




Clinical and molecular characteristics of epidermal growth factor receptor exon 20 insertion mutations in non-small-cell lung cancer

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Abstract

Objective To evaluate the genomic and immune characteristics of non-small-cell lung cancer (NSCLC) patients with epidermal growth factor receptor (EGFR) exon 20 insertion (ex20ins) mutations from a retrospective dataset with molecular spectrum, tumor mutational burden (TMB), and programmed death-ligand 1 (PD-L1) expression, as well as to evaluate the efficacy of immune checkpoint inhibitors (ICIs).

Methods A total of 283 patients with EGFR ex20ins mutations who were diagnosed with NSCLC at our hospital from August 2013 to September 2020 were enrolled in this single-center retrospective study.

Results Among the 283 patients with EGFR ex20ins mutations, 182 patients received next-generation sequencing (NGS) test, and 51 different subtypes of insertion variants were recorded. The most common mutations were A767_V769dup (21.4%), S768_D770dup (19.2%) and A763_Y764insFQEA (7.1%). The most common co-occurring mutations were EGFR amplification (37.9%), TP53 mutation (35.0%) and PIK3CA mutation (8.7%). PD-L1 status was available for 141 patients, and 75.9% (107/141) of these samples showed negative PD-L1 expression. In the 36 cases with TMB tested by NGS, the median TMB was 4.6 mutations/Mb. Then 12 patients received ICIs monotherapy or combination therapy. No severe adverse events were observed.

Conclusion Low PD-L1 expression and TMB were observed in NSCLC patients harboring EGFR ex20ins mutations. Further investigations are needed to confirm the therapeutic sensitivity of ICIs in this subgroup of EGFR mutations.

Keywords EGFR exon 20 insertion mutations · Non-small-cell lung cancer · Genomic analysis · Programmed death-ligand 1 expression · Tumor mutational burden · Immunotherapy

Introduction

The advance in our understanding of epidermal growth factor receptor (EGFR) superfamily has led to the development of molecular subgroups of non-small-cell lung cancer (NSCLC) and novel therapies besides traditional surgery and chemoradiotherapy [1]. As for EGFR gene mutations, the deletion of the inner frame in exon 19 and a point mutation (L858R) in exon 21 are the most commonly EGFR activating mutations which account for 85% of patients with EGFR mutations [2]. Both mutations confer high sensitivity to small-molecule EGFR tyrosine kinase inhibitors

(TKIs) such as erlotinib, gefitinib, afatinib, osimertinib, and dacomitinib [2]. Currently, approximately 8.2% of EGFR mutations in NSCLC are classified as uncommon subtypes, such as G719X, L861Q, S768I and EGFR exon 20 insertions (ex20ins), and their outcomes are not quite the same [3, 4]. Ex20ins mutations account for up to 4% of all EGFR mutations [5] and define a distinct subset of lung adenocarcinoma, which are characterized by a poor response to the first and second-generation EGFR TKIs such as gefitinib, erlotinib and afatinib [5–8], with a median progression-free survival (PFS) of 2.7 months and a median overall survival of 9.2 months [7]. Therefore, the development of an EGFR TKI that can more effectively target NSCLC with EGFR ex20ins mutations is of paramount importance. Osimertinib and novel EGFR inhibitors such as Mobocertinib (TAK788) or poziotinib have demonstrated pre-clinical activity in vitro against EGFR ex20ins [9–11]. Furthermore, they have been reported clinical activity and efficacy in this uncommon

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mutation subtype [12–14]. Up to now, only EGFR/c-Met double anti-rybrevant (amivantamab-VMJW, JNJ-6732) has been accelerated approval for the treatment of NSCLC patients harboring EGFR ex20ins mutations who have progressed after platinum-based chemotherapy by the U.S. FDA [15]. However, as the first-line treatment, conventional cytotoxic chemotherapy might be a better option for this mutation similar to the treatment of EGFR wild-type NSCLC [2, 16]. Therefore, in addition to molecular targeted drugs, it is very important to determine the clinical characteristics and new treatment strategies of these patients.

Immune checkpoint inhibitors (ICIs) have dramatically changed the therapeutic landscape for patients with NSCLC, especially those without sensitizing EGFR mutations or ALK translocations [17–19]. However, patients with EGFR positive NSCLC do not derive a significant benefit from ICIs therapy [20–22]. Furthermore, it is unclear whether such treatment is also without benefit in patients with uncommon EGFR mutations including EGFR ex20ins [3]. The EGFR exon 20 mutation (T790M excluded) patients demonstrated relatively higher PFS and objective response rate (ORR) to immunotherapy than those with common EGFR (PFS: 4.0 vs. 1.9 months; ORR: 19% vs. 0%; $P < 0.05$) [23]. While a real-world data from China do not suggest additional clinical benefit of ICIs for EGFR ex20ins mutations [24]. Since only a subset of patients with lung cancer respond to ICIs, it is necessary to develop clinically practical tools to identify the subset of patients most likely to derive clinical benefit [25]. The Food and Drug Administration has approved programmed death-ligand 1 (PD-L1) expression as predictive biomarker in NSCLC [17], whereas EGFR mutation appears to be a negative predictive factor [25, 26]. Tumor mutational burden (TMB) is also a predictive biomarker in several tumor types including NSCLC patients treated with ICIs [25].

Evidence about the efficacy of ICIs in NSCLC harboring EGFR ex20ins mutations is scarce [3, 26]. Therefore, it is important to further explore the clinical features and treatment response of such patients. Hence, this study was designed to evaluate the genomic and immune characteristics of NSCLC patients with EGFR ex20ins mutations from a retrospective dataset with molecular spectrum, TMB, and PD-L1 expression, as well as to evaluate the efficacy of ICIs.

Materials and methods

Patients

A total of 283 patients with EGFR ex20ins mutations who were diagnosed with NSCLC at our hospital from August 2013 to September 2020 were enrolled in this retrospective study. The patients' clinical data were obtained from

electronic medical record database, including age, gender, histological subtype, the levels of PD-L1 expression in tumors, genomic status, TMB, disease stage, ECOG score and smoking status. The tumor-node-metastasis (TNM) staging at diagnosis was classified using version 8th of the TNM staging system. This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University.

The median age of the patients was 59 years old, with a range of 25–90 years old. Among them, 130 patients (45.9%) were male and 153 patients (54.1%) were female. In addition, 70 patients (24.7%) were smokers, and 200 patients (70.7%) were non-smokers. Furthermore, 220 patients (77.7%) had a good Eastern Cooperative Oncology Group (ECOG) performance status (score 0 or 1) at the onset of the disease. Histological examination revealed that the cohort consisted of 94.0% (266/283) adenocarcinoma samples and 6.0% (17/283) non-adenocarcinoma samples. Meanwhile, 76 patients (26.8%) were diagnosed with stage I–II, 190 patients (67.1%) were diagnosed with stage III–IV, while the stage of 17 patients (6.0%) were not documented. Clinical demographics of this cohort was shown in Table 1.

Genomic analysis

Over the course of time of this study, 184 patients received targeted next-generation sequencing (NGS) to determine the genomic status by physician's choices, and the tumor specimen of the remaining 99 patients were tested by real-time polymerase chain reaction (RT-PCR) through direct sequencing of EGFR from exon 18 to exon 21 by physician's choices. TMB was tested by NGS in 36 individuals which was characterized as the number of somatic base substitution or indel alterations per megabase (Mb) per previously described method [27].

Tumor PD-L1 analysis

Tumor tissues from core biopsies, surgically resected samples, or cytological specimens were used to perform PD-L1 immunohistochemistry testing. PD-L1 immunohistochemistry was graded by a tumor proportion score (TPS) system. PD-L1 TPS, which is the percentage of tumor cells showing partial or complete membrane staining, was calculated.

Immunotherapy

Outcomes from ICIs (Pembrolizumab, Nivolumab, Camrelizumab, Sintilimab or Toripalimab) as single agents or in combination with chemotherapy or angiogenesis inhibitor were captured. Efficacy of ICIs based on the *Response Evaluation Criteria in Solid Tumors version 1.1* was evaluated.

Table 1 The clinicopathological factors in NSCLC patients with EGFR ex20ins ($n=283$)

Item	Number (%)
Median (range) age (year)	59 (25–90)
Gender	
Male	130 (45.9)
Female	153 (54.1%)
Smoking status	
Never	200 (70.7)
Smoker	70 (24.7)
Unkown	13 (4.6)
ECOG score (point)	
0–1	220 (77.7)
2–4	50 (17.7)
Unkown	13 (4.6)
Histologic subtype	
Adenocarcinoma	266 (94.0)
Adenosquamous	7 (2.5)
Undefined pathology	5 (1.8)
Poorly differentiated carcinoma	3 (1.1)
Squamous carcinoma	2 (0.7)
TNM stage	
I	70 (24.7)
II	6 (2.1)
III	34 (12.0)
IV	156 (55.1)
Unkown	17 (6.0)

NSCLC non-small-cell lung cancer; EGFR ex20ins epidermal growth factor receptor exon 20 insertion; ECOG Eastern Cooperative Oncology Group; TNM tumor-node-metastasis

The adverse events were evaluated according to *Common Terminology Criteria for Adverse Events (version 4.03)*.

Statistical analysis

Statistical analysis was performed using SPSS[®] software, version 25 (IBM Corp, Armonk, NY, USA). Descriptive statistics summarized the patients' characteristics including the median, frequency, and percentage for categorical variables. The Chi square test was used to compare categorical characteristics. When $P < 0.05$, the difference was statistically significant.

Results

Genomic analysis

Over the course of time of this study, all the patients received targeted NGS or RT-PCR by physician's choices. Of note, 182 patients had specific and available data of amino acid

sequence changes by NGS testing, while 2 patients had no available data of amino acid sequence changes by NGS. The remaining 99 patients had unknown amino acid sequence changes because insertion mutations were identified just by RT-PCR testing. A total of 51 different subtypes of insertion variants were recorded (Fig. 1). Most commonly were A767_V769dup (39/182, 21.4%), S768_D770dup (35/182, 19.2%), A763_Y764insFQEA (13/182, 7.1%), H773_V774insAH (8/182, 4.4%) and N771_H773dup (8/182, 4.4%) (Fig. 2). Among the 103 EGFR ex20ins cases with co-occurring genetic alterations, EGFR amplifications were the most common genetic alterations and were detected in 37.9% (39/103) of the samples. TP53 alterations were detected in 35.0% (36/103) of the samples. PIK3CA mutations were detected in 8.7% (9/103) of the tested samples. MET amplification mutations were detected in 6.8% (7/103) of the tested samples (Fig. 3). Of note, among all patients, three patients harbored additional EGFR activating mutations (2 of EGFR 19del and 1 of EGFR L858R).

PD-L1 expression and TMB

PD-L1 was found to be located on the membrane of tumor cells. PD-L1 status was available for 141 patients. In this study, membrane PD-L1 expression on tumor cells was defined by TPSs of $\leq 1\%$, 1%–49%, and $\geq 50\%$, respectively. In addition, 75.9% (107/141) of the samples showed negative PD-L1 expression, and 24.1% (34/141) of the NSCLC patients showed positive PD-L1 expression including two cases with a PD-L1 TPS of less than 1%. Moreover, 23 cases showed PD-L1 TPS of 1%–49%, 5 cases showed PD-L1 TPS of no less than 50%, and 4 cases with positive PD-L1 expression showed no detailed percentage. There were no significant differences between PD-L1 expression and clinical parameters including age, gender, smoking status, ECOG score, histological subtype or TNM stage ($P > 0.05$; Table 2). In the 36 cases with TMB tested by NGS, the average TMB of EGFR ex20ins was low (mean 5.3, median 4.6, range between 0.8 and 13.8 mutations/Mb), among which only 2 cases were more than 10 mutations/Mb [1].

Outcomes and adverse events with ICIs

The treatment information was collected until December 2020. Of the 283 patients with EGFR ex20ins mutations of NSCLC, 12 patients received ICIs monotherapy or combined with other agents (chemotherapy or angiogenesis inhibitor) when the disease developed into stage IV (Sintilimab $n=7$, Camrelizumab $n=2$, Toripalimab $n=2$, Pembrolizumab $n=1$). Three patients received ICIs as first-line therapy, six patients received ICIs as second-line therapy, and all these patients received platinum-based chemotherapy with or without bevacizumab as a first-line drug treatment.

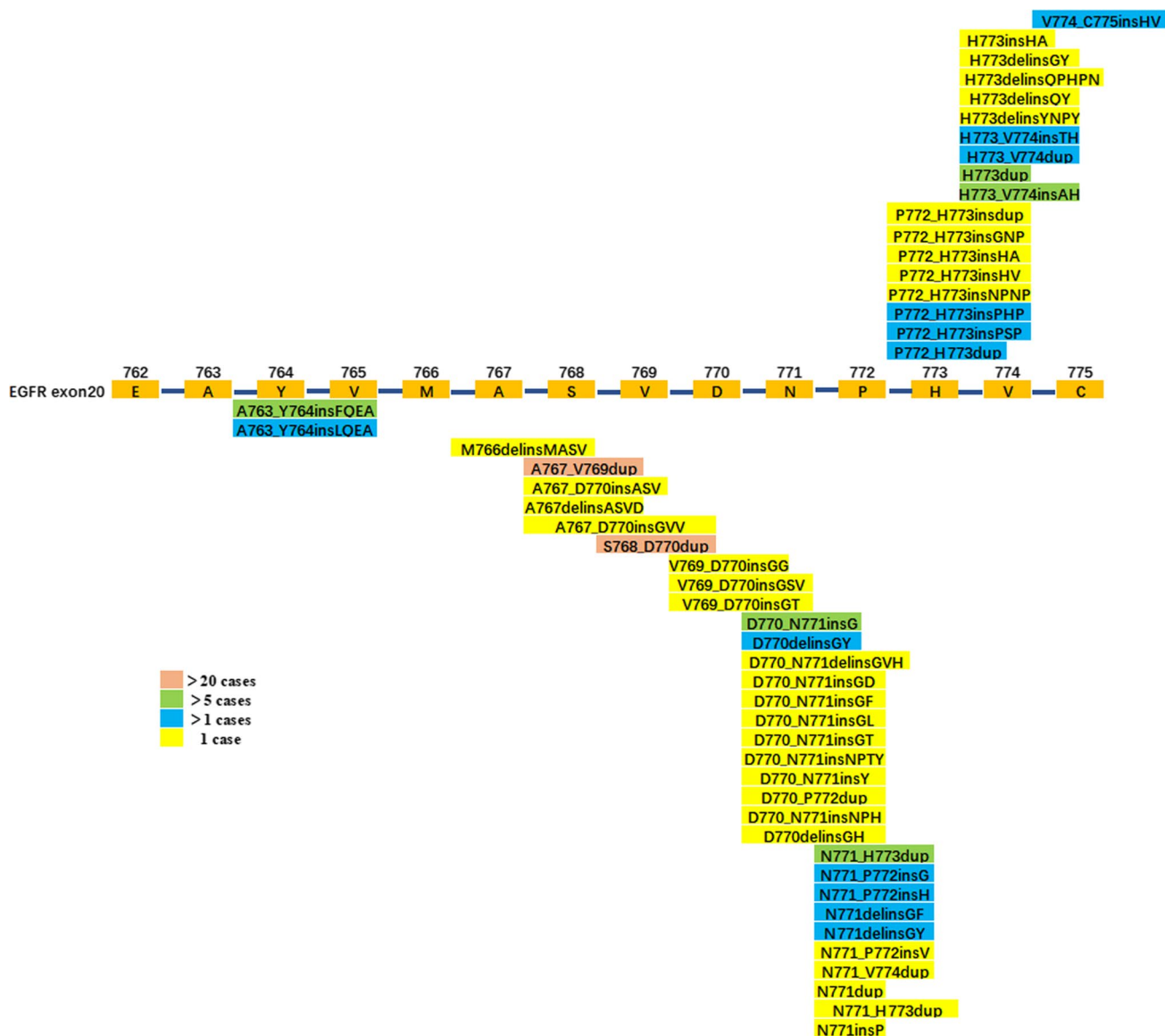


Fig. 1 Schematic of genomic positions of EGFR exon 20 insertions detected by NGS. EGFR amino acids positions were indicated. *EGFR* epidermal growth factor receptor; *NGS* next-generation sequencing

Three patients received ICIs as more than third-line therapy. All these three patients received at least one cytotoxic chemotherapy regimen including platinum-based chemotherapy with or without angiogenesis inhibitor (bevacizumab or anlotinib) as prior treatment. Among these three patients, two individuals received EGFR-TKI after chemotherapy, including one treated with osimertinib (P11) and one treated with poziotinib (P12) (Table 3). Among the 12 patients treated with ICIs, no patients achieved complete response, 2 patients achieved partial response, 8 patients achieved stable disease, 1 patient achieved progressive disease, and 1 patient was unevaluable when treated with ICIs (Fig. 4). The PFS ranged from 1.5 months to 5 months. Grade 1 reactive cutaneous capillary endothelial proliferation occurred in one

patient, and immune-related adverse events of all grades of other patients were not documented in the electronic medical record database.

Discussion

In this study, the largest known dataset of EGFR ex20ins NSCLC of 283 cases were presented. In terms of clinical characteristics, the majority of these patients (67.1%) were at advanced stage III–IV when they were diagnosed. EGFR ex20ins appeared to be associated with non-smoking status. This is in line with the study of Wu et al. that uncommon

Fig. 2 Frequency of different EGFR exon 20 insertions detected by NGS. Each alteration was shown as insertion and percentage. *EGFR* epidermal growth factor receptor; *NGS* next-generation sequencing

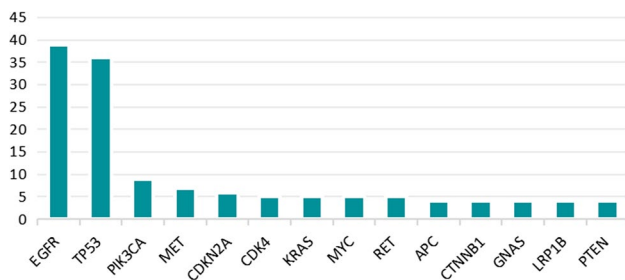
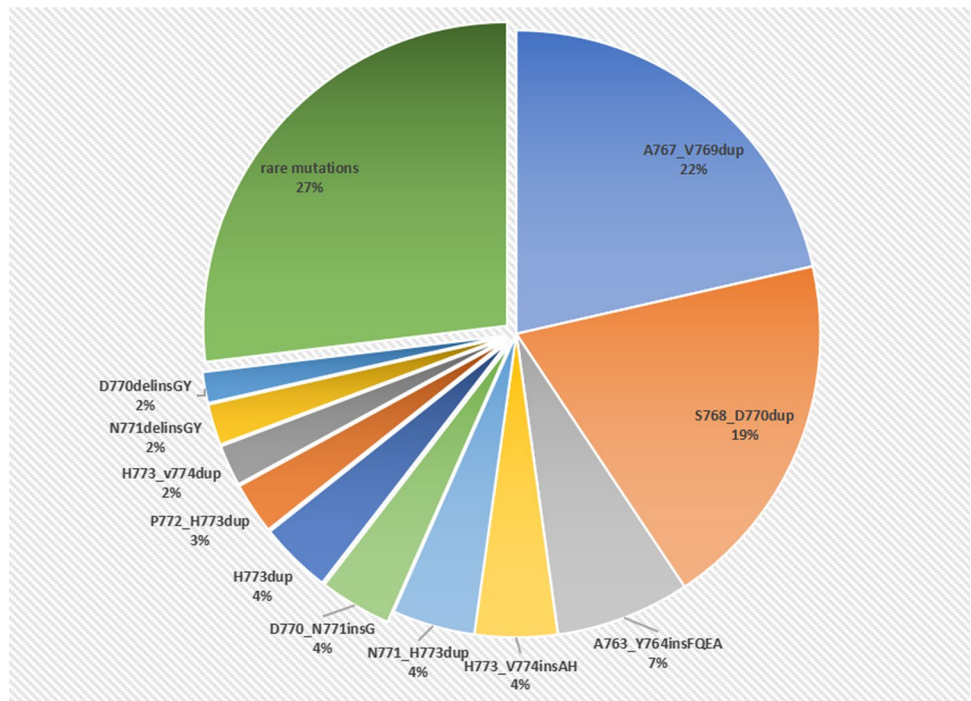


Fig. 3 Co-occurring genomic alterations with a frequency $\geq 4\%$ in NSCLC harboring EGFR exon 20 insertions. *NSCLC* non-small-cell lung cancer; *EGFR* epidermal growth factor receptor

EGFR mutations are more common among never-smokers [5].

Among the 182 patients received NGS test with recorded subtypes of insertion variants, we identified 51 unique molecular subtypes of EGFR ex20ins while Riess et al. identified 64 unique subtypes who reported 263 cases of EGFR ex20ins from the Foundation Medicine Inc database [27]. The most frequently molecular subtypes occurring were A767_V769dup (21.4%) and S768_D770dup (19.2%) represent about 40% of all detected EGFR ex20ins variants in our series, the frequency of these two subtypes was similar with Riess’s study [27]. EGFR ex20ins mutations induce a steric hindrance of the drug-binding pocket, which prevents binding of EGFR TKI [28]. However, not all EGFR ex20ins mutations have the same degree of resistance, and based on preclinical and clinical data, EGFR ex20ins

Table 2 Associations of PD-L1 expression with clinicopathological factors in NSCLC patients with EGFR ex20ins

Item	PD-L1–	PD-L1 +	P
Age (year)			0.413
< 65 (n = 100)	74	26	
≥ 65 (n = 41)	33	8	
Gender			0.299
Male (n = 72)	52	20	
Female (n = 69)	55	14	
Smoking status			0.907
Never (n = 98)	75	23	
Smoker (n = 41)	31	10	
ECOG score (point)			0.371
0–1 (n = 115)	86	29	
2–4 (n = 24)	20	4	
Histologic subtype			0.130
Adenocarcinoma (n = 135)	104	31	
Non-adenocarcinoma (n = 6)	3	3	
TNM stage			0.571
I–II (n = 40)	32	8	
III–IV (n = 98)	74	24	

PD-L1 programmed death-ligand 1; *NSCLC* non-small-cell lung cancer; *EGFR ex20ins* epidermal growth factor receptor exon 20 insertion; Eastern Cooperative Oncology Group; *TNM* tumor-node-metastasis

A763_Y7764insFQEA is generally considered the unique variant sensitive to first or second generation EGFR TKI [27, 29]. This mutation was the third most common molecular

Table 3 Details of patients receiving ICIs

Patient	PD-L1 expression	TMB (mutation/ Mb)	EGFR ex20ins mutation	therapy line	Treatment	Efficacy evaluation	PFS (month)	Adverse events
P1	NE	NE	S768_D770dup	First line	Sintilimab + Pemetrexed + Platinum	NE	NE	NE
P2	Negative	NE	N771_H773dup	First line	Sintilimab + Anlotinib	PR	3.5	None
P3	Negative	NE	NE	First line	Camrelizumab + apatinib + Pemetrexed + Platinum	PR	4.0	Grade 1 RCCEP
P4	Negative	NE	NE	Second line	Sintilimab	SD	1.5	None
P5	Negative	10.3	A767_V769dup	Second line	Pembrolizumab + Bevacizumab + Paclitaxel + Platinum	SD	2.0	None
P6	10%	NE	A763_Y764insFQEA	Second line	Toripalimab + Bevacizumab + Docetaxel + Platinum	SD	5.0	None
P7	NE	NE	H773delinsQY	Second line	Toripalimab + Pemetrexed	SD	1.5	None
P8	NE	11.96	S768_D770dup	Second line	Sintilimab + Anlotinib	SD	4.0	None
P9	Negative	7.01	A767_V769dup	Second line	Camrelizumab + Anlotinib	SD	2.0	None
P10	Negative	NE	N771delinsGY	Third line	Sintilimab + Anlotinib	SD	3.0	None
P11	NE	2.63	D770_N771insGF	Fifth line	Sintilimab	SD	3.5	None
P12	< 1%	4.6	H773insHA	Sixth line	Etoposide + Sintilimab + Anlotinib	PD	1.5	None

ICIs immune checkpoint inhibitors; PD-L1 programmed death-ligand 1; TMB tumor mutational burden; EGFR ex20ins epidermal growth factor receptor exon 20 insertion; PFS progression-free survival; NE not evaluated; PR: partial response; SD stable disease; PD progressive disease; RCCEP reactive cutaneous capillary endothelial proliferation

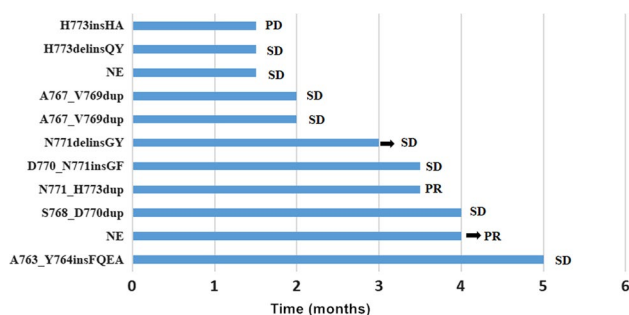


Fig. 4 Swimmer plot for duration of disease stability or response to ICIs treatment in patients with exon-20 mutations of EGFR. Bar length indicates the duration of ICIs treatment for each patient, with the best response observed before treatment failure indicating on the right. The origin corresponds to treatment start date, and the arrow indicates an ongoing response at the time of data censoring. ICIs immune checkpoint inhibitors; EGFR epidermal growth factor receptor; NE not evaluated, PD progressive disease; SD stable disease; PR partial response

subtype and comprised 7.1% (13/182) of all EGFR ex20ins in this study. These data suggested that knowledge of the specific EGFR ex20ins variant might have potential clinical implications for making treatment decisions and promote the exploration of efficacy of conventional TKI or even potent newer generation EGFR TKIs.

Although almost all EGFR ex20ins mutations are mutually exclusive with other mutations, in our dataset, more than half of (103/182) patients tested by NGS had co-existing genetic alterations [28]. EGFR amplification was the most common co-occurring mutation in this study like Riess's report [27]. TP53 alteration was the second most common co-mutation detected in 35.0% (36/103) of the samples, which indicated a decreased efficacy of EGFR TKI in NSCLC patients [30]. Some series have reported co-occurring genomic alterations affecting mutations in *CDKN2A* and *CDKN2B* (22% and 16%, respectively), *NKX2-1* (14%), *RBI* (11%) [27] and *PIK3CA* [24, 31, 32]. While those above co-existing genetic alterations were tested with less than 10% of our samples. Of note, 3 (3/103, 3%) patients were detected with EGFR 19del or 21 L858R mutation in

this study. Similarly, only 0.6% of Chinese NSCLC patients harboring EGFR ex20ins had co-occurrence of a common sensitizing EGFR mutations in a real-world study [24]. However, one cohort in Hispanic patients reported that up to one-third of EGFR ex20ins NSCLC shared a common EGFR sensitizing mutation, which conferred a better prognosis [32]. The information of different EGFR ex20ins variants and different co-mutations indicated the different efficacy to EGFR TKI, thus we need further and arduous exploration to overcome the resistance. Despite the complexity and limitations, results from clinical trials with selective EGFR ex20ins TKI have been eagerly awaited and they represent an important progress towards the identification of an effective therapeutic option for NSCLC patients with EGFR ex20ins, an area of high unmet medical need [28].

Besides the progress of EGFR TKI, ICI with PD-1 or PD-L1 antibodies has revolutionized the treatment of NSCLC and improved survival outcomes for many lung cancer patients [17]. PD-L1 expression and TMB are the most studied and validated predictive biomarkers that respond to ICIs [33]. Higher PD-L1 expression in tumor cells is correlated with higher response rate to anti-PD-1/PD-L1 therapy in NSCLC [26]. In this study, PD-L1 expression was positive in 24.1% (34/141) samples, which was lower than Chen et al. of 49.0% (24/49) that in tumor cells of EGFR ex20ins [3]. Furthermore, the PD-L1 positive expression (24.1%) of EGFR ex20ins was higher than that of mutant-type EGFR (18.4%) but lower than that of wild-type EGFR (39.1%) [34]. The positive PD-L1 expression in EGFR ex20ins lung tumors ranges from 1 to 90%. Although majority patients' PD-L1 expression were negative, high PD-L1 expression ($\geq 50\%$) occurred in five cases were also discovered. In addition to PD-L1 expression, TMB also appears to be associated with clinical benefit to PD-1/PD-L1 antibodies, potentially relating to an increase in neoantigen specific T-cell activity [27]. In the limited number of 36 patients with TMB testing, the median TMB was as low as 4.6 mutations/Mb, which was higher than that of common sensitizing EGFR mutations (3.6/Mb), while lower than that of wild-type EGFR patients (8.1/Mb) [35]. Smoking is associated with increased mutation load of multiple distinct mutational signatures in different cancers types including lung adenocarcinoma [36]. Low TMB of EGFR ex20ins in this study likely reflected non-tobacco associated carcinogenesis, while there was no significant correlation between PD-L1 expression and smoking status. Although low PD-L1 expression [18, 19] or low TMB [37] in the presence of oncogenic driver mutations should not preclude ICI efficacy, it suggests comparable lack of benefit to single agent PD-1/PD-L1 antibodies in EGFR ex20ins NSCLC, as in NSCLC harboring more common EGFR mutations, maybe combination with other agents such as chemotherapy or angiogenesis inhibitor is an effective therapeutic strategy.

In the 283 cases, only limited 12 patients received ICIs or combined with chemotherapy or angiogenesis inhibitor. However, this is the largest reported dataset of EGFR ex20ins NSCLC who received ICIs. Conventional cytotoxic chemotherapy was still the main therapy of these 12 patients. Tumor regression of only two patients who received combined ICIs as first-line therapy achieved partial response. In the remaining nine patients who received ICIs as second-line therapy or more than second-line therapy, the tumor size was not significantly reduced. Eight patients achieved stable disease, and one patient achieved progressive disease. This indicated a better effect on objective response for combined treatment naive than previously treated patients [19, 38]. As for adverse events, no severe immune related adverse events or super-progression were observed, so ICIs may be a choice for EGFR ex20ins' patients. Due to the small sample size and heterogeneous regimens, additional studies are necessary to further evaluate the efficacy of ICIs in the EGFR exon20ins population. Maybe combination therapy is better, indeed it remains unknown whether ICI monotherapy or in combination could be suitable in this subset of patients as EGFR mutant tumors were excluded from randomized phase III clinical trials assessing the role of ICIs in first-line setting of advanced NSCLC patients [19, 39].

There are still some limitations in this study. As a retrospective analysis, the number of patients received ICIs was limited and immunotherapy regimens of patients were heterogeneous. Moreover, information about PD-L1 expression and TMB of every patient who received ICIs were inadequate, and we did not analyze the value of PD-L1 expression and TMB in guiding the application of ICIs. Whether the different subtypes of insertion variants or co-existing genetic alterations will affect the efficacy of immunotherapy is also need to be determined. Hence, future prospective investigation is necessary to explore the best time or mode of ICIs and biomarkers for EGFR ex20ins NSCLC patients.

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Authors' contributions DG and XL designed the study and prepared the first draft of the paper. XL is guarantor. QG and SH contributed to the acquisition of the data. HZ and SG were responsible for statistical analysis of the data. QG and SH were responsible for interpretation of data. All authors revised the paper critically for intellectual content and approved the final version. All authors agree to be accountable for the work and to ensure that any questions relating to the accuracy and integrity of the paper are investigated and properly resolved.

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Data availability All data generated or analysed during this study are included in this published article.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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Consent to participate Not applicable.

Consent for publication Not applicable.

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