Original Study

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Clinical Impact of Rare and Compound Mutations of Epidermal Growth Factor Receptor in Patients With Non–Small-Cell Lung Cancer

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Abstract

Lung cancer samples were analyzed for epidermal growth factor receptor (*EGFR*) mutations, and the disease of patients with a rare *EGFR* mutation did not respond to first-generation tyrosine kinase inhibitors (TKIs), except for a single patient harboring the mutation p.G874D. In contrast, the disease of all patients with compound mutations responded to TKIs. After assessing data from database and literature searches, we found that clinical relevance for rare and compound *EGFR* mutations remains limited.

Background: Standard therapy of advanced non–small-cell lung cancer harboring an activating mutation in the epidermal growth factor receptor (*EGFR*) gene is treatment with tyrosine kinase inhibitors (TKI). However, for rare and compound mutations of the *EGFR* gene, the clinical evidence of TKI therapy is still unclear. **Patients and Methods:** A total of 2906 lung cancer samples were analyzed for *EGFR* mutations during routine analysis between 2010 and 2017. The samples have been investigated by Sanger sequencing and since 2014 by next-generation sequencing. **Results:** We detected *EGFR* mutations in 408 specimens (14%). Among these, we found 41 samples with rare and 22 with compound mutations. In these 63 samples, 56 different rare *EGFR* mutations occurred. Information about the clinical outcome was available for 37. Among those with rare mutations, only one patient harboring the mutation p.G874D had disease that responded to first-generation TKI therapy. In contrast, the disease of all patients with compound mutations responded to first- or second-generation TKI therapy. Furthermore, we collected data on clinical relevance regarding TKI therapy from different databases and from an additional literature search, and only found data for 36 of the 56 detected rare mutations. **Conclusion:** Information about the clinical outcome of patients with rare and compound *EGFR* mutations remains limited. At present, second- and third-generation TKIs are available, which may represent new treatment strategies for these patients. Therefore, it is becoming increasingly important to maintain databases concerning rare *EGFR* mutations.

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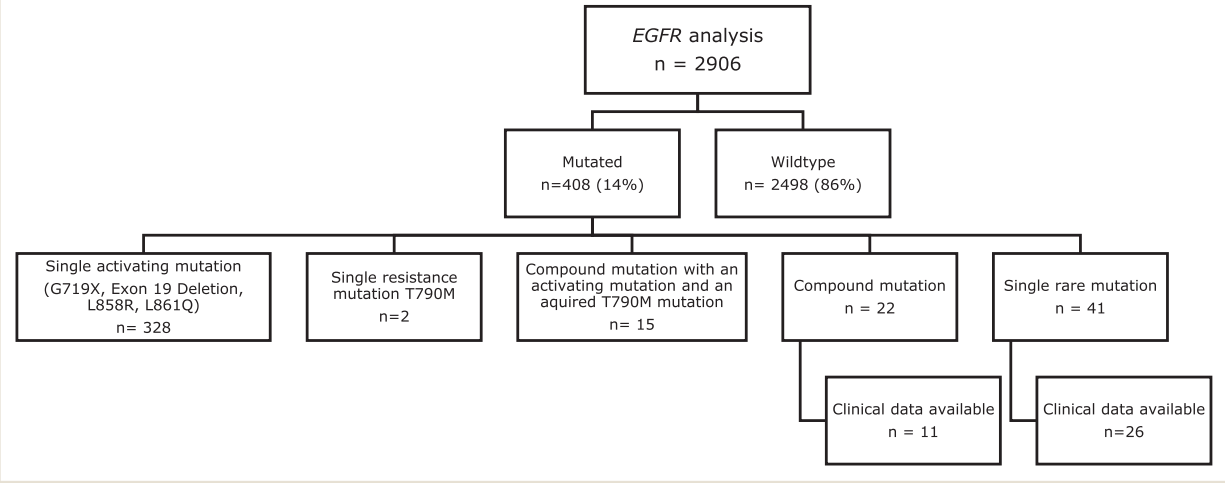
Figure 1 Tested *EGFR* Samples From June 2010 to June 2017



Abbreviation: EGFR = epidermal growth factor receptor.

Introduction

According to the World Health Organization, lung cancer is the most common cancer worldwide. It is of one of the most aggressive cancers and has the highest mortality rate.[1] The main subtype (80-85%) is non−small-cell lung cancer (NSCLC), which includes the histologic subtypes of adenocarcinoma, squamous-cell carcinoma, and large-cell carcinoma.

In addition to adjuvant chemotherapy, epidermal growth factor receptor (*EGFR*) targeting therapies with tyrosine kinase inhibitors (TKI) such as gefitinib, erlotinib, and afatinib, and more recently osimertinib, are provided to treat patients with NSCLC that harbors an activating mutation in the *EGFR* gene. These mutations occur in approximately 10% of white patients,[2-4] with higher frequencies in female patients, in adenocarcinomas, and in never smokers. Short in-frame deletions in exon 19 and the point mutation L858R in exon 21 comprise 85% to 90% of all sensitizing mutations.[5-9] Other less sensitizing and rare mutations are located in exon 18 (p.G719X), exon 20 (p.S768I), and exon 21 (p.L861Q). Moreover, mutations conferring resistance to TKI exist, such as p.T790M.[10] For the resistance mutation p.T790M, the new TKI osimertinib is available.[4,11]

Nevertheless, there are many more rare mutations for which sensitivity to TKI has either not been documented or not well documented. This is because many clinical studies excluded patients with rare *EGFR* mutations.[7]

The DIRECT (DNA-Mutations Inventory to Refine and Enhance Cancer Treatment) database is part of My Cancer Genome and provides information about clinically relevant somatic mutations in lung cancer,[12] but this database has not been updated since 2015 and the data cannot be accessed directly for all listed mutations. New helpful databases for mutations in cancer in general are DoCM (Database of Curated Mutations, http://docm.info/) and CIViC (Clinical Interpretations of Variants in Cancer, https://civicdb.org/). DoCM includes all current databases: CIViC Knowledgebase, Kin-Driver, Drug Gene Knowledge Database, Oncomap Variants, My Cancer Genome, Pan-Cancer Recurrent Hotspots, the WashU hematologic malignancy mutation list, and the literature.[13] For all reported mutations, it is possible to view the linked references and/or database directly, but the clinical relevance is only shown for common mutations. In contrast, the CIViC database focuses particularly on clinical relevance and is an open database to which everyone can contribute.[14]

In addition, there are many publications and reviews about the clinical outcome of rare *EGFR* mutations in NSCLC, which might be not considered in the abovementioned databases. Generally all published mutations are listed in the COSMIC (Catalogue of Somatic Mutations in Cancer, https://cancer.sanger.ac.uk/cosmic) database.[15] The COSMIC database is updated regularly and includes details about drug resistance since 2016.[16]

In order to gain more insights into the efficacy of TKI on rare and compound *EGFR* mutations, we screened our in-house database from the last 7 years for these mutations (excluding cases with the secondary resistance mutation p.T790M). Within the 408 *EGFR*-mutated samples in our cohort, 41 samples contained a rare *EGFR* mutation with unknown or uncertain effect on TKI sensitivity. An additional 22 samples had compound mutations with at least one rare *EGFR* mutation. For these 63 samples, information about the clinical outcome of the affected patients was collected. In addition, we interrogated the current status of the mutations in the abovementioned databases and performed a literature search.

Patients and Methods

Mutational Analysis of EGFR *Gene*

A total of 2906 lung cancer samples were analyzed for *EGFR* mutations during routine analysis at the Institute of Pathology at the Charité in Berlin between June 2010 and June 2017.

Sanger DNA sequencing of exons 18, 19, and 21 of the *EGFR* gene was used until 2014. Sequences of the primer used for PCR

Table 1 Types of Rare *EGFR* Mutations

Exon	Mutation
Exon 18	p.P699L (1)
	p.A702T (1)
	p.E709_T710delinsD (1)
	p.K713T (1)
	p.G724C (1)
	p.T725M (1)
Exon 19	p.L730R (1)
	p.E734V (1)
	p.G735D (1)
	p.P741S (1)
	p.A743V (1)
	p.L747P (2)
	p.E749Q (1)
Exon 20	p.V765M (1)
	p.A767_V769dup (5)
	p.S768_V769delinsIL (1)
	p.S768_D770dup (1)
	p.D770_N771delinsP (1)
	p.N771_H773dup (1)
	p.N771delinsGY (1)
	p.H773dup (1)
	p.H773_V774delinsLM (1)
	p.G779F (1)
	p.Q791H (1)
Exon 21	p.R831C (1)
	p.H835fs*55 (1)
	p.A840T (1)
	p.A840V (1)
	p.V843I (1)
	p.V843L (1)
	p.T847I (1)
	p.P848L (1)
	p.D855Y (1)
	p.G857E (1)
	p.G863S (1)
	p.G874D (1)

Numbers in parentheses indicate number of patients with mutation.
Abbreviation: EGFR = epidermal growth factor receptor.

Table 2 Types of Compound Mutations in *EGFR* Gene (Excluding T790M Mutation)

Mutation 1	Mutation 2
p.N700S	p.T783A (1)
p.N700S	p.S784F (1)
p.E709G	p.L858R (1)[a]
p.G719C[a]	p.S768I (2)
p.G719C[a]	p.L833_V834delinsFL (1)
p.G719C[a]	p.L861Q (1)
p.G719A[a]	p.L861Q (1)
p.G719A[a]	p.L861R (2)
p.S720F	p.L861Q (1)
p.G721D	p.E746_A750del (1)[a]
P. G724S	p.S768I (1)
p.T725M	p.K728E (1)
p.V738F	p.L858R (1)[a]
p.E746_R748del[a]	p.A750P (1)
p.E746_A750del[a]	p.T751P (1)
p.T751_I759del[a]	p.L798F (1)
p.L833V	p.H835L (1)
p.L833_V834delinsFL	p.L858R (1)[a]
p.V834L	p.L858R (1)[a]
p.L858R[a]	p.A871E (1)

Numbers in parentheses indicate numbers of patients with mutations.
Abbreviation: EGFR = epidermal growth factor receptor.
[a]Common tyrosine kinase inhibitor–sensitive mutations.

and sequencing are listed in Supplemental Table 1 in the online version. From 2014, next-generation sequencing was used, also including exon 20 in the analysis. Libraries for next-generation sequencing were prepared with the Ion AmpliSeq Colon and Lung Cancer or Ion AmpliSeq Cancer Hotspot Panel (Life Technologies). Sequencing was performed on an Ion Torrent PGM or S5-XL device. All methods have been described elsewhere.[17,18]

Patient Characteristics

All available clinicopathologic patient characteristics are listed in Supplemental Tables 2 and 3 in the online version, including patient age and sex, histology of the cancer sample, tissue distribution, disease stage, and information about possible medication received before *EGFR* analysis. Our retrospective study relied on tissue from the routine diagnostic archive. All patients had provided consent for their tissue specimens to be used for research purposes.

Results

EGFR *Mutations Detected in Lung Cancer Samples*

In 408 (14%) of the 2906 analyzed NSCLC samples, an *EGFR* mutation was detected. The most common mutations were deletions in exon 19 in 180 samples (44.1%). In 125 samples (30.6%), the mutations p.L858R or p.L861Q in exon 21 were detected. Twenty-three samples (5.6%) harbored a mutation in codon G719 of exon 18. Another 2 samples (0.5%) had a single p.T790M mutation in exon 20. Finally, 41 samples (10.0%) showed a rare mutation of the *EGFR* gene with unknown or uncertain effect to TKI therapy. Also, 37 samples (9.1%) had compound mutations. In 15 (3.7%) of these 37 samples, the combined mutations consisted of a possible activating mutation and an acquired p.T790M mutation. These samples were excluded from our study; therefore, 22 samples with compound mutations (5.4%) were maintained in the study.

In total, in 1.4% of all 2906 analyzed lung cancer samples, a rare mutation and in 0.8% a compound mutation (excluding samples with an acquired p.T790M mutation) were detected (Figure 1).

Characteristics of Rare and Compound EGFR *Mutations*

Overall, we detected 36 different rare single mutations in 41 samples. The mutation p.L747P was detected twice, and 5 different samples harbored the mutation p.A767_V769dup (Table 1).

For the 22 cases of compound mutations, a total of 44 mutations (2 mutations per case) were detected, but not all of these were rare

Table 3 Characteristics of Patients With Rare Mutations (N = 41 or 40)

Characteristic	Value
Sex	
Female	19 (46.3)
Male	22 (53.7)
Age (Y)	
Median	63
Range	35-92
Histology	
Adenocarcinoma	38 (92.7)
Squamous-cell carcinoma	1 (2.4)
Unknown	2 (4.9)

Data are presented as n (%) unless otherwise indicated.
[a]For one sample, age of patient was not reported.

(Table 2). TKI-sensitizing mutations were present together with a rare mutation in 16 samples (7 p.G719X, 4 exon 19 deletions, and 5 p.L858R mutations). Additionally, some rare mutations were detected in 2 (p.N700S, p.L833_V834delinsFL and p.L861R) or in 3 samples (p.S768I, p.L861Q). The rare mutation p.T725M was detected as a single mutation in one patient and in combination with a second mutation p.K728E in another patient. Thus, another 20 rare *EGFR* mutations were detected in the 22 patients with compound mutations.

In total, 56 different rare mutations of the *EGFR* gene were detected in our study.

Clinical Characteristics of Patients With Rare and Compound Mutations

The median age at diagnosis of patients with a single rare mutation was 63 years (range, 35-92 years), with 19 female (46.3%) and 22 male (53.7%) patients (Table 3). In 38 samples (92.7%) an adenocarcinoma and in 1 sample (2.4%) a squamous-cell carcinoma was diagnosed. The remaining 2 samples (4.9%) were classified as NSCLC only by external pathologist.

The median age of patients with compound mutations was 63 years (range, 41-78 years). Fourteen patients were female (63.6%) and 8 patients male (36.4%) (Table 4). Twenty-one samples were adenocarcinomas (95.5%), and 1 sample (4.5%) was classified as NSCLC without any further specification by the external sender.

Table 4 Patient Characteristics of Compound Mutations (N = 22)

Characteristic	Value
Sex	
Female	14 (63.6)
Male	8 (36.4)
Age (Y)	
Median	63
Range	41-78
Histology	
Adenocarcinoma	21 (95.5)
Squamous-cell carcinoma	0
Unknown	1 (4.5)

Data are presented as n (%) unless otherwise indicated.

Clinical Outcome

Information on the outcome of TKI therapy was available for 37 of the 63 patients with a detected rare or compound *EGFR* mutation. The remaining 26 patients were from external hospitals.

Five of the 37 patients died before medication could be provided. Fifteen patients received radio- or chemotherapy instead of TKI, 1 patient refused to undergo any therapy, and the remaining 16 patients were treated with erlotinib, gefitinib, or afatinib (Table 5).

Seven patients, who harbored a single rare mutation and were treated with erlotinib or gefitinib, had disease that did not respond to therapy. One patient experienced a strong adverse reaction to afatinib, and the medication had to be withheld after 10 days, so the response behavior could not be evaluated. Only one patient with a rare *EGFR* mutation (p.G874D) had disease that responded to TKI therapy.

In contrast, the disease of all 7 patients treated with TKI and harboring a compound mutation, consisting of a rare and a sensitizing mutation, responded to the therapy with erlotinib, gefitinib, or afatinib.

Response to TKI According to Database and Literature Search

All 56 detected rare mutations of our cohort were searched in the CIViC, DoCM, and COSMIC databases and by literature query in PubMed as single mutation because the databases generally do not offer information on compound mutations. Still, for many mutations, the results were obtained in patients with compound mutations (Table 6).

For 20 of the 56 different detected mutations, no results about TKI response in lung cancer samples were available in any database or publication. For 2 of these mutations, which have never been described in any lung cancer sample, p.A702T and p.G874D, to our knowledge, our study is the first to note the clinical response to TKI therapy.

According to our database and literature searches, and according to the results of our study, we categorized the 56 detected mutations as follows: (1) rare mutations that may respond to TKIs; (2) rare mutations that often occur with a secondary mutation (sensitizing or second rare mutation); (3) rare mutations with nonresponding behavior toward TKIs; and (4) rare mutations with unknown clinical evidence (Table 6). For category 1, four rare mutations responded to TKI therapy according to our research (results of at least 3 cases and response rate > 50%): p.V765M, p.V843I, p.L861R, and p.L861Q. For category 2, eleven mutations in our study occurred mostly with a secondary mutation (> 60% of the reported cases): p.P699L, p.E709X, p.S720F, p.G724S, p.A750P, p.S768I, p.R831C, p.L833V, p.V834L, p.H835L, and p.A871E. On the basis of the results of our study and a search of the literature, compound mutations generally show sensitivity toward TKI therapy, especially if they consist of a rare and a TKI-sensitive mutation. Two rare *EGFR* mutations in one tumor sample correlated with a nonresponding behavior to TKI (eg, p.P699L and G804K or P699L and T854I). The mutations p.G724S and p.A750P also did

Table 5 Clinical Outcomes

Mutation	Age (Years)	Sex	Smoking Status	Medication	Medication Dose (mg)	Duration	PFS	OS	Response	Additional Information
Single Mutation										
p.P699L	68	M	Nonsmoker	Erlotinib	150	3 mo	0 mo	11 mo	PD	Massive tumor progression
p.A702T	56	F	Smoker	Gefitinib	250	3 mo	—	3 mo	PD	Massive tumor progression
p.E709_T710delinsD	60	M	Nonsmoker	Erlotinib	150	1 mo	1 mo	3 mo	PD	
p.G724C	64	M	Smoker	—	—	—	—	3 mo	—	RT and simultaneous CT
p.T725M	49	M	Smoker	—	—	—	—	1 mo	—	Massive tumor progression after diagnosis, died 1 month later; no medication possible
p.L730R	57	F	Ex-smoker	Afatinib	20	10 d	0 mo	1 mo	NA	Strong adverse drug reaction; termination of medication after 10 days
p.A743V	72	M	NA	—	—	—	—	0 mo	—	Died after diagnosis; no medication possible
p.L747P	37	F	Smoker	Erlotinib	150	3 mo	3 mo	NA	PD	
p.E749Q	50	M	Smoker	Erlotinib	150	3 mo	0 mo	3 mo	PD	
p.A767_V769dup	76	F	Nonsmoker	—	—	—	—	NA	—	RT
p.S768_V769delinsIL	69	F	Nonsmoker	—	—	—	—	NA	—	CT: SD
p.S768_D770dup	64	F	Nonsmoker	—	—	—	—	NA	—	RT
p.V769_D770dup	73	F	NA	—	—	—	—	NA	—	RT, CT, and/or CRT: SD
p.D770_N771delinsP	73	F	Nonsmoker	—	—	—	—	NA	—	CT: PD
p.N772delinsGY	80	F	NA	—	—	—	—	0 mo	—	Died after diagnosis; no medication possible
p.H773dup	70	F	Ex-smoker	—	—	—	—	NA	—	CT: SD
p.H773_V774delinsLM	41	M	Smoker	—	—	—	—	NA	—	CT
p.G779F	66	F	Ex-smoker	—	—	—	—	NA	—	CT
p.Q791H	92	M	NA					NA		Refused any therapy
p.H835fs*55	48	M	Smoker	—	—	—	—	NA	—	RT
p.A840T	78	M	NA	—	—	—	—	0 mo	—	Died after diagnosis; no medication possible
p.V843L	60	F	Smoker	—	—	—	—	22 mo	—	Adjuvant CRT
p.V843I	75	M	Ex-smoker	—	—	—	—	NA	—	RT: response
p.P848L	69	M	Ex-smoker	Erlotinib	150	4 mo	4 mo	6 mo	PD	
p.G863S	56	M	Smoker	Erlotinib	150	3 mo	0 mo	7 mo	PD	Massive tumor progression (+ 35%)
p.G874D	35	M	Smoker	Erlotinib	150	48 mo	>5 y	Still alive[a]	CR	Erlotinib provided only intermittently after 4 years
Compound Mutation										
p.N700S, p.S784F	49	M	Nonsmoker	—	—	—	—	21 mo	—	CT: PD
p.E709G, p.L858R	51	F	Nonsmoker	—	—	—	—	NA	—	Carboplatin/vinorelbine therapy: PD; docetaxel: PD
p.G719C, p.S768I	48	M	Smoker	—	—	—	—	NA	—	CRT: PD

Table 5 Continued

Mutation	Age (Years)	Sex	Smoking Status	Medication	Medication Dose (mg)	Duration	PFS	OS	Response	Additional Information
p.G719C, p.L861Q	61	F	Ex-smoker	Gefitinib	250	13 mo	10 mo	24 mo	PR	
p.G719A, p.L861R	75	M	Smoker	Erlotinib	150	6 mo	6 mo	6 mo	PR	
p.G721D, p.E746_A750del	63	M	Ex-smoker	Gefitinib	250	13 mo	13 mo	NA	PR	Second-medication erlotinib: SD
p.T725M, p.K728E	63	M	Smoker	—	—	—	—	0 mo	—	Died after diagnosis; no medication possible
p.V738F, p.L858R	72	F	Ex-smoker	Gefitinib	250	16 mo	16 mo	19 mo	PR	Additional T790M during medication
p.T751_I759del, L798F	62	F	Nonsmoker	Afatinib	40	24 mo	24 mo	Still alive[a]	PR	RT after tumor progression
p.L833_V834delinsFL, p.L858R	72	F	Smoker	Erlotinib	150	29 mo	29 mo	NA	PR	
p.V834L, p.L858R	77	F	Ex-smoker	Afatinib	40	11 mo	10 mo	Still alive[a]	PR	2 months' RT after tumor progression, after RT continuation of afatinib medication

Abbreviations: CR = complete response; CRT = chemoradiotherapy; CT = chemotherapy; NA = not available; OS = overall survival; PD = progressive disease; PFS = progression-free survival; PR = partial response; RT = reverse transcription; SD = stable disease; TKI = tyrosine kinase inhibitor.
[a]Alive as of September 2018.

not respond to TKI therapy in combination with a sensitizing in-frame deletion of exon 19 mutation.

For category 3, for the following rare mutations, the prognosis is described to be very poor (results of at least 3 cases and response rate < 40%): p.E709_T710delinsD, p.L747P, p.E749Q, p.A767_V769dup, p.S768_D770dup, complex deletions or insertions in exon 20 in general, p.S784F, p.P848L, and p.G857E. For category 4, for the remaining 27 mutations, the clinical evidence remains unclear because only one or two cases have been reported so far or because no clinical data were available at all: p.N700S, p.A702T, p.K713T, p.G721D, p.G724C, p.T725M, p.K728E, p.L730R, p.E734V, p.G735D, p.V738F, p.P741S, p.A743V, p.T751P, p.G779F, p.T783A, p.Q791H, p.L798F, p.L833_V834delinsFL, p.H835fs*55, p.A840T, p.A840V, p.V843L, p.T847I, p.D855Y, p.G863S, and p.G874D.

PolyPhen-2 Analysis

We used PolyPhen-2 software (http://genetics.bwh.harvard.edu/pph2/) to predict the possible impact of an amino acid substitution on the stability and function of a protein.[89] We analyzed all 45 rare point mutations of our study, the 2 common point mutations p.L719X and p.L858R, and the resistance mutation p.T790M (Supplemental Table 4 in the online version). For 43 rare mutations, it was predicted that they were probably or possibly damaging, whereas the 2 mutations p.N700S and p.G863S were predicted to be benign. In contrast, the common and activating mutations p.G719A and p.L858R were also predicted to be probably damaging, just as was the well-known resistance mutation p.T790M.

Discussion

In our routine diagnostic cohort of samples from patients treated between June 2010 and June 2017, we detected an *EGFR* mutation rate of 14%, which is in line with other studies.[90] In 10.0% of all *EGFR*-mutated samples, rare *EGFR* mutations (n = 41) and in 5.4% compound mutations (n = 22, excluding cases of p.T790M) were detected. According to the literature, 85% to 90% of all *EGFR* mutations are common exon 19 deletions or the point mutation L858R in exon 21.[5-9] Thus, the amount of rare and compound mutations in our study is also in accordance with other studies.

The data on clinical outcome for TKI therapy in patients with rare mutations in the *EGFR* gene have been extensively completed in the last few years. Still, for many rare *EGFR* mutations, no data are available. In our study, we could find no data on TKI sensitivity and clinical outcome in the literature for 36% of the detected rare mutations. We used the CIViC, DoCM, and COSMIC database and PubMed for literature searches. The CIViC database is an open database that focuses on the clinical evidence of all reported mutations, but the database currently does not contain as many variations as the COSMIC database. In contrast, in the COSMIC database, possible drug response is not directly accessible. DoCM contains the fewest number of rare *EGFR* mutations and seems to have not been updated since 2016.

One reason why many mutations have never been reported could be because the disease of most patients with rare *EGFR* mutations was not treated with a TKI, because the US Food and Drug Administration only recommends TKI therapy for well-known

Table 6 Data of TKI Response for All Detected Rare Mutations of *EGFR* Gene Exon 18 to 21 Based on Database Research, Literature Research, and Results of This Study

Exon of *EGFR* Gene	Mutation	CIViC		DoCM		COSMIC Database		Additional Literature Research		This Study	Clinical Evidence
		Clinical Response	Literature	Clinical Response	Literature	Clinical Response	Literature	Clinical Response	Literature		
Exon 18	p.P699L	—	—	—	—	Compound mutation with G804K: no response (1)	19	Compound mutation with T854I: PD (1)	20	PD (1)	Compound mutation
	p.N700S	—	—	—	—	—	—	—	—	—	Unclear
	p.A702T	—	—	—	—	—	—	—	—	PD (1)	Unclear
	p.E709G	—	—	—	—	Compound mutation with L858R: PR (8), SD (2), PD (1), compound mutation with G719X: PR (2), SD (1), PD (1), compound mutation with del(19): PR (1)	21-23	Compound mutation with L858R: SD (1), PR (2), PD (1), compound mutation with G719C: PD (1), E709X in general: Response	24, 25	—	Compound mutation
	p.E709_T710delinsD	SD (1), PD (1), response (1)	25, 26	—	—	SD (1), PD (1), response (2)	25, 26, 27	PD (4), PR (1), SD (2)	22, 28, 29, 30	PD (1)	Resistant toward TKI
	p.K713T	—	—	—	—	—	—	—	—	—	Unclear
	p.S720F	PD (1)	28	—	—	Compound mutation with G719A: PR (1)	31	Compound mutation with G719A: PR (1)	25	—	Compound mutation
	p.G721D	—	—	—	—	PD (1)	32	Theoretical study: resistance mutation	24	Compound with mutation with del(19): PR (1)	Unclear
	p.G724C	—	—	—	—	—	—	—	—	—	Unclear
	p.G724S	Sensitive to cetuximab (colon cancer)	33	Sensitive to cetuximab (colon cancer)	33	No response (1); compound mutation with S768I: SD (1); compound mutation with del(19): no response (1)	27, 34, 35	—	—	—	Compound mutation
	p.T725M	Theoretical study: activating *EGFR* mutation	36	—	—	—		Compound mutation with G719A: SD (1)	28	—	Unclear
	p.K728E	—	—	—	—	—	—	No response (1)	37	—	Unclear
Exon 19	p.L730R	—	—	—	—	—	—	—	—	—	Unclear
	p.E734V	—	—	—	—	—	—	—	—	—	Unclear
	p.G735D	—	—	—	—	—	—	—	—	—	Unclear
	p.V738F	—	—	—	—	—	—	—	—	Compound mutation with L858R: PR (1)	Unclear
	p.P741S	—	—	—	—	—	—	—	—	—	Unclear
	p.A743V	—	—	—	—	PD (1)	8	—	—	—	Unclear
	p.L747P	PD (2)	25	—	—	SD (1), no response (1), PD (2)	38-40	—	—	PD (1)	Resistant toward TKI

Table 6 Continued

Exon of *EGFR* Gene	Mutation	CIViC		DoCM		COSMIC Database		Additional Literature Research		This Study	Clinical Evidence
		Clinical Response	Literature	Clinical Response	Literature	Clinical Response	Literature	Clinical Response	Literature		
	p.E749Q	—	—	Compound mutation with del(19) and A750P: PR (1)	41	—	—	No response (1)	42	PD (1)	Resistant toward TKI
	p.A750P	—	—	Compound mutation with del 19: PD (1), compound mutation with del(19) and T790M: PD (1)	43, 44, 45	Compound mutation with del(19): PR (1)	31	—	—	—	Compound mutation
	p.T751P	—	—	—	—	—	—	—	—	—	Unclear
Exon 20	p.V765M	—	—	—	—	PR (3)	41	Response (1)	24	—	Sensitive to TKI
	p.A767_V769dup	PD (3)	46	—	—	—	—	SD (3), PD (5)	8, 21, 30, 47	—	Resistant toward TKI
	p.S768I	PR (1), SD (1), PD (2), compound mutation with del(19) or L858R or G719C: Response (4), compound mutation with T790M: no response (1), as Compound mutation with sensitizing mutation: Response	48-53	All theoretical studies or in vitro studies; no patient samples, but FDA approved mutation (erlotinib, afatinib)	45, 54, 55, 56	PR (1), compound mutation with G719X: PR (4), SD (1) and PD (2), compound mutation with L858R: SD (2), PR (4)	35,52, 57-62	No further literature research necessary		—	Compound mutation
	p.S768_D770dup	No response (1)	63	—	—	—	—	PD (8), SD (1), PR (2)	8, 30, 47, 64	—	Resistant toward TKI
	p.S768_ V769delinsIL	Exon 20 insertion in general: no response (21), response (4)	28, 53, 65	—	—	—	—	—	—	—	Exon 20 insertions are resistant toward TKI in general
	p.D770_ N771delinsP			—	—	—	—	—	—	—	
	p.N771_H773dup			—	—	—	—	PD (1)	92	—	
	p.N771delinsGY			—	—	PD (1)	66	—	—	—	
	p.H773dup			—	—	—	—	—	—	—	
	p.H773_ V774delinsLM			—	—	—	—	—	—	—	
	p.G779F	—	—	—	—	—	—	—	—	—	Unclear
	p.T783A	—	—	—	—	—	—	—	—	—	Unclear
	p.S784F	PD (2)	25, 67	—	—	PD (1), compound mutation with V768M: SD (1)	67, 68	—	—	—	Resistant toward TKI
	p.Q791H	—	—	—	—	—	—	—	—	—	Unclear
	p.L798F	—	—	—	—	—	—	—	—	compound with mutation with del(19): PR (1)	Unclear

Table 6 Continued

Exon of *EGFR* Gene	Mutation	CIViC		DoCM		COSMIC Database		Additional Literature Research		This Study	Clinical Evidence
		Clinical Response	Literature	Clinical Response	Literature	Clinical Response	Literature	Clinical Response	Literature		
Exon 21	p.R831C	—	—	—	—	Compound mutation with L861X: PR (1), SD (1)	25	—	—	—	Compound mutation
	p.L833V	—	—	—	—	Compound mutation with L858R: PR (2), compound mutation with G719A and V834C: CR (1), compound mutation with del(19) and H835L: SD (1), compound mutation with H835L: Response (1), compound mutation with KRAS mutation: PD (1), compound mutation with T790M: no response (1)	21,58,69-72	Compound mutation with H835L: PR (1), compound mutation with E709K and H835L: PR (1)	73, 74	—	Compound mutation
	p.L833_V834delinsFL	—	—	—	—	—	—	—	—	Compound mutation with L858R: PR (1)	Unclear
	p.V834L	—	—	—	—	Compound mutation with del(19): PR (1), compound mutation with G719A and L833V: CR (1)	21, 58	Compound mutation with L858R: PR (1)	25	Compound mutation with L858R: PR (1)	Compound mutation
	p.H835fs*55	—	—	—	—	—	—	—	—	—	Unclear
	p.H835L	—	—	—	—	Compound mutation with del(19) and L833V: SD (1), compound mutation with L833V: Response (1)	58, 71	Compound mutation with L833V: PR (1), compound mutation with E709K and L833V: PR (1)	73, 74	—	Compound mutation
	p.A840T	—	—	—	—	PD (1)	69	—	—	—	Unclear
	p.A840V	—	—	—	—	—	—	—	—	—	Unclear
	p.V843I	—	—	—	—	PR (1), response (1), SD (1), compound mutation with L858R: SD (1)	38, 75, 76	No response (1), SD (1)	28, 77	—	Sensitive to TKI and germ line
	p.V843L	—	—	—	—	—	—	—	—	—	Unclear
	p.T847I	PD (1)	25	—	—	—	—	—	—	—	Unclear
	p.P848L	Cell line: no response (1)	78	—	—	Compound mutation with del(19): PR (1)	21	SD (2)	28, 29	PD (1)	Resistant toward TKI
	p.D855Y	—	—	—	—	—	—	—	—	—	Unclear
	p.G857E	—	—	—	—	SD (2)	75, 79	PD (2)	20, 80	—	Resistant toward TKI
	p.L861Q	PR (4), SD (1)	25	FDA approved mutation (erlotinib, afatinib)	5,55,56, 81-84	115 total treated samples; mainly response	5, 23, 41, 57, 85, 86	No further literature research necessary		Compound mutation with G719C: PR (1)	Sensitive to TKI

Table 6 Continued

Exon of *EGFR* Gene	Mutation	CIViC		DoCM		COSMIC Database		Additional Literature Research		This Study	Clinical Evidence
		Clinical Response	Literature	Clinical Response	Literature	Clinical Response	Literature	Clinical Response	Literature		
	p.L861R	PR (1), Resistance to Crizotinib (1)	25, 87	FDA approved mutation (erlotinib, afatinib)	55, 56	PD (1), compound mutation with G719D: response (1), compound mutation with R831C: PR (2)	25, 48, 67, 88	SD (1)	64	Compound mutation with G719A: PR (1)	Sensitive to TKI
	p.G863S	—	—	—	—	SD (1), compound mutation with T847A: PD (1)	75	—	—	PD (1)	Unclear
	p.A871E	—	—	—	—	—	—	Compound mutation with L858R: PR (1), PD (1)	25, 73	—	Compound mutation
	p.G874D	—	—	—	—	—	—	—	—	CR (1)	Unclear

Numbers in parentheses indicate number of patients. Last access of databases and PubMed for literature research was May 31, 2018.
Abbreviations: CIViC = Clinical Interpretations of Variants in Cancer; COSMIC = Catalogue of Somatic Mutations in Cancer; CR = complete response; DoCM = Database of Curated Mutations; EGFR = epidermal growth factor receptor; FDA = US Food and Drug Administration; PD = progressive disease; PR = partial response; SD = stable disease; TKI = tyrosine kinase inhibitor.

sensitizing mutations. In our study, only half of the patients received TKI treatment, and the other half received radio- or chemotherapy instead. Another reason for the unsatisfactory result of our database and literature searches may be that the obtained data of rare *EGFR* mutations were not published. This may be because *EGFR* analysis and clinical treatment are normally not performed in the same department in many hospitals. Therefore, the classification of the *EGFR* mutation and the clinical outcome of the patient are often not collected together. In our study, we merged clinical and molecular pathologic data for 37 (59%) of 63 patients.

To obtain more results for rare mutations, the response to TKI therapy has also been evaluated in different in silico studies and cell-based assays.[24,36,54,73,78] These methods can be helpful for a better understanding of the clinical significance of rare *EGFR* mutations and for the prediction of response to novel TKIs. Nevertheless, especially cell-based assays are time intensive and high in cost. Therefore, these methods are not very useful in the daily practice of deciding on medication to treat NSCLC.

In our study, 7 patients harboring a single rare *EGFR* mutation had disease that did not respond to first-generation TKIs and resulted in a short overall survival compared to patients who received first-generation TKIs and who harbored a common *EGFR* mutation, as reported by previous studies.[4] These results are in line with other studies, in which inferior response rates and a shorter overall survival were reported for rare *EGFR* mutations.[28,29,57,91-93] In contrast, one patient with the mutation p.G874D had disease that responded to therapy with erlotinib. To our knowledge, this mutation has not been reported to date. Therefore, it is difficult to decide if this mutation can be classified as an activating *EGFR* mutation.

However, 7 patients harboring a complex mutation, consisting of a known TKI-sensitizing mutation and a rare mutation, had disease that responded to first- or second-generation TKI therapy in our study. These results confirm the results of other studies as well as the result of our database search, which report that the response to TKI therapy for patients with a compound mutation composed of a TKI-sensitizing mutation and a rare mutation is comparable to that of patients with a single sensitizing mutation,[58,94-96] and even better compared to patients with a single rare *EGFR* mutation.[9] Only Kim et al[97] reported poor response to TKI therapy in patients with compound mutations of the *EGFR* gene.

The PolyPhen-2 analysis we performed predicted damaging effects for 43 of the 45 rare point *EGFR* mutations we found (similar to the known pathologically relevant mutations p.G719A, p.L858R, and p.T790M). Thus, almost all *EGFR* mutations we found affect the stability of the protein. Previous studies also used 3-dimensional drug–protein interaction modeling to better understand changes in protein structures caused by mutations in the *EGFR* gene and their variable drug response.[98-101] Sickmier et al,[101] for instance, demonstrated that the resistance mutation p.S468R directly blocks the *EGFR* domain III, where cetuximab binds to the *EGF* receptor. These data suggest that poor response to TKI therapy for the rare oncogenic *EGFR* mutations we found are likely associated with steric changes preventing the binding of the drug.

Thus, most patients with rare *EGFR* mutations have disease that does not respond to first-generation TKIs. Still, with more than 50% of the patients harboring a common sensitizing mutation, a

secondary resistance mutation (eg, p.T790M, p.L747S, p.D761Y, or p.T854A) occurs.[43,102] Also, primary resistance in patients with a sensitizing mutation can be possible, caused by the presence of a secondary resistance mutation in Kirsten rat sarcoma viral oncogene (*KRAS*),[27,103-105] *PIK3CA*,[27,106] or v-Raf murine sarcoma viral oncogene homolog B (*BRAF*),[107] or a mesenchymal−epithelial transition (*MET*) gene amplification.[106,108] Consequently, a rare *EGFR* mutation should not be a criterion to exclude these patients from receiving TKI therapy in general.

Today, many different treatment options for patients with *EGFR*-mutated NSCLC are available. Afatinib, a second-generation TKI, is associated with better response rates for patients with rare mutations of the *EGFR* gene,[65,80] but it also shows good therapeutic efficacy in patients with common *EGFR* mutations.[109] Furthermore, combination therapies are also possible. Girard[4] in 2018 reported that first- or second-generation TKIs followed by osimertinib, an irreversible third-generation TKI, may improve survival outcome compared to osimertinib alone. Further, poziotinib may be a new potent TKI for exon 20 mutations in general.[110,111] However, discussions about whether patients benefit most from "best therapy first" (based on overall survival) or combination therapy are ongoing. Thus, personalized medicine for NSCLC patients with rare or compound mutations of the *EGFR* gene needs more individual assessment, and it is likely that future TKIs will provide benefit for patients with rare mutations. Therefore, we encourage all clinicians to publish their data on the clinical outcomes of patients with rare and compound *EGFR* mutations.

Our study expands the knowledge about the clinical response behavior to first- or second-generation TKIs for 8 single rare and 7 compound mutation of the *EGFR* gene. Additionally, we report for the first time the clinical outcome after TKI treatment for 2 rare *EGFR* mutations (p.A702T and p.G874D).

Conclusion

Patients with a rare *EGFR* mutation had disease that did not respond to first-generation TKIs in our cohort, except for a patient harboring the mutation p.G874D. In contrast, compound mutations consisting of a rare and a common sensitizing mutation responded to first- or second-generation TKIs.

The increasing landscape of *EGFR* mutations, including possible resistance mutations to different TKIs, the amount of different available TKIs, and many other factors influencing the response behavior (eg, additional mutation in *KRAS, PIK3CA, BRAF*), indicates that the clinical outcome of all patients with an *EGFR*-mutated NSCLC treated with first-, second-, or third-generation TKIs should be reported and collected in a free, accessible database for a better understanding of the clinical evidence.

Clinical Practice Points

- In our cohort, only one patient with a rare *EGFR* mutation (p.G874D) had disease that responded to TKI therapy with erlotinib, whereas the disease of all other patients with rare *EGFR* mutations did not respond to first-generation TKI therapy. In contrast, all patients with compound mutations, consisting of a common and a rare *EGFR* mutation, had disease that responded to first- or second-generation TKI therapy.
- We report for the first time the clinical outcome after TKI treatment for 2 rare *EGFR* mutations (p.A702T and p.G874D).
- Additional database and literature searches revealed that information about clinical evidence regarding TKI therapy remains limited. This points to the importance of systematically making available the clinical outcome for rare *EGFR* mutations to generate a solid basis for clinical decision making.

Disclosure

The authors have stated that they have no conflict of interest.

Supplemental Data

Supplemental tables accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2019.04.012.

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Supplemental Data

Supplemental Table 1 Primer Sequences Used for PCR and Sequencing

Gene	Forward (5′-3′)	Reverse (5′-3′)
EGFR, exon 18	GCTGAGGTGACCCTTGTCTC	ACAGCTTGCAAGGACTCTGG
EGFR, exon 19	ATGTGGCACCATCTCACAATTGCC	CCACACAGCAAAGCAGAAACTCAC
EGFR, exon 21	GCAGAGCTTCTTCCCATGATGA	GCTGACCTAAAGCCACCTCCT

Abbreviation: EGFR = epidermal growth factor receptor.

Supplemental Table 2 Additional Clinicopathologic Data of Cases of Rare *EGFR* Mutation

EGFR Mutation	Age (Years)	Sex	Histology	Tissue Distribution	Disease Stage	Additional Information
p.P699L	68	M	NSCLC	No further data available		
p.A702T	56	F	ADC	Lung	IV	No medication before analysis
p.E709_T710delinsD	60	M	ADC	Lung	IV	No medication before analysis
p.K713T	78	F	ADC	No further data available		
p.G724C	64	M	ADC	Metastasis	IIIB	No medication before analysis
p.T725M	49	M	ADC	Metastasis	IV	No medication before analysis
p.L730R	57	F	ADC	Lung	IV	Chemotherapy before analysis
p.E734V	54	F	NSCLC	Lung	IV	No medication before analysis
p.G735D	NA	F	ADC	No further data available		
p.P741S	65	M	ADC	No further data available		
p.A743V	72	M	ADC	Lung	IV	No medication before analysis
p.L747P	59	F	ADC	No further data available		
p.L747P	37	M	ADC	Lung (recurred)	IV	Chemotherapy before analysis
p.E749Q	50	M	ADC	Lung	NA	No medication before analysis
p.V765M	56	M	ADC	No further data available		
p.A767_V769dup	72	M	ADC	No further data available		
p.A767_V769dup	73	F	ADC	No further data available		
p.A767_V769dup	76	F	ADC	No further data available		
p.A767_V769dup	68	M	ADC	Metastasis	NA	NA
p.A767_V769dup	76	F	ADC	Lung	IV	No medication before analysis
p.S768_V769delinslL	69	F	ADC	Lung	IV	No medication before analysis
p.S768_D770 dup	64	F	ADC	Lung	IV	No medication before analysis
p.D770_N771delinsP	73	F	ADC	Lung (recurred)	IV	Chemotherapy before analysis
p.N771_H773dup	59	F	ADC	No further data available		
p.N772delinsGY	80	F	ADC	Metastasis	IV	No medication before analysis
p.H773dup	70	F	ADC	Metastasis	IV	No medication before analysis
p.H773_V774delinsLM	41	M	ADC	Metastasis	IV	No medication before analysis
p.G779F	66	F	ADC	Lung	IV	No medication before analysis
p.Q791H	92	M	ADC	No further data available		
p.R831C	57	M	ADC	No further data available		
p.H835fs*35	48	M	ADC	Lung	NA	No medication before analysis
p.A840T	78	M	ADC	Metastasis	IV	NA
p.A840V	66	M	ADC	Lung	IV	Chemotherapy before analysis
p.V843I	75	M	ADC	Lung	I	NA
p.V843L	60	F	ADC	Lung	IIIA	No medication before analysis
p.T847I	55	M	ADC	No further data available		
p.P848L	69	M	ADC	No further data available		
p.D855Y	70	M	SCC	No further data available		
p.G857E	46	F	ADC	Lung	IV	No medication before analysis
p.G863S	56	M	ADC	No further data available		
p.G874D	35	M	ADC	Metastasis	NA	No medication before analysis

Abbreviations: ADC = adenocarcinoma; EGFR = epidermal growth factor receptor; NA = not available; NSCLC = non–small-cell lung cancer; SCC = squamous-cell carcinoma.

Supplemental Table 3 Additional Clinicopathologic Data of Cases of Compound *EGFR* Mutation

Mutation 1	Mutation 2	Age (Years)	Sex	Histology	Tissue Distribution	Disease Stage	Additional Information
p.N700S	p.T783A	72	F	ADC		No further data available	
p.N700S	p.S784F	49	M	ADC	Lung	IV	No medication before analysis
p.E709G	p.L858R	51	F	ADC	NA	IV	NA
p.G719C	p.S768I	48	M	ADC	Lung (recurred)	IV	Chemotherapy before analysis
p.G719C	p.S768I	41	F	ADC	Lung	IV	Radiotherapy before analysis
p.G719C	p.L833_V834delinsFL	64	F	ADC		No further data available	
p.G719C	p.L861Q	61	F	ADC	Lung	IV	No medication before analysis
p.G719A	p.L861Q	54	F	ADC		No further data available	
p.G719A	p.L861R	70	F	ADC		No further data available	
p.G719A	p.L861R	75	M	ADC	Metastasis	IV	No medication before analysis
p.S720F	p.L861Q	78	F	ADC		No further data available	
p.G721D	p.E746_A750del	63	M	ADC	NA	IV	NA
p.G724S	p.S768I	55	F	ADC	Lung	IIIA	NA
p.T725M	p.K728E	63	M	ADC		No further data available	
p.V738F	p.L858R	72	F	ADC		No further data available	
p.E746_R748del	p.A750P	67	M	ADC		No further data available	
p.E746_A750del	p.T751P	70	M	ADC		No further data available	
p.T751_I759del	p.L798F	62	F	ADC	NA	NA	Chemotherapy before analysis
p.L833V	p.H835L	49	F	ADC	Metastasis	NA	NA
p.L833_V834delinsFL	p.L858R	72	F	ADC	Lung	IV	No medication before analysis
p.V834L	p.L858R	77	F	ADC	Lung	IV	No medication before analysis
p.L858R	p.A871E	77	M	NSCLC		No further data available	

Abbreviations: ADC = adenocarcinoma; EGFR = epidermal growth factor receptor; NA = not available; NSCLC = non–small-cell lung cancer.

Supplemental Table 4 Results of PolyPhen-2 Analysis for Rare and Common *EGFR* Single-Point Mutations

EGFR Mutation	HumDiv		HumVar	
	Score	Damaging Effect	Score	Damaging Effect
p.P699L	0.996	Probably damaging	0.908	Possibly damaging
p.N700S	0.949	Possibly damaging	0.398	Benign
p.A702T	0.967	Probably damaging	0.534	Possibly damaging
p.E709G	0.998	Probably damaging	0.959	Probably damaging
p.K713T	0.999	Probably damaging	0.996	Probably damaging
p.G719A[a]	1.000	Probably damaging	0.995	Probably damaging
p.S720F	0.959	Probably damaging	0.718	Possibly damaging
p.G721D	1.000	Probably damaging	0.999	Probably damaging
p.G724C	1.000	Probably damaging	1.000	Probably damaging
p.G724S	1.000	Probably damaging	1.000	Probably damaging
p.T725M	0.999	Probably damaging	0.984	Probably damaging
p.K728E	1.000	Probably damaging	0.987	Probably damaging
p.L730R	0.982	Probably damaging	0.787	Possibly damaging
p.E734V	0.999	Probably damaging	0.989	Probably damaging
p.G735D	0.999	Probably damaging	0.993	Probably damaging
p.V738F	0.987	Probably damaging	0.843	Possibly damaging
p.P741S	1.000	Probably damaging	0.999	Probably damaging
p.A743V	1.000	Probably damaging	0.999	Probably damaging
p.L747P	1.000	Probably damaging	1.000	Probably damaging
p.E749Q	0.975	Probably damaging	0.748	Possibly damaging
p.A750P	0.995	Probably damaging	0.912	Probably damaging
p.T751P	0.801	Possibly damaging	0.588	Possibly damaging
p.V765M	1.000	Probably damaging	0.945	Probably damaging
p.S768I	1.000	Probably damaging	0.999	Probably damaging
p.G779F	1.000	Probably damaging	1.000	Probably damaging
p.T783A	0.620	Possibly damaging	0.615	Possibly damaging
p.S784F	0.998	Probably damaging	0.992	Probably damaging
p.T790M	1.000	Probably damaging	1.000	Probably damaging
p.Q791H	1.000	Probably damaging	1.000	Probably damaging
p.L798F	1.000	Probably damaging	1.000	Probably damaging
p.R831C	1.000	Probably damaging	0.99	Probably damaging
p.L833V	0.949	Possibly damaging	0.823	Possibly damaging
p.V834L	0.998	Probably damaging	0.992	Probably damaging
p.H835L	1.000	Probably damaging	1.000	Probably damaging
p.A840T	1.000	Probably damaging	1.000	Probably damaging
p.A840V	1.000	Probably damaging	0.991	Probably damaging
p.V843I	0.999	Probably damaging	0.946	Probably damaging
p.V843L	0.999	Probably damaging	0.992	Probably damaging
p.T847I	0.997	Probably damaging	0.947	Probably damaging
p.P848L	1.000	Probably damaging	1.000	Probably damaging
p.D855Y	1.000	Probably damaging	1.000	Probably damaging
p.G857E	1.000	Probably damaging	1.000	Probably damaging
p.L858R[a]	1.000	Probably damaging	0.999	Probably damaging
p.L861Q	0.999	Probably damaging	0.973	Probably damaging
p.L861R	1.000	Probably damaging	0.996	Probably damaging
p.G863S	0.317	Benign	0.208	Benign
p.A871E	0.999	Probably damaging	0.963	Probably damaging
p.G874D	0.999	Probably damaging	0.975	Probably damaging

Abbreviation: EGFR = epidermal growth factor receptor.
[a]Common.