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Molecular characteristics and responses to EGFR tyrosine kinase inhibitors in non-small cell lung cancer patients with *EGFR* exon 19 insertions

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Abstract

Background Epidermal growth factor receptor (*EGFR*) exon 19 insertions (19ins) represent a unique subclass of exon 19 alterations that has a relatively low frequency. Here, we aimed to elucidate the molecular characteristics and response to EGFR tyrosine kinase inhibitors (EGFR-TKIs) in lung cancer patients with *EGFR* 19ins.

Methods Next-generation sequencing was performed to profile the molecular characteristics of 83 non-small cell lung cancer (NSCLC) patients with *EGFR* 19ins. Detailed molecular profiling and efficacy analyses were performed on these patients, with comparisons to 68 *EGFR* 19 deletion (19del) patients. Potential resistance mechanisms were also explored.

Results The prevalence of *EGFR* 19ins mutations was 0.17% of all the primary NSCLC patients. *EGFR* 19ins variants identified were I740_K745dup (86.7%) and K745_E746insVPVAIK (13.3%). Concurrent mutations frequently observed were in *TP53* (50.6%), *CDKN2A* (12.0%), *PIK3CA* (10.8%), *LRP1B* (8.4%), and *SMAD4* (8.4%). Notably, *CTNNB1* was significantly associated with 19ins ($p = 0.043$). Efficacy analysis showed median progression-free survival (mPFS) for *EGFR* 19ins patients receiving first-line EGFR-TKI treatment was significantly shorter than for *EGFR* 19del patients (hazard ratio (HR) 1.98, $p = 0.005$). Gefitinib was significantly less effective compared to other first-generation TKIs (HR 19.86, $p < 0.001$). Furthermore, osimertinib did not generate favorable outcomes as 19dels in the first-line setting either ($p = 0.025$). Post-treatment samples revealed higher occurrences of *TP53* mutations (84.6%) and presence of *EGFR* T790M (23.1%) at progression, with case studies highlighting osimertinib's limited efficacy post-first-line treatment.

Conclusions Comprehensive analysis of *EGFR* 19ins in lung cancer patients revealed genomic characteristics and clinical response, helping better inform clinical action and might facilitate the development of more precise therapeutic options for patients with these uncommon driver mutations.

Keywords NSCLC, EGFR, Exon 19 insertions, TKI

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Background

The epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have demonstrated profound clinical efficacies in non-small cell lung cancer (NSCLC) patients with *EGFR* exon 19 deletion (19del) or L858R mutations, showing superior survival benefit over platinum-based chemotherapy and fewer side effects [1]. Besides 19del and L858R, which account for 85% of *EGFR* sensitive mutations, extensive research has uncovered a range of uncommon *EGFR* mutations in NSCLC, including G719X, S768I, L861Q, T790M, and exon 20 insertions (20ins) and their corresponding clinical efficacy to different EGFR-TKIs.

In addition to these well-studied *EGFR* mutations, there is a distinct rare subtype within exon 19 alterations, the *EGFR* exon19 insertion (19ins). This is a particularly rare mutation, affecting only 0.03–0.15% of all lung cancer patients and 0.23% of *EGFR* mutations in the East Asian population [2–6]. The *EGFR* 19ins mutations can be confused with the more common *EGFR* exon 19 deletions (19dels) and are sometimes categorized in the 19del subtype in data analysis [7]. Exon 19 deletions usually begin at amino acids Glu746 or Leu747 and end at Ala750 through Pro753, resulting in in-frame deletion of three to eight amino acids [8]. In contrast, almost all 19ins involve an in-frame insertion of six amino acids [9]. Due to the low prevalence and similarities to 19dels, the understanding of *EGFR* 19ins remains limited, particularly in terms of their genomic characteristics and their associations with clinical outcomes following treatment with TKIs. Previous studies primarily consisted of case reports or small cohort studies, showing that EGFR-TKIs are effective in NSCLC patients with *EGFR* 19ins with varying efficacies [10]. In vitro studies showed that patients with *EGFR* 19ins only exhibit moderate sensitivity to TKIs, which is less sensitive than 19del [9, 11]. Additionally, there has been inconclusive evidence regarding the effectiveness of second and third generation TKIs [2–4, 12–17]. Overall, for this mutation group, there is a notable scarcity of detailed reports on the mutation profiles, concurrent genetic alterations, and the mechanisms of resistance to EGFR-TKIs. Furthermore, comprehensive clinical analysis supporting the efficacy of different EGFR-TKIs is also limited, highlighting a significant gap in the current research.

In this study, we collected the largest cohort of NSCLC patients with *EGFR* 19ins. By conducting detailed molecular and clinical analysis, we aim to elucidate the molecular characteristics and provide therapeutic insights into the treatment of this specific subtype of NSCLC patients.

Methods

Patient sample and clinical data collection

Tumor specimens were collected from *EGFR*-mutated NSCLC patients as a routine diagnosis between June 2015 and May 2020. Qualified samples were subjected to targeted next-generation sequencing by a CLIA-certified (Clinical Laboratory Improvement Amendments) and CAP-accredited (College of American Pathologists) clinical testing laboratory (Nanjing Geneseeq Technology Inc., Nanjing, China) using validated comprehensive genomic profiling panels. Formalin-fixed paraffin-embedded (FFPE) tissue samples were confirmed by pathologists from the centralized clinical testing center before genetic testing. Clinical characteristics and treatment history were extracted from medical records. Progression-free survival (PFS) was defined from the date of treatment initiation to the date of disease progression or last follow-up. This study was approved by the Medical Ethics Committee of Nanjing Geneseeq Medical Laboratory (NSJB-MEC-2024–02). The study was performed in accordance with the Declaration of Helsinki and written consent forms were obtained from all patients before sample collection.

Targeted next-generation sequencing

DNA extraction, library construction, and targeted capture enrichment were carried out following standard protocols as previously described with modifications [18]. FFPE samples were de-paraffinized first with xylene before genomic DNA extraction using QIAamp DNA FFPE Tissue Kit (Qiagen Cat. No. 56404) according to the manufacturer's instructions. Genomic DNA extracted from tumor samples was qualified using Nanodrop2000 (Thermo Fisher Scientific, Waltham, MA), then quantified using the dsDNA HS assay kit on a Qubit 3.0 fluorometer (Life Technology, US) according to the manufacturer's recommendations. Targeted NGS (Next Generation Sequencing) libraries were prepared using the KAPA Hyper Prep kit (KAPA Biosystems) with an optimized manufacturer's protocol for different sample types. Targeted capture enrichment was performed as previously described [18]. According to the manufacturer's instructions, the target-enriched library was then sequenced on HiSeq4000 NGS platforms (Illumina).

Mutation calling

Sequencing data was first demultiplexed and subjected to FASTQ file quality control using Trimmomatic [19]. Only data without extra nucleotide bases and passed quality control (QC above 15) were subjected to the following analyses. Raw reads were mapped to the reference Human Genome (hg19) using Burrows-Wheeler Aligner

(BWA-mem, v0.7.12; https://github.com/lh3/bwa/tree/master/bwakit). Genome Analysis Toolkit (GETK 3.4.0; https://software.broadinstitute.org/gatk/) was employed to perform local realignment around the insertions/deletions (INDELs) and base quality score recalibration. Picard was used to remove PCR (Polymerase Chain Reaction) duplicates. VarScan2 was applied to detect single-nucleotide variations (SNVs) and INDELs. SNVs were filtered out if the mutant allele frequency (MAF) was less than 1% for tumor tissue and 0.3% for plasma samples.

Statistical methods

Statistical analyses and graphical illustrations in this study were generated using the R Project for Statistical Computing (version 3.4.0). To minimize the impact of clinical characteristics on the comparison of molecular and efficacy features between the 19ins and 19del groups, we performed propensity score matching using the R package MatchIt v4.5.5 (http://gking.harvard.edu/matchit). Characteristics, including age, sex, staging, and pathological subtypes, were balanced between the two groups to obtain corresponding 19del control patients from a retrospective population. Chromosome instability (CIS) was estimated using the percentage of targeted genome area with copy number variations. Intratumor heterogeneity (ITH) was computed using a previously established algorithm [20]. Fisher's exact tests were used to test the categorical variables between groups. Kaplan–Meier curves were used to analyze PFS of various patient groups, and the statistical difference was compared using the log-rank test. A two-sided P value of less than 0.05 was considered significant for all tests unless indicated otherwise ($*P<0.05$, $0.01<**P<0.05$, $***P<0.001$).

Results

Patient overview

We retrospectively reviewed 30,637 Chinese NSCLC patients with *EGFR* activating mutations detected by capture-based targeted NGS genetic testing from 2015 to 2020. Of these, we identified 83 patients with the *EGFR* 19ins mutation, corresponding to a prevalence of 0.17%. The median age at diagnosis for these 83 patients was 56 years old (range 26–81), with a female predominance of 54.2% compared to 45.8% males. All patients were diagnosed with lung adenocarcinoma (LUAD), with 35 of them documented as having stage IV cancer (Table 1). In this study, molecular profile analysis was conducted on all 83 patients with *EGFR* 19ins mutation and 68 patients with *EGFR* exon19 deletion from our database using propensity score matching method for comparison. There were no significant differences in clinical characteristics, including age, sex, and stage, between patients with *EGFR* 19ins and *EGFR* 19del (Additional file 2: Table S1).

Table 1 Clinical characteristics of patients

	19ins ($N=83$)
Age at diagnosis	
≤ 56	39 (47.0%)
> 56	39 (47.0%)
Unknown	5 (6.0%)
Median (range)	56 (26–81)
Sex	
Female	45 (54.2%)
Male	38 (45.8%)
Histology type	
LUAD	83 (100%)
Stage of diagnosis	
I–III	18 (21.7%)
IV	35 (42.2%)
Unknown	30 (36.1%)
EGFR 19ins	
I740_K745dup	72 (86.7%)
K745_E746insVPVAIK	11 (13.3%)

Additionally, within our cohort, 17 patients received first-line EGFR-TKI treatment, and clinical efficacy analysis was performed on these patients (Fig. 1A). The median follow-up time for these patients was 7.8 months (range 2.0–36.0 months).

Molecular profiles and concurrent mutations in *EGFR* 19ins

The *EGFR* 19ins variants we identified were 18 base pairs in length and resulted in two subtypes. These variants were positioned amino acids upstream of the common 19del locations. One primary subtype contains a duplication of the amino acid sequence IPVAIK from Leu740, annotated as I740_K745dup. This subtype accounted for 86.7% of cases (72/83), which was the same mutation as I744_K745insKIPVAI or K745_E746insIPVAIK, as annotated previously [11]. The other variant identified was K745_E746insVPVAIK, which accounted for 13.3% of cases (11/83) (Fig. 1B). No other activating *EGFR* mutations occurred concurrently with *EGFR* 19ins variants.

For all 83 samples, *TP53* showed the highest prevalence of 50.6%, followed by *CDKN2A* (12.0%), *PIK3CA* (10.8%), *LRP1B* (8.4%), and *SMAD4* (8.4%), similar to the molecular profile of frequently altered genes in *EGFR* 19del patients (Fig. 2A). Additionally, we found that *CTNNB1* was significantly associated with *EGFR* 19del compared to 19ins mutations ($p=0.043$). We also observed enrichment of *CDK4* ($p=0.014$) and *PTEN* ($p=0.043$) with *EGFR* K745_E746insVPVAI (Fig. 2B). No significant differences were identified for chromosome instability (CIS), tumor mutation burden (TMB), or intratumor

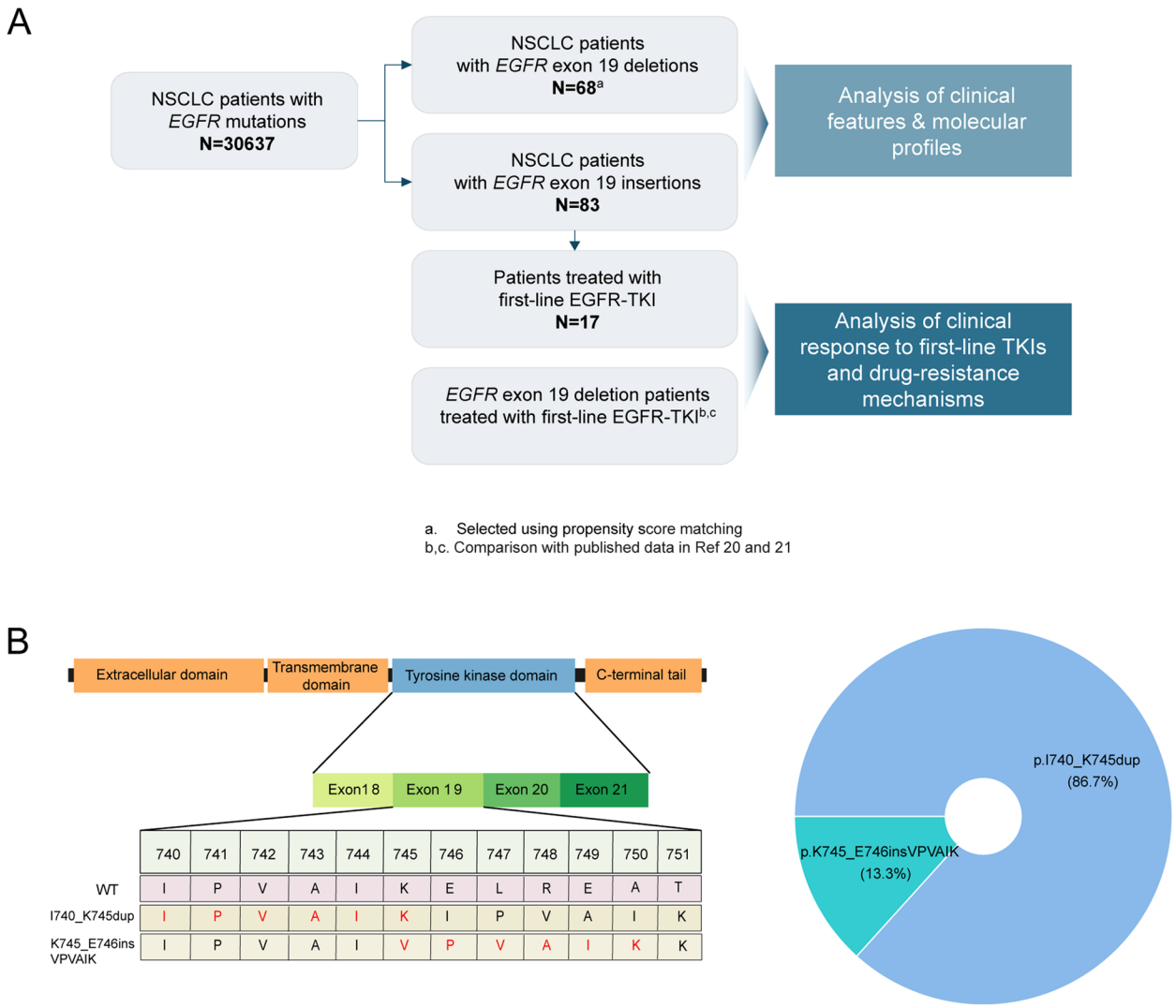

Fig. 1 Study design and molecular characteristics of *EGFR* 19ins. **A** Study workflow. **B** The location of *EGFR* 19ins in the entire *EGFR* and the distribution of two *EGFR* 19ins subtypes

heterogeneity (ITH) between *EGFR* 19del and *EGFR* 19ins (Additional file 1: Fig. S1).

Prognosis of NSCLC patients with *EGFR* 19ins treated with EGFR-TKIs

We investigated the response to EGFR-TKI in patients harboring *EGFR* 19ins with available follow-up data. Seventeen patients received first-line treatment with EGFR-TKIs. Of these, 13 patients were treated with first-generation EGFR-TKIs, specifically gefitinib, erlotinib, and icotinib. One individual was treated with the second-generation EGFR-TKI, afatinib, while three received the third-generation EGFR-TKI, osimertinib, either as monotherapy or in combination with chemotherapy or anti-angiogenesis treatment (Table 2). First, we examined the overall efficacy of first-line EGFR-TKIs between *EGFR* 19ins and 19del patients. Its outcome for *EGFR* 19ins patients was significantly worse than patients with *EGFR* 19del receiving first-line TKI in a previous study [21] (median PFS (mPFS), 7.8 m vs 13.0 m; hazard ratio (HR), 1.98, 95% confidence interval (CI), 1.19–3.27; $p=0.005$) (Fig. 3A). Between the two 19ins subtypes, K745_E746insIPVAIK showed better outcome comparing to I740_K745dup in response to first-line TKI treatment (mPFS, 7.6 m vs 13.0 m; HR, 3.17, 95% CI, 0.68–14.79; $p=0.125$) (Fig. 3B), and similarly in the first-generation subgroup (Additional file 1: Fig. S2D).

We then assessed the efficacy of specific TKI used as first-line treatment of *EGFR* 19ins patients. We found that gefitinib was the least effective among all TKIs (Fig. 3C).

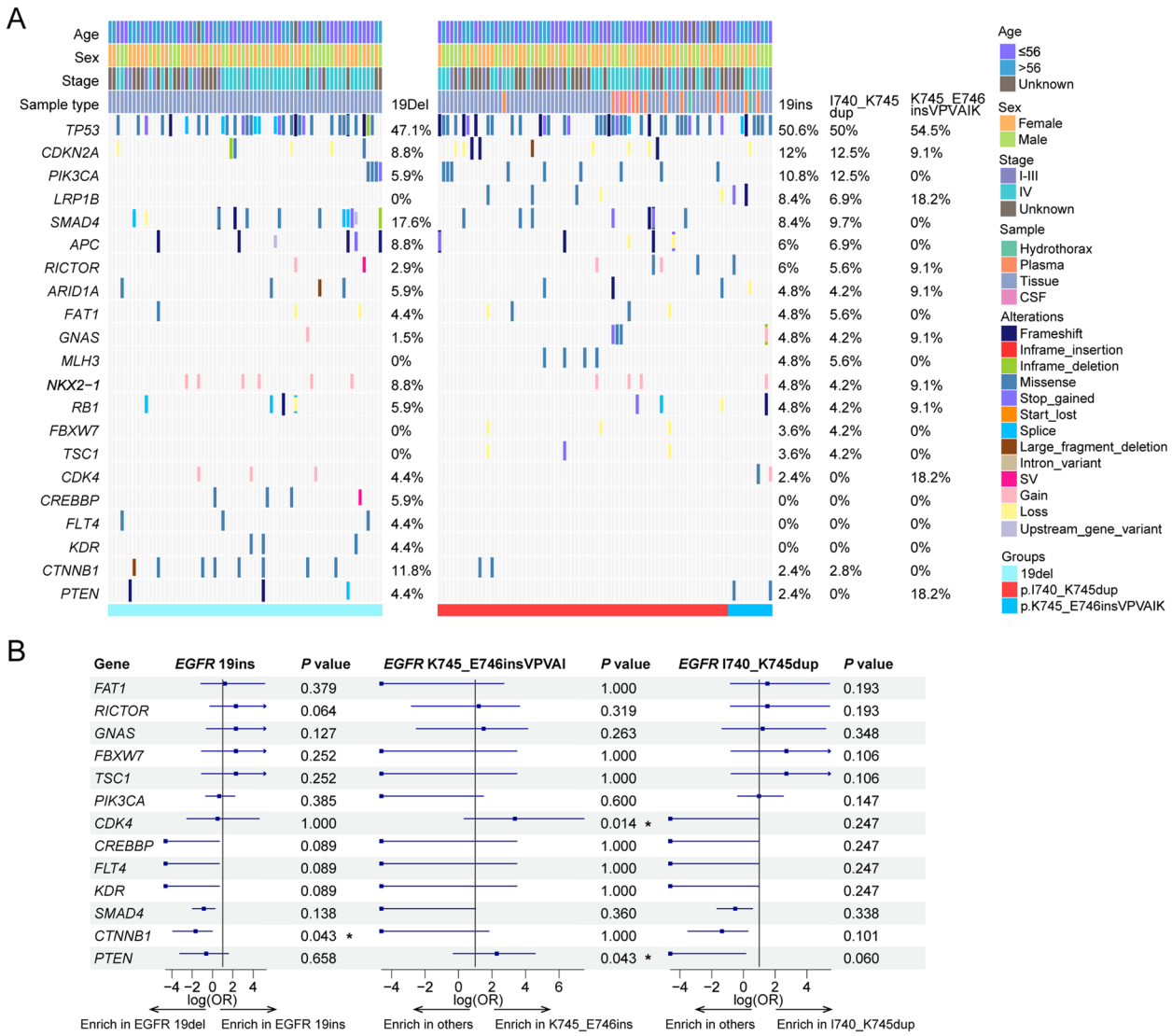

Fig. 2 Mutational landscape of *EGFR* 19ins. **A** The mutational landscape showing the frequency of top concurrent mutations with *EGFR* 19ins and with *EGFR* 19del in NSCLC patients. Each column represented one patient. Clinical characteristics of patients were shown at the top. The frequency of each gene alteration was listed on the right. **B** Forest plot showing the enrichment of different somatic mutations in patients with *EGFR* 19ins subtypes I740_K745dup and K745_E746insVPVAIK and *EGFR* 19del. *P* values are derived from Fisher's exact test

The mPFS of first-generation TKIs, erlotinib, icotinib, and gefitinib, were 22.7, 10.9, and 5.0 months, respectively (Fig. 3D), with gefitinib being significantly worse than erlotinib/icotinib group (HR, 19.86, 95% CI, 2.20–179.24; $p < 0.001$) (Fig. 3E). To confirm this finding, the response of 15 patients with *EGFR* 19ins treated with first-line EGFR-TKI from previous studies was investigated [2–4, 9–12, 15, 16, 23–25] (Additional file 2: Table S2). Similarly, gefitinib demonstrated worse survival compared to erlotinib/icotinib (mPFS 5.0 m vs. 18.0 m, HR, 2.77, 95% CI, 0.90–8.51; $p = 0.065$) (Fig. 3F). Notably, osimertinib also failed to achieve desired first-line benefit compared to *EGFR* 19 del patients, with mPFS of 7.8 m comparing to 19.0 m in the 19del patients [22] ($p = 0.025$) (Fig. 3C, G). In our cohort, erlotinib provided the greatest survival benefit, with a PFS of 36 months as a first-line treatment, and more than 11 months in second or later lines, suggesting potential advantage compared to other EGFR-TKIs (Fig. 3C, Table 2).

Potential resistance mechanisms of TKI in patients with *EGFR* 19ins

To illustrate potential resistance mechanisms of EGFR-TKI treatment in patients with 19ins, we compared

Table 2 Treatment outcomes for patients with *EGFR* exon 19 insertion mutations undergoing TKI therapy

ID	Sex	Histological subtype	Stage	Mutant	1st line drug	PFS1 (month)	2nd line drug	PFS2 (month)	3rd line drug	PFS3(month)
P9	Female	LUAD	IV	I740_K745dup	Gefitinib	2	Erlotinib + Bevacuzumab	11	Osimertinib	1.4
P28	Male	LUAD	IV	I740_K745dup	Gefitinib	2.5	-	-	-	-
P17	Male	LUAD	IV	I740_K745dup	Gefitinib	5	-	-	-	-
P46	Male	LUAD	IV	I740_K745dup	Gefitinib	7	-	-	-	-
P16	Male	LUAD	IV	I740_K745dup	Icotinib	6	Afatinib	8	-	-
P50	Female	LUAD	IV	I740_K745dup	Icotinib	7	Icotinib + chemotherapy	6	Erlotinib + chemotherapy	12
P44	Female	LUAD	IV	I740_K745dup	Icotinib	10.6	-	-	-	-
P60	Male	LUAD	IV	I740_K745dup	Icotinib	11.2	-	-	-	-
P11	Female	LUAD	IV	I740_K745dup	Icotinib	14.6	-	-	-	-
P54	Female	LUAD	IV	I740_K745dup	Erlotinib	9.4	-	-	-	-
P39	Male	LUAD	Unknown	I740_K745dup	Afatinib	12.5	-	-	-	-
P68	Male	LUAD	IV	I740_K745dup	Osimertinib	7.4	-	-	-	-
P22	Female	LUAD	IV	I740_K745dup	Osimertinib + Bevacuzumab	7.8				
P15	Female	LUAD	IV	I740_K745dup	Osimertinib + chemotherapy	10.6	-	-	-	-
P30	Male	LUAD	IV	K745_E746insVPVAIK	Gefitinib	5.6	-	-	-	-
P69	Male	LUAD	IV	K745_E746insVPVAIK	Icotinib	13	Osimertinib	5.5	-	-
P76	Male	LUAD	IV	K745_E746insVPVAIK	Erlotinib	36	-	-	-	-

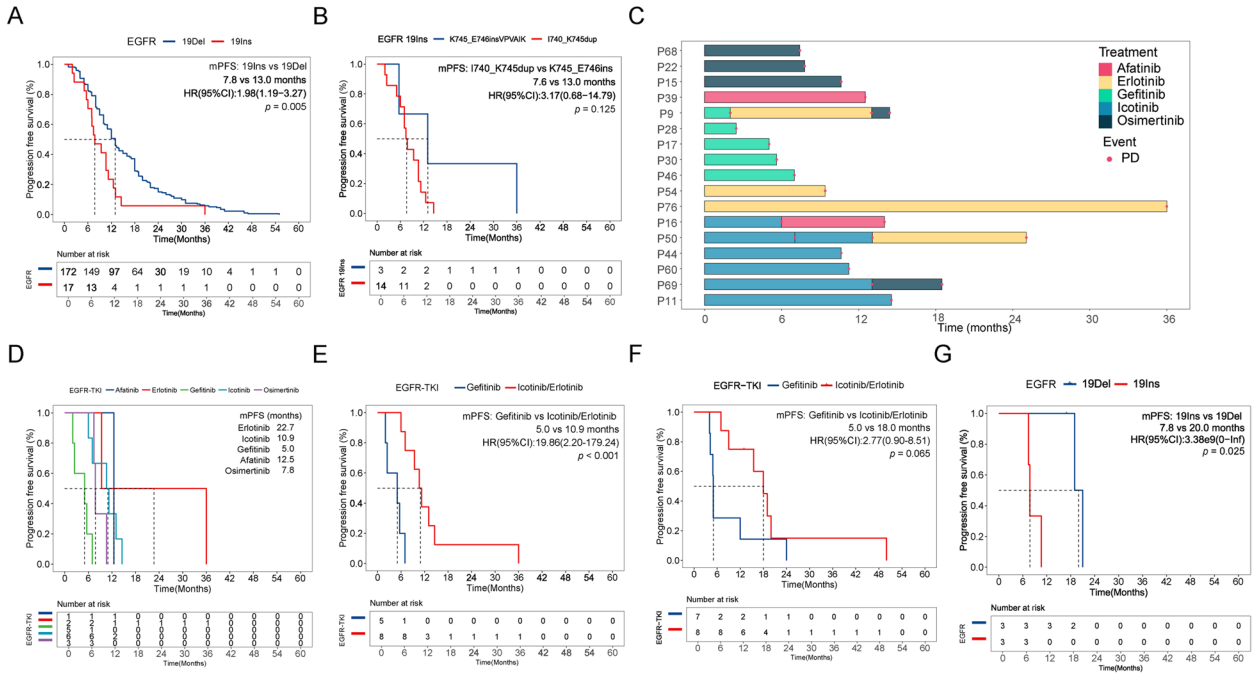

Fig. 3 Comparison of prognosis to EGFR-TKIs in NSCLC patients with different types of *EGFR* 19ins. **A** Kaplan–Meier curve comparing progression-free survival of NSCLC patients with *EGFR* 19ins and *EGFR* 19del [21] receiving first-line EGFR-TKI treatment. **B** Kaplan–Meier curve comparing progression-free survival of two *EGFR* 19ins subtypes after first-line EGFR-TKI treatment. **C** Swimming plot illustrating treatment history of 17 *EGFR* 19ins patients. **D** Kaplan–Meier curve comparing progression-free survival of different EGFR-TKIs in *EGFR* 19ins patients. **E** Kaplan–Meier curve comparing progression-free survival of gefitinib versus other two first-generation EGFR-TKIs (erlotinib and icotinib) in our cohort. **F** Kaplan–Meier curve comparing progression-free survival of gefitinib versus other two first-generation EGFR-TKIs (erlotinib and icotinib) in previous studies. **G** Kaplan–Meier curve comparing progression-free survival of *EGFR* 19ins or *EGFR* 19del patients treated with osimertinib [22]

genomic profiles of samples collected pre- EGFR-TKI treatment (baseline) and post treatment at disease progression (TKI-PD). TKI-PD samples had significantly higher occurrence of *TP53* mutations (84.6% vs. 42.2%, $p=0.011$) and *EGFR* T790M (23.1% vs 0%, $p=0.009$) compared to those at baseline. Moreover, *GNAS* and *MYC* mutations were significantly enriched in TKI-PD samples with the prevalence of 30.8% ($p=0.002$) and 23.1% ($p=0.009$), respectively, compared to none at baseline (Fig. 4A).

Three patients with the acquired T790M indicated that osimertinib can be used as subsequent treatment (Fig. 4B). Among them, only Patient 69 received T790M-guided osimertinib treatment after disease progression following first-line icotinib. However, this only resulted in six months of PFS. *RB1* loss of function mutation, along with alterations in *MYC* and *GNAS*, detected in the post-osimertinib sample probably contributed to the limited efficacy of second-line treatment (Fig. 4B). Moreover, for P39 and P50, *MYC*, *GNAS*, and the common bypass pathway genes, PIK3CA, were also detected, raising concerns of the effectiveness if these patients were to use osimertinib (Fig. 4B). Further assessment is necessary to determine the optimal regimen for 19ins patients who have relapsed after first-line TKIs.

Another interesting case of tumor development associated with *EGFR* 19ins was observed in a patient treated with first-line osimertinib. In January 2021, Patient 22 was diagnosed with stage IVA LUAD in the right lung with brain metastasis. At baseline, NGS analysis identified *EGFR* I740_K745dup in the tissue sample. The patient received osimertinib combined with seven cycles of bevacizumab, which initially reduced the right hilar mass and decreased left occipital lobe enhancement by June 2021. Clinical disease progression was determined in September 2021 with a PFS of 7.9 months. Interestingly, post-progression NGS showed an increased allele frequency of *EGFR* I740_K745dup in the tissue sample with a new *EGFR* E746_A750del mutation detected in the bronchoalveolar lavage fluid. The emergence of this new *EGFR* activating mutation suggested the potential development of a new tumor clone or lesion under the pressure of drug treatment. Treatment was changed to osimertinib combined with anlotinib for 11 months. Unfortunately, the patient died in August 2022 after palliative treatment (Fig. 4C, Additional file 1: Fig. S3).

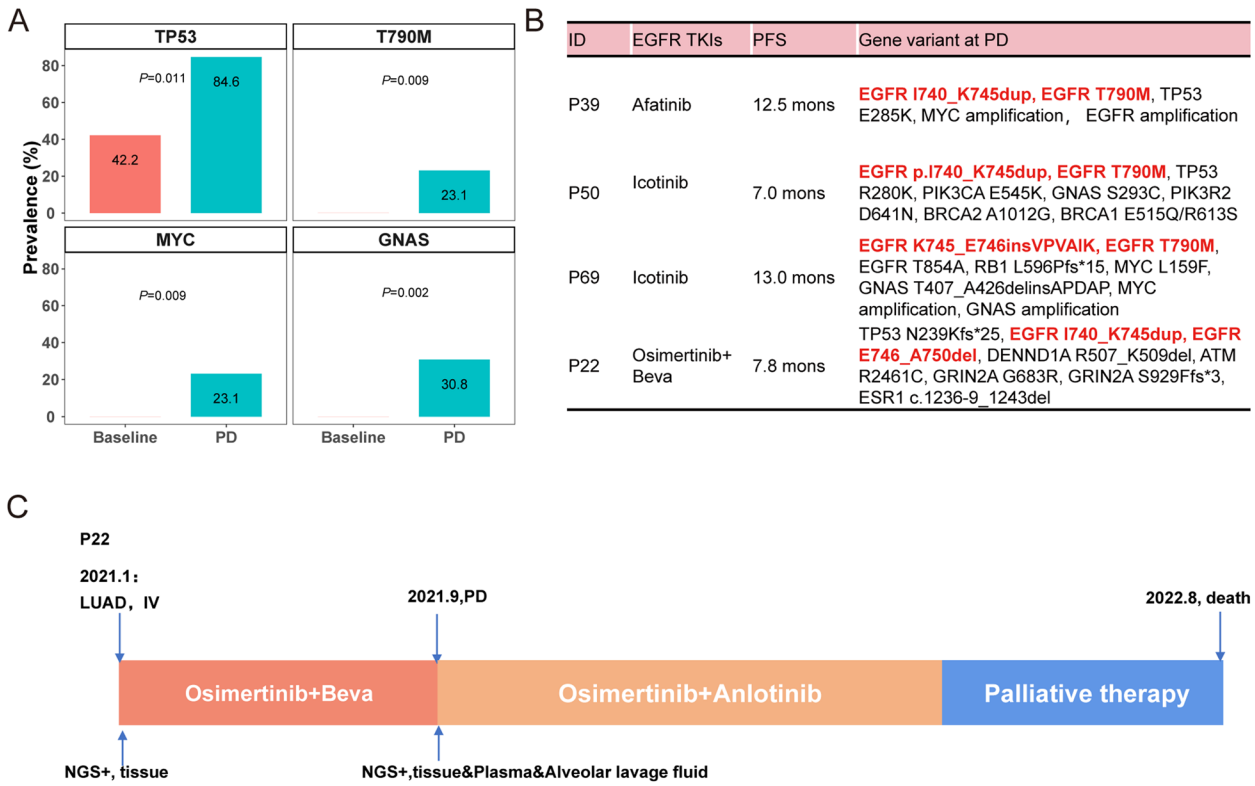

Fig. 4 Resistance mechanisms to EGFR-TKI treatment in *EGFR* 19ins patients. **A** Enrichment of genetic alteration in TKI-PD samples in comparison to baseline samples. **B** List of genetic mutations detected in the TKI-PD samples of four cases of patients treated with first-line afatinib, icotinib, or osimertinib. **C** Detailed treatment history of Patient 22

Discussion

In this study, we retrospectively analyzed targeted next-generation sequencing results from 83 NSCLC patients with *EGFR* 19ins, highlighting the molecular landscape, responses to various EGFR-TKIs, and potential resistance mechanisms.

The infrequency of *EGFR* 19ins has limited previous research primarily to case reports or small sample studies. In this study, we presented the largest cohort of *EGFR* 19ins to date, with a similarly low prevalence of less than 0.2% of NSCLC cases [2–6]. Notably, *EGFR* 19ins mutations can be mistaken for *EGFR* 19del mutations due to their similar in-frame variant types and proximal amino acid changes. However, these are two fundamentally different types of mutations. *EGFR* 19ins mutations almost always involve an in-frame insertion of six amino acids, whereas the length of 19del mutations can vary. Comparing to Sanger sequencing or PCR-based test, NGS has proven to be more sensitive in identifying 19ins mutations [9, 25].

There are three reported subtypes of 19ins mutations. Our study identified I740_K745dup (also annotated as K745_E746insIPVAIK or I744_K745insKIPVAI in other literatures) and K745_E746insVPVAIK subtypes. The I740_K745dup subtype accounted for more than 80% of all 19ins mutations, consistent with findings across different studies [11]. However, K745_E746insTPVAIK was not detected in our cohort, potentially due to demographic differences [9].

Previous reports have shown that patients with *EGFR* 19ins do respond to various EGFR-TKIs, albeit with widely varying PFS [2, 11]. Our study comprehensively evaluated the response to EGFR-TKIs in patients with *EGFR* 19ins mutations, focusing on first-line treatments. We compared the efficacy of various generations of EGFR-TKIs between patients with 19ins and 19del, and between the two identified subtypes within the 19ins cohort. We revealed that the mPFS for *EGFR* 19ins patients was significantly inferior to that of patients harboring *EGFR* 19del mutations, both in our cohort and in historical data, indicating that *EGFR* 19ins might be a poor prognostic marker in *EGFR*-mutant NSCLCs. This was supported by previous in vitro evidence as well [9, 11]. Within the 19ins group, the K745_E746insIPVAIK subtype exhibited a better mPFS than the I740_K745dup subtype. While this finding did not reach statistical significance, possibly due to the small sample size, it suggests a nuanced

heterogeneity within 19ins mutations that warrants further exploration with larger cohorts.

Among all evaluated first-generation TKIs, gefitinib demonstrated the poorest efficacy as a first-line treatment, a finding consistent in both our cohort and previous reports, despite showing partial response [2, 4, 10]. The mPFS of five months with gefitinib in patients with 19ins was markedly shorter than that observed in patient with classic *EGFR* mutations [26]. Icotinib yielded a few cases with poor outcomes but overall showed comparable efficacy to other *EGFR* mutations [27]. In contrast, while cell-line evidence suggested an unfavorable therapeutic window for erlotinib, clinical data from our study and others indicated that erlotinib may be the most effective option for patients with 19ins. Several clinical studies have reported impressive PFS ranging from 16 to 50 months with first-line erlotinib [9, 11], which were comparable to or even better than the mPFS of erlotinib observed in classic *EGFR* mutation subgroups [28, 29]. This positions erlotinib as a preferential up-front treatment choice as it may provide a substantial survival advantage for this subgroup of patients. These findings also emphasize the importance of evaluating different EGFR-TKIs individually to identify the most effective treatment options. Even among first-generation TKIs, their efficacy can vary significantly for these uncommon *EGFR* mutations.

For untreated *EGFR*-sensitive patients harboring *EGFR* 19del and L858R mutations, osimertinib has proven efficacy with an mPFS of 18.9 months on its own and 25.5 months when combined with chemotherapy [30, 31]. However, the effectiveness of osimertinib in *EGFR* 19ins subgroup remains inconclusive. There has been a report of two patients with *EGFR* 19ins who responded to osimertinib within four and eight weeks of treatment, respectively, suggesting potential benefit of osimertinib in this patient subgroup [17]. However, long term survival outcome of osimertinib has rarely been reported. The only documented case revealed nine months of survival with first-line osimertinib monotherapy [11]. In this study, osimertinib did not produce the anticipated first-line benefits in the 19ins cohort. The three patients who received osimertinib or its combination therapies displayed significantly inferior survival compared to the highly efficacious outcomes in *EGFR* 19dels.

From a structural perspective, although the proline residue at Leu747 of all *EGFR* 19ins introduces constitutive activation of EGFR, a later study classified *EGFR* 19ins mutations in the P-loop αC-helix compressing (PACC) group, a structure–function-based subgroup that alters the orientation of the P-loop or αC-helix [7, 9]. This structural change might impact drug binding. Mutations within this group were found to be sensitive to second-generation TKIs but resistant to osimertinib. In vitro experiments found that exon 19ins were only intermediately sensitivity to gefitinib whereas afatinib was the most sensitive TKI [9, 32]. Despite cell line evidence suggesting a favorable response to afatinib, the clinical application has been limited, with only a few cases reporting PFS of 12.5 and 14 months, as documented in our study and others [9]. Importantly, our study also provided critical insights into the potential resistance mechanisms to EGFR-TKIs in patients harboring *EGFR* 19ins mutations by comparing genomic profiles before and after EGFR-TKI treatment. We identified significantly higher occurrences of *EGFR* T790M, *TP53*, *GNAS*, and *MYC* mutations, mutations in TKI-PD samples, indicating these alterations as key resistance mechanisms in diminishing TKI efficacy. Exemplifying these findings, three patients acquired T790M mutations after icotinib or afatinib treatment, indicating osimertinib as a subsequent treatment. The emergence of *EGFR* T790M in these patients suggested that osimertinib can be arranged as a second-line option, although not being desirable as a first-line option. However, *RB1*, *MYC*, and *GNAS* alterations developed post-osimertinib in Patient 69, and common bypass pathway genes, such as *PIK3CA*, detected in Patients 39 and 50, raised concerns about the effectiveness of second-line osimertinib. In the intriguing case of Patient 22, who was initially treated with first-line osimertinib plus bevacizumab, a new *EGFR* E746_A750del mutation emerged upon tumor progression, suggesting new tumor clone development under treatment pressure. This case also emphasizes the need for regular genomic monitoring and adaptive treatment strategies to address evolving resistance mechanisms. Overall, acquired resistance associated with 19ins was not well studied. A few studies have found concurrent MET overexpression or *PI3KCA* with *EGFR* 19ins could potentially lead to intrinsic resistance to EGFR-TKIs [13, 25]. Our findings reiterate the complexity of managing 19ins mutations and the necessity for careful considerations when tailoring therapeutic approaches, including selection for initial treatment and combination therapies and novel agents, to improve patient outcomes.

While we aim to suggest ideal treatment strategies for patients with *EGFR* 19 insertions, we must acknowledge the limitations of our study, particularly the small cohort size with available efficacy data. The low frequency of *EGFR* 19ins mutations complicated definitive conclusions that could influence clinical treatment options and resulted in inadequate statistical power of subgroup analyses. Additionally, real-world survival data may be skewed due to irregular follow-up schedules, affecting the reliability of some of our findings. Prospective clinical trials provide the most robust clinical evidence for

therapeutic options. However, it is worth noting that patients with *EGFR* exon 19 insertions are largely underrepresented in these trials due to their scarcity. Those focusing on uncommon mutations often prioritize the more prevalent types, such as *EGFR* ex20 insertions and G719X mutations [33, 34]. Moreover, despite an increasing number of trials demonstrating prolonged survival with TKI combination therapies with chemotherapy or anti-angiogenesis agents [31, 35], we lack sufficient clinical evidence to definitively recommend combination regimens for 19ins patients. For example, in our study, the combination of bevacizumab with osimertinib yielded only 7.8 months of PFS in one patient. Future investigations are essential to identify the optimal treatment for *EGFR* 19ins patients. Importantly, clinical trials of existing and novel TKIs should incorporate a dedicated arm for this patient population to strengthen the clinical evidence supporting their treatment and assess the need for combinations with chemotherapy, anti-angiogenesis therapy, or radiotherapy.

Conclusions

Our comprehensive analysis of *EGFR* 19ins in lung cancer patients revealed genomic characteristics and clinical response. Our findings help better inform clinical action and might facilitate the development of more precise therapeutic options for patients with these uncommon driver mutations.

Abbreviations

19del	*EGFR* Exon 19 deletion
20ins	*EGFR* Exon 20 insertions
CAP	College of American Pathologists
CIS	Chromosome instability
CLIA	Clinical Laboratory Improvement Amendments
EGFR	Epidermal growth factor receptor
FFPE	Formalin-fixed paraffin-embedded
HR	Hazard ratio
INDELs	Insertions/deletions
ITH	Intratumor heterogeneity
LUAD	Lung adenocarcinoma
MAF	Mutant allele frequency
NGS	Next-generation sequencing
NSCLC	Non-small cell lung cancer
PACC	P-loop αC-helix compressing
PCR	Polymerase chain reaction
PD	Progressive disease
PFS	Progression-free survival
SNVs	Single-nucleotide variations
TKI	Tyrosine kinase inhibitor
TMB	Tumor mutation burden

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-04075-1.

Additional file 1: Fig. S1. Comparison of chromosome instability (CIS), tumor mutation burden (TMB), or intratumor heterogeneity (ITH) between *EGFR* 19del and *EGFR* 19ins subgroups. P values are derived using Wilcoxon rank-sum test. Fig. S2. Treatment history of EGFR TKIs in patients with *EGFR* 19ins. A. Distribution of EGFR TKIs used in patients with *EGFR* 19ins. B. Kaplan–Meier curves comparing progression-free survival of *EGFR* 19ins patients treated with first-generation EGFR TKIs and those treated with second or third generation TKIs. C. Kaplan–Meier curves of progression-free survival in NSCLC patients with *EGFR* 19ins treated with erlotinib, gefitinib, or icotinib as first-line treatment, respectively. D. Kaplan–Meier curves comparing progression-free survival in NSCLC patients with *EGFR* 19ins subtypes treated first-generation TKIs. E. Kaplan–Meier curve of progression-free survival in NSCLC patients with *EGFR* 19ins treated with gefitinib, erlotinib or icotinib and afatinib or osimertinib as first-line treatment, respectively. Fig.S3. Medical images depicting the temporal evolution of metastatic disease in Patient 22. Head MRIs are on the left and chest CTs on the right, both presented in chronological order. The head MRIs, conducted on January 5, 2021, June 24, 2021, and September 28, 2021, illustrate the progression of ring-enhancing lesions, with notable observations in the left parietal and occipital lobes, as well as the corpus callosum. The chest CTs, dated January 8, 2021, June 23, 2021, and September 27, 2021, demonstrate changes in a right hilar mass, ground-glass nodules, and mediastinal lymphadenopathy, as well as the emergence of new lesions and pleural effusions over time. MRI, Magnetic Resonance Imaging; CT, Computed Tomography.

Additional file 2: Table S1. Clinical characteristics of patients with *EGFR* 19ins and 19del. Table S2. Cases of the *EGFR* 19 insertion mutations reported in the literature.

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Authors' contributions

YS3, JM, TW, and YS2 contributed to conceptualization; YL, YN, and FL contributed to methodology; YS3, XW, and JP contributed to formal analysis; YS3, JM, and YN contributed to validation; YL, LH, and TW contributed to resources; YS3, JP contributed to data curation; YS3, YL, YS1, and YC contributed to writing—original draft; YS3, YL, YS1, and YC contributed to writing—review and editing; JM, YS3, XW, and JP contributed to project administration; YL, YN, and YS3 contributed to funding acquisition of the manuscript. All authors have read and approved the final manuscript.

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Data availability

Sequencing data for this study can be obtained by contacting the corresponding author at lyjhappysy@163.com.

Declarations

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Nanjing Geneseeq Medical Laboratory (NSJB-MEC-2024–02). Written consent forms were obtained from all patients before sample collection.

Consent for publication

Not applicable.

Competing interests

Yan Shi, Yedan Chen, Xiaoying Wu, Jiaohui Pang, and Yang Shao are employees of Nanjing Geneseeq Technology Inc. All other authors have no conflict of interest to declare.

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