Clinical Cancer Research

Combination of Metformin and Gefitinib as First-Line Therapy for Nondiabetic Advanced NSCLC Patients with EGFR Mutations: A Randomized, Double-Blind Phase II Trial

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Abstract

Purpose: Preclinical and retrospective studies suggested a role for metformin in sensitizing patients who have diabetes with non–small cell lung cancer (NSCLC) to EGFR tyrosine kinase inhibitors (TKIs). We therefore examined its effects in combination with gefitinib in patients without diabetes harboring EGFR mutations (EGFRm).

Patients and Methods: A total of 224 patients without diabetes with treatment-naïve stage IIIB–IV EGFRm NSCLC were randomly assigned in a 1:1 ratio to receive gefitinib plus either metformin or placebo. The primary endpoint was progression-free survival (PFS) rate at 1 year and secondary endpoints included overall survival (OS), PFS, objective response rate (ORR), and safety. Serum levels of IL6 were also examined in an exploratory analysis.

Results: The median duration of follow-up was 19.15 months. The estimated 1-year PFS rates were 41.2% [95% confidence interval (CI), 30.0–52.2] with gefitinib plus metformin and 42.9% (95% CI, 32.6–52.7) with gefitinib plus placebo ($P = 0.6268$). Median PFS (10.3 months vs. 11.4 months) and median OS (22.0 months vs. 27.5 months) were numerically lower in the metformin group, while ORRs were similar between the two arms (66% vs. 66.7%). No significant treatment group differences were detected across all subgroups with respect to PFS, including those with elevated levels of IL6. Metformin combined with gefitinib resulted in a remarkably higher incidence of diarrhea compared with the control arm (78.38% vs. 43.24%).

Conclusions: Our study showed that addition of metformin resulted in nonsignificantly worse outcomes and increased toxicity and hence does not support its concurrent use with first-line EGFR-TKI therapy in patients without diabetes with EGFRm NSCLC.

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Introduction

The advent of targeted therapy has revolutionized non–small cell lung cancer (NSCLC) management, however, a considerable percentage of patients still succumb to disease progression due to *de novo* or acquired resistance (1, 2). Strategies to sensitize patients to anticancer agents are therefore requisite and repositioning already existing drugs for cancer treatment offers a safe and cost-efficient solution in this regard.

Metformin, a cornerstone of type II diabetes treatment, holds promise for anticancer treatment with its established safety profile (3). Preclinical studies from our group as well as others suggested an antineoplastic activity of metformin across several cancer types in both cell lines and animal models (4–8). These observations were confirmed by epidemiologic and clinical research where metformin intake appeared to be associated with a lower risk of cancer and improved outcomes among patients without diabetes afflicted with various malignancies such as breast, prostate, gastric, endometrial and, in particular, lung cancer (9–14). In advanced NSCLC, several lines of evidence have demonstrated synergistic actions of metformin with standard therapy. When given alongside chemoradiotherapy or platinum-based chemotherapy (no radiotherapy), metformin significantly prolonged progression-free survival (PFS) or both PFS and overall survival (OS) as compared with patients receiving no or

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Translational Relevance

Metformin has emerged as a potential anticancer agent in a variety of cancers, including non–small cell lung cancer (NSCLC). Our observational data and another prospective study demonstrated enhanced efficacy of EGFR-tyrosine kinase inhibitors (TKI) upon addition of metformin in patients with and without diabetes, respectively. Despite extensive investigation, our understanding of metformin's role in NSCLC management is mainly based on studies with a retrospective design, a small dataset, or a heterogeneous study population. The only two other prospective studies on combined use of metformin and TKIs were either single-armed or not rigidly controlled. This led us to specifically compare the effects of metformin versus placebo combined with gefitinib in patients without diabetes with previously untreated NSCLC harboring EGFR mutations in a double-blinded, randomized, and placebo-controlled manner. With numerically worse outcomes and increased toxicity, our study does not support the concurrent use of metformin and EGFR-TKI in this population.

other concurrent antidiabetic treatments (15, 16). More importantly, metformin was able to sensitize patients with NSCLC to therapies targeting oncogenic driver mutations. Our retrospective study revealed that in patients with stage III–IV NSCLC diabetes carrying activating EGFR mutations (EGFRm), concurrent use of metformin and an EGFR-tyrosine kinase inhibitor (TKI) conferred superior results over TKI alone in terms of both median PFS (mPFS, 19.0 months vs. 8.0 months) and median OS (mOS, 32.0 months vs. 23.0 months; ref. 17). The enhanced sensitivity to EGFR-TKI therapy, according to our preclinical observations, may have stemmed from metformin-mediated reversal of epithelial–mesenchymal transition and inhibition of IL6/STAT3 signaling (8). In addition to patients with NSCLC copresenting with diabetes, a synergy between metformin and EGFR-TKIs was also observed in nondiabetic populations, as claimed by a prospective study presented at the 2018 American Society of Clinical Oncology Annual Meeting (ASCO Annual Meeting), where the addition of metformin to standard EGFR-TKI therapy led to a significant increase in mPFS and overall response rate (ORR), and nearly doubled the mOS in patients without diabetes (18). Collectively, these studies advocated inclusion of metformin as a sensitizer in the treatment regimen of advanced NSCLC, especially in conjunction with EGFR-TKIs.

However, the findings described above merit further investigation, because they were either based on retrospective studies with small or heterogeneous populations or were obtained from patients already taking metformin for diabetes. Little is known about metformin's activity in previously untreated patients without diabetes, especially in the context of TKI treatment of patients carrying the EGFRm (17–19). Therefore, prospective investigations with a larger sample size and a more rigorous design are justified to decipher the precise role of metformin in this population. We for the first time conducted a multicenter, double-blinded, randomized, placebo-controlled phase II trial to evaluate the efficacy and safety of metformin in combination with EGFR-TKI therapy as first-line treatment for nondiabetic patients with advanced EGFRm NSCLC.

Patients and Methods

Patients

Patients were recruited at nine hospitals in China and study entry was restricted to patients ages 18–75 who had: histocytologically confirmed metastatic or unresectable locally advanced NSCLC with at least one radiographically measurable lesion per RECIST 1.1; EGFR activating mutations as determined using amplification refractory mutation system-PCR and may include exon 19 deletion, exon 21 L858R mutation, and other rare mutations as specified in Table 1; an estimated life expectancy longer than 12 weeks; and an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2. Patients were excluded if they had: type I or II diabetes (diagnosed according to fasting blood sugar and hemoglobin A1c levels and confirmed using tests such as oral glucose tolerance test); prior exposure to anticancer treatment; hypersensitivity to metformin; and uncontrolled brain metastases. A full set of eligibility criteria are provided at www.clinicaltrials.gov (NCT01864681) and in a previously published protocol (20). All patients provided informed consent before the initiation of any trial-specific treatment. The study protocol was approved by each site's institutional review board and the trial was undertaken in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Table 1. Basic characteristics

Characteristics	Gefitinib + metformin	Gefitinib + placebo	*P*
N	112	111	
Age, years			
Mean (SD)	59.6 (10.09)	58.3 (9.00)	0.2882
Median (min, max)	59.5 (35, 78)	59.0 (32, 76)	
Sex			
Male	46 (41.1%)	48 (43.2%)	0.7426
Female	66 (58.9%)	63 (56.8%)	
WHO performance status			0.6887
0	25 (22.3%)	27 (24.3%)	
1	73 (65.2%)	74 (66.7%)	
2	14 (12.5%)	10 (9.0%)	
Disease stage			0.6090
IIIB	8 (7.1%)	10 (9.0%)	
IV	104 (92.9%)	101 (91.0%)	
Histologic type			0.1708
Adenocarcinoma	104 (92.9%)	108 (97.3%)	
Squamous	1 (0.9%)	1 (0.9%)	
NOS	7 (6.3%)	2 (1.8%)	
Smoking status			0.9368
Former smoker	27 (24.1%)	25 (22.5%)	
Current smoker	1 (0.9%)	1 (0.9%)	
Never smoker	84 (75.0%)	85 (76.6%)	
EGFR mutation types			0.3031
Exon 19 deletion	54 (48.21%)	61 (54.46%)	
L858R	53 (47.32%)	43 (38.39%)	
Others	5[a] (4.46%)	7[b] (6.25%)	

NOTE: Data are median (range) or *n* (%) unless otherwise specified. One patient in the placebo group withdrew consent at the time of randomization and was therefore removed from this population.

Abbreviation: NOS, not a specific histologic type.

[a]Consists of one patient with L861Q, one patient with an exon 20 insertion, one patient with G719X, one patient with an exon 19 deletion plus T790M, and one patient with an exon 19 deletion plus L858R.

[b]Consists of one patient with L861Q, one patient with L861Q plus G719X, one patient with G719X, one patient with G719X plus S768I, two patients with an exon 19 deletion plus T790M, and one patient with an exon 19 deletion plus L858R.

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Study design and treatment

Eligible patients were randomly assigned, in a 1:1 ratio, to receive gefitinib plus either metformin or placebo (produced by Salvage Pharmaceutical). Randomization was performed using permuted blocks (block size of four) via a computer-generated system. Sequences were generated using Clinical Information Management System-Central Randomization System (Mingke Ltd.). All parties involved in the study remained blind to treatment allocation until database locking on June 6, 2018, unless there was an emergency that required unblinding and treatment by investigators. Study treatment was initiated within 2 weeks of randomization. Gefitinib (AstraZeneca) was given orally at a daily dose of 250 mg. Metformin or placebo was initiated orally with meals at 500 mg per day during the first week of treatment. After 1 week, the dose was increased to 1,000 mg as the first dose of the day and 500 mg as the second dose. After another week, the dose was further escalated to 1,000 mg of metformin twice a day. In case of intolerable side effects, the dose of metformin or placebo was reduced to 50% of the maximum dose.

Radiological assessments were performed every 8 weeks according to RECIST version 1.1 and were centrally reviewed. Patients were also monitored for safety every 4 weeks in the first 2 months, and every 8 weeks thereafter. Adverse events were graded in accordance with the NCI Common Terminology Criteria for Adverse Events version 4.0. Treatment was continued until disease progression, intolerable toxicity, or withdrawal of consent. Upon treatment discontinuation, patients were subjected to monthly survival follow-up until 2 years after randomization and treatment beyond dropout/study completion was at the discretion of investigators. Meanwhile, 3 mL of blood sample was collected from each patient at baseline, and serum IL6 levels were measured using electro-chemiluminescence immunoassays (cobas 601, Roche Diagnostics), where a level of $\geq$7 pg/mL was regarded as abnormal according to the reference range provided by the manufacturer.

Outcome assessment

The primary endpoint was the rate of PFS (defined as the time from randomization to disease progression or death by any cause) at 1 year. Secondary endpoints included OS (the time from randomization to death by any cause), PFS, ORR (the percentage of patients with complete or partial response as per RECIST version 1.1 on two consecutive evaluations at least 4 weeks apart), and disease control rate (DCR, the proportion of patients that have had a complete or partial response or stable disease for at least 6 weeks before disease progression). Safety was also monitored as a secondary endpoint. PFSs of subgroups with abnormal and normal IL6 levels at baseline ($\geq$7 pg/mL vs. <7 pg/mL) in both treatment arms were also analyzed as an exploratory endpoint.

Statistical analysis

The sample size was determined on the basis of the primary endpoint of 1-year PFS rate. We estimated that a sample of at least 200 patients would ensure 80% power for the trial to detect a 15% increase in 1-year PFS rate favoring gefitinib plus metformin over gefitinib plus placebo, with a two-sided type I error of 0.05 by log-rank test. For baseline characteristics, categorical variables were summarized by frequency and percentages and examined using χ^2 or Fisher exact test for treatment group comparability, and continuous variables were summarized by summary statistics (means and SDs) and examined using Student *t* test. The statistical analyses on efficacy were performed in a modified intention-to-treat (ITT) population (patients with at least one dose of study medications and at least one therapeutic evaluation). Safety analyses were done in all randomized patients who took at least one dose of study medications. The 1-year PFS rate and the corresponding 95% confidence intervals (CIs) for each treatment group were calculated using the Greenwood formula. For PFS or OS analyses, Kaplan–Meier curves were compared using log-rank test, and the hazard ratios (HRs) were determined via Cox regression. The ORR and DCR of the two groups along with their 95% CIs were analyzed with the Clopper–Pearson method and were compared using Cochran–Mantel–Haenszel test. All reported *P* values were two-tailed, and a $P < 0.05$ was considered statistically significant. Statistical analyses were performed with SAS version 9.4 (SAS Institute).

Results

Patient characteristics

Between August 12, 2013, and December 14, 2015, 949 patients with stage IIIB–IV EGFRm NSCLC were screened and 224 patients who met eligibility criteria were randomly assigned to receive gefitinib with either metformin ($n = 112$) or placebo ($n = 112$; Fig. 1). Twenty-two patients were excluded from efficacy analysis for not taking study treatment, consent withdrawal, or lost to follow-up, hence the modified ITT population consisted of 97 patients in the metformin group and 105 in the placebo group. Baseline characteristics were balanced between the two groups, except that the metformin group had a numerically lower rate of EGFR exon 19 deletion (48.21% vs. 54.46%) and a numerically higher rate of EGFR L858R mutation (47.32% vs. 38.39%) than the placebo arm (Table 1). The median age of the patients was 59.6 years and most patients were nonsmokers (75.0%). The majority of patients had a stage IV disease and adenocarcinoma was the predominant pathologic type in both groups. Brain metastasis was not observed in either group. Median duration of follow-up was 19.15 months (IQR 12.99–28.44).

Efficacy

Average drug exposure was shorter with metformin than with placebo (10.9 vs. 12.6 months). Gefitinib was also given for a shorter period of time in the metformin group (11.6 vs. 13 months). As of June 6, 2018, 143 PFS events and 110 deaths (65 and 56 in the metformin group, and 78 and 54 in the placebo group, respectively) had occurred. Efficacy was evaluated on the modified ITT population as described above. The two groups had similar outcomes as depicted in Fig. 2. The estimated percentage of patients who were alive and had no disease progression at 1 year was 41.2% (95% CI, 30.0–52.2) in the metformin group versus 42.9% (95% CI, 32.6–52.7) in the placebo group ($P = 0.6268$) and the mPFS was 10.3 months (95% CI, 8.4–13.0) in the patients receiving metformin versus 11.4 months (95% CI, 10.0–12.2) in the patients given placebo (HR, 1.04; 95% CI, 0.75–1.45; log-rank $P = 0.8087$; Fig. 2A). Likewise, no significant difference in mOS was detected between the metformin group (22.0 months; 95% CI, 19.0–31.5) and the placebo group (27.5 months, 95% CI 22.8–31.5; HR 1.15; 95% CI 0.79–1.68; log-rank $P = 0.4571$; Fig. 2B). Moreover, addition of metformin led to comparable ORRs [66% (95% CI, 55.7–75.3) vs. 66.7%

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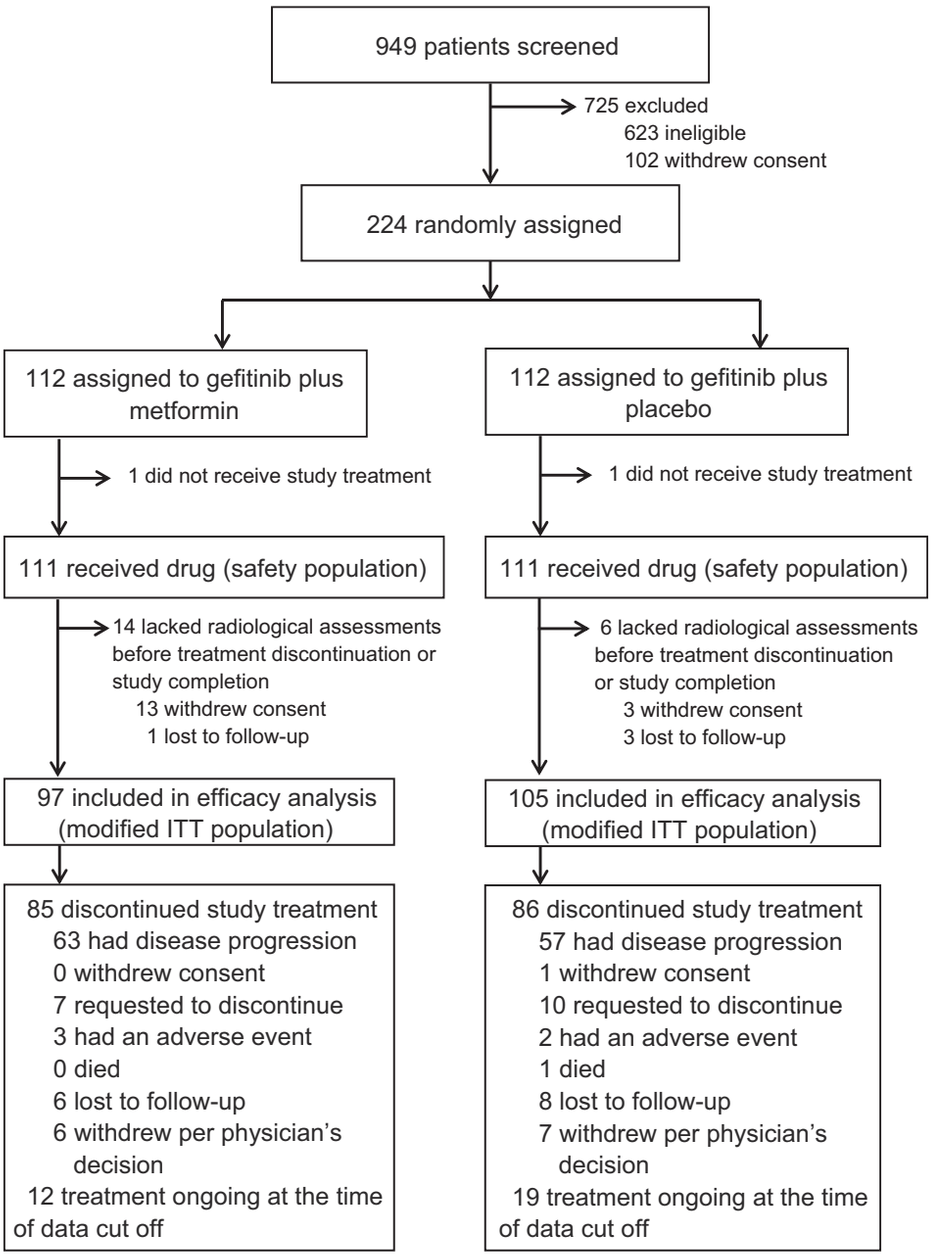

Figure 1.
A trial profile showing the flow of participants from enrollment. All patients who were evaluable for efficacy analyses were included in the ITT population; all patients who received at least one dose of study treatment were included for safety analyses.

(95% CI, 56.8–75.6; OR, 0.97; $P = 1.00$] as well as similar DCRs [97.9% (95% CI, 92.7–99) vs. 97.1% (95% CI, 91.9–99.4; OR, 1.40; $P = 1.00$; Table 2]. When stratified according to baseline characteristics, none of the subpopulations displayed significantly different outcomes, including those with different EGFR mutation subtypes (Fig. 3; Supplementary Fig. S1). Baseline IL6 level was investigated in particular as a potential predictive biomarker, because an elevated level of IL6 was reported to be associated with TKI resistance and metformin-based combination therapy could effectively abrogate this resistance in xenografts by suppressing IL6 signaling pathways (8). However, this trend was not reproduced in patients with abnormal baseline serum levels of IL6 ($\geq$7 pg/mL), as in this subpopulation, patients taking metformin had an mPFS of 8.4 months versus 11.5 months for those taking placebo ($P = 0.5539$; Supplementary Fig. S2). Besides, when baseline IL6 levels other than 7pg/mL were tested as cut-off values, no significant interaction between IL6 cut-off values and treatment was detected for either PFS or OS, as demonstrated by the forest plots and P values for interaction (Supplementary Fig. S3). Also, we followed the change in IL6 levels over time during follow-up for a subset of patients and as shown in Supplementary Fig. S4, at each timepoint, there was no statistically significant difference in IL6 levels between the two treatment arms.

Safety

Treatment-related adverse events occurred in 91.89% of the patients receiving gefitinib plus metformin and in 82.88% of the

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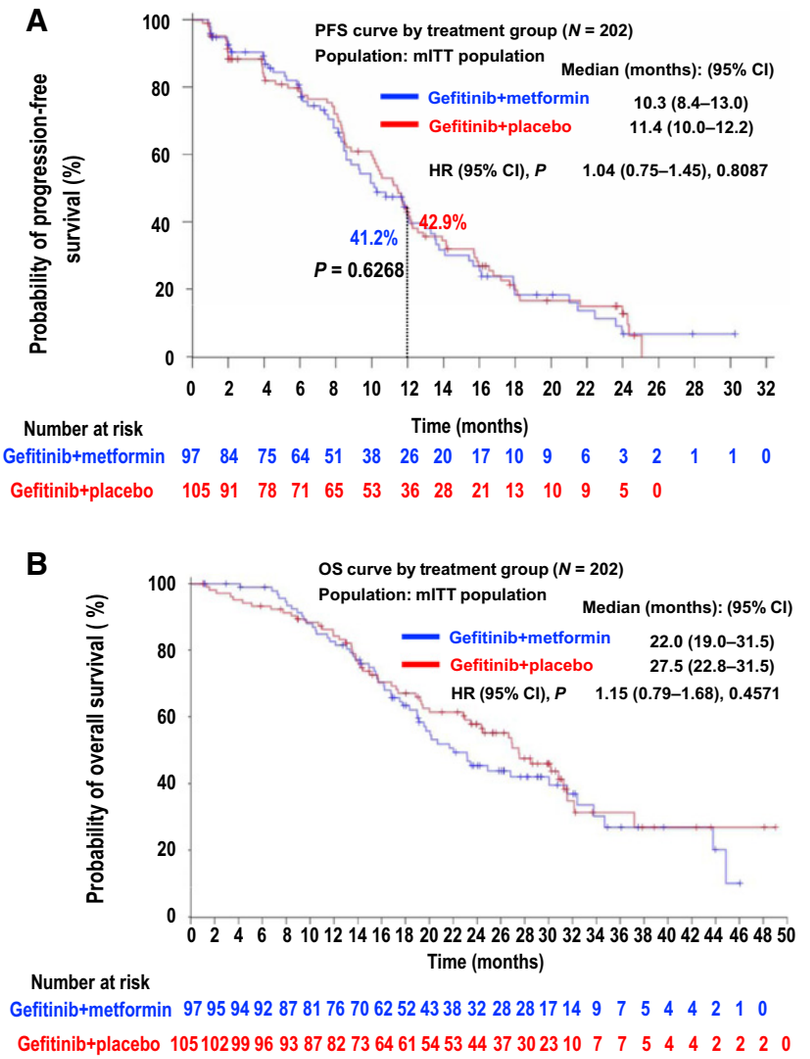

Figure 2.

A and **B,** Kaplan–Meier estimates of PFS and OS according to treatment group in the ITT population. +, data censored at the last time the patient was known to be alive and without disease progression (**A**), and data censored at the last time the patient was known to be alive (**B**), respectively. PFS was evaluated according to RECIST, version 1.1, by means of blinded, independent, and central radiologic review. The ITT population included all patients who had at least one dose of study treatment and at least one radiological assessment. mITT, modified intention to treat.

patients receiving gefitinib plus placebo (Table 3). The two groups shared similar safety profiles except for a slightly higher incidence of grade 3–4 adverse events in the metformin group (23.42% vs. 18.92%). Twenty-two patients receiving metformin required a dose reduction due to intolerable toxicity and three discontinued prematurely, while 10 patients given placebo had a dose reduction and two discontinued treatment. The most commonly reported grade 3–4 adverse events were diarrhea and rash in both groups. Of note, diarrhea occurred at a much higher frequency among patients taking metformin (78.38% vs. 43.24%). No interstitial lung disease was detected in either group. One of the patients on placebo experienced a serious adverse event with a sharp increase in the levels of alanine aminotransferase (ALT, grade 4) and aspartate aminotransferase (AST, grade 4), and after treatment with polyene phosphatidylcholine and reduced glutathione for 3 weeks, both ALT and AST returned to normal levels.

Discussion

Our study showed that incorporation of metformin into standard gefitinib therapy did not prolong PFS or OS in treatment naïve nondiabetic patients with EGFRm NSCLC, neither did it elicit an increase in response to gefitinib. Rather, the mPFS and mOS appeared inferior in the gefitinib plus metformin arm, although the differences were not statistically significant. Analyses of prespecified subgroups revealed no treatment group differences across subgroups, including those stratified on the basis of various baseline serum IL6 levels. In line with previous studies, metformin resulted in a higher incidence of diarrhea.

Over the last decade, repurposing metformin has drawn tremendous attention as an economical way to improve the efficacy of/overcome the resistance to existing anticancer treatment. Metformin uptake by patients with diabetes who have cancer was often associated with survival benefits, especially in breast, prostate, colorectal, endometrial, and lung cancer (9, 11, 12). However, this notion was challenged by a few other studies where metformin failed to show any auxiliary anticancer activities (21–23). The controversies in literature could have resulted from the retrospective nature and small datasets of these studies and most of them also included heterogeneous populations, that is, patients with/without diabetes, with different stages of NSCLC, with different lines of previous treatment, or with different concurrent treatment regimens. In addition, no prior studies specifically addressed application of metformin in

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Table 2. Summary of responses in the ITT population

Variable	Gefitinib + metformin ($n = 97$)	Gefitinib + placebo ($n = 105$)
Best overall response, No. (%)		
Complete response	3 (3.1)	4 (3.8)
Partial response	61 (62.9)	66 (62.9)
Stable disease	31 (32.0)	32 (30.5)
Progressive disease	2 (2.1)	3 (2.9)
Objective response		
No. of patients	64	70
% of patients (95% CI)	66.0 (55.7–75.3)	66.7 (56.8–75.6)
Estimated OR (95% CI)	0.97 (0.52–1.81)	
P	1.0000	
Disease control		
No. of patients	95	102
% of patients (95% CI)	97.9 (92.7–99.7)	97.1 (91.9–99.4)
Estimated OR (95% CI)	1.40 (0.16–17.04)	
P	1.0000	

NOTE: The ITT population included all patients who had at least one dose of study treatment and at least one radiological assessment. Objective response was defined as complete or partial response, as assessed by means of blinded, independent, and central radiologic review according to RECIST, version 1.1. Disease control was defined as complete response, partial response, or stable disease for ≥6 weeks, prior to any disease progression event. The CIs for ORs were calculated using Clopper–Pearson exact method. *P* values were calculated using Fisher exact test. An OR of >1 favors the metformin group.

conjunction with TKI therapy in patients without diabetes. Such ambiguity could only be resolved by prospective trials with a well-defined study population. This work is one of the first prospective efforts to evaluate metformin combined with targeted therapy in nondiabetic patients with NSCLC. Double-blinded and placebo-controlled, our study enrolled treatment-naïve patients and selected gefitinib as the only concurrent TKI, all of which minimized biases or variability. Indeed, the mPFS, mOS, and ORR of our control group were consistent with historical data on gefitinib efficacy (24). The sharp increase in diarrhea incidence accompanying metformin intake also indicated sound patient adherence to the study protocol because metformin is known to cause gastrointestinal disorders. The higher level of toxicity observed in the metformin group could also be partly attributed to the fact that metformin could increase the concentration of gefitinib in blood (25).

Currently, there are over 200 ongoing trials applying metformin in patients without diabetes who have cancer (http://clinicaltrials.gov/). A trial (NCT03071705) recently presented at the 2018 ASCO Annual Meeting caught our special attention, because it also prospectively evaluated combined use of metformin and EGFR-TKIs in patients without diabetes who have NSCLC. In contrast to our findings, this study claimed that addition of metformin to standard EGFR-TKI therapy significantly increased PFS (13.1 months vs. 9.9 months; $P = 0.011$), OS (31.7 months vs. 17.5 months; $P = 0.019$), and ORR (71% vs. 54.3%; $P = 0.042$) in patients without diabetes (18). However, these data should be interpreted with caution, because this study was neither blinded nor placebo controlled. Prior anticancer treatment was also allowed, which might have confounded data interpretation, especially when metformin was shown to enhance the efficacy of radiation/chemotherapy previously. Second, the patients in NCT03071705 had a relatively high rate of metformin

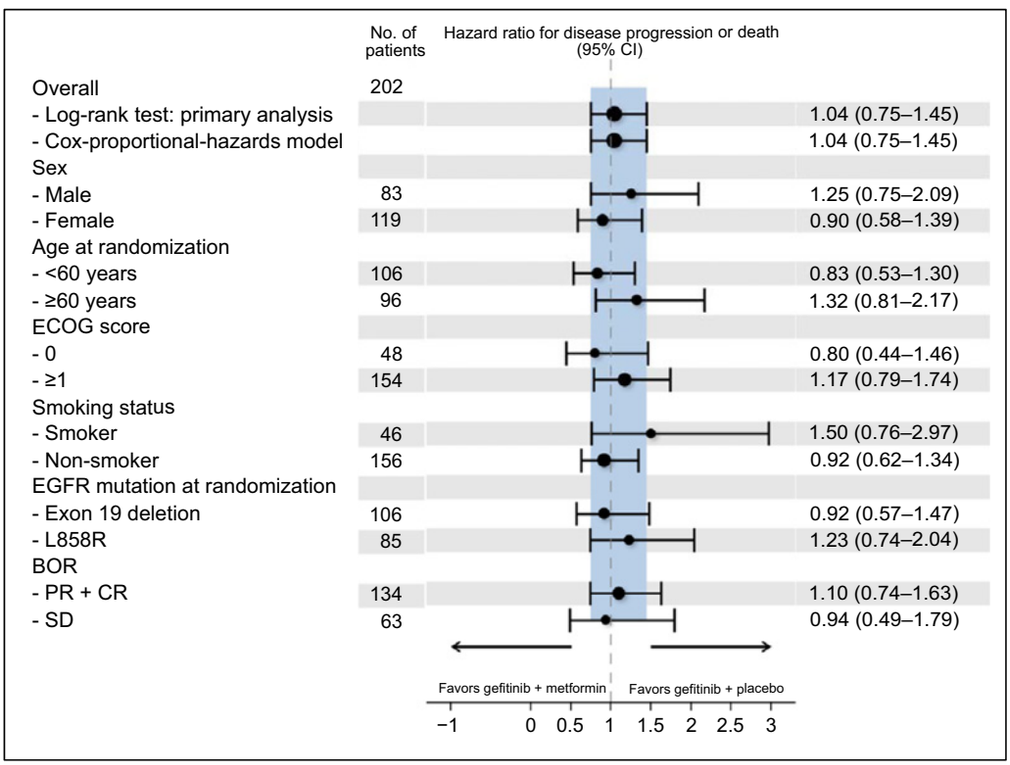

Figure 3.
Analyses of PFS in key subgroups. BOR, best overall response; CR, complete response; PR, partial response; SD, stable disease.

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Table 3. Treatment-related adverse events according to treatment group

Characteristics	Gefitinib + metformin ($N = 111$)					Gefitinib + placebo ($N = 111$)				
	Total	Grade 1	Grade 2	Grade 3	Grade 4	Total	Grade 1	Grade 2	Grade 3	Grade 4
Number of patients (%)										
Any adverse event	102 (91.89)	41 (36.94)	35 (31.53)	23 (20.72)	3 (2.70)	92 (82.88)	37 (33.33)	34 (30.63)	17 (15.32)	4 (3.60)
Diarrhea	87 (78.38)	40 (36.04)	33 (29.73)	13 (11.71)	1 (0.90)	48 (43.24)	24 (21.62)	17 (15.32)	7 (6.31)	0 (0.00)
Rash	59 (53.15)	37 (33.33)	18 (16.22)	4 (3.60)	0 (0.00)	70 (63.06)	44 (39.64)	20 (18.02)	5 (4.50)	1 (0.90)
Increased ALT	31 (27.93)	19 (17.12)	8 (7.21)	4 (3.60)	0 (0.00)	26 (23.42)	14 (12.61)	7 (6.31)	3 (2.70)	2 (1.80)
Increased AST	24 (21.62)	14 (12.61)	8 (7.21)	1 (0.90)	1 (0.90)	25 (22.52)	12 (10.81)	7 (6.31)	4 (3.60)	2 (1.80)
Cough	14 (12.61)	8 (7.21)	4 (3.60)	2 (1.80)	0 (0.00)	15 (13.51)	6 (5.41)	3 (2.70)	6 (5.41)	0 (0.00)
Vomiting	13 (11.71)	7 (6.31)	4 (3.60)	1 (0.90)	1 (0.90)	9 (8.11)	6 (5.41)	3 (2.70)	0 (0.00)	0 (0.00)
Increased LDH	12 (10.81)	7 (6.31)	5 (4.50)	0 (0.00)	0 (0.00)	10 (9.01)	5 (4.50)	3 (2.70)	1 (0.90)	1 (0.90)
Dizziness	11 (9.91)	6 (5.41)	5 (4.50)	0 (0.00)	0 (0.00)	6 (5.41)	4 (3.60)	2 (1.80)	0 (0.00)	0 (0.00)
Oral ulceration	10 (9.01)	5 (4.50)	3 (2.70)	2 (1.80)	0 (0.00)	11 (9.91)	5 (4.50)	3 (2.70)	3 (2.70)	0 (0.00)
Pruritus	7 (6.31)	4 (3.60)	2 (1.80)	1 (0.90)	0 (0.00)	16 (14.41)	9 (8.11)	7 (6.31)	0 (0.00)	0 (0.00)

Abbreviation: LDH, lactate dehydrogenase.

discontinuation (23.2%) and a short follow-up (12.9 ± 10.9 months) and this could have potentially compromised data validity. In our study, patients were followed up for a median of 19.15 months (IQR, 12.99–28.44) and only 3.1% of metformin users discontinued treatment early. We intentionally chose gefitinib to minimize toxicity-induced dose reduction or termination, because metformin was found to increase the concentrations of TKI drugs in blood and the MTD of gefitinib is well above its effective dose (25). However, in NCT03071705, patients were also allowed to take erlotinib (17.1%) or afatinib (42.9%), for which the recommended doses were very close to their MTDs, rendering patients at higher risk of adverse events. Possible concerns over the study design of NCT03071705 could be reflected in the remarkably lower OS and ORR of the TKI monotherapy group in comparison with historical data on TKI efficacy (24, 26–29). Another ongoing trial METformin in Advanced Lung cancer by Fasano and colleagues also attempted to assess the safety and activity of metformin combined with erlotinib in patients without diabetes who have NSCLC, but it differed from our study in that it is a single-arm I/II trial study focused on second-line treatment of patients who carry the EGFR wild-type mutation and the safety run-in part has determined the MTD of metformin at 1,500 mg/day while efficacy analysis still goes on (19, 30).

The negative results of our study were not completely unexpected, as a cohort study on patients with type II diabetes who have NSCLC in the United States' military health system have already shown that improved outcomes were only observed among early-stage patients or those who started metformin before the NSCLC diagnosis (31). This study, however, enrolled late-stage patients who never took metformin previously. It is possible that patients might have responded differently if they were exposed to metformin for an extended period of time. Likewise, the dose of metformin might not have been high enough for it to take effect because the antiproliferative effect of metformin was dose-dependent *in vitro* and clinical data from patients with pancreatic cancer demonstrated that only patients with high plasma concentrations (>1 mg/L) of metformin could gain survival benefits (32, 33). However, given the rise in diarrhea incidence, it was not an option to further increase the dose of metformin. In addition to metformin exposure, some patients might have harbored deficiency in the *LKB1* gene, which encodes a tumor suppressor required for AMP-activated protein kinase (AMPK) activation (via phosphorylation of AMPK at Thr172), which is a critical step in metformin's anticancer mechanism, and this gene is mutated in 20%–30% of Caucasian patients with NSCLC (34, 35). In the Chinese population, *LKB1* was previously shown to be mutated in 10.5% of patients who have the *EGFR* mutation and with lung adenocarcinoma (6/57, one with somatic del50L_53D deletion and five harboring germline F354L polymorphism; ref. 36). Although published literature showed conflicting evidence regarding the role of germline F354L substitution in carcinogenesis, it was demonstrated previously to cause AMPK pathway dysregulation (37). We could not exclude the possibility that inactivation of LKB1 in some patients might have impaired metformin's ability to potentiate the AMPK-dependent repression of prooncogenic pathways, as corroborated by the fact that the synergy between metformin and gefitinib was strictly dependent on the presence of wild-type LKB1 in NSCLC cells (7). It was also possible that the relatively high phosphorylation level of AMPK in patients without diabetes, as regulated by glucose level, might have partially overshadowed the antitumoral effects of metformin (38). At last, even though metformin was suggested to mediate reversal of EGFR-TKI resistance by downregulating IL6 signaling, subgroups defined as having elevated levels of IL6 according to various baseline cutoff values failed to display improved outcomes in the metformin group versus the placebo group, and follow-up of IL6 levels at multiple timepoints also showed no statistically significant differences between the two treatment arms at each timepoint. This might be explained by the fact that IL6 concentrations are increased in an autocrine manner upon treatment with EGFR-TKI and the suppression of IL6 by metformin might have been offset by the effects of gefitinib on IL6 (39).

Although rigorously designed and controlled, our study does have some limitations. First, stratification according to EGFR mutation subtypes was not performed. Back in 2013 when the trial was started, it was unknown whether patients with exon 19del or L858R would respond differently to the same EGFR-TKI. Second, patients were not stratified by the status of *LKB1* or other genes, because genotyping beyond *EGFR* testing was not readily available when the study was started back in 2013. Moreover, as discussed above, therapeutic monitoring of metformin levels might have been necessary to ensure adequate drug exposure. That said, this study improved our understanding of metformin's effects in patients without diabetes who have lung cancer and will help inform decision making by practitioners.

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Conclusions

Taken together, cotreatment with metformin resulted in nonsignificantly worse outcomes but increased the risk of diarrhea; therefore, our study does not support combined use of metformin and EGFR-TKI therapy in patients who are treatment-naïve with advanced EGFRm NSCLC who do not copresent with diabetes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: L. Li, Y. He

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