Lung Cancer 162 (2021) 140–146

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

A phase I/II study of osimertinib in *EGFR* exon 20 insertion mutation-positive non-small cell lung cancer

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ARTICLE INFO

Keywords:
Osimertinib
EGFR exon 20 insertion
Non-small cell lung cancer
Pharmacokinetics
IC_{50}

ABSTRACT

Objectives: Several preclinical data proposed a potential efficacy of osimertinib, a third-generation EGFR tyrosine kinase inhibitor, for *EGFR* exon 20 insertion (*EGFR* ex20ins)-positive non-small cell lung cancer (NSCLC). However, reported case series and a retrospective study proposed controversial efficacy. The efficacy of osimertinib in *EGFR* ex20ins-positive NSCLC have not been well evaluated in prospective clinical trials. In this study, we performed a prospective, single-arm, multi-center, open-label, non-randomized phase I/II study to evaluate efficacy of osimertinib for *EGFR* ex20ins-positive NSCLC.

Materials and methods: From August 2018 to January 2020, 14 NSCLC patients with *EGFR* ex20ins were enrolled, of whom 2 were excluded because they did not meet the inclusion criteria. Efficacy and safety of 80 mg osimertinib were evaluated. In addition, we performed a translational exploratory study to clarify the association of mutation type-specific drug sensitivity, osimertinib pharmacokinetic data, and clinical efficacy.

Results: Of the evaluated patients, none experienced objective response, 7 experienced stable disease (58.3%), and 5 experienced disease progression (41.7%). The median progression free survival (PFS) was 3.8 months, and the median overall survival was 15.8 months. Interestingly, the exploratory study demonstrated statistically significant positive correlation between plasma osimertinib concentration/*in vitro* IC_{50} ratio and PFS ($R = 0.9912$, $P = 0.0001$), highlighting the mutation type-specific concentration-dependent efficacy of osimertinib for *EGFR* ex20ins-positive NSCLC.

Conclusions: Regular dose, 80 mg/day, of osimertinib has limited clinical activity in NSCLC patients with *EGFR* ex20ins. The translational study proposed the potential efficacy of higher dose osimertinib in a subgroup of *EGFR* ex20ins-positive NSCLC.

Abbreviations: *EGFR* ex20ins, *EGFR* exon 20 insertion; NSCLC, non-small cell lung cancer; PFS, progression free survival; AUC, area under the curve.

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https://doi.org/10.1016/j.lungcan.2021.10.006
Received 9 September 2021; Received in revised form 12 October 2021; Accepted 12 October 2021
Available online 16 October 2021

1. Introduction

EGFR exon 20 insertion (*EGFR* ex20ins) mutations comprise about 4–12% [1–4] of *EGFR* mutations, which is the third most common category of mutations detected in NSCLC. More than 60 types of *EGFR* exon 20 insertion mutations have been reported till date [5]. In-frame insertions at exon 20 push the C-helix into its inward position and promote the active conformation of EGFR, which induce ligand-independent activation of EGFR [2]. In clear contrast to common *EGFR* mutations such as the point mutation in exon 21 L858R and in-frame deletions in exon 19 of *EGFR*, *EGFR* ex20ins mutations are known to be resistant to clinically available first and second generation EGFR tyrosine kinase inhibitors (EGFR-TKIs). However, exceptional sensitivity of *EGFR* A763_Y764insFQEA to first and second EGFR-TKIs have also been reported [2,5].

In the LUX-LUNG trial, afatinib, a 2nd generation EGFR-TKI, demonstrated limited efficacy for patients with *EGFR* ex20ins-positive NSCLC with progression-free survival (PFS) of 2.7 months and overall survival (OS) of 9.2 months [6], highlighting the aggressive nature of this subgroup of NSCLC.

Osimertinib, a third generation EGFR-TKI, demonstrated a meaningful efficacy in a FLAURA trial in NSCLC patients harboring common *EGFR* mutations with a PFS of 18.9 months and OS of 38.6 months [7]. In addition, the safety of osimertinib has been reported in multiple clinical trials [7,8] and is widely accepted in clinics. However, the clinical efficacy of osimertinib for *EGFR* ex20ins-positive NSCLC has not been well evaluated in a prospective study.

We proposed a potential efficacy of osimertinib in *EGFR* ex20ins-positive NSCLC patients, using a preclinical model [9,10], although variation of sensitivity is prevalent among *EGFR* ex20ins mutations [11]. The difference of *in vitro* IC_{50} values of osimertinib between wildtype and *EGFR* ex20ins mutations was identified as a therapeutic window, providing a preclinical rationale to use osimertinib for treatment of patients with *EGFR* ex20ins-positive NSCLC. For example, the IC_{50} value of osimertinib for *EGFR* A767_V769dupASV, one of the most frequently reported *EGFR* ex20ins mutations, was 321 nM, whereas that of wildtype *EGFR* was 1271 nM [11]. We also reported the variable sensitivity data among *EGFR* exon 20 insertion mutations. For example, the IC_{50} value was 33 nM for A763_Y764insFQEA, while that of P772_H773insHA was 710 nM. These preclinical data proposed a potential efficacy of osimertinib in a subgroup of *EGFR* ex20ins-positive NSCLC.

Several case reports revealed the efficacy of osimertinib in *EGFR* ex20ins-positive NSCLC, although controversial. [12–15]. Furthermore, recently, a retrospective cohort study data was reported from Netherland's study group [16] in which 21 patients were treated mainly with osimertinib 80 mg once daily. The reported objective response rate (ORR) was 5% and PFS was 3.6 months. The disease control rate (DCR) at three months was 71%. Median OS was 8.7 months, indicating limited efficacy of osimertinib.

However, the efficacy of osimertinib has not been well evaluated in a prospective study. Although several prospective trials have evaluated the efficacy of osimertinib for patients with *EGFR* ex20ins [17,18], considering the variability in types of *EGFR* ex20ins mutations, further evaluation with different patient populations may provide additional insights into the treatment of *EGFR* ex20ins positive NSCLC. In addition, considering the variable sensitivity and insensitivity patterns of *EGFR* ex20ins mutations to osimertinib observed in preclinical studies, data regarding the association among mutation types, osimertinib pharmacokinetics, and clinical efficacy may propose additional insights important for the development of treatment strategy using osimertinib. Therefore, we performed a prospective phase I/II trial to evaluate the efficacy of osimertinib for *EGFR* ex20ins-positive NSCLC. In addition, we performed a translational exploratory study to evaluate the association of mutation-type specific osimertinib sensitivity, plasma osimertinib concentration and clinical efficacy.

2. Material and methods

2.1. Study design and sample size estimation

This was a prospective, single-arm, multi-center, Simon's two-stage phase I/II clinical trial (UMIN000031929). Patients received osimertinib 80 mg once daily until they met the termination criteria. Primary end point was ORR, assessed via Response Evaluation Criteria in Solid Tumors version 1.1. Secondary end points were PFS, OS, and safety profile. The PFS and OS were defined as the time from the day of enrollment to the day of disease progression or death, respectively.

With reference to previous studies, assuming that the expected response rate in the primary endpoint was 36%. The threshold response rate was set to 10%. In this study, to suppress inflation of Type I error applied Simon's two-stage method (minmax design). The required number of cases in first stage and second stage were 12 and 9, respectively, to have 90% power with a one-sided significance level of 5%. If one patient experienced response at the first stage, the trial was expected to proceed to the second stage.

2.2. Patient population/Eligibility criteria

Patients with advanced or metastatic NSCLC with *EGFR* ex20ins mutation who have a history of 0 to 3 regimens of chemotherapy, were enrolled. Patients with history of EGFR-TKI treatment (gefitinib, erlotinib, afatinib, dacomitinib) could be included if the EGFR-TKI treatment did not show any clinical benefit. Patients with *EGFR* mutations, such as exon 19 deletion, L858R, T790M, G719X, L861Q were excluded. Other criteria included Eastern Cooperative Oncology Group performance status 0–1, age 20 years or older, adequate hematological, hepatic, and renal functions, and a life expectancy of 12 weeks or more at the start of this trial. This study was approved by the institutional review board of institutions included in this study. All patients provided written informed consent before this study.

2.3. Efficacy and safety assessments

All patients who received at least one dose of osimertinib were included in the efficacy and safety assessment. Patients with measurable disease at baseline and had been re-examined were evaluated for efficacy analysis. Radiographic tumor assessments were completed every 4–8 weeks. Response was assessed by the investigators according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. Adverse events were recorded from the time of informed consent and were followed for 28 days after the last dose of osimertinib was administered. Adverse events were graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

2.4. Determination of specific genotypes of EGFR ex20ins mutations

Three out of 12 patients participated in the Multi-institutional Lung Cancer Genomic Screening Project (LC-SCRUM-Asia) and their DNA samples from primary lung cancer specimen or pleural effusion were analyzed by a targeted next generation sequencing assay, Oncomine Comprehensive Assay version 3 (Thermo Fisher Scientific, Waltham, MA). On the other hand, for the rest of the patients, no specific genotype was identified and they were simply diagnosed with *EGFR* ex20ins mutations by *in vitro* diagnostic-marked assays such as the cobas *EGFR* Mutation Test v2 kit (Roche Diagnostics) and the therascreen *EGFR* RGQ PCR kit (Qiagen). To determine the specific types of *EGFR* ex20ins mutations, liquid biopsy was performed on these patients. The purified cfDNA was isolated using AVENIO cfDNA Expanded kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. The cfDNA concentration was quantified using the Qubit dsDNA High Sensitivity Assay kit (Thermo Fisher Scientific, Waltham, MA). Additionally, sample quality was confirmed using the Tape Station High Sensitivity D1000

(Agilent, Santa Clara, CA). Sequencing libraries were prepared using the AVENIO cfDNA Library Prep sub-Kit (Roche, Basel, Switzerland). Library pools were sequenced using the NextSeq 500/550 High Output kit v2.5 (Illumina, San Diego CA). Data was analyzed with the AVENIO ctDNA Analysis Software (version 2.0.0).

2.5. Quantification of plasma osimertinib concentrations

Four weeks since initiating the patients on osimertinib, venous blood samples were obtained for pharmacokinetic analysis before and at 1, 3, 6, 12 and 24 h after administration. Peak, trough, and mean plasma osimertinib concentrations were measured by Covance (Indianapolis, IN).

2.6. Association of pharmacokinetic plasma osimertinib concentration to IC_{50} ratio and clinical efficacy

The ratio between plasma osimertinib concentrations, area under the curve (AUC) and trough, and *in vitro* IC_{50} values of corresponding mutations was calculated. IC_{50} values for corresponding mutations were obtained from our previously reported data [11] except for *EGFR* H773_V774insH. The reported IC_{50} values for A767_V769dupASV, S768_D770dupSVD, H773_V774insAH, and D770_N771insG were 321, 66, 710 and 101 nM, respectively. For *EGFR* H773_V774insH, a Ba/F3 cell line was established [2] and MTS cell proliferation assay was performed. The IC_{50} value was determined as more than 10 μM. However, for calculation of the ratio, the IC_{50} value of *EGFR* H773_V774insH was used as 10 μM. The Pearson's correlation test was performed to examine the correlation between the ratio and PFS.

2.7. Statistical analyses

For the analysis of primary endpoint, the ORR was indicated by the rate of responses. The PFS and OS were calculated by Kaplan-Meier method for the analysis of secondary endpoints. The best percentage change of targeted tumor burden was shown by waterfall plot, and the duration of response was shown by swimmer plot. The variables of background of patients were evaluated by the proportion for categorical variables and the summary statistics for continuous variables.

For the exploratory study, the association between IC_{50} values, AUC, trough osimertinib concentration, AUC standardized by IC_{50} values, trough osimertinib concentration standardized by IC_{50} values and PFS was evaluated by calculating correlation coefficient. The same was also studied by representation through a scatter plot. The significance level was set to 0.05 (two-sided) or 0.025 (one-sided).

All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, North Carolina, USA).

3. Results

3.1. Patient characteristics and treatment

Between Aug 2018 to Jan 2020, 14 patients were enrolled in this study. Patient #02 and #04 were excluded after the enrollment because the trial office noticed that they did not meet the inclusion criteria. The characteristics of 12 patients evaluated in this study are summarized in Table 1 and Supplementary Table S1. Median age was 66.5 years (range 22–84) and 58.3% of patients were female. No patient was treated with EGFR-TKIs before the enrollment. Two patients received osimertinib as first line treatment and 7 patients as second line treatment. Clinical genotyping, liquid biopsy, and NGS analysis determined the mutation types of 9 patients. Three patients harbored *EGFR* A767_V769dupASV, two patients harbored H773_V774insH, one patient harbored S768_D770dupSVD, one patient harbored D770_N771insG, one patient harbored H773_V774insAH and one patient harbored D770delinsGY mutations. Although reported to be *EGFR* exon 20 insertion mutation-positive by PCR-based *EGFR* mutation analysis, mutation types could not be determined in three patients.

Table 1
Basic characteristics of the patients included in this study.

	No. of patients	(%)
Total enrolled	12	
Aged (years)		
Median (range)	66.5 (22–84)	
Sex		
Male	5	41.7
Female	7	58.3
ECOG performance status (pre-treatment)		
0	8	66.7
1	4	33.3
Histology		
Adenocarcinoma	11	91.7
Others	1	8.3
Disease stage		
III A	1	8.3
III C	1	8.3
IV A	3	25
IV B	7	58.3
Regimen number		
1st	2	16.7
2nd	7	58.3
3rd	2	16.7
4th	1	8.3
EGFR mutation status		
A767_V769dupASV	3	25
H773_V774insH	2	16.7
S768_D770dupSVD	1	8.3
D770_N771insG	1	8.3
H773_V774insAH	1	8.3
D770delinsGY	1	8.3
Not determined	3	25

Abbreviations: ECOG = Eastern Cooperative Oncology Group.

3.2. Interim efficacy analysis: The first stage in Simon's two-stage design

The interim analysis, evaluating the status of the 12 patients according to the first stage of Simon's two-stage minimax design, was presented to the Independent Data Monitoring Committee (IDMC) in September 2020. None out of the 12 patients achieved objective response, thus the stopping threshold of at most one patient achieving Complete Response (CR) or Partial Response (PR) was crossed. Based on the result, the IDMC recommended to discontinue recruitment of patients at 80 mg dose, and if continued, to amend the protocol and consider increasing the dose. Following the IDMCs recommendation, the trial steering committee finally decided to stop recruitment into the trial with the completion of the first stage.

3.3. Efficacy analysis

Of the 12 evaluated patients, none experienced response to osimertinib. Seven patients experienced Stable Disease (SD). These data indicated 0% Objective Response Rate (ORR) and 58.3% DCR. Three patients with *EGFR* A767_V769dupASV, one patient with S768_D770dupSVD, one patient with D770_N771insG, and one patient with H773_V774insAH experienced SD. On the other hand, two patients with *EGFR* H773_V774insH experienced Progressive Disease (PD). The waterfall, swimmers and spider plot are shown in Fig. 1**A, 1B, and Supplement Data 1**. Despite no response, 8 out of 12 (75%) patients experienced a decrease in tumor size. Median PFS time was 3.8 months and median OS time was 15.8 months (Fig. 1**C and 1D)**. In three patients, lung cancer remained stable for more than 6 months, although two patients with *EGFR* H773_V774insH experienced PD. These data indicate variable efficacy pattern among patients with *EGFR* ex20ins-positive NSCLC although no objective response was observed. In summary, the efficacy data indicated limited efficacy of osimertinib 80 mg in

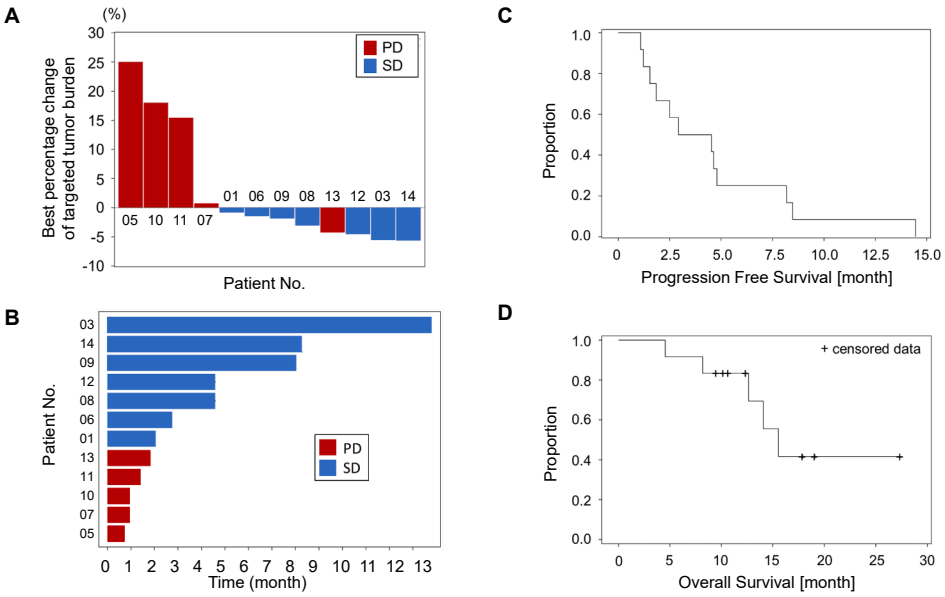

Fig. 1. Efficacy analysis data of this study. (A) Best percentage change of targeted tumor burden. (B) Duration of response. (C) A Kaplan-Meier curve displaying progression free survival. (D) A Kaplan-Meier curve displaying overall survival.

EGFR ex20ins-positive NSCLC.

3.4. Exploratory study

To develop rationale treatment strategy for *EGFR* ex20ins-positive NSCLC, an exploratory study was conducted. Pharmacokinetic analysis determined trough, mean, and peak osimertinib concentrations and AUC of osimertinib in ten patients (Fig. 2A, Table 2). Trough and mean plasma osimertinib concentrations were 466.9 and 591.9 nM, respectively, which is consistent with the previous osimertinib phase I study for common *EGFR*-mutation positive NSCLC [19]. Pharmacokinetic parameters, IC_{50} values and clinical efficacy data are summarized in Table 2. To take variability of mutation-type specific osimertinib sensitivity of *EGFR* ex20ins mutations into account, the correlation between *in vitro* IC_{50} values and PFS was examined. However, no correlation was observed (Fig. 2**B**). No correlation was also observed between AUC or trough concentration of osimertinib alone and PFS (Fig. 2**C, 2D**). Further, to combine the pharmacokinetic data and *in vitro* osimertinib sensitivity data, we calculated ratios between AUC of osimertinib and IC_{50} value (AUC/IC_{50}) and between trough osimertinib concentration and IC_{50} value (trough osimertinib concentration/IC_{50}). Interestingly, clear and statistically significant correlations were observed between

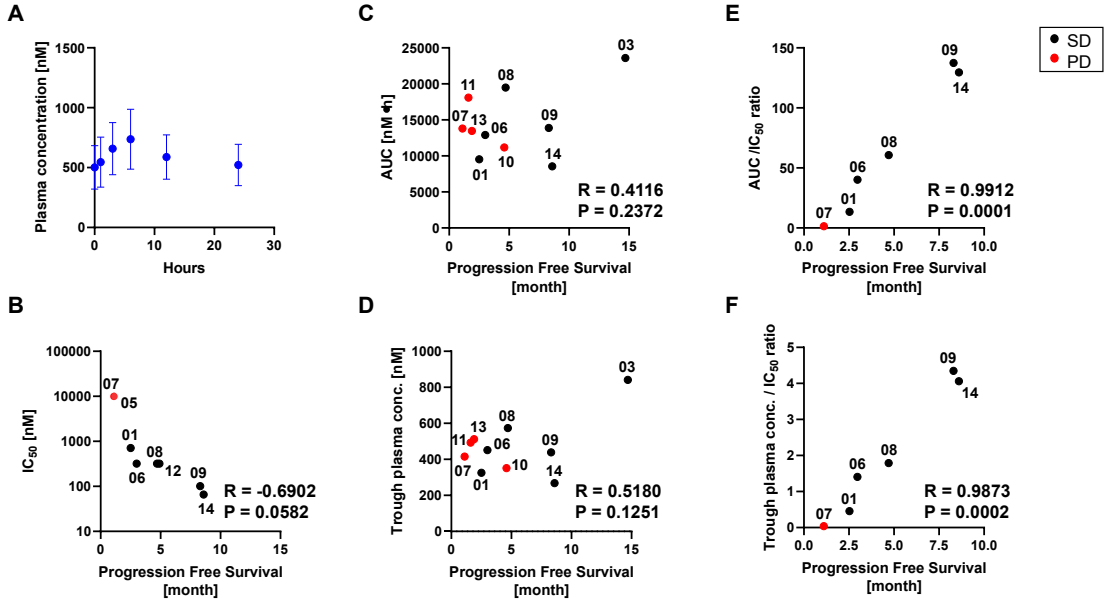

Fig. 2. Results of exploratory study. (A) Plasma osimertinib concentrations (nM) at 0, 1, 3, 6, 12 and 24 h after osimertinib administration. Error bars indicate standard deviation. (B) The association between IC_{50} values (nM) and progression free survival (months). (C) The association between AUC (nM • hour) and progression free survival (months). (D) The association between trough osimertinib concentration (nM) and progression free survival (months). (E) The association between AUC/IC_{50} value and progression free survival (months). (F) The association between trough osimertinib concentration/ IC_{50} value and progression free survival (months). The Pearson's correlation test was performed to calculate R for (B) to (F).

Table 2
EGFR mutation type, IC_{50} *in vitro*, pharmacokinetic parameters and clinical efficacy of osimertinib in each patient.

Patient No.	EGFR mutation type	IC_{50} *in vitro* (nM)	Trough plasma concentration (nM)	AUC (nM·h)	Objective Response	Progression Free Survival (months)
01	H773_V774insAH	710	325	9530.5	SD	2.5
03	Not determined	NA	841	23,601	SD	14.5
05	H773_V774insH	greater than10000	NA	NA	PD	1.2
06	A767_V769dupASV	321	451	12901.5	SD	2.9
07	H773_V774insH	greater than10000	415	13803.5	PD	1.1
08	A767_V769dupASV	321	574	19,486	SD	4.6
09	D770_N771insG	101	439	13,879	SD	8.2
10	D770 delinsGY	NA	351	11,169	PD	4.5
11	Not determined	NA	493	18103.5	PD	1.5
12	A767_V769dupASV	321	NA	NA	SD	4.8
13	Not determined	NA	512	13,461	PD	1.8
14	S768_D770dupSVD	66	268	8554	SD	8.5

Abbreviations: NA: not available; SD: stable disease; PD: progressive disease.

AUC/IC_{50} and PFS (R = 0.9912, P = 0.0001) **(**Fig. 2**E)** and between trough osimertinib concentration/IC_{50} and PFS (R = 0.9873, P = 0.0002) **(**Fig. 2**F).** Moderate but not significant correlation of trough osimertinib concentration to IC_{50} ratio and best percentage of targeted tumor burden and no correlation of trough osimertinib concentration to IC_{50} ratio and OS were observed **(Supplement Data 2).** These data indicate the mutation type-specific concentration-dependent efficacy of osimertinib for *EGFR* ex20ins-positive NSCLC in mutation-type specific manner and provide a rationale evidence to increase dose intensity of osimertinib for treatment of *EGFR* ex20ins-positive NSCLC.

3.5. Safety analysis

In general, osimertinib treatment was well tolerated. The safety profile of this study is summarized in Table 3. Grade 3 paronychia, increased γ-glutamyl transpeptidase (GGT), decreased neutrophil count, decreased white blood cell count, pulmonary embolism and rush were observed in one patient. Dose reduction to 40 mg was necessary for two patients with drug-induced liver injury and skin rash. Safety profile of osimertinib 80 mg is well accepted in clinics. Moreover, our data also supported the tolerability of osimertinib 80 mg in *EGFR* ex20ins-positive NSCLC patients.

Table 3
List of adverse events observed in this study.

CTCAE SOC (System Organ Class)	CTCAE Terms	Any Grade	(%)	Serious	>Grade 3
Gastrointestinal disorders	Constipation	2	16.7	0	0
	Diarrhea	4	33.3	0	0
	Nausea	4	33.3	0	0
	Colon polyp	1	8.7	1	1
Hepatobiliary disorders	Drug-indeced liver injury	1	8.3	1	0
Infections and infestations	Cystitis	2	16.7	0	0
	Paronychia	1	8.3	0	1
Investigations	Creatinine increased	3	25	0	0
	GGT increased	1	8.3	0	1
	Neutrophil count decreased	2	16.7	0	1
	Platelet count decreased	5	41.7	0	0
	White blood cell decreased	2	16.7	0	1
Psychiatric disorders	Insomnia	2	16.7	0	0
Vascular disorders	Pulmonary embolism	1	8.3	1	1
Skin and subcutaneous tissue disorders	Rash acneiform	3	25	0	0
	Dry skin	6	50	0	0
	Eczema	2	16.7	0	0
	Rash	3	25	0	1

This list contains Gr 1 and 2 adverse events occurred in greater than 10% of patients overall, serious adverse event and Gr3, 4, 5 adverse events.

4. Discussion

Recently, multiple inhibitors have been developed for *EGFR* ex20ins-positive NSCLC. These include poziotinib, mobocertinib (TAK-788), CLN-081, BDTX-189, luminespib, and amivantamab [5]. Poziotinib, a small and flexible molecule, demonstrated ORR of 43% and median PFS of 5.5 month. However, toxicity was substantial with 60% grade 3 or greater adverse events [5]. Mobocertinib (TAK-788) showed ORR of 43% and median PFS of 7.3 month with 40% treatment related grade 3 or greater toxicity [20]. Amivantamab, a bispecific antibody against EGFR and MET [21], demonstrated ORR of 40%, median PFS of 8.3 month, and median OS of 22.8 month [22]. The FDA granted approval to amivantamab in May 2021 and mobocertinib in September 2021 as inhibitors for *EGFR* ex20ins-positive NSCLC, which will provide important treatment options for patients with *EGFR* ex20ins-positive NSCLC. Multiple clinical trials are ongoing and further approval is expected in the near future. However, to improve the prognosis of *EGFR* ex20ins-positive NSCLC patients, further development of multiple treatment options is mandatory.

In this prospective phase I/II study, no response was observed in all the 12 enrolled patients and PFS was 3.8 months, indicating the limited efficacy of osimertinib 80 mg/day for *EGFR* ex20ins-positive NSCLC. We conducted an exploratory study using pharmacokinetic and *in vitro* drug sensitivity data, which enabled direct comparison of mutation-type specific sensitivity, osimertinib plasma concentrations, and clinical efficacy. This provided a precious dataset for development of treatment strategy for this cumbersome subgroup of NSCLC. Importantly, the translational exploratory study demonstrated statistically significant correlation between osimertinib pharmacokinetic data relative to mutation-type specific sensitivity data and PFS, although the number of examined patients was small (n = 6).

Two patients, one with *EGFR* S768_D770dupSVD and one with *EGFR* D770_N771insG mutations, whose trough osimertinib concentration to mutation-type specific IC_{50} ratio was approximately 4, indicating almost 4 times higher trough osimertinib concentration than IC_{50} value, experienced a longer SD of 8.5 and 8.2 months, respectively. In two patients with *EGFR* A767_V769dupASV mutation, which comprises about 20% of *EGFR* ex20ins mutations, the ratio was determined to be approximately 2 and they experienced SD with moderate PFS. On the other hand, two patients with *EGFR* H773_V774insH, whose mutation-type specific IC_{50} value was above 10000 nM, experienced PD, indicating that this mutation type is one of the most resistant to osimertinib. Among *EGFR* ex20ins mutations, *EGFR* A767_V769dupASV and S768_D770dupSVD are the two of the most common type of mutations, comprising about 30–40% of all *EGFR* ex20ins mutations combined [1,5]. In conclusion,

these data propose the possibility that a higher dose of osimertinib demonstrates more potent efficacy in a subgroup of *EGFR* ex20ins-positive NSCLC.

A phase I study of osimertinib for patients with common *EGFR* mutation-positive NSCLC reported the serum concentrations of osimertinib to be at variable levels, from 20 mg to 240 mg [19]. In the study, no dose-limiting toxicity was observed during the 28-day evaluation period at any dose levels. Therefore, a maximum tolerated dose was not determined. The maximum serum concentrations were 106.3, 305.1, 635.4, 1006 and 1520 nM for 20, 40, 80, 160, and 240 mg dose levels, respectively. Considering the pharmacokinetic data and safety profile of the phase I study, a higher dose of osimertinib, 160 mg or more, may be tolerable for patients with *EGFR* ex20ins mutations. Recently, the results of two phase II trials investigating the efficacy of osimertinib in NSCLC patients with *EGFR* ex20ins mutations have been reported [17,18]. One was a trial with a standard dose of osimertinib and the other was a trial with higher dose of 160 mg. In the former trial, there were no objective responses, however, in the latter trial, the data indicated an ORR of 24%, DCR of 82%, and PFS of 9.6 months. These data, together with our exploratory data, strongly support the fact that a higher dose of osimertinib will be clinically effective for treating NSCLC patients with subset of *EGFR* ex20ins mutations. Moreover, although the number was small and none of them achieved objective response, the exploratory data suggest that the clinical efficacy of osimertinib on *EGFR* ex20ins mutation, in terms of PFS, is determined by the IC_{50} value of each genotype and plasma concentration of osimertinib in each patient. Therefore, it may be useful to measure the trough plasma concentration of osimertinib to predict the efficacy in each patient.

To summarize, we performed a prospective trial to evaluate the efficacy of osimertinib 80 mg for *EGFR* ex20ins-positive NSCLC. Although no response was observed, we gathered an evidence for development of an effective treatment strategy using a higher dose of osimertinib.

5. Disclosures

HY has potential financial conflicts as funding to conduct this clinical trial to the institution from AstraZeneca (AZ), research grant for lung cancer research from Nippon Boehringer Ingelheim (BI) and honoraria from AZ, Taiho pharmaceutical Co., Ltd (Taiho), Nippon BI and Bristol-Myers Squibb (BMS) Japan. EI has potential financial conflicts as grants from Pfizer, Janssen pharmaceutical K.K., Ono pharmaceutical Co., Ltd. (Ono), Eli Lilly (Lilly), MSD, Takeda pharmaceutical Co., Ltd. (Takeda), and BMS and honoraria from Pfizer, AZ, Chugai pharmaceutical Co., Ltd. (Chugai), Lilly, Janssen pharmaceutical K.K., MSD, BMS, Ono, Nippon Kayaku Co., Ltd., BI. JSK has potential financial conflicts as grants from Lilly. YZ has potential financial conflicts as grants from MSD, Merck, AZ and honoraria from Lilly, AZ, BI, Chugai, BMS, Ono, MSD, Taiho, Takeda. ST has potential financial conflicts as grants from Lilly, Ono, LOXO oncology, Amgen and Incyte and honoraria from Novartis. MM has potential financial conflicts as Support for attending meetings from AZ. KH has potential financial conflicts as grants from MSD, AZ, Chugai, Lilly, BMS and honoraria from Pfizer, AZ, Chugai, Lilly, Takeda, MSD, BMS, Ono, NipponKayaku, Taiho, BI. SM has potential financial conflicts as honoraria from AZ. KK has potential financial conflicts as grants from Taiho, Ono, Chugai, BMS, BI, Takeda, Novartis, Kyorin pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Pfizer, Nippon Kayaku Co., Ltd., MSD, Merck, Shionogi Co., Ltd and honoraria from Pfizer, AZ, Chugai, Ltd., Lilly, Daiichi Sankyo Co., Ltd., MSD, BMS, Ono, Novartis, Taiho, BI. SY has potential financial conflicts as grants from Chugai, BI, and Pfizer and honoraria for lecture from AZ, Chugai, BI, and Pfizer. KG has potential financial conflicts as grants to institution from AZ K.K., Amgen Astellas BioPharma K.K., Amgen K.K., BI Japan, Inc., BMS K.K., Bayer Yakuhin, Ltd., Chugai, DAIICHI SANKYO Co., Ltd., Eisai Co., Ltd., Lilly Japan K.K., Ignyta,Inc. Janssen Pharmaceutical K.K., KISSEI PHARMACEUTICAL CO., LTD., Kyowa Kirin Co., Ltd., Loxo Oncology, Inc., MEDICAL & BIOLOGICAL LABORATORIES CO., LTD., Merck Biopharma Co., Ltd., Merus N.V., MSD K.K., NEC Corporation., Novartis Pharma K.K., Ono, Pfizer Japan Inc., Sumitomo Dainippon Pharma Co., Ltd., Spectrum Pharmaceuticals, Inc., Sysmex Corporation., Haihe Biopharma Co., Ltd., Taiho, Takeda, Turning Point Therapeutics, Inc. and honoraria from AZ, Amgen Astellas BioPharma K.K., Amgen Inc., Amoy Diagnosties Co., Ltd., BI Japan, Inc., BMS K.K., Bayer Yakuhin, Ltd., Chugai, DAIICHI SANKYO Co., Ltd., Eisai Co., Ltd., Eli Lilly Japan K.K., Guardant Health Inc., Janssen Pharmaceutical K.K., Kyowa Kirin Co., Ltd., Life Technologies Japan Ltd., MSD K.K., Novartis Pharma K.K., Ono Pharmaceutical Co., Ltd., Otsuka Pharmaceutical Co., Ltd., Pfizer Japan Inc., Taiho, Takeda. KS has potential financial conflicts as honoraria from AZ, Chugai, Ono, BMS Japan, MSD Oncology, Lilly Japan, Taiho, Nippon Kayaku and Novartis Pharma and as research funding from AZ, Nippon BI and Taiho.

Funding

This study was funded by AstraZeneca. This work was supported in part by the Japan Society for the Promotion of Science Grant to K.S. (19H03671).

CRediT authorship contribution statement

Hiroyuki Yasuda: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Eiki Ichihara:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Jun Sakakibara-Konishi:** Investigation. **Yoshitaka Zenke:** Investigation. **Shinji Takeuchi:** Investigation. **Masahiro Morise:** Investigation. **Katsuyuki Hotta:** Investigation. **Mineyoshi Sato:** Investigation. **Shingo Matsumoto:** Investigation. **Azusa Tanimoto:** Investigation. **Reiko Matsuzawa:** Investigation. **Katuyuki Kiura:** Investigation. **Yuta Takashima:** Investigation. **Seiji Yano:** Investigation. **Junji Koyama:** Investigation. **Takahiro Fukushima:** Investigation, Writing – review & editing. **Junko Hamamoto:** Investigation, Writing – review & editing. **Hideki Terai:** Investigation, Writing – review & editing. **Shinnosuke Ikemura:** Investigation, Writing – review & editing. **Ryo Takemura:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Writing – review & editing. **Koichi Goto:** Conceptualization, Investigation. **Kenzo Soejima:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank all the patients who participated in this trial and their families, staffs in Clinical and Translational Research Center, Keio University Hospital, Dr. Nobuhiko Seki, Prof. Katsuhiko Naoki and Prof. Inoue as IDMC members, and AstraZeneca for supporting the trial.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lungcan.2021.10.006.

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