ORIGINAL ARTICLE

Afatinib as a Potential Therapeutic Option for Patients With NSCLC With *EGFR* G724S

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Received 28 January 2021; revised 17 May 2021; accepted 17 May 2021
Available online - 24 May 2021

ABSTRACT

Introduction: *EGFR* G724S has been described to mediate resistance to first- and third-generation EGFR tyrosine kinase inhibitors (TKIs). In vitro experiments have provided compelling evidence that G724S retains sensitivity for afatinib. Nevertheless, limited data have reported the clinical efficacy of afatinib in patients with NSCLC harboring G724S mutation.

Methods: We identified 52 patients with NSCLC with *EGFR* G724S from an inhouse database and comprehensively profiled their concurrent mutation statuses. Treatments and clinical outcomes were also collected.

Results: Of 52 G724S-positive patients, 39 harbored concomitant *EGFR* exon 19 deletion (19del), and all 37 of the 39 patients who had available clinical data were detected with a G724S mutation after receiving EGFR TKIs. A rare variant of 19del E746_S752delinsV co-occurred with G724S the most frequently (n = 29), whereas 7 of 10 patients with concomitant *EGFR* exon 20 mutation were TKI treatment naive. S768I was the most common mutation in exon 20 (n = 7). One patient harbored a concomitant *EGFR* exon 21 mutation, and two lacked co-occurring *EGFR* mutations. A total of 23 patients provided valid clinical outcome data, of whom eight were treated with afatinib after the emergence of G724S, whereas 15 received non-afatinib treatment (alternative EGFR TKI, chemotherapy, or best supportive care). The disease control rate in afatinib-treated patients (n = 8) reached 100% with a median progression-free survival of 4.5 months, significantly longer than that of non–afatinib-treated (n = 15, 1.7 mo, hazard ratio [HR] = 0.32, $p = 0.037$) and alternative EGFR TKI-treated (n = 11, 1.8 mo, HR = 0.28, $p = 0.042$) patients. In the subset who had progressed on osimertinib, afatinib also yielded a superior progression-free survival (6.2 mo) than non-afatinib therapies (1.0 mo, HR = 0.04, $p = 0.005$) and alternative EGFR TKIs (1.8 mo, HR = 0.06, $p = 0.033$). Analysis of acquired mutations at afatinib progression revealed re-emergence of *EGFR* T790M or *MET* amplification as the potential mechanism of afatinib resistance.

Conclusions: *EGFR* G724S emerges as a resistant mutation against EGFR TKI preferentially in the context of a rare variant of 19del, whereas it might mediate differential mechanisms in the context of exon 20 mutation. We also found that afatinib could be a potential therapeutic option for patients with NSCLC with G724S.

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Disclosure: The authors declare no conflict of interest.

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Cite this article as: Wei Y, Jiang B, Liu S, et al. Afatinib as a potential therapeutic option for patients with NSCLC with *EGFR* G724S. *JTO Clin Res Rep*. 2021;2:100193.

ISSN: 2666-3643

https://doi.org/10.1016/j.jtocrr.2021.100193

Keywords: EGFR G724S; *EGFR* 19 deletion; Non–small Cell Lung Cancer; Afatinib

Introduction

The use of EGFR tyrosine kinase inhibitors (TKIs) has dramatically altered the therapeutic routine for patients with NSCLC and substantially improved the prognosis of *EGFR*-mutant population.[1,2] First-generation EGFR TKI, such as erlotinib or gefitinib, was designed to target EGFR-activating mutations in the tyrosine kinase domain (exon19 deletion (del) or exon21 L858R). Afatinib, a second-generation EGFR TKI selectively and irreversibly blocking ErbB family (including EGFR and HER2), also has clinical activity in patients with NSCLC with uncommon *EGFR* mutation.[3,4] Nevertheless, a secondary *EGFR* T790M gatekeeper mutation often emerges, leading to acquired resistance toward EGFR TKIs.[5,6] To overcome the resistance induced by T790M, third-generation EGFR TKIs, such as osimertinib, have been developed. Osimertinib has recently been approved as first-line therapy for *EGFR*-mutated metastatic NSCLC.[7] Unfortunately, patients treated with osimertinib often inevitably acquire resistance with the emergence of *EGFR* C797S as the most well-described mechanism.[8,9]

EGFR exon 18 G724S is a rare mutation and has recently been described in some case reports mediating the resistance to third-generation EGFR TKIs.[10-12] Both in vitro and in vivo studies have revealed that G724S limits the activity of third-generation EGFR TKIs.[13] Li et al.[14] reported G724S arising in 0.43% (5 of 1170) of osimertinib treatment-naive patients with NSCLC and revealed its association with the resistance to first-generation EGFR TKIs. In vitro experiments have provided compelling evidence that *EGFR* G724S retains its sensitivity for second-generation inhibitors, including afatinib.[13,15] Nevertheless, owing to its rarity, comprehensive characterization of *EGFR* G724S mutation is still lacking and limited data have reported the clinical efficacy of afatinib in patients with NSCLC harboring this rare mutation.

In this study, we identified 52 patients with lung cancer harboring *EGFR* G724S mutation from our inhouse database, aiming to comprehensively profile their concurrent mutation statuses in *EGFR* and other genes and explore potential mechanisms mediated by G724S. We also investigated patients' treatment responses and survival outcomes and explored the mechanism of afatinib resistance in G724S-positive patients.

Materials and Methods

Patient Information

We retrospectively reviewed the genomic profiling data of 42,316 patients with lung cancer from an inhouse database (BR) and identified 52 patients harboring *EGFR* G724S mutation (Supplementary Fig. 1). Formalin-fixed, paraffin-embedded tissue, plasma, cerebrospinal fluid, or pleural fluid samples of these patients were sequenced using a capture-based targeted panel including 520 cancer-related genes or a panel consisting 168 lung cancer genes (Burning Rock Biotech, Guangzhou, People's Republic of China). Clinical characteristics and treatment histories of patients were also retrospectively collected. The study was approved by the institutional review board of Sun Yat-sen University Cancer Center (B2020-323-01). Owing to the retrospective nature of the study, patient's informed consent was waived.

DNA Library Preparation and Sequencing

DNA was extracted using a QIAamp DNA formalin-fixed, paraffin-embedded tissue kit (Qiagen, Venlo, The Netherlands) or a QIAamp circulating nucleic acid kit (Qiagen) accordingly. DNA was sheared using Covaris M220. End repair and A tailing were followed by adaptor ligation. Ligated fragments of 200 to 400 base pairs were selected by beads (Agencourt AMPure XP kit; Beckman Coulter, Brea, CA), hybridized with RNA probe, purified by magnetic beads, and amplified by polymerase chain reaction. Indexed libraries were sequenced on a NextSeq500 (Illumina, San Diego, CA) with pair-end reads.

Sequencing Data analysis

All reads were trimmed for adapters and mapped to the reference human genome (hg19) using the Burrows-Wheeler Aligner version (v.)0.7.10.[16] Local alignment optimization, duplication marking, and variant calling were performed using the Genome Analysis Tool Kit v.3.2,[17] Picard and VarScan v.2.4.3.[18] Variants were filtered using the VarScan fpfilter pipeline, and loci with depth less than 100 were filtered out. Variants with population frequency more than 0.1% in the ExAC, 1000 Genomes, dbSNP, or ESP6500SI-V2 databases were grouped as single-nucleotide polymorphisms and excluded from further analysis. The remaining variants were annotated with ANNOVAR (2016-02-01 release)[19] and SnpEff v.3.6.[20]

Clinical Data Collection and Evaluation of Clinical Outcomes

Clinicopathologic data and treatment histories of *EGFR* G724S-positive patients were retrospectively collected. Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors version

1.1. Investigator-assessed objective response and survival outcomes were analyzed. Progression-free survival (PFS) and overall survival were defined from the start of afatinib therapy until the date of progression and date of death or last follow-up, respectively. A total of 23 patients had clinical outcomes available, and eight of them were treated with afatinib. Acquired resistance mechanisms to afatinib therapy were also described for three patients who had rebiopsy at afatinib progression (Supplementary Fig. 1).

Statistical Analysis

Statistical analysis was performed using R version 3.3.3 software. Pearson's chi-square test was performed to compare the prevalence difference in groups. Kaplan-Meier analysis was used to estimate survival functions, and a log-rank test was used to determine the difference in the survival curves between groups. In addition, p value less than 0.05 was considered to be statistically significant.

Results

G724S Preferentially Co-Occurring With a Rare Variant of EGFR Exon 19 Del Mediates Resistance to EGFR TKI

Of the 52 *EGFR* G724S-positive patients, 48 were diagnosed with adenocarcinomas, one with adenosquamous carcinoma, and one with small cell carcinoma. The histopathology classification of the remaining two patients was unclear. A variant of *EGFR* exon 19 del E746_S752delinsV co-occurred most frequently with G724S (n = 29, 55.8%), which was only observed in 7 of 860 (0.8%) of Memorial Sloan Kettering Cancer Center (MSKCC) NSCLCs ($p < 0.001$) and 4 of 1144 (0.3%) of The Cancer Genome Atlas (TCGA) lung cancers ($p < 0.001$) (Fig. 1*A* and *B*). Other co-occurring exon 19 del variants accounted for 19.2%, including T751_I759delinsN/S (n = 5), S752_I759del (n = 2), L747_S752del (n = 2), and A750_I759delinsPT (n = 1), which were also rarely found in MSKCC and TCGA (Fig. 1*B* and *C*). The mutation S768I in exon 20 occurred in 13.5%

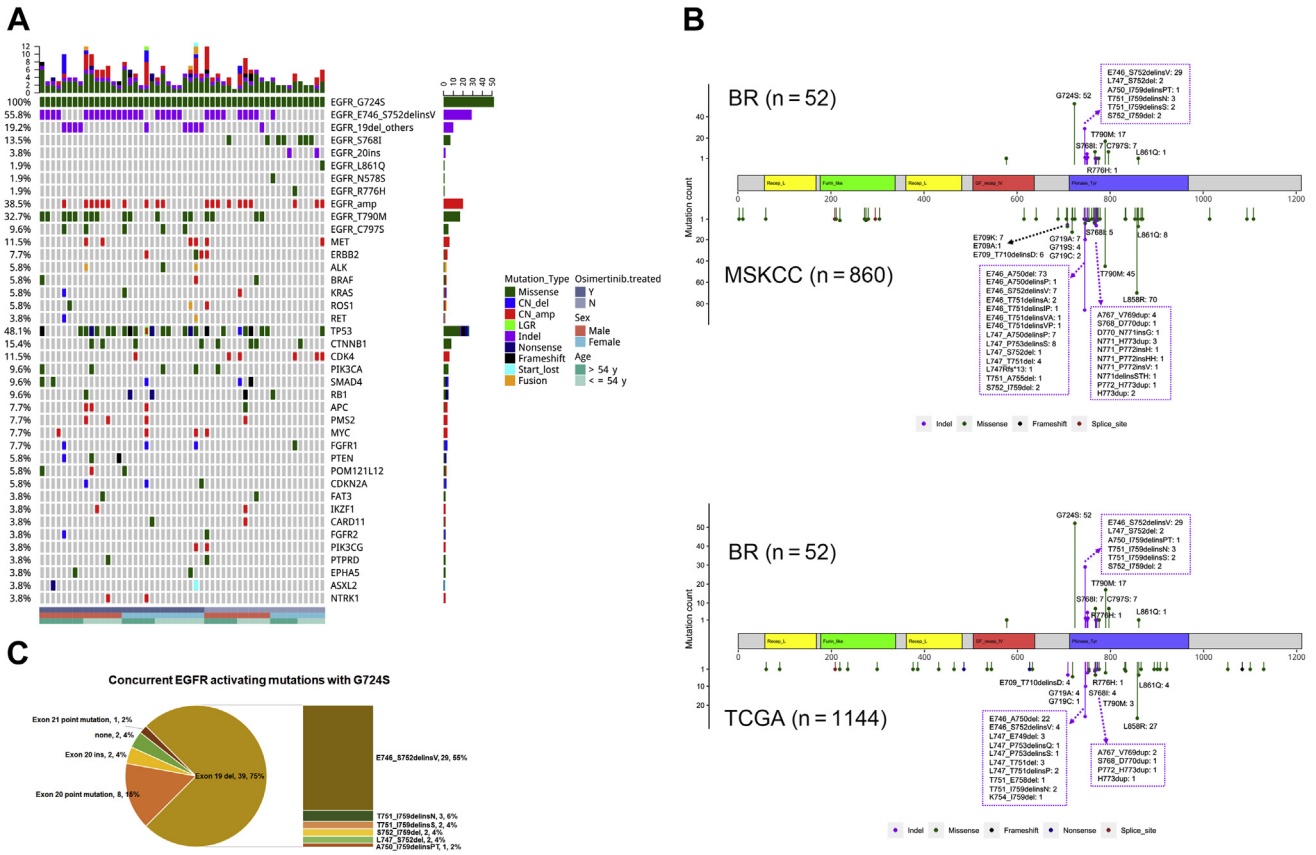

Figure 1. The genomic profiles of *EGFR* G724S-positive lung cancers. (*A*) Oncoprint of *EGFR* and concurrent mutations (n = 52). (*B*) Comparisons of *EGFR* mutational spectra in G724S-positive lung cancers from an inhouse (BR) database (n = 52) versus NSCLCs from MSKCC (n = 860) and pan-lung cancer from TCGA (n = 1144). (*C*) The distribution of *EGFR*-activating mutations co-occurring with G724S. amp, amplification; del, deletion; Indel, insertion and deletion; ins, insertion; MSKCC, Memorial Sloan Kettering Cancer Center; N, no; TCGA, The Cancer Genome Atlas; Y, yes.

of G724S-positive lung cancers (n = 7), compared with 0.3% to 0.5% in unselected lung cancers in TCGA and MSKCC (Fig. 1*A* and *B*). We also observed two 20 exon insertion variants N771dup (n = 1) and V769_D770insGT (n = 1) and two missense mutations in exon 20 (R776H, n = 1) and exon 21 (L861Q, n = 1), co-occurring with G724S (Fig. 2*A* and *B*). Two patients lacked a concurrent *EGFR* mutation (Fig. 1*A*). Collectively, 75% (n = 39) of G724S-positive patients harbored a concurrent mutation of exon 19 del and insertions (delins) and 15% (n = 8) carried a concomitant exon 20 point mutation. The exon 20 insertion and exon 21 point mutation were present in 4% (n = 2) and 2% (n = 1) of patients, respectively (Fig. 1*C*). Of note, none of the G724S-positive patients harbored the common mutation *EGFR* L858R. In comparison, the exon19 del and exon21 point mutation comprised 44% and 43% of all *EGFR* mutations, respectively, in our whole cohort without selection (Supplementary Fig. 2). E746_S752delinsV, the most enriched exon19 del variant in the G724S-positive cohort, only accounted for 3.2% of all exon19 dels with a prevalence of 0.5% in the total cohort of 42,316 patients, which is similar to that of 0.3% to 0.5% in MSKCC and TCGA. The distribution of the G724S among each of the different *EGFR* mutations and variants was also evaluated (Supplementary Table 1). We observed a significantly higher frequency of G724S in patients harboring exon 20 point mutation than those with other types of *EGFR* mutations (2.77% versus 0.26%, $p < 0.001$). Among different exon19 del variants, G724S occurred the most frequently in the context of A750_I759delinsPT (16.67%) and E746_S752delinsV (12.83%). The prevalence of G724S was significantly higher in the context of E746_S752delinsV than other exon19 del variants (12.83% versus 4.5%, $p = 0.003$).

A

Previous EGFR TKI exposure	Concurrent EGFR activating mutation	No. of patient	% of pts with T790M
None	Exon 20 point mutation	5	0
	Exon 20 ins	2	0
	None	1	0
First generation	Exon 19 del	7	43%
	Exon 20 point mutation	3	0
	Exon 21 point mutation	1	0
Second generation	Exon 19 del	1	0
Third generation	Exon 19 del	2	50%
First and Third generation	Exon 19 del	27	44%
	None	1	0
Unknown	Exon 19 del	2	0

B

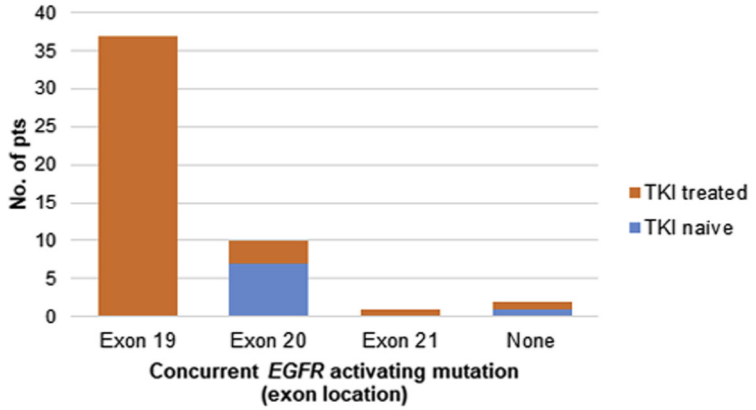

Figure 2. The association between previous EGFR TKI exposure and the type of concurrent *EGFR*-activating mutation. (*A*) List of concurrent *EGFR* mutations in Pts treated with different TKIs. (*B*) The proportions of EGFR TKI exposure in Pts with *EGFR*-activating mutation in different exons. del, deletion; ins, insertion; Pts, patients; TKI, tyrosine kinase inhibitor.

In addition to the *EGFR*, other driver genes co-altered in G724S-postive lung cancers consisted of *MET* (n = 6), *ERBB2* (n = 4), *ALK* (n = 3), *BRAF* (n = 3), *ROS* (n = 3), and *RET* (n = 2) (Fig. 1*A*). *TP53* alterations co-occurred the most frequently (48.1%), followed by *CTNNB1* (15.4%).

We also investigated the time point when *EGFR* G724S emerged and its association with the type of concurrent *EGFR* activating mutation. G724S was detected in 11 patients after progression on first-generation TKI, with seven of them harboring concomitant exon 19 delins, three harboring exon 20 point mutation, and one with exon 21 point mutation (Fig. 2*A*). G724S was detected in one patient after progression on second-generation TKI afatinib accompanied by an exon 19 delins. A total of 30 patients had G724S identified after progression on third-generation TKI, 28 of whom also had received first-generation TKI. All 30 of them carried concurrent exon 19 delins except for one. Eight patients (five with exon 20 point mutation, two with exon 20 insertion, and one without concurrent *EGFR* mutation) were TKI treatment naive before the emergence of G724S. T790M was detected in 40% to 50% of patients harboring co-occurring exon 19 delins who had received TKI treatment. Two patients with exon 19 delins had unknown TKI exposure status. Interestingly, all patients harboring *EGFR* 19 exon delins with known TKI exposure status (n = 37) received EGFR TKI treatment before G724S arising, whereas 7 of 10 patients harboring exon 20 mutations were TKI treatment naive ($p < 0.001$, Fig. 2*B*). This phenomenon suggests that the emergence of *EGFR* G724S mediates the TKI resistance preferentially in the context of *EGFR*-activating mutation exon 19 del, whereas the G724S co-occurring with a mutation in exon 20 is more likely a primary mutation.

The Clinical Outcomes of EGFR G724S-Positive Patients

Of the 52 G724S-positive patients, 23 provided valid data for clinical outcomes with eight of them treated with afatinib after the emergence of *EGFR* G724S (Fig. 3*A*). The remaining received non-afatinib treatment, including alternative EGFR TKI, chemotherapy, or best supportive care (Supplementary Table 2). The survival curves revealed that afatinib treatment resulted in a significantly longer PFS in G724S-positive patients than non-afatinib therapy (4.5 versus 1.7 mo, hazard ratio [HR] = 0.32, $p = 0.037$) (Fig. 3*B*). All the eight afatinib-treated patients achieved stable disease, resulting in a disease control rate of 100% (Table 1). Among the 15 non-afatinib-treated patients, 11 received alternative EGFR TKI (Supplementary Table 2). A longer median PFS (mPFS) was also observed in the afatinib group compared with the alternative EGFR TKI group (4.5 versus 1.8 mo, HR = 0.28, $p = 0.035$, Fig. 3*C*). Among the eight afatinib-treated patients, G724S was identified from two treatment-naive patients (baseline), one patient after gefitinib failure and five patients after osimertinib failure (Table 1), in concurrence with other rare *EGFR* mutations, including *EGFR* exon 20 insertion (n = 1), *EGFR* S768I (n = 2), and E746_S752delinsV (19delins; n = 5) (Fig. 3*A*). Interestingly, all the five patients who acquired G724S at osimertinib progression had co-occurring *EGFR* exon 19delins (Table 1). Patient 24 harboring baseline G724S in concurrence with *EGFR* S768I had the longest PFS of 7.0 months (ongoing) after the first-line treatment of afatinib. Nevertheless, the other patient harboring baseline G724S (patient 2) only obtained a PFS of 2.4 months after afatinib treatment who carried a concurrent *EGFR* exon 20 insertion (Table 1, Fig. 3*D*).

In the subset of 13 patients who had received and progressed on osimertinib treatment, afatinib also yielded a superior PFS (n = 5, 6.2 mo) than non-afatinib therapies (n = 8, 1.0 mo, HR = 0.04, $p = 0.005$, Fig. 3*E*) and alternative EGFR TKIs (n = 5, 1.8 mo, HR = 0.06, $p = 0.033$, Fig. 3*F*). Of note, two of the five afatinib-treated patients (P3 and P32) remained on afatinib with a PFS of 5.1 and 5.7 months (and counting), respectively (Table 1, Fig. 3*D*).

Mechanisms of Afatinib Resistance in G724S-Positive Patients

Analysis of acquired mutation profile at afatinib progression revealed re-emergence of *EGFR* T790M as the mechanism of afatinib resistance in two of the three patients who had available rebiopsy samples. Patient 31, a female patient with baseline *EGFR* exon 19delins who progressed on osimertinib after 28.4 months, lost *EGFR* T790M and acquired G724S at osimertinib progression. After receiving afatinib for 6.2 months, *EGFR* G724S was undetectable but *EGFR* T790M re-emerged which mediated her resistance to afatinib (Fig. 4*A*). Furthermore, patient 38, a male patient with baseline *EGFR* exon 19delins who acquired G724S and lost T790M at osimertinib progression, eventually acquired resistance to afatinib within 2.7 months of treatment. He had the re-emergence of T790M and acquisition of *EGFR* C797S and *BRAF* V600E while retaining *EGFR* G724S and 19delins (Fig. 4*B*). In contrast, patient 2, a female patient with stage IV recurrent lung adenocarcinoma, received afatinib as first-line treatment, who had baseline *EGFR* 20 exon insertion (N771dup) in concomitant with G724S. She acquired *MET* amplification at afatinib progression and retained the *EGFR* G724S and N771dup (Fig. 4*C*), revealing a differential afatinib resistance

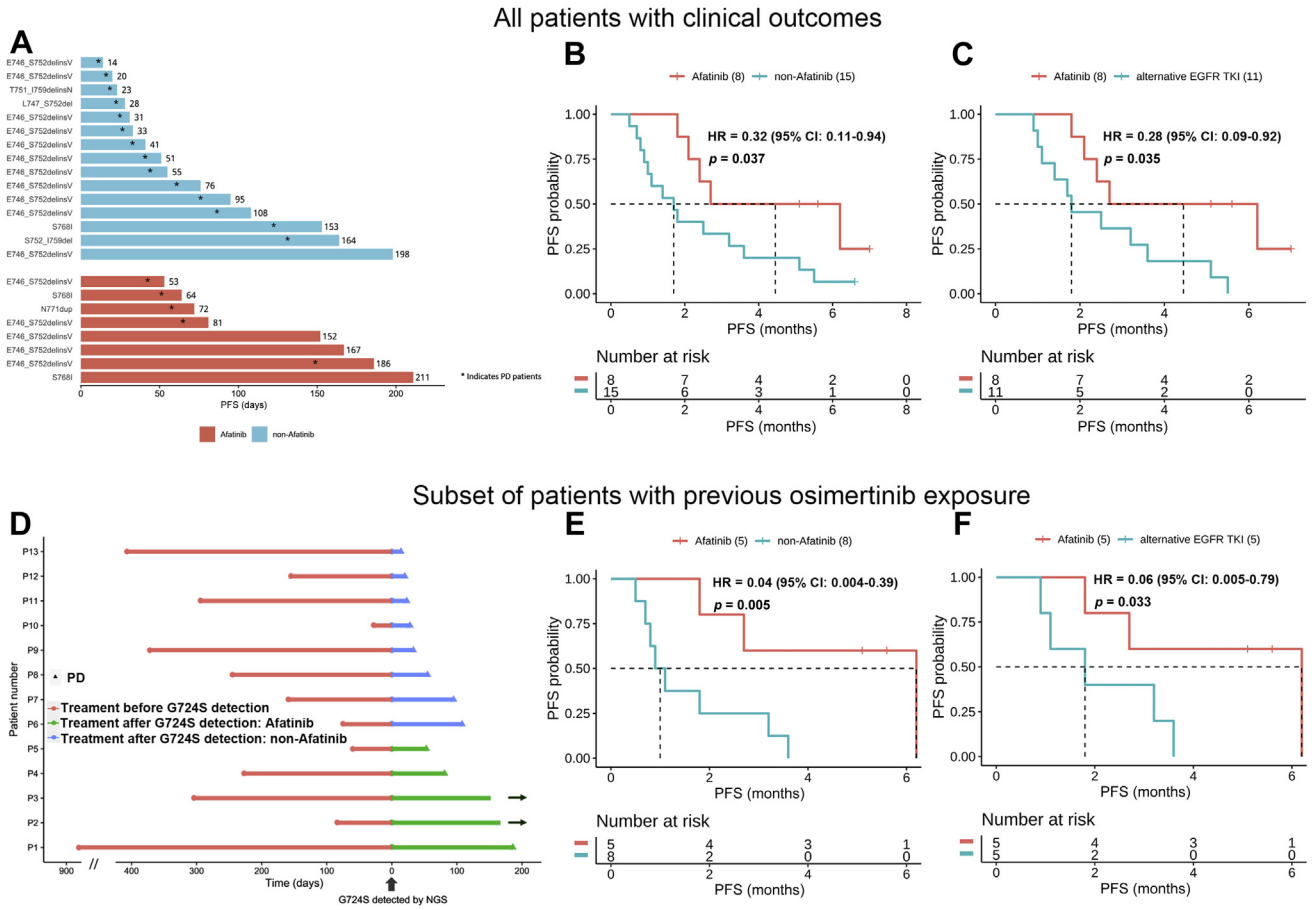

Figure 3. The clinical outcomes of patients with *EGFR* G724S-positive lung cancers. (*A*) PFS according to the type of co-occurring *EGFR*-activating mutations. (*B*) Kaplan-Meier curves for PFS in patients treated with afatinib versus those with non-afatinib treatments. (*C*) Kaplan-Meier curves for PFS in patients treated with afatinib versus those with other TKIs. (*D*) Treatment time of the patients with previous osimertinib exposure. (*E*) Kaplan-Meier curves for PFS in patients treated with afatinib versus non-afatinib therapy post-osimertinib progression. (*F*) Kaplan-Meier curves for PFS in patients treated with afatinib versus other TKIs post-osimertinib progression. *p* value was adjusted by sex and age. CI, confidence interval; HR, hazard ratio; NGS, next-generation sequencing; PFS, progression-free survival; TKI, tyrosine kinase inhibitor.

mechanism in treatment-naive compared with osimertinib-treated patients with *EGFR* G724S.

Discussion

Our study revealed *EGFR* G724S emerging primarily after the progression of third-generation TKI (n = 30) and partially after the failure of first-generation TKI (n = 11). Recent case reports have revealed the potential role of G724S in acquired resistance to osimertinib[10-12,21] and first-generation TKI[14] in lung adenocarcinoma. Fassunke et al.[13] revealed the emergence or persistence of *EGFR* G724S in osimertinib-resistant clones and described increasing G724S frequency accompanied by declining *EGFR* T790M under third-generation TKI treatment. This phenomenon was also observed in our study (Fig. 4*B*) and in other case reports.[12,21] Moreover, in vitro study revealed that *EGFR* G724S limits the activity of erlotinib, and both in vitro and in vivo experiments suggested that G724S confers resistance against third-generation TKI by inducing a conformational change in the glycine-rich loop, which reduces the binding affinity of third-generation TKI.[13,15]

EGFR exon 19 del and L858R are the two activating mutations that most often occur in NSCLCs with approximately equal prevalence.[22] Intriguingly, our data revealed that G724S emerges as a resistant mutation against TKI (especially third-generation) preferentially in the context of exon 19 del but not in concurrence with L858R, which is concordant with previous reports.[10-12,21] Furthermore, the most frequent exon 19 del co-occurring with G724S was a rare variant E746_S752delinsV (55.8%, Fig. 1*B*), which only accounts for less than 2% of exon 19 cases,[22,23] whereas E746_A750del, the most common 19 exon del (~67%), was not identified from our G724S-positive cohort. Studies have revealed that G724S reduces the binding affinity of osimertinib selectively in the context of exon 19 del rather than L868R.[13,15] More interestingly, it has been suggested that

Table 1. Characteristics of Eight Cases With Lung Adenocarcinoma Who Received Afatinib Treatment After the Emergence of *EGFR* G724S

P	Sex	Age, y	Clinical Stage	Concurrent *EGFR* Mutation	Line of Afatinib	Previous TKI	Best Response	PFS (mo)	PD Status	OS (mo)	OS Status
P2	Female	44	IVb	N771dup+amp	1	NA	Stable disease	2.4	Yes	2.4	No
P24	Male	63	IVa	S768I	1	NA	Stable disease	7.0	No	7.0	No
P10	Female	52	IV	S768I	2	Gefitinib	Stable disease	2.1	Yes	2.7	Yes
P3	Male	49	IVa	E746_S752delinsV	3	Erlotinib, osimertinib	Stable disease	5.1	No	5.1	No
P31	Female	62	IV	E746_S752delinsV	3	Erlotinib, osimertinib	Stable disease	6.2	Yes	6.2	No
P32	Female	55	IVb	E746_S752delinsV	4	Erlotinib, osimertinib	Stable disease	5.6	No	5.6	No
P38	Male	48	IVb	E746_S752delinsV	3	Erlotinib, osimertinib	Stable disease	2.7	Yes	3.6	Yes
P39	Male	50	IV	E746_S752delinsV	5	Gefitinib, osimertinib	Stable disease	1.8	Yes	21.4	No

amp, amplification; delins, deletion and insertion; NA, not applicable; OS, overall survival; P, patient; PD, progressive disease; PFS, progression-free survival; TKI, tyrosine kinase inhibitor.

E746_S752delinsV/G724S double mutant enhances the dimerization-dependent αC-helix inward conformation compared with E746_S752delinsV, whereas E746_A750del/G724S reduces the dimerization-dependent activation versus E746_A750del, which might explain the unexpected enrichment of E746_S752delinsV/G724S double mutant.[15]

G724S also occurred in the context of *EGFR* exon 20 mutation in approximately 20% of cases (Fig. 1*C*). Unlike exon 19 del, from the current but limited data, it seemed that most patients harboring exon 20 mutation lacked exposure to TKI before G724S arising (Fig. 2*B*). This observation highlights a distinct underlying mechanism mediated by G724S in the context of exon 20 mutation, which merits further elucidation.

We also identified a single G724S in two patients without other *EGFR*-activating mutations (Fig. 1*A*). G724S has been suggested as an independent oncogenic mutation potentially. G724S single mutant also reveals sensitivity to erlotinib, afatinib, and osimertinib. Furthermore, unlike exon 19Del/G724S double mutant, G724S single mutant does not mediate osimertinib resistance.[15] In our study, one of the single G724S was identified in a treatment-naive patient with resected adenocarcinoma, which supports the oncogenic role of single G724S. The other case with recurrent adenocarcinoma received gefitinib for 1 year followed by 1 year of chemotherapy, and osimertinib was administrated subsequently but immediately failed. Although the single *EGFR* G724S was detected after the progression on osimertinib, it is still uncertain when the G724S first emerged owing to the lack of genomic profile before osimertinib failure. Therefore, the role of single *EGFR* G724S in tumorigenesis and TKI resistance remains elusive.

Both in vitro and in vivo studies have revealed that Ex19Del/G724S retains sensitivity to afatinib.[13,15] A case report of a patient with lung adenocarcinoma with *EGFR* 19 del/G724S achieved stable disease to the combination of osimertinib and afatinib after osimertinib failure but experienced progressive disease within 2 months.[21] A recent study also described a case with acquired G724S in the context of *EGFR* E746_S752delinsV, who achieved PR after afatinib monotherapy with a PFS of more than 3.8 months.[11] Oztan et al.[12] reported two cases of stage IV lung adenocarcinomas harboring *EGFR* G724S concomitantly with exon9 del. One patient received carboplatin and pemetrexed and the other was treated with nivolumab, but neither of the regimens revealed efficacy. Complementing the limited clinical evidence, our study further supports a better survival after afatinib than other treatments including alternative TKIs in *EGFR* G724S-positive patients with lung cancers (HR = 0.33, $p = 0.04$, Fig. 3*B*), and the survival advantage seems more significant in the osimertinib-resistant subset (HR = 0.04, $p = 0.006$, Fig. 3*E*). The disease control rate in afatinib-treated patients reached 100% regardless of the genotype of pre-existing *EGFR* mutation (Table 1).

In addition to the well-described *EGFR* 19 del, our data revealed that G724S-positive patients in the context of S768I or 20 exon insertion also achieved stable disease to afatinib. Notably, of the two patients who received afatinib as the first-line treatment, patient 24 with concurrent S768I had the longest PFS of 7.0 months

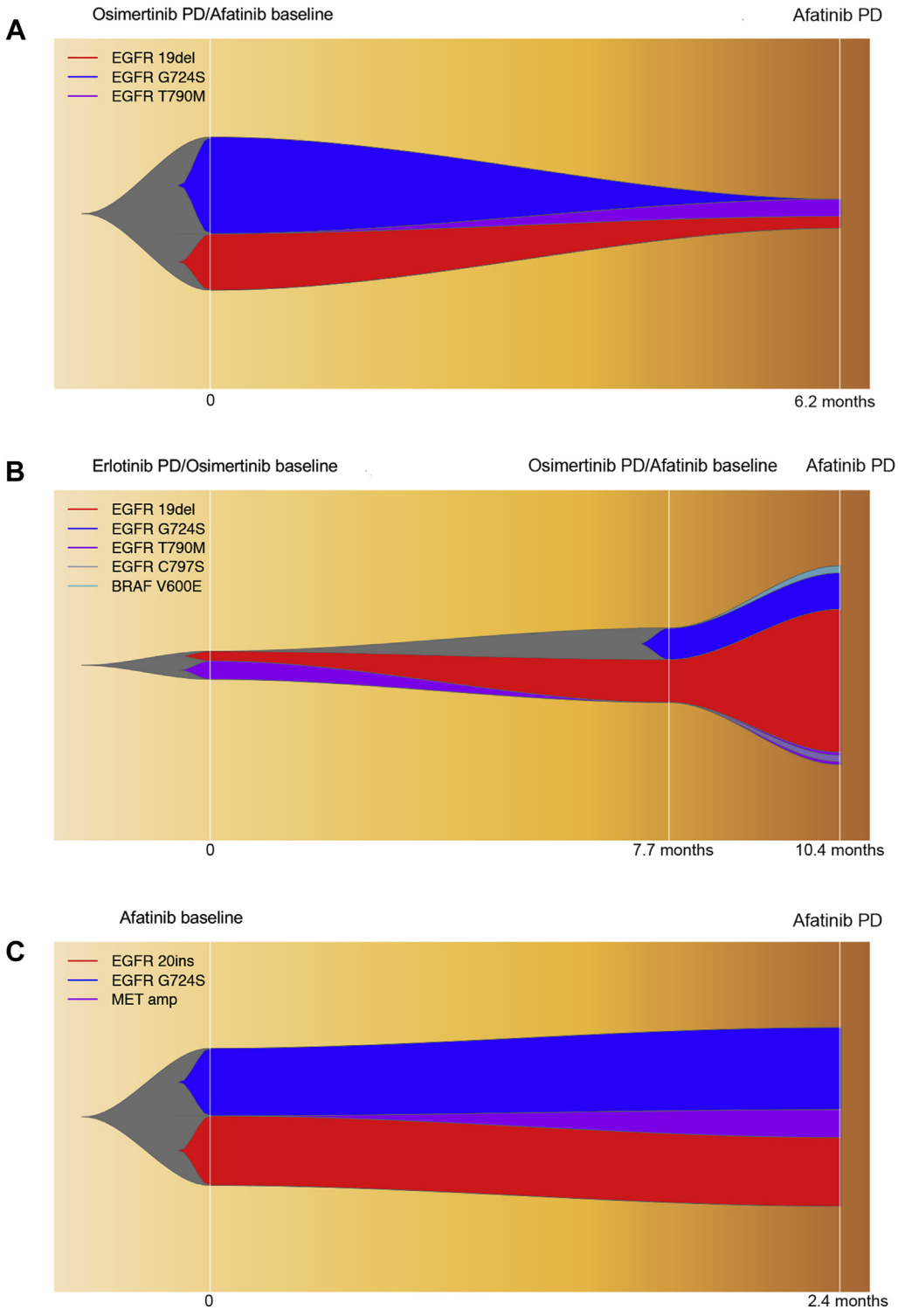

Figure 4. The dynamic changes of plasma mutational profile in three cases treated with afatinib. (*A*) P31; (*B*) P38; and (*C*) P2. amp, amplification; del, deletion; ins, insertion; P, patient; PD, progressive disease.

and patient 2 with 20 exon insertion progressed rapidly with a PFS of 2.4 months (Table 1). Concordantly, the LUX-Lung study reported an inferior response to afatinib in patients with 20 exon insertion (objective response rate = 8.7%, mPFS = 2.7 mo) than those with other *EGFR* mutation, whereas patients with S768I revealed an objective response rate of 100% and mPFS of 14.7 months.[4] Nevertheless, patient 10 experienced rapid disease progression who also harbored S768I but received afatinib treatment after progressing on gefitinib

(Table 1). Collectively, our results suggest a potentially better response to afatinib in TKI-naive patients with *EGFR* S768I/G724S.

T790M has been revealed as major resistance mechanism on afatinib treatment in *EGFR* 19del- or L858R-mutant patients.[5,24] Our study also revealed that *EGFR* T790M re-emerged as the mechanism of afatinib resistance in two osimertinib-treated patients, accompanied by the disappearance of G724S or the acquisition of other resistant mutations (*EGFR* C797S and *BRAF* V600E) (Fig. 4*A* and *B*), whereas *MET* amplification might mediate the acquired resistance in the patients with afatinib as first-line treatment (Fig. 4*C*). Peled et al.[21] reported a decline of C724S clone accompanied with the afatinib and osimertinib combinational treatment and a slight increase of C724S plus emergence of C797S on progression on the combination. Diverse mechanisms observed might be partially attributable to the different regimens administered but also indicate the complexity of acquiring resistance to afatinib in G724S-positive patients.

Our study also has limitations. Despite the large screening cohort, we only identified 52 *EGFR* G724S-positive patients owing to the rarity of this mutation. In addition, the treatment information and clinical outcomes were retrospectively obtained from half of the patients, with only eight treated with afatinib. The small number of patients and the retrospective nature of the study weaken the strength of our finding on the efficacy of afatinib. Furthermore, we were unable to statistically identity genomic modifiers (such as co-occurring *EGFR*-activating variants or other gene alterations) that might be associated with clinical outcomes because of the limited number of patients with therapeutic information. Further well-designed studies with large cohorts are warranted to evaluate the efficacy of afatinib and explore predictive and prognostic biomarkers for patients with advanced NSCLC harboring *EGFR* G724S.

In conclusion, our study reveals that G724S emerges as a resistant mutation against TKI preferentially in the context of a rare variant of *EGFR* exon 19 del and provides clinical evidence that afatinib monotherapy could be a potential therapeutic option for patients with NSCLC with *EGFR* G724S.

Availability of Data and Materials

The data sets used or analyzed during the current study are available from the corresponding author on reasonable request. The data supporting our trial will be found at the online Research Data Deposit website (http://www.researchdata.org.cn) (Research Data Deposit number: RDDA2020001827).

CRediT Authorship Contribution Statement

Yang Wei: Conceptualization, Investigation, Writing—review and editing.

Shulin Liu: Investigation, Resources, Writing—review and editing.

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Wenfeng Fang: Resources, Writing—review and editing.

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Xin Li: Resources, Writing—review and editing.

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Acknowledgments

This work was supported by the Natural Science Foundation of Guangdong Province of People's Republic of China (2018A0303130243) and the China International Medical Foundation (z-2018-32-190013). The authors appreciate the assistance in data analyses and manuscript writing from Drs. Jianxing Xiang, Min Li, and Lin Shao from Burning Rock Biotech.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2021.100193.

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