Review Article

EGFR exon 20 insertion mutations in non-small cell lung cancer

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Abstract: Mutations in the epidermal growth factor receptor (*EGFR*) gene are the most common targetable genomic drivers of non-small cell lung cancer (NSCLC). 90% of the EGFR mutations comprise of EGFR exon 19 deletion and exon 21 L858R mutation, while EGFR exon 20 insertion (EGFR Ex20Ins) is the third most common type of EGFR mutation. Currently, studies on EGFR Ex20Ins are relatively scarce and limited. The frequency of EGFR Ex20Ins mutations in NSCLC was 1–10%. Patients harboring EGFR Ex20Ins exhibited similar clinical characteristics except for poorer prognosis as compared to patients with sensitizing mutations in EGFR. Conventional TKIs have poor efficacy in a majority of EGFR Ex20Ins subtypes. Chemotherapy remains the preferred treatment for advanced NSCLC patients harboring EGFR Ex20Ins. However, some novel inhibitors are considered as putative candidates. This review focuses on the structural and biochemical features, clinical characteristics, treatments, and prognosis of EGFR Ex20Ins in NSCLC.

Keywords: EGFR exon 20 insertion mutations; non-small cell lung cancer (NSCLC); EGFR-TKIs; structural features; clinical features; treatment; prognosis

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Introduction

The incidence and mortality of lung cancer rank first among malignant tumors as it caused 1.76 million deaths worldwide in 2018 (1). Lung cancer is the most important epidemic of the 21th century, currently representing the leading cause of cancer-related deaths worldwide (2). Non-small cell lung cancer (NSCLC) comprises approximately 80–85% of all lung cancers (3,4). Epidermal growth factor receptor (*EGFR*) mutation is the most widely studied in NSCLC; it targets exons 18–21 of the gene that encode part of the tyrosine kinase domain of the receptor. Approximately, 90% of the EGFR mutations comprise of EGFR exon 19 deletion and exon 21 L858R mutation, which are sensitive to tyrosine kinase inhibitors (TKIs), and the EGFR exon 19 deletion and exon 21 L858R mutations are prevalent in non-smoking Asian females with adenocarcinoma. EGFR exon 20 insertions (EGFR Ex20Ins) constitute about 1–10% of all the EGFR mutation types, and hence, are the third most common EGFR mutations associated with poor response to TKI treatment. Hitherto, 122 types of EGFR Ex20Ins have been discovered. The most common subtype is Asp770_ Asn771ins, followed by Val769_ Asp770ins (5). Similar to patients with common sensitizing EGFR mutations, those harboring the EGFR exon 20 insertion mutations are usually non-smoking females with adenocarcinoma

(6-10). EGFR Ex20Ins are associated with *de novo* resistance to targeted EGFR inhibitors and correlate with a poor patient prognosis. Although standard chemotherapy is yet preferred for these patients, some novel EGFR inhibitors are emerging. Drugs, such as Poziotinib, TAK-788, TAS-6417, AUY-922, and JNJ-372, show some effects in clinical trials but need further studies. Also, immunotherapy may be hopeful, necessitating further investigation. The median survival of NSCLC patients with EGFR exon 20 insertion mutations was similar to the EGFR wild-type patients and shorter than those with common EGFR mutations (10). The structural and biochemical features, clinical characteristics, treatments, and prognosis of EGFR exon 20 insertion mutation in NSCLC are reviewed below.

Structural and biological features

EGFR is a member of the ErbB family, which includes HER1 (erbB1), HER2 (erbB2), HER3 (erbB3), and HER4 (erbB4). It is widely distributed in mammalian epithelial cells, fibroblasts, glial cells, keratinocytes, and other cell surfaces, and the EGFR signaling pathway plays a major role in physiological processes such as cell growth (11). The structure is mainly composed of three parts: an extracellular domain, a transmembrane region, and an intracellular tyrosine kinase domain. The *EGFR* gene, length 192 kbp, is composed of 28 exons and localized in the 7p21-14 region of the short arm of chromosome 7. Most mutations occur in exons 18–21, and different types of mutations exert varied effects on the clinical efficacy of EGFR TKIs. Deletions in exon 19 and the L858R substitution in exon 21 are the two most common mutations in EGFR and are sensitive to TKIs. EGFR Ex20Ins mutations are the third most typical EGFR mutation types, which are known to be associated with resistance to common TKIs, such as gefitinib and erlotinib. Hitherto, 122 types of EGFR exon 20 insertion mutations have been identified in Met766-Cys775 after C-helix and a few in G1u762-Tyr764 in C-helix. Among these, 20.5% insertions begin after amino acid Val769, 28.7% after Asp770, 17.2% after Pro772, and 14% after His773 (5). The most common mutation is Asp770_Asn771ins, followed by Va1769_Asp770ins, Asp770_Asn771ins, A1a767_Va1769, Va1769_Asp770ins, and Ser768_Asp770, with similar insertion sequences. EGFR Ex20Ins is a highly heterogeneous family of activating mutations with complex differences between the molecular structures, biological characteristics, and response to EGFR TKIs. The EGFR exon 20 mutations were analyzed previously by *in vitro* systems (8). The study selected two mutations that lie within the C-helix (A763_Y764insFQEA [structurally identical to D761_E762insEAFQ] and Y764_V765insHH) and five mutations that lie at the end of the helix or within the loop (M766_A767insAI, A767_V769dupASV [identical to V769_D770insASV], D770_N771insNPG, D770_N771insSVD [identical to S768_D770dupSVD], and H773_V774insH [identical to P772_H773insH]). Overall, these mutations accounted for more than half of the reported EGFR exon 20 insertions (8). Moreover, in the dose-response experiments, A767_V769dupASV lacked deactivation of phosphorylated EGFR, downstream targets [phosphatidylinositol-3-kinases (PI3K)/protein kinase B (AKT)/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK)] and the upregulation of the apoptotic cascade, as measured by the levels of BIM post-treatment with 1 μM or submicromolar concentrations of erlotinib. On the contrary, A763_Y764insFQEA inhibits downstream phosphorylation and upregulates the level of Bcl-2 interacting mediator of cell death (BIM) and ultimately leads to cell death. The resulting atypical A763_Y764insFQEA is inhibited by erlotinib concentrations <0.1 μM and the 50% inhibitory concentrations (IC50) of the other structural type of EGFR exon 20 insertions exceed 2μM of erlotinib. This phenomenon indicated that the treatment with gefitinib/erlotinib is effective on EGFR Ex20Ins mutation of A763_Y764insFQEA, whereas other mutations are less sensitive.

Beau-Faller *et al.* (6) analyzed the rare mutations of EGFR exon 18 and EGFR exon 20 in 10117 NSCLC patients in a retrospective study. EGFR exon 20 insertions accounted for 4% of all the EGFR mutations; however, their forms and locations vary. Exon back-end insertion (a767-c775) was associated with EGFR-TKI resistance, and front-end insertion (e762-y764) was associated with EGFR-TKI disease control. Thus, these mutations on the loop following the C-helix are not sensitive to TKI therapy but are sensitive to the treatment before insertion of the C-ring.

Clinical features and frequency

Although EGFR exon 20 insertion mutation is the third most common type of EGFR mutation after exon 19 deletions and L858R, to date only a few studies have reported these tumors, and a majority were limited to the East Asian populations. Except the sensitivity to the EGFR TKIs, the clinical and pathological characteristics of NSCLC patients harboring the EGFR Ex20Ins mutation

Table 1 Frequency of EGFR exon 20 insertion mutations in patients with NSCLC

Author	Year	Number of EGFR mutations	Number of EGFR exon 20 insertion mutations	Frequency of the EGFR 20 insertion mutations (%)	Reference
Arcila *et al.*	2009–2011	367	33	9%	(9)
Oxnard *et al.*	2004–2012	1,086	27	2.5%	(10)
Byeon *et al.*	2009–2017	3,539	56	1.6%	(12)
Tu *et al.*	2007–2014	1,837	67	3.6%	(15)
Kuiper *et al.*	2006–2014	240	23	9.6%	(16)

EGFR, epidermal growth factor receptor.

seem to closely match those of patients with classic EGFR mutations. The majority of the studies demonstrated that patients harboring EGFR Ex20Ins mutations are normally non-smoking females with adenocarcinoma. Arcila *et al.* (9) reported that the median age of 33 patients with EGFR Ex20Ins was 66 (range, 38–85 years). The cohort comprised of 22 female patients (67%), 16 patients who were never smokers (48%), and 4 patients were Asian (12%). Eighteen patients were in stage I–II and 15 were in stage III–IV. The study showed that EGFR exon 20 insertions were more common in non-smoking patients than in those lacking EGFR exon 20 insertions (P<0.0001), albeit no significant difference was detected in the sex, age, race, or the stage at diagnosis. Oxnard *et al.* (10) reported that the median age of 27 patients with EGFR 20 insertions was 60 (range, 25–80) years; 19 patients were females (70%) (P=0.24), 15 patients never smoked (56%) (P<0.001), and 23 patients were Asian (85%) (P=0.02). The study showed that EGFR exon 20 insertions were common in non-smoking and Asian patients. Byeon *et al.* (12) reported that the median age of 27 advanced NSCLC patients with EGFR Ex20Ins in Korea was 60 (range, 43–75). Of these, 11 patients were females (40.7%), 21 patients were never smokers (77.8%), and 26 patients had adenocarcinomas (96.3%). The study showed that EGFR exon 20 insertions were more common in non-smoking patients, and the majority of the cases presented adenocarcinoma. Patients harboring the EGFR Ex20Ins mutations have clinical features similar to those with the other common EGFR mutations but with poor prognosis. The EGFR exon 20 insertion mutations were common in never smokers and Asian patients (13). The median overall survival (OS) of patients harboring the EGFR Ex20Ins mutations was 16 months, similar to that of patients with wild-type EGFR but shorter than that of patients with common EGFR mutations (10). Another study demonstrated that compared to other common EGFR mutations, Ex20Ins mutations have obvious clinicopathological features: diagnosis at a significantly younger age, shorter relapse-free survival, higher ratio in never smokers, less dependence on EGFR status, and different pathway activation patterns (14). In conclusion, a high prevalence of EGFR exon 20 insertions is similar to that of common EGFR mutations among females, non-smokers, and adenocarcinoma patients.

EGFR Ex20Ins mutation is considered as a rare subset of EGFR mutations and is ineffective against common TKIs. The true frequency of EGFR Ex20Ins mutations in NSCLC is unknown, and the prevalence of these mutations vary depending on the genotyping technique. The frequency of insertions of EGFR exon 20 mutations is varied, and the frequency of occurrence varies worldwide. According to the reports, the frequency of exon 20 insertion mutations in NSCLC ranges from 1–10% (15,16), and 4% is widely reported (12,17). *Table 1* summarizes the frequency of EGFR Ex20Ins mutations in patients with NSCLC in some studies.

Treatments

First- and second-generation EGFR-TKIs

Preclinical experiments have shown that clinically achievable doses of neither first-generation EGFR TKIs (gefitinib and erlotinib) nor second-generation EGFR TKIs (afatinib, dacomitinib, and neratinib) are effective for most of the EGFR exon 20 insertion mutant protein (5). Cells harboring the common EGFR exon 20 insertion mutation have a higher IC_{50} to EGFR TKIs than the classical EGFR mutations (5,18-21). In line with preclinical data, NSCLC patients with EGFR Ex20Ins mutations rarely respond to first- and second-generation EGFR-TKIs, with an objective

response rate (ORR) between 0 and 29% and progression-free survival (PFS) <3 months (6,8,10,12,22-26). However, some specific subtypes of EGFR Ex20Ins mutations such as A763_Y764insFQEA mutation exhibit a clinical response to EGFR-TKIs. Yasuda *et al.* established a Ba/F3 cell line model expressing distinct representative EGFR Ex20Ins mutations; especially A763_Y764insFQEA mutation has a high sensitivity to erlotinib (8). The present retrospective study included 19 advanced NSCLC patients harboring various EGFR exon 20 insertion mutations treated with gefitinib or erlotinib, 3 patients with A763_Y764insFQEA mutation achieved a significantly higher response rate by erlotinib monotherapy as compared to those with the other subtype mutations (66.7% *vs.* 0%, P=0.0175). Moreover, D770delinsGY mutation is known to be sensitive to dacomitinib *in vitro* (27). This phenomenon was further confirmed in the corresponding clinical trial that the only one patient showing partial response (PR) to dacomitinib harbored the D770delinsGY mutation (26). Subsequently, the subtypes of EGFR exon 20 insertion mutations described above are relatively rare; and therefore, the first- and second-generation EGFR-TKIs might not be valuable; occasionally, next generation sequencing (NGS) might help in selecting the specific mutation subtype.

Osimertinib

Osimertinib is a third-generation EGFR TKI targeting the T790M mutation while inheriting the high selectivity for EGFR exon 19 deletion/21 L858R mutation, which has been recommended as first-line therapy for advanced NSCLC patients with classical EGFR mutations (28). The curative effect of osimertinib in metastatic NSCLC patients with EGFR exon 20 insertion mutation has yet to be fully assessed. A preclinical study evaluated the antitumor activity of osimertinib and its metabolite (AZ5104) against the two most prevalent subtypes of Ex20Ins (Ex20Ins D770_N771insSVD and Ex20Ins V769_D770insASV, accounting for approximately 40% of all Ex20ins mutation forms) by clustered regularly interspaced short palindromic repeat (CRISPR) editing technology and xenograft models (29). Both osimertinib and AZ5104 demonstrated a high potency of inhibition of experimental cell lines *in vitro* and shrinkage of tumor in vivo. Based on the potential of osimertinib against Ex20Ins, a single-arm, non-randomized multicenter phase II study (NCT03414814) of osimertinib for locally advanced or metastatic NSCLC patients with EGFR Ex20Ins who failed in standard chemotherapy is currently underway. A phase I study (NCT02496663) was recently reported in 2019 American Society of Clinical Oncology (ASCO) (30), wherein a combination of osimertinib and EGFR monoclonal antibody necitumumab showed an ORR of 50% in four advanced NSCLC patients harboring EGFR Ex20Ins; however, further studies are essential to substantiate these findings.

Chemotherapy

Due to resistance to first- and second-generation EGFR-TKIs in EGFR Ex20Ins, platinum-based chemotherapy is considered as a standard treatment for advanced NSCLC patients harboring Ex20Ins. Byeon *et al.* reviewed the clinical outcomes of 22 patients with advanced NSCLC harboring EGFR Ex20Ins, who received first-line platinum-based chemotherapy (12). Half of these patients achieved PR, and the PFS was 4.2 months. Naidoo *et al.* (13) conducted a retrospective study enrolling 1882 patients with metastatic lung adenocarcinoma, in which 46 patients carried EGFR Ex20Ins while 258 patients had EGFR exon 19 deletion/L858R mutation. No significant difference was observed in response to conventional chemotherapy in patients with EGFR exon 20 insertions as compared to those with EGFR exon 19 deletion or L858R point mutation, and the median OS was 26 *vs.* 31 months, respectively (P=0.53). Real-world treatment outcome of advanced Chinese NSCLC EGFR Ex20Ins patients was evaluated in a multi-center retrospective study (31), wherein 165 cases were enrolled, and median PFS was significantly longer in patients who received first-line standard chemotherapy than EGFR-TKIs (6.4 *vs.* 2.9 months, P<0.001). Moreover, the median PFS of second-line TKIs therapy was shorter than the second-line paclitaxel-based chemotherapy (2.0 *vs.* 4.1 months, P<0.001). These phenomena suggested that when the effective drugs targeting EGFR Ex20Ins are unavailable, the platinum-based doublet chemotherapy is preferred for advanced NSCLC lung adenocarcinoma patients harboring the EGFR exon 20 insertion.

Cetuximab-based therapies

Cetuximab is a monoclonal antibody that blocks the EGFR signaling pathways extracellularly by binding to EGFR domain and interfering with the formation of the dimerization domain sterically (32). In the structural modeling analysis by Tsigelny *et al.*, D770_P772del_insKG and D770>GY (both are subtypes of EGFR exon

20 insertions) enhances the electrostatic energy between EGFR subunits, which in turn, strengthens the stability of the activated dimerization domain (32). Thus, the cetuximab-based combination of the two targeted drug therapies or chemotherapy can improve the prognosis of patients with EGFR exon 20 insertion mutations. Another retrospective study reported two cases that corroborated the hypothesis (33). A 75-year-old male patient from phase I trial with stage IV lung adenocarcinoma harboring the EGFR Ex20 D770>GY showed PR to cetuximab combined with erlotinib, and the same was maintained for 42 months. The other male patient aged 38 years with advanced NSCLC harboring Ex20 D770_P772del_insKG also achieved PR at 6 months after cetuximab plus chemotherapy and bevacizumab. In addition, Hasegawa *et al.* evaluated the efficacy of cetuximab combined with afatinib or osimertinib for four representative subtypes (A763_Y764insFQEA, Y764_V765insHH, A767_V769dupASV, and D770_N771insNPG) of EGFR Ex20Ins *in vivo* and *in vitro* (34). Except for A763_Y764insFQEA, a subtype mentioned above was recognized to be sensitive to EGFR-TKIs, and the proliferation of Ba/F3 cells transduced with the other three Ex20Ins mutations was not inhibited under different cetuximab concentrations. However, the addition of afatinib or osimertinib to cetuximab reversed the response of Ba/F3 cells carrying the EGFR Ex20Ins to cetuximab alone and had a lower IC_{50} as compared to monotherapy, while erlotinib plus cetuximab did not reproduce an analogous effect. Similarly, tumors carrying either A767_V769dupASV or Y764_V765insHH transplanted in nude mice regressed significantly by afatinib plus cetuximab ($P<0.05$). Consecutively, a study from the Dutch Cancer Institute reported that a combination of cetuximab with afatinib exhibited PR in 3/4 advanced NSCLC patients harboring EGFR Ex20Ins, with a median PFS of 5.4 months (35). Cetuximab-containing therapies exhibit specific potential against EGFR Ex20Ins but require additional prospective data.

Drugs in clinical trials

Poziotinib

Poziotinib (NOV120101, HM781-36B) is a novel, oral, irreversible inhibitor targeting exon 20 insertion of EGFR and human epidermal growth factor receptor 2 (HER2); the quinazoline structure of the drug is similar to that of afatinib and dacomitinib (36). The 3D modeling performed by Robichaux *et al.* indicated that a small terminal group and a flexible core made poziotinib valuable for altered steric conformation resulted from Ex20 insertions. Also, the free energy of binding (G) into the drug-binding pocket of poziotinib was lower than that of afatinib, i.e., poziotinib bound to EGFR Ex20Ins more tightly than afatinib (36). Additionally, the potency of poziotinib in EGFR Ex20Ins mutated Ba/F3 cell line was approximately 100-fold that of osimertinib with an average IC50 value of 1.0 nm. Strikingly, in the genetically-engineered mouse models, poziotinib exhibited anti-Ex20Ins efficiency *in vivo* with 80% shrinkage of the tumor volume, which was in sharp contrast to the 35% expansion of the tumor volume under afatinib. Moreover, durable tumor regression was observed for 12 weeks in genetically engineered mouse models (GEMMs), and EGFR Ex20Ins was treated by poziotinib. The weakened potency of poziotinib in cell lines carrying either C797S mutation, the osimertinib-acquired resistance mechanisms or epithelial-to-mesenchymal transition (EMT) led to the hypothesis that C797S and EMT potentially mediated the resistance to poziotinib (36). The data from a single-arm phase II study (NCT03066206) were recently updated in 2018 World Conference on Lung Cancer (WCLC). In this case, 50 pretreated patients with metastatic NSCLC harboring EGFR Ex20 mutation (46 Ex20Ins and 4 point mutations) received poziotinib alone 16 mg qd po. The potency of poziotinib countered Ex20 mutation in 44 evaluable patients with a confirmed ORR of 43% and median PFS of 5.5 months with tolerable toxicity. However, data from the subgroup analysis on EGFR Ex20Ins cohort were crucial. Currently, another large multicenter phase II single-arm study (NCT03318939) evaluating the efficacy of poziotinib in advanced NSCLC treatment-naive patients with EGFR Ex20Ins is recruiting subjects. In summary, poziotinib is the most promising candidate drug in clinical application targeting EGFR Ex20Ins but needs further studies.

TAK-788

TAK-788 (AP32788), an oral EGFR inhibitor targeting Ex20Ins, is currently evaluated in metastatic NSCLC patients harboring EGFR Ex20Ins (37). In Ba/F3 cell lines carrying 14 variants of EGFR mutations, TAK-788 inhibited all the eight activating subtypes of Ex20Ins *in vitro* with a significantly lower IC_{50} than that in wild-type EGFR. Also, the shrinkage of tumor containing an EGFR Ex20Ins implanted in mice was confirmed. The data from a phase

1/2 open-label, multicenter study (NCT02716116) were recently updated in the 2019 ASCO Annual Meeting. In the expanded cohort (n=26, TAK-788 at 160 mg qd), 23/24 evaluable patients showed varying degrees of regression of target lesion [median best percent decrease, 32.6% (PR=14, SD=9)] (38). The most common adverse event (AE) was diarrhea (85%), followed by rash (43%) and nausea (41%); the toxicity was well-tolerated.

TAS6417

TAS6417 is a newly developed EGFR-TKI designed to target exon 20 insertion; its molecular architecture (6-methyl-8,9-dihydropyrimido[5,4-b] indolizine core) differs from the other currently known small-molecule EGFR-TKIs (39). Based on the selectivity on EGFR Ex20 insertion, TAS6417 was confirmed to bind irreversibly to the ATP binding site on the EGFR hinge region. Engineering the Ba/F3 cells carrying wild-type EGFR (WT EGFR) or mutant EGFR, Hasako *et al.* demonstrated that TAS6417 specifically inhibited the various variants of EGFR Ex20 insertion including V769_D770insASV, D770_N771insSVD, H773_V774insNPH, A763_Y764insFQEA, and D770_N771insG, which accounted for >50% of all the subtypes of Ex20Ins (39). In addition, TAS6417 exhibits a satisfactory anti-tumor effect in a variety of *in vivo* models. These findings support the clinical evaluation of TAS6417 as an effective candidate drug for advanced NSCLC patients harboring EGFR Ex20Ins.

AUY922

Piotrowska *et al.* (40) reported the final results of a phase II trial evaluating the activity of Hsp90 inhibitor luminespib (AUY922) in NSCLC patients with EGFR exon 20 insertion mutations. A total of 29 cases of stage IV NSCLC with EGFR Ex20Ins mutation was included in the trial. Among these patients, the ORR was 17%, the median PFS was 2.9 months (95% CI: 1.4–5.6), and median OS was 13 months (95% CI: 4.9–19.5). This study achieved the primary endpoint, suggesting that Hsp90 inhibitor might be an effective treatment for advanced NSCLC patients with EGFR Ex20Ins mutation. However, further studies on luminespib and other hsp90 inhibitors in NSCLC patients harboring EGFR exon 20 insertions are essential.

JNJ-372

JNJ-372 is an EGFR-cMet bispecific antibody-blocking ligand that binds to promote the receptor degradation. An ongoing phase I study (NCT02609776) evaluating the safety, pharmacokinetics, and activity of JNJ-372 in EFGR-mutant advanced NSCLC was reported in 2019 ASCO (41). Among the response-evaluable patients, 6/20 patients harboring Ex20Ins exhibited an optimal response of PR in a time-dependent manner. Enrollment in the dose expansion study is ongoing.

Immunotherapy

Programmed cell death protein 1 (PD-1) and programmed cell death 1 ligand 1 (PD-L1) has revolutionized the treatment of NSCLC and improved the survival outcomes of lung cancer patients (42). However, in patients with target-driven mutations, PD-1 and PD-L1 showed low response rates. Currently, the prognosis of patients with EGFR Ex20Ins mutation after immune checkpoint blockade has not been reported. Compared to classic EGFR mutants, EGFR exon 20 insertion mutations demonstrated a high disease control rate (DCR) and high ORR at 6 and 12 months. Also, the PFS and OS of EGFR exon 20 insertion mutation are higher than that of classic EGFR mutants (43). From 2014 to 2018, 36 patients with EGFR exon 20 insertion mutations were treated with immune checkpoint inhibitors. The disease control rate was 36% at 6 months and 11% at 12 months. Among them, 1 patient obtained complete response (CR), 8 patients obtained PR, 9 patients obtained stabled disease (SD), 15 patients obtained progressive disease (PD), and 3 patients were not available (NA); the PFS was 2.9 months. In conclusion, compared to classic EGFR mutations, EGFR exon 20 mutations are associated with improved efficacy of immunological checkpoint inhibitors. The tumor mutation burden (TMB) refers to the number of somatic mutations in the tumor genome. Previous retrospective studies have found that TMB has a significant predictive effect on the immunotherapy for 27 tumor types. Jonathan *et al.* (44) reported that <4% (10/263) patients with EGFR exon 20 insertions had an intermediate-high (10–20 mutations/Mb) TMB, and only 0.7% (2/263) patients had high (> 20 mutations/Mb) TMB. The average TMB was low (mean

4.3, median 3.6, range 0–40.3 mutations/Mb) in EGFR exon 20 insertions, which was lower than that for EGFR-WT NSCLC. Therefore, we can speculate that NSCLC patients with EGFR exon 20 insertions do not benefit from single immunotherapy.

Prognosis

According to the relevant literature reports, EGFR Ex20Ins mutations indicate poor prognosis (12,45,46). Tu *et al.* (15) found that compared to other EGFR mutations, those in exon 20 are associated with a low response rate (RR) and short PFS. A total of 67 patients with EGFR Ex20Ins were assessed in the Guangdong Lung Cancer Institute in 2007–2014; the median PFS of patients harboring the EGFR Ex20Ins mutation was 2.7 months and the median overall survival (mOS) was 9.2 months. One retrospective subgroup analysis (47) reported that the prognosis of exon 20 insertion mutations is poor. In the study, 227/580 patients exhibited EGFR TKI sensitization activation mutation; 20 presented exon 20 insertion mutation and 333 were negative for the EGFR/ALK sensitization activation mutation. The mOS of Ex20Ins mutations was 5 months (95% CI: 0.17–9.8 months), the OS of EGFR TKI-sensitizing activating mutations was 16.1 months (95% CI: 12.8–19.5 months), and the OS of EGFR/ALK mutation-negative in patients was 10 months (47). The present study showed that compared to the EGFR exon 20 insertion mutations, the mOS was significantly better for the EGFR TKI-sensitizing activating mutations (P<0.001, log-rank test) and the EGFR/ALK-negating mutations (P=0.037, log-rank test). Some studies reported that the OS in patients with exon 20 mutation was 16–26 months (6,13,25). The retrospective study by Naidoo *et al.* (13) identified 1,882 patients with stage IV lung adenocarcinomas, of which, 46 patients harbored EGFR Ex20Ins. The study found that compared to the sensitized patients with EGFR-TKI mutations, patients with stage IV lung adenocarcinoma exhibited EGFR Ex20Ins with a similar mOS (26 *vs.* 31 months, P=0.53). Although the response to targeted therapy was poor, the study showed that the OS in patients with EGFR Ex20Ins mutations was equal to those harboring the EGFR-sensitizing mutations. This phenomenon might be attributed to the unique biological characteristics of the EGFR Ex20Ins mutation.

Conclusion

In summary, the third most common EGFR-mutant type after EGFR exon 19 deletion and exon 21 L858R mutation was EGFR Ex20Ins mutation exhibiting various degrees of primary resistance to conventional EGFR-TKIs. Metastatic NSCLC patients with EGFR Ex20Ins have poorer prognosis as compared to other common EGFR mutations. Currently, standard chemotherapy is preferred for the most advanced NSCLC patients harboring EGFR Ex20Ins. NGS detection might be helpful as some subtypes located at the front-end insertion are sensitive to EGFR-TKIs. In addition, the combination of EGFR-TKI and monoclonal antibody warrants further studies. Novel inhibitors such as poziotinib, TAK-788, and TAS-6417 seem to be promising candidates in the future.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2020.03.10). The authors have no conflicts of interest to declare.

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

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