Lung Cancer 98 (2016) 1–8

Lung Cancer

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

Tumor marker analyses from the phase III, placebo-controlled, FASTACT-2 study of intercalated erlotinib with gemcitabine/platinum in the first-line treatment of advanced non-small-cell lung cancer☆

Tony Mok[a,*], Guia Ladrera[b], Vichien Srimuninnimit[c], Virote Sriuranpong[d], Chong-Jen Yu[e], Sumitra Thongprasert[f], Jennifer Sandoval-Tan[g], Jin Soo Lee[h], Fatima Fuerte[i], David S. Shames[j], Barbara Klughammer[k], Matt Truman[k], Pablo Perez-Moreno[k], Yi-Long Wu[l,*]

[a] State Key Laboratory of Southern China, Department of Clinical Oncology, Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China
[b] Lung Center of the Philippines, Quezon Ave., Diliman, Quezon City, Metro Manila, Philippines
[c] Siriraj Hospital, 2 Wanglang Road, Bangkok, Bangkoknoi 10700, Thailand
[d] King Chulalongkorn Memorial Hospital, 1873 Rama 4 Road, Bangkok, Pathumwan 10330, Thailand
[e] National Taiwan University Hospital, No. 7, Zhongshan S Rd., Zhongzheng District, Taipei, Taiwan
[f] Faculty of Medicine, Chiangmai University, Chiang Mai, Thailand
[g] Philippine General Hospital, Taft Avenue Ermita, Brgy 670 Zone 72, Manila, 1000 Metro Manila, Philippines
[h] National Cancer Center, Goyang, Republic of Korea
[i] Rizal Medical Center, Pasig Blvd, Pasig, Metro Manila, Philippines
[j] Oncology Biomarker Development, Genentech Inc., South San Francisco, CA 94080, United States
[k] F. Hoffmann-La Roche Ltd., Basel, Switzerland
[l] Guangdong Lung Cancer Institute, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China

ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form 20 April 2016
Accepted 30 April 2016

Keywords:
NSCLC
Biomarkers
EGFR
Erlotinib

ABSTRACT

Objectives: The FASTACT-2 study of intercalated erlotinib with chemotherapy in Asian patients found that *EGFR* mutations were the main driver behind the significant progression-free survival (PFS) benefit noted in the overall population. Further exploratory biomarker analyses were conducted to provide additional insight.

Materials and methods: This multicenter, randomized, placebo-controlled, double-blind, phase III study investigated intercalated first-line erlotinib or placebo with gemcitabine/platinum, followed by maintenance erlotinib or placebo, for patients with stage IIIB/IV non-small cell lung cancer (NSCLC). Provision of samples for biomarker analysis was encouraged but not mandatory. The following biomarkers were analyzed (in order of priority): *EGFR* mutation by cobas® test, *KRAS* mutation by cobas® *KRAS* test, HER2 by immunohistochemistry (IHC), HER3 by IHC, ERCC1 by IHC, *EGFR* gene copy number by fluorescence in-situ hybridization (FISH) and EGFR by IHC. All subgroups were assessed for PFS (primary endpoint), overall survival (OS), non-progression rate and objective response rate.

Results: Overall, 256 patients provided samples for analysis. Considerable overlap was noted among biomarkers, except for *EGFR* and *KRAS* mutations, which are mutually exclusive. Other than *EGFR* mutations ($p < 0.0001$), no other biomarkers were significantly predictive of outcomes in a treatment-by-biomarker interaction test, although ERCC1 IHC-positive status was predictive of improved OS for the erlotinib arm versus placebo in *EGFR* wild-type patients (median 18.4 vs 9.5 months; hazard ratio [HR] HR = 0.32, 95% confidence intervals [CI]: 0.14–0.69, $p = 0.0024$).

Abbreviations: EGFR, epidermal growth factor receptor; TKIs, tyrosine-kinase inhibitors; NSCLC, non-small-cell lung cancer; PFS, progression-free survival; FISH, fluorescence in-situ hybridization; IHC, immunohistochemistry; ERCC1, excision repair cross-complementation group 1; ECOG, Eastern Cooperative Oncology Group; PS, performance status; RECIST, Response Evaluation Criteria in Solid Tumors; OS, overall survival; NPR, non-progression rate; ORR, objective response rate; FACT-L, Functional Assessment of Cancer Therapy—Lung (quality of life questionnaire).

☆ These data were previously presented at the European Society for Medical Oncology Annual Congress 2012, 28 September-2 October, Vienna, Austria.

* Corresponding authors.
E-mail addresses: tony@clo.cuhk.edu.hk (T. Mok), syylwu@live.cn (Y.-L. Wu).

http://dx.doi.org/10.1016/j.lungcan.2016.04.023

Conclusion: Activating *EGFR* mutations were predictive for improved treatment outcomes with a first-line intercalated regimen of chemotherapy and erlotinib in NSCLC. ERCC1 status may have some predictive value in *EGFR* wild-type disease, but requires further investigation.

1. Introduction

Activating mutations in the epidermal growth factor receptor *(EGFR)* are now validated as predictive biomarkers for EGFR tyrosine-kinase inhibitors (TKIs) as first-line treatment of patients with locally advanced or metastatic non-small-cell lung cancer (NSCLC) [1–3] and, ideally, all patients should be tested at initial diagnosis. However, *EGFR* mutation analysis is still not available for all patients and, in many cases, treatment decisions are made while *EGFR* mutation status is unknown [4,5]. Prevalence of *EGFR* testing varies geographically and over time. In a retrospective study of 1503 patients in Korea, the proportion of patients undergoing *EGFR* testing evolved from 23.3% (between January 2007 and July 2008) to 63.5% (between October 2009 and July 2010) [6], while in Canada in 2010/2011, it was estimated that only 38% of potentially eligible patients had *EGFR* testing initiated [7].

Patients with unknown *EGFR* mutation status should generally be treated with systemic chemotherapy, but a potential alternative option is sequential combination of chemotherapy and an EGFR TKI, particularly in countries with high rates of *EGFR* mutations [8,9]. FASTACT-2 (First-line Asian Sequential Tarceva and Chemotherapy Trial) was a large, confirmatory, phase III trial of sequential chemotherapy and erlotinib in a non-selected population of Asian patients with advanced NSCLC. FASTACT-2 met its primary endpoint: patients treated with chemotherapy intercalated with erlotinib had significantly longer progression-free survival (PFS) than patients treated with chemotherapy intercalated with placebo (median PFS 7.6 vs 6.0 months; hazard ratio [HR] = 0.57, 95% confidence interval [CI]: 0.47–0.69; $p < 0.0001$) [9]. Previously reported biomarker analyses of FASTACT-2 confirmed that only patients with *EGFR* mutation-positive NSCLC benefited significantly from intercalated chemotherapy and erlotinib (median PFS 16.8 months vs 6.9 months; HR = 0.25, 95% CI: 0.16–0.39; $p < 0.0001$). Additional exploratory analysis of important biomarkers was conducted on baseline tumor samples. These were markers that had been associated with clinical outcomes for EGFR TKIs in NSCLC or other cancers (*KRAS* mutations, *EGFR* gene copy number by fluorescence in-situ hybridization [FISH] and EGFR expression by immunohistochemistry [IHC]) [10–12] and markers for which there was scientific rationale to anticipate prognostic or predictive effects (HER2 protein expression by IHC, HER3 expression by IHC, and excision repair cross-complementation group 1 [ERCC1] expression by IHC) (see Suppl. Table S1 Table S1 for further details). The current report summarizes the prevalence and overlap of these biomarkers, and their relationships with efficacy outcomes in the FASTACT-2 study.

2. Methods

2.1. Study design and population

FASTACT-2 was a multicenter, randomized, placebo-controlled, double-blind, phase III study of intercalated erlotinib or placebo with gemcitabine plus either carboplatin or cisplatin (at investigators' discretion), followed by maintenance erlotinib or placebo, as first-line treatment in patients with stage IIIB/IV NSCLC. The study was undertaken in 28 Asian centers across China, Hong Kong, Indonesia, South Korea, the Philippines, Taiwan, and Thailand. Patients aged 18 years and older, with stage IIIB/IV NSCLC, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1 and measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST version 1.0) were eligible. Patients were randomly assigned to treatment in a 1:1 ratio and were stratified by disease stage, tumor histology, smoking status, and chemotherapy regimen. The full study methodology has been described previously [9]. Patients were randomized to receive six cycles of gemcitabine plus platinum with either sequential erlotinib or placebo on days 15–28 of each cycle. Patients who did not progress during the six cycles of sequential treatment continued to receive erlotinib or placebo until disease progression, unacceptable toxicity or death. At disease progression, treatment was unblinded; patients in the placebo group had the option to be crossed over to open-label erlotinib; patients in the erlotinib group could receive further treatment at the discretion of the investigator.

FASTACT-2 was approved by the institutional review board or ethics committee of each participating center and was performed in accordance with the principles of the Declaration of Helsinki and Guidelines for Good Clinical Practice. All patients provided written informed consent prior to any study-related procedure. The trial was registered on ClinicalTrials.gov (identifier NCT00883779).

2.2. Biomarker analysis

Patients provided separate consent for biomarker analysis. Tumor samples from first diagnosis or from biopsy at least 14 days prior to first dose of study drug could be provided for biomarker analysis (10–20 slides of a formalin-fixed, paraffin-embedded sample for histological procedures and 10 slides for cytological procedures).

Biomarkers analyzed were (in order of priority): *EGFR* mutation, *KRAS* mutation, HER2 by IHC, HER3 by IHC, ERCC1 by IHC, *EGFR* gene copy number by FISH and EGFR by IHC. The methodologies and criteria used are shown in Suppl. Table S2.

2.3. Statistical analyses

The primary endpoint of the study was PFS. Secondary endpoints included overall survival (OS); PFS and OS in subgroups; non-progression rate (NPR); objective response rate (ORR); duration of response; and quality of life (FACT-L). Disease control rate at 16 weeks was assessed in a post-hoc analysis. The methods and results for these outcomes have been reported previously [9]. In this report, we analyzed PFS and OS by *EGFR* and *KRAS* mutation status, EGFR protein expression status and *EGFR* gene copy status (prespecified secondary analyses). The evaluation of tumor biomarkers and correlation with treatment and outcomes was an exploratory objective. All patients who provided samples suitable for analysis were included in the analysis of biomarker data. The study was not powered for biomarker analysis; a sample size of 450 patients was estimated based on detecting an HR of 0.75 for PFS at 80% power with a 2-sided log-rank test and an α level of 5% (documented in Wu et al., 2013).

A multivariate model of all patients was generated using a stepwise selection procedure for PFS and OS, using the following covariates: treatment, age, ECOG PS, sex, disease stage, disease

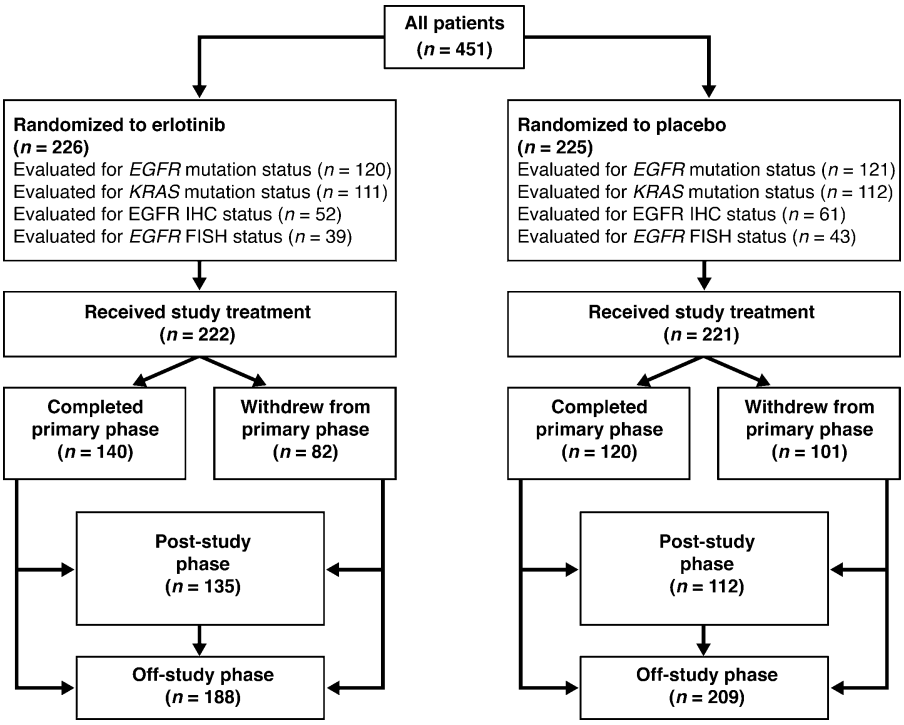

Fig. 1. CONSORT diagram.

histology, smoking status, chemotherapy regimen, *EGFR* mutation status, EGFR IHC status, HER2 status and HER3 status. Covariates could enter the model if they were significant at the 0.1 level and were dropped from the model if their significance fell below the 0.1 level. Treatment by biomarker interactions were assessed by adding appropriate terms to the final derived model. Patients must have had a result for all selected covariates to be included in these analyses.

PFS and OS by biomarker status were assessed by use of the Kaplan–Meier methods, with treatment effect expressed as a HR and two-sided 95% CI. There was no adjustment for multiple testing. ORR and NPR were analyzed by logistic regression and expressed as percentage differences between treatment groups with 95% CI. Biomarker subgroup analyses were presented graphically using forest plots. Statistical analyses were carried out using SAS (version 8.2). The analysis was undertaken at a cut-off date of 22 June 2012.

3. Results

A total of 256 patients provided tissue specimens that were suitable for exploratory evaluation (129 in the chemotherapy plus erlotinib group and 127 in the chemotherapy plus placebo group) (Table 1). Patient disposition in the study is shown in Fig. 1. Of the 241 patients evaluated for *EGFR* mutation, eight had single resistant mutations in exon 20 (one patient with T790M; one with S768I and six with insertion mutations) and were therefore excluded from the *EGFR* mutation data analyses. Of the 233 remaining patients, 97 (41.6%) were confirmed as having *EGFR* mutation-positive status and 136 (58.4%) had *EGFR* wild-type status. Other biomarker subgroups are detailed in Table 1. All biomarkers were balanced between the treatment groups. Considerable overlap was noted among the various biomarker subgroups, with the exception of *EGFR* and *KRAS* mutations, which are typically mutually exclusive (Fig. 2). In addition, some patients had more than one *EGFR* mutation (Suppl. Table S3) Statistically significant associations between biomarker subgroups were observed for *EGFR* mutations and *EGFR* FISH ($p = 0.002$); EGFR IHC and ERCC1 IHC ($p = 0.016$); EGFR IHC and HER2 IHC ($p = 0.025$); and HER2 IHC and HER3 IHC ($p = 0.006$).

3.1. Clinical characteristics

Baseline patient characteristics for the overall population have been described previously [9]. The biomarker evaluable population had similar baseline characteristics to the overall population. Clinical characteristics were balanced between the treatment groups

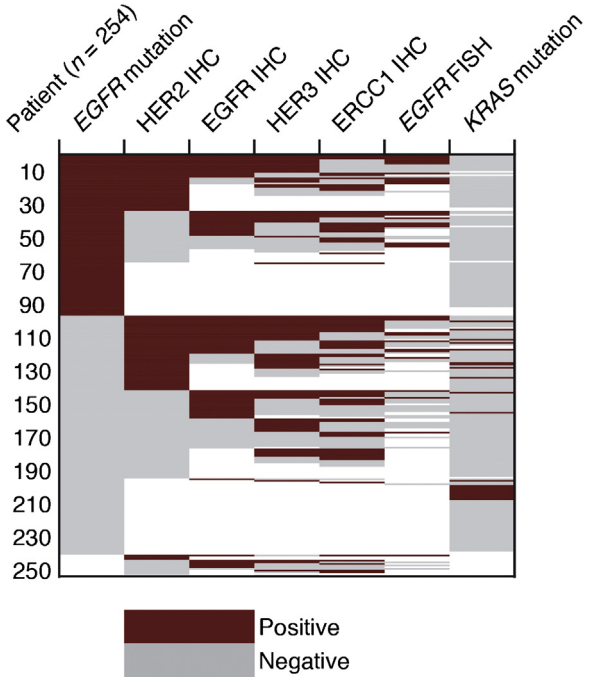

Fig. 2. Overlap of biomarkers in patients with tumor marker data in FASTACT-2.

Table 1
Baseline patient characteristics for the overall population and the biomarker-evaluable populations.

	All patients (*n* = 451)		All biomarker-evaluable patients (*n* = 256)		*EGFR* Mut+ (*n* = 97)		*EGFR* WT (*n* = 136)		*EGFR* unknown (*n* = 210)		*KRAS* Mut+ (*n* = 21)		*KRAS* WT (*n* = 202)		EGFR IHC+ (*n* = 76)		EGFR IHC– (*n* = 37)		*EGFR* FISH+ (*n* = 34)		*EGFR* FISH– (*n* = 48)		HER2 IHC + (*n* = 82)		HER2 IHC– (*n* = 93)	
	GCE *n* = 226	GCP *n* = 225	GCE *n* = 129	GCP *n* = 127	GCE *n* = 49	GCP *n* = 48	GCE *n* = 69	GCP *n* = 67	GCE *n* = 106	GCP *n* = 104	GCE *n* = 10	GCP *n* = 11	GCE *n* = 101	GCP *n* = 101	GCE *n* = 40	GCP *n* = 36	GCE *n* = 12	GCP *n* = 25	GCE *n* = 14	GCP *n* = 20	GCE *n* = 25	GCP *n* = 23	GCE *n* = 41	GCP *n* = 41	GCE *n* = 45	GCP *n* = 48
Sex, %																										
Male	58	62	53	61	43	48	59	76	66	63	80	73	51	60	53	58	58	60	50	65	44	52	56	61	56	56
Female	42	38	47	39	57	52	41	24	34	37	20	27	49	40	48	42	42	40	50	35	56	48	44	39	44	44
Disease stage, %																										
IIIB	9	11	8	9	2	4	16	12	8	13	10	0	12	10	3	3	17	20	0	10	4	9	7	10	13	8
IV	91	89	92	91	98	96	84	88	92	87	90	100	88	90	98	97	83	80	100	90	96	91	93	90	87	92
ECOG PS, %																										
0	26	26	26	21	27	26	30	25	24	27	20	36	31	25	28	33	33	20	36	30	32	30	34	25	18	19
1	74	74	74	79	73	74	70	75	76	73	80	64	69	75	73	67	67	80	64	70	68	70	66	75	82	81
Smoking status, %																										
Current smoker	29	29	26	29	16	15	32	39	33	31	60	55	22	24	23	17	17	28	21	20	20	9	27	34	24	23
Former smoker	22	23	19	21	12	17	25	30	25	23	20	18	20	26	23	31	42	20	29	35	16	30	24	22	18	21
Never smoker	50	48	55	50	71	69	43	31	42	46	20	27	58	50	55	53	42	52	50	45	64	61	49	44	58	56
Histology, %																										
Adenocarcinoma	77	75	80	75	92	92	70	67	75	70	90	91	76	78	80	75	83	88	86	90	76	74	90	93	76	69
Non-adenocarcinoma	23	25	20	25	8	8	30	33	25	30	10	9	24	22	20	25	17	12	14	10	24	26	10	7	24	31

	HER3 IHC+ (*n* = 71)		HER3 IHC– (*n* = 70)		ERCC1+ (*n* = 70)		ERCC1– (*n* = 71)	
	GC-E *n* = 39	GC-P *n* = 32	GC-E *n* = 29	GC-P *n* = 41	GC-E *n* = 35	GC-P *n* = 35	GC-E *n* = 34	GC-P *n* = 37
Sex, %								
Male	49	63	55	51	49	57	53	57
Female	51	38	45	49	51	43	47	43
Disease stage, %								
IIIB	8	13	3	5	6	9	12	8
IV	92	88	97	95	94	91	88	92
ECOG PS, %								
0	31	28	24	25	29	18	26	35
1	69	72	76	75	71	82	74	65
Smoking status, %								
Current smoker	18	34	28	20	11	26	29	22
Former smoker	23	22	21	22	20	23	24	22
Never smoker	59	44	52	59	69	51	47	57
Histology, %								
Adenocarcinoma	90	84	66	76	77	66	82	95
Non-adenocarcinoma	10	16	34	24	23	34	18	5

% values rounded to the nearest whole number.
ECOG PS: Eastern Cooperative Oncology Group performance status; Mut+: mutation positive; WT: wild type.

(Table 1). The majority of clinical characteristics for each biomarker subtype were consistent with the overall biomarker population.

3.2. Clinical endpoints by biomarker subgroups

Due to the well-known, dominant biology associated with activating mutations in *EGFR* and sensitivity to erlotinib and gefitinib, an analysis of the individual biomarkers in subgroups both with and without activating mutations was performed.

With the exception of *EGFR* mutations, no other biomarkers were predictive of outcomes with a positive treatment-by-biomarker interaction test (Suppl. Fig. S1A). The prolonged PFS with the intercalated regimen was statistically significant for most (9/15) of the biomarker groups; however, as this analysis did not account for multiple testing and was not hierarchical, the *p* values should be interpreted with caution.

Patients in the following biomarker subgroups had statistically significantly prolonged OS when receiving intercalated chemotherapy plus erlotinib compared with chemotherapy plus placebo: *EGFR* mutation positive, EGFR IHC positive, ERCC1 IHC positive, HER2 IHC positive, HER3 IHC positive, *EGFR* FISH positive (Suppl. Fig. S1B). Again, this analysis does not account for multiple testing and *p* values should be interpreted with caution. It should be noted that these are all the biomarker 'positive' subgroups with the exception of *KRAS* mutation-positive NSCLC (as this group consisted of only 21 patients, achieving statistical significance would be difficult in this analysis). Further data is provided on the HER2 and HER3 subgroups (see Supplementary information).

The pattern of effects of biomarker status on NPR was similar to that seen with PFS, with the exception that patients with *EGFR* unknown and *EGFR* wild-type status NSCLC did not appear to gain benefit from chemotherapy plus erlotinib versus chemotherapy plus placebo (Suppl. Table S4). For ORR, all biomarker subgroups showed a benefit from chemotherapy plus erlotinib versus chemotherapy plus placebo, and the majority (13/15) were statistically significant.

It should be noted that a significant benefit for chemotherapy plus erlotinib compared with chemotherapy plus placebo was observed in PFS, OS and ORR (but not NPR) for the overall subgroup of patients who were evaluated for biomarkers (Suppl. Fig. S1A and B).

3.3. Predictive power of biomarker status in patients with known EGFR mutation-positive or EGFR wild-type NSCLC

Considering *EGFR* mutation to be the most potent predictive biomarker, we analyzed each biomarker subgroup according to *EGFR* mutation status. None of the biomarkers were predictive of PFS or OS in patients with sensitizing *EGFR* mutations (Fig. 3A and B). *EGFR* FISH positivity was predictive of better OS outcomes (Fig. 3D; HR for *EGFR* FISH-positive vs FISH-negative = 0.34, 95% CI: 0.14–0.80; $p = 0.0142$) but not PFS benefit (Fig. 3C); however, this was only for patients with *EGFR* wild-type NSCLC. A significant prolongation of OS was observed for patients with *EGFR* wild-type and ERCC1 IHC-positive status who received chemotherapy plus erlotinib compared with chemotherapy plus placebo (median OS 18.4 vs 9.5 months; HR = 0.32, 95% CI: 0.14–0.69; $p = 0.0024$), but this was not mirrored in the PFS results: median PFS was 7.5 versus 4.6 months (HR = 0.55, 95% CI: 0.27–1.12, $p = 0.0941$) (Fig. 4).

Exploratory multivariate analyses were also carried out; these are presented as Supplementary information.

4. Discussion

FASTACT-2 was the first randomized phase III study to show an improvement in both PFS and OS with a first-line intercalated regiment of chemotherapy and erlotinib, although the benefit was confined largely to a subgroup of patients whose tumor tissue tested positive for an activating *EGFR* mutation (exon 19 deletions and L858R) or had unknown *EGFR* mutation status. The current report reinforces the fact that the predictive power of *EGFR* mutation dominates over other molecular biomarkers for EGFR TKI-treated NSCLC. *KRAS* mutation is known to be generally mutually exclusive of *EGFR* mutation, thus the benefit seen in the *KRAS* wild-type subgroup was best explained by the presence of *EGFR* mutations, as confirmed when the *EGFR* wild-type and *KRAS* wild-type subgroup was analyzed (Fig. 3C and D). EGFR IHC status was not independently predictive of treatment outcomes in this study, as both EGFR IHC-positive and −negative subgroups had significant survival benefits with the intercalated regimen. Similar findings were observed in the IPASS study (first-line gefitinib vs carboplatin/paclitaxel), which reported an interaction *p* value of 0.214 with EGFR expression by IHC [13], in the SATURN study of maintenance erlotinib therapy [14] and in the BR.21 study of second-/third-line therapy [12]. Consistent with previous studies, *EGFR* FISH was not independently predictive of outcome. Survival benefit was greater in the *EGFR* FISH-positive subgroup (HR = 0.27, $p = 0.0112$), compared with the *EGFR* FISH-negative subgroup (HR = 0.65, $p = 0.1554$), but again this was likely due to a higher incidence of *EGFR* mutations in the former subgroup. In the IPASS study, 78% of patients with *EGFR* FISH-positive status tested positive for the *EGFR* mutation, while only 33% of patients who were confirmed to have *EGFR* FISH-negative status had the mutation. In FASTACT-2, similar findings were observed with 21/34 (62%) and 12/48 (25%) patients with *EGFR* FISH-positive and -negative status, respectively, having *EGFR* mutations. Patients with *EGFR* mutation-positive disease who had EGFR IHC-negative status or *EGFR* FISH-negative status failed to attain statistical significant benefit, which may be explained by the small sample size ($n = 12$ in both groups).

The HER/ErbB family of receptor tyrosine kinases are major drivers of cellular growth and proliferation in both normal and cancer cells. Ligand dependent activation of ErbB signaling is mediated through ligand dependent homo- and heterodimerization between members of the receptor family [15]. More specifically, EGFR phosphorylation has been shown to occur through homodimerization between HER1–HER1 and heterodimerization between HER1–HER2, as well as HER1–HER3 [16–18]. It was of interest, therefore, to determine whether any additional clinical activity of erlotinib might be accounted for by overexpression and concomitant activation of EGFR through other ErbB family members. Approximately 40% of patients assessed for HER2 or HER3 status also had *EGFR* activating mutations. No specific association was seen between *EGFR* mutations and HER2 or HER3 expression. Despite the better survival outcomes with the intercalated combination in patients with positive HER2 or HER3 expression (Suppl. Fig. S2), neither of these biomarkers were considered to be independent predictors as their interaction tests were negative.

ERCC1 may be an important factor for the benefit of the intercalated treatment regimen. The ERCC1 protein plays a role in restoring DNA from platinum damage, by its involvement in the nucleotide excision repair pathway and the interstrand cross-link repair pathway [19]. ERCC1 may be a potential predictor of treatment outcome with cytotoxic chemotherapy [19–21], although results have been mixed and there have been some controversies regarding the quality of ERCC1 reagents [22,23]. Generally patients with low ERCC1 expression were reported to have better tumor response and longer survival with platinum-based regimens [24]. The observation in FASTACT-2 was contrary to current thinking, finding that patients with ERCC1 IHC-positive status, among the *EGFR* wild-type population, benefited more from the intercalated regimen than patients with ERCC1 IHC-negative status. A pre-clinical study on the poten-

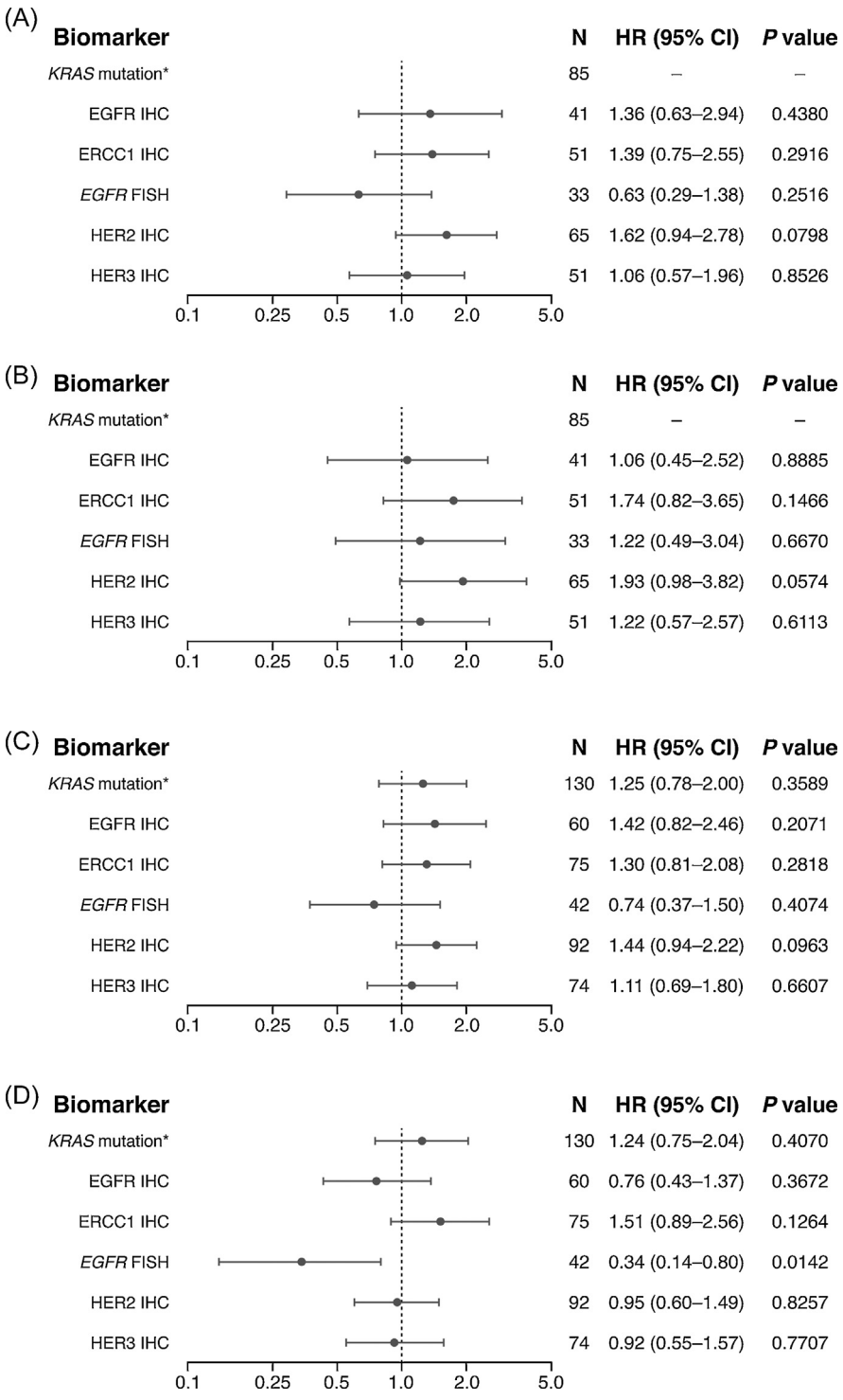

Fig. 3. Forest plots of (A) PFS and (B) OS for patients with *EGFR* mutation-positive NSCLC by biomarker subgroups, and (C) PFS and (D) OS for patients with *EGFR* wild-type NSCLC by biomarker subgroups.
*No patients with *EGFR* mutation-positive disease had a concurrent *KRAS* mutation.

tial impact of EGFR TKIs on ERCC1 expression noted a progressive reduction of ERCC1 expression within 72 h of EGFR TKI exposure in H358 and H1993 cell lines. As a result, the IC_{50} of the studied H358 and H1993 cell lines was reduced, which implied an increased sensitivity to cisplatin in EGFR TKI-treated cells [25]. One potential explanation for the clinical observation was that the EGFR TKI component of the intercalated combination may have reduced ERCC1 expression in the ERCC1 IHC-positive patients, thus showing a superior effect over the same patient group with exposure only to chemotherapy. This hypothesis warrants further translational investigation.

A number of factors should be considered when interpreting the results of this study, as tumor sample collection was optional and samples were available in less than half of the patients enrolled, which may not be representative of the study population. Also, as the biomarker analyses were exploratory in nature, the study was

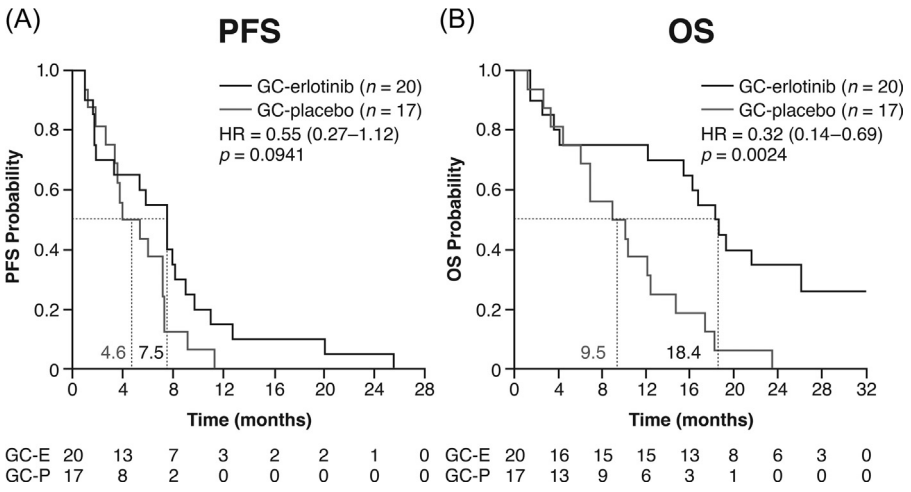

Fig. 4. Kaplan–Meier plots for (A) PFS and (B) OS in patients with *EGFR* wild-type and ERCC1 IHC-positive status.

not statistically powered for this. Additionally, at the time of study design, we aimed to evaluate the most relevant biomarkers, but some of these markers have since been shown to be outdated and have been removed from clinical practice, e.g. EGFR protein expression and *EGFR* gene copy number according to Colorado score [26]. However, recent research showed that *EGFR* gene copy number according to READ MAX [27] could be a valuable asset in identifying benefit from erlotinib treatment in *EGFR*-WT disease.

5. Conclusion

In conclusion, *EGFR* mutation remains the main predictive biomarker for better treatment outcomes with a first-line intercalated combination regimen of chemotherapy and erlotinib for NSCLC. Protein expression of HER2 and HER3 were not independently predictive of treatment benefit, but ERCC1 expression was predictive of treatment outcomes in patients with *EGFR* wild-type disease. This observation is hypothesis generating and warrants further research for validation.

Conflict of interest

BK and PPM have stock ownership and are employees of F. Hoffman-La Roche. TM has received consultancy fees from AstraZeneca, BI, Clovis Oncology, Eisai, Eli Lilly, Janssen, Roche/GNE, MSE, Merck Soreno, Amgen, Novartis, GSK, Pfizer, Biomarin, Novartis and has received payments from Astra Zeneca, Pfizer, Merck Soreno, BI, Eli Lilly, Amgen, Roche. DS is an employee of Genentech and has patents with Genentech Inc., UT Southwestern Medical Center at Dallas and royalties from Alnylam Pharmaceuticals and has stock ownership in Roche Holdings. MT is an employee of Roche Pharmaceuticals and has received consultancy fees from Vifor Pharmaceuticals, Aurinia Pharmaceuticals. YLW has received payments from Roche, AstraZeneca, Eli Lilly, Sanofi. JT participated in a Speakers bureau for Roche. V Sriuranpong received payment for travel from Roche. JSL's institution received a grant from Roche Korea in the past. For the remaining authors none were declared.

Funding

This trial was designed, funded by and monitored by F. Hoffmann-La Roche. Data were collected by the investigators, collated by F. Hoffmann-La Roche (via the CRO, Tata Consultancy Services [TCS], India) and all analysis and interpretation of the data was carried out by Roche Molecular Diagnostics, Roche Global Medical Affairs (Biometrics) and HistoGeneX, with input from the authors. The initial draft of the manuscript was reviewed and commented on by all authors, and by employees of F. Hoffmann-La Roche. The corresponding author had full access to the study data and took full responsibility for the final decision to submit the paper.

Acknowledgments

The study was sponsored by F. Hoffmann-La Roche Ltd., the Chinese Thoracic Oncology Group (CTONG0902), and the Lung Cancer Research Group. Support for third-party writing assistance for this manuscript was funded by F. Hoffmann-La Roche Ltd.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.lungcan.2016.04.023.

References

[1] T. Mok, Y.L. Wu, S. Thongprasert, C.H. Yang, D.T. Chu, N. Saijo, et al., Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma, N. Engl. J. Med. 361 (2009) 947–957.
[2] C. Zhou, Y.L. Wu, G. Chen, J. Feng, X.Q. Liu, C. Wang, et al., Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study, Lancet Oncol. 12 (2011) 735–742.
[3] R. Rosell, E. Carcerency, R. Gervais, A. Vergnenegre, B. Massuti, E. Felip, et al., Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced *EGFR* mutation-positive non-small cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial, Lancet Oncol. 13 (2012) 239–246.
[4] C. Xue, Z. Hu, W. Jiang, Y. Zhao, F. Xu, Y. Huang, et al., National survey of the medical treatment status for non-small cell lung cancer (NSCLC) in China, Lung Cancer 77 (2012) 371–375.
[5] C. Xu, Q. Zhou, Y.L. Wu, Can EGFR TKIs be used in first-line treatment for advanced non-small cell lung cancer based on selection according to clinical factors? A literature-based meta-analysis, J. Haematol. Oncol. 5 (2012) 62–77.
[6] Y.L. Choi, J.M. Sun, J. Cho, S. Rampal, J. Han, B. Parasuraman, et al., EGFR mutation testing in patients with advanced non-small cell lung cancer: a comprehensive evaluation of real-world practice in an East Asian tertiary hospital, PLoS One 8 (2013) e56011.
[7] P.M. Ellis, S. Verma, S. Sehdev, J. Younas, N. Leighl, Implementation of a national *EGFR* testing strategy in a publicly funded health system, J. Thorac. Oncol. 6 (2011) S1513.
[8] T.S. Mok, Y.L. Wu, C.J. Yu, C. Zhou, Y.M. Chen, L. Zhang, et al., Randomized, placebo-controlled, phase II study of sequential erlotinib and chemotherapy as first-line treatment for advanced non-small-cell lung cancer, J. Clin. Oncol. 27 (2009) 5080–5087.

[9] J.L. Wu, J.S. Lee, S. Thongprasert, C.J. Yu, L. Zhang, G. Ladrera, et al., Intercalated combination of chemotherapy and erlotinib for patients with advanced stage non-small-cell lung cancer (FASTACT-2): a randomised, double-blind trial, Lancet Oncol. 14 (2013) 777–786.
[10] D.A. Eberhard, B.E. Johnson, L.C. Amler, A.D. Goddard, S.L. Heldens, R.S. Herbst, et al., Mutations in the epidermal growth factor receptor and in *KRAS* are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib, J. Clin. Oncol. 23 (2005) 5900–5909.
[11] W. Brugger, N. Triller, M. Blasinska-Morawiec, S. Curescu, R. Sakalauskas, G.M. Manikhas, et al., Prospective molecular marker analyses of EGFR and KRAS from a randomized, placebo-controlled study of erlotinib maintenance therapy in advanced non-small-cell lung cancer, J. Clin. Oncol. 29 (2011) 4113–4120.
[12] C.-Q. Zhu, G. da Cunha Santos, K. Ding, A. Sakurada, J. Cutz, C, N. Liu, et al., Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21, J. Clin. Oncol. 26 (2008) 428–475.
[13] M. Fukuoka, Y.-L. Wu, S. Thongprasert, P. Sunpaweravong, S.S. Leong, V. Sriuranpong, et al., Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS), J. Clin. Oncol. 29 (2011) 2866–2874.
[14] J. Mazières, W. Brugger, F. Cappuzzo, P. Middel, A. Frosch, I. Bara, et al., Evaluation of EGFR protein expression by immunohistochemistry using H-score and the magnification rule: re-analysis of the SATURN study, Lung Cancer 82 (2013) 231–237.
[15] H. Shankaran, Y. Zhang, Y. Tan, H. Resat, Model-based analysis of HER activation in cells co-expressing EGFR, HER2 and HER3, PLoS Comput. Biol. 9 (8) (2013) e1003201.
[16] G. Schaefer, L. Haber, L.M. Crocker, S. Shia, L. Shao, D. Dowbenko, et al., A two-in-one antibody against HER3 and EGFR has superior inhibitory activity compared with monospecific antibodies, Cancer Cell 20 (2011) 472–486.
[17] T.R. Wilson, D.Y. Lee, L. Berry, D.S. Shames, J. Settleman, Neuregulin-1-mediated autocrine signaling underlies sensitivity to HER2 kinase inhibitors in a subset of human cancers, Cancer Cell 20 (2011) 158–172.
[18] D.S. Shames, J. Carbon, K. Walter, A.M. Jubb, C. Kozlowski, T. Januario, et al., High heregulin expression is associated with activated HER3 and may define an actionable biomarker in patients with squamous cell carcinomas of the head and neck, PLoS One 8 (2013) e56765.
[19] E. Felip, P. Martinez, Can sensitivity to cytotoxic chemotherapy be predicted by biomarkers? Ann. Oncol. 23 (2012) 189–192.
[20] K. Azuma, Y. Komohara, T. Sasada, Y. Terazaki, J. Ikeda, T. Hoshino, et al., Excision repair cross-complementation group 1 predicts progression-free and overall survival in non-small cell lung cancer patients treated with platinum-based chemotherapy, Cancer Sci. 98 (2007) 1336–1343.
[21] P. Ceppi, M. Volante, S. Novello, I. Rapa, K.D. Danenberg, P.V. Danenberg, et al., ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine, Ann. Oncol. 17 (2006) 1818–1825.
[22] K.A. Olaussen, A. Dunant, P. Fouret, E. Brambilla, F. André, V. Haddad, et al., DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy, N. Engl. J. Med. 355 (2006) 983–991.
[23] L. Friboulet, K.A. Olaussen, J.P. Pignon, F.A. Shepherd, M.S. Tsao, S. Graziano, et al., ERCC1 isoform expression and DNA repair in non-small-cell lung cancer, N. Engl. J. Med. 368 (2013) 1101–1110.
[24] D. Yan, P. Wei, G. An, W. Chen, Prognostic potential of ERCC1 protein expression and clinicopathologic factors in stage III/N2 non-small cell lung cancer, J. Cardiothorac. Surg. 8 (2013) 149.
[25] L.H.T. Cheong, C.W.C. Hui, F. Xu, T.S.K. Mok, C.H. Wong, Down-regulation of ERCC1 by EGFR-TKI improves platinum-based chemotherapy efficacy for non-small cell lung cancer (NSCLC), J. Thorac. Oncol. 9 (Suppl. 9) (2014) S7–52.
[26] F.R. Hirsch, M. Verella-Garcia, P.A. Bunn Jr., W.A. Franklin, R. Dziadziuszko, Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small-cell lung cancer, J. Clin. Oncol. 24 (2006) 5034–5042.
[27] J. Moecks, D. Soulieres, B. Klughammer, ReadMax—a novel reading and scoring approach for *EGFR* gene copy number to predict therapeutic benefit of erlotinib treatment in *EGFR* wild-type non-small-cell lung cancer, J. Pathol. Clin. Res. 1 (2015) 134–143.