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EGFR exon 20 insertions in advanced lung adenocarcinomas: clinical outcomes and response to erlotinib

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Abstract

Background—*EGFR* exon 20 insertions (exon20ins) represent approximately 10% of *EGFR*-mutant lung adenocarcinomas and are associated with resistance to *EGFR* tyrosine kinase inhibitors (TKIs). Clinical outcomes compared to patients with sensitizing *EGFR* mutations are not well-established.

Methods—Patients with stage IV lung adenocarcinomas with *EGFR* exon20ins were identified through routine molecular testing. Clinico-pathologic data were collected. We measured overall survival (OS) from diagnosis of stage IV disease, and in patients treated with EGFR TKIs, time to progression (TTP) on erlotinib.

Results—1882 patients with stage IV lung adenocarcinomas were identified: 46 patients had *EGFR* exon20ins (2%) and 258 patients had an *EGFR* exon 19 deletion (exon19del)/L858R point mutation (14%). Among 11 patients with lung adenocarcinomas with *EGFR* exon20ins who received erlotinib, 3 patients (25%) had a partial response (FQEA=1, ASV=1, unknown variant=1). TTP on erlotinib for patients with *EGFR* exon20ins vs. *EGFR* exon19del/L858R was 3

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months vs. 12 months (p<0.01). Responses to chemotherapy were similar in patients with lung adenocarcinomas with *EGFR* exon20ins and with exon19del/L858R. Median OS from diagnosis of stage IV disease for patients *EGFR* exon20ins vs. *EGFR* exon19del/L858R was 26 months (95% CI: 19-Not reached, n=46) vs. 31 months (95% CI: 28-33, n=258) (p=0.53).

Conclusions—The majority of patients with advanced lung adenocarcinomas harboring an *EGFR* exon20ins, do not respond to EGFR TKI therapy. Standard chemotherapy should be utilized as first-line therapy. These patients have an OS similar to patients with sensitizing *EGFR* mutations. Individuals with certain variants such as FQEA and ASV may respond to erlotinib.

Introduction

Therapeutic targeting of *EGFR* with EGFR tyrosine kinase inhibitors (TKIs) has demonstrated efficacy in lung adenocarcinomas, with the presence of an *EGFR* mutation within the tyrosine kinase domain predicting response to EGFR TKIs [1-3]. In particular, *EGFR* exon 19 deletions (exon19del) and the L858R point mutation in exon 21 are sensitizing mutations that result in favorable responses to therapy with both reversible and irreversible EGFR TKIs[1, 2, 4-10]. Over time, through the institution of routine molecular profiling of lung adenocarcinomas, rarer mutations in the *EGFR* tyrosine kinase domain began to emerge[11-15], with uncertain responsiveness to EGFR TKIs.

Retrospective data initially suggested that *EGFR* exon 20 insertions (exon20ins) conferred resistance to EGFR TKI therapy, and thus this subset of patients has been omitted from many prospective clinical studies [9, 10, 16]. Published retrospective studies investigating this genotypic subset of lung adenocarcinoma have demonstrated that *EGFR* exon20ins occur more commonly in patients who are female, Asian and never-smokers, similar to patients whose tumors harbor classically sensitizing *EGFR* mutations [12, 13, 17-21]. Response to EGFR TKI therapy for these patients has been explored in small retrospective series ranging from 2-25 patients[12, 17-22]. These studies demonstrate mixed results with regard to objective response rate to EGFR TKI, progression-free survival and overall survival.

There is preclinical data to suggest that cells containing certain *EGFR* exon20ins variants may have a similar affinity for EGFR TKI as cells harboring *EGFR* exon19del and L858R. The *EGFR* exon20ins variant A763_Y764insFQEA was studied in vitro, and was the only *EGFR* exon20ins harboring cell line inhibited by erlotinib at concentrations of less than 0.1uM. Yasuda and colleagues subsequently examined the crystal structure of cells harboring *EGFR* exon20ins, and through kinetic studies and conformational analysis, demonstrated that other *EGFR* exon20ins such as D770_N771insNPG have a reduced affinity and sensitivity to EGFR TKI, similar to wild-type EGFR[17].

In light of increasing knowledge of the function and structural differences between rarer subtypes of EGFR mutations including *EGFR* exon20ins variants, further studies are needed to examine differential responses to EGFR TKIs and overall survival in patients who harbor these mutations. This retrospective study aims to assess the clinico-pathologic features, response to EGFR TKI therapy, and overall survival from diagnosis of stage IV disease of patients with stage IV lung adenocarcinomas that harbor *EGFR* exon20ins.

Methods

Study population and Data collection

Patients with lung adenocarcinomas at MSKCC whose tumors underwent routine molecular diagnostic testing between 2009 and 2013 were identified using programmatically abstracted elements from diagnostic molecular pathology reports and tumor registration data available via a web-based application. An MSKCC Institutional Review Board and Privacy Board waiver was obtained to facilitate retrospective collection of clinico-pathologic data. The results were reviewed to identify patients with metastatic disease. Clinical data collected were age, gender, Karnofsky performance status (KPS) and smoking history (current/former smoker, never smoker). Pathologic data included *EGFR* mutation position, exon20ins length, exact amino acid sequence, and the presence or absence of concurrent mutations. Treatment data extracted included the types of treatment, number of lines of therapy, receipt of EGFR TKI, duration of treatment, and radiologic response to therapy. As these patients were not on an official protocol, imaging frequency was variable, and took place on average every 2-3 months. Patients with incomplete or unknown treatment data were excluded from treatment analyses.

Molecular testing

Detection of known sensitizing mutations in *EGFR* (exon19del and L858R) was carried out by a combination of fragment analysis and mass spectroscopy genotyping, using previously described methods [14, 23]. All patients with advanced lung adenocarcinoma assessed at MSKCC underwent mass spectrometry genotyping (Sequenom) of *EGFR, KRAS, BRAF, ERBB2/ HER2, NRAS, AKT, MAP2K1,* and *PIK3CA,* as previously described [23]. This involves a series of multiplexed assays that assess for the presence of 92 non-synonymous point mutations in 6 multiplex reactions. *EGFR* exon20ins were identified using fragment analysis. If an *EGFR* exon20ins was detected, Sanger sequencing was completed to confirm the presence of the mutation and identify the insertion position and sequence. Initial screening was conducted by a sizing assay using primers FW1:50-TCTTCACCTGGAAGGGGTCCA-30 and REV1:50-Fam-TGCCACCTCCACTCCGTCTA-30). Positive cases were characterized by Sanger sequencing using primers FW1:50-CATTCATGCGTCTTCACCTG- 30 and REV1:50-GTATAGGGGTACCGTTTGAG-30[14, 24].

Statistical analyses

Overall survival (OS) and time to progression (TTP) on TKI therapy were calculated using the Kaplan-Meier method. OS was defined as time from diagnosis of stage IV disease, to the death from any cause. TTP was defined as time from commencement of EGFR TKI to radiologic progression. For both OS and TTP analyses, patients who did not experience the event of interest during the study time were censored at the time of data cut-off, December 2013. OS and TTP were compared across groups using log-rank test. Clinical characteristics of those patients with stage IV lung adenocarcinoma harboring an *EGFR* exon19del and L858R, were compared to those with *EGFR* exon20ins using Fisher's exact test. Pathologic characteristics are presented descriptively.

Results

Clinical characteristics

In patients identified with stage IV lung adenocarcinoma (n=1882), 258 patients (14%) had tumors that possessed an *EGFR* exon19del or L858R point mutation, and 46 patients (2%) had an *EGFR* exon20ins. The clinical characteristics of these two groups of patients were similar and are summarized in Table 1. Patients with stage IV lung adenocarcinoma with *EGFR* exon20ins were older (median age= 67years, range= 10 years) than those with *EGFR* exon19del or L858R (median age= 63 years, range= 12years) (p=0.01). No significant differences were detected between these groups of patients with regard to sex, smoking history, ethnicity or performance status.

Pathologic characteristics

The molecular characteristics of the patients with stage IV lung adenocarcinoma with *EGFR* exon20ins (n=46) are detailed in Table 2. Base pair length ranged from 3-12bp, where 61% (n=28/46) of patients had tumors with a 9bp insertion. All patients with *EGFR* exon20ins identified by fragment analysis, subsequently underwent Sanger sequencing. Sequencing on 1 case failed, and one case had very low tumor content such that the previously identified *EGFR* mutation could not be confidently characterized. Seventeen unique *EGFR* exon20ins variants were identified, and are depicted in Table 2. The variants most frequently seen were: D770_N771insSVD (n=11/46, 24%) and V769_D770insASV (n=10/46, 21%).

Of the 46 patients with stage IV lung adenocarcinoma with an *EGFR* exon20ins, 2 patients with D770_N771insSVD and 1 patient with a V769_D770insASV variant had concurrent *PI3K* mutations. Two patients with lung cancers harboring *EGFR* exon20ins had multiple synchronous lung cancers, with two resected lung adenocarcinomas per patient. In these two patients, one lesion harbored an *EGFR* exon20ins, and the second resected lesions contained a *KRAS* Q61H mutation (exon20ins: H773_v774insNPH) and a *KRAS* G12D mutation (exon20ins: V774_C775insHV), respectively.

Treatment and Survival data

Treatment data was available for all 46 patients with tumors harboring an *EGFR* exon20ins. Three patients did not receive systemic therapy: 2 received supportive care only, 1 patient declined therapy. The median number of lines of therapy for the entire cohort was 2 (range: 0-7). In patients who received erlotinib (n=11), the median number of lines of therapy was 3 (range: 1-7), and in those who did not receive erlotinib (n=35) was 2 (range: 0-5). Of the 11 patients treated with erlotinib, they received the following systemic therapies: platinum doublet+/−maintenance (n=7/11: platinum/pemetrexed=5, platinum/taxane=2), single agent chemotherapy (n=5/11: docetaxel=2, gemcitabine=2, pemetrexed=1), immunotherapy (n=2/11), cetuximab (n=1/11). Patients who did not receive erlotinib (n=35), received similar types of systemic therapy: platinum doublet+/−maintenance (n=28/35: platinum/pemetrexed= 25, platinum/taxane=3), single agent chemotherapy (n=17/35: docetaxel=8, gemcitabine=7, pemetrexed=2) and immunotherapy (n=2/35).

The most common systemic therapies received by the entire cohort were: platinum doublet chemotherapy (76%, n=35/46: platinum/pemetrexed= 30, platinum/taxane=5), single agent chemotherapy (48%, n=22/46: docetaxel=10, gemcitabine=9, pemetrexed=3), immunotherapy (9%, n=4/46: 2=nivolumab, 1=MPDL3280A, 1=pembrolizumab). The response rates to the systemic therapies received by patients with *EGFR* exon20ins were: platinum doublet chemotherapy: 63% (n=22/35), single agent chemotherapy: 32% (n=7/22), immunotherapy: 50% (n=2/4) and cetuximab: 0% (n=0/1). The response rates for the three most common regimens were: platinum doublet= 63% (n=22/35), docetaxel= 30%, (n=3/10) and gemcitabine=22% (n=2/9). The median duration of therapy for the three most common regimens were: platinum doublet= 6 months (range: 1-36), docetaxel= 3 months (range: 1-9), gemcitabine= 2 months (range: 1-18).

Eleven patients were treated with erlotinib at the discretion of the treating physician, and none of them harbored a concurrent mutation. Three of these 11 patients were reported in a previous publication, and were among the 8 patients who did not respond to erlotinib [11]. All treatments received by these 11 patients are depicted in Figure 3, where chemotherapy refers to both single agent or combination chemotherapy, and ‘other’ includes immunotherapy and the biologic agents bevacizumab and cetuximab. Treatment response to erlotinib and corresponding molecular data for the 11 patients are detailed in Table 4. Three patients with tumors harboring *EGFR* exon20ins (n=3/11) had a partial response to erlotinib. The median TTP for the 11 patients treated with erlotinib was shorter compared to patients with advanced lung adenocarcinomas cancers harboring sensitizing *EGFR* mutations treated with erlotinib (2.5 mo vs. 12.2 mo, $p<0.001$) (Figure 1). The patient with a mutation in the C-helix of exon 20 (patient 1 in Table 4, A763_Y764insFQEA) exhibited a partial response to therapy and a short TTP, but had an overall survival from diagnosis of stage IV disease of 26 months. Patient 2 harbored a mutation in exon 20 outside the C-helix (V769_D770insASV), and exhibited a partial response to EGFR TKI, prolonged TTP of 20 months and an OS of 24 months. The third patient who responded to erlotinib had a short-lived partial response to therapy, a median OS of 11.1 months, and unfortunately tumor tissue failed sequencing so the exact *EGFR* exon20ins variant present is unknown (patient 11 in Table 4). The median OS from the date of stage IV diagnosis for patients with stage IV lung adenocarcinoma with *EGFR* exon20ins, compared to patients with sensitizing *EGFR* mutations were similar (26months versus 31 months, $p=0.53$) (Figure 2). The three patients with concurrent *EGFR* exon20ins and *PIK3CA* mutations did not receive erlotinib, and their survival from date of diagnosis until death was 33.2 months (D770_N771insSVD), 38 months (D770_N771insSVD), and 2.8 months (V769_D770insASV) respectively.

Discussion

The diagnosis and management of advanced lung adenocarcinomas has changed dramatically in the last decade. Diagnostic molecular testing has defined molecular subsets that have improved responses and survival with the use of targeted therapy, compared to a purely histology-based classification and the use of cytotoxic chemotherapy alone. As broader molecular testing becomes routine, we are identifying rarer genetic alterations and further subclassifying gene mutations previously identified, such as exon20ins in the *EGFR* gene in lung adenocarcinomas.

This study explores the clinical and molecular characteristics, response to targeted therapy, and survival in patients with advanced lung adenocarcinomas harboring *EGFR* exon20ins. In this series, we report that advanced lung adenocarcinomas with *EGFR* exon20ins have an incidence of 2%, which is similar to the 0.4-0.9% reported in published studies [11, 12, 18-21]. These patients have similar clinical characteristics to patients with more common sensitizing mutations in EGFR, but demonstrate an objective response rate of 11% (0-50%) and short PFS of 2.4 months (2.3-2.5 months) with EGFR TKI therapy in published studies. In the 11 patients with *EGFR* exon20ins treated with erlotinib in this study, we report an objective response rate of 27% and a median TTP of 2.5 months. A swimmer's plot of the treatment course of these patients (Figure 3) demonstrates that in all cases except one (V769_D770insASV), treatment with erlotinib was received for a very short period on time. Erlotinib therapy thus does not appear to make a meaningful contribution to the total treatments received. Moreover, even in patients with *EGFR* exon20ins who achieved responses to erlotinib (n=3/11), these responses were short-lived in all but one case (V769_D770insASV) (Figure 2). These findings are corroborated in small series of between 2 and 25 patients with stage IV lung adenocarcinomas with *EGFR* exon20ins treated with EGFR TKI, where the pooled median PFS is approximately 2.4 months [19-21] (Table 3). Thus, most patients with *EGFR* exon20ins spent the majority of their treatment time receiving chemotherapy. Therefore, we would advocate chemotherapy as standard first-line therapy for patients with advanced lung adenocarcinoma, harboring an *EGFR* exon20ins.

When examining the treatment history of patients in these series, it is notable that patients with *EGFR* exon20ins had comparable response rates to systemic chemotherapy when compared to all patients with lung adenocarcinomas and specifically compared to patients with EGFR-mutant lung adenocarcinomas in published phase III studies[4, 25]. The median time on platinum doublet chemotherapy in our series was 6 months, which was similar to the median progression-free survival reported in the subset of patients with EGFR-mutant lung cancers who received carboplatin and paclitaxel in a phase III study[4]. The response rates to standard systemic therapy for patients in our series are slightly better than historical controls (all-comers and those with sensitizing *EGFR* mutations), but include a very small number of patients, which preclude any definitive conclusions.

Response rates to combination chemotherapy in patients with lung adenocarcinomas with *EGFR* exon20ins have been reported in one previous publication, where a response rate of 58% was seen with combination chemotherapy in patients with available imaging (n=7/12)[19]. These findings, together with the observations from the current study which are not based on prospective evaluation, would need to be confirmed in a future study.

Our study is also one of few to report the specific amino acid sequences of *EGFR* exon20ins, and the relationship with response to EGFR TKIs. Insertion sequence variants have been described in previous studies, with up to 13 sequence variants reported [17, 18, 20, 26]. This series reports 17 variants of *EGFR* exon20ins, with two variants not previously described (D770_N771insGV and H773_V774insY). We corroborate the novel funding reported by Yasuda and colleagues, stating that the presence of an *EGFR* exon20ins variant *EGFR*-A763_Y764insFQEA can predict for response to EGFR TKI therapy, and thus has distinct behavior compared to the other *EGFR* exon20ins variants. In

our report of one patient, and in their report of two patients whose tumors harbored the FQEA variant, all three patients demonstrated partial responses to erlotinib and improved OS [17, 27]. In our series, this patient had a short-lived partial response and TTP on erlotinib, and an extended OS from diagnosis of stage IV disease. We identified one additional patient with an *EGFR* exon20ins variant (V769_D770insASV), traditionally thought to confer resistance to EGFR TKIs, who demonstrated a partial response to erlotinib, extended TTP and OS. Therefore, certain *EGFR* exon20ins variants may predict for response to EGFR TKI, and should be considered as a treatment option in patient's whose tumors harbor these variants.

The presence of other concurrent molecular alterations may contribute to response or lack of response to EGFR TKIs. Patients with *EGFR*-mutant lung adenocarcinomas whose tumors harbor concurrent *PI3K* mutations have demonstrated poorer outcomes and variable response to EGFR TKI [28-30]. This phenomenon is also seen in other molecularly driven subsets of lung adenocarcinomas such as *KRAS*-mutant lung adenocarcinomas. In *KRAS*-mutant tumors, the presence of a concurrent *LKB1* (*STK11*) mutation portends a poorer prognosis, and may confer resistance to targeted therapies such as mTOR and MEK inhibition [31-37]. Two of our three patients with concurrent *EGFR* exon20ins and *PI3K* mutations had extended OS, and all three did not receive TKI. Further comprehensive analysis is needed to understand how additional molecular alterations may augment response to EGFR TKIs.

Interestingly, despite a generally poor response to targeted therapy, this study also demonstrates that patients with stage IV lung adenocarcinomas with *EGFR* exon20ins, had median OS of 26 months. This is similar to the cohort of patients who harbored sensitizing EGFR mutations (31 months, exon19del and L858R, n=258), and is consistent with a pooled median OS of 9.5-48 months for patients with stage IV lung adenocarcinomas, with *EGFR* exon20ins [12, 18, 19] (Table 3). The underlying reason for this improved median OS is not currently known, and may point to unique disease biology of lung adenocarcinomas with *EGFR* exon20ins.

Lung adenocarcinomas with *EGFR* exon20ins comprise 2% of all lung adenocarcinomas, which is a larger subset than those that harbor RET or ROS1 rearrangements, in which extensive therapeutic studies are underway. Similar efforts are needed to develop molecularly targeted agents that specifically target *EGFR* exon20ins, as traditional EGFR TKIs are generally ineffective. To date, one ongoing clinical trial has focused on this molecular subset and is assessing the utility of an HSP90 inhibitor AUY992 in this population (NCT01854034).

In conclusion, stage IV lung adenocarcinoma with *EGFR* exon20ins are a unique subset of lung adenocarcinomas. There are a large number of *EGFR* exon20ins variants, and we identified and report two new *EGFR* exon20ins sequence variants in this study. Patients with these tumors have clinical characteristics similar to patients with lung adenocarcinomas that harbor *EGFR* exon19del and L858R, and most insertion variants are resistant to erlotinib. We corroborate previous findings that A763_Y764insFQEA is an *EGFR* exon20ins variant that predicts response to EGFR TKI, and we identify a potential second sensitive variant,

V769_D770insASV although previous published reports suggest this variant confers EGFR TKI resistance[12, 17, 19]. Additional concurrent genetic alterations may elucidate this differential response to EGFR TKI seen. For patients with other *EGFR* exon20ins sequence variants, treatment with erlotinib as first-line treatment is not recommended. We recommend that these patients be treated with chemotherapy in the first-line setting. Interestingly, despite a generally poor response to erlotinib in this study, the median OS of these patients was prolonged, and similar to patients whose tumors harbored sensitizing *EGFR* mutations. The underlying biology accounting for this clinical behavior is currently unknown. As we begin to further understand the functional significance of specific *EGFR* exon20ins, it is likely that these aberrations will be further subdivided into groups of insertion variants which may respond differently to EGFR TKI therapy, and have distinct clinical behaviors.

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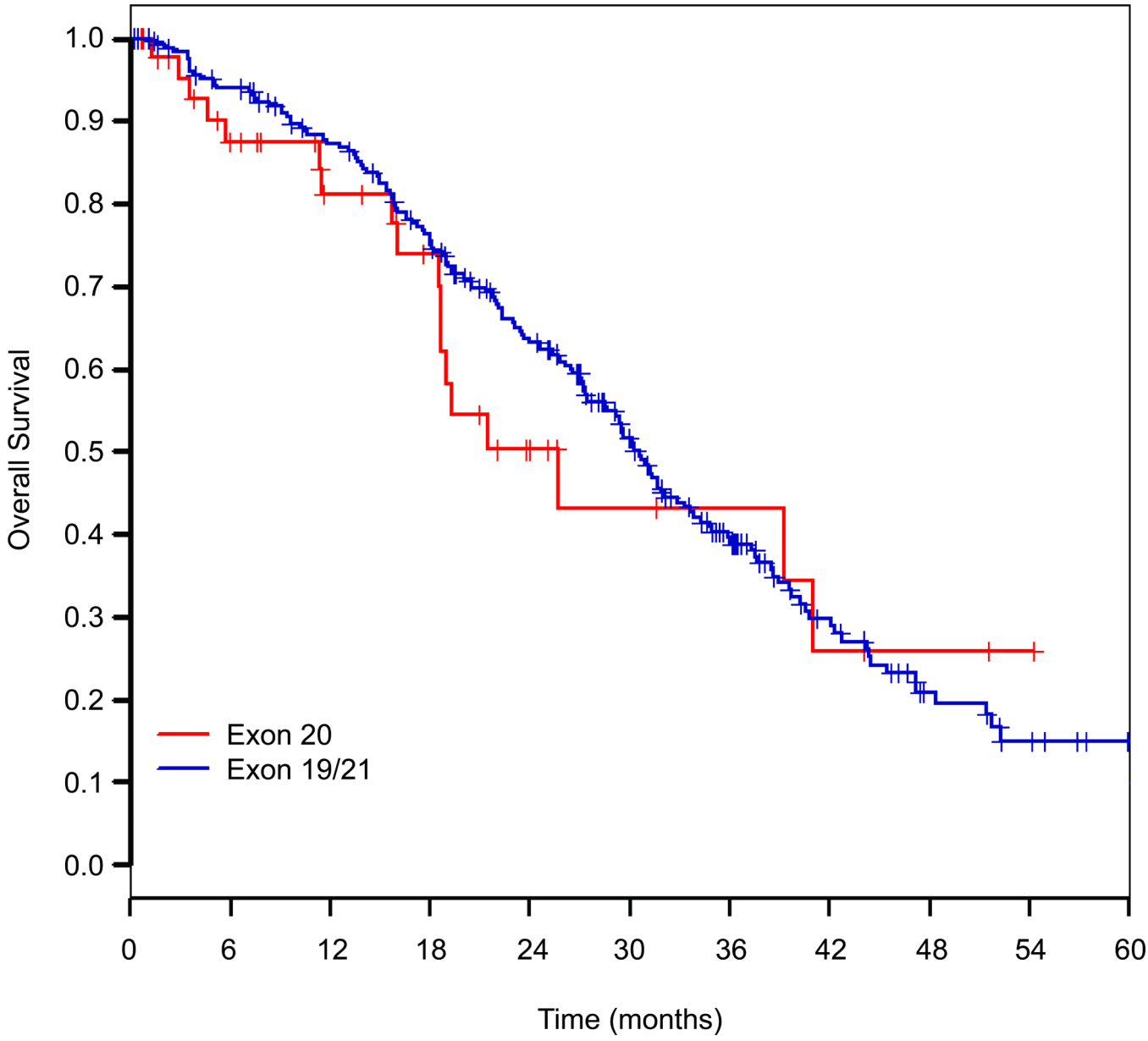

Figure 1.

Kaplan Meier curve depicting time to progression with EGFR tyrosine kinase inhibitor therapy, for patients with stage IV EGFR-mutant lung adenocarcinoma harboring an *EGFR* exon 19 deletion or L858R point mutation versus an *EGFR* exon 20 insertion. TTP= time to progression, del= deletion

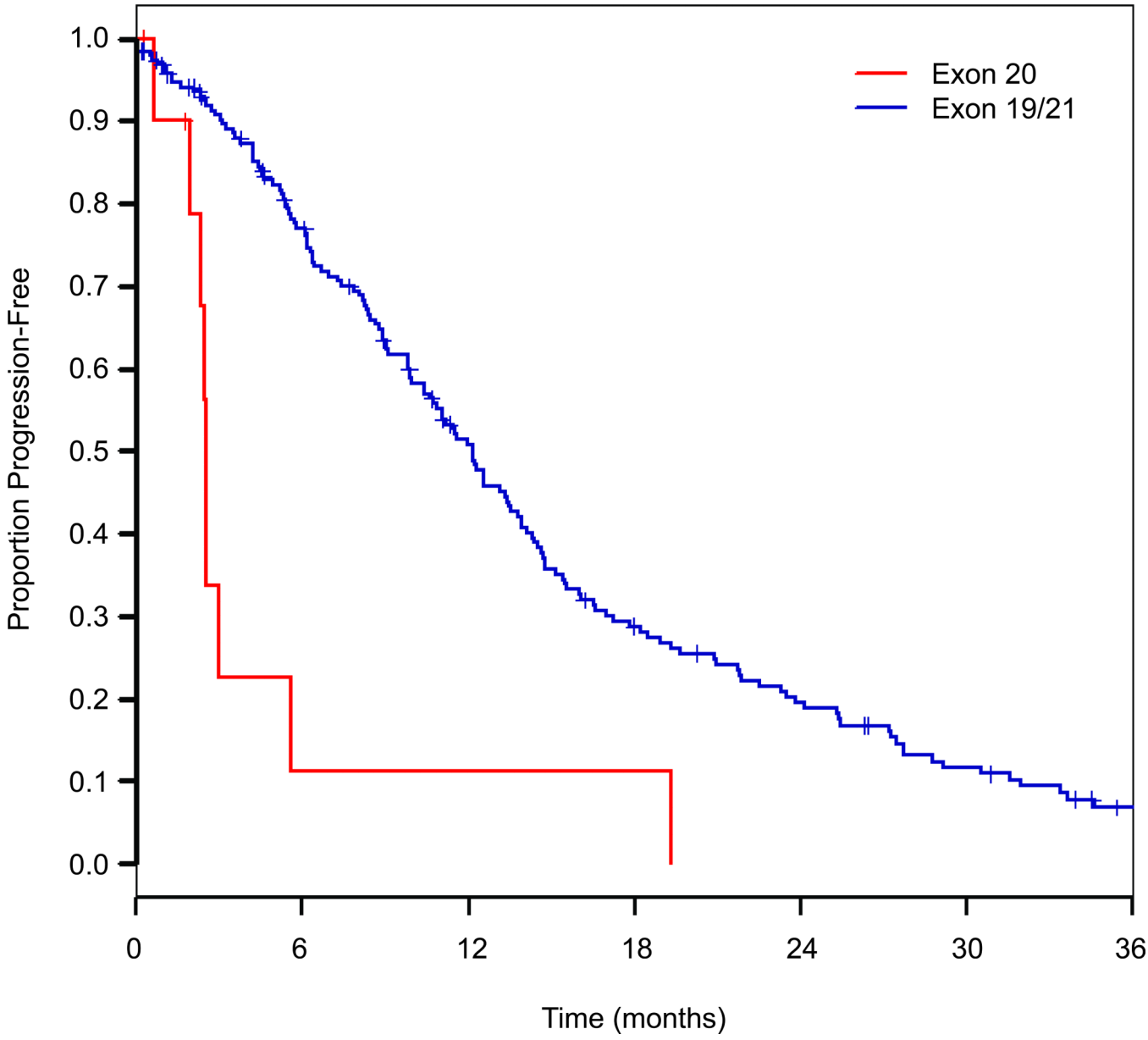

Figure 2.

Kaplan Meier curve depicting overall survival from date of stage IV diagnosis, for patients with stage IV EGFR-mutant lung adenocarcinoma harboring an *EGFR* exon 19 deletion or L858R point mutation versus an *EGFR* exon 20 insertion. OS= overall survival, del= deletion.

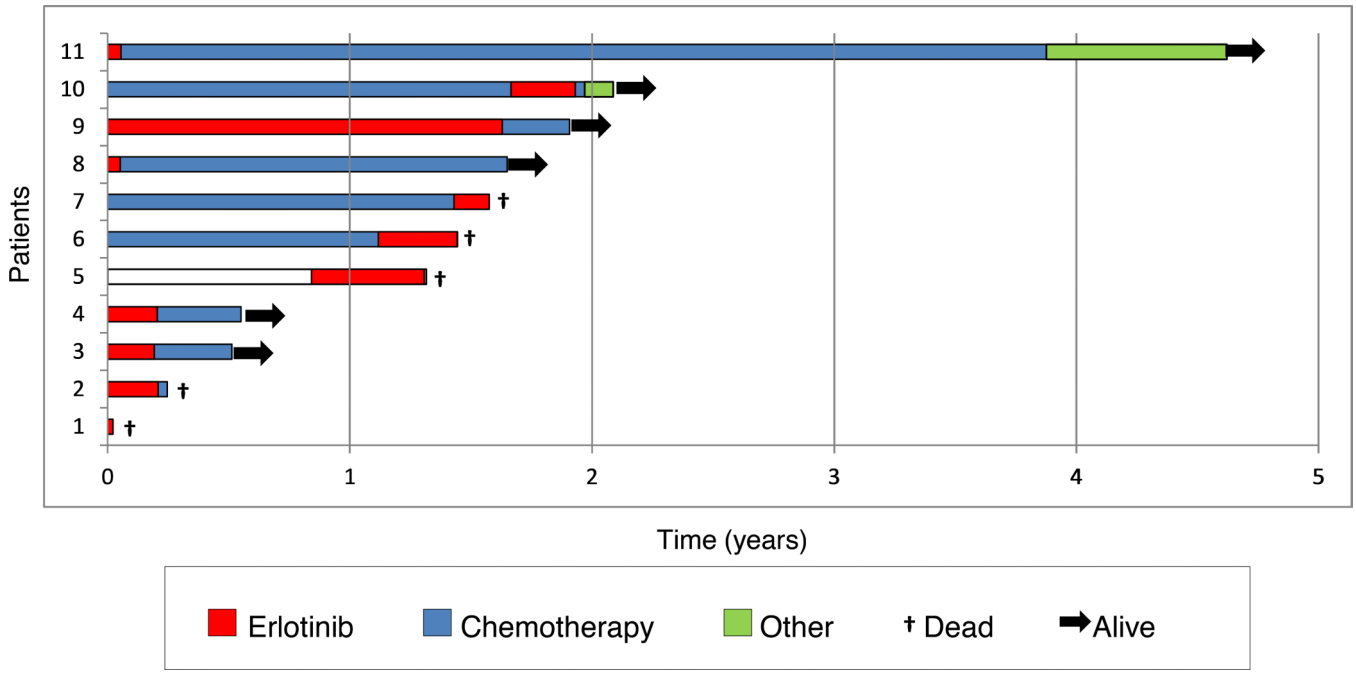

Figure 3.

Swimmer plot depicting the treatment course of 11 patients with advanced lung adenocarcinoma with an *EGFR* exon 20 insertion, who were treated with erlotinib. Chemotherapy= single agent or doublet chemotherapy, Other= immunotherapy or biologic agents: bevacizumab or cetuximab.

Table 1

Clinical Characteristics of Patients with Stage IV Lung Adenocarcinoma with an *EGFR* exon 19 deletion/ L858R vs. an *EGFR* exon 20 insertion

	Exon 19 deletion/L858R (n= 258)	Exon 20 insertion (n=46)	P value
Sex			
Male	86 (33)	19 (41)	0.32
Female	172 (67)	27 (59)	
Mean Age (Range)	63 (60-69)	67 (62-69)	0.01
Smoking status			
Never	150 (58)	26 (57)	0.87
Former/Current	108 (42)	20 (43)	
Ethnicity			
Caucasian	174 (67)	33 (72)	0.31
Asian	42 (16)	9 (20)	
Black	15 (6)	3 (7)	
Other	27 (10)	1 (2)	
Karnofsky PS*			
>80	212 (82)	41 (89)	0.21
<or equal 80	46 (18)	5 (11)	

*PS= performance status. These cases include those previously reported by Arcila et al[13]

Table 2

EGFR exon 20 insertion variants in Patients with Stage IV Lung Adenocarcinoma

Insertion Variant	Number of cases	Concurrent Mutations	Insertion Sequence
A763_Y764insFQEA	1	None	c.2990_2992 ins TCCAGGAAGCCT
A767_S768insTLA	1	None	c.2302_2303 ins CGCTGGCCA
V769_D770insASV	10	PIK3CA (c.1633 G>A)	c.2308_2309 ins CCAGCGTGG
V769_D770insGE	1	None	c.2308_2309 ins GCGAGG
V774_C775insHV	1	None	c.2321_2322 ins CCACGT
N771_P772insH	1	None	c.2314_2315 ins ACC
N771_P772insN	1	None	c.2314_2315 ins ACC
P772_H773insNP	1	None	c.2316_2317 ins AACCCC
P772_H773insNPH	1	None	c.2316_2317 ins GACAACCCC
D770_N771insSVD	11	PIK3CA (p.E545K, C.1633 G>A) PIK3CA H1047R	c.2311_2312 ins GCGTGGACA
D770_N771insGV	1	None	Not available
D770_N771insGT	1	None	c.2310_2311 ins GGCACA
H773_v774insNPH	4	None	c.2319_2320 ins AACCCCCAC
H773_V774insPH	2	None	C2319_2320 ins CCCCAC
H773_V774insAH	2	None	c.2320_2321 ins CTCACG
H773_V774insH	4	None	c.2319_2326 ins CAC
H773_V774insY	1	None	c.2319_2320 ins TAC
Failed sequencing	1		
Insufficient Tissue	1		

Table 3

Response and Survival Data for Patients with Stage IV Lung Adenocarcinoma with *EGFR* exon 20 insertions, treated with EGFR TKI

Pt	Amino Acid sequence	Concurrent Mutation	Best Radiologic Response	TTP (mo)	OS (mo)
1	A763_Y764insFQEA	No	PR*	3.2	25
2	V769_D770insASV	No	PR*	19.8	24
3	D770_N77insSVD	No	PD	0.2	3
4	V769_D770insASV	No	PD	5.6	19
5	H773_v774insNPH	No	PD	2.5	8
6	V769_D770insASV	No	PD	0.6	21
7	D770_N771insGT	No	SD	0.7	55
8	D770_N771insSVD	No	PD	2.3	10
9	H773_V774inAH	No	SD	2.5	3
10	Failed sequencing	No	PD	3.9	19
11	Insufficient DNA	No	PR*	1.7	19

TTP= Time to Progression, OS= Overall Survival, PR*= Partial Response, SD= Stable disease, PD= progressive disease

Table 4

Reported Studies of EGFR TKI Response and Survival in Patients with Advanced Lung Adenocarcinomas with an *EGFR* exon 20 insertion

Study	EGFR Exon 20 insertions /total lung cancers tested (n/total, %)	Patients with stage IV disease (n)	Number of Patients treated with EGFR TKI for stage IV disease (TKI)	Objective Response Rate (% evaluable, n)	PFS with EGFR TKI (mo)	Median OS (mo)
Sasaki et al[20]	7/332 (2%)	2	2 (n=2 gefitinib)	0% (n=0/2)	Not reported	Not reported
Wu et al[21]	10/515 (2%)	14	14 (n=14 gefinitib)	29% (n=4/14)	2.3	Not reported
Arcila et al[18]	33/600 (6%)	15	5 (n=2 erlotinib +chemo) (n=3 erlotinib alone, 4 with available imaging)	50% (n=2/4)	Not reported	48
Oxnard et al[19]	27/1086 (2.5%)	19	8 (n=8, erlotinib alone, 5 with available imaging)	0% (n=0/5)	2.4	16.5
Yasuda et al[17]	19(100%)	19	19 (n=9, erlotinib alone) (n=10, gefinitinib alone)	11% (n=2/19)	Not reported	Not reported
Beau-Faller et al[12]	41/10117 (0.4%)	25	25 (n= 9, erlotinib/ gefitinib first-line) (n= 15, erlotinib/ gefitinib second line) (n=1, erlotinib/ gefitinib third-line)	8% (n=2/25)	Not reported	9.5
Lund-Iverson et al[22]	7/119* (6% of EGFR-mutants)*	7	3 (n=1, gefitinib (n=2, erlotinib)	0% (n=0/3)	Not reported	Not reported
Naidoo et al (current study)	46/1882 (2.4%)	46	11 (n=11, erlotinib)	27% (n=3/11)	2.5	26
Total/Median (Range)	**185/15321 (1.2%)**	**140**	**84 (2-25)**	**11% (0-50%)**	**2.4 (2.3-2.5)**	**16.5 (9.5-48)**

PFS= progression-free survival, OS= overall survival, PD= progressive disease, SD= stable disease, PR= partial response.

*Only in EGFR mutant patients in this study, total number of patients tested for EGFR not reported.