Factors associated with a poor response to gefitinib in the NEJ002 study: Smoking and the L858R mutation

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A B S T R A C T
Introduction: Epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) treatment is the standard therapy for non-small cell lung cancer (NSCLC) harbouring EGFR-activating mutations. The NEJ002 phase 3 clinical trial demonstrated the efficacy of EGFR-TKI: gefitinib was significantly superior in both progression-free survival (PFS) and objective response rate (ORR) than carboplatin plus paclitaxel. However, several cases showed no response. In this study, we performed further analysis of the characteristics of these non-responders.

Methods: Available data from NEJ002 on maximum changes in tumour size were obtained from 103 cases (90.4%) and 110 cases (96.5%) in the carboplatin–paclitaxel and gefitinib groups, respectively. Waterfall plots of maximum tumour size changes were created for non-responders.

Results: Five (4.9%) and 9 (8.2%) cases in the carboplatin–paclitaxel and gefitinib groups were non-responders, respectively. The mean pack years of the non-responders in the carboplatin–paclitaxel and gefitinib groups were 0.33 and 31.7, respectively. The ORR of total smokers (61.5%) and heavy smokers (over 40 pack years, 52.6%) in the gefitinib group were significantly lower compared to people who have never smoked (80.0%) (P=0.044 and P=0.020, respectively). Smoker cases also showed a tendency towards lower PFS and overall survival (OS). In addition, the EGFR common mutation types did not affect PFS and OS in gefitinib-treated cases in NEJ002. However, in this study, the ORR and waterfall plots showed that gefitinib-treated non-responders who had a deletion in exon 19 in the EGFR gene exhibited a tendency towards a higher response compared to those with a L858R mutation.

Conclusions: NSCLC patients with a smoking history or the EGFR L858R mutation may demonstrate a poorer response to gefitinib treatment.

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1. Introduction

Lung cancer is the leading cause of cancer death worldwide. Most lung cancer patients are diagnosed in the advanced stages of the disease; thus, despite a significant improvement in the treatment for this malignancy, the prognosis remains poor [1]. Recent studies have demonstrated driver gene mutations, which promote the development of lung cancer [2]. In 2004, epidermal growth factor receptor (EGFR)-activating mutations were discovered in lung cancer by two different groups [3,4]. Subsequently, EGFR-TKI treatment was established as the standard treatment for lung cancer harbouring EGFR mutations based on the results of pivotal trials [5,6].

Currently, the clinically available EGFR-TKIs are gefitinib, erlotinib, and afatinib. In Japan, the North East Japan Study Group (NEJ) demonstrated the efficacy of gefitinib treatment [6]. This study revealed significantly higher objective response rates (ORR) and longer progression-free survival (PFS) of patients with gefitinib treatment compared to patients treated with carboplatin plus paclitaxel, which is the standard cytotoxic chemotherapy (73.7%, 10.8 months vs. 30.7%, 5.4 months, respectively) [6,7]. Although there was no difference in overall survival (OS) (27.7 months for the gefitinib group vs. 26.6 months for the carboplatin plus paclitaxel group), this was assumed to be due to a high crossover rate because gefitinib was administered as a second-line therapy to most patients who received unsuccessful first-line chemotherapy [7]. Smoking history and type of EGFR common mutations (exon 19 deletion or L858R point mutation) did not affect the OS of each treatment group [7].

Gefitinib treatment for EGFR mutation-positive lung cancer demonstrated a significantly higher ORR; however, we observed several cases that showed a poor treatment response. Using data collected from the pivotal NEJ002 study, we analysed the characteristics of these poor response cases or non-responders.

2. Methods

2.1. Patient population

This was a retrospective analysis of clinical data obtained from 230 patients from the NEJ002 study. The eligibility criteria were previously described in the NEJ002 study [6]. Briefly, the criteria included the presence of advanced non-small cell lung cancer (NSCLC) harbouring sensitive EGFR mutations, the absence of the resistant EGFR mutation T790M (in which threonine at amino acid 790 was substituted by methionine), no history of chemotherapy, and an age of 75 years or younger. From March 2006 to May 2009, 230 patients were enrolled in the NEJ002 study.

2.2. Study design and treatment

After the exclusion of 2 patients, gefitinib was administered to 114 patients, and the other 114 patients were allocated to receive carboplatin plus paclitaxel. Prior to randomisation, patients were stratified according to sex, clinical stage of NSCLC (IIIB, IV, or postoperative relapse), and institution. Eligible patients were randomly assigned to receive gefitinib (at a dose of 250 mg per day orally) or carboplatin (at a dose equivalent to an area under the concentration–time curve of 6) plus paclitaxel (at a dose of 200 mg per square metre of body surface area). Gefitinib was administered until disease progression, development of intolerable toxic effects, or withdrawal of consent. Carboplatin plus paclitaxel were both administered on the first day of every 3-week cycle for at least three cycles. Retrospective analysis was performed using the currently available data. The available data on maximum changes in the tumour target lesion size from baseline were evaluated in 103 patients (90.4%) and 110 patients (96.5%) in the carboplatin plus paclitaxel and gefitinib groups, respectively. Seven patients in the carboplatin plus paclitaxel group and 1 patient in the gefitinib group could not be evaluated for treatment response [6]. The remaining 4 patients in the carboplatin plus paclitaxel group and 3 patients in the gefitinib group showed that the tumour progression after each treatment made the tumour-target-lesion immeasurable. Progression of atelectasis or increased pleural effusion occurred in most of the cases.

2.3. Clinical assessments

An assessment of the maximum changes in tumour size was performed using data for the evaluation of ORR with computed tomography (CT) every 2 months. Unidirectional measurements were adopted on the basis of the Response Evaluation Criteria in Solid Tumours (RECIST, version 1.0). We defined a non-responder as a patient whose tumour-target-lesion size showed no change or increased despite the administration of each treatment during complete first-line treatment. Treatment response and PFS were determined by an external review of the CT scans by experts who were blinded to the treatment assignments. OS was evaluated for the period from the date of randomisation to the date of death.

2.4. Statistical analysis

The smoking pack years between two the groups were compared using the Wilcoxon rank sum test. The ORR was compared using Fisher’s exact test. Kaplan–Meier survival curves were drawn for PFS and OS and were compared using the log-rank test. Each analysis was performed using a two-sided, 5% significance level and a 95% confidence interval using SAS for Windows software (release 9.1.3, SAS Institute, Cary, NC).

3. Results

3.1. Fourteen cases showed no response to either treatment

Waterfall plots showing maximum changes in the tumour target lesion size from baseline are indicated in Fig. 1A (lower). As previously demonstrated in the NEJ002 study, in which gefitinib treatment showed a higher response rate than carboplatin–paclitaxel treatment, the gefitinib group had more cases that showed a partial and complete response to the treatment compared to the carboplatin–paclitaxel group. However, 5 patients (4.9%) in the carboplatin–paclitaxel group and 9 patients (8.2%) in the gefitinib group showed no response and instead experienced no decrease in tumour size or an increased tumour size (Table 1). We analysed the characteristics of these non-responder cases for specific predictive factors of response to treatment.

3.2. Non-responders to gefitinib treatment showed a tendency towards higher smoking pack years than the carboplatin plus paclitaxel group

The number of smoking pack years of each case is indicated in Fig. 1A (upper). When only non-responders were evaluated, those in the gefitinib treatment group showed a tendency towards higher smoking pack years. The mean pack years of cigarette smoking of the non-responders in the carboplatin plus paclitaxel and gefitinib groups were 0.3 and 31.7, respectively ($P = 0.164$, Fig. 1B).

Among the 9 non-responders of the gefitinib treatment group, 4 of the subjects were never smokers (Table 1). Case GC-007 showed a long duration of stable disease, which indicated the partial efficacy of gefitinib. Case GC-054 had an exon 18 minor mutation in
the EGFR gene. Our group previously published data on the poor treatment response to gefitinib in patients with minor mutations [8]. Both case GC-194 and case GC-063 discontinued gefitinib treatment due to serious adverse event, including drug-induced lung disease and liver dysfunction, respectively. In contrast, among the remaining 5 patients who had a smoking history, only 1 patient had an exon 18 minor mutation, and the other patients had no episodes of serious adverse events. In the carboplatin plus paclitaxel group, only 1 patient ceased the first-line treatment due to the onset of ileus, and the remaining non-responders did not show any specific clinical characteristics.

3.3. PFS and OS of the gefitinib-treated smoker group showed a tendency towards poor prognosis

The ORR of the gefitinib group was 73.7% [6]. When divided into 2 groups by smoking history, the ORR of the smoker group was significantly lower than the never smoker group (61.5% vs. 80.0%, P=0.044, Table 2). Moreover, the ORR of the heavy smoker group (over 40 pack years) was 52.6% and significantly lower than the non-smoker group (P=0.020).

Kaplan–Meier curves of the PFS and OS are shown in Fig. 2A and B. Although not statistically significant, the smoker cases showed a tendency towards lower PFS and OS compared to the non-smoker cases (P=0.074 and P=0.164, respectively).

3.4. NSCLC patients with the EGFR L858R mutation showed a relatively poor response to gefitinib compared to patients with an exon 19 deletion mutation

Although we previously reported the PFS and OS of the gefitinib treated exon 19 deletion mutant group did not show any difference compared to the L858R mutation group [6,7], the types of EGFR mutations may also be an important predictive factor of the treatment response, as shown in Table 1, which depicts the non-responders’ EGFR mutation status. Namely, three non-responders (GC-007, 011, 194) with gefitinib treatment, who showed increases of over 20% in tumour growth from baseline, had a L858R mutation. A comparison of the patients based on EGFR common activating mutations, L858R and exon 19 deletion, revealed that ORR (Table 3) and the maximum tumour size change from baseline (Fig. 3A and B) in gefitinib-treated patients and indicated that the L858R mutation was worse than an exon 19 deletion mutation. In contrast, patients who received carboplatin plus paclitaxel did not show any differences.

Table 1

| Individual non-responders cases from NEJ002. Non-responder denotes patients who never had decrease in the size of measurable lesion during first-line treatment. |
|---|---|---|---|---|---|---|---|---|---|
| Case No. | Maximum change | Sex | Age | ECOG-PS | Histology | Stage | EGFR mutation | Smoking pack years | Response | Duration | OS (month) |
| Carbobplat + paclitaxel | | | | | | | | | | | |
| GC-068 | +9.7 | Female | 72 | 1 | AD | IV | Exon 19 deletion | 0 | PD | 0.9 | 43.7 |
| GC-176 | +2.7 | Female | 69 | 1 | AD | IV | Exon 19 deletion | 0 | SD | 1.6 | 25.6 |
| GC-001 | 0 | Female | 72 | 1 | AD | IV | G719S | 0 | SD | 1.9 | 9.8 |
| GC-077 | 0 | Male | 71 | 1 | AD | IV | Exon 19 deletion | 0 | SD | 1.7 | 16.4 |
| GC-220 | 0 | Male | 75 | 1 | AD | IV | Exon 19 deletion | 1.65 | NE | 0.8 | 20.6 |
| Gefitinib | | | | | | | | | | | |
| GC-007 | +33.3 | Female | 70 | 1 | AD | IIIB | L858R | 0 | SD | 22.0 | 53.6 |
| GC-011 | +32.1 | Male | 56 | 1 | AD | Relapse | L858R | 60 | PD | 2.3 | 21.9 |
| GC-194 | +22.2 | Female | 60 | 1 | AD | IV | L858R | 0 | PD | 1.1 | 1.7 |
| GC-054 | +21.1 | Male | 68 | 1 | AD | IV | G719C | 0 | PD | 1.9 | 11.8 |
| GC-158 | +8.8 | Male | 65 | 0 | AD | IV | Exon 19 deletion | 40 | SD | 2.3 | 27.6 |
| GC-183 | +7.8 | Male | 63 | 0 | AD | IIIB | Exon 18 | 86 | PD | 2.2 | 5.7 |
| GC-195 | +7.6 | Male | 51 | 1 | AD | IV | Exon 19 deletion | 62 | PD | 2.0 | 10.9 |
| GC-031 | +2.4 | Male | 64 | 1 | AD | IV | Exon 19 deletion | 37.5 | PD | 0.3 | 10.8 |
| GC-063 | 0 | Female | 67 | 0 | AD-SQC | IIIB | Exon 19 deletion | 0 | SD | 1.2 | 37.1 |

AD: adenocarcinoma; AD-SQC: adenosquamous carcinoma; PD: progressive disease; SD: stable disease; NE: not evaluated.

a Maximum change from baseline during the first-line treatment (%).

b Duration from entry to maximum size (month).
Table 2
Response of cases categorised by smoking history in the gefitinib treatment group.

<table>
<thead>
<tr>
<th>Gefitinib Treatment Group</th>
<th>Non Smoker</th>
<th>Smoker Total</th>
<th>Light Smoker</th>
<th>Heavy Smoker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Under 40 pack years</td>
<td>Over 40 pack years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39 (100.0%)</td>
<td>20 (100.0%)</td>
<td>19 (100.0%)</td>
</tr>
<tr>
<td>CR</td>
<td>5 (6.7%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>PR</td>
<td>55 (73.3%)</td>
<td>24 (61.5%)</td>
<td>14 (70.0%)</td>
<td>10 (52.6%)</td>
</tr>
<tr>
<td>SD</td>
<td>9 (12.0%)</td>
<td>9 (23.1%)</td>
<td>5 (25.0%)</td>
<td>4 (21.1%)</td>
</tr>
<tr>
<td>PD</td>
<td>6 (8.0%)</td>
<td>5 (12.8%)</td>
<td>1 (5.0%)</td>
<td>4 (21.1%)</td>
</tr>
<tr>
<td>NE</td>
<td>0 (0.0%)</td>
<td>1 (2.6%)</td>
<td>0 (0.0%)</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td>CR + PR</td>
<td>60 (80.0%)</td>
<td>24 (61.5%)</td>
<td>14 (70.0%)</td>
<td>10 (52.6%)</td>
</tr>
<tr>
<td>95% CI</td>
<td>(69.2%, 88.4%)</td>
<td>(44.6%, 76.6%)</td>
<td>(45.7%, 88.1%)</td>
<td>(28.9%, 75.6%)</td>
</tr>
</tbody>
</table>

Fisher’s exact test

P=0.044
P=0.020

CR: complete response; PR: partial response; PD: progressive disease; SD: stable disease; NE: not evaluated.

Fig. 2. The survival curves of non-smokers and smokers in the gefitinib treatment group of the NEJ002 study, as described by the Kaplan–Meier method and compared using the log-rank test. (A) PFS. (B) OS.

4. Discussion

For NSCLC cases with an EGFR mutation, gefitinib treatment increased both the ORR and PFS more than carboplatin plus paclitaxel treatment. Nevertheless, the number of non-responders to gefitinib treatment was also higher compared to patients treated with carboplatin plus paclitaxel, 9 (8.1%) vs. 5 (4.4%), respectively. Interestingly, non-responders of the gefitinib group, who had neither a serious adverse event nor minor EGFR mutations, had a smoking history. This result indicates that a smoking history may be an important predictive factor for gefitinib treatment. The type of EGFR-activating mutation may also be another predictive factor for the response to gefitinib treatment; NSCLC patients with a L858R mutation exhibited a poorer response to gefitinib compared to patients with an exon 19 deletion mutation.

Most of the EGFR-mutant patients who had a smoking history or L858R mutation showed a better response to gefitinib compared to carboplatin plus paclitaxel. However, the response rate was significantly lower, particularly in the heavy smoker group compared to the non-smoker group.

Several studies indicated that NSCLC patients harbouring EGFR mutations with many smoking pack years showed a relatively poor response to EGFR-TKI treatment [9–11]. Several mechanisms have been proposed to explain the poorer response to EGFR-TKI in patients with a smoking history. One group found that cigarette smoking induced EGFR posttranslational changes [12] and that the Src oncogene may confer resistance to treatment [13]. Another group demonstrated that activation of the nicotinic acetylcholine receptor by cigarette smoking induced EGFR-TKI resistance [14]. Furthermore, many chemicals contained in cigarette smoke have a high activity of mutagenesis [15]. Consistent with this finding, the rate of gene alteration in smoker patients with NSCLC harbouring EGFR mutations was considerably higher compared to non-smokers [16,17]. Moreover, lung cancer cells derived from lung
Recent studies have shown that certain mutations in the EGFR gene can drive tumour growth and be targeted by EGFR-TKIs. These mutations are often found in the L858R exon 19 deletion, which can be considered a "driver" mutation. However, other mutations, known as "passenger" mutations, can also be found in the same region.

To determine the efficacy of treatment, patients were categorised based on the type of EGFR mutation. The Table below shows the response of cases categorised by the types of EGFR common mutation.

<table>
<thead>
<tr>
<th>Gefitinib</th>
<th>Carboplatin+paclitaxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>L858R exon 19 deletion</td>
<td>L858R exon 19 deletion</td>
</tr>
<tr>
<td>Total</td>
<td>49 (100.0%)</td>
</tr>
<tr>
<td>CR</td>
<td>1 (2.0%)</td>
</tr>
<tr>
<td>PR</td>
<td>32 (65.3%)</td>
</tr>
<tr>
<td>SD</td>
<td>11 (22.4%)</td>
</tr>
<tr>
<td>PD</td>
<td>4 (8.2%)</td>
</tr>
<tr>
<td>NE</td>
<td>1 (2.0%)</td>
</tr>
<tr>
<td>CR + PR</td>
<td>33 (67.3%)</td>
</tr>
<tr>
<td>95%CI</td>
<td>(52.5%, 80.1%)</td>
</tr>
</tbody>
</table>

Fisher’s exact test

\[ P = 0.074 \quad P = 1.000 \]

of heavy smokers contained "driver" EGFR mutations and many other "passenger" gene mutations. These passenger genes may modify signal transduction pathways that render cell death more difficult to induce by treatment with EGFR-TKI alone.

Recently, treatment of other clinically available EGFR-TKIs, such as erlotinib and afatinib, in NSCLC patients with an exon 19 deletion showed a higher response than those with the L858R mutation [18,19]. However, for gefitinib phase 3 studies, the type of EGFR mutation did not affect PFS and OS [5–7]. In the present study, we found that gefitinib treatment also showed a tendency towards a favourable response in patients with an exon 19 deletion mutation based on an evaluation of short-term responses, such as ORR and the maximum change in tumour size from baseline. If the response of gefitinib treatment was affected by EGFR subtypes, then all three EGFR-TKIs demonstrated a higher treatment response in patients with an exon 19 deletion compared to those with the L858R mutation to varying degrees. There was no difference in the half maximal inhibitory concentration (IC50) of gefitinib given to cancer cell lines harbouring an exon 19 deletion and those with the L858R mutation [20,21]. However, a recent study revealed that the crystal structure of the L858R mutation is more stable in maintaining the active form than the exon 19 deletion mutation [22]. The rationale underlying these differences in response to EGFR-TKI may be explained by their activating mechanism.

In this study, we found candidate predictive factors of the response to gefitinib treatment. Due to the high efficacy of gefitinib treatment, the number of non-responders was very small. To confirm the results of this study, additional data on non-responders to EGFR-TKI treatment should be collected for further analysis.

5. Conclusion

In this study, on the basis of the characteristics of non-responders to gefitinib in the NEJ002 study, we found two potential factors for a poor response to EGFR-TKI treatment. Patients who had a smoking history showed a significantly lower response rate to gefitinib treatment. Gefitinib treatment may be more effective in patients with an exon 19 deletion than those with the L858R mutation. To clarify these relationships, further studies using additional data on non-responders are needed.
Conflict of interest

Dr. Maemondo, Dr. Inoue, Dr. Oizumi, Dr. Gemma, Dr. Hagiwara, and Dr. Nukiwa received a lecture fee from AstraZeneca Pharmaceutical for this work that is under consideration for publication. Dr. Maemondo participated on the advisory board. Dr. Kinoshita, Dr. Saijo, and Dr. Morita received a lecture fee from AstraZeneca Pharmaceutical for other work. All of the remaining authors have declared no conflicts of interest.

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