Review Article

Multiple mutations in the *EGFR* gene in lung cancer: a systematic review

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Background: Lung cancer is a public health problem worldwide. Currently, identifying genetic mutations in the epidermal growth factor receptor (*EGFR*) has brought significant changes in diagnosing and managing patients with lung cancer. The presence of multiple mutations, defined as the presence of more than one *EGFR* mutation, has been reported in a few studies. Therefore, we carried out this systematic review to describe the most common multiple mutations in the *EGFR* gene.

Methods: We conduct a systematic review of descriptive studies, cohorts, and clinical trials published in Scopus, PubMed, Scielo, and Virtual Health Library literature. The inclusion criteria for the systematic review were descriptive studies, cohorts, and clinical trials with the presence of multiple mutations in the *EGFR* gene. It was followed the Preferred Reporting Items for Systematic Meta-Analyses (PRISMA) guidelines.

Results: In the systematic review, 41 articles were included. Four hundred and forty-six cases with multiple mutations in the *EGFR* gene were found (0.95% of the patients included in the studies). The most prevalent dual mutations observed were T790M + L858R and deletions in exon 19 + T790M. Triple mutations were found in 9 cases (2.017%). According to reports, the presence of T790M mutation in the multiple mutations has been associated with poor clinical outcomes.

Discussion: The presence of multiple mutations in the *EGFR* gene is rare. It is of great importance to consider the T790M mutation since it generates resistance to pharmacological management and has worse outcomes. The most important limitation was that clinical information data and follow-up could not be collected in a large percentage of patients. Therefore, future work should be focused on clinical characteristics, follow-up and repercussions in the treatment of patients with multiple mutations.

Keywords: Epidermal growth factor receptor (*EGFR*); multiple mutations; lung cancer; systematic review

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Introduction

Lung cancer is a public health problem worldwide. In 2021, it was estimated that around 235,760 new cases of lung neoplasms had been diagnosed, contributing to 131,880 deaths secondary to cancer. Overall, lung cancer causes more deaths than breast, prostate, colorectal, and brain cancers combined (1,2). According to recent data from GLOBOCAN, lung cancer (11.4%) is the second most diagnosed neoplasm after breast cancer in women (11.7%) and the leading cause of cancer death, accounting for 18.0% of the total

cancer deaths (3). Among the lung cancer subtypes, non-small cell lung cancer (NSCLC) accounts for 85% of cases (4,5).

In recent years, identifying genetic mutations in the epidermal growth factor receptor (*EGFR*) has brought significant changes in diagnosing and managing patients with lung cancer (6). The *EGFR* gene is located on chromosome 7p11.2 and contains 28 exons. The protein it encodes has an extracellular domain, a transmembrane domain, and a cytoplasmic domain (7). The cytoplasmic domain (also called the tyrosine kinase domain) is responsible for the phosphorylation of its downstream targets and self-regulation. The impact of mutations in the *EGFR* gene falls on cell proliferation and differentiation regulation, hence its association with cancer (8,9).

The incidence of *EGFR* mutations differs significantly according to ethnicity, with incidences of 10–15% reported in European and North American populations, 26% in Latin America (10), and up to 62% in Asian people (11). In a recent study published by Suda *et al.* (12), in which 13,951 samples were obtained from patients with lung cancer, it was shown that the rate of smoking was higher in patients with the mutation in the *EGFR* gene (P=0.02). Deletion of exon 19 and the missense mutations in exon 21 (L858R) are the most common mutations of the *EGFR* gene (80–90%). They are considered common mutations sensitive to treatment with tyrosine kinase inhibitors (TKIs) (13). The use of *EGFR* TKIs has been first-line therapy in treating NSCLC with *EGFR* mutations (14). Patients with advanced NSCLC with TKIs-sensitive *EGFR* mutations may develop progressive disease after 12 months, mainly from secondary *EGFR* mutations leading to TKIs resistance (15). The main mutation after TKIs management is the substitution of the threonine residue at position 790 with methionine (T790M) within exon 20 of the *EGFR* gene, corresponding to approximately half of the cases of acquired resistance to TKIs (16,17).

The presence of multiple mutations, defined as the presence of more than one *EGFR* mutation, has not been much studied, and the clinical impact is uncertain. Therefore, we carried out this systematic review to describe the most common multiple mutations in the *EGFR* gene with clinical data collection. We present the following article according to the Preferred Reporting Items for Systematic Meta-Analyses (PRISMA) reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-235/rc).

Methods

Search strategy

We conduct a systematic review of descriptive studies, cohorts, and clinical trials published in Scopus, PubMed, Scielo, and Virtual Health Library literature. No limits were placed on the language or publication period in the search. The search was performed with the following terms: lung AND EGFR AND ("dual" or "multiple" AND "mutation").

The PRISMA guidelines were followed for data extraction, analysis, and reporting (18). The search was carried out in December 2021. We consider papers available in the following languages: English and Spanish.

Study selection and data extraction

The inclusion criteria for the systematic review were descriptive studies, cohorts, and clinical trials with the presence of multiple mutations in the *EGFR* gene. Two reviewers (JJC and JPC) screened all the titles and abstracts from the publications and performed an eligibility assessment. Retrieved articles were rejected if the eligibility criteria were not met. A third reviewer (RPM) was consulted when eligibility criteria were unclear. Hand searches were performed from the papers that seemed to be relevant.

The extracted data from each article, when possible, were age, gender, smoking status, staging of the tumor, pathologic description of the tumor, kind of pathological sample, molecular method of diagnosis, history of TKIs use and resistance, and survival. All cases were analyzed individually and organized according to the exon with the lowest denomination present in the multiple mutations.

Data synthesis and analysis

Univariate analysis was applied to determine the distribution of clinical and pathological findings.

Results

Systematic review literature

We identified 533 original articles in the databases. Three hundred and thirty-seven duplicates were identified. One hundred and ninety-six articles, including clinical trials, analytical studies, and descriptive studies, were evaluated for eligibility. Finally, 41 articles including descriptive studies

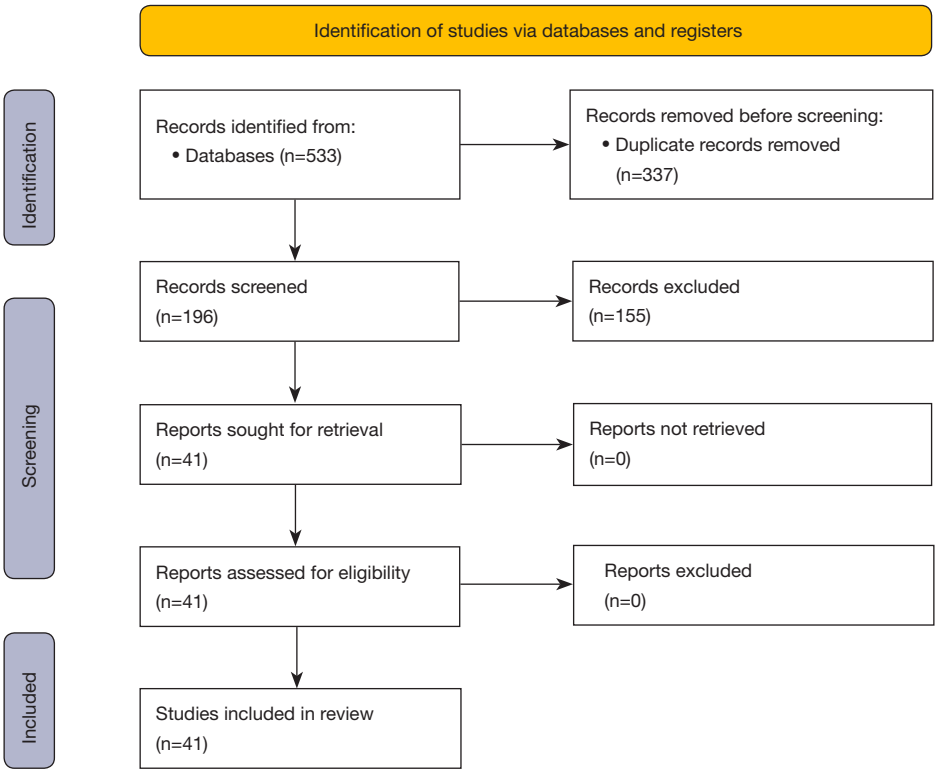

Figure 1 Flow chart of the systematic literature review.

which consist of (6,9,19-39) and (40-51) (n=35, 85.36%), cohorts (52) (n=1, 2.43%), and clinical trials (53-57) (n=5, 12.19%) were included that contained data that met the eligibility criteria (*Figure 1*).

Multiple mutations data

Four hundred and forty-six cases with multiple mutations in the *EGFR* gene were found out of 46,679 samples included in the systematic review (0.95% of the total) (*Tables 1,2*). Although not all the articles mentioned the histological type of cancer, according to the report's information, it was possible to collect 125 cases of adenocarcinoma, 7 cases of adenosquamous carcinomas, 13 cases described as non-adenocarcinoma, and 1 case as bronchoalveolar carcinoma (previous terminology). The majority of samples were obtained after surgical (7 articles) or needle biopsy procedures (7 articles).

From the data of the patients included with follow-up, it was possible to obtain that the average progression-free survival (PFS) was 8 months (n=29 patients), and the overall survival (OS) was 19.3 months (n=24 patients).

The molecular techniques for the detection of *EGFR* gene mutations were: the Amplification Refractory Mutation System (ARMS) (1 article, 89 patients), methods based on polymerase chain reaction (PCR) (13 articles, 61 patients), MiSeq system (3 articles, 12 patients), TruSeq Amplicon-Cancer Panel (1 article, 6 patients), and next generation sequencing (NGS) (2 articles, 2 patients).

The exon most involved in multiple mutations was exon 20 (n=382, 85.65% of the total), followed by exon 21 (n=243, 54.48%) and exon 19 (n=172, 38.56%). The most prevalent dual mutations observed were T790M (*de novo* and acquired)/L858R (n=158, 35.42%) and del19/T790M (*de novo* and acquired) (n=140, 31.39%). Only one case was reported in exon 16 (detected by NGS) (48). Other information on dual mutations is present in *Table 3*.

In 181 cases (*Table 1*), we could obtain more clinical information in patients with multiple mutations in the *EGFR* gene. The average age was 61.2 years, being more frequent in women (n=91/149, 61.07%) and people without a history of smoking (n=105/133, 78.9%). In 175 cases (not including triple mutations), we could determine what type of multiple mutations were. One hundred cases were from

Table 1 Clinical and sociodemographic data of patients with multiple mutations in the *EGFR* gene

Author	Country/ region	Year	Number of total cases of lung cancer	Number of dual/triple mutations	Dual and triple mutations	Age (years)	Gender	Smoking history	Tumor staging	Pathology	Pathological sample	Molecular techniques for the detection of *EGFR* gene mutations	Previous use of TKI medication	History of TKI resistance	PFS (months)	OS (months)
Huang SF (32)	Taiwan	2004	101	9	del19 (exon 19) + V769M (exon 20)	62	Male	No	Ia	Adenocarcinoma	Surgical sample	PCR	No	Unknown	Unknown	Unknown
					L858R (exon 21) + L838V (exon 21)	59	Female	No	IIIa	Adenocarcinoma			No	Unknown	Unknown	Unknown
					E709A (exon 18) + L858R (exon 21)	76	Male	Yes	IV	Adenocarcinoma			No	Unknown	Unknown	Unknown
					E709G (exon 18) + L858R (exon 21)	50	Female	Yes	IIIa	Adenocarcinoma			No	Unknown	Unknown	Unknown
					L833V (exon 21) + H835L (exon 21)	76	Female	No	Ib	Adenocarcinoma			No	Unknown	Unknown	Unknown
					G719C (exon 18) + S768I (exon 20)	75	Male	No	IIb	Adenocarcinoma			No	Unknown	Unknown	Unknown
					G719S (exon 18) + S768I (exon 20)	71	Female	Yes	IV	Adenocarcinoma			No	Unknown	Unknown	Unknown
					del19 (exon 19) + L861Q (exon 21)	66	Female	No	Unknown	Adenocarcinoma			Yes	No	Unknown	1.9
					W731Stop (exon 19) + H773R (exon 20)	66	Male	No	Unknown	Adenocarcinoma			Yes	Yes	Unknown	1.5
Bean J (19)	United States	2008	48	1	L858R (exon 21) + T854A (exon 21)	69	Female	Yes	Stage IIB	Poorly differentiated adenocarcinoma	Unknown	Rapid PCR-based detection	Yes	Yes	Unknown	Unknown
Wu CC (40)	Taiwan	2008	4,737	10	G719A (exon 18) + S768I (exon 20)	66	Female	No	IV	Adenocarcinoma	Surgical or needle biopsy/aspiration procedure	RT-PCR	Yes	No	1.25	2
					S768I (exon 20) + L858R (exon 21)	45	Female	No	IV	Adenocarcinoma			Yes	No	21.75	20
					R776G (exon 20) + L858R (exon 21)	87	Female	No	IV	Adenocarcinoma			Yes	No	0.25	1.0
					R776H (exon 20) + L858R (exon 21)	52	Male	Yes	IV	Adenocarcinoma			Yes	No	17.5	32
					R776H (exon 20) + L861Q (exon 21)	65	Female	No	IV	Adenocarcinoma			Yes	No	2.25	7
					G779S (exon 20) + L858R (exon 21)	76	Female	No	IV	Adenocarcinoma			Yes	No	15.25	23
					T790M (exon 20) + L858R (exon 21)	80	Female	No	IV	Adenocarcinoma			Yes	No	2.25	7
					T790M (exon 20) + L858R (exon 21)	55	Female	No	IV	Adenocarcinoma			Yes	No	2.25	30
					T790M (exon 20) + L858R (exon 21)	66	Male	Yes	IV	Adenocarcinoma			No	No	Unknown	36
					N771_H773dupNPH (exon 20) + H773_V774insG (exon 20)	78	Female	No	Ib	Adenocarcinoma			No	Unknown	Unknown	17
Jia XL (21)	China	2011	6,990	7	G719S (exon 18) + L747S (exon 19)	49	Male	No	Unknown	Adenosquamous carcinoma	Surgical sample	PCR	No	Unknown	Unknown	Unknown
					del19 (exon 19) + D807N (exon 20)	54	Female	No	Unknown	Adenosquamous carcinoma			No	Unknown	Unknown	Unknown
					del19 (exon 19) + Q787Q (exon 20)	53	Male	No	Unknown	Adenosquamous carcinoma			No	Unknown	Unknown	Unknown
					del19 (exon 19) + K754A (exon 19) + Q787Q (exon 20)	45	Male	No	Unknown	Adenosquamous carcinoma			No	Unknown	Unknown	Unknown
					Q787Q (exon 20) + L858R (exon 21)	63	Female	No	Unknown	Adenosquamous carcinoma			No	Unknown	Unknown	Unknown
					Q787Q (exon 20) + L858R (exon 21)	64	Female	No	Unknown	Adenosquamous carcinoma			No	Unknown	Unknown	Unknown
					Q787Q (exon 21) + H850R (exon 21)	57	Male	Yes	Unknown	Adenosquamous carcinoma			No	Unknown	Unknown	Unknown
Johnson ML (53)	United States	2011	21	12	del19 (exon 19) + T790M (exon 20) (10 cases)	Unknown	Unknown	Unknown	Unknown	Adenocarcinoma	Unknown	Mutation specific PCR-based methods	Yes	Yes	Unknown	Unknown
					T790M (exon 20) + L858R (exon 21) (2 cases)	Unknown	Unknown	Unknown	Unknown	Adenocarcinoma			Yes	Yes	Unknown	Unknown
Kim SH (54)	Korea	2013	16	1	2317-2319 CAC dup (exon 20) + L858R (exon 21)	69	Female	No	Unknown	Adenocarcinoma	Unknown	RT-PCR	No	No	1.0	Unknown
Tsao AS (57)	United States	2013	54	4	del19 (exon 19) + T790M (exon 20)	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	MiSeq system	Yes	Yes	3.8	5.5
					del19 (exon 19) + C797S (exon 20)	Unknown	Unknown	Unknown	Unknown	Unknown			No	No	0.9	1.0
					T790M (exon 20) + L858R (exon 21)	Unknown	Unknown	Unknown	Unknown	Unknown			Yes	Yes	1.9	4.1
					V802I (exon 20) + K852R (exon 21)	Unknown	Unknown	Unknown	Unknown	Unknown			Yes	Yes	2.6	6.3

Table 1 (*continued*)

Table 1 (*continued*)

Author	Country/ region	Year	Number of total cases of lung cancer	Number of dual/triple mutations	Dual and triple mutations	Age (years)	Gender	Smoking history	Tumor staging	Pathology	Pathological sample	Molecular techniques for the detection of *EGFR* gene mutations	Previous use of TKI medication	History of TKI resistance	PFS (months)	OS (months)
Landi L (25)	Italy	2014	96	8	del19 (exon 19) + T790M (exon 20)	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	RGQ PCR kit	No	Unknown	1.5	16.9
					del19 (exon 19) + T790M (exon 20)	Unknown	Unknown	Unknown	Unknown	Unknown			No	Unknown	15.4	19.16
					del19 (exon 19) + T790M (exon 20)	Unknown	Unknown	Unknown	Unknown	Unknown			Yes	Unknown	1.9	2.14
					del19 (exon 19) + T790M (exon 20)	Unknown	Unknown	Unknown	Unknown	Unknown			Yes	Unknown	2.20	5.32
					del19 (exon 19) + T790M (exon 20)	Unknown	Unknown	Unknown	Unknown	Unknown			Yes	Unknown	8	9.5
					del19 (exon 19) + T790M (exon 20)	Unknown	Unknown	Unknown	Unknown	Unknown			Yes	Unknown	3.8	3.91
					T790M (exon 20) + unspecified mutation (exon 21)	Unknown	Unknown	Unknown	Unknown	Unknown			Yes	Unknown	5.1	10.35
					del19 (exon 19) + T790M (exon 20)	Unknown	Unknown	Unknown	Unknown	Unknown			Yes	Unknown	1.1	2.5
Shan L (29)	China	2015	92	2	del19 (exon 19) + T790M (exon 20)	Unknown	Unknown	Unknown	Unknown	Unknown	Surgical or needle biopsy procedure	Unknown	Yes	Unknown	20.5	Unknown
					del19 (exon 19) + exon 20 ins	Unknown	Unknown	Unknown	Unknown	Unknown			Yes	Unknown	4.2	Unknown
Svaton M (33)	Czech Republic	2015	1	1	G719X (exon 18) + S768I (exon 20)	63	Female	Yes	IV	Adenocarcinoma	Unknown	RT-PCR	No	Yes	Unknown	Unknown
Lou Y (27)	United States	2016	427	5	G719S (exon 18) + T790M (exon 20)	51	Female	No	IV, T4N2M1	Adenocarcinoma	Unknown	PCR amplifications	No	Unknown	Unknown	Unknown
					del19 (exon 19) + T790M (exon 20)	71	Male	No	IV, T2N3M1	Adenocarcinoma			No	Unknown	Unknown	Unknown
					T790M (exon 20) + L858R (exon 21)	62	Female	No	IV, T2N0M1	Bronchoalveolar carcinoma			No	Unknown	Unknown	Unknown
					del19 (exon 19) + T790M (exon 20) + L858R (exon 21)	53	Female	No	IIIA, T4N0M0	Adenocarcinoma			No	Unknown	Unknown	Unknown
					T790M (exon 20) + L858R (exon 21)	34	Female	No	IV, T4N3M1	Adenocarcinoma			No	Unknown	Unknown	Unknown
Ortiz-Cuaran S (28)	Germany	2016	7	7	del19 (exon 19) + T790M (exon 20)	71	Female	Unknown	Unknown	Adenocarcinoma	Biopsy tissue sample	MiSeq system	No	Yes	Unknown	Unknown
					T790M (exon 20) + L861Q (exon 21)	64	Female	Unknown	Unknown	Adenocarcinoma			No	Yes	Unknown	Unknown
					del19 (exon 19) + T790M (exon 20)	54	Female	Unknown	Unknown	Adenocarcinoma			No	Yes	Unknown	Unknown
					T790M (exon 20) + L858R (exon 21)	57	Male	Unknown	Unknown	Adenocarcinoma			No	Yes	Unknown	Unknown
					del19 (exon 19) + T790M (exon 20)	60	Male	Unknown	Unknown	Adenocarcinoma			No	Yes	Unknown	Unknown
					del19 (exon 19) + T790M (exon 20)	56	Female	Unknown	Unknown	Adenocarcinoma			No	Yes	Unknown	Unknown
					del19 (exon 19) + T790M (exon 20)	51	Female	Unknown	Unknown	Adenocarcinoma			No	Yes	Unknown	Unknown
Jiang L (22)	China	2017	297	1	del19 (exon 19) + T790M (exon 20)	Unknown	Unknown	Unknown	Unknown	Adenocarcinoma	Unknown	Unknown	Unknown	Yes	Unknown	Unknown
Kadabur L (23)	India	2017	6	4	Unspecified mutation (exon 21) + L858R (exon 21)	56	Female	No	Unknown	Adenocarcinoma	Biopsy tissue sample	RT-PCR	No	Unknown	Unknown	Unknown
					Unspecified mutation (exon 21) + L858R (exon 21)	50	Female	No	Unknown	Adenocarcinoma	Biopsy tissue sample		No	Unknown	Unknown	Unknown
					T790M (exon 20) + L858R (exon 21)	40	Female	No	Unknown	Adenocarcinoma	Needle aspiration cytology		No	Unknown	Unknown	Unknown
					T790M (exon 20) + L858R (exon 21)	54	Unknown	Yes	No	Adenocarcinoma	Biopsy tissue sample		No	Unknown	Unknown	Unknown
Suryavanshi M (30)	India	2017	76	1	T790M (exon 20) + L858R (exon 21)	54	Male	Unknown	Unknown	Adenocarcinoma	Biopsy tissue sample	RGQ PCR kit	Yes	Yes	Unknown	Unknown

Table 1 (*continued*)

Table 1 (*continued*)

Author	Country/ region	Year	Number of total cases of lung cancer	Number of dual/triple mutations	Dual and triple mutations	Age (years)	Gender	Smoking history	Tumor staging	Pathology	Pathological sample	Molecular techniques for the detection of *EGFR* gene mutations	Previous use of TKI medication	History of TKI resistance	PFS (months)	OS (months)
van Veggel B (56)	Netherlands	2018	8	6	del19 (exon 19) + T790M (exon 20)	62	Female	Unknown	IV	Unknown	Unknown	TruSeq Amplicon-Cancer Panel (Illumina)	Yes	Yes	1.2	Unknown
					T790M (exon 20) + L858R (exon 21)	70	Male	Unknown	IV	Unknown			Yes	Yes	1.4	Unknown
					del19 (exon 19) + T790M (exon 20)	79	Female	Unknown	IV	Unknown			Yes	Unknown	1.3	Unknown
					del19 (exon 19) + T790M (exon 20)	37	Female	Unknown	IV	Unknown			Yes	No	1.4	Unknown
					del19 (exon 19) + T790M (exon 20)	66	Female	Unknown	IV	Unknown			Yes	Yes	1.4	Unknown
					T790M (exon 20) + L858R (exon 21)	72	Female	Unknown	IV	Unknown			Yes	Yes	2.1	Unknown
Zaemes J (31)	United States	2018	1	1	G719S (exon 18) + S768I (exon 20)	82	Female	No	Unknown	Adenocarcinoma	Biopsy tissue sample	TruSight tumor panel on the Illumina MiSeq	No	No	Unknown	Unknown
Gao X (52)	China	2019	16,347	89	del19 (exon 19) + T790M (exon 20) (22 cases)	<60 (10 cases), >60 (12 cases)	Female (12 cases), male (10 cases)	No (17 cases), yes (4 cases)	IA (13 cases), IB–IIIA (6 cases)	Adenocarcinoma (20 cases), non-adenocarcinoma (1 case)	Unknown	The ARMS	No	Unknown	Unknown	Unknown
					T790M (exon 20) + L858R (exon 21) (63 cases)	<60 (26 cases), >60 (37 cases)	Female (37 cases), male (26 cases)	No (51 cases), yes (12 cases)	IA (33 cases), IB–IIIA (27 cases)	Adenocarcinoma (49 cases), non-adenocarcinoma (12 cases)			No	Unknown	Unknown	Unknown
					T790M (exon 20) + L861Q (exon 21)	Unknown	Unknown	Unknown	Unknown	Unknown			No	Unknown	Unknown	Unknown
					G719X (exon 18) + T790M (exon 20)	Unknown	Unknown	Unknown	Unknown	Unknown			No	Unknown	Unknown	Unknown
					del19 (exon 19) + T790M (exon 20) + L858R (exon 21) (2 cases)	Unknown	Unknown	Unknown	Unknown	Unknown			No	Unknown	Unknown	Unknown
Hu Y (20)	China	2019	1	1	del19 (exon 19) + T790M (exon 20)	43	Male	No	IIIa, T1N2M0	Adenocarcinoma	Surgical sample	NGS	Yes	Yes	Unknown	Unknown
Zhang Z (50)	China	2020	13	8	T790M (exon 20) + L858R (exon 21)	Unknown	Male	Yes	Unknown	Unknown	Unknown	Unknown	Yes	Unknown	Unknown	Unknown
					T790M (exon 20) + L858R (exon 21)	Unknown	Female	No	Unknown	Unknown			Yes	Unknown	Unknown	Unknown
					del19 (exon 19) + T790M (exon 20)	Unknown	Male	Yes	Unknown	Unknown			Yes	Unknown	Unknown	Unknown
					del19 (exon 19) + T790M (exon 20)	Unknown	Female	No	Unknown	Unknown			Yes	Unknown	Unknown	Unknown
					del19 (exon 19) + T790M (exon 20)	Unknown	Male	No	Unknown	Unknown			Yes	Unknown	Unknown	Unknown
					E709K (exon 18) + L858R (exon 21)	Unknown	Female	No	Unknown	Unknown			Yes	Unknown	Unknown	Unknown
					T790M (exon 20) + H805L (exon 20) + L858R (exon 21)	Unknown	Male	No	Unknown	Unknown			Unknown	Unknown	Unknown	Unknown
					T790M (exon 20) + L858R (exon 21) + D1012E (unknown exon)	Unknown	Male	Yes	Unknown	Unknown			Unknown	Unknown	Unknown	Unknown
Lahmadi M (24)	Algeria	2021	58	2	del19 (exon 19) + L858R (exon 21)	Unknown	Male	Unknown	Unknown	Adenocarcinoma	Surgical or needle biopsy procedure	Mutation specific PCR-based methods	Unknown	Unknown	Unknown	Unknown
					del19 (exon 19) + L858R (exon 21)	Unknown	Female	Unknown	Unknown	Adenocarcinoma			Unknown	Unknown	Unknown	Unknown
Bie Y (48)	China	2021	1	1	L643V (exon 16) + del19 (exon 19)	60	Female	No	I, pT1N0M0	Adenocarcinoma	Surgical sample	NGS	No	Yes	Unknown	Unknown

EGFR, epidermal growth factor receptor; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; RGQ, Rotor-Gene Q; ARMS, Amplification Refractory Mutation System; TKI, tyrosine kinase inhibitor; NGS, next generation sequencing; PFS, progression-free survival; OS, overall survival.

Table 2 Other multiple mutations

Author	Country/ region	Year	Number of total cases of lung cancer	Number of dual/triple mutations	Dual and triple mutations
Kosaka T (45)	Japan	2004	277	4	G719X (exon 18) + E709H (exon 18) S768I (exon 20) + L858R (exon 21) R776C (exon 20) + L858R (exon 21) T790M (exon 20) + L858R (exon 21)
Shigematsu H (46)	Japan, Taiwan, United States and Australia	2005	617	3	G719S (exon 18)+ S768I (exon 20) I715S (unknown exon) + S720F (unknown exon) + L861Q (exon 21) G719C (exon 18) + E709V (exon 18) + L718L (unknown exon)
Yokoyama T (41)	Japan	2006	264	7	G719S (exon 18) + S768I (exon 20) G719C (exon 18) + S768I (exon 20) S768I (exon 20) + V769L (exon 20) S768I (exon 20)+ V774M (exon 20) E709K (exon 18) + L858R (exon 21) E709G (exon 18) + L858R (exon 21) E709A (exon 18) + L858R (exon 21)
Janjigian YY (55)	Netherlands and United States	2014	201	73	del19 (exon 19) + T790M (exon 20) (44 cases) T790M (exon 20) + L858R (exon 21) (24 cases) G719S (exon 18) + T790M (exon 20) G719A (exon 18) + T790M (exon 20) G719C (exon 18) + T790M (exon 20) S768I (exon 20) + T790M (exon 20) T790M (exon 20) + L861Q (exon 21)
Li B (26)	China	2015	100	3	Unspecified mutation (exon 19) + unspecified mutation (exon 21) (3 cases)
Dolesova L (9)	Slovakia	2015	450	1	S768I (exon 20) + L858R (exon 21)
Udupa KS (42)	India	2015	85	2	G719X (exon 18) + L858R (exon 21) G719X (exon 18) + L858R (exon 21)
Ardakani NM (35)	Australia	2016	493	10	del19 (exon 19) + T790M (exon 20) E709K (exon 18) + L858R (exon 21) E709G (exon 18) + L858R (exon 21) G719A (exon 18) + S768I (exon 20) G719A (exon 18) + S768I (exon 20) T790M (exon 20) + L858R (exon 21) T790M (exon 20) + L858R (exon 21) S768I (exon 20) + L858R (exon 21) R776C (exon 20) + L858R (exon 21) E709G (exon 18) + L858R (exon 21)
Labbé C (38)	Canada	2017	105	5	Unspecified mutation (exon 20) + unspecified mutation (exon 21) Unspecified mutation (exon 20) + unspecified mutation (exon 21) Unspecified mutation (exon 19) + unspecified mutation (exon 20) Unspecified mutation (exon 18) + unspecified mutation (exon 21) Unspecified mutation (exon 18) + unspecified mutation (exon 21)
Choi YW (37)	Korea	2018	60	4	Unspecified mutation (exon 18) + L858R (exon 21) del19 (exon 19) + L858R (exon 21) Unspecified mutation (exon 18) + del19 (exon 19) Unspecified mutation (exon 18) + unspecified mutation (exon 20)
Wei Y (34)	China	2018	41	4	S768I (exon 20) + L858R (exon 21) T790M (exon 20) + L858R (exon 21) T790M (exon 20) + L858R (exon 21) del19 (exon 19) + T790M (exon 20)

Table 2 (*continued*)

Table 2 (*continued*)

Author	Country/ region	Year	Number of total cases of lung cancer	Number of dual/triple mutations	Dual and triple mutations
Rana V (44)	India	2018	152	1	del19 (exon 19) + L858R (exon 21)
Kate S (51)	India	2019	1,260	44	del19 (exon 19) + T790M (exon 20) (17 cases) T790M (exon 20) + L858R (exon 21) (15 cases) G719X (exon 18) + S768I (exon 20) (3 cases) S768I (exon 20) + L858R (exon 21) (2 cases) G719X (exon 18) + T790M (exon 20) G779S (exon 20) + L858R (exon 21) del19 (exon 19) + exon 20 ins L858R (exon 21) + L861Q (exon 21) T790M (exon 20) + S768I (exon 20) T790M (exon 20) + L861I (exon 21) G719X (exon 18) + S768I (exon 20) + L858R (exon 21)
Wang X (6)	United States	2019	307	1	T790M (exon 20) + L858R (exon 21)
Chen K (36)	China	2020	600	5	G719X (exon 18) + L861Q (exon 21) G719X (exon 18) + S768I (exon 20) T790M (exon 20) + L858R (exon 21) T790M (exon 20) + L858R (exon 21) del19 (exon 19) + L858R (exon 21)
Nakra T (39)	India	2020	3,436	19	del19 (exon 19) + T790M (exon 20) del19 (exon 19) + L858R (exon 21) D761Y (exon 19) + L858R (exon 21) Unspecified mutation (exon 19) + L858R (exon 21) T790M (exon 20) + L858R (exon 21) G719X (exon 18) + L858R (exon 21) G719X (exon 18) + L861Q (exon 21) G719X (exon 18) + Unspecified mutation (exon 20) S768I (exon 20) + L858R (exon 21) G719X (exon 18) + unspecified mutation (exon 19) G719X (exon 18) + T790M (exon 20) S768I (exon 20) + L858R (exon 21) L858R (exon 21) + G810V (unspecified exon) E734K (exon 19) + L858R (exon 21) Unspecified mutation (exon 19) + unspecified mutation (exon 20) del19 (exon 19) + del19 (exon 19) G719X (exon 18) + E734K (exon 19) G719X (exon 18) + S768I (exon 20) L858R (exon 21) + A871G (unspecified exon)
Tang Y (43)	China	2020	1,293	51	T790M (exon 20) + L858R (exon 21) (31 patients) del19 (exon 19) + T790M (exon 20) (20 patients)
Brindel A (49)	France	2020	7,539	27	G719X (exon 18) + S768I (exon 20) (9 cases) G719X (exon 18) + E709X (exon 18) (7 cases) E709X (exon 18) + L858R (exon 21) (4 cases) S768I (exon 20) + L858R (exon 21) (3 cases) G719X (exon 18) + L858R (exon 21) (2 cases) G719X (exon 18) + L861Q (exon 21) (2 cases)
Cruz Castellanos P (47)	Spain	2021	1	1	G719X (exon 18) + L858R (exon 21)

Table 3 Most frequent dual mutations according to exon location

First mutation	Second mutation	N (cases)
Exon 16		
L643V (exon 16)	del19 (exon 19)	1
Exon 18		
G719X (exon 18)	S768I (exon 20)	15
	E709X (exon 18)	7
	L858R (exon 21)	6
	L861Q (exon 21)	4
	T790M (exon 20)	3
	E709H (exon 18)	1
	E734K (exon 19)	1
	Unspecified mutation (exon 19)	1
	Unspecified mutation (exon 20)	1
G719S (exon 18)	S768I (exon 20)	4
	T790M (exon 20)	2
	L747S (exon 19)	1
G719A (exon 18)	S768I (exon 20)	3
	T790M (exon 20)	1
G709G (exon 18)	L858R (exon 21)	4
E709X (exon 18)	L858R (exon 21)	4
Unspecified mutation (exon 18)	Unspecified mutation (exon 21)	2
	del19 (exon 19)	1
	L858R (exon 21)	1
	Unspecified mutation (exon 20)	1
E709K (exon 18)	L858R (exon 21)	3
G719C (exon 18)	S768I (exon 20)	2
	T790M (exon 20)	1
E709A (exon 18)	L858R (exon 21)	2
Total number		71
Exon 19		
del19 (exon 19)	T790M (exon 20)	140
	L858R (exon 21)	6
	Exon 20 ins	2
	D807N (exon 20)	1
	Q787Q (exon 20)	1
	C797S (exon 20)	1
	L861Q (exon 21)	1
	del19 (exon 19)	1
	V769M (exon 20)	1

Table 3 (*continued*)

Table 3 (*continued*)

First mutation	Second mutation	N (cases)
Unspecified mutation (exon 19)	Unspecified mutation (exon 21)	4
	Unspecified mutation (exon 20)	2
W731Stop (exon 19)	H773R (exon 20)	1
D761Y (exon 19)	L858R (exon 21)	1
E734K (exon 19)	L858R (exon 21)	1
Total number		154
Exon 20		
T790M (exon 20)	L858R (exon 21)	158
	L861Q (exon 21)	3
	S768I (exon 20)	2
	L861I (exon 21)	1
	Unspecified mutation (exon 21)	1
S768I (exon 20)	L858R (exon 21)	12
	V769L (exon 20)	1
	V774M (exon 20)	1
Q787Q (exon 20)	L858R (exon 21)	2
	H850R (exon 21)	1
R776C (exon 20)	L858R (exon 21)	2
R776G (exon 20)	L858R (exon 21)	1
R776H (exon 20)	L858R (exon 21)	1
	L861Q (exon 21)	1
G779S (exon 20)	L858R (exon 21)	2
Unspecified mutation (exon 20)	Unspecified mutation (exon 21)	2
N771_H773dupNPH (exon 20)	H773_V774insG (exon 20)	1
2317-2319 CAC dup (exon 20)	L858R (exon 21)	1
V802I (exon 20)	K852R (exon 21)	1
Total number		194
Exon 21		
L858R (exon 21)	L838V (exon 21)	1
	T854A (exon 21)	1
	Unspecified mutation (exon 21)	2
	L861Q (exon 21)	1
	G810V (unspecified exon)	1
	A871G (unspecified exon)	1
L833V (exon 21)	H835L (exon 21)	1
Total number		8

Table 4 Triple mutations

Triple mutations	N
del19 (exon 19), T790M (exon 20), L858R (exon 21)	3
del19 (exon 19), K754A (exon 19), Q787Q (exon 20)	1
T790M (exon 20), H805L (exon 20), L858R (exon 21)	1
T790M (exon 20), L858R (exon 21), D1012E (unknown exon)	1
I715S (unknown exon), S720F (unknown exon), L861Q (exon 21)	1
G719C (exon 18), E709V (exon 18), L718L (unknown exon)	1
G719X (exon 18), S768I (exon 20), L858R (exon 21)	1
Total number	9

patients in the group with any *EGFR* gene mutation (except deletion in exon 19, L858R, and *de novo* T790M) plus a *de novo* mutation T790M in the *EGFR* gene, 60 cases from the group with the presence of a deletion in exon 19 or L858R, and any *EGFR* gene mutation (except deletion in exon 19, L858R, and *de novo* T790M), 12 cases from the group of a combination of any *EGFR* gene mutation (except deletion in exon 19, L858R, and *de novo* T790M) and two cases of combination of deletion in exon 19 and L858R mutation.

In 140 of the 175 patients, the T790M mutation of exon 20 was present, of which 100 cases were *de novo*, and 40 patients were acquired after TKI treatment. In the group of *de novo* T790M mutations, it can be established from the cases with clinical information that the mean age was 55.18 years, none was previously managed with TKIs, and the PFS and OS were 8.45 months (1 article, 2 patients) and 24.02 months (2 articles, 3 patients), respectively. However, survival data is limited to a small number of cases. In the case of patients with T790M acquired mutation, the average age was 61.69 years; 90% of the subjects had previous management with TKIs, where the majority were first-generation TKIs. In this group of patients, more information on patients was available, and the PFS was 3.62 months (5 articles, 17 patients) and OS 8.03 months (3 articles, 10 patients).

Triple mutations were found in 9 cases (2.017%). In 5 of them, the dual T790M/L858R mutations were associated with a third variable mutation. Interestingly, in two instances, triple mutations were composed of the most frequent mutations found in our study: del19/T790M/L858R. The cases are described in greater detail in *Table 4*.

Discussion

Multiple mutations in the *EGFR* gene are a rare event. We found 446 of 46,679 patients with multiple mutations in the present review. These mutations were reported mainly in exons 18 to 21. Only one case reported mutation in exon 16 (48). We included patients from different ethnicity, predominantly Asian and European. The most common multiple mutation was the L858R mutation in exon 21 with the T790M in exon 20 (*de novo* and acquired). In contrast to Kate *et al.* (51), the most common multiple mutations were a deletion in exon 19 with the T790M mutation in exon 20. In our study, there were 158 patients with the L858R and T790M mutations, from which detailed information could be obtained in some of them. Gao *et al.* (52) reported 63 cases, of which 37 (58.7%) patients were older than 60 years, 37 cases were women (58.7%), most of these patients had no history of smoking (80.9%), and in 49 cases (77.7%) were adenocarcinomas.

The most common single mutation associated with dual mutations was the mutation in exon 20 (T790M) (*de novo* and acquired), with 305 cases in total. In the patients with the T790M mutation that we were able to collect data from (138 patients), we found that patients with the acquired T790M mutation are more frequent with the subgroup of patients with a deletion in exon 19. In contrast, *de novo* mutations are more abundant in patients with the L858R mutation. Liang *et al.* (58) analyzed 25 studies including 1,770 patients; they observed that T790M was more frequent in exon 19 deletion than in L858R among patients with acquired resistance to TKIs.

According to previous data, around 73% of lung cancers with the T790M mutation have a second mutation in the *EGFR* gene (59). In our study, the T790M mutation (including *de novo* and acquired cases) was present in 69.05% (n=310/446) of all multiple mutations. This mutation is usually detected in NSCLC patients previously exposed to TKIs, where approximately half of the cases developed resistance to this type of drug (39). The study by Singh *et al.* (60). showed that the coexistence of the *de novo* *EGFR* T790M mutation with any other *EGFR* mutation had primary resistance to first- and second-generation *EGFR* TKIs. A hypothesis suggests that the T790M mutation by itself could be a weak oncogene, requiring a secondary mutation to enhance cancer development (59). However, clinical follow-up data are limited to a few patients. We found that the PFS and OS from patients with acquired T790M mutation plus other mutations (PFS: 3.6 months, OS: 8.2 months) are lower than patients with *de novo* T790M mutation plus other mutations (PFS: 8.4 months, OS: 24 months). In the study by Lin *et al.* (61), it was shown

that patients with acquired multiple mutations with the presence of the T790M mutation have a lower median PFS and OS compared to patients without this mutation (median PFS 2.9 *vs.* 9.7 months; median OS 17.8 *vs.* 31 months). Liang *et al.* (58) suggested that patients with T790M mutation and deletion in exon 19 may be more likely to benefit from osimertinib than those with L858R; hence first-generation TKIs may be less recommended in patients with exon 19 deletion. Recently Moriya *et al.* (62) conducted a study showing that management with a third-generation TKI such as osimertinib achieved a longer PFS than first- and second-generation TKIs. The meta-analysis presented by Liu *et al.* (63) showed that the acquired T790M mutation was not predictive of a superior OS. However, unlike the study by Lin *et al.* (61) and the present study, it was demonstrated that the acquired T790M mutation had a higher PFS and OS compared to patients with the *de novo* T790M mutation. It should be noted that these data are not explicitly extracted from cases with multiple *EGFR* mutations. Also, the clinical behavior of patients with uncommon *EGFR* mutations against TKIs is variable; for example, the S768I mutation is resistant to first-generation TKIs (gefitinib/erlotinib), while insertions in the exon 20 confer resistance to first and second-generation TKIs (afatinib) (64).

In vitro studies have observed that dual mutations reduce the response to TKIs compared with one mutation (65,66). A recent systematic review by Attili *et al.* (67) described that the objective response rate (ORR) and the PFS are different in patients with dual common and uncommon mutations treated with first-generation or second-generation TKIs. They defined common mutation as the deletion in exon 19 or L858R mutation; and uncommon mutation as any mutation but L858R, exon 19 deletions, or *de novo* T790M mutation. They found that patients with uncommon dual mutations had less ORR and PFS than common dual mutations. They concluded that in patients with common dual mutations and dual mutations of common and uncommon, the similar overall response and survival outcomes with any TKIs are similar to a single common *EGFR* mutation. At the same time, the patients with uncommon mutations have higher benefits with second and third-generation TKIs.

The nine cases with triple mutations in the world literature are interesting. It was possible only to obtain information from four patients from our analysis. It is striking to see that a large percentage of the mutations involved are the most common mutations, including exon 19 deletions, L858R and T790M; which raises the hypothesis that with the development of last generation TKIs, the tumor biologically must give "third hits" to maintain its oncogenic activity. Especially, third-generation TKIs have shown better efficacy and safety profiles than other inhibitors. However, their resistance mechanism is more heterogeneous than that of other inhibitors, and in many cases, the resistance mechanism has not yet been identified (68).

This article has limitations. The most important was that clinical information data could not be collected in a large percentage of patients. In addition, where data were available, the type of information was not uniform, which prevented the extraction of accurate epidemiological data, especially regarding patient follow-up. Also, in general, when studying multiple *EGFR* gene mutations in patients with lung neoplasms, there is a risk that a mutation may be missed because molecular screening techniques are used in many medical centers and not specific genotyping methods (69). In our study, we mainly analyzed patients with deletions in exon 19; however, according to a recent study by Chen *et al.* (70), there are a large number of variants in this type of mutation (different starting locations and with or without amino acid insertion/substitution) that may have clinical repercussions, for which we consider it essential to take this into account for future studies.

Conclusions

According to our analysis, multiple mutations in the *EGFR* gene are found in less than 1% of the samples studied in patients with lung neoplasms. The most frequent dual mutations were T790M with L858R and exon 19 deletions with T790M. The most common single mutation associated with dual mutations was the mutation in exon 20 (T790M) (*de novo* and acquired). Acquired T790M mutations are more frequent in the subgroup of patients with a deletion in exon 19, whereas *de novo* mutations are more abundant in patients with the L858R mutation. The presence of T790M mutation showed poor outcomes in the follow-up of patients.

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Footnote

Reporting Checklist: The authors have completed the PRISMA reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-235/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-235/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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