In the treatment of non-small cell lung cancers (NSCLCs) with epidermal growth factor receptor (EGFR) mutations, EGFR-tyrosine kinase inhibitor (EGFR-TKI) is a key drug that can prolong the survival of patients. However, resistance to EGFR-TKIs has become a new problem that must be solved. All patients with NSCLCs with an EGFR mutation who have been treated with first- and second-generation EGFR-TKIs eventually exhibit disease progression, even if they have had a long period of response to the EGFR-TKI treatment. To overcome this resistance, various mechanisms for the acquisition of the resistance to first- and second-generation EGFR-TKIs have been identified, including second site mutation in EGFR, bypass mechanism of other pathways (such as MET amplification, PI3KCA mutation, and BRAF mutation), and transformation to small cell lung cancer. T790M second site mutation is the most frequently detected mechanism in recurrent EGFR mutant NSCLCs after first-line EGFR-TKI treatment. This second site mutation is substitution of threonine to methionine at the 790 site of exon 20, leading structural change in the ATP binding site of EGFR. T790M is called a “gate-keeper mutation” and is responsible for approximately 60% of acquired resistance to first- and second-generation EGFR-TKIs.

To overcome this most frequent mechanism of resistance, third-generation EGFR-TKIs have been investigated, and some of these compounds have now fully conquered this problem. Osimertinib, CO-1686 (rociletinib), HM61713 (olmutinib), and other third-generation EGFR-TKIs (EGF816, ASP8273) selectively bind to the ATP binding site of T790M mutant EGFR, and their efficacy has been shown in both in vitro and in vivo studies (1,2). Based on the results of a phase 3 trial, which showed a response rate (RR) of approximately 71%, and a progression-free survival (PFS) period of 10.1 months (3), osimertinib obtained FDA and EMA approval for the treatment of T790M-positive EGFR mutant NSCLC.

However, third-generation EGFR-TKIs have also created the next problem: acquired resistance to themselves. In this article, we would like to describe this problem in detail and to discuss the promising strategies that are now being investigated by many researchers, focusing on brigatinib, an ALK inhibitor that shows activity toward osimertinib-resistant EGFR mutant tumor cells.

**Mechanisms of resistance to third-generation EGFR-TKIs**

Tumors with double mutations consisting of T790M and a driver mutation that are treated with a third-generation EGFR-TKI eventually exhibit disease progression, even though they initially responded to the TKI. The mechanism responsible for this resistance to third-generation EGFR-TKIs is now being investigated, and several mechanisms that are similar to the resistance to first- and second-generation EGFR-TKIs conferred by the T790M mutation...
have been found. The study of cell-free DNA in the AURA trial detected a C797S substitution in 6 out of 15 specimens from AZD9291 (osimertinib)-resistant patients and the loss of the T790M mutation in 4 of the other specimens (4). Similar data were reported in an in vitro study investigating third-generation EGFR-TKI-resistant cell lines (5). The study detected 3 major acquired mutations (L718Q, L844, and C797S) that occurred during treatment with WZ4002, CO-1686, and AZD9291, although only the C797S mutation led to AZD9291 resistance. Other than these EGFR point substitutions, HER2 amplification, cMET amplification, and other mechanisms were also detected in osimertinib-resistant tumors (6). As osimertinib is now used in clinical practice, the mechanisms of resistance are expected to become much more heterogeneous.

Similar to the situation surrounding the acquisition of the T790M mutation after first- and second-generation EGFR-TKI treatment, a strategy to overcome the resistance conferred by the C797S mutation, which is thought to be the most frequently acquired mechanism of resistance to third-generation EGFR-TKI treatment, is now attracting our concerns. The cysteine residue at the 797 site of the ATP binding pocket of EGFR forms a covalent bond with third-generation EGFR-TKIs, and the substitution of cysteine to serine at the 797 site obstructs the bonding, resulting in resistance to those TKIs. To overcome this mechanism of resistance, an EGFR allostERIC inhibitor (EAI)-045, which binds to an allostERIC pocket of the EGFR structure, has been discovered and the efficacy of this inhibitor against C797S/T790M/L858R triple mutant NSCLC cells when administered in combination with cetuximab has been shown (7). However, the inhibitor was not effective against C797S/T790M/del19 triple mutant NSCLC cells, since C797S/T790M/del19 triple mutant EGFR has a different structure at the allosteric pocket from that of C797S/T790M/L858R triple mutant EGFR. A “fourth-generation EGFR-TKI” that is effective against both C797S/T790M/L858R and C797S/T790M/del19 triple mutant NSCLCs has not yet been discovered, and further investigation is expected.

**Brigatinib, an ALK inhibitor harboring some activity toward T790M mutant EGFR**

Brigatinib was first investigated as an ALK inhibitor and is effective against ALK-rearranged NSCLCs that are resistant to the ALK-TKI, crizotinib (8). This compound exhibited selectivity for the L1196M substitution in translocated ALK, which is known to be a frequent mechanism of resistance to crizotinib, in an in vitro study, and phase 1/2 trials have shown a relatively high RR of 50–70% for patients with ALK-rearranged NSCLC (9). A randomized phase 2 trial targeting ALK-rearranged NSCLCs previously treated with crizotinib is presently ongoing.

**Efficacy of brigatinib against C797S triple mutation in a preclinical study**

Regarding the problem that EAI-045 is not effective against C797S/T790M/del19 triple mutant NSCLC cells, Uchibori et al. screened 30 drugs to identify agents with activity against C797S/T790M/del19 triple mutant NSCLC cells and discovered that brigatinib, an ALK inhibitor, exhibited such activity. Though brigatinib was already known to have some efficacy against T790M double mutant NSCLCs, as described above, this was the first report to indicate that brigatinib is also active against C797S/T790M/del19 triple mutant NSCLC cells, as well as T790M/del19 double mutant NSCLC cells.

In the in vitro part of this study, brigatinib showed its activity against C797S/T790M/del19 triple mutant NSCLC cells, and the growth of C797S/T790M/del19 triple mutant xenograft tumors in nude mice was also inhibited by brigatinib. Furthermore, an in silico simulation showed that
brigatinib binds to the ATP binding pocket of C797S triple mutant EGFR. This result is interesting in that brigatinib is the first TKI to be reported to bind to the ATP binding pocket of C797S triple mutant EGFR and to exert activity against both C797S/T790M/del19 triple mutant NSCLC cells and C797S/T790M/L858R triple mutant cells. Thus, brigatinib might be a promising TKI for overcoming the resistance conferred by the C797S mutation, potentially enabling it to be called a “fourth-generation EGFR-TKI”.

However, the activity of brigatinib against C797S/T790M/L858R triple mutation could not be confirmed in this preclinical in vitro study. Brigatinib was effective against C797S/T790M/L858R triple mutant cell lines to some extent, but its activity was less potent than that against C797S/T790M/del19 triple mutant cell lines. Furthermore, an in vivo study using xenografts was not performed for C797S/T790M/L858R triple mutant tumors. To confirm the efficacy of brigatinib against C797S triple mutant NSCLCs, an in vivo study similar to that used for C797S/T790M/del19 triple mutant tumors might also be needed for C797S/T790M/L858R triple mutant tumors.

**Assessable results in clinical trials**

As seen in the preclinical study, brigatinib is a prospective agent to overcome the resistance conferred by the C797S mutation. To confirm the efficacy of brigatinib in patients with C797S triple mutant NSCLCs, a phase 1/2 trial to study the efficacy and toxicity of brigatinib against ALK-rearranged NSCLCs would provide us with useful information (9). As described above, 2 (5.6%) of the 36 patients with an assessable response exhibited a partial response, while 14 (38.9%) showed stable disease. Since most of the patients had progressive disease after first-line EGFR-TKI treatment and approximately half of them had the T790M mutation, an RR of 5.6% suggests a limited benefit. Of course, this study did not include patients with tumors resistant to third-generation EGFR-TKIs, but the limited efficacy of brigatinib against T790M double mutant NSCLCs predicts a limited efficacy against C797S triple mutant NSCLCs in clinical use, since the preclinical data for the activities of brigatinib were similar for both tumor cell types. Further investigation is needed to elucidate the clinical benefit of brigatinib against EGFR mutant NSCLCs.

**Combination therapy with cetuximab**

The combination of an EGFR-TKI and an anti-EGFR monoclonal antibody was first tried using afatinib, a second-generation EGFR-TKI, and cetuximab, with the aim of overcoming the resistance conferred by T790M mutation acquired during first-generation EGFR-TKI treatment. Afatinib is a pan-HER inhibitor, and afatinib monotherapy only yielded a RR of 7% after first-generation TKI treatment in the LUX-Lung 1 trial (10), failing to overcome the resistance conferred by T790M mutation. Despite this limited efficacy, the combination of afatinib with cetuximab was shown to be beneficial against T790M mutant NSCLCs in both a clinical trial and preclinical studies. The phase 1b trial of this combination regimen, which enrolled patients who had been previously treated with first-generation EGFR-TKI, reported a RR of 32% and of 25% against T790M-positive and T790M-negative NSCLCs, respectively (11). While the benefit of afatinib seemed to be strengthened by its combination with cetuximab, the adverse events (AEs) in this trial were also severer than those for the monotherapy, with grade 3 and 4 AEs reported in 44% and 2% of the patients, respectively. Nevertheless, this clinical trial showed the potential benefit, as well as the potential toxicity, of combination with an anti-EGFR monoclonal antibody for EGFR-TKI treatment.

As expected based on the results for afatinib, the combination of brigatinib and cetuximab in a preclinical study showed a higher efficacy than brigatinib monotherapy alone. This result is promising, since the strategy of combining an EGFR-TKI with an anti-EGFR monoclonal antibody might play an important role in overcoming TKI resistance. However, further investigation in clinical trials is needed to confirm the efficacy and toxicity of such regimens.

Treatments for EGFR mutant NSCLCs have changed dramatically since gefitinib first began to be used clinically in 2002. In 2016, osimertinib was approved in the US and other countries, and it is now used clinically for the treatment of T790M-positive EGFR mutant NSCLCs. As more and more patients are now being treated with osimertinib, further investigations to overcome mechanisms of resistance to osimertinib are likely to be needed. In addition, data from the FLAURA trial, a phase 3 trial comparing first-generation EGFR-TKIs and osimertinib as a first line therapy for patients with EGFR mutant NSCLCs, has now been analyzed, and a longer PFS of 18.9 months was obtained for the osimertinib arm than 10.2 months for the gefitinib arm (12). The results of this trial suggest that osimertinib might be used as a first-line treatment for EGFR mutant NSCLCs in the near future. The mechanism of resistance to osimertinib in first-line use...
has not yet been reported, and we are unsure to what degree C797S mutation may contribute to resistance to first-line osimertinib. The importance of studies on brigatinib might change depending on the percentage of resistant tumors bearing the C797S mutation.

For now, however, C797S mutation is the most frequent mechanism of resistance to osimertinib, and a means of overcoming this mechanism is likely to be an unavoidable issue for the treatment of EGFR mutant NSCLC. The preclinical study examining the efficacy of brigatinib against C797S triple mutant NSCLCs suggested that brigatinib might be a promising agent for overcoming resistance conferred by the C797S mutation, and it has the potential to become a “fourth-generation EGFR-TKI”. However, its activity toward tumor cells harboring the EGFR driver mutation was less potent in in vitro studies than those of other TKIs, such as gefitinib and osimertinib, and the efficacy of brigatinib monotherapy against EGFR mutant NSCLCs was limited in a clinical trial. To overcome the resistance conferred by the C797S mutation, a structural study of brigatinib might provide hints for the creation of other TKIs with an affinity to C797S mutant tumor cells, and a combination regimen with cetuximab should be evaluated in clinical trials in the near future. Even if the C797S mutation is not detected in tumor cells from patients receiving osimertinib as a first-line treatment, strategies similar to that of the brigatinib study might yield promising agents and regimens to overcome TKI resistance.

**Acknowledgements**

None.

**Footnote**

*Conflicts of Interest:* S. Kanda reports research fund from AstraZeneca and Bristol-Myers Squibb outside the submitted work. The other author has no conflicts of interest to declare.

**References**


**Cite this article as:** Noda-Narita S, Kanda S. Overcoming resistance to third-generation epidermal growth factor receptor tyrosine kinase inhibitor in non-small cell lung cancer. Transl Cancer Res 2017;6(Suppl 7):S1187-S1190. doi: 10.21037/tcr.2017.09.04