Editorial Commentary

Fourth-generation epidermal growth factor receptor-tyrosine kinases inhibitors: hope and challenges

Chunxia Su[1], Shi-Yong Sun[2]

[1]Department of Oncology, Shanghai Pulmonary Hospital & Thoracic Cancer Institute, Tongji University School of Medicine, Shanghai, China; [2]Department of Hematology and Medical Oncology, Emory University School of Medicine and Winship Cancer Institute, Atlanta, GA, USA

Correspondence to: Chunxia Su, MD. Department of Oncology, Shanghai Pulmonary Hospital & Thoracic Cancer Institute, Tongji University School of Medicine, 507 Zhenming Road, Shanghai 200082, China. Email: susu_mail@126.com; Shi-Yong Sun, PhD. Department of Hematology and Medical Oncology, Emory University School of Medicine and Winship Cancer Institute, 1365-C Clifton Road, C3088, Atlanta, GA 30322, USA. Email: ssun@emory.edu.

Comment on: Lim SM, Fujino T, Kim C, *et al.* BBT-176, a Novel Fourth-Generation Tyrosine Kinase Inhibitor for Osimertinib-Resistant EGFR Mutations in Non-Small Cell Lung Cancer. Clin Cancer Res 2023;29:3004-16.

Keywords: Epidermal growth factor receptor (EGFR); EGFR-tyrosine kinase inhibitors (EGFR-TKIs); osimertinib; fourth-generation; lung cancer

Submitted Mar 13, 2024. Accepted for publication Jun 26, 2024. Published online Jul 25, 2024.
doi: 10.21037/tcr-24-406
View this article at: https://dx.doi.org/10.21037/tcr-24-406

Epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer (NSCLC) are among the most common driver mutations, particularly in certain populations, such as Asian patients and non-smokers. Deletions in exon 19 and the L858R point mutation in exon 21 are the most frequent aberrations and collectively account for over 80% of all EGFR mutations (1). In the past two decades, the emergence of targeted therapy has profoundly changed the treatment strategy for patients with advanced driver gene-positive NSCLC. As an important therapeutic target for NSCLC, EGFR-tyrosine kinase inhibitors (EGFR-TKIs) have completely rewritten the diagnosis and treatment of patients with EGFR mutant NSCLC (2). First-generation (e.g., gefitinib and erlotinib) and second-generation (e.g., afatinib) EGFR-TKIs have demonstrated increased response rates and progression-free survival (PFS) compared to traditional chemotherapy. Third-generation EGFR-TKIs such as osimertinib were developed primarily for overcoming acquired resistance to earlier generations of EGFR-TKIs due to T790M resistance mutation, a common mechanism of resistance to early generations of EGFR-TKIs (3). Osimertinib has also shown efficacy as a first-line treatment in EGFR-mutated NSCLC patients, due to its ability to target both common activating EGFR mutations and the T790M resistance mutation. However, patients treated with these third-generation EGFR-TKIs still inevitably develop disease progression with complex mechanisms of acquired resistance, resulting in treatment failure. Emergence of acquired resistance to osimertinib has posed a significant challenge in the treatment of NSCLC. Several mechanisms of osimertinib resistance have been identified (4), among which, acquisition of the resistance mutation, C797S, which inhibits the binding of osimertinib to the EGFR protein, is a primary mechanism, particularly when osimertinib is used as a second-line treatment option. Hence, it sounds logical to develop next generation of high-affinity reversible EGFR-TKIs aiming for C797S mutation.

Overview of fourth-generation EGFR inhibitors

BLU945 is a fourth-generation EGFR-TKI that specifically targets T790M/C797S co-mutations and other T790M resistance mutations secondary to osimertinib resistance. The SYMPHONY (NCT04862780) study is a phase I/II study of patients with metastatic EGFR-mutated NSCLC who have previously received TKI treatment. It aims to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary anti-tumor activity of BLU-945 alone and BLU-945 combined with osimertinib

in patients with EGFR-mutated NSCLC (5). However, it cannot effectively inhibit the compound mutations of 19del/L858R and C797S, and had limited efficacy of single drug in the sequent-line population (6). And the development of BLU-945 has not continued EAI045 inhibits the activator subunit of EGFR, to which the monomeric EGFR molecule is sensitive. Both *in vitro* and *in vivo* experiments confirmed that the combination of EAI045 and cetuximab showed ideal tumor inhibitory effects in L858R/T790M±C797S conditions (7). However, it is worth noting that EAI045 is ineffective in monotherapy, possibly because dimerization of EGFR after activation prevents small molecule inhibitors from entering the allosteric binding site, thus limiting further clinical application. JBJ-04-125-02 (8) and JBJ-09-063 (9) are both improved product of EAI045. The improved pharmacological properties have taken a key step in the clinical development of fourth-generation EGFR inhibitors and showed a good inhibitory effect on EGFR mutated tumors, especially in combination with a third-generation EGFR-TKI.

TQB-3804 is another fourth-generation EGFR-TKI. Its *in vitro* study results were firstly reported at the 2019 American Association for Cancer Research conference, indicating that it could target EGFR T790M/C797S, L858R/T790M/C797S, L858R/T790M with better enzyme inhibitory activity. The above conclusions were also verified through CDX and PDX *in vivo* models, providing a preclinical support for the potential of TQB-3804 in the treatment of NSCLC patients relapsed to third-generation EGFR-TKIs due to C797S mutation (10). A phase I trial of TQB3804 is ongoing (NCT04128085).

H002, unveiled as a fourth-generation EGFR-TKI, distinguishes itself with pronounced selectivity with potent inhibition across various EGFR mutations in cellular and animal models. Its initial safety in rats and dogs has been established, alongside its capability to penetrate the blood-brain barrier (11). Phase I/II clinical trials of H002 are currently underway (NCT05519293).

In a preclinical study testing the efficacy of EGFR-TKI, BI-4020, in an EGFR-Del19/T790M/C797S mutant NSCLC model with daily oral administration of 10 mg/kg BI-4020, it was reported that BI-4020 induced strong regression in all 10 tumor models and all mice tolerated the treatment well (12). Further preclinical and clinical studies remain to be conducted.

BDTX-1535 mainly targets patient groups with osimertinib-resistant mutations (C797S) and other driver mutations. Unlike most previously reported fourth-generation EGFR inhibitors that can only be used for late-line treatment (EGFR C797S), BDTX-1535 has the potential to be used as a first-line treatment (applicable to driver mutations such as L858R, Ex19del, L718Q, L747P, and E709V). Animal experiments show that BDTX-1535 has excellent brain penetration ability (13). In 2023, a phase I clinical trial (NCT05256290) data of BDTX-1535 showed that among 12 evaluable patients, six tumors were evaluated as partial response (PR), overall response rate (ORR) reached 50%, and disease control rate (DCR) was 100% (14).

THE-349 is a fourth-generation TKI targeting full-spectrum inhibition of EGFR mutations and CNS metastasis. It demonstrated potent and highly selective inhibition of all major EGFR single, double, and triple mutants in cell experiments. At the same time, it showed deep and long-lasting tumor shrinkage effects in osimertinib-resistant PDX and NSCLC tumor models (15).

In another preclinical study, professor Ding obtained JND3229 through selective screening of the compound library. The activity results showed that their activity against EGFRL858R/T790M/C797S reached 5.8 nM, and the cell activity also reached 0.51 μM, which showed promising anti-tumor activity as well (16).

Other fourth-generation EGFR-TKIs like JIN-A02 (17), LS-106 (18), BAY2927088, and so on also show good anti-tumor activity and needs further exploration. The summary of fourth-generation EGFR-TKIs was displayed in *Table 1*.

Development and preclinical evaluation of BBT-176

BBT-176 is another orally available fourth-generation EGFR-TKI targeting triple-mutant EGFR C797S-related mutant forms non-covalently (19). As a reversible ATP-competitive inhibitor designed for overcoming C797S-mediated acquired resistance of osimertinib, BBT-176 has a distinct binding mode into triple mutant (19Del/T790M/C797S or L858R/T790M/C797S). BBT-176's core structure engages with the EGFR kinase domain through hydrogen bonds, salt bridges, and hydrophobic interactions, critical for its inhibitory action. Structural alterations in the kinase domain's active and inactive states affect the positioning of Lys745, Asp855, and Gly857, altering their interactions. The unique interaction between BBT-176's sulfone group and Lys745 may enhance its potency against triple-mutant EGFR, offering selective inhibition over the wild-type (19).

In the recent study by Lim *et al.* (19), BBT-176 was shown to have potent nanomolar range efficacy against

Table 1 Summary of fourth-generation EGFR-TKIs

Fourth-generation EGFR-TKIs	Targets	Phase	Clinical trial
BLU945	Del19/L858R/T790M/C797S	Phase I/II	NCT04862780
EAI045	L858R/T790M±C797S	Preclinical	–
TQB-3804	Del19/T790M/C797S or L858R/T790M/C797S	Phase I	NCT04128085
H002	Del19/L858R±T790M±C797S	Phase I/IIa	NCT05519293
BI-4020	Del19/T790M/C797S	Preclinical	–
BDTX-1535	Del19/L858R/T790M/C797S and others	Phase I/II	NCT05256290
THE-349	Del19/L858R±T790M±C797S	Preclinical	–
JND3229	Del19/L858R/T790M/C797S	Preclinical	–
JIN-A02	Del19/L858R/T790M±C797S	Phase I/II	NCT05394831
LS-106	Del19/L858R/T790M±C797S	Preclinical	–
BAY2927088	EGFR/HER2	Phase I/II	NCT05099172

EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2.

EGFR mutants, including 19Del/T790M/C797S, 19Del/C797S, and L858R/C797S, and the growth of cell lines carrying these mutations with weak antiproliferation activities in EGFR wild-type cancer cells, outperforming osimertinib in both biological and cell-based assays. The N-ethyl-N-nitrosourea (ENU) mutagenesis assay suggested that BBT-176 treatment did not introduce any secondary mutations in the EGFR gene and the BBT-176 resistant cells were still dependent on EGFR signaling for survival, highlighting its unique feature. The anti-EGFR antibody cetuximab can enhance the activity of a first- or second-generation EGFR-TKI. Cetuximab shows growth inhibitory activity against a variety of mutated Ba/F3 cells, but is ineffective against T790M secondary mutant cells. The presence of cetuximab can increase the activity of BBT-176, especially against cells with triple mutations of 19Del/T790M/C797S, in which the half-maximal inhibitory concentration (IC_{50}) values were reduced by 50 folds compared to its solo administration. Downstream signaling was enhanced in combination treatment. The effects of BBT-176 were also tested in mice bearing Ba/F3 EGFR 19Del/C797S or Ba/F3 EGFR 19Del/T790M/C797S xenografts and PDX models, 90 mg/kg/day showed potential antitumor efficacy and tolerable adverse reactions.

Clinical studies and efficacy of BBT-176

The phase I/II, open-label clinical trial of BBT-176 (NCT04820023) is designed to investigate the safety and tolerability of BBT-176 (part 1) and to evaluate the anti-tumor activity of BBT-176 (part 2) in patients with advanced NSCLC who progressed following prior therapy with an EGFR-TKI. As the first-in-human study of BBT-176, the enrollment of a total of 45 patients were planned.

The 2022 World Conference on Lung Cancer (WCLC) meeting was the debut of the BBT-176 clinical study, with phase I clinical trial data reported (NCT04820023). A total of 18 patients were enrolled, of which five patients had EGFR triple mutations (exon 19 del/T790M/C797S or L858R/T790M/C797S) detected in blood samples. Judging from the response of these five patients to treatment, only one PR and four stable disease (SD) (20).

As the study going on, the study presented in the recent study by Lim *et al.* revealed that 25 patients received BBT-176 on a once-daily dosage schedule in escalating cohorts, exhibiting favorable tolerance and a safety profile akin to other EGFR inhibitors (19). Grade ≥3 treatment-related adverse events (TRAEs) were notable at a 320 mg daily dose, predominantly gastrointestinal toxicities. As reported, the investigation continues with dose escalation beginning at 160 mg administered bi-daily. It is worth noting that circulating tumor DNA (ctDNA) was used in this study to explore and excavate subsequent drug resistance mechanisms. Although in the ctDNA analysis of the two cases presented, the authors were temporarily unable to identify non-EGFR mechanisms of acquired resistance. As

the concept of precise diagnosis and treatment of malignant tumors continues to deepen, multiplex tissue sequencing can match NSCLC with an increasing number of life-extending targeted therapies. However, in reality, patients cannot undergo multiplexed tumor sequencing due to unavailable tissue or sequencing failure. Sequencing of ctDNA in plasma can similarly identify targeted alterations. As a valuable information carrier for clinical decision-making in the microscopic world, its potential to guide treatment of lung cancer has received widespread attention.

As the study progresses, as of March 2023, 44 patients have received BBT-176 treatment. Triple-mutated EGFR genes (exon 19 del/T790M/C797S or L858R/T790M/C797S) were detected in the serum of 11 patients. Drug exposure was clearly proportional to dose. Most of the TRAEs were level 1 or 2, and the drug safety is acceptable. Of note was one case of neutropenia (grade 4) in the 600 mg QD cohort. One additional case of interstitial lung disease (grade 4) occurred in the BID cohort.

Future perspectives and challenges with BBT-176

The targets of most fourth-generation EGFR-TKIs have focused on the EGFR gene and its C797S site mutation, meaning that the use of these fourth-generation drugs after osimertinib resistance still requires genetic testing to determine whether there are expected resistant mutations eligible for the use of fourth-generation EGFR-TKIs (21). The exploration of BBT-176 highlights the application value of non-invasive biopsy such as ctDNA in fourth-generation EGFR-TKIs.

In the study by Lim *et al.* (19), engineered cell lines from Ba/F3, a murine pro-B cell line, were primarily used for generating their preliminary data although it was done as other studies did. This limitation also raises a challenge for the entire field, lack of epithelium-derived NSCLC cell lines with natural acquisition of C797S mutation post a prolonged treatment with osimertinib or other third-generation EGFR-TKIs. Hence, it is urgent and important to generate these cell lines valuable for preclinical study of fourth-generation EGFR-TKIs in the future.

When summarizing clinical studies on fourth-generation EGFR-TKIs, it is evident that the majority of these drugs are still in the preclinical stage, while only a limited number have progressed to phase I/II trials. One notable example is EAI045, the first allosteric TKI targeting T790M and C797S mutations in EGFR. Despite early reports of promising preclinical activity in 2017, clinical trials for EAI045 have not been initiated as of today. Similarly, JBJ-04-125-02, another fourth-generation drug, emerged relatively early, but no clinical trials have been conducted for this compound to date. While there are ongoing phase I (NCT04128085) and phase II (NCT04180150) trials for TQB3804, no relevant data have been released thus far, leaving its clinical potential to be determined in the future. As for the BBT-176, the protagonist of this article, cannot escape the fate of stopping research and development, the clinical trial of BBT-176 was terminated. The BBT-207 of the same company is under development (NCTNCT05920135), which has shown excellent anti-tumor effects against double mutations including C797S and triple mutations in the preclinical stage.

The development process of fourth-generation EGFR-TKIs, particularly those targeting C790S mutation only, to address EGFR on-target resistance poses several challenges and limitations. One major hurdle is the slow progress in clinical research, which contributes to the prolonged timeline for these drugs to reach the phase III stage, where their clinical benefit can be definitively established. The extensive testing required in phase III trials adds to the overall time and effort required for these drugs to become available to patients.

In addition to the slow development process, fourth-generation EGFR-TKIs also face challenges in patient population screening and available patient population. For example, if a fourth-generation drug like BBT-176 exclusively targets the C797S mutation population, there may already be multiple treatment options available for this specific subgroup of patients. This raises the question of whether introducing a new fourth-generation drug would provide a significant advantage over existing treatments in terms of efficacy and patient outcomes. Under the best scenario of the drug with clinically acceptable efficacy, the available patient population eligible for the treatment is a concern giving the low mutation prevalence of C797S (5–7%) in patients who accept osimertinib as a first-line therapy. This should be particularly an issue in the Western country where there are only about 10–15% patients with EGFR mutant NSCLC who are eligible for the treatment with EGFR-TKIs.

For some fourth-generation EGFR-TKIs that target both C797S resistance mutation and other common activating mutation such as 19del and L858R, they would need to compete with osimertinib, a breakthrough drug that has shown remarkable efficacy in this patient population

with the common activation mutations. The FLAURA-2 and MARIPOSA studies respectively explored the efficacy of different combination modes of osimertinib in the first-line treatment of patients with EGFR mutations, and found that combination chemotherapy or EGFR-mesenchymal-epithelial transition (EGFR-MET) bispecific antibodies can improve the efficacy of osimertinib. It provides a reference for the treatment mode after resistance, but it still needs further exploration in preclinical and clinical studies. Conducting clinical research in such a scenario becomes more challenging due to the need for comparative studies against an established and highly effective treatment option like osimertinib. This poses higher risks and increased difficulty in demonstrating the superiority of fourth-generation EGFR-TKIs in terms of efficacy, safety, and overall clinical benefit.

To overcome these challenges, careful consideration and strategic planning are necessary during the development of fourth-generation EGFR-TKIs. This includes identifying specific patient populations that would benefit the most from these drugs, exploring novel mechanisms of action that offer advantages over existing treatments, and conducting rigorous clinical trials that effectively demonstrate the superiority and clinical relevance of fourth-generation EGFR-TKIs in the context of EGFR-positive NSCLC. Overall, fourth-generation EGFR-TKIs that target both C797S resistance mutation and other common activating mutation such as 19del and L858R may have a hope albeit with many challenges.

Acknowledgments

Funding: This study was supported by the National Natural Science Foundation of China (Nos. 82072568 and 82373320).

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Translational Cancer Research*. The article has undergone external peer review.

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-406/prf

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-406/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. da Cunha Santos G, Shepherd FA, Tsao MS. EGFR mutations and lung cancer. Annu Rev Pathol 2011;6:49-69.
2. Lee DH. Treatments for EGFR-mutant non-small cell lung cancer (NSCLC): The road to a success, paved with failures. Pharmacol Ther 2017;174:1-21.
3. Ramalingam SS, Vansteenkiste J, Planchard D, et al. Overall Survival with Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. N Engl J Med 2020;382:41-50.
4. Leonetti A, Sharma S, Minari R, et al. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. Br J Cancer 2019;121:725-37.
5. Eno MS, Brubaker JD, Campbell JE, et al. Discovery of BLU-945, a Reversible, Potent, and Wild-Type-Sparing Next-Generation EGFR Mutant Inhibitor for Treatment-Resistant Non-Small-Cell Lung Cancer. J Med Chem 2022;65:9662-77.
6. Elamin YY, Nagasaka M, Shum E, et al. BLU-945 monotherapy and in combination with osimertinib (OSI) in previously treated patients with advanced EGFR-mutant (EGFRm) NSCLC in the phase 1/2 SYMPHONY study. J Clin Oncol 2023;41:9011.
7. Jia Y, Yun CH, Park E, et al. Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. Nature 2016;534:129-32.
8. To C, Jang J, Chen T, et al. Single and Dual Targeting of Mutant EGFR with an Allosteric Inhibitor. Cancer Discov 2019;9:926-43.
9. To C, Beyett TS, Jang J, et al. An allosteric inhibitor

against the therapy-resistant mutant forms of EGFR in non-small cell lung cancer. Nat Cancer 2022;3:402-17.
10. Liu X, Zhang X, Yang L, et al. Preclinical evaluation of TQB3804, a potent EGFR C797S inhibitor. Cancer Res 2019;79:1320.
11. Huang W, Zhu L, Yan X, et al. H002: A wide spectrum, highly selective fourth-generation EGFR inhibitor overcoming resistance harboring C797S mutation in NSCLC. Cancer Res 2022;82:5461.
12. Engelhardt H, Böse D, Petronczki M, et al. Start Selective and Rigidify: The Discovery Path toward a Next Generation of EGFR Tyrosine Kinase Inhibitors. J Med Chem 2019;62:10272-93.
13. Lucas MC, Merchant M, O'Connor M, et al. 27MO BDTX-1535, a CNS penetrant, irreversible inhibitor of intrinsic and acquired resistance EGFR mutations, demonstrates preclinical efficacy in NSCLC and GBM PDX models. Ann Oncol 2022;33:S14.
14. Dardenne E, O'Connor M, Nilsson M, et al. BDTX-1535: A MasterKey EGFR inhibitor targeting classical and non-classical oncogenic driver mutations and the C797S acquired resistance mutation to address the evolving molecular landscape of EGFR mutant NSCLC. Cancer Res 2024;84:1229.
15. Zhang S, Nadworny S, Huang WS, et al. Abstract B167: Preclinical characterization of THE-349, a mutant-selective, CNS-active, fourth-generation EGFR inhibitor to overcome T790M- and C797S-mediated resistance in NSCLC. Mol Cancer Ther 2023;22:B167.
16. Lu X, Zhang T, Zhu SJ, et al. Discovery of JND3229 as a New EGFR(C797S) Mutant Inhibitor with In Vivo Monodrug Efficacy. ACS Med Chem Lett 2018;9:1123-7.
17. Yun MR, Yu MR, Duggirala KB, et al. 999P JIN-A02, a fourth-generation, highly effective tyrosine kinase inhibitor with intracranial activity, targeting EGFR C797S mutations in NSCLC. Ann Oncol 2022;33:S1010-1.
18. Liu Y, Lai M, Li S, et al. LS-106, a novel EGFR inhibitor targeting C797S, exhibits antitumor activities both in vitro and in vivo. Cancer Sci 2022;113:709-20.
19. Lim SM, Fujino T, Kim C, et al. BBT-176, a Novel Fourth-Generation Tyrosine Kinase Inhibitor for Osimertinib-Resistant EGFR Mutations in Non-Small Cell Lung Cancer. Clin Cancer Res 2023;29:3004-16.
20. Lim SM, Ahn JS, Hong MH, et al. MA07. 09 BBT-176, a 4th generation EGFR TKI, for Progressed NSCLC after EGFR TKI Therapy: PK, Safety and Efficacy from Phase 1 Study. J Thorac Oncol 2022;17:S70-1.
21. Lu X, Yu L, Zhang Z, et al. Targeting EGFR(L858R/T790M) and EGFR(L858R/T790M/C797S) resistance mutations in NSCLC: Current developments in medicinal chemistry. Med Res Rev 2018;38:1550-81.

Cite this article as: Su C, Sun SY. Fourth-generation epidermal growth factor receptor-tyrosine kinases inhibitors: hope and challenges. Transl Cancer Res 2024;13(8):3929-3934. doi: 10.21037/tcr-24-406

