**Case Reports**

**Good Clinical Response to Gefitinib in a Non-small Cell Lung Cancer Patient Harboring a Rare Somatic Epidermal Growth Factor Gene Point Mutation; Codon 768 AGC > ATC in Exon 20 (S768I)**

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Recently, two small-molecule kinase inhibitors targeting epidermal growth factor receptor have proven effective in the treatment of non-small cell lung cancer. There are specific activating mutations within the tyrosine kinase domain of epidermal growth factor receptor related to the sensitivity of tyrosine kinase inhibitors. However, it is unknown whether rare mutations in the N-lobe (exons 18–20) and the C-lobe (exon 21) of the epidermal growth factor receptor kinase domain other than L858R in exon 21 and the in-frame deletion in exon 19 may predict the effectiveness of epidermal growth factor receptor—tyrosine kinase inhibitors. We reported a case of non-small cell lung cancer harboring a rare epidermal growth factor somatic mutation, codon 768 AGC > ATC in exon 20 (S768I), who showed a good clinical response to gefitinib. Therefore, we may suggest that this rare mutation (S768I in exon 20) may not be an insensitive epidermal growth factor receptor somatic mutation in vivo.

**Key words:** epidermal growth factor receptor — tyrosine kinase inhibitor — non-small-cell lung cancer

**BACKGROUND**

Lung cancer is a major cause of cancer-related mortality worldwide and is expected to remain a major health problem for the foreseeable future. Chemotherapy is the cornerstone for the management of the disease; however, therapeutic impact on patient survival has been modest. Recent discovery have provided greater understanding of the molecular basis of the disease and the success of the two small-molecule kinase inhibitors (TKIs), gefitinib (Iressa®, AstraZeneca, Stanhope gate City, London, UK) and erlotinib (Tarceva®, OSI Pharmaceuticals, Melville City, NY, USA), against epidermal growth factor receptor (EGFR) in the treatment of non-small cell lung cancer (NSCLC) has provided evidence for the effectiveness of the strategy.

Many reports have indicated that EGFR–TKIs have significant efficacy in specific sub-groups of patients, such as the Asian population, patients with adenocarcinomas, non-smokers and females (1–3), and have indicated that the presence of somatic mutations in the EGFR is a strong predictor for both clinical and *in vitro* sensitivity to EGFR–TKIs (2–18).

Since Lynch et al. have identified specific activating mutations within the tyrosine kinase domain of EGFR (12), the missense mutation L858R in exon 21 and the in-frame deletion in exon 19, nested around the amino acid residues 747–750 of the EGFR polypeptide, account for >85%; of all clinically important mutations related to TKI sensitivity (2). Other recurrent but far less common EGFR mutations
known to be associated with sensitivity to EGFR–TKIs include the G719 mutations in exon 18 and the L861
mutations in exon 21. Making use of the association between EGFR mutations and clinical response to TKIs, the detection of EGFR mutations in tumor tissues has been applied for predicting the response of TKI treatment and hence guiding the treatment for advanced NSCLC.

A panel of 30 EGFR kinase domain mutations that were recently reported in NSCLC patients was cloned and expressed for analysis of kinase activity, transforming potential and drug sensitivity. These mutations affect the N-lobe (exons 18–20) and the C-lobe (exon 21) of the EGFR kinase domain (19); however, it is not uncertain whether rare mutation in the N-lobe and the C-lobe of the EGFR kinase domain other than L858R in exon 21 and the in-frame deletion in exon 19 may be a predictor of effectiveness with EGFR–TKIs.

We report, for the first time, a good clinical response with gefitinib of NSCLC harboring rare EGFR smatic mutation; codon 768 point mutation AGC > ATC (S768I).

CASE REPORT

The patient was an 81-year-old Japanese man with an existing smoking history of 37 pack-years. He suffered from prolonged left back pain on our first visit. Evaluation at another facility revealed two small nodules in the right lung, two small nodules in the left lung, measuring < 2 cm in diameter and left pleural effusion. Pathologic examination of specimens obtained by trans-bronchial biopsy from the left lung mass revealed adenocarcinoma (Fig. 1a–d) and the disease stage is cT1N0M1b. For choosing therapeutic strategy, we investigated EGFR gene mutation status of trans-bronchial biopsy specimen with direct sequencing method and detected S768I in exon 20; codon 768 point mutation AGC > ATC (S768I).

Cancer cells were obtained from paraffin-embedded biopsy specimens by manual micro-dissection. The definition of small specimen is that the quantity of the specimen is sufficient to make a pathologic diagnosis in other words, about several hundreds at maximum of tumor cells is necessary. Formalin-fixed paraffin-embedded tissue was cut in 6–8 μm sections, and mounted on pre-treated glass slides. Non-cancer cells and necrotic parts were manually removed from the slide under the microscope. The slides were de-paraffinized, then DNA was extracted with phenol–chloroform and ethanol precipitation. Exons 18, 19 and 21 were amplified by a polymerase chain reaction (PCR). Exons 18, 19 and 21 were selected for examination because most of the reported mutations known to relate to the effect of EGFR–TKIs in NSCLC have been described in these loci. Primers and cycling conditions for PCR amplification were performed by a modification of previously published methods (12,14). Sequencing reactions were electrophoresed on the ABI PRISM 3100 (Applied Biosystems, Foster City,

Figure 1. High-power magnification of a tumor specimen of the left lung nodule shows adenocarcinoma (a) positive for TTF-1 (100%) (b), Ki-67 index 15% (c), p53 partially positive (50%) (d).
Direct sequencing of the PCR products was performed in both sense and antisense directions.

On the basis of the results of molecular analysis (Fig. 2), because the result of EGFR gene mutation was not TKI-sensitive mutation and poor performance status (PS3), the patient received vinorelbine 25 mg/m² intravenously on Day 1 of a 3-week cycle, other than platinum doublet regimens, docetaxel monotherapy or EGFR–TKIs. Both the multiple smaller pulmonary lesions and left pleural effusion showed stable disease, as assessed by response evaluation criteria in solid tumors (RECIST) (20). He has remained on medication vinorelbine up to six course with stable disease.

Four months later since last chemotherapy, the left dominant pulmonary lesion increased in size from 20 to 39 mm and other pulmonary nodules revealed and he received the oral EGFR-tyrosine kinase inhibitor, gefitinib as the second line treatment. The rapidly growing left solitary lesion decreased in size from 39 to 19 mm and the multiple smaller pulmonary lesions disappeared which showed partial response, as assessed RECIST (Fig. 3a and b). Grade 2 dermatitis occurred in the course of gefitinib treatment which was not aggravated in steroid external use. He has remained now on gefitinib medication and has been without evidence of disease progression for 461 days.

**DISCUSSION**

In this case, we selected vinorelbine monotherapy for the first-line treatment because the result of EGFR gene mutation was not TKI-sensitive mutation such as L858R in exon 21 and the in-frame deletion in exon 19. He has remained on medication vinorelbine up to six course with stable disease; however, according to disease progression, he received gefitinib as the second-line treatment for his poor PS. Surprisingly, both the rapidly growing left solitary lesion and the multiple smaller pulmonary lesions showed partial response and he has remained on gefitinib medication and has been without evidence of disease progression for more than 1 year.

Kancha et al. have identified four sets of mutations based on their drug sensitivity profiles: (i) mutations that are very sensitive to all three drugs tested with IC50 values in the...
low nanomolar range (L858R and Del 747–753insS), (ii) mutations that are less sensitive to gefitinib (IC$_{50}$ > 100 nmol/L) but sensitive to both erlotinib and AEE788 (G719S, V742A and R776C; IC$_{50}$ < 100 nmol/L), (iii) mutations that are less sensitive to both gefitinib and erlotinib but sensitive to AEE788 (D761N, S768I, S847F, L838V and L861Q) and (iv) mutations that are resistant to all three drugs tested (N826S and T790M) (21). S768I in exon 20 categorized to mutations that are less sensitive to gefitinib in vitro activity. This type of mutation has been reported as very rare case and also this mutation has been associated with insensitive to EGFR TKI inhibitor in vitro and in vivo. Asahina et al. reported that S768I and V769L mutations are associated with insensitive to EGFR–TKI in the patient sample (22).

However, in vitro results reported by Kancha et al. (21) dose not consider ‘oncogene addiction’ even though this system showed growth factor-independent growth. Thus, it can be possible that S768I mutation is drug sensitive. Generally, it is not surprising that the sensitivity to some agent in vitro differ from that in vivo. For example, L861Q, categorized as less sensitive to gefitinib and erlotinib in Kancha’s study (21), has been considered as ‘sensitive mutation’ from the early days according to clinical results of NSCLC patients (23). Therefore, it is important to examine the sensitivity of EGFR–TKI in patients with NSCLC harboring such rare mutations.

Limitations of our case study include it is hard to know whether the cancer clone remained the same just before the gefitinib treatment and it is not clear that this type of mutation is directly associated with the EGFR sensitivity. However, in our case, a good clinical response was shown, which may be the reason not to quit EGFR–TKI therapy against previous untreated NSCLC cases harboring insensitive EGFR gene mutation.

In this report, we showed the NSCLC case harboring EGFR rare somatic mutation who showed a good clinical response to gefitinib for the first time. We may suggest this rare mutation (S768I in exon 20) may not be an insensitive EGFR somatic mutation in vivo, regardless of the previous reported result of in vitro sensitivity (19).

Conflict of interest statement

None declared.

References

