www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 41), pp: 71358-71370

Review

The resistance mechanisms and treatment strategies for *EGFR*-mutant advanced non-small-cell lung cancer

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Keywords: *EGFR mutation, advanced NSCLC, EGFR-TKIs resistance mechanism, precision medicine*

Received: *May 04, 2017* ***Accepted:*** *August 06, 2017* ***Published:*** *August 17, 2017*

ABSTRACT

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) have been established as the standard therapy for *EGFR*-sensitizing mutant advanced non-small-cell lung cancer (NSCLC). However, patients ultimately develop resistance to these drugs. There are several mechanisms of both primary and secondary resistance to EGFR-TKIs. The primary resistance mechanisms include point mutations in exon 18, deletions or insertions in exon 19, insertions, duplications and point mutations in exon 20 and point mutation in exon 21 of *EGFR* gene. Secondary resistance to EGFR-TKIs is due to emergence of T790M mutation, activation of alternative signaling pathways, bypassing downstream signaling pathways and histological transformation. Strategies to overcome these intrinsic and acquired resistance mechanisms are complex. With the development of the precision medicine for advanced NSCLC, available systemic and local treatment options have expanded, requiring new clinical algorithms that take into account resistance mechanism. Though combination therapy is emerging as the standard of to overcome resistance mechanisms. Personalized treatment modalities based on molecular diagnosis and monitoring is essential for disease management. Emerging data from the ongoing clinical trials on combination therapy of third generation TKIs and antibodies in *EGFR* mutant NSCLC are promising for better survival outcomes.

INTRODUCTION

The development of epidermal growth factor receptor (EGFR)-targeted tyrosine kinase inhibitors (TKI) has led to significant advances in patients with tumors harboring *EGFR* mutations (*EGFR*m). Approximately 50% of Asian patients with NSCLC have *EGFR* mutations [1]. In patients with sensitizing mutations of *EGFR*, first-line TKI treatment has good response rates of 50 to 80% compared to conventional chemotherapy that has response rate of 30%.Despite the demonstrated benefits of EGFR-targeting therapies, not all patients respond to treatment, moreover, patients who respond to EGFR-TKIs ultimately develop resistance to these drugs with the median progression-free survival (mPFS) around 9 to 13 months [2, 3]. Despite *EGFR* sensitizing mutations which show good response to EGFR TKIs, other *EGFR* mutations or pathways may play a role in development of resistance to EGFR TKIs. There are various mechanisms for development of resistance to EGFR TKIs. These include emergence of secondary mutation T790M, an amino acid substitution at position 790 in EGFR from a threonine to a methionine (T790M), bypass signaling activation , phenotype transition and aberrant downstream signaling pathways [4, 5]. Approximately 50% of patients who respond well initially to TKIs develop resistance due to the occurrence of secondary mutation T790M, This is the most common mechanism of acquired resistance to EGFR-TKIs [6, 7]. Till recently, no optimal therapy was available for NSCLC patients with T790M mutation in China. Hence, new clinical algorithms that take into account resistance mechanism and clinical pattern are warranted. The aim of this paper is to provide a comprehensive review of mechanisms of resistance to EGFR-TKIs and the potential treatment strategies to overcome this resistance in patients with advanced NSCLC.

The resistance mechanisms of EGFR-TKI

Primary resistance

Primary, intrinsic or de novo resistance is defined as the failure to respond to the treatment at the first time after receiving TKI and presents no obvious symptoms improvement, disease control or overall survival (OS) [8, 9]. Primary resistance could be categorized into four classes: TKI resistance in the presence of a drug-sensitizing EGFR mutations, drug resistant EGFR mutations, genomic alterations along with EGFR mutations and EGFR wild-type tumors. The common mutations that confer primary resistance to TKIs are the result of non-sensitive EGFR mutation. Exon 19 mutations L747S/D761Y, exon 20 mutation T790M and exon 21 mutation T854A are some of the mutations that are associated with primary resistance [5]. Approximately 4% subgroup harbors insertions in *EGFR* exon 20 that account for intrinsic resistance [10]. *In vitro* studies showed that exon 20 `mutations were less sensitive to TKIs compared to exon 19 deletion and exon 21 point mutation (L868R) [11]. A concomitant T790M mutation in TKI naïve patients could also be the potential cause for intrinsic resistance [12]. Despite *EGFR*-related mutations, other mutations localized at the genes affecting EGFR downstream signaling might also lead to intrinsic resistance. Deletion of tumor suppressor gene *PTEN* or *PIK3CA* mutation can lead to aberrant activation of PI3K/AKT pathway [13, 14]. Other mechanisms, the crosstalk of EGFR and IGF1R pathway , the activation of NFκB pathway the polymorphisms of pro-apoptotic protein BIM, are also considered as the potential mechanisms for intrinsic resistance [15–17]. Increased expression of HGF, increases binding of MET-mediated activation of the PI3K–AKT pathway, thus hindering the ability of an EGFR TKI to inhibit this signaling pathway. The role of MET in primary resistance owing to increased HGF activation of MET is through GAB1 [21, 22]. IPASS clinical trial demonstrated insensitivity to gefitinib in tumors negative for EGFR mutations [24]. Studies have also reported role of *KRAS* mutations, *BRAF* mutations, *ALK* translocation and overexpression of HGF in primary resistance to EGFR TKIs [18–21]. BRAF mutations are present in approximately 2–3% EGFR wild-type tumor also confer intrinsic resistance due to the presence of other somatic mutations in genes encoding signaling molecules [19, 23]. Approximately 5% of tumors harbor ALK translocations that are reported to be insensitive to EGFR TKIs [20, 25].

Acquired resistance

A clinical definition of acquired resistance to EGFR TKIs has been proposed that is systemic progression (by RECIST or WHO criteria) after a complete or partial response or ≥ 6 months of stable disease after treatment with a targeted therapy [26, 27].

Secondary mutations of EGFR gene

The secondary mutations of *EGFR* gene that cause EGFR-TKI acquired resistance include T790M (exon 20), L747S (exon 19), D761Y (exon19) and T854A (exon 21) [28–30]. The most common mechanism of acquired resistance is due to emergence of T790M mutation, approximately 50% of NSCLC patients develop resistance to TKIs [31, 32]. Potential mechanism by which T790M causes resistance to EGFR-TKIs is by increasing the affinity to ATP [9, 33]. The mutation, results in substitution of threonine to methionine at the "gatekeeper" amino acid 790, leading to a bulkier side thus causing TKI medications to lose potency; This causes steric hindrance and the affinity of ATP at EGFR kinase domain recovers, which may lead to kinase phosphorylation and thereby activating the signaling pathways associated with tumor progression [34].

Bypass signaling activation

The tumor enhancing effects caused by *EGFR*-sensitizing mutations has impact on the activation of downstream signaling pathway [35]. Despite blocking the downstream signaling pathways by EGFR-TKI, other signaling pathway, such as *c-Met*, could initiated and facilitate tumor progression [36]. Several studies reported approximately 5–20% subsets harboring *c-Met* gene amplification in TKI resistant NSCLC patients [7, 37–39]. Additionally, *c-Met* gene amplification can be an independent to T790M to confer resistance to EGFR-TKIs[40]. *c-Met* gene amplification activates ERBB3 and thus activates the downstream PI3K/AKT pathway causing gefitinib and erlotinib resistance [41]. Therefore *c-Met* gene amplification could bypass the inhibited EGFR phosphorylation kinase pathway and activate downstream signal transduction, thus facilitating the tumor cells proliferation and developing resistance to EGFR-TKI. Other mechanisms of TKI resistance include *Her-2* amplification *MAK* amplification and *BRAF* (G469A and V600E)mutation [18, 42–43], *Her-2* amplification is reported in 10–20% of NSCLCs [44–47] and BRAF mutation is reported in 1–4% of NSCLCs [23, 48–50].

Phenotype transition

Phenotype transition includes transition to small cell lung cancer (SCLC) and epithelial mesenchymal transition (EMT). Sequist *et al.* found that 14% advanced NSCLC patients harboring *EGFR*-sensitizing mutations transformed into SCLC after TKI treatment [38]. Similar studies demonstrated SCLC transformation after TKI treatment in advanced NSCLC patients harboring *EGFR*-sensitizing mutations [51–53]. Therefore, it can be speculated that NSCLC transforming into SCLC might be one of the mechanisms of acquired EGFR-TKI and moreover, these SCLC also harbors similar EGFR mutations prior to transformation. In addition,

Uramoto *et al.* indicated that about 40% (4/9) of patients with EGFR-TKI resistance presented epithelial cell transformation to mesenchymal cell, thus forming tumor stem cells, reducing the dependence on EGFR signaling pathway, and eventually leading to tumor malignance progression or metastasis [54].

Other causes

Several other EGFR-TKI resistance mechanisms have been identified including: downstream signaling pathway activation, such as the overexpression of insulin-like growth factors-1 receptor (IGF-1R). TKI could induce the formation of heterodimer of EGFR and IFG-1R in NSCLC cellular membrane, activate IFG-1R and its downstream signal transduction (PI3K/Akt), enhance anti-apoptosis effect and hence develop resistance to TKI [55].

Treatment strategies for EGFR-TKI resistance

Treatment strategies for intrinsic EGFR-TKI resistance

Based on the mechanisms of EGFR intrinsic resistance caused by the concomitant gene mutations, combination therapy may be one potential solution. The combination of EGFR-targeted inhibitors and IGFIR inhibitors could potentially overcome resistance [56]. In mutant *EGFR* NSCLC patients, BCL-2 antibody triggers TKI-mediated apoptosis improving TKI efficacy [57, 58]. In patients with early disease stage (resected stage II-IIIA) with EGFR mutated NSCLC adjuvant chemotherapy/TKI treatment or both could be considered as an effective treatment. In patients with chemotherapy-naïve advanced EGFR mutated NSCLC chemotherapy or TKI treatment before after chemotherapy or concurrently could be an effective treatment [59]. In patients with late stage disease combination therapy has shown to be beneficial to prevent or delay emergence of resistance. Similarly, combination therapy is also seen to be effective in patients with other genomic alterations occurring with EGFR mutations [60, 61].

Treatment strategies for acquired resistance

Treatment strategies based on clinical assessment

Yang *et al.* classified tumor progression after EGFR-TKI failure into three modes as gradual progression, local progression, and dramatic progression based on radiological and clinical examination results [62]. Disease control (lasting ≥ 6 months) with no significant increment in tumor burden in comparison with earlier assessment and asymptomatic status of pre-existing item (Symptom scored ≤ 1) with EGFR-TKI treatment is defines as gradual progression [63–65]. Disease control lasting ≥ 3 months with EGFR-TKI treatment with PD due to solitary extracranial lesion or limitation in intracranial lesions and asymptomatic status or stability of pre-existing item (Symptom scored ≤ 1) is defined as local progression [66–70]. Disease control (lasting ≥ 3 months) with rapid progression of multiple targeted lesions compared to previous assessment, or progressive involvement of non-target lesions with a score > 2 along with deterioration of any pre-existing item or new item (Symptom scored 2) after TKI treatment is defined as dramatic progression [64–66]. Yang et al. classification of clinical modes of EGFR-TKI failure could plausibility be beneficial in planning treatment modalities and predicting survival benefits in management of these patients [62].

In patients with gradual progression failing EGFR-TKI treatment, continuation of EGFR-TKI is recommended as TKI treatment after progression also showed good median PFS (14.9) months and could delay salvage therapy [71]. In patients with local progression, it is recommended to continue TKI therapy in conjunction with local therapy [62]. Yu *et al.* reported median OS up to 41 months in combination therapy of local therapy (surgical resection, radiofrequency ablation, or chemotherapy) combined with EGFR-TKI treatment after acquired resistance to EGFR-TKIs [72]. Gomez et al. reported improved progression free survival with local consolidated therapy with or without maintenance therapy compared to maintenance therapy alone [73]. However, there is still no sufficient evidence proving that local therapy in conjunction with EGFR-TKI is superior to local therapy alone or EGFR-TKI mono-therapy, respectively. In patients with dramatic progression, it is not recommended to continue TKI medications. As histological transformation to small-cell lung carcinoma occurs during active treatment with targeted therapy, in dynamic progression it is recommended to do biopsy to capture these histological and phenotypic changes as this could impact the chemotherapy options [38, 74–75]. Few studies suggest to continue chemotherapy after patients acquiring resistance [76, 77]. Kuo *et al.* reported higher remission rate (48.7% *vs* 21.4%), longer medium PFS (5.1 months *vs* 1.8 months) and medium OS (12.7 months *vs* 7 months) after receiving taxane-based chemotherapy than patients in nontaxane regimen [76] IMPRESS, a phase 3 randomized trial showed that progression-free survival was not prolonged in patients on continuation of gefitinib after disease progression compared to platinum-based doublet chemotherapy suggesting the later to be an gold-standard in such settings [78]. Nevertheless, more prospective studies are warranted for evaluating optimum chemotherapy strategy in this subset of patients.

Gandara et al. classified progressive disease (PD) into three categories as 1) CNS Sanctuary PD 2) Oligo-PD and Systemic PD in the settings of acquired resistance to EGFR-TKIs. These subtyping has implications in clinical settings and patient management [79]. In CNS sanctuary PD local therapy (surgical resection, focused radiotherapy or whole brain radiotherapy,) with continuation of TKIs is recommended. This approach had

implications in extending PFS in this subtype of patients as reported by Gomez et al. in an randomized controlled trial and observational studies. [73, 80–81]. In oligo-PD a similar approach of treatment is seen to be beneficial with combination of local therapy and continuation of TKIs [73, 81]. In systemic PD several treatment strategies are available which include switching therapy or continuing with the same TKI to anticipation of slowing PD and continuation of the same TKI with addition of other therapies [3, 82–83].

Targeted therapy based on acquired resistance mechanisms

Treatment strategies targeting secondary EGFR mutations

With the development of molecular biology, molecular targeted therapy based on the molecular mechanism of EGFR-TKI and targeted diverse resistance mechanisms has become the new direction fir advanced NSCLC precision medicine.

Emergence of *EGFR* T790M mutation is the preliminary mechanism of the acquired TKI resistance to the first-generation EGFR-TKIs (erlotinib and gefitinib). Although, the second-generation EGFR-TKIs (afatinib and dacomitinib) showed activity overcoming T790M mutation resistance in *in vitro* studies, clinical studies had disappointing results. Studies showed second-generation EGFR-TKI medications (afatinib and dacomitinib) had a remission rate lower than 10% in patients with TKI resistant advanced NSCLC [84, 85]. Since the efficacy of second-generation TKI monotherapy was not satisfactory, combination therapy has been explored. Janjigian *et al.* found that afatinib combined with cetuximab in treating EGFR-TKI resistant NSCLC patients harboring *EGFR*-sensitizing mutations could achieve an ORR of 29% and PFS of 5.7 months. However, the ORR between T790M-positive and T790M-negative patients was similar (32% vs 25%, P=0.341) and the overall efficacy was far from satisfaction, and the rate of side effects was high in combination medications, with the most common being rash (90%), diarrhea (71%), and stomatitis (56%) [86]. In two more studies, the combination therapy of cetuximab with erlotinib or gefitinib had no effect on OR [87, 88].

Compared to the second-generation TKIs, the third-generation EGFR-TKI selectively target T790M mutations, and irreversibly bind to EGFR ATP pocket, inhibiting EGFR kinase phosphorylation and the activation of downstream tumor signaling pathway. To date, the third-generation EGFR-TKI medications includes Osimertinib (AZD9291), CO-1686, HM61713, EGF816, ASP8273 and China local Avitinib [89–92]. Osimertinib (AZD9291) is small molecule, with high selective and irreversible suppressive effects on both NSCLC *EGFR*-sensitizing and T790M resistance mutations [89]. Osimertinib was approved for the treatment of advanced NSCLC patients with *EGFR* T790M mutations and acquired TKI resistance by FDA in November, 2015 [93]. In 2016, Osimertinib was further approved by EMA for the treatment of locally advanced or metastasis NSCLC patients with *EGFR* T790M mutation [94]. Recently, osimertinib has been approved in China by China food and Drug administration (CFDA) for metastatic or locally advanced NSCLC with T790M mutation [95]. Osimertinib has been approved for clinical usage in USA, EU, Japan, Canada, Switzerland, Israel, Korea and Mexico, mainly based on two AURA clinical trials [96–99]. In these two trials, a total of 411 patients with first-generation TKI resistance and EGFR T790M mutation were administrated with Osimertinib at the dosage of 80 mg/d. Patients with T790M mutations demonstrated response rate of 66–71% and median PFS of 9.6–10.1 months. For these patients, the median PFS in standard chemotherapy is estimated to be less than half a year [96, 97]. The preliminary data on osimertinib as first-line treatment in EGFR mutated treatment naïve NSCLC patients showed median progression free survival of 19.3 months (95% CI, 13.7, Not Calculable) with disease control rate of 97% (95% CI 88.5–99.6) and progression free survival in 55% of patients at 18 months with manageable safety profile [100]. The ongoing FLAURA (NCT02296125), a randomized, double-blind phase III trial to assess the efficacy and safety of osimertinib versus erlotinib/gefitinib as first-line therapy in EGFR mutated locally advance NSCLC are much awaited to position osimertinib as frontline therapy [101]. TATOON (NCT02143466), a multi-arm, phase 1b, study assessing the safety and tolerability of osimertinib with durvalumab versus osimertinib monotherapy has been suspended on the grounds of safety due to increased incidence of interstitial lung disease [102, 103]. In contrast, another third-generation EGFR-TKI, CO-1686, that had selective potency to T790M showed ORR of 28–34% in T790M positive patients [104]. However, CO-1686 and HM6171 are withdrawn from development due to poor efficacy outcomes [105, 106]. In addition, preliminary data on HM61713 revealed an ORR of 62% in T790M positive patients [107]. Other third-generation TKIs EGF816, ASP8273 and Avitinib are still in different stages of clinical development. Third-generation TKIs impact on clinical outcomes in EGFR mutated NSCLC patients are summarised in Table 1. Long-term follow-up studies are warranted to determine the efficacy of these third-generation EGFR-TKI.

Treatment strategies targeted at non-EGFR related secondary mutations

Besides T790M mutation, there are other mechanisms accounting for the resistance in nearly half of lung cancer patients, i.e. *c-Met* amplification, *Her-2* amplification, *PIK3CA* mutations and *BRAF* mutations.

Table 1: Third generation TKIs impact on clinical outcomes of EGFR T790M mutation positive and negative NSCLC patients

Study group	Treatment strategy (TKI-naïve/post-TKI)	Treatment	T790M positive				T790M Negative			
			ORR	DCR	Median PFS	Other outcomes	ORR	DCR	Median PFS	Other outcomes
Janne *et al*. [72]	Post TKI	Osimertinib (AZD9291)	61%	95%	9.6 months	–	21%	61%	6 months	–
Oxnard GR *et al.* [73]	Post TKI	Osimertinib (AZD9291)	62%	–	9.7 months	–	26%	–	3.4 months	–
Park *et al.*, [78]	Post TKI	HM61713	62%	91%	–	–	–	–	–	–
Yang J *et al.*, [74]	Post TKI	Osimertinib (AZD9291)	71%	–	9.7 months	DoR: 9.6 months	–	–	–	–
Wu YL et al, [81]	Post TKI	Avitinib (AC0010)	44%	85%	–	–	–	–	–	–

'–': not reported.

For these patients, combination therapy of inhibiting EGFR pathway together with bypass pathways signaling might overcome the acquired resistance. A phase II study investigated the therapeutic efficacy of a cMET inhibitor, INC280 or Capmatinib in combination with gefitinib for NSCLC patients with EGFR-TKI resistance and *cMET* positive, which demonstrated ORR of 18% (12/65) and DR of 62% (40/65) [108]. Neal *et al.* reported a phase II study combining Cabozantinib (MET inhibitor) and Erlotinib showed a longer median PFS, as compared with Erlotinib monotherapy (3.9 month vs 1.9 month) [109]. In addition, a Phase I clinical trial showed that the combination therapy of Erlotinib and PI3K inhibitor (BKM120) was well tolerated in advanced NSCLC patients with *EGFR*-sensitizing mutations and acquired resistance [110].

Cisplatin and etoposide-based therapy could be considered in patients with phenotypic modulation to SCLC [111]. The third-generation EGFR-TKI also somehow demonstrated efficacy in acquired-resistant patients with unclear mechanism. AURA phase-I trial indicated that Osimertinib had certain efficacy in T790M mutation-negative NSCLC patients with EGFR-TKI resistance, the subgroup at the dosage of 120mg/d demonstrating a 30% response rate. However, it should be noted that there are significant individual variations [112]. This might be attributed to tumor heterogeneity and the technical limitations in detecting T790M mutations. Different mechanisms of acquired resistance might occur in different specimens from the same *loci*, or from the different *loci*. Furthermore, even if for the potential T790M mutation-positive clone, when the concentration is lower than detection level, the result could show as negative. Hence, in AURA Phase I study, the demonstrated efficacy of Osimertinib in TKI resistant patients without EGFR T790M mutations might actually because of the undetectable T790M mutation clones. Therefore, it is still controversial regarding the effect of Osimertinib on TKI resistance induced by non-T790M mutations. Moreover, IMPRESS study showed that combination treatment of gefitinib and chemotherapy (cisplatin and pemetrexed) resulted in a PFS of 6.7 months in advanced NSCLC patients with TKI resistance T790M mutation negative patients [113]. Large scale studies are warranted to study the continuation of first-generation TKI in conjunction with chemotherapy which might be an alternative option for this subset of patients with *EGFR*-T790M mutation TKI resistant patients.

Diagnosis and monitoring of EGFR-TKI resistance

Lung cancer management guidelines recommend molecular testing as a standard of care in patients with NSCLC [114–117]. As mutations, play an important role in determining personalized treatment, molecular mechanisms underlying these mutations is essential. Due to the intrinsic and acquired resistance mechanisms to EGFR-TKIs, dynamic monitoring of these mutations will be beneficial for early diagnosis and administration of effective drugs to overcome these resistance mechanisms and improve survival outcomes. Repeat biopsy is not feasible for monitoring disease and treatment response due to its limitations in terms of practicality, invasiveness, risk, availability of tissue, heterogeneity of tissue and advanced disease condition. Circulating tumor DNA (ctDNA) has emerged as a biomarker for real-time monitoring due to its less invasiveness, practicality and accessibility

Table 2: Summary of clinical trials on combination therapy of EGFR-TKI with antibodies for treatment of NSCLC

Trial number	Indication/EGFR Mutation status	Phase	Drug	Treatment	Sponsor
NCT02454933	T790M positive advanced NSCLC	III	Osimertinib MEDI4736	Osimertinib And MEDI4736 Vs Osimertinib monotherapy	AstraZeneca
NCT02143466	Advanced EGFR mutation positive NSCLC	I	Osimertinib AZD6094 MEDI4736	Osimertinib and AZD6094 Osimertinib and selumetinib Osimertinib and MEDI4736 Vs AZD6094 monotherapy	AstraZeneca
NCT02789345	Advanced T790M mutation positive NSCLC	I	Ramucirumab Necitumumab Osimertinib	Ramucirumab or Necitumumab in combination with Osimertinib A	Eli Lilly and Company
NCT02040064	EGFR mutant NSCLC	I	Gefitinib Tremelimumab	Gefitinib and Tremelimumab	Gustave Roussy, Cancer Campus, Grand Paris
NCT03054038	Advanced EGFR mutant NSCLC	I	Afatinib Necitumumab	Afatinib and Necitumumab	Vanderbilt-Ingram Cancer Center
NCT02716311	EGFR mutated NSCLC	II	Afatinib Cetuximab	Afatinib and Cetuximab	Intergroupe Francophone de Cancerologie Thoracique

[118–120]. Several studies have shown concordance between tissue and blood based testing [118, 119, 121]. In sight of FDA approval for blood based testing using cobas *EGFR* mutation test for detecting EGFR mutations, it is practically possible to provide personalized treatment [122, 123]. Dynamic monitoring of T790M mutation plays an important role in patient management by early administration of third generation TKIs.

It is reported that the T790M mutation is detectable in plasma much earlier than the clinical manifestation of disease progression (PD) which is aided by dynamic monitoring of T790M mutation. Lee *et al.* monitored *EGFR* mutations during gefitinib or erlotinib treatment in plasma samples (N=367) revealed T790M detection rate of 28.6% in patients with PD. Among these 16.3% patients were detected with T790M mutation 2–12 months before PD and 12.2% during PD suggesting the importance of serial monitoring using plasma especially in patients with TKI resistance [124]. Similarly another study evaluated the clinical outcomes using quantification and dynamic monitoring of T790M mutation in plasma (N = 117) showed mutations in 47% of patients who developed TKI resistance (first or second line TKI) and in half of these positive patients the median time of early detection was 2.2 months before PD. Patients with detectable T790M showed lower OS compared to T790M negative group (26.9 months vs NA), moreover this positivity was associated with poor OS outcomes as identified by analysis of univariate and multivariate Cox proportional hazard model (HR = 1.716, 95% CI:1.014–2.903, P = 0.0443) [125]. Marchetti *et al.* reported early detection (at 35 days) of T790M in plasma after initiating erlotinib in slow responders, indicating development of early resistance [126]. These studies emphasize the importance of dynamic monitoring in treatment response and planning treatment strategies in these resistant NSCLC patients.

Present research and future perspective

With the wide application of the third-generation EGFR-TKIs, it is demanded to develop the strategies overcoming acquired resistance to the third-generation EGFR-TKIs. To date, some ongoing clinical trials are engaged to determine the superiority of the combination of the third-generation EGFR-TKIs (Osimertinib, CO-1686 or others) with other agents (antibodies to BCL-2, MET or MEK), to third-generation EGFR-TKIs monotherapy. Moreover, studies testing EGFR-TKIs combined with EGFR monoclonal antibody are also currently ongoing. Table 2 summarizes the clinical trials on combination therapy of EGFR-TKIs with antibodies for treatment of NSCLC. These studies would spread the lights on the treatment of EGFR-TKI resistant NSCLC patients in the near future.

CONCLUSIONS

EGFR-TKI is the standard treatment for advanced NSCLC patients harboring *EGFR*-sensitizing mutations. However, intrinsic and acquired resistance is unavoidable. Currently the key treatment approach for EGFR-TKI resistance is to be based on EGFR-TKI resistance mechanism and clinical implications. However, due to tumor heterogeneity and the limitation of second biopsy, targeted at one single resistance mechanism might be insufficient. Therefore, the combination overcoming multiple resistance mechanisms is the new direction under the era of precision medicine.

ACKNOWLEDGMENTS

The authors acknowledge Dr Anuradha Nalli (PhD) and Dr Amit Bhat (PhD) (Indegene, Bangalore, India) for providing medical writing support and technical assistance in the development of this manuscript.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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