Abstract - Somatic mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR) gene are common in lung cancers patients who are never-smokers, and many of these EGFR-mutant patients have shown good responses to the 1st-generation EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib in clinical trials since 2003. Unfortunately, almost all patients with advanced NSCLC who responded to either 1st-generation EGFR TKIs (e.g., gefitinib, erlotinib) or the 2nd-generation EGFR TKIs (e.g., afatinib, dacomitinib) will eventually develop acquired resistance, while ~50-60% will develop T790M mutation in the EGFR kinase domain. Third-generation EGFR TKIs osimertinib has thus been developed and approved in late 2016 as the treatment choice for patients with the T790M mutation who have had disease progression following prior EGFR TKI therapies. However, acquired resistance to osimertinib will also develop and can become complex to overcome. Progression on osimertinib after prior EGFR-TKI treatment (i.e., after 1st-2nd line EGFR TKIs) revealed that the biology of patients who retain T790M and those who lose T790M can be very different. For those who retain T790M, about 50% develop C797S mutation as well, which prevents osimertinib from binding to the ATP cleft. However, those who lost T790M may have a broad range of non-EGFR resistance mechanisms such as MET,mutations in PI3 kinase, HER2 amplification, KRAS, BRAF, etc. Only in cases where C797S mutation is on the trans-site of T790M, dual-EGFR TKIs treatment (e.g., erlotinib combined with osimertinib) can be effective for some time. There is thus an urgent clinical need worldwide to sort out what are the latest and effective treatment options to overcome acquired resistance to osimertinib for EGFR-mutant NSCLC patients after prior EGFR-TKI treatments, as will be discussed in this paper.

Keywords - Acquired Resistance, Afatinib, Cetuximab, EGFR (epidermal growth factor receptors), Erlotinib, Gefitinib, Non-Small-Cell Lung Cancer (NSCLC), Never-Smoker’s lung cancer, Osimertinib, Tyrosine Kinase Inhibitor (TKI)

I. INTRODUCTION

Lung cancer is by far the leading cause of cancer-related death worldwide among both men and women. In the US, more people die of lung cancer than of colon, breast, and prostate cancers combined every year [1]. There are three main types of lung cancer: non-small cell lung cancer (NSCLC), ~85% of lung cancers, where adenocarcinoma, squamous cell carcinoma, and large cell carcinoma are all subtypes; small cell lung cancer (SCLC), ~10%-15% of lung cancers, tends to be more aggressive; and lung carcinoid tumor (slow and rarely spread). It is still true that smoking is the leading risk factor for lung cancer, but non-smokers/never-smokers do get lung cancer at an alarming rate. It was estimated that more than 40,000 cases of lung cancer in the US are diagnosed each year in non-smokers, and these can be caused by exposure to radon, second-hand smoke, air pollution, exposure to certain cancer-causing agents at work, etc. [1]. However, the percentage of lung cancer patients who are never-smokers are clearly on the rise in the US. One recent study shows the percentage of NSCLC patients who never smoked increased from 8.0% to 14.9% from 1990–1995 to 2011–2013 (P<0.001); the increase was consistent across all three hospitals surveyed. The percentage of women with NSCLC who never smoked also more than doubled from 10.2% to 22.1% from 1990 through 2013 (P=0.001), and the percentage of men with NSCLC who never smoked only slightly increased from 6.6% to 8.9% during the same period (P=0.006) [2]. If we categorize the never-smoker’s lung cancer as a separate cancer, it was already ranked 7th in the US in 2008 for killing most cancer patients then, and it may now rank higher [3]. Globally, China has been hit particularly hard by lung cancer: more than 700,000 new cases of lung cancer are diagnosed in China every year, and over 600,000 death due to lung cancer yearly [4]. Air pollution and smoking are thought to be the leading causes of lung cancer in China - for example, smoking rates in China among males are far higher than in many countries; Chinese men constitute about a tenth of the world’s population but smoke one-third of the world’s cigarettes [5]. There were nearly 4.3 million new cancer patients in China in 2015, including 730,000 cases of lung cancer, accounting for 36 percent of the world's total. Cancer is responsible for about 25% of Chinese deaths, and an estimated 300 million Chinese people are smokers, but there had been a rapid increase in a form of lung cancer that develops deep in the lung and is not associated with tobacco use among Chinese as...
well[6]. It is thus clear that smoking or second-hand smoking are often not the causes for many female NSCLC patients, especially for Asian young female patients. For example, an alarming trend has been reported that 90% of the female new lung cancer patients diagnosed in Taiwan are never-smokers [7]!

Recently, scientists have found that the majority of non-smoker lung cancer patients had developed somatic mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR) gene. These patients can also be very responsive to the 1st-generation EGFR tyrosine kinase inhibitors (TKIs) such as to gefitinib (Iressa®) as found in clinical trials since 2003. EGFR, a receptor tyrosine kinase (RTK) mutated or overexpressed in many types of cancers, plays a key role in tumor cell proliferation and vascularization. Since 15%-65% of NSCLC adenocarcinoma express EGFR mutations depending on various factors such as race, sex, ethnicity and smoking history, EGFR has become a key therapeutic target for treating NSCLC adenocarcinoma, especially for non-smokers/never-smokers [8]. For patients of advanced NSCLC with EGFR-mutations, we now know 1st-line treatment using an EGFR TKI is superior to traditional chemotherapy with a significantly longer PFS (Progression-Free Survival) time, and it is also much better in terms of the response rate than the immunotherapy using PD-1/PD-L1 check-point inhibitors [9]. Therefore, EGFR mutation testing should be mandatory for NSCLC patients. However, about 10% patients not tested EGFR-positive clinically also responded well to EGFR-targeting TKIs, suggesting that selection of treatment based solely on somatic mutations using an FDA approved EGFR test kit is not always adequate. Unfortunately, patients with advanced NSCLC who responded to either the treatment of 1st-generation TKIs (i.e., gefitinib or erlotinib) or the 2nd-generation TKIs (e.g., afatinib or dacomitinib) will usually develop acquired drug resistance within a few years, some from further mutation on the EGFR protein. Preclinical studies as well as patients’ re-biopsies have revealed that the EGFR T790M point mutations arise in ~50-60% of these cases, as the cancer cells acquired the abilities to avoid apoptosis and continue proliferation as secondary-resistant cancer cells. Therefore, third-generation EGFR TKIs such as osimertinib (Tagrisso®) have been developed to address this acquired resistance, and osimertinib was approved by the FDA in late 2016 as the treatment choice for patients with the acquired T790M mutation following prior EGFR TKI therapies; it has later also been approved in 2018 as a 1st-line treatment option for EGFR-mutant NSCLC patients[10]. However, the acquired resistance to osimertinib also tends to develop within a year or two, and it is very complex, so oncologists world-wide are looking for the next effective clinical treatment options. Pre-treated NSCLC patients who progress on 2nd/3rd/4th-line osimertinib revealed that the biology of patients who retain T790M and those who lose T790M can be fundamentally different [11]. Those who retain T790M appear to have developed another acquired mutation: about half of those patients will develop C797S mutation, which prevents osimertinib from binding at the ATP cleft. However, loss of EGFR T790M mutation was associated with early treatment failure and patients can develop a broad range of different resistance mechanisms, including mesenchymal-epithelial transition factor (MET) amplifications and mutations in PI3 kinase, KRAS, fusions in RET, BRAF, FGFR3, as well as in ALK, NTRK, and ROS1, and even small cell transformation[11]. In tumors that retain T790M, the PFS time on osimertinib can often exceed 1 year. Patients who lost T790M, however, may have significantly lower PFS on osimertinib, suggesting that those competing mechanisms of resistance were already present in those patients when T790M was detected. Only in some specific cases where the C797S mutation developed on the trans-site of the T790M, new dual-TKIs treatment such as erlotinib combined with osimertinib appears to be effective in controlling the disease progression for some time[12]. Therefore, a bit more data is urgently needed to address the latest treatment options to the acquired resistance induced by osimertinib on pre-treated EGFR-mutant NSCLC patients, which will be the focus of this paper. Note in this work even though we do not specifically address the acquired resistance to osimertinib when it is used as the 1st-line treatment for EGFR-mutant NSCLC, many of these acquired resistance mechanisms and their corresponding treatment options discussed later in this paper will be very similar or identical for the acquired resistance to 1st-line osimertinib.

II. EGFR AND T790M MUTATIONS

The human epidermal growth factor receptors (EGFR/HER-1; or ErbB-1/ERBB-1) are small transmembrane glycoprotein as a member of the protein kinase superfamily. These proteins are mostly found on the surface of cells, as they bind ligands such as epidermal growth factor (EGF) and transforming growth factor α (TGFα) circulating in the blood. Binding of the EGFR to a ligand induces receptor dimerization and tyrosine autophosphorylation that leads to cell proliferation in a strictly controlled manner. However, in many cancer cells such as lung adenocarcinoma, EGFR is either mutated and/or abundantly over-expressed such that the EGFR biological processes that normally stimulate cell growth become constantly active, leading to uncontrolled tumors proliferation with insufficient apoptosis. Thus, EGFR-targeted small-molecule TKIs that can travel across the cellular membrane and bind to the ATP-binding sites at the kinase domain of EGFR can effectively block the...
activation of downstream signaling induced by EGFR, and these TKI shave demonstrated strong efficacy for treating EGFR-mutant NSCLC patients. The popular first-generation EGFR-TKIs for NSCLC are Tarceva® (erlotinib), Iressa® (gefitinib), and Icotinib (only available in China). These TKIs are particularly effective to inhibit the most common types of EGFR mutations in NSCLC (exon 19 deletions or exon 21 L858R substitution). The popular second-generation EGFR-TKIs are Gilotrif® (afatinib) and Vizimpro® (dacomitinib), which form irreversible covalent-bonding to the ATP sites of the ErbB family and are more potent pan-HER inhibitors than the first-generation EGFR-TKIs, making them more effective and especially to some uncommon EGFR mutations such as the G719 mutation (point mutation in exon 18), etc.

However, almost all patients treated with 1st or 2nd generation EGFR TKIs will eventually develop acquired resistance, and thus the third-generation EGFR TKI has been developed to address this problem; the more notable ones are Tagrisso® (osimertinib); CO1686 (rociletinib); and HM61713 (olmutinib). Of all 3rd-gen EGFR TKIs, osimertinib is by far the most popular and important one, captured the lion’s share of this key market. Fig. 1 shows some pictures of a lung nodule (adenocarcinoma) at a patient’s right upper lobe; a pair of the EGFR transmembrane protein with the L858R mutation; and also the additional acquired resistance T790M mutation from the 1st or 2nd-generation EGFR TKI treatment. As illustrated in Fig. 1, this T790M mutation can be detected as a “2nd-site mutation” in more than 50-60% of all EGFR-mutant NSCLC patients pretreated with 1st/2nd-gen EGFR TKIs [14,15]. These 3rd-gen EGFR TKIs are thus specifically designed to irreversibly inhibit EGFR T790M. The T790M mutation occurs within exon 20, which encodes part of the kinase domain of EGFR, and results in an amino acid substitution at position 790 in EGFR, from a threonine (T) to a methionine (M). Osimertinib irreversibly inhibits mutant EGFR, including EGFR T790M, with much less activity against wild-type EGFR and thus it shows significantly less toxicities vs. the 1st/2nd-gen EGFR TKIs. Early results from the phase I trial of osimertinib already demonstrated an impressive objective response rate (ORR) of 64% in the 107 NSCLC patients with EGFR T790M+ tumors [15,16]. Note osimertinib has also demonstrated efficacy in cohorts who did not have the EGFR T790M-mutation, but the ORR in that case is not as high [16]. Arandomized, international, open-label, phase 3 trial showed osimertinib had significantly greater efficacy than platinum therapy plus pemetrexed patients with T790M+ advanced NSCLC, including those with CNS (central nervous system) metastases. The median PFS was significantly longer with osimertinib than with platinum therapy plus pemetrexed (10.1 vs. 4.4 months). The ORR was also significantly better with osimertinib (71%) than with platinum therapy plus pemetrexed (31%) [17]. It is thus clear thatosimertinib can potently inhibit both the EGFR mutations and the acquired EGFR T790M+ mutations in NSCLC patients pretreated with 1st/2nd-gen EGFR-TKIs.

III. MECHANISMS OF ACQUIRED RESISTANCE FROM OSIMERTINIB IN EGFR TKIPRE-TREATED NSCLC

Unfortunately, almost all EGFR-mutant NSCLC patients will eventually develop acquired resistance from osimertinib, which is what we will discuss next. Oxnard et al. [19] has reported that by looking at 143 EGFR-mutant NSCLC patients who received osimertinib after acquired resistance to prior EGFR TKI and were EGFR T790M positive in either tumor or plasma, loss of the EGFR T790M mutation was common on resistance to osimertinib and was associated with early treatment failure and development of a range of competing resistance mechanisms, such as the MET amplification (~10%), small cell transformation (17%) and some novel ones such as acquired KRAS mutations and targetable gene fusions. Fig. 2 plots these various mechanisms reported from [19]. One can see 13 patients retained T790M mutation, while 28 lost T790M mutation. Among the 13 patients (32%) whom maintained T790M, EGFR C797S mutation was also seen in 9 patients (22%). However, among the 28 patients (68%) who lost T790M, various competing resistance mechanisms was detected, and we still have 11 patients (26.8%) whose mutations (other than the original EGFR mutations) were not detected. Time to osimertinib treatment discontinuation was shorter in patients who lost T790M mutation (6.1 vs 15.2 months), and this suggests emergence of pre-existing resistant clones in tumors. These data demonstrated
clinical evidence of the heterogeneity and complexity of acquired resistance in post-oximertinib EGFR-mutant NSCLC.

Fig. 2 Mechanisms of acquired resistance after osimertinib treatment on 41 EGFR-mutant NSCLC patients pre-treated with 1st/2nd generation EGFR-TKIs; data sourced from Ref. [19]. Each patient maintains his/her founder EGFR mutation. Note patients with HER2 amplification happened not to be present in this cohort, but it can be of ~10% patients.

Clinically, how do we then deal with these many diverse and many EGFR-independent mechanisms of post-osimertinib acquired resistance? In general, if thenovel mutations developed cannot be inhibited by targeted therapy, the traditional platinum-based chemotherapy (say, carboplatin + pemetrex) will be used; after that, one can re-challenge the EGFR TKI again as it is rare for EGFR-mutant patients to lose EGFR mutation. However, there are also some promising and potentially better personalized treatment options that we list below:

1. For patients with EGFR-Dependent acquired resistance; e.g., “triple-mutant” patients with C797S, T790M and EGFR mutations: the combination of osimertinib with many other agents are currently being hotly pursued in clinical trials, such as osimertinib + necitumumab (NCT02496663) can be promising. This is because necitumumab (Portrazza®) is a fully human IgG1 monoclonal antibody directed against the EGFR and can bind to and block the ligand binding site of EGFR on the extracellular domain. This kind of novel treatment option targeting the inhibition of more than one receptor of the entire ERBB family for EGFR-mutant NSCLC has shown promises for overcoming the acquired resistance from 1st/2nd-generation EGFR TKIs. For example, cetuximab (Erbitux®) is an anti-EGFR monoclonal antibody that binds to the extracellular domain of EGFR, thereby preventing its activation and subsequent dimerization. When cetuximab is used in combination with afatinib, they both inhibit the EGFR pathway and this dual-inhibition has demonstrated a synergistic potency for anti-tumor activity, and induced antibody-dependent cell cytotoxicity (ADCC) in patients [20]. This combo has been studied in patients with advanced EGFR-mutant NSCLC progressed on prior 1st-generation EGFR TKI treatment and achieved a 32% ORR and a 4.6 month median PFS time in 54 patients, and they are effective on both T790M and T790M patients [21]. Since necitumumab acts similarly as cetuximab, osimertinib + necitumumab trial is quite promising.

Note some latest preclinical data have suggested that EGFR-mutant late-stage NSCLC might even be “cured” by using multiple drugs combo to inhibit the entire ERBB pathways, such as with a pan-HER TKI (e.g., osimertinib or afatinib) in combination with EGFR antibodies (cetuximab or necitumumab) for inhibiting EGFR, together with a monoclonal antibody (mAb) trastuzumab (Herceptin) that inhibits HER2, and another mAb U3-1402 that inhibits HER3 [22]. As described above for the afatinib + cetuximab combo, this type of multiple inhibition of the ERBB family has proven to be effective in overcoming the acquired resistance of 1st/2nd-generation EGFR TKIs clinically with manageable toxicity, but very little clinical data has been gathered for its efficacy against the acquired resistance induced by the 3rd-generation TKI osimertinib. Also, triplet therapy with afatinib, cetuximab, and bevacizumab (Avastin; avascular endothelial growth factor (VEGF) inhibitor) was reported to induce deep remission in EGFR-mutant NSCLC, but appears to have difficulty overcoming osimertinib resistance in NSCLC harboring EGFR-T790M, and thus an alternative triplet therapy with osimertinib, cetuximab, and bevacizumab may be worth exploring [23]. Therefore, future clinical trials using multiple inhibition of the ERBB family such as the “3xmAB” approach that includes a triplet containing cetuximab, trastuzumab, U3-1402 and/or bevacizumab with osimertinib inhibit C797S-expressing tumors can be of critical importance to NSCLC patients worldwide, and even a subset of ERBB inhibition on HER3 deserves to be investigated carefully (NCT03260491) [22].

Also, the C797S mutation alone is considered very challenging for osimertinib inhibit –as mentioned before, only in some specific cases where the C797S mutation developed on the trans-site of the T790M, dual-TKIs using erlotinib andosimertinib can be effective for some time [12]. Thus, to overcome the C797S mutation which occurs in ~30% of patients, and in particular those happened on the cis-site of T790M, fourth-generation EGFR-TKIs are being developed, such as the allosteric EAI045 agent that targets both the T790M and C797S EGFR mutants [24]. All current 1st/2nd/3rd generation EGFR TKIs target the ATP-site of the EGFR kinase, but EAI045 is intended to be non-ATP-competitive and mutant selective. When EAI045 is in combination with cetuximab, they are effective preclinically against NSCLC driven by L858R/T790M EGFR and by the triple-mutant L858R/T790M/C797S...
EGFR[24]. Unfortunately, no clinical trial with EAI045 is available at this time.

Brigatinib, the dual EGFR-ALK inhibitor, especially when used in combination of with an anti-EGFR antibody, has shown to be a powerful candidate to overcome on C797S/T790M/EGFR triple mutants preclinically since 2017 [25]. Computational simulation demonstrates that brigatinib fits into the ATP-binding pocket of triple-mutant EGFR, and the efficacy of brigatinib is enhanced considerably by combination with an anti-EGFR antibody because of the decrease of surface and total EGFR expression. Latest clinical data on brigatinib used in EGFR-mutant NSCLC indicated brigatinib + cetuximab canindeed effectively target concomitant EGFR-T790M and C797S mutations in cis[26]. Even though this combo treatment is not available to most patients yet as brigatinib is still mainly recognized as an anaplastic lymphoma kinase (ALK) inhibitor, we believe it can be highly interesting to triple-mutant EGFR patients.

2. For patients with EGFR-Independent acquired resistance, e.g. MET amplification: Mesenchymal-epithelial transition (MET) amplifications is a common mechanism of resistance to EGFR-TKI treatment, observed in ~20-30% of EGFR-mutant NSCLCs after osimertinib. Latest results from two dose-expansion arms of the TATTON trial (NCT02143466) found that combining the investigational c-MET inhibitor savolitinib (volitinib, HMPL-504, AZD6094) with osimertinib yielded impressive partial responses in NSCLC patients who developed MET-driven resistance to EGFR-directed therapies. In one cohort from the phase Ib study, NSCLC patients with MET-based resistance to 1st/2nd-generation EGFR TKIs following treatment with savolitinib+osimertinib had an ORR=52%, with median duration of response of 7.1 months [27]. And in a second cohort of the trial on EGFR-mutant, MET-amplified NSCLC after progression on prior 3rd-generation EGFR TKIs (including osimertinib), an ORR=28% was achieved for savolitinib+osimertinib-treated patients with a median duration of response of 9.7 months [28]. Another promising Phase 2 clinical trial using anti-EGFR/c-Met bispecific antibody JNJ-61186372 that targets cMet and EGFR mutations is recruiting (NCT02609776); patients must be chemo naïve but progression on osimertinib is not required.

There are also several novel clinical trials involving osimertinib combo therapies that can be effective to curb post-osimertinib EGFR-independent acquired resistance, such as: osimertinib + olapalumab (a monoclonal antibody against the ectoenzyme CD73, NCT03381274); osimertinib + selumetinib (ATP-independent inhibitor of mitogen-activated protein kinase kinase (MEK or MAPK/ERK kinase) 1 and 2, NCT02143466); osimertinib + navitoclax (B-cell leukemia 2 (Bcl-2) family protein inhibitor, NCT02520778); osimertinib + sapanisertib (mTOR1/2 inhibitor, NCT02537722); osimertinib + CDK 4/6 inhibitors (G1T38, NCT03455829); osimertinib + AXL inhibitor (DS-1205c, NCT03255083); etc. One novel trial NCT03073785 uses highly-selective RET (rearranged during transfection) inhibitor BLU-667 as it built on previous success using osimertinib and BLU-667 to have effectively treated two EGFR-mutant NSCLC patients with post-osimertinib RET-mediated resistance: one scan after 8 weeks revealed a RECIST tumor shrinkage of 78% [29]. That study also suggests MEK inhibitor trametinib but not the BRAF inhibitor dabrafenib overcome acquired resistance from the post-osimertinib BRAF fusion.

Finally, we also like to strongly caution against the concurrent use of immunotherapy with an EGFR-TKI, as pneumonitis (inflammation of the lungs) is a serious live-threatening problem for the combination of PD-1/PD-L1 checkpoint inhibitors with EGFR-TKIs, such as durvalumab+ osimertinib one arm of the TATTON trial had to be terminated early because of increased incidence of interstitial lung disease-like events [30]. There is also no clear clinical data showing benefits on the role of immunotherapy for NSCLC patients with EGFR as the oncogenic driver. Therefore, we will strongly caution against using concurrent immunotherapy with EGFR-TKIs to treat the post-osimertinib acquired resistance on EGFR-mutant patients. The traditional platinum-based chemotherapy is still a viable treatment option post-osimertinib; however, for patients who had used chemotherapy combined with immunotherapy such as those in the IMPower150 trial, we would recommend to wait for a few months before starting EGFR-TKIs for fear of elevated risks of pneumonitis [31].

REFERENCES

[1] https://www.cancer.org/content/dam/CRC/PDF/Public/8703.00.pdf


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HuH. et al., AACR Annual Meeting 2019, Mar. 29 - Apr. 3, Atlanta, Georgia, USA https://www.abstractsonline.com/pp8/?fbclid=IwAR1kW-suj69Ckh1qRCQTYRZJmtTPumDCmBybUeiWDOoqzNKxslyE4p#/6812 presenatation9826

Sequist L. V. et al., AACR Annual Meeting 2019, Mar. 29 - Apr. 3, Atlanta, Georgia, USAhttps://www.abstractsonline.com/pp8/?fbclid=IwAR1kWSu69Ckh1qRCQTYRZJmtTPumDCmBybUeiWDOoqzNKxslyE4p#/6812presentation9827

