ORIGINAL ARTICLE

Examining Treatment Outcomes with Erlotinib in Patients with Advanced Non-Small Cell Lung Cancer Whose Tumors Harbor Uncommon *EGFR* Mutations

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Received 9 September 2015; revised 16 December 2015; accepted 17 December 2015
Available online - 7 January 2016

ABSTRACT

Introduction: Exon 19 deletions and the exon 21 L858R mutation of the epidermal growth factor receptor gene (*EGFR*) predict activity of EGFR tyrosine kinase inhibitors, including erlotinib; however, the ability of less common *EGFR* mutations to predict efficacy of erlotinib is unclear.

Methods: The efficacy of erlotinib in individual patients with rare *EGFR* mutations from the MERIT, SATURN, TITAN, TRUST, ATLAS, BeTa, and FASTACT-2 trials was analyzed and compared with data from the literature.

Results: In the patients tested for biomarkers, the frequency of rare mutations identified here ranged from 1.7% (eight of 467) in the SATURN study to 7.4% (27 of 364) in ATLAS. Some rare mutations were associated with greater clinical benefit from EGFR tyrosine kinase inhibitor therapy or improved prognosis independent of treatment, whereas others appeared to have a poorer prognosis. In particular, exon 18 G719 mutations, exon 19 K757R and E746G mutations, the exon 20 S768I mutation, and the exon 21 G836S mutation appeared to confer a good outcome with erlotinib treatment, whereas exon 18 S720I showed a particularly poor outcome. Owing to the small number of patients with each mutation, however, it is difficult to confirm whether these rare mutations do indeed confer sensitivity or resistance to erlotinib.

Conclusions: Erlotinib can have different efficacy depending on the specific *EGFR* mutation. More research is needed to create a central database such as the My Cancer Genome database of rare mutations to definitively confirm whether these mutations are activating, resistant, or neutral.

Keywords: NSCLC; Erlotinib; *EGFR* mutations; Uncommon; Rare

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***Disclosure:** Drs. Delmar, Klughammer, Shames, and Wu are employees of F. Hoffmann-La Roche and own stock in F. Hoffmann-La Roche. Drs. Klingelschmitt, Spleiss, and Yue are employees of F. Hoffmann-La Roche. Dr. Ciuleanu has recieved an honorarium from F. Hoffmann-La Roche. Dr. Mok has consulted for and recieved honoraria for symposia and advisory boards for F. Hoffmann-La Roche. Dr. Reck has served as an advisor for F. Hoffmann-La Roche.*

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ISSN: 1556-0864

http://dx.doi.org/10.1016/j.jtho.2015.12.107

Introduction

The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) erlotinib has been at the forefront of changes in treatment practice for advanced non–small cell lung cancer (NSCLC) over the past 10 years. Erlotinib was initially approved for use in the second- or third-line setting in an unselected population because of statistically significant improvements in overall survival (OS) versus placebo.[1] Erlotinib was subsequently approved for maintenance treatment of unselected patients with NSCLC as a result of data from the SATURN trial.[2] Finally, on the basis of compelling evidence from several clinical trials of single-agent erlotinib versus chemotherapy,[3–5] erlotinib was also approved for the first-line treatment of patients whose tumors harbor activating mutations of the epidermal growth factor receptor gene (*EGFR*).

The most common activating mutations used to guide treatment decisions in NSCLC are deletions in exon 19 of *EGFR* and the specific point mutation L858R in exon 21; however, many testing methods are actually able to identify other less common activating mutations, as well as the most frequent resistance mutations (e.g., T790M in exon 20). Genetic profiling suggests that some patients with advanced NSCLC whose tumors harbor these less common mutations of *EGFR* may derive a greater or lesser benefit from treatment with erlotinib or other EGFR TKIs (e.g., afatinib, gefitinib) than do those with classical activating mutations, but full mutation profiling is still in its infancy.

To allow truly personalized treatment, a greater understanding of these "rare" mutations is required to facilitate individual patient profiling and accurate prediction of response to EGFR TKI therapy. Examining existing data and tumor samples can provide valuable guidance in building these disease profiles for application to clinical practice, particularly in cases in which the number of patients with a specific mutation is insufficient to carry out a full clinical trial. De Pas et al. investigated the frequency of rare mutations in 681 patients with NSCLC who were screened between 2006 and 2010. Of the 99 patients with *EGFR* mutations, 18 harbored rare mutations. Of these patients, 10 were treated with erlotinib or gefitinib, with varying responses depending on the specific mutations.[6] In an observational, prospective cohort of 188 NSCLC patients, common and rare mutations were assessed with regard to EGFR TKI response by Arrieta et al. Patients with rare mutations had significantly ($p < 0.001$) lower response rates to EGFR TKIs than did those with common mutations.[7] Similarly, Chiu et al.[8] examined 639 patients with activating *EGFR* mutations (478 with common mutations and 161 with rare mutations). When patients were treated with EFGR TKIs, the response rate was significantly lower for those with rare mutations than for those with common mutations (41.6% versus 66.5%, $p = 0.001$) and the median progression-free survival (PFS) was also lower (7.7 versus 11.4 months, $p = 0.001$).[8] A retrospective analysis by Baek et al. also reported that patients with rare or complex mutations had inferior response and survival when treated with EGFR TKIs compared with patients whose tumors had common *EGFR* mutations.[9]

In a post hoc analysis from three clinical studies of afatinib (LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6), 75 of 600 patients (12%) had rare *EGFR* mutations, and after afatinib treatment, the median PFS varied depending on the type of mutation.[10] Patients with point mutations or duplications had a median PFS of 10.7 months and a median OS of 19.4 months, whereas patients with de novo T790M mutations had a median PFS of 2.9 months and a median OS of 14.9 months. Patients with an exon 20 insertion had a median PFS of 2.7 months and median OS of 9.2 months.[10]

Here we use a case report style to report individual patient data from patients with rare mutations as assessed from baseline tumor samples in several well-controlled clinical studies of erlotinib to assess the efficacy outcomes for patients with uncommon mutations.

Methods

Clinical Studies Included in This Analysis

Data from the SATURN (NCT00556712), TITAN (NCT00556322), TRUST (NCT00949910), ATLAS (NCT00257608), BeTa (NCT00130728), FASTACT-2 (NCT00883779), and MERIT (BO18279) studies were assessed in this analysis. MERIT was a single-arm, open-label, phase II gene expression profiling study that aimed to identify candidate genes in order to predict outcomes in patients receiving erlotinib.[11] The TRUST study was a phase IV expanded access study that assessed efficacy and safety outcomes with erlotinib (regardless of line of therapy) in a large patient population reflective of real-life clinical practice (>7000 patients).[12] Biomarker samples were obtained where possible and several prior analyses were carried out, including analyses of *EGFR* mutations.[13,14] SATURN and TITAN were twin phase III studies conducted in the post–first-line setting. Patients were initially enrolled into a chemotherapy run-in phase, after which they could be enrolled into either SATURN (if their disease had not yet progressed) or TITAN (if their disease had progressed during the initial cycles of first-line chemotherapy). Patients in SATURN were randomized to receive either erlotinib maintenance therapy or placebo,[2] whereas those in TITAN were randomized to receive either second-line erlotinib or chemotherapy (docetaxel or pemetrexed, at the investigators' discretion).[15] Tumor sampling was mandatory in both

studies, and extensive biomarker analyses have been reported.[15,16] FASTACT-2 was a phase III, randomized, controlled study assessing the efficacy and safety of an intercalated regimen of erlotinib and first-line chemotherapy versus intercalated placebo and chemotherapy in Asian patients with advanced NSCLC. Patients were not selected on the basis of *EGFR* mutation status, but biomarker analysis did demonstrate that the significant efficacy benefit of intercalated erlotinib was driven by this patient subgroup.[17] BeTa was a phase III randomized study evaluating bevacizumab plus erlotinib for the treatment of recurrent or refractory NSCLC. Patients were not selected on the basis of *EGFR* mutation status, but tumor biopsy material was requested at study entry for biomarker analysis.[18] The phase III ATLAS study assessed bevacizumab with or without erlotinib in the maintenance treatment setting.[19] Provision of tumor tissue for biomarker assessment was optional.

Mutation Testing Methodology

Tumor sampling was mandatory for MERIT, SATURN, and TITAN, and optional for TRUST, ATLAS, BeTa, and FASTACT-2; informed consent was obtained from all patients contributing samples. *EGFR* mutation testing was carried out using different methods across the different clinical studies. For MERIT and TRUST, microdissection (manual or laser capture) of tumor cells was carried out on formalin-fixed paraffin-embedded tissue samples. DNA was then extracted from the microdissected cells, and *EGFR* exons 18 through 21 were amplified by polymerase chain reaction (PCR) and then sequenced for *EGFR* mutation status assessment. In SATURN and TITAN, DNA lysates were obtained from macrodissected or microdissected tissue samples; these samples had to have at least 5000 tumor cells and a minimum 60% tumor cell content. Exons 18 through 21 were amplified by PCR, with multiple independent products sequenced. Mutations other than the most commonly known resistance and activating mutations had to be identified in at least two PCR products to be confirmed. For FASTACT-2, *EGFR* mutation analysis was carried out using the cobas 4800 system (Roche Molecular Diagnostics, Pleasanton, CA), which is able to detect the most common activating and resistance mutations, as well as less common mutations in exons 18 through 21. In addition, blood samples were tested using the cobas 4800 *EGFR* mutation blood test (in development). In the ATLAS study, analyses of *EGFR* mutations in exons 18 through 21 were performed with Somatic Mutation Scanning by Surveyor Endonuclease Digestion and Analysis on WAVE HS (Transgenomics Inc., Omaha, NE) using cells captured by manual macrodissection or laser microdissection. In the BeTa study, *EGFR* mutations in exons 18 through 21 were analyzed by denaturing high-performance liquid chromatography (Transgenomics Inc.).

Results

To effectively assess the efficacy of erlotinib in patients with the rare mutations identified, it is important to consider the results for the intent-to-treat population and, where available, the results for subgroups with classical *EGFR* mutations from each study shown in Supplementary Table 1.

Individual Patient Data for Rare Mutations

Efficacy results for individual patients with rare mutations in MERIT, SATURN, TITAN, TRUST, ATLAS, BeTa, and FASTACT-2 are presented in Tables 1 through 4 (Table 1, rare mutations in exon 18; Table 2, rare mutations in exon 19; Table 3, rare mutations in exon 20; and Table 4, rare mutations in exon 21). In the samples tested for biomarkers, the frequency of rare mutations identified in this analysis ranged from 1.7% (eight of 467) in the SATURN study to 7.4% (27 of 364) in ATLAS. Some mutations were associated with greater clinical benefit from EGFR TKI therapy or with improved prognosis regardless of treatment, showing both predictive and prognostic effects of certain rare mutations, whereas others appeared to have a detrimental effect on survival. Some of these rare mutations are detailed in the following sections, evaluating the results from this analysis in comparison with data from the literature.

Exon 18 Mutations

G719. One of the more frequently observed "rare" mutations is the point mutation at G719 (n = 10 of 24 exon 18 mutations in this analysis). The mutation to cysteine (G719C) appears to confer a PFS and OS benefit, with one patient from the SATURN study reporting a partial response, PFS of 500 days, and OS of 795 days (>2 years). When this mutation was combined with the K714N mutation in one patient in BeTa, an OS of 973 days (>2.5 years) was observed. A point mutation to alanine was also seen at G719. In SATURN, a G719A mutation plus T725M resulted in a PFS of 169 days and OS of 343 days, whereas in TRUST, a patient with G719A had a PFS of 463 days and OS of 488 days. A patient in FASTACT-2 with an unknown G719 mutation achieved an OS of 1008 days (>2.5 years). When compared with the PFS and OS results from the overall populations of each study, the G719A mutation appeared to be sensitizing, but to a lesser extent than the G719C mutation.

Literature Review. Substitutions at G719 with alanine or cysteine have been shown to confer sensitivity to EGFR TKIs in several previous studies. This is because the point

Table 1. Efficacy Data for Rare Mutations in Exon 18 of *EGFR*

Protocol	Age, y	Sex	Race	ECOG PS	Tx Line	Smoking Status	First Trial Medication	Actual Mutation Exon 18	Response	PFS, d	*OS*, d	D or C
TITAN	64	Male	White	0	2	Current	Docetaxel	P.G719A[a]	PD	25	25	C
SATURN	58	Male	White	1	1	Former	Erlotinib	P.E709A; P.G719S	SD	253	509	C
SATURN	58	Female	Asian	1	1	Never	Erlotinib	P.G719C	PR	500	795	C
SATURN	59	Male	White	0	1	Current	Erlotinib	P.G719A; P.T725M	SD	169	343	D
MERIT	50	Male	Asian	1	2	Never	Erlotinib	P.E709K; P.G719A	PD	42	259	D
TRUST	67	Female	White	1	2	Never	Erlotinib	P.G719A[b]	SD	463	488	D
TRUST	50	Female	Asian	1	2	Never	Erlotinib	P.E709_T710DELINSD	PD	38	52	D
TRUST	38	Male	Asian	2	2	Never	Erlotinib	P.V689M[b]	SD	491	873	D
FASTACT-2	61	Female	Asian	0	1	Never	Carboplatin/gemcitabine	P.G719UNK[c]	SD	225	1084	D
FASTACT-2	63	Female	Asian	1	1	Never	Carboplatin/gemcitabine	P.G719UNK[d]	PR	130	1008	D
ATLAS	72	Male	White	1	1	Former	Bevacizumab + erlotinib	P.L707S[e]	PD	42	235	C
ATLAS	41	Male	White	1	1	Current	Bevacizumab + erlotinib	P.I715V	SD	413	413	C
ATLAS	73	Male	White	1	1	Former	Bevacizumab + erlotinib	P.S720P	PD	78	285	C
ATLAS	67	Female	Black	1	1	Former	Bevacizumab + erlotinib	T727STOP	PD	1	464	D
ATLAS	61	Male	White	1	1	Current	Bevacizumab + erlotinib	P.S720P	SD	109	302	C
ATLAS	64	Male	White	1	1	Current	Bevacizumab + erlotinib	P.F723L	PD	81	311	C
ATLAS	73	Female	Hispanic	1	1	Current	Bevacizumab	P.G719C	SD	453	673	D
ATLAS	70	Male	Hispanic	0	1	Former	Bevacizumab	P.G719A	PD	84	227	C
ATLAS	59	Male	White	1	1	Former	Bevacizumab + erlotinib	P.S720F	PD	2	89	C
ATLAS	70	Male	Asian	1	1	Former	Bevacizumab + erlotinib	P.V736A	NE	177	183	D
BeTa	77	Female	White	1	2/3	Former	Erlotinib + placebo	P.K714N; P.G719C	SD	359	973	C
BeTa	74	Female	White	0	2/3	Former	Erlotinib + placebo	P.Y727C	SD	191	269	D
BeTa	64	Female	White	0	2/3	Former	Erlotinib + bevacizumab	P.I706T; P.G874S	SD	423	444	C
BeTa	61	Female	White	0	2/3	Former	Erlotinib + placebo	P.A722V[f]	PD	39	429	C

[a]Exon 21 L833F mutation in addition.
[b]Exon 20 mutation in addition.
[c]Patient received second-line erlotinib.
[d]Patient received second-line gefitinib.
[e]Exon 19 N7565 mutation in addition.
[f]Exon 19 deletion in addition.
C, censored; D, death; ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor gene; NE, nonevaluable; OS, overall survival; PFS, progression-free survival; PR, partial response; PD, progressive disease; SD, stable disease; Tx, treatment.

Table 2. Efficacy Data for Rare Mutations in Exon 19 of *EGFR*

Protocol	Age, y	Sex	Race	ECOG PS	Tx Line	Smoking Status	First Trial Medication	Actual Mutation Exon 19	Response	PFS, d	OS, d	D or C
TITAN	69	Male	White	0	2	Current	Docetaxel	P.D761N	SD	101	178	D
MERIT	77	Female	Asian	2	2	Former	Erlotinib	P.I744V	PD	82	136	D
MERIT	73	Female	Asian	1	2	Never	Erlotinib	P.L747FS	PD	122	211	D
MERIT	41	Male	Asian	1	2	Never	Erlotinib	P.K757R	SD	250	435	D
ATLAS	72	Male	White	1	1	Former	Bevacizumab + erlotinib	P.N756S[a]	PD	42	235	C
BeTa	58	Male	White	1	2/3	Former	Bevacizumab + erlotinib	P.K745E[b]	NE	85	282	D
BeTa	63	Female	White	0	2/3	Never	Erlotinib + placebo	P.E746G	NE	415	462	C
BeTa	54	Male	White	0	2/3	Former	Bevacizumab + erlotinib	P.V717A	SD	79	292	D
BeTa	67	Female	White	1	2/3	Former	Erlotinib + placebo	P.A755T[c]	SD	215	329	C

[a]Exon 18 L7675 mutation in addition.
[b]Y827C, V845M, H870Y exon 21 mutations in addition.
[c]V851A exon 21 mutation in addition.
C, censored; D, death; ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor gene; NE, nonevaluable; OS, overall survival; PFS, progression-free survival; PR, partial response; PD, progressive disease; SD, stable disease; Tx, treatment.

mutation alters the P-loop of the receptor structure to allow the receptor a higher affinity to adenosine triphosphate (ATP), therefore favoring activation.[20] Han et al.[9] reported three gefitinib-treated patients with G719A mutations. Of these patients, two achieved partial responses, with one patient reporting a time to progression of 22 months (≈669 days) and an OS longer than 26 months (≈791 days).[21] Point mutations at G719 resulted in 15 patients achieving a response rate of 53.3%, median PFS of 8.1 months (≈247 days), and median OS of 16.4 months (≈499 days) when treated with gefitinib or erlotinib.[22] Baek et al. reported that in a subgroup in which six of 11 patients had G719 mutations, median PFS was 5.1 months (≈155 days) and median OS was 18.3 months (≈557 days) in a retrospective analysis.[9] When patients with point mutations at G719 (n = 18) were treated with afatinib, they achieved an objective response rate (ORR) of 77.8%, PFS of 13.8 months, and OS of 26.9 months in a study by Yang et al.[10] Chui et al. reported that 78 of 161 (48.4%) patients with rare mutations had a single G719 mutation. After treatment with gefitinib or erlotinib, the ORR was 36.8% with a disease control rate (DCR) of 72.4%. However, a compound mutation of G719 + L861Q (exon 21) (5.6%) or G719 + S768I (exon 20) (6.2%) demonstrated a higher ORR (88.9% and 50%, respectively) and a greater DCR (100% for both).[8]

S720. Poor response and short OS were observed with the S720P mutation, with an OS less than 1 year seen in two patients from the ATLAS study (285 and 302 days). One patient from ATLAS with a S720F mutation had an OS of 89 days, suggesting that this mutation may be associated with particularly poor survival outcomes; however, a larger sample size would be needed to confirm such an association as this result could be due to comorbidities or certain other patient characteristics.

Literature Review. Very little has been published regarding mutations at S720. In a study of mutation type in patients treated with gefitinib, however, a female Asian nonsmoker with the S720P mutation had a median PFS of 13.2 months (≈402 days) and median OS of 20.5 months (≈624 days).[23] This suggests that tumors with the same mutation may react differently to different TKIs; however, the small sample size limits interpretation.

Exon 19 Mutations

Response and survival varied considerably between the different rare exon 19 mutations. Mutations that appeared sensitizing to EGFR TKIs were K757R (n = 1) and E746G (n = 1), which resulted in a PFS of 250 and 415 days in MERIT and BeTa, respectively, and an OS of more than 1 year (435 days in MERIT and 462 days in

Table 3. Efficacy Data for Rare Mutations in Exon 20 of *EGFR*

Protocol	Age, y	Sex	Race	ECOG PS	Tx Line	Smoking Status	First Trial Medication	Actual Mutation Exon 20	Response	PFS, d	OS, d	D or C
TITAN	54	Male	Asian	1	2	Current	Docetaxel	P.A767_S768INSSVSS	PD	17	64	D
TITAN	56	Male	Asian	1	2	Never	Docetaxel	P.M766_A767INSASV	SD	81	115	D
SATURN	64	Female	White	1	1	Former	Erlotinib	P.T790M	PD	47	134	C
SATURN	53	Female	White	1	1	Never	Erlotinib	P.D770_N771INSSVD	SD	84	599	C
SATURN	51	Female	White	1	1	Never	Erlotinib	P.D770_N771INSSVD	PR	128	881	C
TRUST	67	Male	White	2	2	Never	Erlotinib	P.ALA767_VAL769DUP	SD	71	155	D
TRUST	60	Male	White	2	2	Never	Erlotinib	P.A763_Y764INSFQEA	PR	532	731	D
TRUST	67	Female	White	1	2	Never	Erlotinib	P.T790M[a]	SD	463	488	D
TRUST	70	Male	White	1	2	Never	Erlotinib	P.H773_V774INSAH	PD	53	390	D
TRUST	62	Female	Asian	1	2	Never	Erlotinib	P.S768I; P.V774M	SD	166	1106	C
TRUST	85	Female	White	1	2	Never	Erlotinib	P.V769_D770INSASV	NE	642	642	D
TRUST	38	Male	Asian	2	2	Never	Erlotinib	P.T790M[a]	SD	491	873	D
FASTACT-2	61	Female	Asian	0	1	Never	Carboplatin/gemcitabine	P.S768I[a]	SD	225	1084[b]	D
FASTACT-2	62	Male	Asian	0	1	Never	Carboplatin/gemcitabine + erlotinib	P.S768I	PR	371	1909[b]	C
FASTACT-2	56	Female	Asian	1	1	Never	Carboplatin/gemcitabine	P.S768I	SD	169	939	D
ATLAS	57	Male	White	1	1	Former	Bevacizumab	P.A763V[b]	CR	689	827	D
ATLAS	71	Male	White	1	1	Former	Bevacizumab + erlotinib	P.P772S	NE	75	75	C
ATLAS	33	Female	Black	1	1	Never	Bevacizumab + erlotinib	P.D746A[b]	PR	424	435	D
ATLAS	58	Male	Asian	1	1	Current	Bevacizumab + erlotinib	P.V774M	PD	36	82	C
BeTa	61	Male	White	1	2/3	Former	Erlotinib + bevacizumab	P.K860E	PD	39	127	D
BeTa	67	Female	White	1	2/3	Never	Erlotinib + placebo	INS	PD	42	354	D
BeTa	70	Male	White	1	2/3	Former	Erlotinib + placebo	T790M	PR	296	498	D
BeTa	69	Male	White	0	2/3	Never	Erlotinib + placebo	T790M[c]	PD	40	665	C
BeTa	60	Female	White	1	2/3	Former	Erlotinib + placebo	T790M[b]	NE	27	27	D
BeTa	50	Female	White	1	2/3	Former	Erlotinib + bevacizumab	S768I; V769L	PR	83	125	D

[a]Exon 18 mutation in addition.
[b]Exon 19 deletion in addition.
[c]Exon 21 mutation in addition.
C, censored; CR, complete response; D, death; ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor gene; NE, nonevaluable; OS, overall survival; PFS, progression-free survival; PR, partial response; PD, progressive disease; SD, stable disease; Tx, treatment.

Table 4. Efficacy Data for Rare Mutations in Exon 21 of *EGFR*

Protocol	Age, y	Sex	Race	ECOG PS	Tx Line	Smoking Status	First Trial Medication	Actual Mutation Exon 21	Response	PFS, d	OS, d	D or C
TITAN	60	Male	Asian	1	2	Never	Erlotinib	P.L861Q	PD	18	18	D
TITAN	51	Male	White	1	2	Current	Pemetrexed	P.P848L	PD	50	173	D
TITAN	64	Male	White	0	2	Current	Docetaxel	P.L833F[a]	PD	25	25	C
SATURN	47	Female	White	0	1	Current	Erlotinib	P.P848L	SD	78	655	C
SATURN	63	Male	White	0	1	Former	Placebo	P.H835L; P.L833V	SD	85	308	D
TRUST	42	Female	White	2	2	Current	Erlotinib	P.D837N	SD	52	52	D
TRUST	56	Male	White	2	2	Current	Erlotinib	P.K860N	SD	354	354	D
FASTACT-2	48	Female	Asian	1	1	Never	Carboplatin/gemcitabine	P.L861Q	PR	141	175	D
ATLAS	63	Female	White	1	1	Former	Bevacizumab	P.L858Q; P.V834A	PD	36	137	C
ATLAS	62	Female	White	1	1	Current	Bevacizumab + erlotinib	P.R841G; P.A859V	PD	39	52	C
ATLAS	78	Male	White	1	1	Former	Bevacizumab + erlotinib	P.H870Y	SD	85	691	D
ATLAS	52	Female	White	1	1	Current	Bevacizumab + erlotinib	P.L858R[b]	SD	126	728	D
ATLAS	53	Female	White	0	1	Former	Bevacizumab + erlotinib	P.L858R; L861P	PD	212	348	C
ATLAS	69	Female	White	1	1	Former	Bevacizumab + erlotinib	P.A840V; P.A859T	PR	85	407	C
ATLAS	23	Male	White	0	1	Never	Bevacizumab	P.G836S	PD	46	763	D
ATLAS	72	Female	White	1	1	Current	Bevacizumab + erlotinib	P.G836S	PR	429	642	D
ATLAS	88	Male	White	0	1	Former	Bevacizumab + erlotinib	P.A871V	SD	125	313	D
ATLAS	43	Female	White	1	1	Former	Bevacizumab	P.V843I[b]	SD	127	511	D
ATLAS	70	Male	White	1	1	Former	Bevacizumab	P.Q849S	NE	78	184	C
ATLAS	71	Female	Black	0	1	Current	Bevacizumab	P.G824D; P.V851A	PD	78	293	C
BeTa	58	Male	White	1	2/3	Former	Erlotinib + bevacizumab	Y827C; V845M; H870Y[c]	NE	85	282	D
BeTa	61	Female	Hispanic	1	2/3	Never	Erlotinib + placebo	P.H835R	PR	417	460	C
BeTa	69	Male	White	0	2/3	Never	Erlotinib + placebo	P.L858R[b]	PD	40	665	C
BeTa	69	Male	White	1	2/3	Current	Erlotinib + placebo	P.R831C; P.T854I[d]	SD	116	116	D
BeTa	64	Female	White	0	2/3	Former	Erlotinib + bevacizumab	P.G874S[e]	SD	423	444	C
BeTa	72	Male	White	1	2/3	Former	Erlotinib + bevacizumab	P.L861Q	SD	234	339	C
BeTa	47	Female	Black	1	2/3	Former	Erlotinib + placebo	P.T854I	PD	39	104	D
BeTa	67	Female	White	1	2/3	Former	Erlotinib + placebo	P.V851A[f]	SD	215	329	C
BeTa	64	Female	White	0	2/3	Former	Erlotinib + bevacizumab	P.L838V; P.L858R	SD	211	430	C
BeTa	78	Female	White	0	2/3	Former	Erlotinib + bevacizumab	P.A859T	NE	36	687	C
BeTa	53	Female	White	0	2/3	Former	Erlotinib + bevacizumab	P.D855V	SD	173	259	C

[a]Exon 18 mutation in addition.
[b]Exon 20 T790M mutation in addition.
[c]Exon 19 K745E in addition.
[d]Exon 19 deletion in addition.
[e]Exon 18 I706T mutation in addition.
[f]Exon 19 A755T mutation in addition.
C, censored; D, death; ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor gene; NE, nonevaluable; OS, overall survival; PFS, progression-free survival; PR, partial response; PD, progressive disease; SD, stable disease; Tx, treatment.

BeTa). Other exon 19 mutations generally had shorter PFS and OS than the previously mentioned mutations, with a best response of progression or stable disease. However, these results were comparable with the results for the overall study population, so EGFR TKI treatment is unlikely to be of reduced benefit in these patients.

Literature Review. To our knowledge, no previous publications have reported K757R mutations. The E746G mutation was reported in combination with an L861Q mutation in a study by Wu et al., with the patient achieving a partial response to EGFR TKI therapy.[22] A study by Peng et al.[24] reported deletions at E746 in two patients who both received gefitinib treatment. One patient had a stable disease status, a PFS of 8 months, and an OS of more than 8 months, and the other patient achieved a partial response, a PFS of 15 months, and an OS of more than 58 months.[24] Exon 19 insertions, L747F and P733L, have both been reported as sensitizing rare exon 19 mutations[20] and several rare mutations in exon 19 have been reported as resistance mutations, including D761Y, E746V, and L747S, showing the varied responses that different point mutations in one exon can elicit.[20] The L747F mutation may confer sensitivity by inhibiting the αC helix adopting the inactive position,[25] whereas the D761Y resistance mutation appears to confer resistance by altering the receptor interaction with ATP.[20] In two case studies by Agbarya et al. and Chan et al., data from female patients with p.K745_E746insIPVAIK exon 19 insertions were reported, and in both cases the patients responded to EGFR TKI treatment.[26,27]

Exon 20 Mutations

T790M is known to cause EGFR TKI resistance,[28] which is generally consistent with the short PFS seen in patients with this mutation in our analysis. When combined with exon 18 mutations, however, a response of stable disease and a PFS of more than 1 year was seen in two patients from the TRUST study. In the case of double mutations, however, the mutant allele frequency must be considered because if the mutant allele frequency for T790M were to be particularly low when in combination with another mutation, the outcome would be skewed toward the other mutation, although the presence of the T790M resistance mutation would still be detected. S768I appeared to confer sensitivity to erlotinib, with a patient from FASTACT-2 reaching an OS of 1909 days (>5 years) and a female Asian patient from the TRUST study with an additional V774M mutation having an OS of 1106 days (>3 years). Across the exon 20 mutations reported, eight appeared to be insertions. P.A767_S768INSSVSS and P.M766_A767INSASV from the TITAN study were associated with short PFS and OS. Conversely, the P.D770_N771INSSVD mutation was associated with longer OS (599 and 881 days) in two patients in the SATURN study.

Literature Review. Masago et al. published a case report of a patient with an S768I mutation who had achieved a partial response and PFS of more than 1 year while receiving second-line gefitinib treatment.[29] Other reports have identified the S768I mutation as a resistance mutation[22,30] or as a mutation less sensitive to erlotinib or gefitinib than to dual EGFR and the vascular EGFR inhibitor AEE788.[31] Chiu et al. reported seven patients with the S768I mutation who achieved an ORR of 33.3% and DCR of 66.7%, although patients with a compound S768 mutation (n = 10) showed a PFS of 11.9 months compared with 6.5 months for the single mutation.[8] A study in Norway identified three patients with S768I mutations, two of whom went on to receive EGFR TKI treatment. One patient had a partial response to first-line gefitinib, with a treatment time of 14 months, whereas the other patient received second-line erlotinib for 1 month until disease progression.[32] The conflicting reports highlight how difficult it can be to obtain a consensus on whether a mutation is resistant, activating, or neutral when so few cases are reported. Exon 20 mutations may function by altering the kinase domain conformation.[20]

Although many exon 20 mutations have been reported as conferring resistance to erlotinib by altering the P-loop and thus reducing the ATP-binding pocket,[25] a retrospective study by Naidoo et al. reported a V769_770insASV insertion that resulted in a PFS of 19.8 months ($\approx$602 days) and an OS of 24 months ($\approx$730 days), and a patient with a D770_N771insGT insertion achieving an OS of 55 months ($\approx$1674 days), thus demonstrating the variability of rare mutation effects.[33] Another retrospective study analyzed 1086 patients for *EGFR* mutations and identified 27 patients (2.5%) with exon 20 insertions, with the most common variant being V769_D770insASV.[34] In this study, exon 20 insertions were associated with a median PFS of 16 months and were more commonly found in never-smokers and Asian patients.[34]

Exon 21 Mutations

Response varied among the specific exon 21 mutations. Generally rare mutations in exon 21 resulted in a PFS of less than 6 months (182 days) and an OS of less than 1 year, with disease progression or stable disease reported as the best response. However, P848L, H870Y, G836S, and L858R + T790M mutations all resulted in an OS of longer than 1.5 years (547 days), with the G836S mutation also providing a PFS of longer than 1 year (>365 days) and partial response observed as the best overall response. The patient with the H870Y mutation

(OS 691 days) and another patient with a G836S mutation (OS 763 days) both continued to receive erlotinib treatment after progression, which could be linked to their longer OS.

Literature Review. As with our analysis, many rare mutations in exon 21 have been identified with varying responses.[20] The L861Q mutation has been investigated extensively and is reported to be a sensitizing mutation that increases kinase activity, whereas L861R and L862V have been reported as resistance mutations.[6] In the NEJ002 study, OS was significantly lower for patients with rare mutations (G719X or L861Q) than for those with common *EGFR* mutations ($p = 0.002$). Patients with rare *EGFR* mutations (G719X or L861Q) treated with gefitinib had a significantly shorter OS (11.9 versus 29.3 months; $p < 0.001$). Interestingly, OS was similar between patients with uncommon and common mutations when treated with carboplatin-paclitaxel ($p = 0.358$).[35]

Discussion

Although the classical *EGFR* activating mutations of exon 19 deletions and exon 21 L858R mutations have been proved to act as sensitizing mutations for erlotinib, how other less common *EGFR* mutations affect erlotinib efficacy is unclear. These analyses demonstrate the considerable variation in treatment outcome for patients whose tumors harbor different uncommon *EGFR* mutations. Although the general consensus in the literature is that rare mutations are associated with inferior responses to TKI therapy, our analyses showed that many rare mutations result in efficacy similar to that seen in unselected populations or patients with classical activating mutations; therefore, patients with these mutations should not necessarily be excluded from receiving EGFR TKI therapy. Some rare mutations might even slow tumor growth or metastasis compared with wild-type disease, and patients with these mutations might survive longer even without treatment. Also, some patients with rare mutations had a particularly positive outcome with erlotinib treatment, which may help to identify those uncommon mutations that are potentially predictive of an efficacy benefit with this therapy.

Our analysis reported that exon 18 G719 mutations, exon 19 K757R and E746G mutations, the exon 20 S768I mutation, and the exon 21 G836S mutation appeared to confer a good outcome with erlotinib treatment, whereas exon 18 S720I showed a particularly poor outcome. Because of the small number of patients with each mutation, however, it is difficult to confirm whether these rare mutations do indeed confer sensitivity or resistance to erlotinib. Our results are similar to those of the afatinib analysis of uncommon *EGFR* mutations, which observed tumor responsiveness and prolonged PFS in afatinib-treated patients with G719X mutations and L858R + T790M mutation; however, in contrast to our findings, S786I and S786I + L858R mutations were thought to be sensitizing in the afatinib study.[10] In a retrospective study by Naidoo et al., the exon 20 D770_N7719nsSVD mutation had low OS (3 months [≈91 days] and 10 months [≈302 days])[33]; in this analysis, however, these mutations appeared to confer long OS (599 days and 881 days in two patients in SATURN). This variation between analyses could be due to the mutations having a different effect with different TKIs, or it could simply highlight the difficulty in confirming a class effect with so few cases.

The difficulty in testing the efficacy of targeted agents against specific mutations is that because they occur at extremely rare frequencies, it is practically impossible to enroll enough patients to power a clinical trial except by using large-scale screening and complicated treatment allocation algorithms. This means that analyses of rare mutations are unlikely to reach statistical significance. Even if the same mutation is seen across several studies, the difference in study design, end points, and agents investigated means that it is hard to reliably pool data to gain a consensus on mutation type. Despite these issues in reliability of analysis, rare mutations are important to consider because licensed indications are usually specific to certain common mutations and those who might benefit considerably from a specific targeted therapy could be missing out on effective treatment with the current approach. This can be seen by comparing the U.S. labels for first-line erlotinib and afatinib, on which the indications are specified as exon 19 deletions and exon 21 L858R, with the EU labels, which mention only *EGFR* activating mutations (therefore, rare mutations could be included).

One potential limitation of the current diagnostic approach of testing only for common exon 19 and 21 mutations, as was done in the EURTAC or OPTIMAL studies,[4,5] is that such an approach might miss identifying a number of patients who could have some benefit from EGFR TKI treatment. Additional evidence may be needed to support conclusions regarding the predictive value of specific mutations that are less commonly observed. Another consideration limiting the current approach is that the mutation profile of a patient's tumor may change throughout the course of disease. As the difference between activating, neutral, and resistance mutations can make a sizable difference in how a patient's disease responds to EGFR TKI therapy, it is important to identify as many of these mutations as possible to guide treatment, however infrequent particular mutations are.

Some researchers have taken the reverse approach to that taken in this analysis and have identified

patients with exceptional responses to specific therapies, and then carried out full gene analysis on patients' tumor tissue to identify which mutation may be driving that response.[36] The limitation of this approach is that it can be a time-consuming process and would rely on patients being given off-label experimental treatments to assess whether their tumors respond, which is not an option for many clinicians owing to strict formularies and reimbursement issues. Online registries of oncogenic mutations, such as MyCancerGenome,[37] may act as a central repository of observed outcomes with different treatments in different tumors, but this information is still incomplete and will require further research and knowledge sharing by all cancer researchers and clinicians to become a feasible clinical resource.

The use of more sensitive testing techniques such as next-generation sequencing and the potential use of blood samples to make it simpler to obtain material for testing may in the future make it more feasible to assess both rare mutations and the classical mutations already used for diagnosis. This would be helpful to get a more accurate picture of mutation status for patient selection for upfront TKI treatment.

Conclusions

Further research is clearly needed to better understand the impact of rare mutations in *EGFR* and other genes involved in lung cancer tumorigenesis. As these mutations are increasingly being detected by standard testing methods, it is important to have a central repository of information about the implications of specific mutations for patient prognosis and treatment choice. Large-scale investigation in clinical trials is unlikely to be an option in evaluating treatments in these very small patient subgroups, so clinicians will need to be proactive in finding other methods to determine optimal therapy choice.

Acknowledgements

Support for third-party writing assistance for this manuscript was provided by Joanna Musgrove of Gardiner-Caldwell Communications and funded by F. Hoffmann-La Roche. We would like to thank MyCancerGenome and the DNA-Mutation Inventory to Refine and Enhance Cancer Treatment for aiding in the literature search.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at http://dx.doi.org/10.1016/j.jtho.2015.12.107.

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