Acquired resistance to third-generation EGFR-TKIs and emerging next-generation EGFR inhibitors

Xiaojing Du,1,2 Biwei Yang,1,2 Quanlin An,1 Yehuda G. Assaraf,4,* Xin Cao,1,* and Jinglin Xia1,2,3,5,*

*Correspondence: assaraf@technion.ac.il (Y.G.A.); caox@fudan.edu.cn (X.C.); xiajinglin@fudan.edu.cn (J.X.)

Received: November 23, 2020; Accepted: April 1, 2021; Published Online: April 3, 2021; https://doi.org/10.1016/j.xinn.2021.100103

© 2021 The Author(s). This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Graphical abstract

Public summary

- EGFR gene mutations are detected in about 50% of non-small cell lung cancer (NSCLC) patients worldwide
- The three generations of EGFR tyrosine kinase inhibitors (TKIs) are critical milestones for NSCLC patients
- Like other targeted therapies, new EGFR mutations and coupled drug resistances emerge rapidly after TKI treatment, posing formidable obstacles to cancer management
- The investigational fourth-generation EGFR inhibitors are of great promise, through a number of novel mechanisms, in overcoming these resistances after third-generation TKI treatment, and will bring more benefits to NSCLC patients
Acquired resistance to third-generation EGFR-TKIs and emerging next-generation EGFR inhibitors

Xiaojing Du,1,2,3 Biewei Yang,1,2 Quanlin An,1 Yehuda G. Assaraf,4,6 Xin Cao,1,8 and Jinglin Xia1,2,3,5,6

1 Institute of Clinical Science, Zhongshan Hospital, Fudan University, Shanghai 200032, China
2 Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai 200032, China
3 Institute of Fudan-Minhang Academic Health System, Minhang Hospital, Fudan University, Shanghai 201199, China
4 The Fred Wyszkowski Cancer Research Lab, Department of Biology, Technion-Israel Institute of Technology, Haifa 3200000, Israel
5 The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China
6 Correspondence: assaraf@technion.ac.il (Y.G.A.); caox@fudan.edu.cn (X.C.); xiajinglin@fudan.edu.cn (J.X.)

INTRODUCTION

The discovery that mutations in the EGFR gene are detected in up to 50% of lung adenocarcinoma patients, along with the development of highly efficacious epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), has revolutionized the treatment of this frequently occurring lung malignancy. Indeed, the clinical success of these TKIs constitutes a critical milestone in targeted cancer therapy. Three generations of EGFR-TKIs are currently approved for the treatment of EGFR mutation-positive non-small cell lung cancer (NSCLC). The first-generation TKIs include erlotinib, gefitinib, lapatinib, and icotinib; the second-generation ErbB family blockers include afatinib, neratinib, and dacotinib; whereas osimertinib, approved by the FDA on 2015, is a third-generation TKI targeting EGFR harboring specific mutations. Compared with the first- and second-generation TKIs, third-generation EGFR inhibitors display a significant advantage in terms of patient survival. For example, the median overall survival in NSCLC patients receiving osimertinib reached 38.6 months. Unfortunately, however, like other targeted therapies, new EGFR mutations, as well as additional drug-resistance mechanisms emerge rapidly after treatment, posing formidable obstacles to cancer therapeutics aimed at surmounting this chemoresistance. In this review, we summarize the molecular mechanisms underlying resistance to third-generation EGFR inhibitors and the ongoing efforts to address and overcome this chemoresistance. We also discuss the current status of fourth-generation EGFR inhibitors, which are of great value in overcoming resistance to EGFR inhibitors that appear to have greater therapeutic benefits in the clinic.

Keywords: Cancer; targeted therapy; EGFR inhibitors; drug resistance; mutations; chemoresistance mechanisms; surmounting chemoresistance

EGFR PATHWAY IN CANCER AND LAUNCHED EGFR INHIBITORS

The EGFR family harbors an extracellular domain, a transmembrane region, and an intracellular domain.1 The intracellular kinase domain contains the N-lobe, which is subdivided into nucleotide-binding site, P-loop, C-helix, and C-lobe, including the DFG motif, catalytic residue Asp813, catalytic loop, and A-loop, with the ATP-binding pocket located in the catalytic cleft between the lobes.1 The A-loop folds into a helix under the inactive state and restrains C-helix rotation toward the catalytic cleft (C-helix-out; Figure 1A).1,14 When triggered by ligands, EGFR signal acts an asymmetric dimer, following which the kinase domain turns into a tail-to-head interaction, which shifts the equilibrium to an active state by pushing the C-helix into an active position (C-helix-in; Figure 1B),15 thereby resulting in the subsequent activation of PI3K/AKT, RAS/RAF/MEK/ERK, and STAT3 signaling pathways.1 These pathways regulate pivotal cellular processes, including proliferation, differentiation, survival, and migration, making EGFR signaling susceptible to being hijacked by cancer cells.1,2 Various pre-clinical and clinical studies to date have revealed the driver function of EGFR in various cancer types.1,2

The first-generation EGFR-TKIs (Figure 2), including gefitinib, erlotinib, lapatinib, and icotinib, have been widely used to block EGFR activity in an ATP-competitive and -reversible manner.2 Their cytotoxic effect has been stronger when targeting mutated EGFR than wild-type EGFR (EGFRWT), especially EGFR harboring an L858R mutation as well as EGFR displaying exon19 deletion (Ex19del).2,6 However, almost all cancer patients developed resistance to the first-generation EGFR-TKIs after 10–14 months of treatment; this TKI resistance has been associated with the EGFR T790M mutation in 50%–60% of the cases.16

Over the years, second-generation EGFR-TKIs with more potent inhibitory activity against EGFR have been developed and applied.2 Covalent binding of these TKIs to EGFR at Cys797 residue may lead to irreversible inhibition of the EGFR kinase.2 The second-generation EGFR-TKIs afatinib, neratinib, and dacotinib have shown a higher antitumor activity compared with the first-generation EGFR-TKIs.1 Unfortunately, these EGFR-TKIs continue to be limited in their activity due to the frequent emergence of the drug-resistance...
The cellular proliferation features of EGFR render it heavily implicated in malignant tumors; this has been confirmed by a large amount of pre-clinical and clinical evidence. The majority of aberrant forms of EGFR in malignant tumors emerge as mutations and/or gene amplification, which has been reported to occur in lung cancer, colorectal cancer, squamous cell carcinoma of the head and neck (SCCHN), pancreatic cancer, renal cell cancer, hepatocellular carcinoma, breast cancer, gastric cancer, glioma, and ovarian cancer.

**EGFR-TARGETED THERAPY IN THE CLINIC**

The cellular proliferation features of EGFR render it heavily implicated in malignant tumors; this has been confirmed by a large amount of pre-clinical and clinical evidence. The majority of aberrant forms of EGFR in malignant tumors emerge as mutations and/or gene amplification, which has been reported to occur in lung cancer, colorectal cancer, squamous cell carcinoma of the head and neck (SCCHN), pancreatic cancer, renal cell cancer, hepatocellular carcinoma, breast cancer, gastric cancer, glioma, and ovarian cancer.

For patients with advanced NSCLC with central nervous system (CNS) metastases, the first-/second-generation EGFR-TKIs were ineffective, since the penetration of these drugs into the brain is relatively low. In

---

**EGFR is a major driver gene in NSCLC, the most common lung cancer.**

Precise molecular typing is highly recommended before administration of EGFR-targeted therapy according to the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology. For patients with the common sensitive EGFR mutations (Ex19del and L858R), osimertinib is recommended. The FLAURA study on advanced NSCLC patients with the common sensitive EGFR mutations showed that the median progression-free survival (mPFS) was 10.2 months for the first-generation EGFR-TKIs (gefitinib and erlotinib) and 18.9 months for osimertinib (hazard ratio [HR] = 0.46, p < 0.001). Moreover, the median duration of response (mDoR) was 8.5 months and 17.2 months, and the median overall survival (mOS) was 31.8 months and 38.6 months (HR = 0.8, p = 0.046), respectively.

At 3 years, 28% of patients in the osimertinib group and 9% in the gefitinib and erlotinib group continued with a clinical trial. Osimertinib also has a similar safety profile and lower rates of severe adverse events than the standard EGFR-TKIs. Combined post hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6 clinical trials revealed that afatinib was active in patients with uncommon EGFR mutations, especially NSCLC patients harboring uncommon EGFR mutations.

Four EGFR-targeted monoclonal antibodies (mAbs), cetuximab, panitumumab, nimotuzumab, and denosumab, have been approved by the FDA. Since the mAbs could suppress the ligand-dependent dimerization of EGFRWT and some mutant EGFR via binding to the extracellular domain of EGFR that competes for endogenous ligand binding, they are sometimes also classified as EGFR inhibitors. Combined administration with chemotherapeutic agents is frequently required for EGFR mAbs in the clinic. It is noteworthy that only a fraction of the patients is sensitive to these mAbs, and EGFR-targeted toxicities may restrict their clinical application.

---

**EGFR-TARGETED THERAPY IN THE CLINIC**

The cellular proliferation features of EGFR render it heavily implicated in malignant tumors; this has been confirmed by a large amount of pre-clinical and clinical evidence. The majority of aberrant forms of EGFR in malignant tumors emerge as mutations and/or gene amplification, which has been reported to occur in lung cancer, colorectal cancer, squamous cell carcinoma of the head and neck (SCCHN), pancreatic cancer, renal cell cancer, hepatocellular carcinoma, breast cancer, gastric cancer, glioma, and ovarian cancer.

For patients with advanced NSCLC with central nervous system (CNS) metastases, the first-/second-generation EGFR-TKIs were ineffective, since the penetration of these drugs into the brain is relatively low.
contrast, osimertinib effectively crossed the blood-brain barrier (BBB) due to its lower efflux by human BBB multi-drug efflux pumps than other EGFR-TKIs, which implies the attainment of a higher concentration of osimertinib in the brain. The AURA3 study on CNS metastasis in NSCLC patients with the T790M EGFR mutation demonstrated that CNS ORR was 70% with osimertinib and only 31% with the platinum-pemetrexed combination (odds ratio [OR] = 5.13, p = 0.015); the ORR was 40% and 17% (OR = 3.24, p = 0.014), respectively. The mDoR of CNS was 8.9 months for osimertinib and 5.7 months for platinum-pemetrexed, whereas the mPFS of CNS was 11.7 and 5.6 months (HR = 0.32, p = 0.004), respectively. Moreover, the BLOOM study on EGFR-mutated NSCLC patients with leptomeningeal metastases showed that mPFS and mOS of osimertinib were 8.6 months and 11.0 months, respectively. Among these patients, the neurologic function was ameliorated in 57% (12/21) of patients with an abnormal assessment at baseline, which improved the quality of life and prognosis.

For patients with advanced NSCLC whose sensitive EGFR mutations were found during chemotherapy, completion or interruption of the planned chemotherapeutic regimen was recommended, followed by an administration of osimertinib or the first-/second-generation EGFR-TKIs. When the disease progresses after osimertinib treatment, definitive local therapy combined with continued treatment of osimertinib is recommended for asymptomatic patients and patients with brain metastases or isolated lesions. When the disease progresses after other EGFR-TKI treatment, the detection of the T790M mutation is recommended, and osimertinib is the preferred treatment for patients with this frequent T790M EGFR mutation. As postoperative adjuvant therapy, osimertinib significantly reduces the recurrence rate after the operation by 79%.

Colorectal cancer

EGFR is a crucial target for colorectal cancer. EGFR mAbs are the first group of targeted drugs developed for the treatment of metastatic colorectal cancer.
In patients with wild-type KRAS, Cetuximab combined with FOLFIRI significantly improved mOS (23.5 versus 20.0 months; HR = 0.796, p = 0.0093), mPFS (9.9 versus 8.4 months; HR = 0.696, p = 0.0012), and response rate (RR) (57.3% versus 39.7%; OR = 2.069, p = 0.001) when compared with FOLFIRI alone. Addition of cetuximab to FOLFOX4 remarkably prolonged mPFS (8.3 versus 7.2 months, HR = 0.576, p = 0.0064) than FOLFOX4 alone, resulting in a trend of OS benefit (22.8 versus 18.5 months, HR = 0.855, p = 0.39). Cetuximab plus panitumumab also improved the mPFS of FOLFOX4 (10.0 versus 8.6 months, HR = 0.80, p = 0.01).

Table 1. Clinical progress of the third-generation EGFR-TKIs

| Drugs          | Stage            | Company          | Indications                  | Clinical trials                  | Refs.                           |
|---------------|------------------|------------------|------------------------------|----------------------------------|---------------------------------|
| Osimertinib   | 2015, approved   | AstraZeneca      | NSCLC, other solid cancers   | NCT02296125*, NCT02151981*, NCT02511106*, NCT03521154*, NCT04413201* | Soria et al.17, Ramalingam et al.18, Cho et al.19, Wu et al.20, Yang et al.21, Wu et al.22 |
|               | (US); 2017,      |                  |                              |                                  |                                 |
|               | approved (CN)    |                  |                              |                                  |                                 |
| Olmutinib     | 2016, approved   | Hanmi Pharma     | NSCLC                        | NCT01588145*, NCT02485652*, NCT03228277* | Guardiola et al.23, Wang and Adjei24, Tan et al.25 |
|               | (South Korea)    |                  |                              |                                  |                                 |
| Almonertinib  | 2020, approved   | Hansoh Pharma    | NSCLC                        | NCT04500717*, NCT04500704*      | Wang and Adjei24                |
|               | (CN)             |                  |                              |                                  |                                 |
| Maverlertinib | phase II         | Pfizer           | NSCLC                        | NCT02349633*, Wang and Adjei24, Tan et al.25 |                                    |
| Nazartinib    | phase II         | Novartis         | NSCLC, other solid cancers   | NCT02335944*, NCT03040973*, NCT02923156* | Wang and Adjei24, Tan et al.25 |
| Avitinib      | phase II         | ACEA Pharma      | NSCLC, B cell lymphoma       | NCT03800115*, NCT03060805*      | Wang and Adjei24, Tan et al.25 |
| CK-101        | phase II         | CKPT             | NSCLC, other solid cancers   | NCT02926768*                     | Wang and Adjei24                |
| D-0316        | phase II         | InventisBio      | NSCLC, other solid cancers   | NCT03861156*, NCT04206072*      | Wang and Adjei24                |
| BPI-7711      | phase III        | Beta Pharma      | NSCLC                        | NCT03866499*                     | Wang and Adjei24                |
| SH-1028       | phase III        | Sanhome Pharma   | NSCLC                        | NCT0423983*                      | Wang and Adjei24                |
| ASK120067     | phase III        | Aosaikang Pharma | NSCLC                        | NCT04143607*                     | Wang and Adjei24, Zhang et al.26 |
| ZN-e4         | phase II         | Zeno Pharma      | NSCLC                        | NCT03446417*                     | Wang and Adjei24                |
| Rociletinib   | phase III        | Clovis Oncol     | NSCLC                        | NCT02147990*                     | Wang and Adjei24, Tan et al.25 |
| Naquotinib    | phase I          | Astellas Pharma  | NSCLC                        | NCT02113813*                     | Wang and Adjei24, Tan et al.25 |
| Lazertinib    | phase III        | Yuhan/Genosco/   | NSCLC                        | NCT04248829*                     | Wang and Adjei24                |
|               |                  | Janssen Pharma   |                              |                                  |                                 |
| Alfutinib     | phase III        | Allist Pharma    | NSCLC                        | NCT03787992*                     | Wang and Adjei24                |
| ES-072        | phase I          | Bossan           | NSCLC                        | CTR20180074*                     |                                 |
| TY-9591       | phase I          | TYK Med          | NSCLC                        | NCT04204473*                     |                                 |
| TAS-121       | phase I          | Taiho Pharma     | NSCLC                        | JapicCTI142651C                  |                                 |
| BPI15086      | phase I          | Beta Pharma      | NSCLC                        | NCT02914990*                     |                                 |
| FHND9041      | phase II         | Chuangte Pharma  | NSCLC                        | CTR20191359*                     |                                 |
| C-005         | phase I          | Shuangliang Bio  | NSCLC                        | CTR20191910*                     |                                 |
| BEBT-109      | phase I          | Bebetter Med     | NSCLC                        | CTR20192575*                     |                                 |
| YZJ-0318      | phase I          | Haiyan Pharma    | NSCLC                        | CTR20171646*                     |                                 |
| TQB3456       | phase I          | ChiaTai TianQing | NSCLC                        | NCT03754244*                     | Wang and Adjei24                |
| WZ4002        | pre-clinical     |                  |                              |                                  | Ricordel et al.27               |
| CNX-2006      | pre-clinical     |                  |                              |                                  | Ricordel et al.27               |

CN, China; US, United States; CKPT, Checkpoint Therapeutics; NSCLC, non-small cell lung cancer.

*https://www.clinicaltrials.gov

**https://www.chinadrugtrials.org.cn

***https://www.clinicaltrials.jp

Cancer (mCRC). In patients with wild-type KRAS, Cetuximab combined with FOLFIRI significantly improved mOS (23.5 versus 20.0 months; HR = 0.796, p = 0.0093), mPFS (9.9 versus 8.4 months; HR = 0.696, p = 0.0012), and response rate (RR) (57.3% versus 39.7%; OR = 2.069, p = 0.001) when compared with FOLFIRI alone. Addition of cetuximab to FOLFOX4 remarkably prolonged mPFS (8.3 versus 7.2 months, HR = 0.576, p = 0.0064) than FOLFOX4 alone, resulting in a trend of OS benefit (22.8 versus 18.5 months, HR = 0.855, p = 0.39). Cetuximab plus panitumumab also improved the mPFS of FOLFOX4 (10.0 versus 8.6 months, HR = 0.80, p = 0.01). Interestingly, mCRC originating from the left colon was found to be more sensitive to
Table 2. Aberrant EGFR in different cancers

| Cancer type       | EGFR aberration                                      | EGFR inhibitors approved by FDA in clinic |
|-------------------|------------------------------------------------------|------------------------------------------|
| NSCLC             | 15%–50% mutation (Ex19del, L858R, Ex20ins, G719X, L861G, S768I, E709K, etc.), 32% gene amplification | erlotinib, gefitinib, afatinib, dacomitinib, osimertinib, brigatinib |
| Colorectal cancer | 7%–89% increased gene copy number,<sup>19</sup> <1% mutation<sup>18</sup> | cetuximab, panitumumab |
| SCCHN             | 10%–17% gene amplification, 42% EGFRvIII mutation<sup>61</sup> | cetuximab, nimotuzumab |
| Pancreatic cancer | 55%–69% protein overexpression, 1.5% mutation<sup>16</sup> | erlotinib |
| Renal cell cancer | 38% gene amplification<sup>65</sup> | NA |
| Hepato carcinoma  | 68% protein overexpression<sup>56</sup> | NA |
| Breast cancer     | 14%–64% gene amplification,<sup>57</sup><sup>,58</sup> 3%–11% mutation<sup>46</sup> (<Ex19del, T847I, L858R, G719A, V786M), 13% SNP (T725T, Q787Q) | lapatinib, neratinib, pyrotinib, afatinib |
| Prostate cancer   | 14%–30% protein overexpression,<sup>60</sup><sup>,61</sup> 15% mutation<sup>52</sup> (<Ex19del, V738G, D761G, E709K) | NA |
| Gastric cancer    | 27% protein overexpression,<sup>62</sup> 5.2% mutation<sup>16</sup> (<Y801C, L858R, G863D); 37.9% SNP (<T702A) | NA |
| Gloma             | 40%–45% gene amplification<sup>15</sup> 14% mutation<sup>15</sup> (D464E, G63R, R108K, T263P, A2989G, R224L, E330K, P596L, G585V, L861Q), 30% EGFRvIII mutation<sup>52</sup> | nimotuzumab |
| Ovarian cancer    | 4%–22% gene amplification<sup>68</sup> 23.5% mutation<sup>16</sup> (<Ex19del, T725X, L858R, R832C, E868D, T852M, L705P, S720F, N700S, etc.) | NA |

*The approval of brigatinib for NSCLC is mainly due to itsALK inhibition.

EGFR-targeted mAbs. EGFR targets KRAS (mPFS = 5.6 months), hence deserving further clinical assessment. Another phase II study revealed that cetuximab plus erlotinib was also active in mCRC with wild-type KRAS (mPFS = 5.6 months), hence deserving further clinical assessment.

Squamous cell carcinoma of the head and neck

Cetuximab was the first mAb that showed clinical activity for SCCHN. Cetuximab combined with radiotherapy notably ameliorated the prognosis of SCCHN. The mOS times in the group of cetuximab and radiotherapy group and the group undergoing radiotherapy alone were 49.0 and 29.3 months (HR = 0.74, p = 0.03), mPFS was 17.1 and 12.4 months (HR = 0.70, p = 0.006), and the 5-year survival rate was 45.6% and 36.4%, respectively. Erlotinib, combined with cisplatin and radiotherapy in patients with locally advanced SCCHN, failed to improve PFS. Multi-targeted EGFR-TKIs such as afatinib, dacomitinib, and lapatinib may be effective in SCCHN, although they should also be further assessed for their potential side effects. In recurrent metastatic nasopharyngeal carcinoma after radical radiotherapy, addition of nimotuzumab to chemotherapy resulted in a superior ORR, PFS, and OS than those who did not receive this nimotuzumab addition (88.9% versus 12.5%, p < 0.001; 7.4 versus 2.7 months, p = 0.081; 17.0 versus 8.0 months, p = 0.202). This combination was well tolerated and displayed a potential clinical application prospect in nasopharyngeal carcinoma.

Pancreatic cancer

Combining erlotinib with gemcitabine can improve the prognosis of pancreatic cancer patients, and this combination has been clinically approved. A phase II study of advanced pancreatic cancer with wild-type KRAS showed that nimotuzumab plus gemcitabine could significantly ameliorate patient prognosis. The mOS for combined therapy and gemcitabine alone was 8.6 and 6.0 months (HR = 0.69, p = 0.0341), mPFS was 5.1 and 3.4 months (HR = 0.68, p = 0.0163), and the 1-year OS/PFS rate was 34%–22% and 19%–10% (HR = 0.69, p = 0.03; HR = 0.68, p = 0.02), respectively. Neoadjuvant therapy with intensity-modulated radiotherapy, cetuximab, and gemcitabine was tolerable and increased margin-negative resection rates. Partial locally advanced tumors may be downstaged by applying this combination to allow for complete resection. However, the addition of cetuximab to gemcitabine could not improve prognosis compared with gemcitabine alone, and EGFR inhibitors plus MEK inhibitors were also of finite benefit to advanced pancreatic cancer. Overall, although EGFR-targeted therapy can improve pancreatic cancer prognosis, its clinical benefit is still limited.

Renal cell cancer

A phase II study showed that erlotinib improved disease control and survival outcomes under acceptable toxicity in renal cell cancer, with mOS of 27 months. Unfortunately, gene detection was absent before treatment. Detailed molecular typing may enhance the efficacy of EGFR inhibitors in renal cell cancer. Other clinical trials suggested that erlotinib plus bevacizumab achieved mPFS of 11 months in metastatic clear renal cell cancer. Losartin resulted in longer OS than hormone in EGFR-positive advanced renal cell cancer (46.0 versus 37.9 weeks; HR = 0.69, p = 0.02). These clinical trials highlight the therapeutic potential of EGFR inhibitors in renal cell cancer; however, additional investigations are warranted.

Hepatocellular carcinoma

A phase II study of patients with advanced hepatocellular carcinoma suggested that the mOS of erlotinib was 13 months. Combined erlotinib with bevacizumab achieved more potent activity, prolonging mPFS and mOS to 9 and 15.7 months, respectively. Another phase II study found that lapatinib was well tolerated, while it only had a therapeutic effect on a certain subgroup of patients with advanced hepatocellular carcinoma (mPFS = 1.9 months; mOS = 12.6 months), for whom predictive molecular or clinical characteristics have not been fully clarified.

Breast cancer

HER2 is the main driver gene in breast cancer. EGFR/HER2 target EGFR-TKIs, such as afatinib, neratinib, afatinib, and lapatinib, display an efficacy in breast cancer. For selective EGFR-TKIs, data from a phase II study uncovered a finite efficacy of erlotinib plus bevacizumab in breast cancer. Moreover, another study showed that a combination of gefitinib and anastrozole or trastuzumab could not improve the treatment efficacy in breast cancer. A phase II study of triple-negative breast cancer showed that addition of cetuximab to cisplatin achieved clinical benefit compared with cisplatin alone; mPFS for cetuximab plus cisplatin was 3.7 months and for cisplatin it was 1.5 months (HR = 0.67, p = 0.032), whereas mOS was 12.9 and 9.4 months (HR = 0.82, p = 0.31), respectively. However, these researches also suggested that the application of selective EGFR inhibitors for breast cancer should be further assessed.
Prostate cancer
A phase II study of metastatic prostate cancer showed that erlotinib achieved clinical benefit (decrease or stabilization of prostate-specific antigen [PSA] levels without clinical progression) in 40% of the patients.99 For androgen-resistant metastatic prostate cancer, a phase II study indicated that cetuximab decreased PSA in some patients and prolonged survival time (mOS = 13.3 months).97

Other indications
Multi-target EGFR inhibitors have been applied in other cancer types such as gastric cancer, thyroid cancer, leukemia, and ovarian cancer.2 These EGFR inhibitors may achieve activity by suppressing kinase targets other than EGFR. Nevertheless, selective EGFR inhibitors need further investigation in these cancer types.

MECHANISMS OF ACQUIRED RESISTANCE TO THIRD-GENERATION EGFR-TKIs
Considering the capacity to overcome resistance induced by the T790M EGFR mutation, the third-generation EGFR-TKIs, especially osimertinib, have received a great deal of attention. After approximately 1–2 years of treatment, disease progression occurred in most patients, implying the emergence of a drug-resistant phenotype.96 Mechanisms of the acquired resistance to the third-generation EGFR-TKIs possess significant tumor heterogeneity and can be classified as EGFR-dependent and EGFR-independent in general (Figures 3, 4, and 5; Table 3).

EGFR-dependent mechanisms

**EGFR mutations.** EGFR mutations, especially in exons 18, 20, and 21, account for about one-third of the mechanisms of resistance to the third-generation EGFR-TKIs (Figure 4).25 Cys797 is the most frequent mutation hotspot. Non-Cys797 EGFR mutations often coexist with other EGFR mutations.13,25 These mutations reduce the binding of the third-generation EGFR-TKIs to EGFR via different mechanisms.

**Mutations in EGFR exon 20.** C797S (22%–40% of the mutations) is the most frequent mutation that mediates resistance to the third-generation EGFR-TKIs (Figure 4).25 Cys797 is located at the edge of the ATP-binding pocket in the EGFR kinase domain. Third-generation EGFR-TKIs such as osimertinib and rociletinib are based on a pyrimidine scaffold and covalently bind to Cys797 via a Michael acceptor group.23,102,131 The C797S mutation prevents the formation of the covalent bond and results in drug resistance.23,102,131 Clinical data showed that the C797S mutation is more frequent in the Ex19del mutation.132 Intriguingly, activation of downstream signaling pathways induced by L858R/T790M/C797S mutant EGFR partially requires EGFR dimerization, promoting its sensitivity to cetuximab to a certain extent.132 For patients without the T790M mutation, C797S mutated EGFR moderately responds to the first-generation EGFR-TKIs.95 In patients with the T790M mutation, the colocalization of the T790M and C797S on the allele determines the option of a treatment strategy for overcoming C797S-mediated resistance. If C797S and T790M reside on different alleles (T790M/cis-C797S mutation), a combination of the first- and third-generation EGFR-TKIs may be active.103,104 If residing on the same allele (T790M/ trans-C797S mutation), all clinically approved EGFR inhibitors will fail to surmount drug resistance.133

To overcome the T790M/C797S mutation, the combination therapy of brigatinib with EGFR mAbs or the mutant-selective allosteric inhibitors may be an effective strategy.103,104 Some studies pointed out that the C797G or C797N EGFR mutations also mediated resistance to third-generation EGFR-TKIs.25,134

Next-generation sequencing (NGS) on cell-free DNA (cfDNA) of 93 osimertinib-resistant NSCLC patients revealed Gly796/Cys797 as well as Leu792 EGFR mutations in 24.7% and 10.8% of the cases, respectively.137 Among the L792X mutations including L792F, L792H, L792R, L792Y, L792V, and L792P, the L792H EGFR mutation results in the most potent resistance to osimertinib.135 L792X mutations in the absence of T790M also lead to osimertinib resistance.135 G796X mutations include G796R and G796S.135 Structural analyses uncovered that the L792H and G796R mutations sterically and energetically hinder the binding of osimertinib to EGFR via a similar mechanism.105 The L792H mutation is sensitive to the combination therapy of cetuximab + osimertinib.105 Moreover, docetaxel, a potent antimotic cytotoxic agent, effectively decreased the proliferation of tumor cells with L792H or G796S mutation.105

In patients with T790M mutation, C797S and T790M reside on different alleles (T790M/cis-C797S mutation), a combination of the first- and third-generation EGFR-TKIs may be active.103,104 If residing on the same allele (T790M/ trans-C797S mutation), all clinically approved EGFR inhibitors will fail to surmount drug resistance.133

Next-generation sequencing (NGS) on cell-free DNA (cfDNA) of 93 osimertinib-resistant NSCLC patients revealed Gly796/Cys797 as well as Leu792 EGFR mutations in 24.7% and 10.8% of the cases, respectively.137 Among the L792X mutations including L792F, L792H, L792R, L792Y, L792V, and L792P, the L792H EGFR mutation results in the most potent resistance to osimertinib.135 L792X mutations in the absence of T790M also lead to osimertinib resistance.135 G796X mutations include G796R and G796S.135 Structural analyses uncovered that the L792H and G796R mutations sterically and energetically hinder the binding of osimertinib to EGFR via a similar mechanism.105 The L792H mutation is sensitive to the combination therapy of cetuximab + osimertinib.105 Moreover, docetaxel, a potent antimotic cytotoxic agent, effectively decreased the proliferation of tumor cells with L792H or G796S mutation.105

In patients with T790M mutation, C797S and T790M reside on different alleles (T790M/cis-C797S mutation), a combination of the first- and third-generation EGFR-TKIs may be active.103,104 If residing on the same allele (T790M/ trans-C797S mutation), all clinically approved EGFR inhibitors will fail to surmount drug resistance.133

Next-generation sequencing (NGS) on cell-free DNA (cfDNA) of 93 osimertinib-resistant NSCLC patients revealed Gly796/Cys797 as well as Leu792 EGFR mutations in 24.7% and 10.8% of the cases, respectively.137 Among the L792X mutations including L792F, L792H, L792R, L792Y, L792V, and L792P, the L792H EGFR mutation results in the most potent resistance to osimertinib.135 L792X mutations in the absence of T790M also lead to osimertinib resistance.135 G796X mutations include G796R and G796S.135 Structural analyses uncovered that the L792H and G796R mutations sterically and energetically hinder the binding of osimertinib to EGFR via a similar mechanism.105 The L792H mutation is sensitive to the combination therapy of cetuximab + osimertinib.105 Moreover, docetaxel, a potent antimotic cytotoxic agent, effectively decreased the proliferation of tumor cells with L792H or G796S mutation.105

In patients with T790M mutation, C797S and T790M reside on different alleles (T790M/cis-C797S mutation), a combination of the first- and third-generation EGFR-TKIs may be active.103,104 If residing on the same allele (T790M/ trans-C797S mutation), all clinically approved EGFR inhibitors will fail to surmount drug resistance.133

The C797S mutation occurs in <3% of NSCLC patients after rociletinib treatment.136 A new L798I mutation was found in cis with T790M by circulating tumor DNA (ctDNA) analysis.136 Molecular dynamics simulations revealed that the Asp800 side chain forms a hydrogen bond with the quaternary piperazine NH⁺ of rociletinib in the T790M mutation.136 However, the double T790M/L798I mutation triggered the rotation of rociletinib orientation in EGFR, which hindered the formation of this hydrogen bond, and altered the angle of the Cys797 side chain, which diminished its nucleophilicity, leading to reduction in the binding affinity of rociletinib to EGFR.136

The C797S mutation occurs in <3% of NSCLC patients after rociletinib treatment.136 A new L798I mutation was found in cis with T790M by circulating tumor DNA (ctDNA) analysis.136 Molecular dynamics simulations revealed that the Asp800 side chain forms a hydrogen bond with the quaternary piperazine NH⁺ of rociletinib in the T790M mutation.136 However, the double T790M/L798I mutation triggered the rotation of rociletinib orientation in EGFR, which hindered the formation of this hydrogen bond, and altered the angle of the Cys797 side chain, which diminished its nucleophilicity, leading to reduction in the binding affinity of rociletinib to EGFR.136

Importantly, MET gene amplification was found to be the most frequent mechanism of rociletinib resistance, and patients with multiple pre-existing resistance mechanisms (T790M and MET gene amplification) experience inferior responses.136 Similarly, rociletinib-resistant xenografts develop MET gene amplification that can be overcome with the MET inhibitor crizotinib.136
The Innovation

Cells with EGFR D770insSVD, H773insH, or H773insPH mutations retain sensitivity to osimertinib. When these EGFR insertion mutations are accompanied by E762K, L792I/S, P794S, or G796D mutation, tumor cells acquire resistance to osimertinib. 

**Mutations in EGFR exon 18.** The aforementioned analysis of 93 osimertinib-resistant NSCLC patients also found EGFR Leu718/Gly719 mutations in 9.7% of the patients. Leu718 is located in the ATP-binding pocket of the EGFR kinase domain. L718Q results in more robust resistance to osimertinib than the L792X mutation. The L858R/T790M/cis-L718Q mutation shows similar resistance to osimertinib compared with the L858R/T790M/cis-C797S mutation, but slightly less resistance than the Ex19del/T790M/cis-C797S mutation. The L718Q mutation also induces resistance to osimertinib in the absence of the T790M mutation. Free-energy calculations uncovered that the L718Q mutation does not decrease the affinity of osimertinib to EGFR, whereas Gly719 forms a hydrogen bond with an acylamide warhead of osimertinib and maintains the latter in a specific conformation, which hampers Cys797 alkylation, contributing to the easy replacement of related downstream signaling pathways. Activation of degradation of mutant EGFR may be a promising strategy to overcome this chemoresistance.

**Defective degradation of EGFR.** EGFRWT is internalized into the cytoplasm after activation of the downstream signaling pathway and is degraded in lysosomes. However, clathrin-mediated internalization of mutant EGFR could not induce its degradation, resulting in a continuous activation of its downstream cascades. In osimertinib-resistant cells, clathrin inhibition by pitstop initiated micropinocytosis-dependent internalization of EGFR, which prompted the degradation of mutant EGFR and inhibited activation of related downstream signaling pathways. This study suggested that re-activation of degradation of mutant EGFR may be a promising strategy to overcome resistance to the third-generation EGFR-TKIs, although only further investigations may confirm the clinical efficacy of clathrin inhibition in cancer with resistance to the third-generation EGFR-TKIs.

**EGFR-dependent activation of PKCα.** An intriguing phenomenon whereby EGFR knockdown could reverse drug resistance, which could not be reversed by EGFR-TKIs, has been observed in resistant cells harboring a mutant EGFR. A further study indicated that a new role of activating-mutant EGFR, which is independent of EGFR kinase activity, stimulates the survival of TKI-resistant NSCLC with mutant EGFR. In detail, TKI-induced EGFR dimerizes with other membrane receptors of the tyrosine protein kinase family such as Anexelekto (AXL) as well as HER2. It should be noted that AXL is known to serve as a cancer driver and thus has been associated with poor survival in various aggressive malignancies.

---

**Figure 4. EGFR-dependent mechanisms of resistance to third-generation EGFR-TKIs**

(A) Mutations located in the EGFR kinase domain induce resistance to third-generation EGFR inhibitors. (B) Amplification of the EGFRWT and EGFR. (C) Deletion of the T790M mutation. (D) Clathrin-mediated internalization could not induce the degradation of mutant EGFR. (E) Heterodimerization of EGFR and HER2 or AXL activates PKCα via phosphorylation of Tyr1173. Activated PKCα triggers nuclear translocation of PKCα and activates AKT and NFκB signaling.
The Innovation 2, 100103, May 28, 2021

including NSCLC.\(^{114}\) This heterodimer triggers phosphorylation of Tyr1173 and the activation of PLC\(_{\gamma}2\) (phospholipase \(\gamma\)_2), leading to nuclear translocation of protein kinase \(\mathrm{C}\) (PKC) and the activation of PLC\(_{\gamma}\).\(^{112}\) PKC\(_{\gamma}\) also activates AKT and nuclear factor \(\kappa\)B (NF-\(\kappa\)B) signaling pathways.\(^{112}\) Consistently, combined inhibition of both PKC\(_{\gamma}\) and EGFR brings about robust resistance of resistant NSCLC with EGFR mutations.

**EGFR-independent mechanisms**

**Aberrant activation of downstream EGFR signaling pathways**

Activation of MAPK/ERK signaling pathway. Several alterations, such as NRAS mutations (G12V, G12R, G61K, and E63K), KRAS mutations (G12S, G12A, G61H, G12D, G13D, and A146T), BRAF mutations (G469A and V600E), MEK1 mutations, deletion of neurofibromin 1 (NF1), a GTPase-activating protein which negatively regulates the RAS/MAPK pathway, and gene amplification of v-crk sarcoma virus CT10 oncogene homolog-like (CRKL), provoke the sustained activation of the MAPK/ERK signaling pathway. Deletion of the CRKL gene sustains the activation of the MAPK/ERK signaling pathway. Deletion of the NF1 gene antagonizes AKT, resulting in resistance to the third-generation EGFR-TKIs.\(^{112}\) Combined therapy with MEK inhibitors may effectively overcome drug resistance. The recent development of KRAS inhibitors has been suggested as a new potential strategy to address this drug resistance.\(^{144}\)

**Activation of PI3K/AKT signaling pathway.** Deletion of phosphatase and tensin homolog (PTEN) increased phosphatidylinositol 3-kinase (PI3K) levels and induced hyperactivation of AKT, resulting in resistance to the third-generation EGFR-TKIs.\(^{13,114}\) Peroxisome proliferator-activated receptor-\(\gamma\) (PPAR\(\gamma\)) agonists such as rosiglitazone restore the sensitivity of PTEN-deleted cells displaying resistance to EGFR-TKIs via promotion of autophagy.\(^{114}\) PIK3CA is one of the PI3K isoforms, and PIK3CA mutations abnormally activate AKT. Specifically, the PIK3CA mutations, E545K, E542K, R88Q, N345K, and E418K, have been reported to confer resistance to osimertinib.\(^{27,142,143,145}\) The PIK3CA mutations E545K, E81K, and E418K were also described in ronitolib-resistant patients.\(^{27,145}\)

**Oncogenic fusions.** Studies have found that 3–10% of patients who received second-line treatment of osimertinib developed resistance due to oncogenic fusions, including CDC6-RET, PCBP2-BRAF, AGK-BRAF, FGFR3-TACC3, NTRK1-TPM3, RET-ERC1, NCOA4-RET, G0PC-ROS1, ESYT2-BRAF, and EML4-ALK.\(^{27,128,142,146–149}\) SPTBN1-ALK was found in the first-line treatment of osimertinib but not in second-line treatment.\(^{128,142,143}\) Combination therapy greatly contributes to overcoming oncogenic fusion-dependent drug resistance to third-generation EGFR-TKIs. For instance, selective RET inhibitors, such as BLU-667 plus osimertinib, were effective in CCDC6-RET-mediated drug resistance in NSCLC.\(^{98}\)

**Activation of bypass survival signaling pathways.** MET gene amplification. The MET signaling pathway is the most frequent bypass survival signaling pathway as an acquired resistance mechanism to EGFR-TKIs.\(^{128,142,143,150,151}\) MET activated by hepatocyte growth factor potentiates tumor cell survival through persistent activation of EGFR downstream signaling pathways, boosting the emergence of resistance to EGFR-TKIs.\(^{128,142,143}\) Combination of crizotinib, a multi-targeted MET inhibitor, and osimertinib, or alternatively capmatinib and afatinib, could be active in MET gene amplification-dependent resistance to osimertinib.\(^{115,116}\) This combination also acted on MET exon 14 skipping-mediated resistance to osimertinib in NSCLC with mutant EGFR.\(^{117}\)

**Figure 5. EGFR-independent mechanisms of resistance to third-generation EGFR-TKIs**

(A) Activation of downstream EGFR signaling pathways. NRAS mutation, KRAS mutation, BRAF mutation, MEK1 mutation, deletion of NF1, or amplification of the CRKL gene sustains the activation of the MAPK/ERK signaling pathway. Deletion of the PTEN or PIK3CA mutation results in constitutive activation of the PI3K/AKT signaling pathway. (B) Oncogenic fusions trigger sustained activation of survival-related signaling pathways. (C–G) Amplification of tyrosine kinase receptors including MET (C), HER2/3 (D), IGF1R (E), AXL (F), and FGFR1 (G). (H) Alteration of cell-cycle-related genes. (I) Aberrant phosphorylation of ACK1 enhances the activation of the AKT signaling pathway, leading to decreased BIM levels. (J) Increased nuclear translocation of YAP upregulates the expression of FOXM1, which promotes the expression of SAG members. (K) Epithelial-mesenchymal transition (EMT). (L) Transformation from NSCLC.
HER2/3 gene amplification. Amplification of HER2 arose in 2% or 5% cases that received first-line or second-line osimertinib treatment, respectively.\textsuperscript{124,125} HER2 gene amplification reduced the sensitivity of T790M-mutant cells to osimertinib and rociletinib.\textsuperscript{126} Plasma cfDNA analysis of one patient with the L858R/T790M mutation revealed that the occurrence of exon16-skipping HER2 mutation rendered the patient resistant to osimertinib.\textsuperscript{121} This mutation expresses HER2D16, leading to osimertinib resistance via a steroid receptor coactivator (src)-independent signaling pathway.\textsuperscript{121} Combining osimertinib with afatinib could synergistically repress the growth of tumor cells with HER2D16.\textsuperscript{121} HER3 forms heterodimers with other HER family members and activates the PI3K-AKT signaling pathway, which further contributes to the development of resistance to the third-generation EGFR-TKIs.\textsuperscript{152} A triple mixture of monoclonal antibodies simultaneously targeting EGFR, HER2, and HER3 repressed the growth of T790M-expressing tumors.\textsuperscript{119} This antibody triplet, also containing cetuximab and trastuzumab, inhibited C797S-expressing tumors. Unlike osimertinib, which induces apoptosis, this mAb triplet enhanced the degradation of the three receptors and induced cellular senescence.\textsuperscript{119} Consistently, combined treatment with these three mAbs and subinhibitory doses of osimertinib displayed a synergistic effect and eliminated tumors.

| Resistant mechanisms | Third-generation EGFR-TKIs | Countermeasures |
|----------------------|-----------------------------|-----------------|
| EGFR C797S           | osimertinib                 | first-generation EGFR-TKIs\textsuperscript{99-101} |
|                      | rociletinib                 | fourth-generation EGFR-TKIs\textsuperscript{99,102-104} |
| WZ4002               |                             |                 |
| EGFR C797N/G         | osimertinib                 | NA              |
| EGFR L792H           | osimertinib                 | chemotherapy (docetaxel)\textsuperscript{105} |
| EGFR L792F/R/Y/V/P/I/S | osimertinib             | NA              |
| EGFR G796R           | osimertinib                 | chemotherapy (docetaxel)\textsuperscript{105} |
| EGFR G796S/D         | osimertinib                 | NA              |
| EGFR M766Q           | osimertinib                 | neratinib or poziotinib\textsuperscript{106} |
| EGFR L798I           | rociletinib                 | NA              |
| EGFR E762K           | osimertinib                 | NA              |
| EGFR P794S           | osimertinib                 | NA              |
| EGFR L718Q           | osimertinib                 | afatinib\textsuperscript{107} |
| WZ4002               |                             |                 |
| EGFR L718V           | osimertinib                 | NA              |
| WZ4002               |                             |                 |
| EGFR G724S           | osimertinib                 | second-generation EGFR-TKIs\textsuperscript{106} |
| EGFR L844V           | WZ4002                      | gefitinib\textsuperscript{109} |
|                      | rociletinib                 | afatinib\textsuperscript{109} |
|                      |                             | osimertinib\textsuperscript{109,110} |
| EGFR\textsuperscript{WT} or EGF amplification | osimertinib | EGFR mAbs |
| Deletion of T790M mutation | osimertinib | accompanying mechanism |
| Defective degradation of EGFR | osimertinib | clathrin inhibitors\textsuperscript{111} |
| EGFR-dependent activation of PKC\textsuperscript{a} | osimertinib | PKC\textsuperscript{a} inhibitors\textsuperscript{112} |
| MAPK/ERK signaling pathway | osimertinib | MEK inhibitor\textsuperscript{13} |
|                      | rociletinib                 | SHP2 inhibitor\textsuperscript{13} |
|                      | WZ4002                      |                 |
| PI3K/AKT signaling pathway | osimertinib | PPAR\textgamma agonist\textsuperscript{14} |
|                      | rociletinib                 |                 |
| Oncogene fusions     | osimertinib                 | RET inhibitor\textsuperscript{28} etc. |

| Resistant mechanisms | Third-generation EGFR-TKIs | Countermeasures |
|----------------------|-----------------------------|-----------------|
| MET amplification    | osimertinib                 | MET inhibitor\textsuperscript{115-118} |
|                      | rociletinib                 |                 |
|                      | CNX-2006                    |                 |
| HER2/3 amplification | osimertinib                 | HER2/3 inhibitor (U3-1042)\textsuperscript{110} |
|                      | rociletinib                 |                 |
| Exon16-skipping HER2 mutation | osimertinib | afatinib\textsuperscript{121} |
| IG/IGF1R amplification | osimertinib | IGF/IGF1R inhibitor\textsuperscript{122,123} |
|                      | WZ4002                      |                 |
| GAS6/AXL amplification | osimertinib | AXL degrader\textsuperscript{124} |
|                      | rociletinib                 | AXL inhibitor\textsuperscript{124} |
| Alteration of cell-cycle-related genes | osimertinib | CDC4/6 inhibitor\textsuperscript{125} |
| ACK1 activation      | ASK120067                   | ACK1 inhibitor\textsuperscript{26} |
| SAC components activation | osimertinib | AURKA/AURKB inhibitor\textsuperscript{126,127} |
|                      | rociletinib                 | PLK1 inhibitor\textsuperscript{126} |
|                      |                             | Survivin inhibitor\textsuperscript{126} |
|                      |                             | KSP inhibitor\textsuperscript{126} |
| FGF2/FGFR amplification | osimertinib | FGF2/FGFR1 inhibitor\textsuperscript{128} |
| EMT                  | osimertinib                 | EMT inhibitor (JMF3086)\textsuperscript{129} |
|                      | rociletinib                 | Twist1 inhibitor\textsuperscript{128} |
|                      |                             | ZEB1 inhibitor\textsuperscript{127} |
|                      |                             | YAP inhibitor\textsuperscript{127} |
| SCLC transformation  | osimertinib                 | chemotherapy (etoposide and carboplatin)\textsuperscript{129} |

\textsuperscript{142,143} HER2 gene amplification reduced the sensitivity of T790M-mutant cells to osimertinib and rociletinib. Plasma cfDNA analysis of one patient with the L858R/T790M mutation revealed that the occurrence of exon16-skipping HER2 mutation rendered the patient resistant to osimertinib. This mutation expresses HER2D16, leading to osimertinib resistance via a steroid receptor coactivator (src)-independent signaling pathway. Combining osimertinib with afatinib could synergistically repress the growth of tumor cells with HER2D16. HER3 forms heterodimers with other HER family members and activates the PI3K-AKT signaling pathway, which further contributes to the development of resistance to the third-generation EGFR-TKIs. A triple mixture of monoclonal antibodies simultaneously targeting EGFR, HER2, and HER3 repressed the growth of T790M-expressing tumors. This antibody triplet, also containing cetuximab and trastuzumab, inhibited C797S-expressing tumors. Unlike osimertinib, which induces apoptosis, this mAb triplet enhanced the degradation of the three receptors and induced cellular senescence. Consistently, combined treatment with these three mAbs and subinhibitory doses of osimertinib displayed a synergistic effect and eliminated tumors.
U3-1042 is a promising HER3 inhibitor that has been recently discovered. A phase II study demonstrated that the combination of U3-1042 and osimertinib could significantly ameliorate the patient prognosis. Owing to the characteristics of the HER3 heterodimer, U3-1042 can substantially overcome resistance to the third-generation EGFR-TKIs caused by C797S, MET amplification, BRAF mutations, and other drug-resistance modalities.

**IGF/IGF1R amplification.** Aberrant expression of insulin-like growth factor (IGF)/IGF1 receptor (IGF1R) triggers continuous activation of MAPK/ERK and PI3K/AKT signaling pathways, hence provoking resistance to osimertinib in cancers. Combination with IGF1R or IGF inhibitors could restore osimertinib sensitivity.

**GAS6/AXL amplification.** In lung cancer, AXL and its ligand GAS6 are upregulated after resistance to osimertinib. Degradation of AXL is slower in osimertinib-resistant cells than in drug-sensitive cells. AXL also drives intrinsic resistance to osimertinib. Activated AXL by its ligand GAS6 triggers activation of MEK/ERK and PI3K/AKT pathways and boosts resistance to third-generation EGFR-TKIs. Agents inducing AXL degradation (such as yuanhuadine) or inhibition (BGB324) may be a potentially effective treatment to surmount resistance to osimertinib.

**Alteration of cell-cycle-related genes.** In 12% and 10% of NSCLC patients treated with first-line and second-line osimertinib, cell-cycle-related genes were altered, including gain of CCND, CCNE1, and CDK4/6, as well as CDKN2A E27fs. cdfDNA analysis of 41 osimertinib-treated NSCLC patients discovered that CDC4/6-positive patients had a poor response to osimertinib and shorter PFS. Indeed, CDC4/6 inhibitors such as palbociclib enhanced the response to osimertinib.

**Activation of ACK1-related signaling pathway.** A human phospho-RTK array on ASK120067-resistant lung cancer cells with the T790M mutation revealed that phosphorylation of activated cdc42-associated tyrosine kinase 1 (ACK1) was upregulated in resistant cells. A further study revealed that activation of ACK1 drives resistance to ASK120067 through the AKT-BIM (Bcl-2-like protein 11) signaling pathway.

**Activation of spindle assembly checkpoint components.** A previous study of lung cancer cells indicated that AURKA (Aurora kinase A, one of the spindle assembly checkpoint [SAC] components) activity significantly increased in both osimertinib-resistant and rociletinib-resistant cells compared with parental cells. Activation of AURKA by its coactivator targeting protein for Xklp2 (TPX2), which is upregulated simultaneously, promotes mitosis and suppresses phosphorylation of BIM, hence conferring drug resistance.

**Small cell lung cancer transformation.** The transformation from NSCLC to small cell lung cancer (SCLC) is another escape mechanism of a cancer cell from treatment, which has been confirmed in 14% of patients with acquired resistance to EGFR-TKIs. A study reported that patients harboring this transformation also failed to respond to osimertinib. The levels of both EGFR protein and mRNA were decreased in SCLC. In addition, SCLC did not display EGFR signaling pathway addiction, which may account for EGFR inhibitor resistance. The underlying mechanism of transformation remains unclear. Recent studies pointed out that SCLC transformation may be associated with the inactivation of Rb and p53.

**EXPLORATION OF NEXT-GENERATION EGFR-TKIs**

To overcome the EGFR mutation-mediated resistance to third-generation EGFR-TKIs, great attention have been dedicated since 2015/2016 to explore the next generation of EGFR-TKIs, known as the fourth generation (Table 4). Since the fourth generation of EGFR-TKI is an emerging area, de novo library screening as well as receptor structure-based drug design studies provide original hit compounds, while the evolved structure-activity relationships provide important guidelines and rationales for the discovery and iterative development of new drugs. After years of innovative research, several promising drug candidates have been evaluated in pre-clinical stages and phase I studies.

**EA0105**

A novel EGFR allosteric inhibitor hit compound with a thiazole amide skeleton, EA0101, was discovered by a library screening comprising ~2.5 million compounds displaying special selectivity for mutant EGFR. The IC50 value of EA0101 against L858R and T790M mutant EGFR kinase reached 24 nM, while the IC50 value for the EGFRWT was more than 50 μM. EA0105 emerged from medicinal chemistry-based optimization of EA0101 (Figure 6A). At a concentration of 1 mM ATP, the IC50 of EA0105 to T790M/ L858R mutant kinase was 3 nM, hence being more than 1,000-fold selective when compared with EGFRWT, in which the inhibition is not affected by the concentration of ATP. According to the crystal structure of EA0101 binding to T790M-mutant EGFR, EA0105 may also bind to allosteric sites in the EGFR kinase domain and requires EGFR to stay at the C-helix-out conformation. Molecular dynamics simulations revealed the mechanism underlying the selectivity of EA0105 toward mutant EGFR. In EGFRWT, the Leu858 side chain is stably buried in the hydrophobic allosteric pocket and prevents the binding of EA0105 to EGFR. In L858R mutant EGFR, Arg858 induces the allosteric pocket to open, thus enabling the binding of EA0105 to EGFR. Other similar mutations that could expose the allosteric pocket, such as L851Q, may also bear high affinity toward EA0105. However, EA0105 exhibited poor antitumor activity and incomplete inhibition of EGFR autophosphorylation in cancer cells.

In EGFRWT asymmetric dimers, the C-lobe of the “activator” subunit turned the C-helix located at the N-lobe of the “receiver” subunit into an in-conformation. Hence, only the “receiver” subunit is activated in EGFRWT. In mutant EGFR dimers, both subunits could activate the downstream signaling pathway while EA0105 only binds to one subunit, resulting in incomplete inhibition of mutant EGFR. Combined with other agents that could block EGFR dimerization, they could enhance the efficacy of EA0105. More precisely, cetuximab effectively enhanced the activity of EA0105 toward L858R/T790M/C797S triple EGFR mutant cells.
For the T790M mutant EGFR without L858R, although a decreasing trend occurred with the increasing ATP concentration, EAI045 still displayed a promising inhibition effect on the T790M mutation. However, the aforementioned molecular dynamics simulations could not explain this robust EAI045 inhibition of T790M/C797S EGFR mutation. The crystal structure of EAI045-T790M/C797S/V948R mutant EGFR indicated that EAI045 could bind to a deep pocket between the ATP pocket and the C-helix, thus requiring adequate space created by the C-helix-out rotation (Figure 6A). Amino acids deletion pulls the C-helix toward the ATP-binding pocket and constrains the allosteric site to a small volume, leading to the low affinity of EAI045 to the Ex19del EGFR mutation. However, EAI045 is inactive as a single agent and is only active when combined with EGFR-targeted mAbs, such as cetuximab. Cetuximab is only active when combined with EGFR-targeted mAbs, such as cetuximab. JBJ-04-125-02 also binds to the C-helix in the conformation of EGFR, contributing to its high affinity toward Ex19del/T790M/C797S mutation.

**JBJ-04-125-02**

An iterative process of synthesizing structural analogs of EAI001 was applied, whereby a new compound, designated as JBJ-04-125-02, was obtained (Figure 6A). The crystal structure revealed that, like EAI045, JBJ-04-125-02 also binds to the allosteric pockets in the C-helix-out conformation of EGFR. Intriguingly, JBJ-04-125-02 binding induced a novel conformation of the A-loop that seems to be stabilized by a hydrogen bond between the piperazine group of the compound and Glu865 in the A-loop (Figure 6C). JBJ-04-125-02 displayed a cytotoxic effect on L858R, L858R/T790M, or L858R/T790M/C797S mutations without coadministration of cetuximab. JBJ-04-125-02 also lacks binding affinity toward EGFRWT or the Ex19del mutant. Further research indicated that osimertinib might enhance the binding affinity of JBJ-04-125-02 to EGFR, leading to a more potent antitumor activity. These data suggested that combining a covalent mutant-selective allosteric EGFR-TKI may be a useful treatment strategy for some of the lung cancer patients who are resistant to the third-generation EGFR-TKIs. However, both EAI045 and JBJ-04-125-02 cannot overcome the resistance mediated by the Ex19del/T790M/C797S triple mutant.

**CH7233163**

A novel compound, CH7233163 (IC50 = 0.28 nM), which is capable of overcoming the resistance mediated by the Ex19del/T790M/C797S triple mutation, has been isolated from a massive chemical library (Figure 6A). Compared with EGFRWT, CH7233163 exerted a more selective inhibition on various EGFR mutants (e.g., L858R/T790M/C797S, L858R/T790M, Ex19del/T790M, Ex19del, and L858R). Further tests verified the significant anti-tumor activity of CH7233163 in cancer cells with the Ex19del/T790M/C797S or L858R/T790M/C797S triple mutation. The crystal structure of CH7233163-L858R/T790M/C797S triple mutant EGFR revealed that CH7233163 interacts with the ATP-binding pocket via hydrogen bonds and CH/π interactions but not with the Ser797 residue (Figure 6D). Compared with osimertinib, CH7233163 not only binds to the P-loop and the hinge region but directly interacts with Met790 residues. Compared with EAI001, CH7233163 binds to the C-helix in the conformation of EGFR, contributing to its high affinity toward Ex19del/T790M/C797S mutation.

**Other compounds**

TQB3804 (Figure 6A) and BBT-176 are novel fourth-generation EGFR-TKIs isolated by pharmaceutical enterprises. Preclinical study showed that TQB3804 and BBT-176 displayed an outstanding inhibitory effect on Ex19del/T790M/C797S and L858R/T790M/C797S triple mutation. BLU-945 is another fourth-generation EGFR-TKI targeting the T790M/C797S mutation reported in ESCO. In vitro data suggested that BLU-945 achieved robust inhibition of Ex19del/T790M/C797S and L858R/T790M/C797S triple mutation, rather than EGFRWT. Cell-derived xenograft and patient-derived xenograft models consistently showed that single treatment with BLU-945 or coadministration with osimertinib/gefitinib significantly blocked the

Table 4. Progress of the next-generation EGFR-TKIs

| Drug          | Company            | Targeted mutation            | Active mechanism       | Stage       | Reference          |
|---------------|--------------------|------------------------------|------------------------|-------------|--------------------|
| EAI045        | Novartis           | T790M/C797S, L858R/C797S     | reversible             | pre-clinical| Jia et al.102      |
|               |                    | L858R/T790M/C797S           | non-ATP competitive    |             |                    |
| JBJ-04-125-02 | Johnson & Johnson  | T790M/C797S, L858R/C797S     | reversible             | pre-clinical| To et al.104       |
|               |                    | L858R/T790M/C797S           | non-ATP competitive    |             |                    |
| CH7233163     | Roche Pharma       | T790M/C797S, L858R/C797S     | reversible             | pre-clinical| Kashima et al.161  |
|               |                    | Ex19del/C797S               | ATP competitive        |             |                    |
|               |                    | Ex19del/T790M/C797S         |                        | phase I     | Liu et al.162      |
|               |                    | L858R/T790M/C797S           |                        | phase I     | Pharmabiz163       |
| TQB3804       | ChiaTai TianQing   | Ex19del/T790M/C797S         | NA                     | phase I     | Schalm et al.164   |
|               |                    | L858R/T790M/C797S           |                        | pre-clinical|                   |
| BBT-176       | Bridge Bio         | Ex19del/T790M/C797S         | NA                     | phase I     |                   |
|               |                    | L858R/T790M/C797S           |                        | pre-clinical|                   |
| BLU-945       | Blueprint Medicines| Ex19del/T790M/C797S         | NA                     | pre-clinical|                   |
|               |                    | L858R/T790M/C797S           |                        |             |                   |
| Brigatinib    | TAKEDA Pharma      | Ex19del/T790M/C797S         | reversible             | FDA approved| Uchibori et al.163|
|               |                    | L858R/T790M/C797S           | ATP competitive        |             |                    |

*Multiple-target inhibitor.*
progression of tumors with the Ex19del/T790M/C797S mutation. Brigatinib was identified as a next-generation inhibitor targeting anaplastic lymphoma kinase (ALK). A series of screening data suggested that brigatinib potently inhibits the proliferation of cells harboring the triple EGFR mutation. Moreover, several small-molecule compounds, including the 4-aminopyrazolopyrimidines, tri-substituted imidazoles, and 2-aryl-4-aminquinazolines, have also been reported to be able to surmount C797S mutation-mediated drug resistance. Additional clinical trials for these fourth-generation EGFR-TKIs may offer a promising therapeutic strategy to overcome mutant EGFR-based TKI resistance in various cancers.

SEQUENCING TECHNOLOGY ACCELERATING THE REALIZATION OF EGFR-TARGETED PRECISION THERAPY

Gene sequencing based on tumor tissue may help discover cancer driver genes and target genes for treatment, serving as an ideal “compass” for precision therapy. The development of NGS provides a powerful tool for gene sequence determination, contributing to the molecular typing of tumors and EGFR-targeted precision medicine. Compared with traditional sequencing technologies such as fluorescence in situ hybridization, amplification refractory mutation system PCR (ARMS-PCR), and droplet digital PCR (ddPCR), NGS can detect known and unknown EGFR mutations, gene copy-number variation, gene rearrangement, and other gene abnormalities via large-scale and high-throughput methods. Various driver genes contribute to cancer progression, and there also have been multiple mutations or comutations in different cases. Different driver genes or mutations require different targeted treatments, leading to arduous selection in the clinic. For example, NSCLC with common or uncommon EGFR mutations displays a greatly variable response to different EGFR-TKIs, for which NGS could effectively determine the precise therapeutic strategy and also predict patient prognosis.
heterogeneous and unknown drug-resistance mechanisms greatly limit the clinical application of traditional sequencing technology. In contrast, NGS can identify the mechanism underlying drug resistance and provide effective means to guide the overcoming of resistance to EGFR-TKIs. This could facilitate the transformation of a malignant disease to a chronic disorder and help patients with advanced malignant tumors to achieve long-term survival.

In some cases it is difficult to obtain tumor tissues, such as an unresectable advanced tumor or high-risk biopsy. Liquid biopsy is a newly emerging technique and constitutes a new dawn for patients from whom tumor specimens cannot be obtained. Moreover, compared with a single-lesion needle biopsy, liquid biopsy can better capture the molecular heterogeneity of different clonal populations in a given tumor. ctDNA detection based on liquid biopsy has rapidly become a crucial means of standard tumor biopsy, even a potentially alternative method. Present methods for ctDNA detection comprise cobas PCR, ARMS-PCR, ddPCR, BEAMing, and NGS. The ability of cobas PCR, ARMS-PCR, ddPCR, and BEAMing to detect common EGFR mutations (Ex19del, L858R, and T790M) was assessed, whereby it was found that different methods have their own advantages in detecting different mutations. These four methods could guide individualized medicine based on EGFR inhibitors by detecting known EGFR mutations. Nevertheless, these methods are deficient in their abilities to detect unknown gene alterations, making it difficult to meet the demands of precision medicine therapy. NGS detected ctDNA in 15,000 patients with advanced cancer, consisting of 37% lung cancer, 14% breast cancer, 10% colorectal cancer, and 38% other cancers, and compared that indicated with data from tissues in The Cancer Genome Atlas database, the correlation of EGFR mutations between ctDNA and tissue detection was 92%. These data suggested that ctDNA could also be the biomarker for cancer and guide precision medicine targeting EGFR. In fact, patients who received EGFR therapy can benefit from NGS of ctDNA. In particular, EGFR mutations are detected in ctDNA by NGS, which is more instructive for EGFR-targeted therapy. However, NGS of ctDNA is prone to false negativity due to the low concentration of ctDNA, especially in early-stage tumor or low-burden tumor patients. An American Society of Clinical Oncology report evaluated the guiding significance of ctDNA in urine and blood for rocletinib treatment in NSCLC. Taking histological T790M mutation detection as a standard, plasma and urine sensitivity was 80.9% and 81.1%, respectively. Patients were then divided into three groups according to the type of detected samples, including tissues, plasma, and urine. Based on the results of this detection, patients in each group received rocletinib treatment. The authors found, remarkably, that there was no significant difference in ORR and mDoR among these three groups. Moreover, T790M mutation-negative patients in these three distinct groups of body fluids were not overlapping, indicating that detection of ctDNA from different body fluids may substantially decrease the false-negative rate. Another study showed that the detection of ctDNA in both urine and plasma could effectively identify Ex19del and L858R mutations. Taken together, NGS of multiple body fluids or tissues is conducive to the realization of personalized medicine based on targeted EGFR.

CONCLUSIONS AND FUTURE PERSPECTIVES

EGFR inhibitors have enormous benefit for cancer patients. However, since tumor heterogeneity and genomic instability are hallmarks in tumor biology, the theme of anticancer drug resistance becomes inevitable for such EGFR inhibitors. The present review describes that highly heterogeneous mechanisms mediate resistance of EGFR-mutated tumors to the third-generation EGFR-TKIs. Diverse and distinct mechanisms of chemoresistance emerge and coexist simultaneously. Since distinct resistance mechanisms require different treatment strategies, identification of precise molecular mechanisms of drug resistance is essential in overcoming chemoresistance. The discovery of new resistance mechanisms and selection of the proper treatment strategies is of paramount importance. The emergence of NGS and liquid biopsy provides a powerful tool for the precise surmounting of resistance to EGFR inhibitors. Through the detection of known or unknown resistance mechanisms via dynamic monitoring achieved by applying NGS, accurate overcoming modalities can be developed in the clinic, which is conducive to the long-term survival of cancer patients.

How to improve the efficacy of EGFR inhibitors in cancer treatment is another important issue. Sequential therapy is an effective strategy to enhance the clinical benefit of patients from EGFR inhibitors. Based on the integration and analysis of clinical data from several independent clinical trials, the OS of NSCLC patients who received different sequential therapies has been predicted. It was found that sequential treatment of the first-/second-generation EGFR-TKIs, the third-generation EGFR-TKIs, and chemotherapy or sequential treatment of the third-generation EGFR-TKIs and chemotherapy may be the preferred strategy for NSCLC treatment to achieve longer OS. Another study indicated that sequential treatment of afatinib and osimertinib prolonged the mPFS of NSCLC patients to 21.9 months; also, the mOS values for this sequential treatment were not reached after 4.7 years of follow-up. Compared with the FLAURA study of osimertinib, sequential treatment of afatinib and osimertinib seems to be an optimal choice to maximize OS. A phase IV trial (NCT04413201) that compared osimertinib alone versus afatinib followed by osimertinib is still in the patient recruitment phase. Unfortunately, high-quality RCTs for evaluation of sequential EGFR inhibitor therapy in cancers are still overwhelmingly rare. Moreover, it is unclear whether sequential treatment of osimertinib and the fourth-generation EGFR-TKIs can be used as more efficient therapy. Hence, high-quality clinical trials are urgently needed to assess the efficacy of different sequential EGFR inhibitor therapies.

Combination therapy such as immunotherapy is another meaningful strategy to increase the benefit of patients from EGFR inhibitors. Data from NEJ026, RELAY, and ARTEMIS studies showed that combination of EGFR inhibitors with anti-angiogenic therapy significantly prolonged PFS but not OS. A phase III study indicated that combining EGFR inhibitors with chemotherapy markedly extended PFS and OS. Combination therapy could also effectively overcome resistance to third-generation EGFR-TKIs induced by activation of bypass signaling pathways. For instance, a combination of cetuximab, trastuzumab, and low-dose osimertinib induced degradation of EGFR and HER2 and reduced the abundance of several bypass signaling cascades such as MET, AXL, and HER3, which are able to provoke the onset of resistance to osimertinib. Other combination therapies for combating resistance phenomena have also been discussed herein. Nevertheless, the combination of several drugs has also resulted in more side effects, implying that personalized medicine and timely treatment should be of much concern when using combination therapy in the clinic.

For some rare drug-resistance mechanisms it is difficult to conduct clinical trials with a sufficient number of patients, leading to arduous investigation of potential strategies to overcome drug resistance to third-generation EGFR-TKIs. Furthermore, the aberrant rate and altered form of EGFR are also greatly cancer specific, representing similar problems concerning the small sample size hence being too small to meet the needs of clinical trials for certain cancers. A master protocol is an integrated experimental scheme designed to resolve multiple issues, including an umbrella trial, basket trial, and platform trial. These trials consist of a series of substudies that share key design and implementation elements, yielding a better coordination effect than when they are designed and implemented separately. Therefore, it is of great clinical value to carry out a master protocol related to large-scale clinical trials to evaluate the antitumor effect of the third-generation EGFR-TKIs and customize strategies to overcome drug resistance.

REFERENCES

1. Kumar, A., Petri, E.T., Halmos, B., et al. (2008). Structure and clinical relevance of the epidermal growth factor receptor in human cancer. J. Clin. Oncol. 26, 1742–1751.
2. Guaroldia, S., Varese, M., Sanchez-Navarro, M., et al. (2019). A third shot at EGFR new opportunities in cancer therapy. Trends Pharmacol. Sci. 40, 941–955.
3. Porcelli, L., Giovannetti, E., Assaraf, Y.G., et al. (2014). The EGFR pathway regulates BCRP expression in NSCLC cells: role of erlotinib. Curr. Drug Targets 15, 1322–1330.
4. Assaraf, Y.G., Brozovic, A., Goncalves, A.C., et al. (2019). The multi-factorial nature of clinical multidrug resistance in cancer. Drug Resist. Updat. 46, 100645.
14. Wood, E.R., Truesdale, A.T., McDonald, O.B., et al. (2004). A unique structure for EGFR.
17. Soria, J.C., Ohe, Y., Vansteenkiste, J., et al. (2018). Osimertinib in untreated EGFR-
16. Wu, S.G., and Shih, J.Y. (2018). Management of acquired resistance to EGFR TKI-targeted therapy. Cancer Res. 11, 217–227.
15. Yun, C.H., Boggon, T.J., Li, Y., et al. (2007). Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanisms of activation and insights into different inhibitor sensitivity. Cancer Cell 11, 217–227.
14. Wu, Y.-L., Ahn, M.-J., Garassino, M.C., et al. (2018). CNS efficacy of Osimertinib in EGFR T790M-mutant advanced non-small-cell lung cancer (NSCLC). J. Clin. Oncol. 36, 2323–2423.
13. Yan, X.E., Ayaz, P., Zhu, S.J., et al. (2020). Structural basis of AZD9291 selectivity for epidermal growth factor receptor (EGFR) Inhibitors against C797S resistance in non-small-cell lung cancer harboring uncommon EGFR mutations: a multicenter, open-label, phase II trial (KCSG-LU15-09). J. Clin. Oncol. 38, 488–495.
12. Wu, Y.-L., Ahn, M.-J., Garassino, M.C., et al. (2018). CNS efficacy of osimertinib in patients with T790M-positive advanced non-small-cell lung cancer: data from a randomized phase III trial (AURA3). J. Clin. Oncol. 36, 2702–2709.
11. Zhitomirsky, B., and Assaraf, Y.G. (2016). Lysosomes as mediators of drug resistance in cancer. Drug Res. 52, 100704.
10. Mosca, L., Ili, A., Fazi, F., et al. (2021). Taxanes in cancer treatment: activity, chemoresistance and its overcoming. Drug Resist. Updat. 54, 100742.
9. Zhitomirsky, B., and Assaraf, Y.G. (2016). Lysosomes as mediators of drug resistance in cancer. Drug Res. 52, 24–33.
8. Malapelle, U., Ricciuti, B., Baglivo, S., et al. (2018). Osimertinib. Recent Results Cancer Res. 211, 257–276.
7. Nagano, T., Tachihara, M., and Nishimura, Y. (2018). Mechanism of resistance to epidermal growth factor receptor-tyrosine kinase inhibitors and a potential treatment strategy. Cells 7, 212.
6. Wood, E.R., Truesdale, A.T., McDonald, O.B., et al. (2004). A unique structure for epidermal growth factor receptor factor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. Cancer Res. 64, 6652–6659.
5. Wu, Y.-L., Tsuboi, M., He, J., et al. (2020). Osimertinib in resected EGFR-mutated non-small-cell lung cancer (NSCLC): a pan-mutation-selective EGFR tyrosine kinase inhibitor with a broad spectrum of preclinical activity against clinically relevant EGFR mutations. Mol. Cancer Res. 17, 643–655.
4. Piotrowska, Z., and Sequist, L.V. (2016). Treatment of EGFR-mutant lung cancers after progression in patients receiving first-line EGFR tyrosine kinase inhibitors: a review. JAMA Oncol. 2, 948–954.
3. Recondo, G., Facchini, F., Olauszen, K.A., et al. (2018). Making the first move in EGFR-driven or ALK-driven NSCLC: first-generation or next-generation TKI? Nat. Rev. Clin. Oncol. 15, 694–708.
2. Cappuzzo, F., Hirsch, F.R., Rossi, E., et al. (2005). Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J. Natl. Cancer Inst. 97, 643–655.
1. Yasuda, H., Kobayashi, S., and Costa, D.B. (2012). EGFR exon 20 insertion mutations in other activating mutations. Cancer Res 29, 1004–1006.
60. Nastaly, P., Stoupiec, S., Popeda, M., et al. (2020). EGFR as a stable marker of progres-
tate cancer dissemination to bones. Br. J. Cancer 123, 1767–1774.
61. Schlomn, T., Kistein, P., Iwers, L., et al. (2007). Clinical significance of epidermal
growth factor receptor protein overexpression and gene copy number gains in pros-
tate cancer. Clin. Cancer Res. 13, 6579–6584.
62. Cho, K.S., Lee, J.S., Cho, N.H., et al. (2008). Gene amplification and mutation analysis of
epidermal growth factor receptor in hormone refractory prostate cancer. Prostate 68, 403–408.
63. Kim, M.A., Lee, H.S., Lee, H.E., et al. (2008). EGFR in gastric carcinomas: prognostic
significance of protein overexpression and high gene copy number. Histopathology
52, 738–746.
64. Liu, Z., Liu, L., Li, M., et al. (2011). Epidermal growth factor receptor mutation in
gastric cancer. Pathology 43, 234–238.
65. Eskelsson, O., Rosland, G.V., Solecki, G., et al. (2018). EGFR heterogeneity and im-
lications for therapeutic intervention in glioblastoma. Neuro. Oncol. 20, 743–752.
66. Lee, J.C., Vivanco, I., Beroukhim, R., et al. (2006). Epidermal growth factor receptor
activation in glioblastoma through novel missense mutations in the extracellular
domain. Plos Med. 3, e485.
67. Yang, K., Ren, X., Tao, L., et al. (2019). Prognostic implications of epidermal growth
factor receptor variant III expression and nuclear translocation in Chinese human gli-
omas. Chin. J. Cancer Res. 31, 188–202.
68. Sheng, Q., and Liu, J. (2011). The therapeutic potential of targeting the EGFR family in
epithelial ovarian cancer. Br. J. Cancer 104, 1241–1245.
69. Tanaka, Y., Terai, Y., Tanabe, A., et al. (2011). Prognostic effect of epidermal growth
factor receptor gene mutations and the aberrant phosphorylation of Akt and ERK in
ovarian cancer. Cancer Biol. Ther. 11, 50–57.
70. Yang, J.C.H., Sequist, L.V., Geater, S.L., et al. (2015). Clinical activity of afatinib in pa-
tients with advanced non-small-cell lung cancer harbouring uncommon EGFR muta-
tions: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6.
Lancet Oncol. 16, 830–838.
71. Coldough, N., Chen, K., Johnstroom, P., et al. (2021). Preclinical comparison of the
blood-brain barrier permeability of osimertinib with other EGFR TKIs. Clin. Cancer
Res. 27, 189–201.
72. Van Cutsem, E., Kohne, C.H., Lang, I., et al. (2011). Cetuximab plus irinotecan, fluoro-
uracil, and leucovorin as first-line treatment for metastatic colorectal cancer: up-
dated analysis of overall survival according to tumor KRAS and BRAF mutation sta-
tus. J. Clin. Oncol. 29, 2011–2019.
73. Bokemeyer, C., Bondarenko, I., Hartmann, J.T., et al. (2011). Efficacy according to
biomarker status of cetuximab plus FOLFIRI-4 as first-line treatment for metastatic colorectal
cancer: the OPTIMIST study. Ann. Oncol. 22, 1535–1546.
74. JY, D., S, S., J, C., et al. (2014). Final results from PRIME: randomized phase 3 study of
panitumumab with FOLFIRI4 for first-line treatment of metastatic colorectal cancer.
Ann. Oncol. 25, 1346–1355.
75. Arnold, D., Lueza, B., Douillard, J.Y., et al. (2017). Prognostic and predictive value of
primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated
with chemotherapy and EGFR directed antibodies in six randomized trials. 
Clin. Cancer Res.
76. National Comprehensive Cancer Network (2020). NCCN Clinical Practice Guidelines
in Oncology: Colon Cancer (2020.V2) (NCCN). https://www.nccn.org/professionals/
physician_gls/default.aspx#site.
77. Weickhardt, A.J., Price, T.J., Chong, G., et al. (2012). Dual targeting of the epidermal
growth factor receptor and vascular endothelial growth factor receptor 2 with
everolimus and bevacizumab for refractory colorectal cancer. J. Clin. Oncol.
30, 1473–1479.
78. Bonner, J.A., Harari, P.M., Giralt, J., et al. (2010). Radiotherapy plus cetuximab for lo-
cal-regional advanced head and neck cancer: 5-year survival data from a phase 3
randomised trial. J. Clin. Oncol. 28, 1612–1620.
79. Cathomas, R., Rothermundt, C., Klingbiel, D., et al. (2012). Effect of erlotinib on
response of protein overexpression and high gene copy number. Histopathology
60, 1415–1419.
80. Piotrowska, Z., Izzazaki, H., Lennier, J.K., et al. (2018). Landscape of acquired resis-
tance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined
EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET fusion.
Cancer Discov. 8, 1529–1539.
81. Rangachari, D., To, C., Shplisky, J.E., et al. (2019). EGFR-mutated lung cancers resis-
tant to osimertinib through EGFR C797S respond to first-generation reversible EGFR
inhibitors but eventually acquire EGFR T790M/C797S in preclinical models and clini-
cal samples. J. Thorac. Oncol. 14, 1995–2002.
82. Arulandana, S., Do, H., Musafer, A., et al. (2017). Combination osimertinib and gefitin-
ib in C797S and T790M EGFR-mutated non-small cell lung cancer. J. Thorac.
12, 1728–1732.
83. Zhou, Z., Zhao, Y., Shen, S., et al. (2019). Durable clinical response of lung adenocar-
cinoma harboring EGFR 19Del/T790M/in trans-C797S to combination therapy of
osimertinib and gefitinib. J. Thorac. Oncol.
84. Jia, Y., Yun, C.H., Park, E., et al. (2016). Overcoming EGFR(T790M) and EGFR(C797S)
resistance with mutant-selective allosteric inhibitors. Nature
6057.
107. Starrett, J.H., Guenett, A.A., Cuomo, M.E., et al. (2020). Drug sensitivity and allele specificity of first-line osimertinib resistance EGFR mutations. Cancer Res. 80, 2017–2030.

108. Fassunke, J., Muller, F., Keul, M., et al. (2018). Overcoming EGFR (G724S)-mediated osimertinib resistance through unique binding characteristics of second-generation EGFR inhibitors. Nat. Commun. 9, 4655.

109. Ercan, D., Choi, H.G., Yun, C.H., et al. (2015). EGFR mutations and resistance to irreversible pyrimidine-based EGFR inhibitors. Clin. Cancer Res. 21, 3913–3923.

110. Lee, P.C., Fang, Y.F., Yamaguchi, H., et al. (2018). Targeting PKCdelta as a therapeutic strategy against heterogeneous mechanisms of EGFR inhibitor resistance in EGFR-mutant lung cancer. Cancer Cell 34, 954–969.

111. Sun, Y., Meyers, B.A., Czako, B., et al. (2020). Allosteric SHP2 inhibitor IACS-13909 overcomes EGFR-dependent and EGFR-independent resistance mechanisms towards osimertinib. Cancer Res. 80, 4840–4853.

112. Papadimitrakopoulou, V.A., Wu, Y.L., Han, J.Y., et al. (2018). On-target resistance to the mutant-selective EGFR inhibitor osimertinib can develop in an allele-specific manner dependent on the original EGFR-activating mutation. Cancer Res. 25, 3341–3351.

113. Lee, Y., Kim, T.M., Kim, D.W., et al. (2018). Mechanisms of acquired resistance to AZD9291: a mutation-selective, irreversible EGFR inhibitor. J. Thorac. Oncol. 10, 1736–1744.

114. Brown, B.P., Zhang, Y.K., Westover, D., et al. (2019). On-target resistance to the mutant-selective EGFR inhibitor osimertinib can develop in an allele-specific manner dependent on the original EGFR-activating mutation. Cancer Res. 25, 3341–3351.

115. Papadimitrakopoulou, V.A., Wu, Y.L., Han, J.Y., et al. (2018). Analysis of resistance mechanisms to osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. Ann. Oncol. 29, https://doi.org/10.1093/annonc/mdy483.007.

116. Romaniello, D., Marrocco, I., Belugali Nataraj, N., et al. (2020). Targeting HER3, a catastrophic resistance mechanism to EGFR-TKI treatment in EGFR-mutated non-small cell lung cancer. Cancer Res. 25, 2244–2256.

117. Suwa, K., Offin, M., Schoenfeld, A.J., et al. (2019). Acquired MET exon 14 alteration drives secondary resistance to epidermal growth factor receptor tyrosine kinase inhibitor in EGFR-mutated lung cancer. JCO Precis Oncol 3, https://doi.org/10.1200/PO.19.0011.

118. Sequist, L.V., Han, J.Y., Ahn, M.J., et al. (2020). Osimertinib plus savolitinib in patients with EGFR-mutation-positive, MET-amplified, non-small-cell lung cancer after progression on EGFR tyrosine kinase inhibitors: interim results from a multicentre, open-label, phase 1b study. Lancet Oncol. 21, 373–386.

119. Mancini, M., Gal, H., Gaborit, N., et al. (2018). An oligonucleotide antibody durably overcomes resistance of lung cancer to third-generation EGFR inhibitors. EMBO Mol. Med. 10, 294–308.

120. Li, H.A., Baik, C.S., Gold, K., et al. (2020). LBA42 efficacy and safety of patitumumab deruxtecan (U3-1402), a novel HER3 directed antibody drug conjugate, in patients (pts) with EGFR (mutated) (GFRm) NSCLC. Ann. Oncol. 31, S1189–S1190.

121. Hsu, C.C., Liao, B.C., Liao, W.Y., et al. (2020). Exon 16-skipping HER2 as a novel mechanism of osimertinib resistance in EGFR L858R/T790M-positive non-small-cell lung cancer. J. Thorac. Oncol. 15, 50–61.

122. Oxnard, G.R., Hu, Y., Mileham, K.F., et al. (2018). Assessment of resistance mechanisms and clinical implications in patients with EGFR T790M-positive lung cancer and acquired resistance to osimertinib. JAMA Oncol. 4, 1527–1534.

123. Romaniello, D., Marrocco, I., Belugali Nataraj, N., et al. (2020). Targeting HER3, a catastrophic resistance mechanism to EGFR-TKI treatment in EGFR-mutated non-small cell lung cancer. Cancer Res. 25, 2244–2256.

124. Papadimitrakopoulou, V.A., Wu, Y.L., Han, J.Y., et al. (2018). Analysis of resistance mechanisms to osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. Ann. Oncol. 29, https://doi.org/10.1093/annonc/mdy483.007.

125. Romaniello, D., Marrocco, I., Belugali Nataraj, N., et al. (2020). Targeting HER3, a catastrophic resistance mechanism to EGFR-TKI treatment in EGFR-mutated non-small cell lung cancer. Cancer Res. 25, 2244–2256.

126. Romaniello, D., Marrocco, I., Belugali Nataraj, N., et al. (2020). Targeting HER3, a catastrophic resistance mechanism to EGFR-TKI treatment in EGFR-mutated non-small cell lung cancer. Cancer Res. 25, 2244–2256.

127. Romaniello, D., Marrocco, I., Belugali Nataraj, N., et al. (2020). Targeting HER3, a catastrophic resistance mechanism to EGFR-TKI treatment in EGFR-mutated non-small cell lung cancer. Cancer Res. 25, 2244–2256.
The Innovation

158. Sequist, L.V., Waltman, B.A., Dias-Santagata, D., et al. (2011). Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci. Transl. Med. 3, 75ra26.

159. Lee, J.K., Lee, J., Kim, S., et al. (2017). Clonal history and genetic predictors of transformation into small-cell carcinomas from lung adenocarcinomas. J. Clin. Oncol. 35, 3055–3074.

160. Niederst, M.J., Sequist, L.V., Poirier, J.T., et al. (2015). BRox in resistant EGFR mutant lung adenocarcinomas that transform to small-cell lung cancer. Nat. Commun. 6, 6373.

161. Kashima, K., Kawauchi, H., Tanimura, H., et al. (2020). CHE232163 overcomes osimertinib resistant EGFR Del19/T790M/C797S mutation. Mol. Cancer Ther. 19, 2288–2297.

162. Liu, X.L., Zhang, X.Q., Yang, L., et al. (2019). Preclinical evaluation of TQB3804, a selective 4th generation EGFR TKI for the treatment of EGFR T790M/C797S resistant NSCLC. Ann. Oncol. 31, 5839.

163. Choe, H., Jeon, B.U., Jung, M.E., et al. (2017). Structure-activity relationship study of Pharambiz (2019). Bridge Biotherapeutics seeks approval from US FDA & MFDS in India to begin phase I/II study of BT-176 to treat NSCLC. http://www.pharmabiz.com/NewsDetails.aspx?id=120094&sid=2.

164. Pharambiz (2019). Bridge Biotherapeutics seeks approval from US FDA & MFDS in India to begin phase I/II study of BT-176 to treat NSCLC. http://www.pharmabiz.com/NewsDetails.aspx?id=120094&sid=2.

165. Schalm, S.S., Dineen, T., Lim, S.M., et al. (2020). 1295P BLU-945, a highly potent and selective 4th generation EGFR TKI for the treatment of EGFR T790M/C797S resistant NSCLC. Ann. Oncol. 31, 5839.

166. Lu, X., Smaill, J.B., and Ding, K. (2020). Medicinal chemistry strategies for the development of kinase inhibitors targeting point mutations. J. Med. Chem. 63, 10726–10741.

167. He, J., Zhou, Z., Sun, X., et al. (2021). The new opportunities in medicinal chemistry of fourth-generation EGFR inhibitors to overcome C797S mutation. Eur. J. Med. Chem. 210, 112995.

168. Heitzer, E., Haque, I.S., Roberts, C.E.S., et al. (2018). Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat. Rev. Genet. 19, 1141–1149.

169. Kang, S., Venkatachalam, G., Lim, H.H., et al. (2018). Conformational landscape of the epidermal growth factor receptor kinase reveals a mutant specific allosteric pocket. Chem. Sci. 9, 5212–5222.

170. Lu, J., Smaill, J.B., and Ding, K. (2020). Medicinal chemistry strategies for the development of kinase inhibitors targeting point mutations. J. Med. Chem. 63, 10726–10741.

171. Zhao, J., Lin, G., Zhuo, M., et al. (2020). Next-generation sequencing based mutation analysis in patients with untreated, EGFR-mutated, advanced non-small-cell lung cancer (NEJ026): interim analysis of an open-label, randomised, multicentre, phase 3 trial. Lancet Oncol. 20, 625–635.

172. Nakagawa, K., Karon, E.B., Seto, T., et al. (2019). Ramucirumab plus erlotinib in patients with untreated, EGFR-mutated, advanced non-small-cell lung cancer (RELAY): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 20, 1655–1669.

173. Zhou, Q., Wu, Y.L., Cheng, Y., et al. (2019). CTONG 1509: phase III study of bevacizumab plus erlotinib in untreated Chinese patients with advanced EGFR-mutated NSCLC. Ann. Oncol. 30, https://doi.org/10.1093/annonc/mdz260.002.

174. Noronha, V., Patil, V., Joshi, A., et al. (2019). Gefitinib versus gefitinib plus pemetrexed and carboplatin chemotherapy in EGFR-mutated lung cancer. J. Clin. Oncol. 37, 1234–1236.

175. Kosomi, Y., Morita, S., Sugawara, S., et al. (2019). Gefitinib alone versus gefitinib plus chemotherapy for non-small-cell lung cancer with mutated epidermal growth factor receptor: NEJ009 Study. J. Clin Oncol. 38, 115–123.

176. Romaniello, D., Mazeo, L., Mancini, M., et al. (2018). A combination of approved antibodies overcomes resistance of lung cancer to osimertinib by blocking bypass pathways. Clin. Cancer Res. 24, 5610–5621.

177. Woodcock, J., and LaVange, L.M. (2017). Master protocols to study multiple therapies, multiple diseases, or both. N. Engl. J. Med. 377, 62–70.

ACKNOWLEDGMENTS

The study was supported by the Natural Science Foundation of Shanghai (No. 20ZR141040), the Extraordinary 2025 Elite Project of Fudan University, the National Natural Science Foundation of China (No. 81772590 and 8172393S), the Open Fund of Key Laboratory of Diagnosis and Treatment of Severe Hepato-pancreatic Diseases of Zhejiang Province (No. 2018E10008), and the CAS Interdisciplinary Innovation Team JCTD-2019-07. All figures showing EGFR kinase structure and binding modes were generated using PyMol 2.4.1 (www.pymol.org).

AUTHOR CONTRIBUTIONS

X.D., B.Y., and Q.A. performed data collection and analyses. X.D. and X.C. organized the review and prepared the manuscript. Y.G.A., X.C. and J.X. revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

LEAD CONTACT WEBSITE

http://shmc.fudan.edu.cn/html/viajinglin.html