

Special Article - Lung Cancer

C797S Mutation in Resistance Development to Third-Generation Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitors in Lung Cancer

Tripathi SK and Biswal BK*

Department of Life Science, National Institute of Technology Rourkela, India

***Corresponding author:** Bijesh K Biswal, Department of Life Science, National Institute of Technology Rourkela, Cancer Drug Resistance Laboratory, Odisha-769008, India**Received:** July 17, 2019; **Accepted:** August 01, 2019;**Published:** August 08, 2019**Abstract**

Lung cancer is the most difficult types of cancer to treat. Overexpression of Epidermal Growth Factor Receptor (EGFR) is main causative agent in almost all lung cancer types. All the drugs used for lung cancer treatment have targeted to EGFR mutation. Earlier, first- and second-generation Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitors (EGFR-TKIs) were commonly used drugs for the treatment of EGFR mutant lung cancer patients. The main problem with this treatment is development of drug resistance within a few months of treatment. Recently, third-generation EGFR-TKIs such as osimertinib, rociletinib, olmutinib, etc. have been developed to target and inhibit T790M mutant and acquired resistant EGFR in lung cancer patients. Patients developed resistance to third-generation EGFR-TKIs has C797S mutation. C797S mutation is responsible for loss of sensitivity to third-generation EGFR-TKIs. Considering these observation next-generation EGFR-TKIs are the demand of current scenario which can overcome C797S mediated acquired resistance to third-generation EGFR-TKIs in lung cancer patients. Combinatorial treatment strategy has been emerged as an effective treatment policy to overcome T790M and C797S mutations mediated lung cancer drug resistance.

Keywords: Lung cancer; EGFR; C797S mutation; EGFR-TKIs**Introduction**

Lung cancer is the most difficult types of cancer to treat. Overexpression of Epidermal Growth Factor Receptor (EGFR) is main causative agent in almost 10%-20% of reported lung cancer [1]. Mutations such as deletion in exon 19 to exon 21, L858R, etc. are foremost causative agent for EGFR overexpression. Earlier, first- and second-generation Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitors (EGFR-TKIs) such as erlotinib, gefitinib, afatinib, dacomitinib, etc. were commonly used drugs for the treatment of EGFR mutant lung cancer patients [2-4]. First-generation EGFR-TKIs have ability to target and bind with threonine on 790 position in ATP binding pocket of the EGFR kinase domain. Initially, most of the patients responded very impressively to first-generation EGFR-TKIs drug treatments but unfortunately, patients developed resistance and stop responding within a few months of treatment [5-7]. Several mechanisms are responsible for acquired resistance to first-generation EGFR-TKIs including T790M mutation. Therefore, second-generation EGFR-TKIs were developed that could irreversibly bind to Cysteine 797 in ATP binding pocket of the T790M mutant EGFR kinase domain and sensitized resistant EGFR [8]. However, the major drawback with second-generation EGFR-TKIs is high toxicity (rashes and diarrhea) at required dose for inhibition of wild type EGFR [9]. Recently, third-generation EGFR-TKIs such as osimertinib, rociletinib, olmutinib, etc. have been developed to target and inhibit T790M mutant and acquired resistant EGFR in lung cancer patients [10,11]. These EGFR-TKIs also have covalent binding ability to cysteine 797 in ATP binding pocket of EGFR kinase domain

at very low toxicity. However, new reason to acquired resistance to third-generation EGFR-TKIs named C797S mutation has come in knowledge in recent pre-clinical studies [12]. It has been observed from patient's cell free plasma DNA samples that patients developed resistance to third-generation EGFR-TKIs have C797S mutation [11,13]. Further studies also confirmed that C797S mutation is responsible for loss of sensitivity to third-generation EGFR-TKIs. Considering these observation next-generation EGFR-TKIs are the demand of current scenario which can overcome C797S mediated acquired resistance to third-generation EGFR-TKIs in lung cancer patients. Here, we will abridges the novel C797S mutation mediated acquired resistance to third-generation EGFR-TKIs in lung cancer, based on latest available studies. In this review, we will also focus on possible strategies to overcome C797S mutation mediated acquired resistance to third-generation EGFR-TKIs in lung cancer.

Third-Generation EGFR-Tkis and Lung Cancer

Due to development of resistance to first- and second- generation EGFR-TKIs in lung cancer patients, some novel, irreversible, and target selective third-generation EGFR-TKIs have been developed. These third-generation EGFR-TKIs including rociletinib, osimertinib, olmunitib, etc. can effectively suppress the wild type EGFR at very low toxic dose by binding to C797 residue in ATP binding pocket of EGFR kinase in a covalent manner. However, their main targets are EGFR activating mutation and C797S mutation and they have 100-200 times more prone to bind with mutated EGFR as compared to wild type EGFR. Therefore, third-generation EGFR-TKIs provide

more clinical benefits to patients developed resistance to first- and second-generation EGFR-TKIs. A brief detail of different types of third-generation EGFR-TKIs are as follow.

Osimertinib

Osimertinib, also known as mereletinib or AZD9291 is a cysteine-797 residue (ATP binding site of the EGFR kinase) selective third generation EGFR-TKI that was developed by AstraZeneca Pharmaceuticals [11,13,14]. It has ability to form covalent bond with C797 position in ATP binding site of the EGFR kinase in an irreversible manner [15,16]. Osimertinib has especially designed to target and overcome T790M mutation mediated acquired resistance to the first- and second-generation EGFR-TKIs in lung cancer patients [10]. It selectively targets 200 times more potently, L858R/T790M mutant EGFR as compared to wild-type EGFR [1]. Currently, osimertinib is the only EGFR-TKI drug that has been approved for treatment of lung cancer metastatic patients with EGFR T790M mutation in United States and many other European countries [15-17]. It had been granted approval by FDA and European Commission (EC) in 2015 and 2016, respectively and available for patients in 2017. Osimertinib showed significant antitumor activity in mice xenograft models. Clinical trials also confirmed that osimertinib treatment significantly improved progression-free survival of T790M mutated EGFR positive lung cancer patient's resistance for platinum based chemotherapy [15,16]. Furthermore, *in-vivo* pharmacokinetic study also demonstrated very impressive bioavailability, wide tissue distribution, and moderate clearance properties of osimertinib [15,16]. Targeting ability to bypass downstream signaling pathways of EGFR signaling such as AKT and RAS pathway makes it a better therapeutic for the treatment of EGFR-mutated lung cancer patients.

Rociletinib

Rociletinib, a potent EGFR mutation selective third-generation EGFR-TKI has been developed by Clovis Oncology *Inc.* and had FDA approval in 2014. It is also known as AVL301 and CO1686. Rociletinib shows antineoplastic activity *via* irreversible binding and inhibition of both mutant and wild type EGFR [18]. Mainly, rociletinib targets and inhibits the secondary EGFR mutation (T790M) responsible for acquired drug resistance leading to cell death of these first- and second- generation EGFR-TKIs resistance lung cancer cells [18]. *In-vitro* comparison studies between rociletinib and erlotinib revealed that rociletinib had 22 times more selectivity towards mutant EGFR as compared to erlotinib [19]. Furthermore, these studies were also confirmed in xenograft and transgenic models [19]. Also, rociletinib showed better tumor growth inhibition ability in xenograft models as compared to afatinib. In phase I/II clinical trials of rociletinib (TIGER trials) hyperglycemia (grade III side effect) had been reported in approximately 22% of the patients [20]. Likely, to first- and second-generation EGFR-TKIs, lung cancer patients also gained resistance to rociletinib within few months of medication because of EGFR L798I, MET, PIK3CA, ERRB2, etc. mutations [1]. Studies also suggested some other possible causes of rociletinib resistance including MET amplification, EGFR overexpression, and loss of T790M mutation in lung cancer patients. Unfortunately, it has been observed that osimertinib treatment in rociletinib resistance lung cancer patients causes serious responses; therefore, FDA rejected the use of rociletinib in 2016. Then, Clovis Oncology, *Inc.* also decided to stop all ongoing studies on rociletinib after negative response from FDA.

Olmutinib

Olmutinib (HM61713), another novel third-generation EGFR-TKI has cysteine binding ability near to EGFR kinase domain in an irreversible and covalent manner [15,16]. Olmutinib was developed by Hanmi Pharmaceutical Co. Limited and granted FDA approval in December, 2015 for the treatment of NSCLC patients. Further, olmutinib granted approval by the Korean Ministry of Food and Drug Safety (MFDS) on May, 2016 to treat the locally advanced, metastasized, T790M mutated EGFR positive NSCLC patients [21,22]. It shows promising inhibitory activity against T790M and L858R mutant and exon 19 deleted lung cancer cells [23]. In phase I/II trials, thirty four NSCLC patients of advanced stage harboring acquired resistance to EGFR-TKIs with T790M mutation showed 97.1% disease control rate [24]. Interestingly, olmutinib treatment actively inhibited tumor growth and did not show any side effects in xenograft models of lung cancer cells such as H1975 and HCC827.

Naquotinib

Naquotinib (ASP8273) is one more target selective and irreversible third-generation EGFR-TKIs recently developed by Astellas Pharma Global Development, *Inc* [15]. It also has covalent binding ability with L858R/T790M mutant EGFR in the kinase domain cysteine residue on 797 position [16]. However, naquotinib shows limited activity against NSCLC patients having wild type EGFR. In addition, 14 days treatment of naquotinib promoted complete deterioration of tumor in xenograft mouse models [25]. Naquotinib shows more uniqueness over other third-generation EGFR-TKIs as it responses very nicely on osimertinib and rociletinib EGFR-TKIs resistant NSCLC cell lines [26].

Nazartinib

Nazartinib (EGF816; Novartis Pharmaceuticals) has EGFR mutant selective and irreversible target binding ability and can sensitize the T790M and EGFR mutants NSCLC [27]. It can covalently bind with multiple mutant forms of EGFR leading to inhibition of EGFR-mediated downstream signaling pathways [28]. Studies suggested that nazartinib shows 60 times more selectivity for mutant EGFR over wild type EGFR [15,16]. Nazartinib shows potent antineoplastic activity *Via* both regression and cell death of EGFR-overexpressed tumor cells. Therefore, nazartinib can be a possible novel treatment option in acquired drug resistance lung cancer patients due to T790M mutation.

C797S Mutation and Resistance to Third-Generation EGFR-Tkis

Currently, third-generation EGFR-TKIs are the most preferred drug for the treatment of acquired-resistant patient due to mutant EGFR. Several pre-clinical and clinical trials are still in progress to introduce newly developed third-generation EGFR-TKIs for the treatment of lung cancer patients. Many resistance mechanisms to third-generation EGFR-TKIs have also been described in *in-vitro* and clinical studies [29]. However, Cys797Ser, a point mutation in ATP binding pocket of EGFR tyrosine kinase domain of exon 20 of EGFR results acquired resistance to third-generation EGFR-TKIs in lung cancer patients [30]. C797S mutation abolishes the covalent binding of third-generation EGFR-TKIs to EGFR leading to acquired resistance against them in lung cancer patients [29,31].

Ercan et al. firstly identified this tertiary mutation (C797S) which promote resistance to osimertinib, rociletinib, and olmutinib in Ba/F3 cells [32]. Prior studies have documented that development of C797S mutation accounts approximately 19% acquired resistance to osimertinib and <3% to rociletinib in lung cancer patients [33]. It has also been observed that C797S mutation instigated resistance to third-generation EGFR-TKIs in both *in-vitro* and *in-vivo* models having exon 19 deletion or L858R mutation EGFR or T790M mutated EGFR. Another case report of a 57-year-old never-smoker female stage IV lung adenocarcinoma patient revealed that she acquired resistance to olmutinib because of C797S mutation [34]. To date, C797S mutation with exon 19 deletion has frequently shown in first-generation EGFR-clinical resistant cases [14]. Moreover, double mutant (EGFR activating mutation/C797S) drug resistant cell lines had been found to be sensitive for quinazoline-based EGFR-TKIs including gefitinib and afatinib, whereas triple mutant (EGFR activating mutation/T790M/ C797S) drug resistant cell lines did not show sensitivity for quinazoline-based EGFR-TKIs [32]. Further studies revealed that if these triple mutant cell lines were having C797S and T790M mutations in *trans* form, cell lines might be resistance to third-generation EGFR-TKIs, but showed sensitivity to combination of third- and first-generation EGFR-TKIs [12]. While, triple mutant cells with C797S and T790M mutations in *cis* form did not show sensitivity for any existing EGFR-TKIs, either alone or in combination [12]. Still, there is no information available on reproducibility of this *in-vitro* treatment combination (third- and first-generation EGFR-TKIs) for *trans* C797S at clinical level. In osimertinib trial, Thress and colleagues demonstrated that only one participant of trial had C797S mutation in *trans* form rest all had *cis* form [13]. To date, most of the results on C797S mutation mediated resistance to third-generation EGFR-TKIs are based on *in-vitro* studies, therefore *in-vivo*, pre-clinical, and clinical studies are highly needed. Overall, available studies concluded that C797S mutation may be a possible target to overcome acquired resistance to third-generation EGFR-TKIs in lung cancer patients after exploring at pre-clinical and clinical level.

C797S Mutation as a Novel Target to Overcome Third Generation EGFR-Tkis Resistance

To date, third-generation EGFR-TKIs are highly effective treatment option for patients acquired resistance to first-generation EGFR-TKIs [10,35]. Unfortunately, patients also started to developed resistance to third-generation EGFR-TKIs. Pre-clinical and clinical studies data revealed that C797S mutation in ATP binding domain of tyrosine kinase EGFR is responsible for the loss of sensitivity to third-generation EGFR-TKIs in lung cancer patients [13,34,36,37]. Therefore, targeting C797S mutation and discovery of novel fourth-generation allosteric C797S inhibitors might be a possible strategy for overcoming the acquired resistance to third-generation inhibitors in lung cancer patients. Treatment with EGFR/HER2/HDAC inhibitor (CUDC-101) and broad-spectrum protein kinase inhibitor (PKC412) had inhibited triple mutant cell line (EGFR activating mutation/T790M/C797S) at very low concentration [38]. Considering this observation, it seems that use of non-covalent EGFR inhibitors can re-sensitize C797S mutation mediated drug resistance. Previous studies clearly indicated that single agent may not be sufficient for the treatment of lung cancer due to emergence of resistance against drugs

with in few months of treatment. Currently, combinatorial treatment strategy has also been emerged as an effective treatment policy to overcome T790M and C797S mutations mediated drug resistance. The first allosteric TKIs inhibitor EAI045 has been developed whose binding site is away from the ATP-binding site of tyrosine kinase domain of EGFR. Therefore, treatment with EAI045 can overcome T790M and C797S mutations mediated resistance in lung cancer patients [39]. Further studies suggested that EAI045 was ineffective alone (cannot block receptor dimerization of receptor) but showed full activity against T790M and C797S mutation in combination with cetuximab (a monoclonal antibody that can block EGFR dimerization). The activity of EAI045 has been further characterized in Ba/F3 cells harboring L858R/T790M mutation, which leads to significant reduction in proliferation of Ba/F3 cells [39]. Similar results of EAI045 treatment were seen in Ba/F3 cells harboring L858R/T790M/C797S mutation and in mice having L858R/T790M/C797S tumor xenografts [39]. Uchibori K. and colleagues found that brigatinib treatment was very effective in triple mutant (L858R/T790M/C797S) cells with resistance to osimertinib and its treatment efficacy was enhanced significantly in combination with anti-EGFR antibody [40]. Therefore, brigatinib and anti-EGFR antibody can be used as a combination therapeutic to overcome resistance to third-generation EGFR-TKIs in triple-mutant EGFR. Overall, C797S mutation seems to be an ideal target to re-sensitize the developed resistance to third-generation EGFR-TKIs in lung cancer patients.

Conclusion and Future Prospective

To date, third-generation EGFR-TKIs are the most appropriate treatment option for lung cancer patients harboring resistance to first-generation EGFR-TKIs. Many third-generation EGFR-TKIs has already been approved for the treatment of T790M positive lung cancer patients. While, research on the improvement of existing third-generation EGFR-TKIs and development of other novel fourth-generation EGFR-TKIs are still going on. However, resistance also arises against third-generation EGFR-TKIs, mainly due to C797S mutation. Current approaches suggested that C797S mutation could be easily identified; therefore, it appears as an ideal target to overcome acquired resistance to third-generation EGFR-TKIs in lung cancer patients. Allosteric TKIs inhibitors have also been developed that can overcome C797S mutation mediated resistance in EGFR-TKIs resistant lung cancer patients as a combination therapeutic.

Acknowledgement

The authors thank Ministry of Human Resource and Development (MHRD), Government of India, Department of Science and Technology, Government of Odisha, India (Grant Number: 1201) and National Institute of Technology Rourkela, Odisha, India for providing laboratory and fellowship to SKT.

References

1. Van Der Steen N, Capareello C, Rolfo C, Pauwels P, Peters GJ, Giovannetti E. New developments in the management of non-small-cell lung cancer, focus on rociletinib: what went wrong? *Oncotargets and therapy*. 2016; 9: 6065.
2. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *The lancet oncology*. 2010; 11: 121-128.

3. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *The lancet oncology*. 2012; 13: 239-246.
4. Sequist LV, Yang JCH, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *Journal of clinical oncology*. 2013; 31: 3327-3334.
5. Kobayashi S, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *New England Journal of Medicine*. 2005; 352: 786-792.
6. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS medicine*. 2005; 2: e73.
7. Niederst MJ, Engelman JA. Bypass mechanisms of resistance to receptor tyrosine kinase inhibition in lung cancer. *Sci. Signal*. 2013; 6: re6-re6.
8. Reckamp KL, Giaccone G, Camidge DR, Gadgeel SM, Khuri FR, Engelman JA, et al. A phase 2 trial of dacomitinib (PF-00299804), an oral, irreversible pan-HER (human epidermal growth factor receptor) inhibitor, in patients with advanced non-small cell lung cancer after failure of prior chemotherapy and erlotinib. *Cancer*. 2014; 120: 1145-1154.
9. Camidge DR, Pao W, Sequist LV. Acquired resistance to TKIs in solid tumours: learning from lung cancer. *Nature reviews Clinical oncology*. 2014; 11: 473.
10. Cross DA, Ashton SE, Ghiorghiu S, Eberlein C, Nebhan CA, Spitzler PJ, et al. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer discovery*. 2014; 4: 1046-1061.
11. Helena AY, Tian SK, Drilon AE, Borsu L, Riely GJ, Arcila ME, et al. Acquired resistance of EGFR-mutant lung cancer to a T790M-specific EGFR inhibitor: emergence of a third mutation (C797S) in the EGFR tyrosine kinase domain. *JAMA oncology*. 2015; 1: 982-984.
12. Niederst MJ, Hu H, Mulvey HE, Lockerman EL, Garcia AR, Piotrowska Z, et al. The allelic context of the C797S mutation acquired upon treatment with third-generation EGFR inhibitors impacts sensitivity to subsequent treatment strategies. *Clinical Cancer Research*. 2015; 21: 3924-3933.
13. Thress KS, Paweletz CP, Felip E, Cho BC, Stetson D, Dougherty B, et al. Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. *Nature medicine*. 2015; 21: 560.
14. Jänne PA, Yang JCH, Kim DW, Planchard D, Ohe Y, Ramalingam SS, et al. AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. *New England Journal of Medicine*. 2015; 372: 1689-1699.
15. Cheng H, Nair SK, Murray BW. Recent progress on third generation covalent EGFR inhibitors. *Bioorganic & medicinal chemistry letters*. 2016; 26: 1861-1868.
16. Wang S, Cang S, Liu D. Third-generation inhibitors targeting EGFR T790M mutation in advanced non-small cell lung cancer. *Journal of hematology & oncology*. 2016; 9: 34.
17. Ayeni D, Politi K, Goldberg SB. Emerging agents and new mutations in EGFR-mutant lung cancer. *Clinical Cancer Research*. 2015; 21: 3818-3820.
18. Liu SV, Subramaniam D, Cyriac GC, Abdul-Khalek FJ, Giaccone G. Emerging protein kinase inhibitors for non-small cell lung cancer. *Expert opinion on emerging drugs*. 2014; 19: 51-65.
19. Walter AO, Sjin RTT, Haringsma HJ, Ohashi K, Sun J, Lee K, et al. Discovery of a mutant-selective covalent inhibitor of EGFR that overcomes T790M-mediated resistance in NSCLC. *Cancer discovery*. 2013; 3: 1404-1415.
20. Dhingra K. Rociletinib: has the TIGER lost a few of its stripes? *Oxford University Press*. 2016.
21. Steuer CE, Khuri FR, Ramalingam SS. The next generation of epidermal growth factor receptor tyrosine kinase inhibitors in the treatment of lung cancer. *Cancer*. 2015; 121: E1-E6.
22. Peters S, Zimmermann S, Adjei AA. Oral epidermal growth factor receptor tyrosine kinase inhibitors for the treatment of non-small cell lung cancer: comparative pharmacokinetics and drug-drug interactions. *Cancer treatment reviews*. 2014; 40: 917-926.
23. Kim ES. Olmutinib: first global approval. *Drugs*. 2016; 76: 1153-1157.
24. Park K, Lee JS, Lee KH, Kim J-H, Min YJ, Cho JY, et al. Updated safety and efficacy results from phase I/II study of HM61713 in patients (pts) with EGFR mutation positive Non-Small Cell Lung Cancer (NSCLC) who failed previous EGFR-tyrosine kinase inhibitor (TKI). *American Society of Clinical Oncology*. 2015.
25. Sakagami H, Konagai S, Yamamoto H, Tanaka H, Matsuya T, Mori M, et al. ASP8273, a novel mutant-selective irreversible EGFR inhibitor, inhibits growth of non-small cell lung cancer (NSCLC) cells with EGFR activating and T790M resistance mutations. *AACR*. 2014.
26. Goto Y, Nokihara H, Murakami H, Shimizu T, Seto T, Krivoshik AP, et al. ASP8273, a mutant-selective irreversible EGFR inhibitor in patients (pts) with NSCLC harboring EGFR activating mutations: Preliminary results of first-in-human phase I study in Japan. *American Society of Clinical Oncology*. 2015.
27. Jia Y, Juarez J, Li J, Manuia M, Niederst MJ, Tompkins C, et al. EGF816 exerts anticancer effects in non-small cell lung cancer by irreversibly and selectively targeting primary and acquired activating mutations in the EGF receptor. *Cancer research*. 2016; 76: 1591-1602.
28. Lelais G, Epple R, Marsilje TH, Long YO, McNeill M, Chen B, et al. Discovery of (R, E)-N-(7-Chloro-1-(1-[4-(dimethylamino) but-2-enoyl] azepan-3-yl)-1 H-benzo [d] imidazol-2-yl)-2-methylisonicotinamide (EGF816), a Novel, Potent, and WT Sparing Covalent Inhibitor of Oncogenic (L858R, ex19del) and Resistant (T790M) EGFR Mutants for the Treatment of EGFR Mutant Non-Small-Cell Lung Cancers. *Journal of medicinal chemistry*. 2016; 59: 6671-6689.
29. Eberlein CA, Stetson D, Markovets AA, Al-Kadhimi KJ, Lai Z, Fisher PR, et al. Acquired resistance to the mutant-selective EGFR inhibitor AZD9291 is associated with increased dependence on RAS signaling in preclinical models. *Cancer research*. 2015; 75: 2489-2500.
30. Yu Z, Boggon TJ, Kobayashi S, Jin C, Ma PC, Dowlati A, et al. Resistance to an irreversible epidermal growth factor receptor (EGFR) inhibitor in EGFR-mutant lung cancer reveals novel treatment strategies. *Cancer research*. 2007; 67: 10417-10427.
31. Planchard D, Loriot Y, Andre F, Gobert A, Auger N, Lacroix L, et al. EGFR-independent mechanisms of acquired resistance to AZD9291 in EGFR T790M-positive NSCLC patients. *Annals of Oncology*. 2015; 26: 2073-2078.
32. Ercan D, Choi HG, Yun CH, Capelletti M, Xie T, Eck MJ, et al. EGFR mutations and resistance to irreversible pyrimidine-based EGFR inhibitors. *Clinical cancer research*. 2015; 21: 3913-3923.
33. Xu J, Wang J, Zhang S. Mechanisms of resistance to irreversible epidermal growth factor receptor tyrosine kinase inhibitors and therapeutic strategies in non-small cell lung cancer. *Oncotarget*. 2017; 8: 90557.
34. Song HN, Jung KS, Yoo KH, Cho J, Lee JY, Lim SH, et al. Acquired C797S mutation upon treatment with a T790M-specific third-generation EGFR inhibitor (HM61713) in non-small cell lung cancer. *Journal of Thoracic Oncology*. 2016; 11: e45-e47.
35. Zhou W, Ercan D, Chen L, Yun CH, Li D, Capelletti M, et al. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. *Nature*. 2009; 462: 1070.
36. Chabon JJ, Simmons AD, Lovejoy AF, Esfahani MS, Newman AM, Haringsma HJ, et al. Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. *Nature communications*. 2016; 7: 11815.
37. Ortiz-Cuaran S, Scheffler M, Plenker D, Scheel AH, Fernandez-Cuesta L, Meder L, et al. Heterogeneous mechanisms of primary and acquired resistance to third-generation EGFR inhibitors. *Clinical Cancer Research*. 2016; 22: 4837-4847.

38. Robichaux JP, Nilsson M, Heymach JV. Non-covalent EGFR T790M targeting TKIs inhibit AZD9291 resistant EGFR C797S mutants. AACR. 2017.
39. Jia Y, Yun CH, Park E, Ercan D, Manuia M, Juarez J Xu, et al. Overcoming EGFR (T790M) and EGFR (C797S) resistance with mutant-selective allosteric inhibitors. Nature. 2016; 534: 129.
40. Uchibori K, Inase N, Araki M, Kamada M, Sato S, Okuno Y, et al. Combined with anti-EGFR antibody overcomes osimertinib resistance in EGFR-mutated non-small-cell lung cancer. Nature communications. 2017; 8: 14768.