

Epidermal growth factor receptor exon 20 p.S768I mutation in non-small cell lung carcinoma: A case report combined with a review of the literature and investigation of clinical significance

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Abstract. Epidermal growth factor receptor (EGFR) plays a significant role in non-small cell lung cancer (NSCLC), the most prevalent form of lung cancer worldwide. Therefore, EGFR may be a useful molecular target for personalized therapy utilizing tyrosine kinase inhibitors (TKIs). Somatic activating *EGFR* mutations may be used to identify tumors sensitive to the effects of small-molecule EGFR-TKIs (gefitinib and erlotinib), and alternative, less frequently observed mutations, including the majority of mutations identified within exon 20, may be associated with a lack of response to TKIs. However, due to the comparative rarity of *EGFR* exon 20 mutations, clinical information concerning the association between *EGFR* exon 20 mutations and responsiveness to TKIs has been limited within the relevant literature, particularly for certain rare mutations, including p.S768I. The current study reports the case of a patient with NSCLC harboring a p.S768I mutation in the *EGFR* gene [a substitution at codon 768 of exon 20 (c.2303G>T, p.S768I)], as well as a mutation at codon 719, exon 18 (p.G719A). The relevant literature concerning this rare *EGFR* somatic mutation is also reviewed.

Introduction

Lung cancer is the most common cause of cancer-associated mortality in a number of developed countries (1), and non-small cell lung cancer (NSCLC) is the most prevalent

form of lung cancer worldwide, accounting for 85% of all lung cancer cases (2,3). Epidermal growth factor receptor (EGFR) may play a significant role in NSCLC, and is thus a potential molecular target for personalized therapy with tyrosine kinase inhibitors (TKIs) (4).

Somatic activating *EGFR* mutations, which are clustered within the tyrosine kinase domain, most commonly occur in the form of deletions in exon 19 or p.L858R mutations in exon 21. These somatic activating mutations account for ~85% of all *EGFR* mutations, and may indicate the likely sensitivity of tumors to the effects of small-molecule inhibitors (such as gefitinib and erlotinib) (4-6). Other, less prevalent *EGFR* mutations, including exon 18 p.G719X mutations (3% of all *EGFR* mutations) (7) and exon 21 p.L861Q (2% of all *EGFR* mutations) have been associated with enhanced efficacy of EGFR-TKIs (8). By contrast, alternative classes of *EGFR* mutations may be associated with a lack of response to TKIs, and this is the case for the majority of exon 20 mutations, which account for ~5% of all *EGFR* mutations (9).

EGFR exon 20 mutations occur in patients with clinico-pathological features similar to those of patients with classical *EGFR* mutations (women, non-smokers, adenocarcinomas). Exon 20 mutations encompass the area surrounding amino acid positions Glu762 to Cys775, located in the N-lobe of the kinase domain of EGFR following the C-helix. These mutations induce a pattern of *in vitro* and *in vivo* resistance to EGFR-TKIs (9). A number of mutations in *EGFR* exon 20 are thought to increase the affinity of EGFR for adenosine triphosphate (ATP), thus decreasing the efficacy of TKI inhibition (10). However, due to the comparative rarity of *EGFR* exon 20 mutations, clinical data concerning the association between *EGFR* exon 20 mutations and responsiveness to TKIs has, to the best of our knowledge, been limited so far within the relevant literature, particularly for certain rare mutations, including p.S768I.

The present study reports the case of a patient with NSCLC exhibiting p.S768I in the *EGFR* gene [a substitution at codon 768 of exon 20 (c.2303G>T, p.S768I)], as well as a

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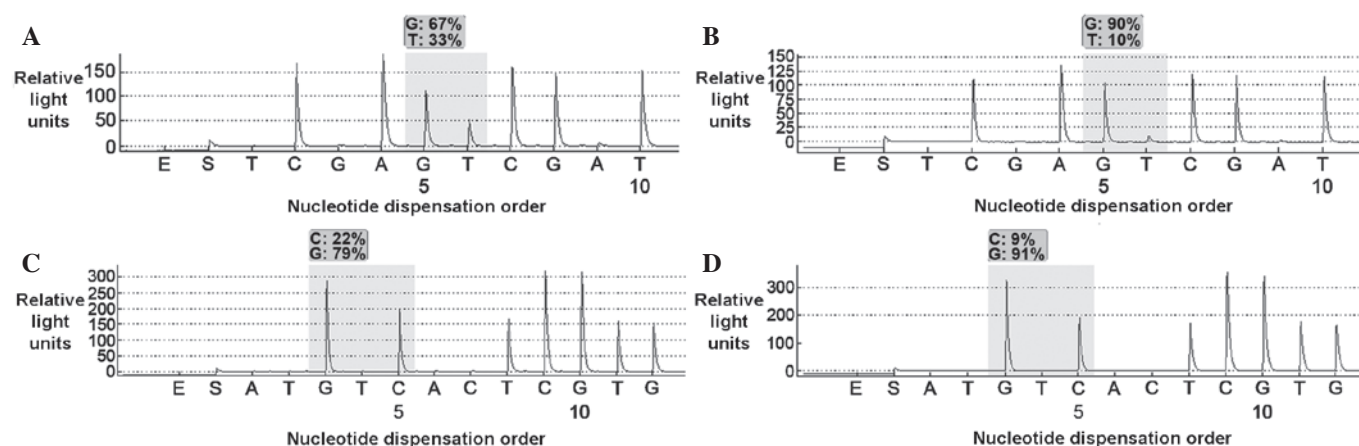


Figure 1. Pyrogram traces obtained following histological and cytological analyses of samples. (A) Pyrogram trace obtained following analysis of the histological sample from the metastatic supraclavicular lymph node, revealing an AGC>ATC mutation (p.S768I) in the second base of codon 768 of *EGFR* exon 20 (allele frequency, 33%). (B) Pyrogram trace obtained following analysis of the cytological sample from pericardial effusion, revealing an AGC>ATC mutation (p.S768I) in the second base of codon 768 of *EGFR* exon 20 (allele frequency, 10%). (C) Pyrogram trace obtained following analysis of the histological sample from the metastatic supraclavicular lymph node, revealing a GGC>GCC mutation (p.G719A) in the second base of codon 719 of *EGFR* exon 18 (allele frequency, 22%). (D) Pyrogram trace obtained following analysis of the cytological sample from pericardial effusion, revealing a GGC>GCC mutation (p.G719A) in the second base of codon 719 of *EGFR* exon 18 (allele frequency, 9%). *EGFR*, epidermal growth factor receptor.

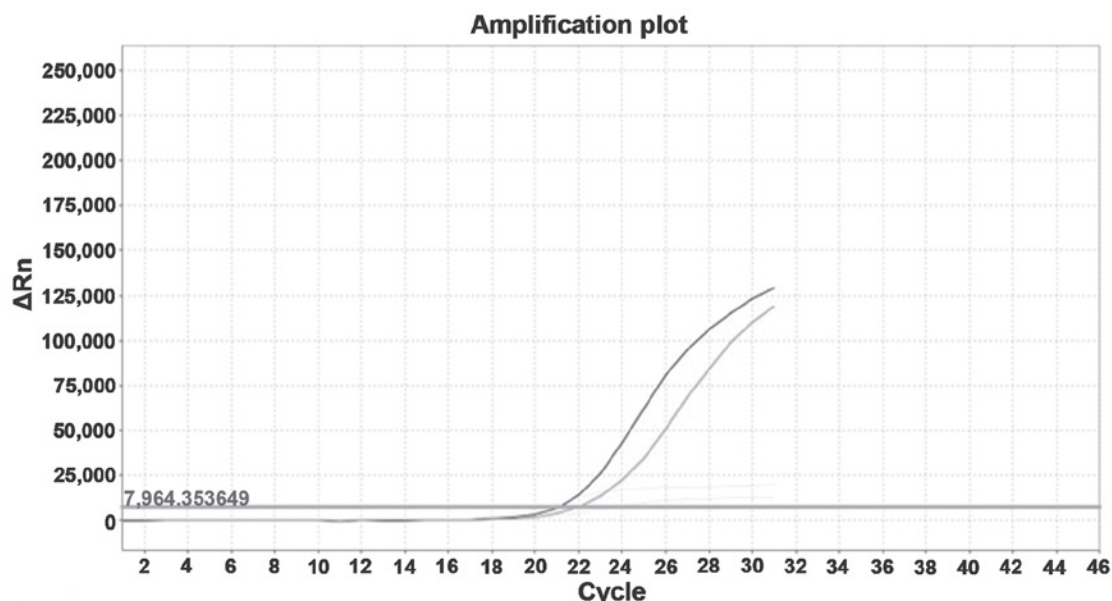


Figure 2. Magnitude of normalized fluorescence signal generated by the reporter at each cycle during polymerase chain reaction amplification of the histological sample from the metastatic supraclavicular lymph node. The ADX-amplification refractory mutation system was used to reveal synchronous mutations at codon 719 of exon 18 (p.G719A) and at codon 768 of exon 20 (p.S768I). ΔRn , baseline-corrected normalized reporter.

mutation at codon 719, exon 18 (p.G719A), in combination with a review of the relevant literature regarding this rare *EGFR* somatic mutation.

Case report

A 48-year-old Asian male was admitted to Cannizzaro Hospital (Catania, Italy) in March 2014, presenting with a poor performance status (PS) and increasing dyspnea. A total body computed tomography (CT) scan revealed a neoformation at the base of the left lung, measuring ~4 cm and extending to the visceral pleura. Furthermore, additional secondary nodules in both lungs, along with pericardial effusion, were identified.

Mediastinal lymphadenopathy and liver metastases were detected. The patient underwent a CT-guided biopsy of the left basal pulmonary lesion, which exhibited the typical histology of an adenocarcinoma, according to well-established World Health Organization criteria (11). The neoplasia consisted of neoplastic glands with focal papillary structures. Immunoreactivity for thyroid transcription factor-1 and napsin A, and negativity for thyroglobulin supported the pulmonary origin of the lesion.

Written informed consent was obtained from the patient for sequencing of the *EGFR* gene and for publication of the case report. The PyroMark Q24 system (Qiagen GmbH, Hilden, Germany) was utilized for pyrosequencing analysis of *EGFR* exons 18-21, using 2.5- μ m sections of formalin

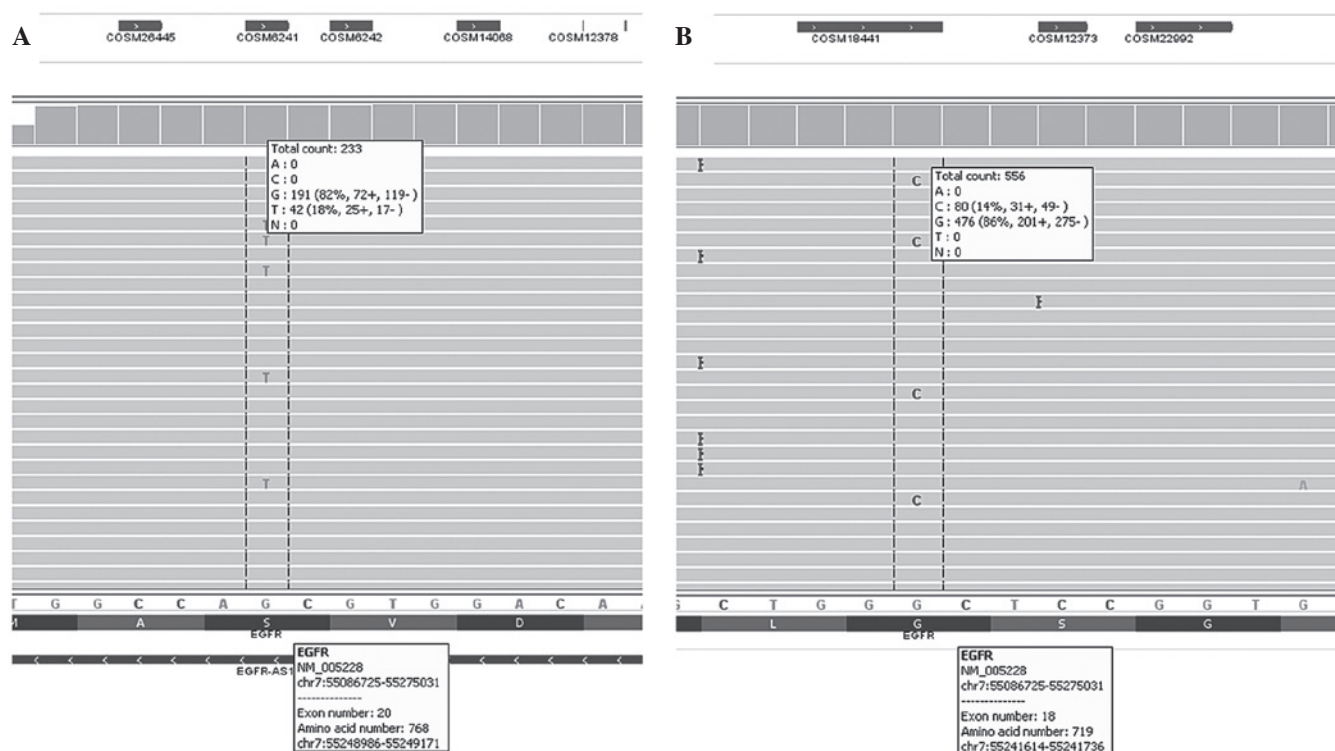


Figure 3. Next-generation sequencing trace obtained using an Ion Torrent™ approach, revealing the coexistence of (A) p.S768I and (B) p.G719A mutations, in epidermal growth factor receptor exons 20 and 18, respectively.

fixed paraffin-embedded tissue from metastatic supraclavicular lymph nodes (whole slide) and thinPrep cytological samples from pericardial effusion. All slides underwent genomic DNA extraction, using QIAamp MinElute spin columns (Qiagen GmbH), according to the manufacturer's instructions, and the sequence of interest was amplified by polymerase chain reaction (PCR; Applied Biosystems GeneAmp® PCR System 9700; Thermo Fisher Scientific, Inc., Foster City, CA, USA). Using a therascreen EGFR Pyro kit (Qiagen GmbH), all hotspot regions (4) of exons of the *EGFR* gene were analyzed, and PyroMark Q24 software (Qiagen, GmbH) was utilized for data analysis.

Pyrosequencing analysis of the full exome of *EGFR* from each sample type, revealed the presence of a rare mutation at codon 768, exon 20 (p.S768I; Fig. 1A and B), as well as a mutation at codon 719, exon 18 (p.G719A; Fig. 1C and D).

Patient DNA was subsequently retested for the presence of a p.S768I mutation in exon 20 of the *EGFR* gene, and its association with a mutation at codon 719, exon 18 (p.G719A). Molecular results were confirmed using the AmoyDx EGFR Mutation Test kit (Amoy Diagnostics Co. Ltd., Xiamen, China) for the detection of somatic mutations in the *EGFR* gene, using the principle of amplified refractory mutation. The assay was performed according to the manufacturer's instructions, using the Applied Biosystems StepOnePlus™ Real-Time PCR system (Thermo Fisher Scientific, Inc.; Fig. 2).

Patient DNA was additionally analyzed using next generation sequencing on an Ion Torrent™ with Applied Biosystems Colon-Lung v2 panel (Thermo Fisher Scientific, Inc.). This analysis identified two mutations: *EGFR* (c.2303G>T, p.S768I) with a target coverage depth of 233 in exon 20, and *EGFR*

(c.2156G>C, p.G719A) in exon 18 with a target coverage depth of 556 (Fig. 3).

Due to conflicting data in the existing literature regarding the effectiveness of EGFR-TKIs in the presence of a p.S768I mutation, and due to the poor PS of the patient, a decision was reached to administer the patient with supportive care only. The patient succumbed to the disease 6 weeks subsequent to diagnosis.

Discussion

EGFR mutations are considered to be a robust predictive biomarker of clinical response to EGFR-TKIs in clinical practice (4). Gefitinib, an EGFR-targeting agent, is an orally active small molecule drug, which has been demonstrated to exhibit antitumor activity in NSCLC. The response of NSCLC to gefitinib has been closely associated with *EGFR* mutations in the kinase domain (4,5); Lynch *et al* (4) suggested that repositioning of critical residues due to such mutations may act to stabilize their interaction with ATP and with gefitinib (its competitive inhibitor), and gefitinib-induced inhibition may thus be enhanced by certain mutations. However, as *EGFR* mutations may occur at varying positions within the kinase domain, the biochemical properties of these mutations and the sensitivity to gefitinib of tumors possessing rare mutations may not be identical (4). Therefore, the association between *EGFR* mutations and sensitivity to EGFR-TKIs in NSCLCs remains controversial, particularly for rare mutations (4-6,7,12-17).

The p.S768I mutation in exon 20 of the *EGFR* gene is a rare mutation that has been identified sporadically in previous studies and is reported to confer reduced sensitivity to gefitinib *in vitro* compared with the two most commonly

Table I. Summary of review of the literature concerning the p.S768I mutation, as well as its associated mutations at alternative *EGFR* codons.

Author (reference no.)	Year	Nationality	Patients, n	<i>EGFR</i> mutants, n	p.S768I mutants, n	Reported mutations	RECIST
Huang <i>et al</i> (13)	2004	Taiwanese	101	39	1	S768I+G719C	
					1	S768I+G719S	
Kosaka <i>et al</i> (12)	2004	Japanese	277	111	1	S768I+V769L	
Shigematsu <i>et al</i> (7)	2005	Japanese, Taiwanese, Australian	617	134	1	S768I+G719S	
Takano <i>et al</i> (31)	2005	Japanese	66	43	1	S768I+L858R	
					1	S768I+G719C	
Asahina <i>et al</i> (27)	2006	Japanese	1	1	1	S768I+V769L	PD
Pugh <i>et al</i> (23)	2007	Japanese	349	102	2	S768I	
					1	S768I+V769L	
					1	S768I+V774M	
		Asian	39	8	1	S768I	PR
Wu <i>et al</i> (20)	2008	Taiwanese	515	253	1	S768I+G719A	PD
					1	S768I+L858R	PR
Masago <i>et al</i> (25)	2010	Japanese	1	1	1	S768I	PR
Wu <i>et al</i> (8)	2011	Taiwanese	1,261	627	2	S768I+L858R	PR
Szumera-Ciećkiewicz <i>et al</i> (26)	2013	Polish	273	29	1	S768I	
Kobayashi <i>et al</i> (29)	2013	Japanese	79	11	2	S768I+G719A	PR
					1	S768I+V769L	PR
Weber <i>et al</i> (24)	2014	Danish	462	57	1	S768I	PD
Pallan <i>et al</i> (28)	2014	British	2	2	2	S768I	PD

EGFR, epidermal growth factor receptor; RECIST, Response Evaluation Criteria In Solid Tumors (37); PR, partial response; PD, progressive disease.

observed types of mutations: Exon 19 deletions and p.L858R mutations (18,19). Due to the relative rarity of *EGFR* exon 20 mutations, clinical data concerning their associations with drug responsiveness are limited, and conflicting data exist regarding the sensitivity to EGFR-TKIs of tumors harboring p.S768I mutations (20,21). The literature review conducted for the present report revealed a limited number of cases involving p.S768I mutations (Table I), and conflicting data with regard to its clinical association with EGFR-TKI efficacy. A notable observation, which was confirmed by the results of the present study, is the association between p.S768I in exon 20 and other *EGFR* mutations, identified frequently in exon 18 and 21 (22). The significance of this molecular/mutational association remains to be elucidated, and may require further investigation. In previous studies where this molecular/mutational association was not observed (23-26), there may have been a lack of utilization of sensitive detection techniques such as next-generation sequencing approaches.

As shown in Table I, Asahina *et al* (27) reported that p.S768I and p.V769L mutations were associated with insensitivity to EGFR-TKIs in the patient cohort investigated. In a Danish patient cohort investigated by Weber *et al* (24), one patient possessed a p.S768I point mutation in exon 20. This

patient exhibited no response to treatment with the EGFR-TKI erlotinib, and succumbed to progressive disease 4 weeks subsequent to the start of treatment. An additional case concerning a Taiwanese patient with progressive disease and harboring two distinct mutations (p.S768I and p.G719A), was identified by Wu *et al* (20), and an a further two cases were reported by Pallan *et al* (28). By contrast, a positive clinical response to gefitinib in an NSCLC patient harboring the rare mutation p.S768I was observed by Masago *et al* (25). Additional previous studies have also reported partial responses to EGFR-TKIs in patients exhibiting p.S768I and other mutations (8,20,23,29,30). In addition, a number of retrospective analyses of *EGFR* mutations (Table I) have investigated the p.S768I mutation; however, the clinical responsiveness to EGFR-TKIs has not been reported (7,12,13,26,31).

In certain *in vitro* studies, a number of mutations have been shown to exhibit distinctive phosphorylation patterns in several C-terminal tyrosine (Tyr) residues of the *EGFR* gene, and have demonstrated varying sensitivities to gefitinib when stably transfected into NSCLC cell lines (19,32). A number of these mutants, including p.S768I, are hyperphosphorylated on the Tyr 1045 residue, which is normally involved in the recruitment of Casitas B-lineage lymphoma

(Cbl) to EGFR and the initiation of Cbl-mediated receptor multi-ubiquitination; mutations at this site are refractory to EGF-induced ubiquitination and degradation (33-35). Gefitinib treatment exerts reduced growth-suppressive effects on cells expressing exon 20 mutations compared with cells expressing exon 19 deletions or L858R mutations, or those expressing the wild-type counterpart (19).

Kancha *et al* (18) identified four sets of *EGFR* mutations based on their drug sensitivity profiles *in vitro*: i) mutations sensitive to all three drugs investigated (gefitinib, erlotinib and AEE788) with half maximal inhibitory concentration (IC_{50}) values in the low nanomolar range (L858R and Del 747-753 insS mutations); ii) mutations exhibiting reduced sensitivity to gefitinib ($IC_{50} > 100$ nmol/l), but sensitivity ($IC_{50} < 100$ nmol/l) to both erlotinib and AEE788 (G719S, V742A and R776C mutations); iii) mutations exhibiting reduced sensitivity to both gefitinib and erlotinib, but sensitivity to AEE788 (D761N, S768I, S748F, L838V and L861Q mutations); and iv) mutations resistant to all three drugs investigated (N826S and T790M mutations).

However, despite the *in vitro* results reported by Kancha *et al* (18), data regarding the clinical significance of all *EGFR* mutations in the literature are unavailable at present. This includes p.S768I and other relatively rare mutations, whose association with EGFR-TKIs remains to be elucidated. Although a number of mutations in exons 18-21 have been identified to be associated with EGFR-TKI resistance, only p.T790M is known for its clinical significance to primary TKI drug resistance. This resistance is caused by a conformational change in the ATP-binding pocket, which increases the affinity of EGFR for its natural substrate, and reduces its affinity for EGFR-TKIs (7,36).

Kancha *et al* (18) categorized p.S768I in exon 20 as a mutation that confers reduced sensitivity to the *in vitro* activity of gefitinib. The relevant literature indicates that this type of mutation is rare, and is associated with insensitivity to EGFR-TKIs *in vitro* and *in vivo*, as previously described by Asahina *et al* (27). However, conflicting results have also been reported regarding the *in vivo* sensitivity of p.S768I mutants to TKIs; Masago *et al* (25), for example, reported a case of a patient with NSCLC harboring the p.S768I mutation who demonstrated a good clinical response to gefitinib.

The present study reported a case of NSCLC harboring a rare *EGFR* somatic mutation, along with the conflicting data from the literature regarding the clinical significance of this mutation. *In vitro* results reported by Kancha *et al* (18) do not consider the 'impact and the influence' of the tumor microenvironment; it is not necessarily notable that the sensitivity to certain drugs *in vitro* differs from that observed *in vivo*. Thus, it may be speculated that the p.S768I mutation is drug sensitive.

In conclusion, further examination of the sensitivity of EGFR-TKIs in a more representative cohort of NSCLC patients harboring a range of rare mutations may be required in order to optimize the individual treatment of patients with such mutations.

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