Resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer

ZHOU Qing (周清)[1*], WEI XueWu(魏雪武)[2,1], GAO Xin (高欣)[3,1], HE YunTing(何韵婷)[3,1] & YANG XiaoRong(杨潇蓉)[2,1]

[1] *Guangdong Lung Cancer Institute, Guangdong General Hospital and Guangdong Academy of Medical Sciences, Guangzhou* 510080, *China*;

[2] *School of Medicine, South China University of Technology, Guangzhou Higher Education Mega Center, Guangzhou 510006, China*;

[3] *Second School of Clinical Medicine, Southern Medical University, Guangzhou 510515, China*

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Abstract Treatment with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) prolongs the overall survival of patients with EGFR-mutated advanced non-small-cell lung cancer (NSCLC). EGFR-TKIs including first-generation (e. g., gefitinib and erlotinib), second-generation (e. g., afatinib and dacomitinib) and third-generation (e. g., osimertinib) drugs are effective for the treatment of EGFR-mutated NSCLC. However, almost all patients exhibit drug failure related to resistance including primary and acquired resistance. Several mechanisms involved in primary and acquired resistance to EGFR-TKIs have been reported recently. Primary resistance to EGFR-TKIs involves point mutations in exon 18, deletions or insertions in exon 19, insertions, duplications and point mutations in exon 20 and a point mutation in exon 21 of the EGFR gene. Acquired resistance to EGFR-TKIs can be characterized into two groups: resistance to first- and second-generation EGFR-TKIs, and resistance to third-generation EGFR-TKIs. The third-generation EGFR-TKI resistance group presents a complex model including *EGFR* C797S mutations, erb-b2 receptor tyrosine kinase 2 gene (ERBB2) amplification, BRAF V600E mutations, ROS1 fusion, and MNNG HOS transforming gene (c-Met) amplification. Personalized diagnosis and monitoring as well as the development of next generation drugs are desperately needed for better survival outcomes in EGFR mutant NSCLC patients. In this article, we review these mechanisms and discuss the latest therapeutic strategies to overcome resistance to EGFR-TKIs.

Keywords Epidermal growth factor receptor tyrosine kinase inhibitors; Non-small cell lung cancer; Primary resistance; Acquired resistance

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1 Introduction

Lung cancer has a high morbidity and mortality rate, and is the leading prevalence of cancer death worldwide [1, 2]. New cases in China account for about one-third of the global community, which makes China the largest country with lung cancer [1]. The rapid development of targeted therapy since the beginning of the 21st century has greatly enhanced the treatment of lung cancer, especially non-small-cell lung cancer (NSCLC) with definite mutation targets. Although EGFR-TKIs make an exciting therapeutic contribution to EGFR-mutated NSCLC, most patients develop resistance to EGFR-TKIs, irrespective of

* Corresponding author (Email: gzzhouqing@126.com; Tel: +86 20 83827812-51221 or 51487; Fax: +86 20 83844620)

the targeted therapeutic drugs used. Thus, improved understanding of mechanisms and treatment strategies of drug resistance is essential for the development of targeted therapy of lung cancer.

2 Resistance underlying in NSCLC

2.1 Primary resistance

TKI drug treatment has achieved great success for NSCLC patients with EGFR mutations. However, there small numbers of patients with *de novo* EGFR mutations present with a poor response to first-line treatment with TKIs. Primary, intrinsic or *de novo* resistance is defined as a failure to respond to first-line TKI treatment [3]. We speculate that the different efficacy of TKIs may be related to the molecular heterogeneity of EGFR mutations, including intratumoral heterogeneity and intertumoral heterogeneity [4]. In our previous study, the LCM system Arcturus was used to microdissect captured individual tumor cells from 10 EGFR-mutant NSCLC patients, and then single-cell sequencing was performed. Intratumoral heterogeneity of EGFR activating mutations in lung adenocarcinoma was confirmed at the single cell level. This result is consistent with a report by Zhang et al. of multi-region tissues and matched circulating tumor DNA (ctDNA) sequencing [5]. Subsequently, we used direct sequencing and the Scorpion amplification refractory mutation system (Scorpions-ARMS) to analyze 100 advanced lung cancer patients treated with EGFR-TKIs. We found significant individual differences in mutant relative abundance at the DNA level in patients with lung cancer, and specific antibody staining of tumor tissues also confirmed differences in mutant relative abundance at the protein level. By comparing the clinical efficacy of patients, it was found that the abundance of EGFR mutants could predict the efficacy of EGFR-TKI, and that low abundance is one mechanism of primary resistance (Fig. 1) [6].

In general, primary resistance can be divided into four classes: TKI resistance in the presence of drug-sensitizing EGFR mutations, drug resistant EGFR mutations, genomic alterations and EGFR mutations, and EGFR wild-type tumors. The response sensitivity of TKIs is impacted by the relative abundance of EGFR mutations [3]. Non-sensitive EGFR mutations, including exon 19 mutations L747S/D761Y, exon 20 *de novo* mutation T790M and exon 21 mutation T854A, are closely related to primary resistance [7, 8]. Genomic alterations along with EGFR mutations include but are not limited to MNNG HOS transforming gene (MET) amplification [9] and KRAS mutants [10]. Among treatment-naive patients with an EGFR mutation, 4.2%—5.0% exhibited a *de novo* T790M mutation [11, 12].

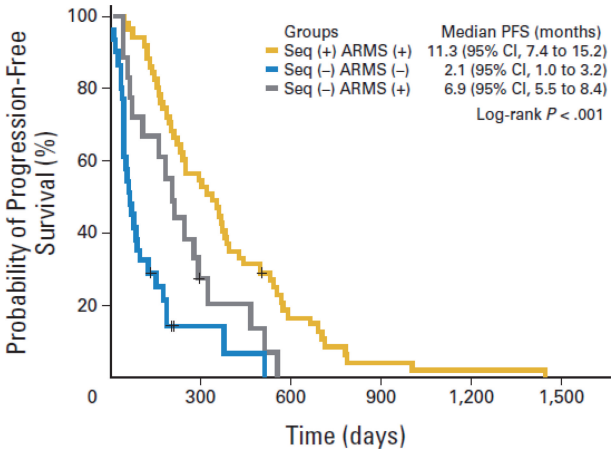

Figure 1 Progression-free survival (PFS) of patients in the various abundance of EGFR mutations [6]. ARMS, Amplification refractory mutation system; Seq, DNA sequencing.

2.2 Acquired resistance

First-generation EGFR-TKIs (e.g., gefitinib and erlotinib) and second-generation EGFR-TKIs (e.g., afatinib and dacomitinib) had an excellent therapeutic effect on NSCLC patients with EGFR exon 19 deletions or exon 21 L858R mutations [13—15]. However, almost all cases experienced disease progress or recurrence after 1 to 2 years of EGFR-TKI treatment that was related to acquired resistance [13—15].

2.2.1 Acquired resistance in first- and second-generation EGFR-TKIs

Overall, 224 consecutive patients with lung cancer who acquired resistance after first- or second-generation EGFR-TKI treatment in our center from 2010 to 2014 were included in the study. High-throughput sequencing technology was used to explore the distribution difference of T790M mutations,

MET amplification, ALK fusion, KRAS and PIK3CA mutations and other acquired resistance mutations between patients with EGFR exon 19 deletions or exon 21 L858R mutations. The total incidence of T790M mutations in this study was 45.1%. Except for the T790M mutation, there were no significant differences in MET amplification, histological transformation, KRAS/PIK3CA mutation, and ALK fusion between the groups. We further analyzed 792 cases and found that the proportion of T790M-resistant mutations in the exon 19 deletion subgroup was significantly higher than that in the L858R mutation group (54.5% vs 37.3%, $P<0.001$). The overall survival of patients with T790M mutations was 36.0 months (95%CI: 30.9—41.2 months), which was significantly higher than that of patients in the MET-positive transformation, histological transformation, and KRAS/PIK3CA/ALK-altered groups. This was the first study to reveal the difference in first-generation EGFR-TKI efficacy between different mutant subtypes from the perspective of resistance heterogeneity, which demonstrated that the distribution of resistant mutations is an independent prognostic factor for TKI resistance in patients with EGFR-sensitive mutations [16].

2.2.2 Acquired resistance in third-generation EGFR-TKIs

Third-generation irreversible EGFR TKIs, designed to target EGFR T790M mutations and EGFR activating mutations, have been developed. The median progression-free survival (PFS) of EGFR T790M-positive patients treated by osimertinib was about 10 months [17]. EGFR TKIs such as olmutinib (HM61713), abivertinib (AC0010), rociletinib (CO-1686), naquotinib (ASP8273), mavelertinib (PF-0647775) and nazartinib (EGF816) also showed excellent therapeutic potential [18—24]. However, even patients with good responses to third-generation EGFR TKIs will develop resistance after 6—10 months [17].

Four osimertinib resistance mechanisms were identified by studies of osimertinib-resistant T790M mutation patients including: (1) the combination of C797S and T790M mutations, and a novel replacement mutation occurring at the junction site of osimertinib, which mutated the cysteine at position 797 to serine (EGFR C797S), thus causing drug connection repression [25]. A C797S mutation in the EGFR tyrosine kinase region was a major resistance mechanism of third-generation irreversible EGFR TKIs [26]; (2) T790M mutation without C797S mutation; (3) lack of T790M mutation and C797S mutation; and (4) loss of T790M mutation [27]. Because only 6 of the 15 patients had acquired C797S mutations, this suggests other mechanisms might mediate the resistance to osimertinib [25]. Song et al. reported the first case of a C797S mutation caused by olmutinib (HM61713), which prevented EGFR inhibition and promoted resistance to third-generations EGFR-TKIs [28]. These studies emphasize the importance of repeated testing of biological samples to evaluate the potential resistance mechanisms of patients with third-generation resistance, and the importance of the results for therapeutic strategies.

A recent study reported a large difference between the mutations of C797S *in cis* or *in trans*. Niederst et al. reported that the allelic context of the C797S mutation had a predictive effect on therapeutic strategies [29]. When C797S and T790M mutations are located in different EGFR alleles, the mutation is termed *in trans*. Cells with these mutations are resistant to third-generation EGFR-TKIs, but are sensitive to the combination of first- and third-generation EGFR-TKIs [30]. Mutations *in cis* are defined as mutations on the same side of the EGFR allele. Neither EGFR-TKIs alone nor EGFR-TKIs in combination inhibit its activity. From the above, the location of the EGFR alleles in which C797S is located has an effect on the outcome of subsequent treatments [29]. However, *in cis* mutations constitute most mutation configurations, and C797S and T790M mutations *in trans* are seen in less than 25% of cases [30].

In addition to C797S mutations, third-generation TKIs also lead to many new mutation types [31]. Loss of T790M mutations, HER2 amplification, c-MET amplification, KRAS G12S kinase mutations, EGFR L718Q mutations, exon 18 G724S29, L718V30, E709K31, G796D mutations, BRAF V600E mutations, PIK3CA mutations, L792H, G796R36 as well as ERBB2 and MET amplification are also considered responsible for the resistance of cases with non-T790M or C797S mutations to third-generation EGFR TKI [32].

However, unlike osimertinib, the major resistance mechanisms of the EGFR-TKI abivertinib include erb-b2 receptor tyrosine kinase 2 gene (ERBB2) amplification, BRAF V600E mutations, ROS1 fusion, and MNNG HOS transforming gene (c-Met), but not EGFR C797S mutations [33]. This may be related to abivertinib targeting c-MET and BCL-2 [34]. We analyzed the heterogeneous landscape of resistance in

32 patients with *EGFR* T790M-positive advanced NSCLC and progression in 31 patients with previous EGFR TKIs who had received abivertinib. Of these, 14% experienced EGFR T790M loss, 13% developed EGFR tertiary mutations including C797S and 34% showed EGFR amplification. CDKN2A, MET, PIK3CA, HER2, TP53, Rb1 and small-cell lung cancer transformation were also found in resistance patients (unpublished data). These results suggested that third-generation EGFR-TKI resistance genetic alterations may be more complicated than that of the first- or second-generation EGFR-TKIs. Therefore, when third-generation EGFR-TKI resistance occurs, multiple pathways might be co-activated inducing many new genetic variants, diversifying genes associated with resistance.

3 Diagnosis, monitoring and treatment strategies for EGFR-TKI resistance

3.1 Diagnosis and monitoring strategies for EGFR-TKI resistance

Liquid biopsy technology makes it possible to monitor mutations and provide early TKI treatments [35]. Monitoring T790M from plasma samples is better than the clinical manifestation of disease progression because of earlier detection is more conducive to the replacement therapy of third-generation TKIs [35]. Moreover, ctDNA can be used for multiple gene detection to identify potentially targetable mechanisms of resistance. A 70-gene cell-free DNA (cfDNA) next-generation sequencing technique was used by Helman et al. to detect 77 EGFR-mutated NSCLCs treated with third-generation TKIs, and 93% and 85% of cases were observed to harbor the initial EGFR activating or EGFR T790M resistance mutations, respectively [35]. Profiling of progression samples showed significant heterogeneity, with different variant types (e.g., mutations, amplifications, and fusions) detected in multiple genes (EGFR, MET, RB1) that may drive resistance in patients, as well as novel alterations such as NTRK1 fusion [35]. Patients with detectable T790M showed markedly shorter OS compared with the T790M negative group (26.9 months vs NA), which indicated the feasibility and importance of monitoring EGFR mutation dynamics in NSCLC patients with TKI treatment [36]. Therefore, dynamic monitoring allows the timely planning of early treatment strategies.

3.2 Treatment strategies for primary EGFR-TKI resistance

Based on the mechanisms of EGFR primary resistance caused by concomitant gene mutations, combination therapy has gained increasing attention. The main causes of primary resistance include low EGFR mutation abundance and abnormal expression or mutation of other genes. Therefore, it is necessary to quantitatively detect EGFR mutation abundance and a wide range of other molecular genes for clinical diagnosis. For naive advanced EGFR mutated NSCLC patients with a low abundance of EGFR mutations and non-target therapy drugs, concurrent or sequential chemotherapy might be an effective treatment [37]. For patients with genomic alterations with recognized targeted therapeutic agents, such as c-MET, T790M mutations, the corresponding targeted therapeutic agent can be directly used for treatment [38].

3.3 Treatment strategies for acquired EGFR-TKI resistance

3.3.1 Treatment strategies based on clinical assessment

The clinical categorization of EGFR-TKI resistance is advantageous when determining strategies for treatment and to predict survival benefit in advanced NSCLC. Based on radiological and clinical examination results, we classified tumor progression after EGFR-TKI failure into three modes to optimal treatment protocols and improve patients' survival: gradual progression, local progression, and dramatic progression [39]. Gomez et al. reported that local consolidated therapy with or without maintenance therapy significantly improved progression free survival compared with maintenance therapy alone [40]. However, whether local therapy combined with EGFR-TKI is better than local therapy alone or EGFR-TKI mono-therapy requires more evidence. Gandara et al. divided progressive disease (PD) into three subtypes: systemic PD, oligo-PD and CNS (central nervous system) sanctuary PD in patients with acquired resistance to EGFR-TKIs based on the sites of progression [41]. The categorization of clinical modes provides a simple, practical, and feasible basis for treatment strategies, and important guidance for re-biopsy time and location for resistance molecular mechanisms research.

3.3.2 Treatment strategies based on EGFR-related acquired resistance mechanisms

3.3.2.1 Treatment strategies targeting T790M mutations

Exon 20 T790M mutations of EGFR are the most common EGFR-TKI resistance mechanism, and are present in 50%—60% of patients with NSCLC after EGFR-TKI treatment and in about 5% of NSCLC patients without treatment [42, 43]. Moreover, the PFS of patients with combined EGFR L858R mutations or 19 exon deletions with T790M mutations before EGFR-TKI treatment was inferior to that of patients with only L858R mutations or 19 exon deletions, but was superior to that of EGFR wild-type patients. This suggests that the primary T790M mutation is an independent predictor and risk factor for the prognosis of first- and second-generation EGFR-TKI efficacy [42, 43].

Osimertinib was the first EGFR-TKI approved by the FDA, EMA and NMPA for the treatment of EGFR T790M-positive patients. AURA studies reported an objective response rate (ORR) of 61%—71% and a median progression-free survival (PFS) of about 10 months in EGFR T790M-positive patients with osimertinib treatment [44]. Osimertinib also showed robust efficacy in the first-line treatment of patients with advanced EGFR mutation-positive NSCLC [45]. Other third-generation EGFR-TKIs such as olmutinib (HM61713), abivertinib (AC0010), rociletinib, ASP8273 and EGF816 have become potential options for T790M mutations [18, 33, 46].

3.3.2.2 Treatment strategies targeting C797S mutations

The proportion of C797S positivity is about 21% in drug resistant EGFR-T790M-positive non-small cell lung cancer patients treated with osimertinib. *In vitro* experiments showed that T790M/C797S mutation *in trans* were sensitive to combination first- and third-generation EGFR-TKIs and this strategy was confirmed in EGFR-mutant lung cancer patients recently [30, 47]. However, because of the heterogeneity of the tumor, not all patients with T790M/C797S mutations *in trans* show benefit from this treatment. In addition, osimertinib combined with erlotinib was ineffective for T790M/C797S mutations *in cis*, resulting in a poor therapeutic effect in patients with concurrent T790M/C797S mutations *in trans* and *in cis* (T790M and C797S are located in the same EGFR allele) [47, 48].

To overcome the T790M/C797S mutation *in cis*, some fourth-generation EGFR-TKIs, such as EAI045 and brigatinib [49], have been screened. When combined with cetuximab, they had a good therapeutic effect against the triple mutation of EGFR L858R/T790M/C797S. However, the study provide limited information because they used mouse models and whether the T790M/C797S mutation is *in cis* or *in trans* is unclear [50]. Recently, we reported a case of osimertinib resistance. When combined with cetuximab, osimertinib blocked EGFR dimerization and achieved initial treatment benefit. The efficacy of brigatinib was markedly enhanced by combination with anti-EGFR antibody associated with a decrease in cell surface and total EGFR expression [49]. Recently, *in vitro* studies indicated that trastuzumab emtansine in combination with osimertinib delayed and overcame resistance to osimertinib in NSCLC EGFR mutated cell lines [51—53]. However, there is still a need to explore feasible treatment combinations for T790M/C797S mutations before the approval of fourth-generation EGFR-TKIs.

3.3.3 Treatment strategies based on non-EGFR related acquired resistance mechanisms

In addition to T790M/C797S mutations and EGFR amplification or loss of the EGFR mutant, there are other non-EGFR mechanisms involved in the resistance in nearly half of NSCLC patients, i.e. c-Met amplification, Her-2 amplification, RAS mutations, PIK3CA mutations and BRAF mutations. For these patients, targeting bypass signaling pathways combined with EGFR-TKIs might overcome the acquired resistance.

3.3.3.1 Treatment strategies targeting c-MET amplification

c-MET amplification accounts for 5%—27% of the first-generation EGFR-TKI resistance mechanism and is also detected in patients with primary resistance and third-generation EGFR-TKI-treated acquired resistance [54—56]. The resistance mechanisms of the c-MET signaling pathway is complex, including c-MET amplification resistance, protein overexpression, MET genomic aberrations, and exon 14 shear mutations [55]. Therefore, for patients with failed EGFR-TKI treatment, MET gene amplification, c-MET protein expression and MET hotspot mutation should be detected. In addition to inhibiting ALK gene mutations, crizotinib has an inhibitory effect on the c-MET pathway, and therefore might be a potential choice for the treatment of MET mutations. A retrospective study showed that patients with

EGFR-TKI resistance and MET overexpression who received crizotinib plus EGFR-TKI or crizotinib monotherapy had an overall ORR and DCR of 50.0% and 85.7%, respectively. The median PFS (mPFS) and mOS of patients receiving crizotinib plus EGFR-TKI were 12.6 months and 24.0 months, respectively [57]. Capmatinib (INC280) and tepotinib are novel c-MET targeting inhibitors. A phase Ib/II study by our team showed that preliminary clinical activity of capmatinib indicated an ORR of 27%.

3.3.3.2 Treatment strategies targeting HER2 mutations

For HER2 amplification, trastuzumab is a potential treatment choice. Trastuzumab emtansine was used to treat EGFR-mutant NSCLC with HER2 mutations in the PC9/HER2c1 xenograft model [51]. De Langen et al. reported the safety and efficacy of trastuzumab and paclitaxel treatment in 24 patients with EGFR mutation who had HER2 expression (IHC 1+) after EGFR-TKI treatment. The ORR was 46%, 67% in patients with HER2 IHC3+, and 100% in patients with HER2 copy number 10+. The median size of tumors was decreased by 42% and the median duration of response was 5.6 months [58].

3.3.3.3 Treatment strategies targeting the RAS signaling pathway

Preclinical studies of osimertinib by Eberlein et al. reported that in patients with acquired resistance, the copy number of WT NRAS and KRAS mutations, including the novel NRAS mutation (E63K), was strongly dependent on the RAS signaling pathway. In addition to treatment with osimertinib, MEK inhibition (treated with selumetinib) also inhibited tumors in transgenic mice [59]. However, further clinical evidence is required.

3.4 Treatment strategies for pathological type transformation

A recent report found that a patient with NSCLC transformed to SCLC with EGFR T790M mutations after undergoing first-generation EGFR-TKI treatment although osimertinib maintained a clinical response for 6 months [60]. This study demonstrated that it is important to detect EGFR T790M mutations in NSCLC patients transformed for other pathological types [60]. However, this patient also developed a C797S mutation, resulting in acquired resistance to osimertinib [60].

4 Challenges and perspectives

The heterogeneity and diversity of drug resistance after EGFR-TKI treatment is a significant clinical problem [35, 61]. Therefore, to overcome the mutations in EGFR-TKI resistance, some new research directions are urgently needed.

In addition to the development of new targets, re-sensitive to target may also be a potential strategy. T790M/C797S mutations *in trans* can be treated with a combination of first- and third-generation EGFR-TKIs. However, after C797S is suppressed, a high frequency of T790M mutations and EGFR exon 19 deletions may appear, forming a new drug-resistant cancer, which needs new treatment strategies. Although EGFR L718V is a novel mutation that is resistant to osimertinib, it is sensitive to second-generation afatinib, which suggests that the different generations of EGFR-TKI are not completely sequential. This means that first- and second-generation TKIs may still be effective after resistance to third-generation EGFR-TKIs [62].

Whether novel mutations co-occur with genetic alterations or post-treatment acquired alterations is important for treatment strategies. The EGFR mutations were often accompanied by TP53, RTK, RAS-MAPK, PI3K, WNT/β-catenin, CDK4/6 and other genetic alterations [63]. Our initial BENEFIT also showed similar results [12]. These combined mutations might make treatment even more difficult, and might be related to TKI treatment resistance. Although the next generation of EGFR-TKIs has beneficial efficacy and safety for C797S mutations, its long-term perspective should be treat newly-acquired mutations.

Although new mutations that occur after treatment continue to be discovered, why tumors evolve new mutations constantly is still unknown. Moreover, the mechanism of third-generation EGFR-TKI resistance is complicated and heterogeneous, and many mutation forms are still unknown; therefore, it is difficult to perform targeted clinical therapy [64]. Thus, future studies should investigate the fundamental driver genes of EGFR mutations. By blocking these driver genes, the occurrence of new mutant forms induced by EGFR-TKIs may be reduced and the time to drug resistance might be delayed. TPX2, Aurora kinase A

and PKCδ are potential driver genes for the evolution of resistance to EGFR-TKI in lung cancer [65, 66]. We should not only study genetic mutations that have already appeared, but also prevent the occurrence of new mutations. Developing new drugs from this perspective is an important current direction.

The next generation of EGFR-TKIs is constantly being developed, but primary and acquired resistance is still unavoidable. Currently, therapeutic strategies are based on the mechanism of EGFR-TKI mutations. However, because of the heterogeneity of tumors and limitations of the detection methods used, we may not be able to identify all the EGFR mutant types of NSCLC. Conquering EGFR mutations requires the provision of next-generation TKIs through precision medicine and research to understand the core driver genes of constant mutations. These studies should be a priority for the treatment of EGFR-TKI resistant NSCLC patients in the future.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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ZHOU Qing

Dr. Zhou is a Professor of Pulmonary Oncology. She is the Deputy Director of the Guangdong Lung Cancer Institute and Director of Division Three of Pulmonary Oncology at Guangdong General Hospital & Guangdong Academy of Medical Sciences, Guangzhou, China. She graduated in 1997 from Norman Bethune Medical University in Jilin Province, China, completed her PhD training in 2006 at Sun Yat-sen University of Medical Sciences, and studied phase I clinical trials in 2009 at the Cancer Center of Colorado University, USA. Her main research interests are multidisciplinary synthetic therapy of lung cancer from basic science to bedside and evidence-based medicine in oncology. She has published more than 50 SCI research papers in the past 5 years and one was published in the *Journal of Clinical Oncology* (IF = 24.8). She leads several scientific research projects including the National Key Research and Development Program of China and National Natural Science Foundation of China. She participates in more than 120 multicenter clinical trials in the lung cancer field and acts as Principle Investigator of 13 clinical trials. She has received the second prize of the National Scientific and Technological Progress Award, first prize of the China Medical Science and Technology Award, as well as first and second prizes of the Guangdong Science and Technology Award.