



# Evaluation of EGFR protein expression by immunohistochemistry using H-score and the magnification rule: Re-analysis of the SATURN study

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## ABSTRACT

**Introduction:** The phase III SATURN study demonstrated that first-line maintenance erlotinib extended progression-free survival (PFS) and overall survival (OS) versus placebo in patients with advanced non-small cell lung cancer (NSCLC). Analysis of epidermal growth factor receptor (EGFR) expression by immunohistochemistry (IHC) found no significant interaction between EGFR IHC status and PFS ( $p = 0.63$ ) or OS ( $p = 0.52$ ). The FLEX study of first-line cetuximab plus chemotherapy demonstrated that EGFR IHC expression was predictive of improved OS with cetuximab when assessed by H-score with a magnification rule. This novel method was used to reassess samples from SATURN.

**Methods:** The H-score method assigned a score of 0–300 to each patient, based on the percentage of cells stained at different intensities viewed at various magnifications. The discriminatory threshold was set at 200, per the FLEX study, and existing samples were re-read and classed as low (H-score < 200) or high ( $\geq 200$ ) EGFR expression. PFS and OS were re-analyzed based on these new classifications.

**Results:** In the overall and *EGFR* wild-type populations, erlotinib provided a consistent survival benefit versus placebo. Hazard ratios (HRs) in the overall population were similar between EGFR IHC-positive and -negative patients for median PFS (HR 0.68 [95% confidence interval (CI) 0.53–0.86] and 0.76 [95% CI 0.62–0.93], respectively) and OS (HR 0.80 [95% CI 0.62–1.05] and 0.80 [95% CI 0.64–1.01] for IHC-positive and IHC-negative, respectively). In the *EGFR* wild-type population, HRs were again similar between EGFR IHC-positive and -negative subpopulations for PFS (HR 0.69 [95% CI 0.51–0.95] and 0.84 [95% CI 0.63–1.12], respectively) and OS (HR 0.78 [95% CI 0.55–1.10] and 0.76 [95% CI 0.55–1.05], respectively).

**Conclusions:** These data suggest that EGFR IHC does not have value as a marker to predict erlotinib benefit in the first-line maintenance setting for advanced NSCLC.

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## 1. Introduction

Cytotoxic chemotherapy treatment for patients with metastatic non-small cell lung cancer (NSCLC) has reached a plateau [1]. Strategies to further improve outcomes for these patients include customized chemotherapy regimens and the integration of targeted therapy. One of the major targets in lung oncogenesis is

the epidermal growth factor receptor (EGFR), which belongs to the ErbB family of transmembrane tyrosine-kinase (TK) receptors. EGFR moderates the activation of a signaling pathway controlling cell proliferation, invasion, metastasis and angiogenesis. This pathway also plays a role in inhibiting apoptosis. Blocking EGFR function has been proven to be an effective treatment strategy across multiple tumor types whether by TK inhibitors (TKIs) such as erlotinib or antibodies such as cetuximab [2–7].

Erlotinib, an orally available EGFR TKI, has proven efficacy as a second- or third-line treatment for NSCLC patients with progressive disease after first-line chemotherapy [2], and as first-line therapy in patients whose tumors harbor activating *EGFR* mutations [3,4]. The efficacy of erlotinib as maintenance therapy in patients with non-progressive disease following

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first-line platinum-doublet chemotherapy for NSCLC was also demonstrated in the double-blind, randomized, phase III SATURN study (BO18192). Erlotinib-treated patients achieved significantly longer progression-free survival (PFS) than placebo-treated patients ( $p < 0.0001$ ), regardless of clinical characteristics [8]. With regards to the co-primary endpoint, namely PFS in patients with high EGFR protein expression as assessed by immunohistochemistry (IHC), PFS was significantly longer in patients with EGFR IHC-positive tumors who received erlotinib versus placebo ( $p < 0.0001$ ). EGFR IHC-positive disease was defined in SATURN as any membrane staining in  $\geq 10\%$  of tumor cells. A prospective biomarker analysis from this study found that the interaction between treatment and EGFR IHC status was not significant for PFS ( $p = 0.63$ ) or overall survival (OS;  $p = 0.52$ ), suggesting no differential effect of erlotinib between IHC-positive and IHC-negative groups [9].

Cetuximab, a chimeric monoclonal antibody targeting EGFR, has also been investigated in advanced NSCLC. In a major phase III clinical trial, the FLEX study, the investigators demonstrated that the addition of first-line cetuximab to cisplatin and vinorelbine significantly improved OS ( $p = 0.044$ ) compared with chemotherapy alone in patients with stage IV NSCLC [6]. In an attempt to increase the clinical benefit–risk ratio of this combination, the investigators examined the expression of EGFR by IHC as a potential predictive factor [10]. They used the H-score method with magnification rule, as previously proposed by Hirsch et al. [11] to define staining intensity across different categories [12]. A score was assigned to each patient on a continuous scale of 0–300 with an outcome-based discriminatory threshold calculated at 200. Based on this categorization, EGFR IHC-positive status (H-score  $\geq 200$ ) was associated with improved OS for patients who received cetuximab, whereas patients with EGFR IHC-negative status (H-score  $< 200$ ) had no OS benefit with cetuximab [10].

We hypothesized that this scoring system with magnification rule might help to predict outcomes in patients treated with EGFR TKIs as maintenance therapy. We therefore re-examined existing available samples from the SATURN study using this alternative EGFR IHC reading and scoring method, to determine whether the new classification would lead to any correlation between EGFR IHC status and survival outcomes with erlotinib in this setting.

## 2. Materials and methods

### 2.1. Study design

Between December 2005 and May 2008, 1949 patients were screened and received platinum-doublet chemotherapy. A total of 889 patients had non-progressive disease after chemotherapy and were suitable for randomization into the SATURN study. Following stratification (according to EGFR IHC status, disease stage, Eastern Cooperative Oncology Group [ECOG] performance status [PS], chemotherapy regimen, smoking status and region), patients were randomized to receive either erlotinib (150 mg/day) or placebo until disease progression or unacceptable toxicity. The SATURN inclusion/exclusion criteria and methodology are further detailed in the original manuscript [8]. The study was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The protocol was approved by local ethics committees at each investigating center. All patients gave informed written consent, both for study participation and the provision of a tumor sample.

### 2.2. IHC analyses

In the pre-specified SATURN study analyses (SATURN protocol-defined EGFR IHC), EGFR protein expression was assessed by IHC with the Dako EGFR PharmDx kit (DakoCytomation, Berkeley, CA).

Samples were classified as EGFR IHC positive if  $\geq 10\%$  of the tumor cells demonstrated membranous staining of any intensity. At the time of the prospective pre-planned analysis, an exploratory H-score-based (without magnification rule) cut-off search was also undertaken to determine a threshold for patient benefit according to EGFR IHC expression. All patients seemed to benefit, therefore a cut-off based on this marker could not be determined (Fig. 1).

The updated H-score method (EGFR IHC by H-score with magnification rule), first developed in 2003 by Hirsch et al. [11] was recently adapted for the FLEX study by Pirker et al. [10]. This method assigns an IHC H-score to each patient on a continuous scale of 0–300, based on the percentage of cells at different staining intensities visualized at different magnifications (unlike the previously used H-score method visualized at one magnification) [10]. Membrane staining was scored according to four categories: 0 for 'no staining', 1+ for 'light staining visible only at high magnification', 2+ for 'intermediate staining' and 3+ for 'dark staining of linear membrane, visible even at low magnification' as seen in Supplementary Fig. 1. The percentage of cells at different staining intensities was determined by visual assessment, with the score calculated using the formula  $1 \times (\% \text{ of } 1+ \text{ cells}) + 2 \times (\% \text{ of } 2+ \text{ cells}) + 3 \times (\% \text{ of } 3+ \text{ cells})$  [10]. As per the FLEX analysis, the outcome-based discriminatory threshold IHC H-score for this analysis was set at 200 and existing samples were re-read and scored according to the above method. Samples were then classified as either low (H-score  $< 200$ ; IHC negative) or high ( $\geq 200$ ; IHC positive) for EGFR protein expression. A secondary analysis was also carried out using the new reading results with the original protocol-defined designation of EGFR IHC-positive status as  $\geq 10\%$  any membrane staining.

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.lungcan.2013.07.016>.

The IHC scoring assessment was performed by a commercial lab, Targos Advance (Kassel, Germany). EGFR IHC scoring of all cases was performed by a senior pathologist. All equivocal cases with H-score between 150 and 250 and any cases with non-specific staining, fixation artifacts or pseudomembranous staining were scored blinded by a second board-certified pathologist. All cases which were found to be discrepant in positive or negative score were reanalyzed and discussed by both pathologists before a final score was given.

### 2.3. Statistical analysis

PFS and OS were analyzed in terms of hazard ratios [HRs] and 95% confidence intervals [CI] by Cox model, with log-rank  $p$ -values to assess significance (by EGFR IHC status using the 'protocol-defined' and 'H-score with magnification rule' methods). The  $p$ -values for 'H-score with magnification rule (200 score cut-off)' and H-score with magnification (10% staining cut-off) were exploratory in nature, as they were not adjusted for multiple testing.

## 3. Results

The prospective SATURN EGFR IHC analysis used samples from 370 and 372 patients in the erlotinib and placebo arms, respectively. The current analysis examined existing available samples from 351 and 361 patients in the erlotinib and placebo arms, respectively.

By applying the H-score with magnification rule using a threshold of 200, we identified 303 patients in the high-score, EGFR IHC-positive group ( $\geq 200$ ) and 409 patients in the low-score, EGFR IHC-negative group ( $< 200$ ). Baseline characteristics were generally

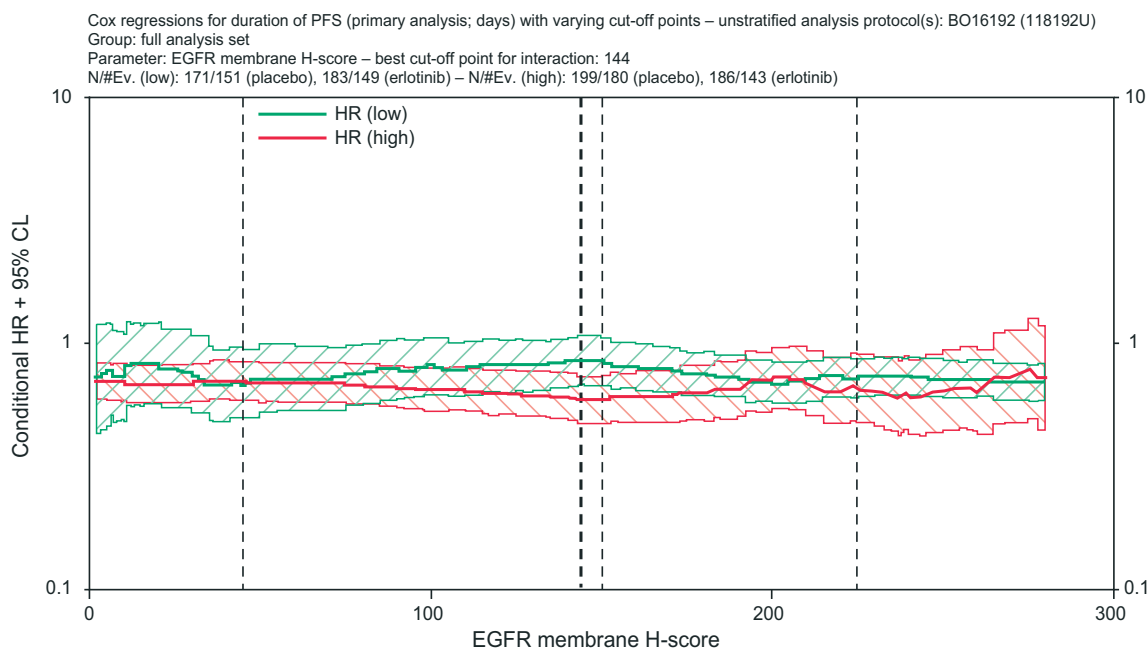


Fig. 1. Evolution chart using H-score (magnification rule not applied).

similar between the overall SATURN population and the EGFR IHC subgroups, however the EGFR IHC-positive group did include more patients with squamous-cell histology compared with the IHC-negative group, as already observed in the FLEX study [6] (Table 1). The patients with EGFR wild-type disease also had similar baseline characteristics to the overall population. Of the 189 patients with EGFR wild-type disease in the placebo arm and the 199 patients with wild-type disease in the erlotinib arm, 181 and 189 patients, respectively, had valid H-score with magnification rule result.

Using the H-score assessment with the magnification rule, the HR in the overall intent-to-treat (ITT) population was similar between patients with EGFR IHC-positive and -negative tumors for median PFS (HR 0.68 and 0.76, respectively) and median OS (HR 0.80 for both IHC scores), showing little difference in PFS or OS between patients with IHC H-score-positive and -negative

status. Despite the difference in categorization, the HRs for median PFS and median OS comparisons were similar between the two scoring methods (Table 2). In the unselected population, erlotinib demonstrated significantly prolonged PFS compared with placebo in patients with high EGFR IHC status regardless of the method used ( $p < 0.0001$  for protocol-defined method and exploratory  $p = 0.0010$  for H-score with magnification rule).

In the EGFR wild-type (WT) population, erlotinib provided a consistent survival benefit versus placebo, regardless of IHC scoring method used (Table 2). The PFS for the population with protocol-defined IHC-positive disease was 12.1 weeks for the erlotinib arm compared with 10.4 weeks for the placebo arm (HR 0.79,  $p = 0.0336$ ; Fig. 2a). For patients with IHC-negative disease, PFS was 10.9 weeks versus 7.1 weeks (HR 0.65,  $p = 0.1146$ ) for erlotinib and placebo, respectively. When assessed by the H-score with magnification

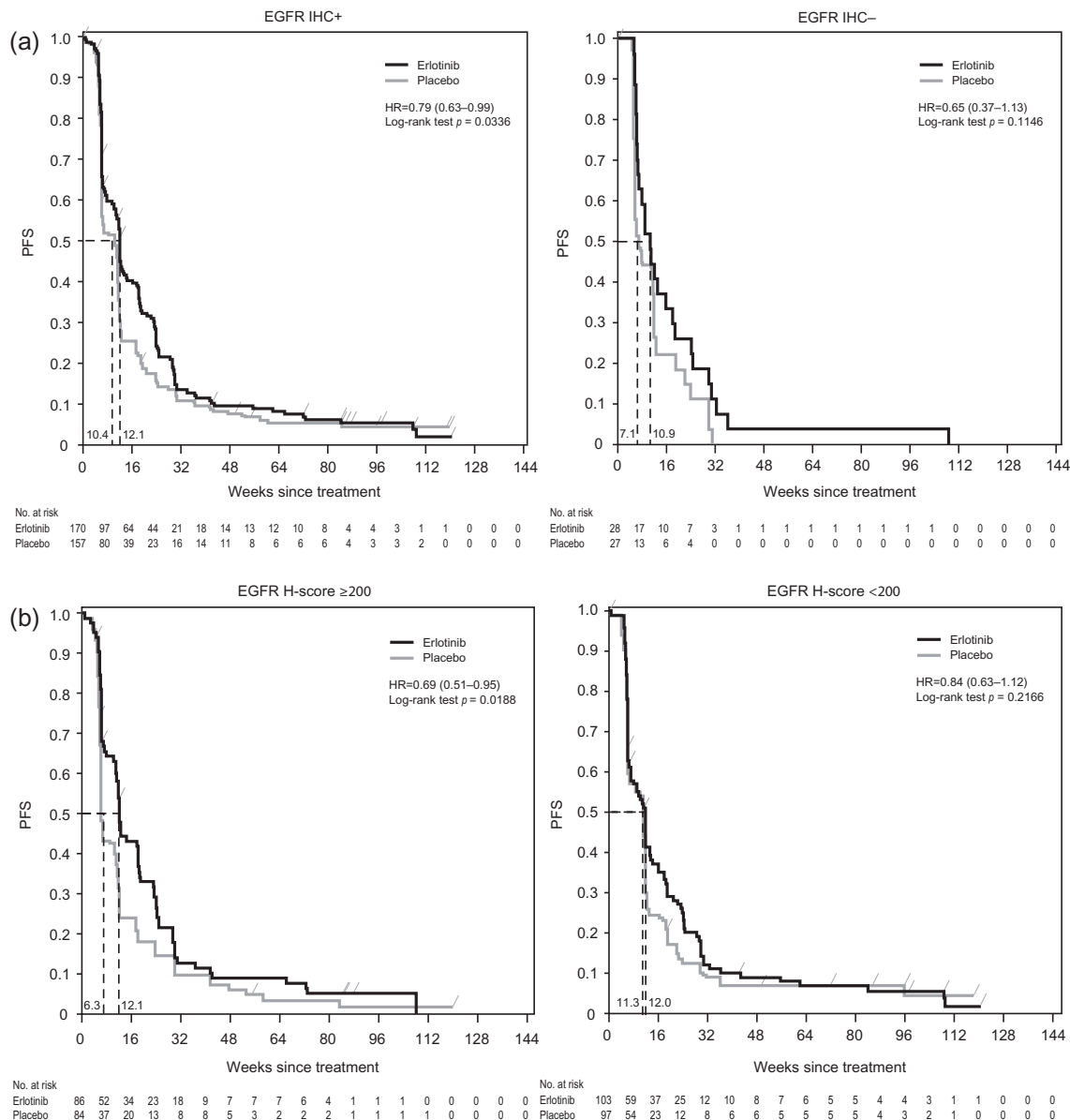
Table 1  
 Baseline characteristics in the overall population and EGFR wild-type population versus the EGFR IHC high and low subgroups.

N (%)	Overall SATURN population		EGFR wild-type population		EGFR IHC low (H-score <200) population		EGFR IHC high (H-score ≥200) population	
	Erlotinib (n=438)	Placebo (n=451)	Erlotinib (n=199)	Placebo (n=189)	Erlotinib (n=205)	Placebo (n=204)	Erlotinib (n=146)	Placebo (n=157)
Characteristic								
Gender								
Male	321 (73)	338 (75)	161 (81)	150 (79)	148 (72)	152 (75)	108 (74)	113 (72)
Female	117 (27)	113 (25)	38 (19)	39 (21)	57 (28)	52 (25)	38 (26)	44 (28)
Median age, years (range)	60 (33–83)	60 (30–81)	60 (35–83)	59 (30–76)	59.2 (33–81)	58.9 (33–81)	59.9 (35–83)	60.6 (30–77)
Disease stage								
IIIB	116 (26)	109 (24)	55 (28)	47 (25)	52 (25)	48 (24)	39 (27)	40 (25)
IV	322 (74)	342 (76)	144 (72)	142 (75)	153 (75)	156 (76)	107 (73)	117 (75)
ECOG PS								
0	135 (31)	145 (32)	64 (32)	69 (37)	61 (30)	68 (33)	50 (34)	49 (31)
1	303 (69)	306 (68)	135 (68)	120 (63)	144 (70)	136 (67)	96 (66)	108 (69)
Histology								
Adenocarcinoma/ Bronchoalveolar carcinoma	205 (47)	198 (44)	83 (42)	76 (40)	113 (55)	96 (47)	56 (38)	61 (39)
Squamous-cell carcinoma	166 (38)	194 (43)	95 (48)	96 (51)	60 (29)	78 (38)	73 (50)	77 (49)
Other	67 (15)	59 (13)	21 (11)	17 (9)	32 (16)	30 (15)	17 (12)	19 (12)
Smoking status								
Current smoker	239 (55)	254 (56)	116 (58)	110 (58)	113 (55)	109 (53)	75 (51)	88 (56)
Former smoker	122 (28)	122 (27)	58 (29)	57 (30)	58 (28)	60 (29)	44 (30)	41 (26)
Never smoker	77 (18)	75 (17)	25 (13)	22 (12)	34 (17)	35 (17)	27 (18)	28 (18)

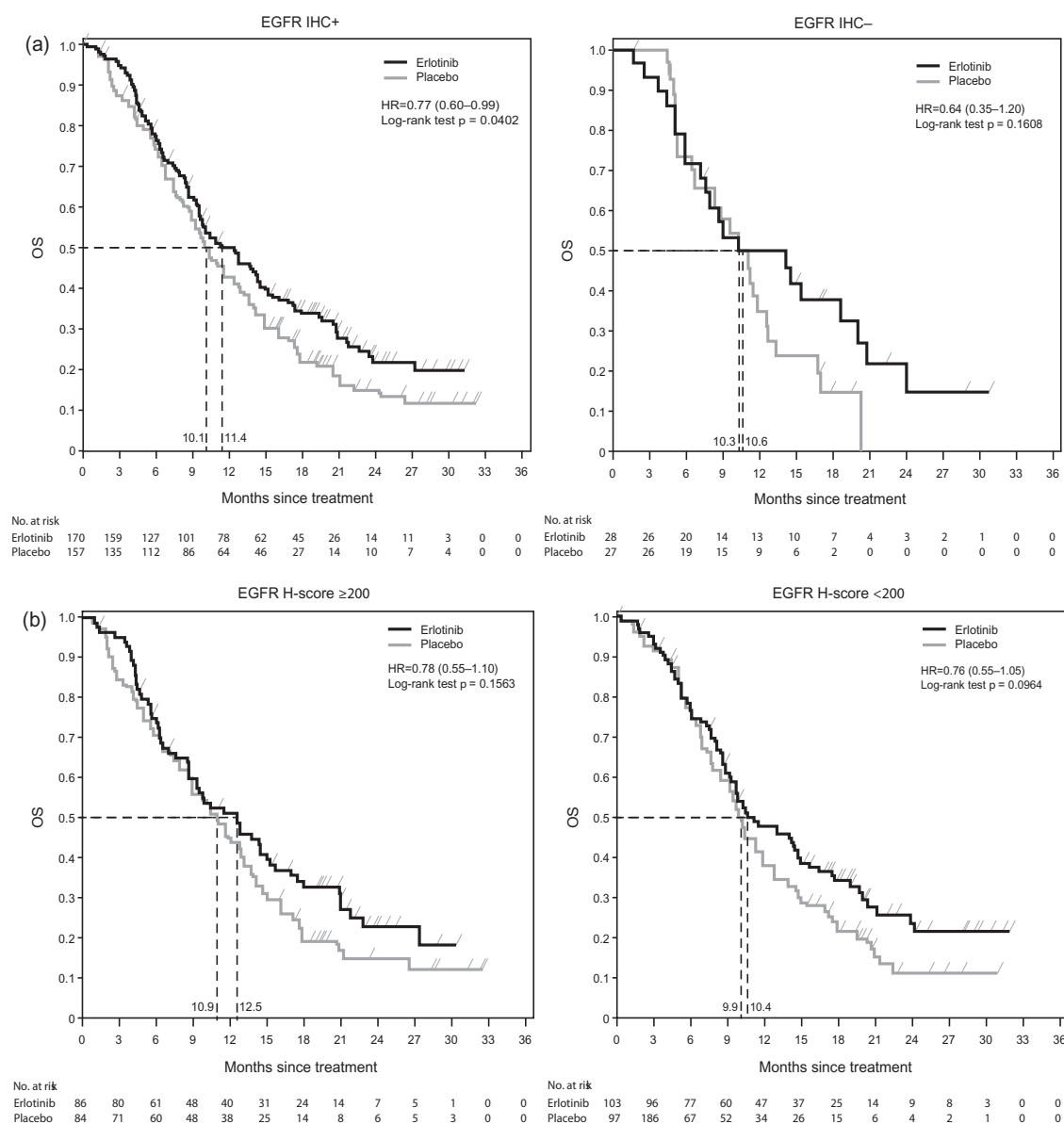
**Table 2**  
Analysis of PFS and OS by EGFR IHC status according to different scoring methods.

	SATURN protocol-defined EGFR IHC+	SATURN protocol-defined EGFR IHC-	EGFR IHC by H-score ≥200 (high)	EGFR IHC by H-score <200 (low)	EGFR IHC by H-score ≥10%	EGFR IHC by H-score <10%
<b>ITT population</b>						
<i>n</i>	Erlotinib: 308 Placebo: 313	Erlotinib: 62 Placebo: 59	Erlotinib: 146 Placebo: 157	Erlotinib: 205 Placebo: 204	Erlotinib: 267 Placebo: 287	Erlotinib: 84 Placebo: 74
PFS, HR (95% CI)	0.69 (0.58–0.82) <i>p</i> < 0.0001	0.77 (0.51–1.14) <i>p</i> = 0.1768	0.68 (0.53–0.86) <i>p</i> = 0.0010	0.76 (0.62–0.93) <i>p</i> = 0.0076	0.71 (0.59–0.84) <i>p</i> < 0.0001	0.79 (0.57–1.10) <i>p</i> = 0.1627
OS, HR (95% CI)	0.77 (0.64–0.93) <i>p</i> = 0.0063	0.91 (0.59–1.38) <i>p</i> = 0.6482	0.80 (0.62–1.05) <i>p</i> = 0.1099	0.80 (0.64–1.01) <i>p</i> = 0.0639	0.78 (0.64–0.96) <i>p</i> = 0.0161	0.85 (0.60–1.22) <i>p</i> = 0.3901
<b>EGFR WT population</b>						
<i>n</i>	Erlotinib: 170 Placebo: 157	Erlotinib: 28 Placebo: 27	Erlotinib: 86 Placebo: 84	Erlotinib: 103 Placebo: 97	Erlotinib: 147 Placebo: 144	Erlotinib: 42 Placebo: 37
PFS, HR (95% CI)	0.79 (0.63–0.99) <i>p</i> = 0.0336	0.65 (0.37–1.13) <i>p</i> = 0.1146	0.69 (0.51–0.95) <i>p</i> = 0.0188	0.84 (0.63–1.12) <i>p</i> = 0.2166	0.78 (0.62–0.99) <i>p</i> = 0.0400	0.69 (0.44–1.10) <i>p</i> = 0.1126
OS, HR (95% CI)	0.77 (0.60–0.99) <i>p</i> = 0.0402	0.64 (0.35–1.20) <i>p</i> = 0.1608	0.78 (0.55–1.10) <i>p</i> = 0.1563	0.76 (0.55–1.05) <i>p</i> = 0.0964	0.78 (0.60–1.02) <i>p</i> = 0.0698	0.72 (0.43–1.19) <i>p</i> = 0.2004

Note: HR < 1 is in favor of erlotinib; all *p*-values are by log-rank test; not adjusted for multiple testing.



**Fig. 2.** (a) PFS in the EGFR WT population according to protocol-defined EGFR IHC status. (b) PFS in the EGFR WT population according to EGFR IHC H-score with magnification.



**Fig. 3.** (a) OS in the EGFR WT population according to protocol-defined EGFR IHC status. (b) OS in the EGFR WT population according to EGFR IHC H-score with magnification.

rule, PFS for patients with IHC-positive disease (score  $\geq 200$ ) was 12.1 weeks in the erlotinib arm and 6.3 weeks in the placebo arm (HR 0.69, exploratory  $p = 0.0188$ ; Fig. 2b). PFS for patients with IHC-negative disease (H-score <200) was 12.0 weeks in the erlotinib arm and 11.3 weeks in the placebo arm (HR 0.84, exploratory  $p = 0.2166$ ; Fig. 2b).

For OS in the EGFR WT population, the patients with protocol-defined IHC-positive disease had a significant benefit with erlotinib versus placebo (HR 0.77,  $p = 0.0402$ ), while assessment by H-score with magnification rule ( $\geq 200$ ) resulted in a HR of 0.78 (exploratory  $p = 0.1563$ ) (Fig. 3a and b). Protocol-defined assessment of patients with IHC-negative disease resulted in a HR of 0.64 ( $p = 0.1608$ ) and when assessed by H-score with magnification rule the HR was 0.76 (exploratory  $p = 0.0964$ ).

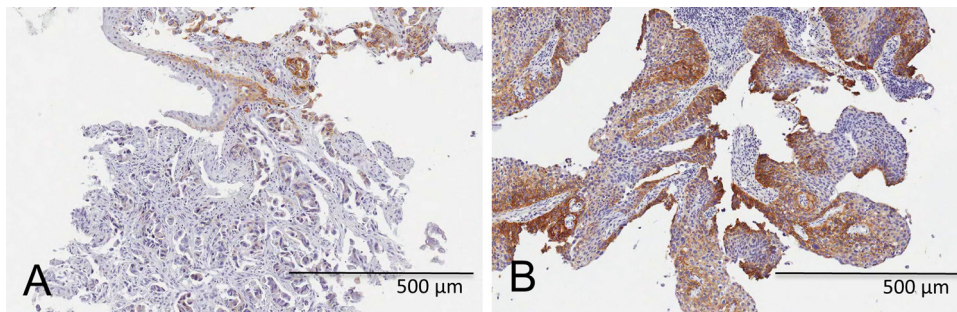
When the protocol-defined scoring system of  $\geq 10\%$  membrane staining of any intensity to define IHC-positive status was applied to the new readings (meaning the H-score with magnification rule readings were assessed as positive if  $\geq 10\%$  of cells had positive-staining without giving any weighting to the magnification used to visualize the staining), HR values were similar to both the original

protocol-derived values and the H-score with magnification values (Table 2).

#### 4. Discussion

Maintenance treatment is now a standard therapeutic strategy in advanced NSCLC, but many challenges still exist, such as identifying the patients who derive the most benefit from continuing anti-cancer treatment until progression. As erlotinib directly targets EGFR and identification of high EGFR protein expression by IHC was recently shown to be predictive of efficacy with the EGFR inhibitor cetuximab in advanced NSCLC, we aimed to apply this test to the cohort of SATURN patients. Re-scoring of EGFR IHC status in SATURN by H-score with the magnification rule found that erlotinib provided similar benefits in terms of PFS or OS for subsets with high or low EGFR expression, in the overall and EGFR WT populations. This was despite clear differences in the categorization of patients by the two different methods into EGFR IHC-positive or -negative subpopulations, as demonstrated by the number of





**Fig. 4.** Two representative cases, which were scored positive according the protocol-defined scoring, but scored negative applying the H-score method with a cut-off of 200 for EGFR expression. Image A shows a case stained heterogenous for EGFR with a H-score of 120 and B another case with a H-score of 180.

patients in each category (protocol-defined IHC positive  $n=621$ , negative  $n=121$ ; H-score with magnification rule high  $n=303$ , low  $n=409$ ). Fig. 4 demonstrates samples that were classed positive by the protocol-defined scoring but were classed negative by the H-score plus magnification rule method. From the evolution chart used in the original IHC analysis (Fig. 1), markedly different outcomes were not expected; however, the use of the magnification rule may have provided more objective guidance to the reading pathologist.

In both studies, EGFR analysis was performed on pretreatment biopsies, but the possibility cannot be excluded that EGFR expression is modified by induction chemotherapy, as has been demonstrated for *EGFR* mutation status [13]. Thus, the level of EGFR expression may have changed from the initial assessment by the time erlotinib was administered in the SATURN study, whereas cetuximab was given first line in the FLEX study. Moreover, patients included in the SATURN study were non-progressive after induction chemotherapy, meaning that chemoresistant patients were not taken into account, in contrast to the FLEX study.

It could be suggested that this negative result is due to a lack of reproducibility of the method. However, this seems unlikely as a recent study showed good reproducibility between training pathologists, with a concordance of 76–91% [14].

Lastly, the most probable explanation relies on the use of a monoclonal antibody in combination with a chemotherapy doublet in the FLEX study versus an EGFR TKI as monotherapy in the SATURN study. The different agents have differences in their mode of action, with one targeting the internal kinase activity of the EGFR and the other targeting the protein externally by an antibody blocking ligand binding. Therefore, the predictive value of EGFR expression could be expected to be different with these agents because of their distinct mechanisms of action. One could speculate that EGFR expression could be more likely to predict the efficacy of antibodies, as part of their anti-tumor effect is mediated through antibody-dependent cellular cytotoxicity, which is directly associated with the presence of EGFR protein [15]. The best predictive marker for cetuximab remains unknown: *KRAS* mutations are known to be associated with cetuximab resistance in colorectal cancer, but no reliable markers are currently available for lung cancer.

The H-score method with magnification rule used retrospectively in the FLEX study of cetuximab reported an OS benefit in patients with EGFR IHC-positive tumors but no benefit in patients with EGFR IHC-negative disease for cetuximab plus chemotherapy versus chemotherapy alone [10]. However, as the cut-off for the H-score threshold was data driven, no dedicated trial has been conducted so far to validate prospectively the H-score method. Of note, in the phase III BMS 099 study of cetuximab and first-line taxane/carboplatin in NSCLC patients, EGFR expression did not predict survival outcomes for cetuximab [16]. When the BMS 099 data was retrospectively analyzed by the same H-score as used in the FLEX

study, EGFR expression again did not predict overall survival or progression-free survival outcomes for cetuximab [17].

For EGFR TKIs, *EGFR* mutations have been proven to be the best biomarker for the prediction of superior efficacy [3,18–20]. The potential use of EGFR expression as a marker has been widely investigated, with conflicting results. High EGFR expression has been previously associated with improved response, longer time to progression and improved survival in NSCLC patients treated with gefitinib [21]. Biomarker analysis of the BR.21 study showed survival among patients with high EGFR expression was longer in the erlotinib arm versus the placebo arm, whereas a limited advantage of erlotinib treatment was seen in patients with EGFR IHC-negative tumors [22]. These results were the basis for the inclusion of PFS in patients with EGFR IHC-positive disease as a co-primary endpoint in SATURN. However, Pérez-Soler et al. reported no correlation between survival and EGFR expression ( $p=0.90$ ) in NSCLC patients treated with erlotinib in the second-/third-line setting [23]. Additionally, Murray et al. demonstrated no correlation between EGFR protein expression and disease control rate in erlotinib-treated patients when staining for total EGFR or phosphorylated EGFR [24]. For the SATURN study, using a positive threshold of  $\geq 10\%$  membrane staining failed to identify any correlation between EGFR expression and patient outcomes. Using a different IHC analysis method (H-score with application of the magnification rule) in the present analysis did not change the correlation between EGFR expression levels and PFS or OS in SATURN. The different results between these studies suggest that the value of EGFR IHC to predict clinical outcomes may vary between different EGFR inhibitors and across different patient populations and treatment settings.

The BioLOGUE advisors recently concluded that EGFR IHC status was weakly prognostic but not predictive of outcomes with erlotinib, and noted that inconsistency across trials meant EGFR IHC was not a suitable biomarker [25]. Assessment of total receptor expression may not be the most accurate indicator of response to EGFR TKIs, as *EGFR* activating mutations are considered to be more important than EGFR protein expression levels. It has been suggested that a combination of IHC and fluorescence in situ hybridization may provide more suitable analysis [24], but this method has not yet been investigated in clinical trials. One reason that previous EGFR IHC studies might not have shown correlations with treatment response may be that the majority of diagnostic antibodies target the external domain of the receptor, while it is mutations in the internal tyrosine-kinase domain that result in the increased response to erlotinib. The use of a diagnostic antibody that targets the internal EGFR domain (such as 5B7) [26] might result in better prediction of response with erlotinib using IHC.

## 5. Conclusion

The results of this re-analysis suggest that EGFR IHC does not accurately predict erlotinib benefit for the overall population or

the *EGFR* WT population in the first-line maintenance setting for advanced NSCLC.

### Conflict of interest statement

Dr Mazieres has received honoraria from Roche, Pfizer, Eli Lilly and Boehringer Ingelheim. Dr Bara and Dr Klingelschmitt are employees of Roche. Dr Klughammer is an employee of Roche and owns stocks in F. Hoffmann-La Roche Ltd. Dr Cappuzzo has received payment for consultancy or advisory roles from Roche. Dr Brugger has received honoraria and payment for consultancy or advisory roles from Roche. Dr Middel has received other remunerations from F. Hoffmann-La Roche Ltd. Dr Frosch has declared no conflicts of interest.

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This trial was designed, funded by and monitored by F. Hoffmann-La Roche Ltd. Data were collected, analyzed and interpreted by F. Hoffmann-La Roche, with input from the authors and investigators. 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.

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