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REVIEW ARTICLE

First-line therapy for advanced non-small cell lung cancer with activating EGFR mutation: is combined EGFR-TKIs and chemotherapy a better choice?

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Abstract
As the standard first-line treatment for advanced non-small cell lung cancer (NSCLC) with activating epidermal growth factor receptor (EGFR) mutation, EGFR-tyrosine kinase inhibitors (EGFR-TKIs) have significantly improved the median progression-free survival (PFS) up to 18.9 months. However, almost all patients eventually develop acquired resistance to EGFR-TKIs, which limits the first-line PFS. To overcome the resistance and improve overall survival, researchers have tried to identify the resistance mechanisms and develop new treatment strategies, among which a combination of EGFR-TKIs and cytotoxic chemotherapy is one of the hotspots. The data from preclinical and clinical studies on combined EGFR-TKIs and chemotherapy have shown very interesting results. Here, we reviewed the available preclinical and clinical studies on first-line EGFR-TKIs–chemotherapy combination in patients with advanced NSCLC harboring activating EGFR mutation, aiming to provide evidences for more potential choices and shed light on clinical treatment.

Keywords Activating EGFR mutation · NSCLC · EGFR-TKIs · Cytotoxic chemotherapy · First-line treatment

Introduction

Lung cancer is the leading cause of cancer death worldwide, with an estimated 1.6 million deaths in 2012 (1.1 million in men and 491,200 deaths in women) [1]. In the total population of lung cancer patients, non-small cell lung cancer (NSCLC) accounts for 80–85%. Most patients are diagnosed with NSCLC at the advanced stage at the first time to presentation. In recent years, driver gene-guided target therapy has rewritten the history of NSCLC treatment. So far, the most well-recognized driver gene for NSCLC is epidermal growth factor receptor (EGFR), which encodes a cell membrane receptor with tyrosine kinase activity. EGFR protein is expressed in most NSCLC cells and plays important roles in promotion of tumor proliferation, angiogenesis, metastatic potential and chemo-resistance, as well as inhibition of apoptosis [2]. Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib, erlotinib, afatinib, dacomitinib, and osimertinib, can inhibit the activity of tyrosine kinase and the primed signaling of EGFR, and, therefore, exert an anti-tumor effect. In several classical phase III randomized controlled clinical trials including IPASS, OPTIMAL, WJTOG3405, NEJ002, ENSURE, EURACT, LUX-LUNG3, LUX-LUNG6, ARCHER 1050, and FLAURA, EGFR-TKIs significantly improved clinical efficacy compared with chemotherapy in advanced NSCLC patients with activating EGFR mutations [3–12]. EGFR mutation is currently the only well-established predictive and prognostic biomarker for EGFR-TKIs' application [3–10]. Based on these trials, the first-line therapy with first-to-third-generation EGFR-TKIs in those patients acquired a median progression-free survival (PFS) of 9.5–18.9 months, disease control rate (DCR) of up to 90%, and a highest median overall survival (OS) of 35.5 months. Therefore, EGFR-TKIs are recommended as the standard first-line treatment for patients with advanced NSCLC harboring activating EGFR mutation [13, 14]. In addition,

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gefitinib, erlotinib, and afatinib have been approved as the standard first-line agents by FDA (US Food and Drug Administration), EMA (European Medicines Agency), and CFDA (China Food and Drug Administration). We are also looking forward to the authorization of dacomitinib and osimertinib as the first-line agents. However, after about 10–18 month initial response, almost all patients eventually develop resistance to EGFR-TKIs, which may be due to the T790M or Cys797Ser (C797S) mutation in EGFR exon 20, MET amplification, hepatocyte growth factor (HGF) overexpression, epithelial–mesenchymal transition (EMT), transformation to small cell lung cancer, or activation of AXL kinase [15–23]. Although osimertinib has been verified to be clinically benefit to patients who are secondary resistant to EGFR-TKIs caused by T790M mutation [24, 25], the PFS is only about 9 months and large amount of patients without T790M mutation have no valid agents. In most regions, osimertinib is still unavailable owing to the regulatory limitation. More importantly, treatment with osimertinib can also cause resistance [23]. Since longer first-line PFS indicates longer OS for advanced NSCLC patients [26], researchers have been trying to identify the resistance mechanisms, develop new targets and agents, as well as investigate new combination strategies of existing agents, such as the combination of EGFR-TKIs and cytotoxic chemotherapy, to get a longer OS.

Theoretically, chemotherapy can destroy the structure and function of DNA, RNA, or protein of cancer cells, which may overcome the tumor heterogenicity, postpone the resistance to EGFR-TKIs, and consequently improve the PFS and overall response rate (ORR) in the first-line treatment of advanced NSCLC patients harboring EGFR mutation when combined with EGFR-TKIs. Therefore, the combination of these two kinds of drugs is one of the hot points in clinical trials.

Many preclinical and clinical studies tried to explore the proper patient selection, regimens, sequence, and interaction mechanisms of EGFR-TKIs–chemotherapy combination for a better clinical outcome. Although there is still no uniform conclusion, these studies showed a promising clinical prospect. Here, we review the available preclinical studies and clinical trials about the combination of EGFR-TKIs and chemotherapy in patients with advanced NSCLC harboring activating EGFR mutation, and aim to provide theoretical and practical evidences for better clinical practices.

Preclinical studies: interaction between EGFR-TKIs and chemotherapy agents

EGFR, encoded by the oncogene C-erbB-1 (HER-1, also named EGFR) and generally expressed in human epidermal and stromal cells, is a cell-surface receptor with constitutive tyrosine kinase activity. When binding to its specific ligands such as epidermal growth factor (EGF) and transforming growth factor-α (TGF-α), EGFR protein will transform from inactive monomer to an active homodimer, which subsequently activates its intrinsic intracellular protein-tyrosine kinase activity, initiates downstream signal transduction cascades (including Ras/Raf/MEK/ERK/MAPK, PI3K/PDK1/Akt, PLC-γ, and JAK/STAT pathway [27, 28]), and regulates critical cellular processes, such as proliferation, differentiation, survival, metabolism, migration, and cell-cycle control [28, 29]. However, the aberrant expression and mutational activation of EGFR lead to tumor development by promoting cancer cell proliferation, adhesion, invasion, metastases, and tumor angiogenesis [2]. In NSCLC cells, high-frequency EGFR mutations include in-frame deletions in exon 19, insertions in exon 20, and point mutations in exons 18 and 21 [30]. EGFR-targeted small-molecule TKIs can block EGFR-mediated downstream signaling and inhibit the malignant tumor behaviors.

Unlike EGFR-TKIs, most cytotoxic agents kill tumor cells by destroying DNA function and structure to interfere with their transcription, translation, mismatch repair, and finally cell mitosis [31]. They have no specific targets and can kill or inhibit all rapidly proliferative cells, regardless of EGFR mutation status.

Although there is still no data on combination of chemotherapy with second/third-generation EGFR-TKIs, some preclinical researches have explored the interaction of first-generation EGFR-TKIs and cytotoxic agents, and implied their clinical application for combination therapy. In this part, we summarize the comprehensive effect and underlying mechanisms of their combination in NSCLC cell lines with activating EGFR mutation. Better understanding of these mechanisms will provide theoretical evidence for their combination and even shed light on further combination of second/third-generation EGFR-TKIs with chemotherapy.

EGFR-TKIs and anti-microtubules agents (AMTAs)

AMTAs, or mitotic inhibitors, such as paclitaxel, docetaxel, and vinorelbine, prevent cells from mitosis and growth by impacting microtubules polymerization, ribosome function, or amino acid supplement. They are cell-cycle-specific agents (CCSAs), which mainly affect the tumor cells in mitotic period (Phase M).

So far, the results from preclinical experiments on EGFR-TKIs–AMTAs combination have consistently pointed to their synergistic anti-proliferation [32–34] and pro-apoptosis [34, 35] activity. It is noteworthy that their anti-proliferation activity is associated with the sequence of EGFR-TKIs and AMTAs delivery [32, 33]. Sequential treatment with

paclitaxel followed by gefitinib produced stronger anti-proliferative effect compared with concurrent or reverse sequence in both EGFR-TKIs resistant and sensitive cell lines [32, 33]. Several mechanisms might be responsible for this phenomenon: (1) the two kinds of drugs have distinct effect on the cell cycles. While Gefitinib exposure caused accumulation of the G1- or G0/G1-phase cells, administration of AMTAs significantly increased the fraction of S- or G2/M-phase cells [32, 35]. G1 or G0/G1 arrest caused by EGFR-TKIs treatment effectively disturbed the function of AMTAs. (2) AMTAs increased the phosphorylation of EGFR and AKT (a downstream signaling molecule of EGFR) [32–35], which may represent a survival response of tumor cells following AMTAs treatment [32, 33]. Interestingly, the increased pEGFR is the exact target for gefitinib. It was also demonstrated that the EGFR phosphorylation caused by AMTAs was potentially promoted by increasing both the transcription rates and activation of TGF-α, a specific ligand of EGFR [33]. (3) The combination of EGFR-TKIs with AMTAs, especially sequential treatment of paclitaxel followed by gefitinib decreased VEGF secretion in H1975 cell line, while single agent did not [32]. Although unmentioned in the article, tumor angiogenesis and proliferation caused by different VEGF isoforms might represent a synergistic mechanism for the combination therapy [36]. However, another study [37] showed that the combination of gefitinib and AMTAs had synergistic effect only in the wild-type NSCLC cell lines but not in EGFR mutation ones. Using the Pgp-overexpressed NCI-H23 sub-clones, they demonstrated that mechanically, gefitinib remarkably enhanced AMTAs sensitivity by blocking Pgp-associated efflux, an active resistance mechanism for AMTAs [37]. Taken together, these data suggest that combined application of EGFR-TKIs and AMTAs might be a potential clinical strategy for NSCLC patients with EGFR mutation.

EGFR-TKIs and anti-metabolites agents

Anti-metabolites agents include pemetrexed, methotrexate, 5-Fluorouracil (5-FU), gemcitabine, etc. Their structures mimic that of natural substances in nucleic acid metabolism. Therefore, they can alter the enzyme function required for cell metabolism and protein synthesis and mainly attack cells in phase S. Pemetrexed is a classical anti-folates drug most frequently used in NSCLC treatment. It inhibits the enzyme activity of thymidylate synthase (TS), dihydrofolate reductase (DHFR), and gylcinamide ribonucleotide formyltransferase (GARFT) which are involved in purine and pyrimidine synthesis [38, 39]. It is reported that resistance to pemetrexed is mainly related to increased TS expression [40].

In multiple studies, the combination of erlotinib/gefitinib and pemetrexed had a synergistic toxicity in NSCLC cell lines with EGFR mutation if exposure to erlotinib/gefitinib before pemetrexed was avoided [41–43]. These studies also found several potential synergistic mechanisms for erlotinib/gefitinib-pemetrexed interaction: (1) pemetrexed induced phosphorylated AKT expression in tumor cells, which was associated with increased phosphorylated EGFR level [41, 42]. Therefore, the addition of erlotinib or LY294002 (a potent PI3K inhibitor) to pemetrexed led to a remarkable increase in growth inhibition compared with pemetrexed alone [42]. (2) Erlotinib significantly reduced TS expression and activity, which were almost completely reversed on its combination with pemetrexed, possibly via down-regulation of E2F-1 (a transcription factor regulating TS gene) expression [41]. (3) Both in vitro and in vivo, gefitinib resistance mediated by T790M mutation or EMT was prevented by its combination with pemetrexed in NSCLC, when pemetrexed was the first treatment, given alone or together with gefitinib [43]. However, the underlying mechanisms need further investigation. It is noteworthy that when exposed before pemetrexed, erlotinib induced cell arrest in G1 phase and prevented them from the cytotoxicity of subsequent pemetrexed exposure [41, 42]. Therefore, the sequence erlotinib before pemetrexed should be avoided. One of these studies also showed that the negative interaction can be avoided by removing erlotinib from the cell culture medium for a sufficient interval (≥ 8 h) before exposure to pemetrexed [42]. Although most results support their synergism, another study only demonstrated the additive or antagonistic effect of gefitinib/pemetrexed combination in NSCLC cell line with EGFR mutation [44].

Furthermore, there were several studies on EGFR-TKIs combination with other anti-metabolites, such as S-1 and gemcitabine.

S-1 (or 5-FU) and gefitinib exerted a synergistic anti-proliferative effect on NSCLC cells with/without EGFR mutation both in vivo and in vitro [45]. It was also demonstrated that gefitinib suppressed the expression of TS, an enzyme determining the tumor cell sensitivity to 5-FU. However, gemcitabine combination with EGFR-TKIs had no synergistic effect in EGFR-mutation NSCLC cell lines H3255, HCC827, and PC-9 [37]. Further investigation is needed to confirm this conclusion.

EGFR-TKIs and platinum agents

Platinum belongs to cell-cycle nonspecific agents (CCNSA). Their interaction with DNA forms intra- or inter-stranded cross links and DNA kinking, which inhibit transcription and result in cell death [46, 47].

Most preclinical studies showed that the combination of EGFR-TKIs and platinum had an antagonistic or (at best) addictive effect on NSCLC cell lines with activating EGFR mutation [34, 44, 48, 49]. Unlike the AMTAs and

anti-metabolites agents, the sequence of drugs exposure did not change the results [34, 49]. In Liu's study [48], the combination of gefitinib and cisplatin induced a higher level of autophagy than monotherapy of either agent. While the addition of CQ, an autophagy inhibitor, reversed this antagonism and even led to synergistic effect through upregulation of the pro-apoptotic protein Bax and down-regulation of the anti-apoptotic protein Bcl-2 expression. Furthermore, it was demonstrated that gefitinib could reduce cisplatin influx in vitro [49].

Collectively, the combination of EGFR-TKIs with cytotoxic agents especially with AMTAs, pemeterexed, and S-1 (or 5-FU) showed promising results, while the combination containing platinum had no synergistic effect in preclinical studies. The underlying mechanisms for EGFR-TKIs and cytotoxic agent interaction are diverse depending on different regimens and sequence which include modulation of cell cycles, EGFR signaling, autophagy, and drug resistance of tumor cells. We also expect preclinical studies on combined cytotoxic agents with second/third-generation EGFR-TKIs. Better understanding of the complex mechanisms will provide theoretical evidences for more reasonable clinical trials' design and clinical application of EGFI-TKI-based combination therapy.

Clinical trials

For the first-line therapy of patients with advanced NSCLC harboring activating EGFR mutation, accumulating clinical trials [3–12] have verified that EGFR-TKIs provide more clinical benefits compared with platinum-based chemotherapy (Table 1). Therefore, those EGFR-TKIs are recommended as the standard first-line therapy for these patients by authoritative guidelines [13, 50, 51]. However, after the initial about 10–18 months' response, almost all patients eventually develop secondary resistance. Although it is approved that osimertinib has clinical activity in patients with EGFR T790M-positive disease who have progressed on an EGFR-TKI [24, 25], these patients only account for about 48–63% [52–55] and the PFS is only about 9 months [24, 25]. More importantly, resistance inevitably develops even after treatment with third-generation EGFR-TKIs. Considering that longer first-line PFS indicates longer OS for advanced NSCLC patients [26], some researchers tried to investigate whether the combination EGFR-TKIs and cytotoxic drugs could prevent or delay the emergence of acquired resistance to EGFR-TKIs therapy and prolong OS as compared to EGFR-TKIs monotherapy. In the first decade, because of unselected patients with a predictive biomarker and the cell-cycle-specific antagonistic effect between EGFR-TKIs and cytotoxic agents, several clinical trials showed that combination therapy did not improve OS compared with chemotherapy alone [56–59]. In recent years, based on the results of preclinical experiments, many clinical trials with more rational design have been completed. Furthermore, most of them showed potential clinical benefit with combination therapy. In this part, we focus on several representative clinical trials to provide evidences for more reasonable clinical choices.

Table 1 ORR, PFS, and OS of clinical trials about EGFR-TKIs vs. chemotherapy

Publication date	Clinical trials		ORR (%)		Median PFS (months)		Median OS (months)	
			TKI	Chemo	TKI	Chemo	TKI	Chemo
2009.12	WJTOG3405		62.1	32.2*	9.6	6.6	35.5	38.8
2011.07	IPASS mut^{+}		71.2	47.3**	9.5	6.3**	21.6	21.9$^{\#}$
2012.01	EURTAC		58	15	9.7	5.2*	19.3	19.5$^{\#}$
2012.11	NEJ002		73.7	30.7*	10.8	5.4*	27.7	26.6$^{\#}$
2013.07	LUX-LUNG3		56	23**	13.6	6.9**	31.6	28.2$^{\#}$
2014.01	LUX-LUNG6		66.9	23.0*	11.0	5.6*	23.6	23.5$^{\#}$
2015.06	ENSURE		62.7	33.6	11.0	5.5*	26.3	25.5$^{\#}$
2015.07	OPTIMAL		83	36*	13.1	4.6*	22.8	27.2$^{\#}$
2017.09	ARCHER 1050	G	72$^{\#}$	–	9.2**	–	–	–
		D	75$^{\#}$	–	14.7**	–	–	–
2017.11	FLAURA	G/E	76$^{\#}$	–	10.2**	–	–	–
		O	80$^{\#}$	–	18.9**	–	–	–

G gefitinib, *D* dacomitinib, *E* erlotinib, *O* osimertinib

*$p < 0.0001$

**$p \leq 0.001$

$^{\#}p > 0.01$

The JMIT study [60] is a randomized phase II trial on gefitinib with or without pemetrexed as first-line therapy in patients with advanced non-squamous (NS) NSCLC harboring activating EGFR mutations. Chemotherapy-naïve patients from East Asia were randomly assigned to open-label pemetrexed (500 mg/m^2 on day 1 of every 21-day cycle) plus gefitinib [250 mg/d ($n=129$)] (P + G arm) or gefitinib alone ($n=66$) at a ratio of 2:1. The primary endpoint was PFS. There was a statistically significant prolongation of PFS in P + G arm (median, 15.8 months; 95% CI, 12.6–18.3 months) than gefitinib-alone arm [median, 10.9 months; 95% CI, 9.7–13.8 months; adjusted hazard ratio (HR), 0.68; 95% CI, 0.48–0.96; one-sided $p=0.014$; two-sided $p=0.029$]. Furthermore, P + G also significantly prolonged time to progressive disease (TtPD) and duration of response (DoR) compared with gefitinib monotherapy. Although grade 3 or 4 study drug–related adverse events (AEs) were more common in the P + G arm compared with the gefitinib-alone arm, toxicities were clinically manageable. Meanwhile, it is noteworthy that the PFS Kaplan–Meier curves for the two arms overlapped on the first 7–8 months, which indicated that there was no benefit with combination strategy in the early time. Therefore, we wonder whether the addition of chemotherapy-to-EGFR-TKIs treatment after the first several months (about 7–8 months) would get more benefits and less adverse events for those patients.

The subgroup analysis of FASTACT-2 study [61] also supports the combined EGFR-TKIs and chemotherapy. This study is a randomized double-blind phase III trial of the intercalated combination of chemotherapy and erlotinib for patients with untreated stage IIIB/IV NSCLC. Patients were randomly assigned at a ratio of 1:1 to receive six cycles of gemcitabine (1250 mg/m^2 on days 1 and 8, intravenously) plus platinum (carboplatin 5 × area under the curve or cisplatin 75 mg/m^2 on day 1, intravenously) with intercalated erlotinib (150 mg/day on days 15–28, orally; chemotherapy plus erlotinib) or placebo orally (chemotherapy plus placebo) every 4 weeks. Patients continued to receive erlotinib or placebo until disease progression (PD) or unacceptable toxicity or death, and all patients in the placebo group were offered second-line erlotinib at the time of progression. The primary endpoint was PFS. The subgroup analysis of patients with activating EGFR mutation in chemotherapy plus erlotinib group ($n=97$) showed that the PFS was 16.8 months and the OS was 31.4 months. Although not formally compared, the PFS with the combination regimen was obviously improved when compared with that of first-generation EGFR-TKIs monotherapy in the previous phase III studies (median PFS, 9.5–13.7 months) (Table 1). The combined regimen caused a minimal increase of toxicities. However, we do not know whether the chemotherapy regiment gemcitabine plus carboplatin was proper to the first-line therapy.

Another three-arm study [62] also showed exciting results. It was a randomized open-label phase II study comparing pemetrexed plus carboplatin and gefitinib to either pemetrexed plus carboplatin or gefitinib alone as first-line therapy for untreated patients with advanced lung adenocarcinoma harboring sensitive EGFR mutations. A total of 121 patients were randomly assigned in a 1:1:1 ratio to receive gefitinib combined with pemetrexed and carboplatin, pemetrexed plus carboplatin, or gefitinib alone. The combination therapy group received pemetrexed (500 mg/m^2 on day 1) plus carboplatin (AUC 5 on day 1) combined with gefitinib (250 mg/day on days 5–21) and repeated every 4 weeks for up to six cycles and then continued to receive pemetrexed combined with gefitinib every 4 weeks. The chemotherapy group received the same chemotherapy regimen as the combination group every 4 weeks for up to six cycles and then continued to receive pemetrexed alone every 4 weeks. The gefitinib group received gefitinib alone. All therapies were continued until PD, unacceptable toxicity or death. The PFS for patients in the combination, chemotherapy, and gefitinib groups was 17.5 (95% CI, 15.3–19.7), 5.7 months (95% CI, 5.2–6.3), and 11.9 (95% CI, 9.1–14.6), respectively, while the ORR were 82.5, 32.5, and 65.9% and the OS were 32.6 (95% CI, 25.5–39.8), 24.3 (95% CI, 17.7–30.1), and 25.8 months (95% CI, 21.3–30.2), respectively. The toxicity profiles showed good tolerance of all patients. However, this trial was a monocenter study, and the results should be interpreted considering this limitation.

The study CALGB30406 [63] is a randomized phase II trial of erlotinib alone or with carboplatin and paclitaxel in patients who were never or light former smokers with advanced lung adenocarcinoma. Patients were randomly assigned to continuous erlotinib (arm A; $n=81$) or in combination with carboplatin and paclitaxel (ECP) (arm B; $n=100$) for six cycles followed by erlotinib alone. Patients in both arms continued to receive erlotinib until PD or unacceptable toxicity. In addition, the primary endpoint was PFS. The subgroup analysis of EGFR-mutation patients of two arms (both $n=33$) indicated that there were no statistical differences between arms A and B in ORR (70 vs. 73%), median PFS (14.1 vs. 17.2 m), or OS (31.3 vs. 38.1 m). However, we cannot ignore the influence of the small sample capacity of subgroup ($n=66$) to the result. In addition, the PFS and OS in combination group had a better tendency.

Based on the results of the study CALGB30406 and NEJ002, the study NEJ005 [64] had been designed. It was a randomized phase II study of concurrent vs. sequential alternating gefitinib and chemotherapy in previously untreated NSCLC patients with activating EGFR mutations, with PFS as the primary endpoint. This study was designed to select a combination regimen for phase III evaluation. There were 80 patients randomly assigned to

concurrent regimen group ($n=41$) and sequential alternating group ($n=39$). Patients in the concurrent regimen group received concurrent gefitinib (250 mg daily) and carboplatin [6 × area under the curve (AUC), day 1]/pemetrexed (500 mg/m^{2,} day 1) in a 3-week cycle for up to 6 cycles, followed by concurrent gefitinib and pemetrexed maintenance until PD, unacceptable toxicity, or death. Patients in the sequential alternating regimen group initially received 8 weeks of gefitinib and then 2 cycles of carboplatin/pemetrexed; this sequential treatment was repeated three times (carboplatin/pemetrexed was repeated for 6 cycles), followed by alternating gefitinib and pemetrexed maintenance. The result showed that the concurrent regimen group produced slightly better PFS (18.3 vs. 15.3 months, $p=0.2$), OS (41.9 vs. 30.7 months, $p=0.042$), and ORR (87.8 vs. 84.6%, $p=0.75$) compared with the sequential alternating group. The most common AEs were neutropenia, anemia, and thrombocytopenia and there is no increase in fatal events in both arms. Although not formally compared in this study, the PFS and OS obtained in response to these protocols were obviously longer than those obtained in response to first-generation EGFR-TKIs monotherapy (Table 1). In addition, the AEs were all manageable. Based on the results, another phase III study NEJ009, which compares the concurrently combined EGFR-TKIs and chemotherapy with standard gefitinib monotherapy in the EGFR-mutated setting, is underway. We expect a more clear and reliable outcome.

Furthermore, in 2015, two single-arm studies in Japan were designed to detect the efficiency and safety of the combination regimens of EGFR-TKIs and chemotherapy as first-line therapy in EGFR-mutated NSCLC patients [65, 66]. In these studies, the median PFS obtained in response to no matter interstitial therapy of gefitinib plus pemetrexed (18.0 months) or sequential treatment of gefitinib plus cisplatin and docetaxel (19.2 months) were obviously longer than that of EGFR-TKIs monotherapy (Table 1). Due to lacking of control group, we only got the survival data without the evidence from statistical difference. In addition, the small sample capacity of these two studies ($n=26$ and 34) was a shortcoming which may influence the results.

In spite of these favorable results, another randomized phase II trial [67] comparing erlotinib with erlotinib intercalated with chemotherapy in first-line therapy for advanced NSCLC made a different voice on this issue. A total of 143 patients from the United States or the United Kingdom were randomly assigned to either erlotinib (erlotinib 150 mg daily orally until PD) or CT + erlotinib arm (paclitaxel 200 mg/m^2 intravenously (IV) and carboplatin dosed by creatinine clearance (AUC 6) IV on day 1 intercalated with erlotinib 150 mg orally on days 2 through 15 every 3 weeks for four cycles followed by erlotinib 150 mg orally until PD). The primary endpoint was 6-month PFS. The results from subgroup analysis showed that patients with activating EGFR mutations treated with erlotinib alone but not combined agents had superior 6-month PFS rates (89 vs. 42%), 12-month OS rates (100 vs. 41.7%), PFS (18.2 months vs. 4.9 months), and RRs (67 vs. 33%) compared with the intercalated therapy arm.

In summary, combination of first-generation EGFR-TKIs with cytotoxic agents showed better clinical results and application prospect than monotherapy. However, owing to the limitation in clinical trial design, further randomized phase III studies are urgently warranted. We also expect canonical clinical data on second/third-generation EGFR-TKIs combined chemotherapy could brighten the future of first-line therapy in advanced NSCLC patients with activating EGFR mutation.

Conclusion

Most of the available preclinical and clinical studies support the first-generation EGFR-TKI-based combination with cytotoxic chemotherapy especially with AMTAs and anti-metabolites as the first-line therapy in advanced NSCLC patients with activating EGFR mutation. Clinically, the PFS with the combination regimens (Table 2) was improved when compared with that of first-generation EGFR-TKI monotherapy. Although the AEs slightly increased in combination regimens treated arms, they were still predictable and clinically manageable. However, the cytotoxic agents of the combination regimens from these trials were different and the sample sizes were limited. In addition, several combination regimens contained two kinds of cytotoxic agents which would decrease the patient compliance and reduce the diversity of alternative agents in followed-line therapy. Therefore, we cannot draw a final conclusion that the combined EGFR-TKIs and cytotoxic agents should replace EGFR-TKIs monotherapy as the first-line therapy in advanced NSCLC with activating EGFR mutation. Further randomized phase III studies comparing EGFR-TKI monotherapy and a combination therapy of EGFR-TKIs and chemotherapy, especially second/third-generation EGFR-TKIs with single cytotoxic agent such as pemetrexed or AMTAs, are warranted. We hope that there will be more evidences to guide our clinical practice.

Table 2 Data of clinical trials about the combination of EGFR-TKIs and chemotherapy

Publication date	Clinical trials	Samples no.		Regimens	PFS (months)	OS (months)	ORR (%)
2011.09	Fred R. Hirsch etc. mut$^+$	35	18	Erlotinib 150 mg daily orally until PD	18.2	–	67
			17	Paclitaxel 200 mg/m2 intravenously (IV) and carboplatin dosed by creatinine clearance (AUC 6) IV on day 1 intercalated with erlotinib 150 mg orally on days 2 through 15 every 3 weeks for four cycles followed by erlotinib 150 mg orally until PD	4.9	–	33
2012.06	CALGB30406 mut$^+$	66	33	Continuous erlotinib alone	14.1	70	31.3
			33	Continuous erlotinib in combination with carboplatin and paclitaxel (ECP) for six cycles followed by erlotinib alone	17.2	73	38.1
2013.06	FASTACT-2 mut$^+$	97		Six cycles of gemcitabine (days 1 and 8) plus platinum (carboplatin on day 1) with intercalated erlotinib (days 15–28) every 4 weeks	16.8	31.4	–
2015.02	NEJ005	80	41	Concurrent gefitinib and carboplatin (day 1)/pemetrexed (day 1) in a 3-week cycle for up to 6 cycles, followed by concurrent gefitinib and pemetrexed maintenance	18.3	87.8	41.9
			39	8 weeks of gefitinib and then 2 cycles of carboplatin/pemetrexed; repeated 3 times (carboplatin/pemetrexed was repeated for 6 cycles), followed by alternating gefitinib and pemetrexed maintenance	15.3	84.6	30.7
2015.06	Yoshimura N etc	26		Pemetrexed (day 1) and gefitinib (days 2–16) every 3 weeks	18.0	32	84.6
2015.06	Kanda S etc	34		Gefitinib (days 1–56), then, after a 2 week drug-free period, three cycles of cisplatin and docetaxel (days 71, 92, and 113), thereafter, gefitinib was re-started on day 134 and continued until disease progression	19.5	48	–
2016.08	JMIT	195	129	Pemetrexed (day 1, every 21 day) plus gefitinib	15.8	–	80
			66	Gefitinib alone	10.9	–	74
2017.09	Han B etc	121	40	Pemetrexed (day 1) plus carboplatin (day 1) combined with gefitinib (days 5–21) and repeated every 4 weeks for up to six cycles and then continued to receive pemetrexed combined with gefitinib every 4 weeks	17.5	82.5	32.6
			40	Pemetrexed (day 1) plus carboplatin (day 1) every 4 weeks for up to six cycles and then continued to receive pemetrexed alone every 4 weeks	5.7	32.5	24.3
			41	Gefitinib alone	11.9	65.9	25.8

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Compliance with ethical standards

Conflict of interest All authors declared that they have no competing interests.

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