Differential efficacy of cisplatin plus pemetrexed between L858R and Del-19 in advanced EGFR-mutant non-squamous non-small cell lung cancer

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Abstract

Background: LUX-Lung 3 showed afatinib improved progression-free survival (PFS) compared with cisplatin plus pemetrexed in patients with epidermal growth factor receptor (EGFR) mutations. In this study, chemotherapy efficacy tended to differ between patients with Leu858Arg (L858R) point mutation and Exon 19 deletion (Del-19); PFS in L858R patients (8.1 months) was greater than in Del-19 patients (5.6 months). We investigated whether there is any difference in efficacy of cisplatin plus pemetrexed between Del-19 and L858R.

Methods: This study is a multicenter retrospective study. We reviewed medical records of patients who had received cisplatin plus pemetrexed as first line chemotherapy. Efficacies were evaluated between EGFR mutation status: Del-19 and L858R. Wild type cases were reference arm only, and not included in any statistical analysis.

Results: Among 304 patients, 78 (25.7%) harbored EGFR mutations: Del-19 (36/78 patients, 46.2%); and L858R (42/78, 53.8%). Median PFS of L858R group (9.4 months, 95% confidence interval [CI]: 7.0–12.6) was significantly longer than Del-19 group (5.5 months, 95% CI: 3.6–8.6) ($p = 0.049$). Response rate (RR) and OS presented no significant difference between L858R and Del-19. In multivariate analysis, EGFR mutation status (L858R versus Del-19) was the only significant factor for longer PFS (Hazard ratio [HR]: 0.78, 95% CI: 0.62–0.98) ($p = 0.033$).

Conclusion: Our study indicated better efficacy of cisplatin plus pemetrexed in L858R than in Del-19 patients. In EGFR-mutant NSCLC, EGFR-TKIs are undoubtedly the premier therapy. However, in second line or later settings, cisplatin plus pemetrexed regimen may confer higher efficacy for L858R patients.

Keywords: Exon 19 deletion, L858R point mutation in exon 21, Non-squamous non-small cell lung cancer, Pemetrexed, Progression-free survival

Background

Lung cancer is now one of the most common malignancies in the world. An estimated 1.8 million new lung cancer patients were diagnosed in 2012, and accounted for about 13% of total newly-diagnosed cancer patients [1]. Approximately 80% of lung cancer is histologically non-small cell lung cancer (NSCLC); most patients are already unresectable on their initial diagnosis and are selected to receive chemotherapy. Platinum doublet regimens were once the primary therapeutic choice for advanced NSCLC, but cytotoxic chemotherapies’ progress has reached a plateau. However, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have improved therapeutic outcomes of EGFR-mutant advanced NSCLC. Exon 19 deletion (Del-19) mutation and Leu858Arg (L858R) point mutation in exon 21 are the most common EGFR mutations in NSCLC. Several clinical randomized phase III trials have demonstrated that EGFR-mutant advanced NSCLC patients treated with EGFR-TKIs obtain
a longer progression-free survival (PFS) than those treated with platinum-based chemotherapy [2–7].

Combined analysis of overall survival (OS) data from two randomized phase III trials, LUX-Lung 3 and LUX-Lung 6, showed that overall survival was improved with the 2nd generation EGFR-TKI afatinib (31.7 months) over standard chemotherapy (20.7 months) for patients with Del-19 mutant NSCLC ($p = 0.0001$) [8]. These results demonstrate afatinib achieved greater effect than standard chemotherapy for Del-19 patients as a whole. Conversely, OS of patients with L858R was not significantly different between afatinib (22.1 months) and standard chemotherapy (26.9 months) ($p = 0.16$). We suppose afatinib exerts greater efficacy in Del-19, and chemotherapy exerts greater efficacy in L858R (OS of Del-19 with chemotherapy: 20.7 months, L858R with chemotherapy: 26.9 months). PFS in L858R and Del-19 patients treated with cisplatin plus gemcitabine were almost equivalent in LUX Lung 6 (5.6 months, both). However, LUX-Lung 3 results suggested cisplatin plus pemetrexed promoted longer PFS in L858R patients (8.1 months) than in Del-19 patients (5.6 months) [9]. L858R patients treated with cisplatin plus pemetrexed may obtain greater PFS than in Del-19. However, there is no verified data on this subject, and the efficacy of cisplatin plus pemetrexed for L858R is unclear now.

In this study, we retrospectively examined the efficacy of cisplatin plus pemetrexed as first line chemotherapy according to EGFR mutation status: Del-19 and L858R, in advanced non-squamous NSCLC.

**Methods**

**Patients**

We screened NSCLC patients treated with cisplatin plus pemetrexed as first line chemotherapy at participating institutions. Results of patient characteristics were analyzed using medical and radiographic records to ascertain age, gender, Eastern Cooperative Oncology Group (ECOG) performance status (PS), smoking history, clinical stage (stage IIIB, IV or recurrence), and histology. We also investigated number of induction and maintenance therapies, and post-treatment. Tumor response was retrospectively evaluated every 2 to 3 cycles to evaluate treatment response and disease progression.

**Statistical analysis**

Response rate (RR) and disease control rate (DCR) were compared between EGFR mutation positive (Del-19 and L858R) patients using Fisher’s exact test. PFS and OS curves were estimated according to the Kaplan-Meier method. PFS and OS were compared between Del-19 and L858R using log-rank test. Independent risk factors were analyzed in multivariate analysis using Cox proportional hazards model. In multivariate analysis, we selected each patients characteristics (age, gender, ECOG PS, smoking history, clinical stage, histology, and EGFR mutation status), and stepdown method was used in model selection to choose predictive variables. Subgroup analysis was performed between Del-19 and L858R. Wild type cases were reference arm only, and not included in any statistical analysis. $P$-values less than 0.05 were considered to be statistically significant. Statistical analysis was performed using JMP 9 software (SAS Institute Inc., Cary, NC, USA).

**Results**

**Patient characteristics**

Between January 2010 and December 2014, 304 patients received cisplatin plus pemetrexed as first line chemotherapy. Among 304 patients, 78 (25.7%) patients harbored EGFR mutations, including Del-19 (36/78 patients, 46.2%)
and L858R (42/78, 53.8%). Their clinical characteristics are shown in Table 1. Median age was 64.0 years (range, 37 to 78 years). Most patients were male (216/304, 71.1%), had a good PS of 0/1 (273/304, 89.8%) and had ever smoked (219/304, 72.0%). Stage IIIB or IV (277/304, 91.1%) and adenocarcinoma (276/304, 90.8%) were predominant. However, EGFR mutations were predominantly female (44/78, 56.4%) and never smoker (50/78, 64.1%). L858R and Del-19 patient characteristics were not significantly different. In this investigation, histological types were limited to: adenocarcinoma; large cell carcinoma; and NSCLC-not otherwise specified (NOS). We defined these 3 histological types as non-squamous. Mean number of induction therapy cycles was 3.7 in Del-19 and 4.0 in L858R, and mean maintenance therapy cycles were 4.1 in Del-19 and 6.0 in L858R. L858R cycle numbers tended to be slightly higher than Del-19, however, no significant differences were observed. In maintenance therapy, interruption for unacceptable toxicity was undertaken in two cases, one Del-19 case and one L858R.

### Treatment efficacy

Results of RR and DCR are summarized in Table 2. Overall RR was 39.7% and RRs were not significantly different between L858R and Del-19. Overall DCR was 85.9% and DCRs were not significantly different between L858R and Del-19 as well.

Median PFS was significantly longer for L858R patients (9.42 months, 95% confidence interval [CI]: 6.97–12.6) than in Del-19 patients (5.52 months, 95% CI: 3.57–8.63) \( (p = 0.049) \) (Fig. 1a). Subgroup analyses of PFS for EGFR mutation status (L858R versus Del-19) are shown in Forest plot (Fig. 2a). In patients under 65 years, with good PS and clinical stage IIIB or IV, L858R mutation was favored over Del-19.

### Table 1. Comparison of patient characteristics

| Patient characteristics | All patients (n = 304) | Del-19 (n = 36) | L858R (n = 42) | p-value (Del-19/L858R) | Wild type (n = 226) |
|-------------------------|------------------------|----------------|----------------|------------------------|---------------------|
| Age (years)             |                        |                |                |                        |                     |
| Median (range)          | 64.0 (37–78)           | 62.5 (41–77)   | 66.0 (38–78)   | 0.18                   | 64.0 (37–78)        |
| < 65                    | 160                    | 20             | 17             | 0.18                   | 123                 |
| ≥ 65                    | 144                    | 16             | 25             | 0.18                   | 103                 |
| Gender                  |                        |                |                |                        |                     |
| Male                    | 216                    | 19             | 15             | 0.13                   | 182                 |
| Female                  | 88                     | 17             | 27             | 0.13                   | 44                  |
| PS (ECOG)               |                        |                |                |                        |                     |
| 0–1                     | 273                    | 35             | 40             | 0.65                   | 198                 |
| 2                       | 31                     | 1              | 2              | 0.65                   | 28                  |
| Smoking history         |                        |                |                |                        |                     |
| Never                   | 85                     | 20             | 30             | 0.15                   | 35                  |
| Ever                    | 219                    | 16             | 12             | 0.15                   | 191                 |
| Clinical stage          |                        |                |                |                        |                     |
| IIIB, IV                | 277                    | 30             | 35             | 1.00                   | 212                 |
| Recurrence              | 27                     | 6              | 7              | 1.00                   | 14                  |
| Histology               |                        |                |                |                        |                     |
| Adenocarcinoma          | 276                    | 34             | 42             | 0.30                   | 200                 |
| Large cell carcinoma    | 4                      | 1              | 0              | 0.30                   | 3                   |
| NSCLC-NOS               | 24                     | 1              | 0              | 0.30                   | 23                  |
| Induction therapy cycles|                        |                |                |                        |                     |
| Mean (range)            | 3.7(1–6)               | 3.7 (1–6)      | 4.0 (1–6)      | 0.23                   | 3.6 (1–6)           |
| Maintenance therapy cycles|                    |                |                |                        |                     |
| Mean (range)            | 3.3(0–30)              | 4.1 (0–30)     | 6.0 (0–22)     | 0.17                   | 2.7 (0–30)          |

Del-19 19 deletion; L858R Leu858Arg; PS performance status
ECOG Eastern Cooperative Oncology Group
NSCLC-NOS non small cell lung cancer-not otherwise specified
Median OS did not significantly differ between L858R (35.6 months, 95% CI: 27.6–54.1) and Del-19 (40.1 months, 95% CI: 27.7–60.0) ($p = 0.64$) (Fig. 1b). Subgroup analyses of OS for EGFR mutation status (L858R versus Del-19) are shown in Forest plot (Fig. 2b). No significant differences were observed.

**Multivariate analyses**

Multivariate analyses were performed to identify independent risk factors using the Cox proportional hazards model. We eliminated the variables of ECOG PS and histology due to small numbers, and all wild type cases from multivariate analyses. In the results of multivariate analysis, EGFR mutation status remained as the only identified independent predictive factor for longer PFS (L858R: hazards ratio: 0.78, 95% CI: 0.62–0.98, $p = 0.033$) (Table 3). Multivariate analyses of OS identified clinical stage as a significant factor (IIIB, IV: hazards ratio: 2.49, 95% CI: 1.37–6.20, $p = 0.001$). However, EGFR mutation status was a not significant prognostic factor for OS in multivariate analysis.

**Post-treatment according to EGFR mutation status**

Details on the post-treatment regimens are given in Table 4. There were no major differences in post-treatment between L858R and Del-19, with clear majorities of each receiving EGFR-TKIs.

**Discussion**

Pemetrexed was approved as a therapeutic drug for malignant mesotheliomas in the United States in February, 2004. For NSCLC, pemetrexed gained supplemental approval in August of that year [11]. Pemetrexed inhibits a folic acid-dependent metabolic pathway necessary for cell replication by replacing folic acid and disrupting cellular activity. Pemetrexed inhibits many enzymes: Thymidylate synthase (TS); Dihydrofolate reductase (DHFR); and Glycinate ribonucleotide formyltransferase (GARFT). Some reports indicate pemetrexed has greater efficacy in non-squamous cell carcinoma than in squamous cell carcinoma [12, 13]. This differential efficacy may be explained by the higher TS expression exhibited by squamous cell carcinoma. In squamous cell carcinoma, the higher expression of TS and activity of Skp2, the enzymes synthesizing thymidine monophosphate (TMP), decreases the efficacy of pemetrexed [14, 15].

Furthermore, in subgroup analysis of international clinical phase III trial (PROFILE1007) aimed at Anaplastic lymphoma kinase (ALK) positive advanced lung cancer, pemetrexed showed higher effect than docetaxel [16]. Shaw et al. reported ALK positive lung cancer minimally expresses TS, and pemetrexed may thus have greater efficacy [17]. Ren et al. reported low TS expression in EGFR-mutant NSCLC too [18]. Giovannetti et al. reported different TS gene expression level among six human NSCLC cell lines. Especially, NCI-H1650 (H1650) harboring EGFR mutations had lower TS gene expression than the other five NSCLC cell lines which expressed wild type EGFR [19]. Cells with EGFR mutations may have greater sensitivity to pemetrexed due to lower TS gene expression levels. However, EGFR mutation was not separated by Del-19 or L858R in these reports. Pemetrexed may exert greater efficacy on L858R patients if TS expression is lesser in L858R than in Del-19. Wu et al. reported pemetrexed-based chemotherapy showed a higher response and longer PFS in EGFR-mutant than in wild type [20]. These results may affirm…

| RR (%) | p-value | DCR (%) | p-value |
|--------|---------|---------|---------|
| All patients ($n = 78$) | 39.7 | | 85.9 | |
| EGFR mutation status | | | |
| Del-19 | 36.1 | 0.54 | 80.6 | 0.21 |
| L858R | 42.9 | | 90.5 | |

**Fig. 1** Kaplan-Meier curves for a progression-free survival and b overall survival among patients in L858R group, Del-19 group, and wild type group.
our study. However, in these reports EGFR mutation was not separated by Del-19 or L858R as well, and no prospective study examining TS expression among differing EGFR-mutants has been reported.

According to preclinical data, X-ray crystallographic analysis of the domain revealed different protein conformations of Del-19 and L858R. Both vary in their activated stability by difference in conformation, and their continuation state of kinase activation after the disruption of dimerization is different also [21]. Reguart et al. reported that biological properties of Del19 and L858R mutations differ, with different patterns of EGFR amplification and EGFR autophosphorylation between cell lines containing each mutation [22]. Other experimental reports show that

![Diagram a](image1.png)
![Diagram b](image2.png)

**Fig. 2** A forest plot for a progression-free survival hazard ratios and b overall survival hazard ratios comparing Del-19 group with L858R group for subgroups stratified by the indicated factors

### Table 3

Multivariate analyses of progression free-survival and overall survival between Del-19 and L858R (n = 78)

| Covariate                                      | Progression-free survival | Overall survival |
|------------------------------------------------|---------------------------|-----------------|
| **Age (<65 vs ≥65)**                           | Hazard ratio | 95% CI | p-value | Hazard ratio | 95% CI | p-value |
| Gender (Male vs Female)                        | 1.13       | 0.89-1.42 | 0.31     |               |         |        |
| Smoking history (Never vs Ever)                | 0.88       | 0.70-1.13 | 0.32     |               |         |        |
| Clinical stage (IIIB, IV vs Recurrence)        | 1.36       | 0.99-1.93 | 0.055    |               |         |        |
| **EGFR mutation status (L858R vs Del-19)**     | 0.78       | 0.62-0.98 | *0.033   |               |         |        |

| Covariate                                      | Overall survival |
|------------------------------------------------|-----------------|
| **Age (<65 vs ≥65)**                           | Hazard ratio | 95% CI | p-value |
| Gender (Male vs Female)                        | 1.21       | 0.87-1.70 | 0.25     |
| Smoking history (Never vs Ever)                | 1.01       | 0.73-1.38 | 0.95     |
| Clinical stage (IIIB, IV vs Recurrence)        | 2.49       | 1.37-6.20 | *0.001   |
| **EGFR mutation status (L858R vs Del-19)**     | 1.14       | 0.83-1.57 | 0.43     |

CI confidence interval

* p < 0.05
biomedically, L858R and Del-19 may be two different things [23, 24]. Carey et al. compared the proliferation abilities of induced wild type, Del-19, and L858R NR6 fibroblasts [25]. According to this report, there are differences in cell proliferation ability among wild type, Del-19 and L858R. Especially in Del-19, high cell proliferation ability has been confirmed. Therefore, Del-19 may be faster in progression speed than L858R. According to this report, L858R and Del-19 may respond differently to pemetrexed.

Various resistance mechanisms to EGFR-TKI treatments, such as ErBB family receptor amplification or other RTK co-amplification may be involved in the differential efficacy between L858R and Del-19. Yu et al. reported that frequencies of Met-amplification and HER-amplification did not differ between L858R and Del-19, although sample size was small [26]. The possible differences between Del-19 and L858R require further research.

In comparison with the results of LUX-Lung 3, PFS of our study was similar. In Del-19, PFS of LUX-Lung 3 was 5.6 months versus 5.52 months in our study. In L858R, PFS of LUX-Lung 3 was 8.1 months versus 9.42 months in our study. The results of our study showed reproducibility. Our study demonstrated significant difference in PFS between L858R and Del-19 treated with pemetrexed, but did not reveal significant difference in OS. EGFR-TKIs were administered post-pemetrexed in approximately 90% of each mutation type, and most have reported higher EGFR-TKI efficacy in Del-19 than in L858R [4–6, 8]. This “catch-up” effect may explain similar OS between L858R and Del-19. Our study covered wild type as reference group in this study. Wild type PFS was 4.62 months, in accordance with past reports [27]. In multivariate analysis of OS, a significant difference was found between recurrence and advanced (clinical stage IIIB or IV) NSCLC. Yoshioka et al. reported that recurrent lung cancer had good prognosis and our study agrees in this respect [28].

Our study presents a few limitations. First, it is retrospective. Second, our sample size is relatively small. However, we were able to examine and obtain pure data by only including first line cisplatin plus pemetrexed treated patients and excluding other regimens (for instance, carboplatin-based or addition of bevacizumab), mirroring the strict patient selection of LUX-Lung 3. Finally, we were not able to perform TS expression immunostaining to conclude any causal link between EGFR mutation type, TS expression and treatment efficacy in this study. Further studies are warranted.

This study is the first report showing a significantly greater efficacy of cisplatin plus pemetrexed in L858R than in Del-19 between EGFR-mutant NSCLC chemotherapy naïve patients. In this study, we examined cisplatin plus pemetrexed regimen in first line chemotherapy, similar to LUX-lung studies. In EGFR-mutant NSCLC, EGFR-TKIs are undoubtedly the premier therapy, with limitations. Third generation EGFR-TKI after acquired resistance to first or second generation EGFR-TKIs represents an efficacious treatment [29], but only in the roughly 50% of patients who express T790 M mutation [30]. Immunotherapy has also attracted investigation in NSCLC [31–33], but it has shown reduced efficacy against EGFR-mutants [34, 35]. Thus, cytotoxic chemotherapy still has a role in treating patients who cannot benefit from further EGFR-TKI exposure or immunotherapy. Furthermore, administering platinum doublet chemotherapy alternating with EGFR-TKI produced longer survivals among EGFR-mutants than EGFR-TKI alone [28]. In addition, some report that EGFR-mutants may be more likely to benefit from cytotoxic chemotherapy than wild type [36, 37].

**Conclusion**

Our study indicated better efficacy of cisplatin plus pemetrexed in L858R than in Del-19 patients. In EGFR-mutant NSCLC, EGFR-TKIs are undoubtedly the premier therapy. However, in second line or later settings, cisplatin plus pemetrexed regimen may confer higher efficacy for L858R patients.

**Abbreviations**

ALK: Anaplastic lymphoma kinase; CI: confidence interval; CT: computed tomography; DCR: disease control rate; Del-19: 19 deletion; DHFR: Dihydrofolate reductase; ECOG: Eastern Cooperative Oncology Group; EGFR: Epidermal growth factor receptor; GARFT: Glycinate ribonucleotide formyltransferase; L858R: Leu858Arg; NOS: not otherwise specified; NSCLC: non-small cell lung cancer; OS: overall survival; PD: progressive disease; PFS: progression-free survival; PR: partial response; PS: performance status; RECIST: Response Evaluation Criteria in Solid Tumors; RR: Response rate; SD: stable disease; TKIs: tyrosine kinase inhibitors; TS: Thymidylate synthase

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**Table 4:** Post-treatment according to EGFR mutation status as second line between Del-19 and L858R (n = 78)

| Post-treatment                        | Del-19 (n = 36) | L858R (n = 42) |
|--------------------------------------|-----------------|----------------|
|                                      | No. of patients | %              | No. of patients | %          |
| Not received                         | 0               | 0%             | 0               | 0%         |
| Received                             |                 |                |                 |            |
| Platinum doublet therapy             | 0               | 0%             | 0               | 0%         |
| Single-agent chemotherapy            | 1               | 2.8%           | 1               | 2.4%       |
| EGFR-TKI therapy                     | 32              | 88.9%          | 38              | 90.5%      |
| Other molecular target drug therapy  | 2               | 5.5%           | 2               | 4.7%       |
| Immunotherapy                        | 0               | 0%             | 0               | 0%         |
| Other                                | 1               | 2.8%           | 0               | 0%         |
| During first line chemotherapy       | 0               | 0%             | 1               | 2.4%       |
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Authors' contributions
Conceptualization and design: All authors. Development of methodology: TK, HY, MT, AT, AH. Collection and analyzed patients data: TK, HY, MT, AT, AH. AO. Research and investigation of data/evidence: TK, HY, MT, AT, AH. Wrote the manuscript: TK, HY, AH. All authors have read the manuscript and given their approval for the submission.

Ethics approval and consent to participate
This study was approved by the Institutional Review Board of each participating institutions, which waived the requirement for informed consent due to the retrospective nature of the analysis.

Competing interests
HY received lecture fees from Chugai, Astra Zeneca, Boeringer Ingelheim, and Eli Lilly. AH received lecture fees from Chugai, Astra Zeneca, Boeringer Ingelheim, and Eli Lilly.

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