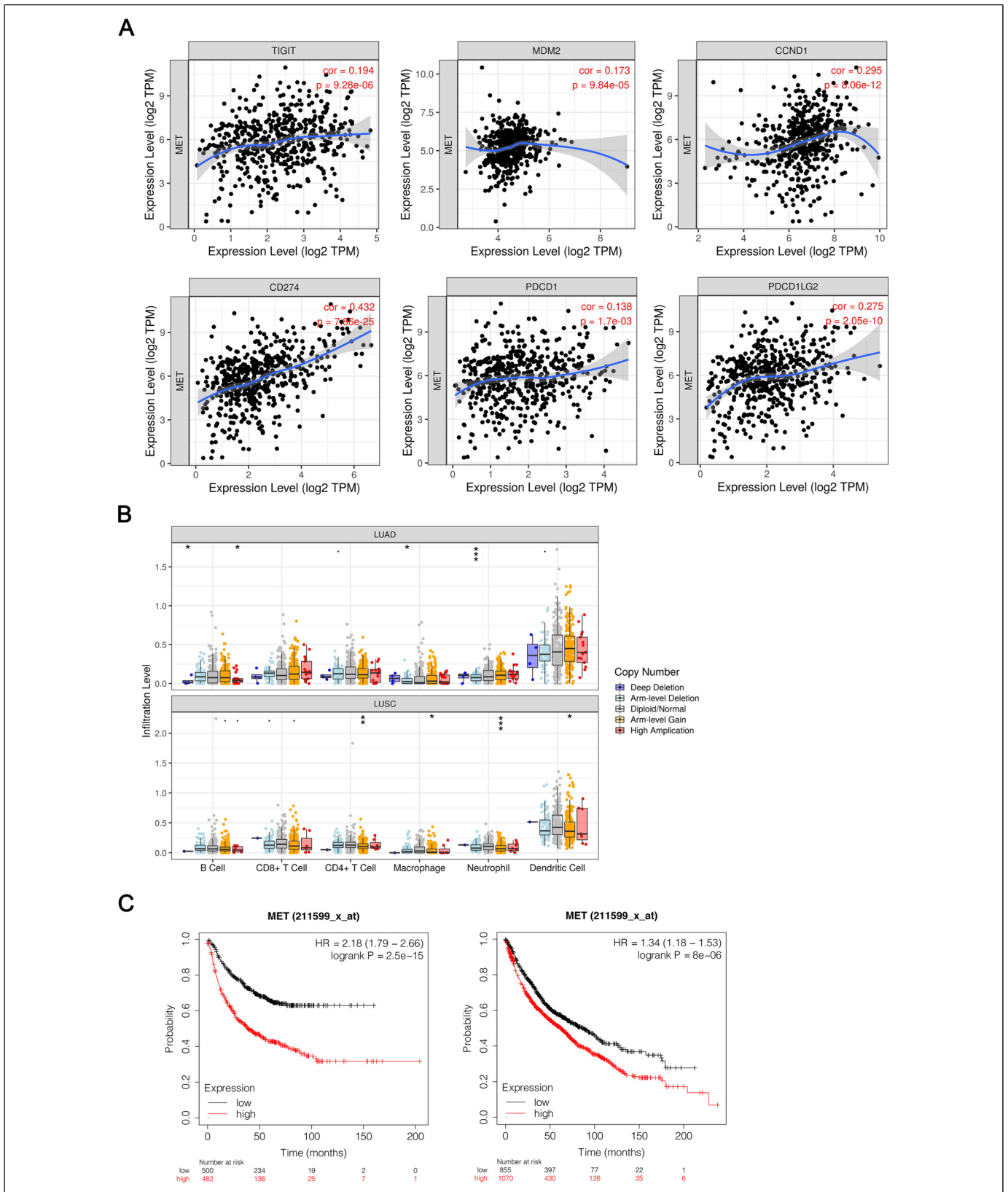
Figure 5. The role of MET in the tumor microenvironment and the prognosis of non-small cell lung cancer (NSCLC). (A) The relationship between the expression of MET and that of a series of molecules; (B) the infiltration level of a variety of tumor-infiltrated cells between patients with different types of *MET* alterations; (C) survival analysis of NSCLC patients according to the MET expression level.

crizotinib treatment, and chemotherapy—to explore the response of NSCLC patients harboring primary *MET* amplification to these therapeutics. The results suggested that crizotinib, which is a MET-TKI, demonstrated better efficacy than the other 2 types of therapeutics, regardless of the treatment line. However, even crizotinib treatment maintained SD for only 4.57 months, according to the meta-analysis based on 90 patients who received this treatment in real-world studies. No significant heterogeneity was detected in this meta-analysis, suggesting no significant difference in the treatment response between patients with different kinds of *MET* alterations. According to this study, primary *MET* amplification seems to be for a predictor of poor response to the therapeutics listed above, and novel therapeutics need to be explored. Clinical trials with patients harboring *MET* alterations might provide these patients with more options.[30] In the GEOMETRY trial, capmatinib (INC280) achieved an inspiring 12.6-month duration of response (DOR) as first-line treatment and a 9.7-month DOR as second-line treatment. Other trials for tepotinib (VISION trial) and savolitinib (NCT02897479) proved the convincing efficacy of these TKIs. Despite the promising efficacy of TKIs in clinical trials, real-world studies in patients harboring primary *MET* amplification are still needed.

For immunotherapy, *MET* amplification was a biomarker for a poor response to ICI treatment in this study. The 2.94-month DOR according to the meta-analysis based on 111 patients was not as satisfactory as the efficacy of the treatment in the real-world study. High expression of PD-L1 has been proven to be associated with a good response to immunotherapy and a good prognosis, as described in the KEYNOTE-189 trial.[31] However, NSCLC patients with *MET* amplification and concurrent high PD-L1 expression did not benefit from ICI treatment in this study. To explore the potential mechanism related to insensitivity to ICIs, we investigated the relationship between MET expression and factors related to a poor response to ICI treatment. The results suggested that MET expression is related to increased expression of molecules that inhibit the immune response in the TIME, such as TIGIT, which induces and maintains immune tolerance, participates in modulating the activation threshold of T-cells and limits the T-cell effector response.[32,33] As a result, the level of tumor tissue infiltration was found to be relatively low in patients with MET amplification. In addition, *MDM2* and *CCND1* are believed to be associated with a poor response to ICI treatment, and *MDM2* amplification is especially likely to induce hyperprogressive disease.[34] In this study, MET was also found to be associated with the expression of these 2 molecules. Further studies are needed to determine the relationship of these molecules.

Acquired *MET* alterations are one of the mechanisms underlying resistance to EGFR-TKIs through the resulting high affinity for RTKs and continuous activation of RTK signaling pathways. For these patients, it is unclear what kind of treatment would have the greatest benefit. According to previous studies, treatment with MET-TKIs such as tepotinib or capmatinib (INC280) plus gefitinib[35,36] achieved a good response in this subpopulation, and the PFS time of patients treated with tepotinib plus gefitinib was 4.9 months. In this study, we performed a survival analysis of patients treated with 3 types of therapeutics and suggested that chemotherapy plus bevacizumab might be the best choice for this subpopulation of patients. Specifically, the efficacy of chemotherapy plus bevacizumab might be better than that of combination therapy with MET-TKIs and EGFR-TKIs. However, the small sample size in the 2 groups of patients limit the accuracy of the analysis and larger scale trials are warranted to verify the results.

Admittedly, several limitations exist in this study. First, given the low incidence of *MET* alterations, the sample size of NSCLC patients harboring primary or acquired *MET* alterations is small, and as a result, the calculation and justification of the sample size selected for this study were not performed. To expand the results in this study, we should perform a meta-analysis based on a larger sample size. In addition, the role of MET in the TIME lacks verification via further experiments.

Conclusion

MET amplification is a biomarker for poor prognosis in NSCLC and correlates with a low response to immunotherapy. For patients harboring primary *MET* amplification, crizotinib or other MET-TKIs is likely to be an optional treatment with worth-expecting efficacy. Chemotherapy plus bevacizumab may benefit patients harboring sensitive *EGFR* mutations and acquired *MET* amplification after the failure of EGFR-TKI treatment. These results warrant further large prospective studies.

Authors' Contributions

Conception/design was planned by Helei Hou and Hong Li; provision of study material or patients was handeled by Junyan Tao, Weihua Yan, Jingjuan Zhu, Hai Zhou, and Helei Hou; collection and/or assembly of data was carried out by Dantong Sun, Junyan Tao, Yingying Sheng, and Chaofan Xue; data analysis and interpretation was done by Dantong Sun, Helei Hou, and Hong Li; manuscript writing was done by Dantong Sun and Helei Hou; final approval of manuscript was done by all authors.

Consent for Publication

All authors approved publication of the article.

Availability of Data and Material

All data and material in this study are available.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

Ethical Approval

This study was approved by the ethical committee of the Affiliated Hospital of Qingdao University (No. 16, Jiangsu Road, 266000, QINGDAO, China) on March 26, 2021, and the approval number is QYFY WZLL 27234.

Informed Consent

Written informed consent was obtained from all patients included in the study.

ORCID iD

Helei Hou 🆔 https://orcid.org/0000-0001-8502-7461

References

1. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*. 2009;361(10):947-957.

2. Solomon BJ, Mok T, Kim DW, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med*. 2014;371(23):2167-2177.

3. Shaw AT, Ou SH, Bang YJ, et al. Crizotinib in ROS1-rearranged non small-cell lung cancer. *N Engl J Med*. 2014;371(21):1963-1971.

4. Wu YL, Herbst RS, Mann H, Rukazenkov Y, Marotti M, Tsuboi M. ADAURA: Phase III, double-blind, randomized study of andomized versus placebo in EGFR mutation-positive early-stage NSCLC after complete surgical resection. *Clin Lung Cancer*. 2018;19(4):e533-e536.

5. Langer CJ, Gadgeel SM, Borghaei H, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: A andomized, phase 2 cohort of the open-label KEYNOTE-021 study. *Lancet Oncol*. 2016;17(11):1497-1508.

6. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med*. 2016;375(19):1823-1833.

7. Tong JH, Yeung SF, Chan AW, et al. MET Amplification and exon 14 splice site mutation define unique molecular subgroups of non-small cell lung carcinoma with poor prognosis. *Clin Cancer Res*. 2016;22(12):3048-3056.

8. Lutterbach B, Zeng Q, Davis LJ, et al. Lung cancer cell lines harboring MET gene amplification are dependent on met for growth and survival. *Cancer Res*. 2007;67(5):2081-2088.

9. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol*. 2003;4(12):915-925.

10. Camidge DR, Otterson GA, Clark JW, et al. Crizotinib in patients with MET-amplified NSCLC. *J Thorac Oncol*. 2021;16(6):1017-1029.

11. Wong SK, Alex D, Bosdet I, et al. MET Exon 14 skipping mutation positive non-small cell lung cancer: Response to systemic therapy. *Lung Cancer*. 2021;154:142-145.

12. Awad MM, Leonardi GC, Kravets S, et al. Impact of MET inhibitors on survival among patients with non-small cell lung cancer harboring MET exon 14 mutations: A retrospective analysis. *Lung Cancer*. 2019;33:96-102.

13. Guisier F, Dubos-Arvis C, Viñas F, et al. Efficacy and safety of anti-PD-1 immunotherapy in patients with advanced NSCLC with BRAF, HER2, or MET mutations or RET translocation: GFPC 01-2018. *J Thorac Oncol*. 2020;15(4):628-636.

14. Mazieres J, Drilon A, Lusque A, et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: Results from the IMMUNOTARGET registry. *Ann Oncol*. 2019;30(8):1321-1328.

15. Sabari JK, Leonardi GC, Shu CA, et al. PD-L1 expression, tumor mutational burden, and response to immunotherapy in patients with MET exon 14 altered lung cancers. *Ann Oncol*. 2018;29(10):2085-2091.

16. Guo Y, Song J, Wang Y, et al. Concurrent genetic alterations and other biomarkers predict treatment efficacy of EGFR-TKIs in EGFR-mutant non-small cell lung cancer: A review. *Front Oncol*. 2020;10:610923.

17. von Elm E, Altman DG, Egger M, et al. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Ann Intern Med*. 2007;147:573-577.

18. Sun D, Zhu Y, Zhao H, et al. Loss of ARID1A expression promotes lung adenocarcinoma metastasis and predicts a poor prognosis. *Cell Oncol (Dordr)*. 2021;44(5):1019-1034.

19. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6(269):pl1.

20. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2(5):401-404.

21. Li T, Fan J, Wang B, et al. TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res*. 2017;77(21):e108-e110.

22. Li B, Severson E, Pignon J-C, et al. Comprehensive analyses of tumor immunity: Implications for cancer immunotherapy. *Genome Biol*. 2016;17(1):174.

23. Ru B, Wong CN, Tong Y, et al. TISIDB: An integrated repository portal for tumor-immune system interactions. *Bioinformatics*. 2019;35(20):4200-4202.

24. Gyorffy B, Surowiak P, Budczies J, Lanczky A. Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer. *PloS One*. 2013;8(12):e82241.

25. Sun D, Hou H, Zhang C, Zhang X. The efficacy and safety of apatinib for refractory malignancies: A review and meta-analysis. *Onco Targets Ther*. 2018;11:6539-6554.

26. Hou H, Sun D, Zhang C, Liu D, Zhang X. ALK Rearrangements as mechanisms of acquired resistance to andomized in EGFR mutant non-small cell lung cancer. *Thorac Cancer*. 2021;12(6):962-969.

27. Sun D, Zhu Y, Zhu J, et al. Primary resistance to first-generation EGFR-TKIs induced by MDM2 amplification in NSCLC. *Mol Med*. 2020;26(1):66.

28. Sun D, Tian L, Zhu Y, et al. Subunits of ARID1 serve as novel biomarkers for the sensitivity to immune checkpoint inhibitors and prognosis of advanced non-small cell lung cancer. *Mol Med*. 2020;26(1):78.

29. Hou H, Zhu H, Zhao H, et al. Comprehensive molecular characterization of young Chinese patients with lung adenocarcinoma identified a distinctive genetic profile. *Oncologist*. 2018;23(9):1008-1015.

30. Tan AC, Loh TJ, Kwang XL, Tan GS, Lim KH, Tan DSW. Novel therapies for metastatic non-small cell lung cancer with MET exon 14 alterations: A spotlight on capmatinib. *Lung Cancer (Auckl)*. 2021;12:11-20.

31. Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. 2018;378(22):2078-2092.

32. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med*. 2000;192(7):1027-1034.

33. Burr ML, Sparbier CE, Chan YC, et al. CMTM6 Maintains the expression of PD-L1 and regulates anti-tumour immunity. *Nature*. 2017;549(7670):101-105.

34. Sun D, Liu D, Liu Q, Hou H. Nivolumab induced hyperprogressive disease in advanced esophageal squamous cell carcinoma. *Cancer Biol Ther*. 2020;21(12):1097-1104.

35. Wu YL, Cheng Y, Zhou J, et al. INSIGHT Investigators. Tepotinib plus gefitinib in patients with EGFR-mutant non-small-cell lung cancer with MET overexpression or MET amplification and acquired resistance to previous EGFR inhibitor (INSIGHT study): An open-label, phase 1b/2, multicentre, andomized trial. *Lancet Respir Med*. 2020;8(11):1132-1143.

36. Wu YL, Zhang L, Kim DW, et al. Phase Ib/II study of capmatinib (INC280) plus gefitinib after failure of epidermal growth factor receptor (EGFR) inhibitor therapy in patients with EGFR-mutated, MET factor-dysregulated non-small-cell lung cancer. *J Clin Oncol*. 2018;36(31):3101-3109.