Article

Predominance of the Rare EGFR Mutation p.L861Q in Tunisian Patients with Non-Small Cell Lung Carcinoma

Rania Abdelmaksoud-Dammak 1, Nihel Ammous-Boukhris 1, Aména Saadallah-Kallel 1, Slim Charfi 2, Souhir Khemiri 3, Rim Khemakhem 4, Nesrin Kallel 4, Wala Ben Kridis-Rejeb 3, Afef Khanfir 3, Ilhem Yangui 4, Jamel Daoud 5 and Raja Mokdad-Gargouri 1,*

Center of Biotechnology of Sfax, Laboratory of Eucaryotes Molecular Biotechnology, University of Sfax BP K 1177, Sfax 3018, Tunisia
2 Department of Anatomopathology, Habib Bourguiba Hospital, Sfax 3002, Tunisia
3 Department of Medical Oncology, Habib Bourguiba Hospital, Sfax 3002, Tunisia
4 Department of Pneumology, Hedi Chaker Hospital, Sfax 3089, Tunisia
5 Department of Radiotherapy, Habib Bourguiba Hospital, Sfax 3002, Tunisia
* Correspondence: raja.gargouri@CBS.RNRT.TN; Tel./Fax: +216-74-874-449

Abstract: Objectives: Several new cancer therapies targeting signaling pathways involved in the growth and progression of cancer cells were developed as personalized medicine. Our study aimed to identify epidermal growth factor receptor (EGFR) mutations for TKI treatment in non-small-cell lung cancer (NSCLC) Tunisian patients. Methods: Analysis of the TKI sensitivity mutations in exons 18 to 21 of the EGFR gene and exon 15 of the B-raf gene was performed in 79 formalin fixed-paraffin embedded (FFPE) NSCLC samples using pyrosequencing. Results: EGFR mutations were detected in 34 cases among 79 (43%), with the predominance of the L861Q in exon 21 found in 35.3% of the cases (12 out of 34). Deletions in exon 19 were found in 8 cases (23.5%), and only one young male patient had the T790M mutation. Three patients harbored composite EGFR mutations (p.E746_A750del/p.L861R, p.E746_S752>V/p.S768I, and p.G719A/p.L861Q). Furthermore, the EGFR mutated status was significantly more frequent in female patients (p = 0.019), in non-smoker patients (p = 0.008), and in patients with metastasis (p = 0.044). Moreover, the B-raf V600E was identified in 5 EGFR negative patients among 39 analyzed samples (13.15%). Conclusion: The p.L861Q localized in exon 21 of the EGFR gene was the most common mutation identified in our patients (35.3%), whereas the “classic” EGFR mutations such as Del19 and p.L858R were found in 23.5% and 11.7% of the cases, respectively. Interestingly, most of p.L861X mutation-carrying patients showed good response to TKI treatment. Altogether, our findings suggest a particular distribution of the EGFR-TKIs sensitivity mutations in Tunisian NSCLC patients.

Keywords: non-small-cell lung carcinoma; epidermal growth factor receptor; mutation; pyrosequencing; tyrosine kinase inhibitors; targeted therapy

1. Introduction

Lung cancer is the most common cause of cancer deaths per year, estimated to be responsible for nearly one in five deaths worldwide, with about 2.2 million cases in 2018 [1]. According to 2020 GLOBOCAN data, lung cancer was the second most commonly diagnosed cancer (11.4%) with 1.8 million evaluated deaths (18%) [1]. In Tunisia, lung cancer is the 2nd most common cancer with around 2929 new cases per year and a frequency of 15.1% [2]. This malignancy is the most lethal cancer with a mortality rate of 22.2% in 2020 [3].

Non-small cell lung cancer (NSCLC) is the major type of lung cancer, identified in 80 to 85% of patients, for which adenocarcinoma is the most common subtype [4]. More than 60% of lung cancer diagnoses are made at an advanced stage (III or IV) and the 5-year overall survival rate for metastatic NSCLC remains very poor [4].
In NSCLC, numerous molecular alterations affecting key signaling pathways have been reported and defined as driver oncogenes. Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein encoded by the ErbB gene and overexpressed in more than 40% of non-small cell lung cancers (NSCLC) [5–9]. EGFR is mutated in 10–20% of lung adenocarcinomas among Caucasian patients and more commonly in young never-smoker Asian women, whereas EGFR mutations are rare in other lung cancer subtypes [10,11].

Several drugs have been developed for EGFR-driven NSCLC and are becoming the standard of care for the first-line treatment of advanced NSCLC with EGFR mutations [12]. Several somatic mutations in the EGFR gene have been identified and classified as conferring sensitivity to the EGFR tyrosine kinase inhibitors (TKIs), which are mainly located in the intracellular tyrosine kinase domain spanning from exon 18 to exon 21 [12]. The “classic” EGFR mutations, namely deletions in exon 19 and L858R in exon 21, account for about 85% of EGFR mutations in NSCLC [13–15]. Patients harboring either of these two mutations represent a classic subtype of NSCLC, with a higher response to EGFR-TKIs and even improved overall survival (OS) rates compared to patients with wild-type EGFR tumors [16–18]. On the other hand, rare mutations including point mutations, deletions, and insertions within exons 18 to 21 of the EGFR gene account for the remaining 15% of the EGFR mutations in NSCLC [13–15].

B-raf is one of three members of the RAF kinase family: A-raf, B-raf, and C-raf, which belongs to the group of serine-threonine kinases and plays a critical role in mitogen-activated protein kinase (MAPK) pathways [19,20]. Mutations in B-raf, mainly the V600E, have been found in different types of cancer, predominantly melanoma and metastatic colorectal cancer with frequencies of 50% and 9%, respectively [21,22]. In NSCLCs, the B-raf V600E was reported in 1 to 3% of the cases and generated a constitutive activation of the MAP pathway, leading to cell growth, proliferation, and resistance to negative modulatory feedback signals [23].

Several inhibitors of the B-raf V600E mutant protein, such as dabrafenib, are recently used in therapy and have shown increased response in patients carrying the V600E mutation, with an average of 5.5 months progression-free survival [24,25].

In Tunisian NSCLC patients, only few studies have investigated the EGFR and B-raf mutations. Using different methods such as immunohistochemistry, QPCR, or NGS, the percentage of EGFR mutations varied from 18.4 to 44% [26–29]. The most frequent mutations were exon 19 deletions and the p.L858R in exon 21 [27–29].

Regarding B-raf mutation, the study of Mezni et al. reported that among 41 patients screened by NGS, only 3 harbored mutations [28].

In order to better investigate the profile of EGFR and B-raf in NSCLC the present study aims to identify the EGFR and B-raf mutations in 79 Tunisian patients. EGFR mutations were correlated with clinicopathological features, treatment, and patient’s survival.

2. Materials and Methods

2.1. Clinical Samples

Between 1 March 2018 and 30 June 2022, 79 tumor samples were collected from Tunisian patients with an advanced lung adenocarcinoma. Clinicopathological features were available for only 69 cases. The average age was 59.54 years (range 24–85 years). In our cohort, 69.56% (48/69) of patients had metastases and 61.21% (44/69) declared a previous or current smoking history. Available characteristics of patients included in this study are shown in Table 1.

All analyses were conducted with respect of patients’ confidentiality and according to procedures approved by the Personal Protection Committee (PPC) of UHC Habib Bourguiba of Sfax, Tunisia, responsible for ethics in research. All samples were histologically analyzed by a pathologist, the percent of tumor cells was determined, and each sample was classified according to the WHO classification of lung cancer.
Table 1. Clinical pathological characteristics of patients.

| Patients’ Characteristics | Number (%) |
|---------------------------|------------|
| Total                     | 79         |
| Age (Years)               | 69         |
| Median                    | 59.54      |
| Range                     | 24–85      |
| Gender                    | 79         |
| Male                      | 65 (82.27) |
| Female                    | 14 (17.72) |
| Smoking history           | 69         |
| Never-smoker              | 24 (34.78) |
| Former/current smoker     | 44 (61.21) |
| Histological Type         | 56         |
| Adenocarcinoma            | 51 (91.07) |
| Epidermoid carcinoma      | 4 (7.14)   |
| Pleomorphic carcinoma     | 1 (1.78)   |
| Metastasis                | 69         |
| Absence                   | 48 (69.56) |
| Presence                  | 21 (30.43) |

2.2. DNA Extraction and PCR Amplification

Formalin-fixed and paraffin-embedded tissues genomic DNA was extracted from 3–6 (10 µm thick) sequential sections through QIAamp DNA FFPE tissue kit (Qiagen) according to the manufacturer’s instructions and checked for adequacy by NanoDrop. Primers used were previously developed in our lab and each sample was analyzed using both our primers and the Therascreen EGFR Pyro Kit primers (Qiagen) for validation. In brief, when using our own developed primers, PCR reactions were performed on 100 ng of DNA in a total volume of 50 µL containing 5× buffer, 200 µM dNTP, 0.5 µM of each primer, and 0.2U Taq polymerase (Takara), with the following cycling conditions: 95 °C for 5 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 56/60 °C for 30 s, extension at 72 °C for 30 s. A final extension at 72 °C for 5 min was finally performed. PCR of the Therascreen EGFR Pyro Kit were performed according to the manufacturer directives (Qiagen). Amplicons were analyzed by gel electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized by ultraviolet trans-illumination.

2.3. Pyrosequencing Analysis

PCR products were incubated under shaking with binding buffer (40 µL) and added with sepharose beads (1 µL) covered by streptavidin. Then, PCR products were washed with 70% ethanol, denatured with denaturation solution (Qiagen), and re-washed with wash solution (Qiagen). A pyrosequencing reaction was then performed for AQ mode in a total of 25 µL, including 24.2 µL of annealing buffer and 0.8 µL of sequencing primer (final concentration 0.3 µM). Pyrosequencing assays were performed on a PyroMark Q24 MDx using PyroMark Gold reagents (Qiagen). Assays for mutation analysis in exons 18, 19, 20, and 21 of EGFR and exon 15 of B-raf were performed according to manufacturer’s instructions and nucleotide dispensation order was outlined by the software Q24 2.0. Sequencing primers were generated according to PyroMark Assay Design software version 2.0 (Qiagen). Pyromark Q24 ID version 2.0.8 software was used to generate and automatically analyze pyrograms resulting from sequencing onto PyroMark Q24 ID system.
2.4. Statistical Analysis

The SPSS software Version 20 was used to statistically analyze the association between EGFR and B-raf mutation state and clinicopathological features, and the correlations were assessed by Chi-square test. Statistical significance was set to \( p \leq 0.05 \) in each test.

3. Results

3.1. EGFR and BRAF Mutation Analysis

Mutation screening of exons 18 to 21 of the EGFR gene showed that 34 cases among 79 (43%) were mutated. Among EGFR-positive patients, 35.3% (12 out of 34) carried the p.L861Q mutation, while the p.L861R was identified in only 5 patients (Figure 1, Table 2). Regarding the “classic” mutations, deletions in exon 19 were detected in 23.5% (8/34) of cases with the predominance of the p.E746_A750del (5 among 8 cases, 62.5%), while 11.7% (4 out of 34) carried the p.L858R (Figure 1, Table 2). In addition, composite mutations were identified in 3 patients: p.E746_A750del/p.L861R, p.E746_S752>V/p.S768I, and p.G719A/p.L861Q (Table 2).

![EGFR Mutation Distribution](image)

**Figure 1.** Histogram showing distribution of identified EGFR mutations.

**Table 2.** Identified mutation in exons 18–21 of the EGFR in Tunisian NSCLC patients. *: Deceased before starting the target therapy.

| Patient | Gender | Age | EGFR Alteration     | Metastasis         | Smoking History | Therapy        | Status  | Survival (Months) |
|---------|--------|-----|---------------------|--------------------|-----------------|----------------|---------|------------------|
| P3      | M      | 60  | p.L861Q             | Lymph nodes        | 30 PA           | Chemotherapy   | dead    | 37               |
| P4      | M      | 46  | p.T790M             | Liver, Bones       | 20 PA           | -              | -       | -                |
| P27     | F      | 65  | p.L861Q             | Lung               | No              | Chemotherapy + Erlotinib | alive | 40               |
| P34     | M      | 72  | p.L861R             | No                 | 25 PA           | Chemotherapy   | dead    | 18               |
| P35     | M      | 72  | p.L861R             | No                 | 30 PA           | -              | -       | -                |
| P36     | M      | 50  | p.E746_A750del/p.L861R | Bones            | 40 PA           | Erlotinib      | alive   | 15               |
| P37     | M      | 60  | p.G719S             | Brain              | No              | Chemotherapy   | dead    | 35               |
| P40     | M      | 72  | p.G719A             | Lung               | No              | -              | -       | -                |
| P42     | M      | 63  | p.E746_A750del      | Bones              | 60 PA           | Chemotherapy   | alive   | 17               |
| P104    | M      | -   | p.L861Q             | -                  | 20 PA           | -              | -       | -                |
| P105    | M      | -   | p.E746_A750del      | Liver              | 40 PA           | -              | -       | -                |
Table 2. Cont.

| Patient | Gender | Age | EGFR Alteration | Metastasis | Smoking History | Therapy | Status | Survival (Months) |
|---------|--------|-----|----------------|------------|----------------|---------|--------|------------------|
| P106    | F      | -   | p.E746_S752>V/p.S768I | Bones     | No             | -       | -      | -                |
| P109    | F      | -   | p.E746_T751I     | Bones     | 10 PA          | -       | -      | -                |
| P112    | M      | -   | p.L861R         | Brain     | 50 PA          | -       | -      | -                |
| P117    | F      | -   | p.L747_T751del  | Bones     | 15 PA          | -       | -      | -                |
| P120    | M      | 66  | p.G719A/p.L861Q | Liver     | No             | -       | -      | -                |
| P121    | M      | 51  | p.L858R         | Lung      | No             | -       | -      | -                |
| P125    | M      | 54  | p.E746_A750del  | Lung      | 30 PA          | -       | -      | -                |
| P128    | M      | -   | p.L858R         | Liver     | 10 PA          | -       | -      | -                |
| P134    | M      | 67  | p.L861Q         | No        | No             | -       | -      | -                |
| P135    | M      | 54  | p.G719C         | No        | 40 PA          | -       | -      | -                |
| P136    | M      | 76  | p.E746_A750del  | No        | 20 PA          | Surgery | alive | 7                |
| P137    | F      | 63  | p.L861Q         | No        | No             | -       | -      | -                |
| P139    | M      | 64  | p.G719C         | Adrenal   | Chemotherapy   | Chemotherapy + Erlotinib | dead | 1                |
| P140    | M      | 66  | p.L861Q         | Bones     | Unknown        | Chemotherapy | Chemotherapy + Erlotinib | dead | 3                |
| P145    | M      | 59  | p.L858R         | No        | 50 PA          | *       | dead | 3                |
| P146    | F      | 48  | p.G719A         | Bones     | No             | *       | dead | 4                |
| P148    | F      | 70  | p.L861Q         | No        | No             | -       | -      | -                |
| P149    | F      | 60  | p.L861R         | No        | No             | Erlotinib | alive | 3                |
| P150    | M      | 62  | p.L861Q         | No        | No             | -       | -      | -                |
| P156    | M      | 62  | p.L861Q         | Liver     | 40 PA          | -       | -      | -                |
| P157    | M      | 54  | p.L861Q         | Liver     | No             | Erlotinib | alive | 2                |
| P158    | M      | 56  | p.L861Q         | Liver     | 30 PA          | -       | -      | -                |
| P160    | F      | 75  | p.L858R         | Bones     | No             | Erlotinib | alive | 2                |

Furthermore, 38 EGFR negative patients were screened for the V600E mutation in the B-raf gene by pyrosequencing. Only 5 patients (13.15%) carried the V600E B-raf mutation.

3.2. EGFR Mutations and Clinicopathological Features

EGFR mutation correlated significantly with gender ($p = 0.019$), non-smoking history ($p = 0.008$), and metastasis ($p = 0.044$) (Table 3). In our cohort, 44 patients are current or former smokers and 18 among them carried EGFR mutation. Figure 2a represent mutation distribution according to the number of smoked pack year (PA).

Table 3. EGFR mutations and clinicopathological feature of Tunisian NSCLC.

| Clinical Feature | Overall | Mutation (%) | Wt (%) | $p$-Value |
|-----------------|---------|--------------|--------|-----------|
| Gender          | M       | 65 (82.27)   | 24 (36.9) | 41 (63.1) | 0.019 |
|                 | F       | 14 (17.72)   | 10 (71.4) | 4 (28.6)  |
| Age             | <60     | 26 (32.91)   | 10 (38.5) | 16 (61.5) |
|                 | >60     | 33 (41.7)    | 16 (48.5) | 17 (51.5) | 0.307 |
|                 | Unknown | 20 (25.31)   | 8 (40)   | 12 (60)   |
| Smoking History | Smoker  | 45 (56.6)    | 15 (33.3) | 30 (66.7) | 0.008 |
|                 | Non-smoker | 24 (31.3)  | 16 (61.5) | 10 (38.5) |
|                 | Unknown  | 10 (12)      | 3 (30%)  | 7 (70%)   |
| Histological subtype | Adenocarcinoma | 51 (64.55) | 21 (41.2) | 30 (58.8) |
|                 | Other subtypes | 5 (6.32)   | 4 (80)   | 1 (20)    | 0.646 |
|                 | Unknown  | 23 (29.11)   | 9 (39.1) | 14 (60.9) |
| Metastasis      | Presence | 48 (60.75)   | 26 (54.2) | 22 (45.8) | 0.044 |
|                 | Absence  | 21 (26.58)   | 6 (28.6)  | 15 (71.4) |
|                 | Unknown  | 10 (12)      | 2 (20)   | 8 (80)    |
Further, we noticed that about 50% (10 among 22) of patients with distant metastasis harbored the p.L861Q or p.L861R in EGFR exon 21. All 3 patients with composite mutations had bone or hepatic distant metastasis, as presented in Figure 2b.

3.3. EGFR and BRAF Mutations and Therapy

Regarding anti-cancer treatment, only a few patients carrying EGFR mutations had received TKI-based therapy (Table 4). Indeed, out of the 12 EGFR positive patients, for whom we had access to therapy and follow-up data, only 6 were able to benefit from erlotinib targeted therapy, and most of them (5 patients) harbor the p.L861X, alone or associated to exon 19 deletion.

**Table 4.** Treatment response and survival of patients carrying EGFR mutations.

| Patient | EGFR Alteration | Therapy | Response to Therapy Protocol | Status | Survival (Months) |
|---------|-----------------|---------|------------------------------|--------|------------------|
| P3      | p.L861Q         | Chemotherapy | Good response to chemotherapy then metastasis | died | 37               |
| P27     | p.L861Q         | Chemotherapy + Erlotinib | Complete remission | alive | 40               |
| P34     | p.L861R         | Chemotherapy | Bad response to chemotherapy | died | 18               |
| P36     | p.E746_A750del/p.L861R | Erlotinib | 63% tumor regression then resumption of tumor progression | alive | 15               |
| P37     | p.G719S         | Chemotherapy | Bad response to chemotherapy | died | 35               |
| P42     | p.E746_A750del | Chemotherapy | Good response to chemotherapy | alive | 17               |
| P136    | p.E746_A750del | Surgery | No treatment | alive | 7                |
| P139    | p.G719C         | Chemotherapy | Good response to chemotherapy | alive | 18               |
| P140    | p.L861Q         | Chemotherapy + Erlotinib | Chemotherapy then 2 weeks of erlotinib, died of interstitial lung disease | died | 1                |
| P149    | p.L861R         | Erlotinib | Treated for 1 month | alive | 3                |
| P157    | p.L861Q         | Erlotinib | Treated for 2 months | alive | 2                |
| P160    | p.L858R         | Erlotinib | Treated for 1 month and 2 weeks | alive | 2                |

The first case is a 65-year-old woman (P27), initially diagnosed with a localized form of NSCLC and treated by conventional chemotherapy and radiotherapy. After developing metastasis, she tested positive for the p.L861Q mutation and had received a 1-year long erlotinib therapy. Now she is in complete remission with an OS of 40 months (Table 2).

On the other hand, the 50-year-old patient (P36) had NSCLC with bone metastasis and carried composite EGFR mutations, namely p.E746_A750del/p.L861R, had received 2 months of erlotinib therapy, during which he showed a 63% tumor regression. During the third month of treatment, he displayed a resumption of tumor progression, typical of the appearance of resistant mutations. Having been unable to find those mutations in the circulating tumor DNA, he is currently undergoing chemotherapy while waiting to be re-biopsied and re-tested for these mutations.

Patient P140, who also had NSCLC with bone metastasis, had been treated with several rounds of chemotherapy before being tested for EGFR mutations. After discovering the p.L861Q mutation, he was treated with erlotinib for only 2 weeks, after which he died of interstitial lung disease.

Patients P149, P157, and P160 are still alive and have been on erlotinib therapy for 1 month or 2, and we do not have enough hindsight to judge the effectiveness of erlotinib therapy in their case.

As for patients carrying the B-raf V600E mutation, follow-up and treatment data were available for one patient only. This patient, who is a 70-year-old man, had NSCLC with
hepatic, adrenal, and cerebral metastasis. He was treated with chemotherapy for 5 months before being tested for EGFR and B-raf mutations. Despite having the V600E mutation, he did not have the chance to benefit from targeted therapy, since he passed away 7 months after being initially diagnosed.

**Figure 2.** Distribution of EGFR mutation according to smoking status (a) and metastasis site (b).

### 4. Discussion

In NSCLC, the incidence of EGFR mutations varies considerably in different regions of the world. Several studies reported that the prevalence of EGFR mutations ranged from 11% to 50% [30,31]. A large meta-analysis conducted in 2016 including 456 studies showed significant heterogeneity in all analyzed variables related to the prevalence of EGFR mutations in NSCLC patients [32]. In fact, ethnic backgrounds, patient characteristics, clinical settings, and methodology may contribute to these differences. This was confirmed by numerous reports from frequently assessed populations, showing a huge variability of EGFR mutation frequencies in patients with lung adenocarcinoma, ranging between 6 and 41% in Europe, 3 and 42% in North America, and 20 and 76% in Asia-Pacific [33].

Our studied population, which is predominantly from South Tunisia, had a percentage of 43% EGFR positivity. This rate is comparable to those observed in Asian countries and clearly higher than in Europe and North America. A meta-analysis including Middle Eastern and African studies reported a prevalence of 21.2% of EGFR mutations, varying from 44% in Turkey to 21% in Morocco and 2.1% in Saudi Arabia [34]. In addition, two recent studies from Morocco and Algeria revealed EGFR mutation frequencies of 21.9%, and 39.6%, respectively [35,36]. Analysis of the EGFR TKI sensitivity mutations in Tunisian NSCLC patients showed variable frequencies, depending on the number of analyzed samples, the patient’s selection criteria (clinical and pathological), and the used mutational analysis technique. In fact, while Mraihi et al. found 44% EGFR positive samples using IHC, Dhieb et al. reported only one sample with the E746-A750 del19 mutation, using the same technique [26,27]. Another study evaluating the molecular profile of 87 NSCLC
samples by qPCR or NGS found that 18.4% of patients had EGFR activating mutations (12 cases with the exon 19 deletions and 4 patients carrying the p.L858R) [28]. In addition, Arfaoui et al. showed that 3 out of the 26 analyzed samples harbor two sensitizing mutations (exon 19 deletion and p.G719X) and one exon 20 insertion associated with de novo resistance to targeted EGFR inhibitors and correlate with a poor patient prognosis [29]. Altogether, these findings support the heterogeneity in the prevalence of EGFR mutations among populations.

Interestingly and apart from the variability in the EGFR mutation frequencies, our results showed a particularity in the mutation profile of NSCLC patients. Actually, in-frame deletions of amino acids LREA of exon 19 and the p.L858R mutation are considered the “classic” mutations, accounting for 85% of EGFR mutations [11,12]. In the present study, we found a higher frequency of the p.L861X mutation with 35.3% and 14.7% of cases carrying the p.L861Q and the p.L861R, respectively. In a recent study, John et al. reported that the prevalence of uncommon EGFR mutations varied between 1.0% and 18.2% in Asia and South America [37]. According to this study, the most frequently reported uncommon mutations were G719X (0.9–4.8%), exon 20 insertions (0.8–4.2%), L861X (0.5–3.5%), and S768I (0.5–2.5%). Compared to our results, the p.L861X was more frequent in NSCLC Tunisian patients (50%) with the predominance of the p.L861Q mutation. Interestingly, among the p.L861X patients, 5 out of 7 received TKI-based therapy and showed a good response compared to those who were treated with only chemotherapy. In this context, Chiu et al. concluded that the p.L861Q is somewhat sensitive to TKIs but to a lesser extent than the “classic” mutations [38]. In addition, Liu et al. investigated the sensitivity to six first-in-class TKIs of the rare p.L861Q mutation by establishing two cell lines (EGFR p.L861Q variant and EGFR p.L861Q + exon 19 deletion variant) using the CRISPR-Cas9 gene-editing technology [39]. The authors showed that the EGFR p.L861Q + 19del variant and p.L861Q variant displayed significant sensitivity to TKIs tested particularly to gefitinib [39].

Moreover, a meta-analysis reported that afatinib had good clinical activity in NSCLC with uncommon p.L861Q mutation, with a response rate of 56.3%, a median progression-free survival of 8.2 months, and a median overall survival of 17.1 months [40].

Another recent case report showed the successful treatment of an 83-year-old patient with an uncommon L861Q epidermal growth factor receptor mutation. He was treated with low-dose afatinib, supporting the sensitivity of this mutation to TKI-based therapy [41].

Regarding the “classic” mutations such as deletion in exon 19 and p.L858R, we found that 23.5% of our patients carried deletion in exon 19, which is in line with data from previous studies including those conducted on Tunisian patients [26–36]. However, the p.L858R was identified in only four patients (11.7%), confirming once again the particularity of our cohort.

In addition, three patients harbored composite EGFR mutations, two cases carried an exon 19 deletion associated with p.L861R or p.S768I, and the third patient harbored the p.G719A with the p.L861Q. Composite EGFR mutations are double or multiple mutations of the EGFR tyrosine kinase domain, in which a sensitizing mutation is identified along with another one, usually of unclarified clinical significance [42]. Double mutations are detected in 14 to 18% of NSCLC samples, but their clinical significance remains not clearly characterized [43,44]. Kim et al. concluded that patients with composite EGFR mutations have poor clinical outcomes and should be closely monitored during follow-up [41]. In our study, the follow-up was available for only one patient among three carrying composite EGFR mutations. This 50-year male patient carrying the p.E746_A750del/p.L861R was treated with erlotinib and had a survival rate of 15 months, but he developed resistance to the TKI, suggesting the emergence of resistance mutation. Recently, Liu et al. reported a case of a female patient with lung adenocarcinoma carrying three mutations in EGFR exon 18: p.G724S, p.E709K, and p.V689I. The patient developed resistance to multiple EGFR-TKI and had a short overall survival time [45].

In addition to EGFR, the B-raf gene alterations are also associated with increased kinase activity leading to constitutive activation of the MAP kinase pathway [23]. B-raf
mutations have been reported in about 4% of NSCLC cases and are commonly associated with adenocarcinoma non-small cell lung cancer [46]. \( B\)-raf \( V600E \) mutation specifically occurs in about 1–2% of non-small cell lung cancer patients and most patients harboring this genetic alteration tend to have a smoking history [47]. Our findings are higher than the global data with a mutation frequency reaching 13.5%. In Tunisian patients, Mezni et al. reported only 2 cases harboring the \( V600E \) mutation in a cohort of 41 patients [28]. Two large meta-analyses concluded that there was a significant association between \( B\)-raf \( \) mutations and adenocarcinomas in NSCLC compared with non-ADKs and no significant difference was observed in smoking and stage in patients with \( B\)-raf \( \) mutations [48,49].

Finally, this study has the convenience of being the first Tunisian study enrolling 79 patients that are locally analyzed by pyrosequencing. However, it still has some limitations, such as the limited cohort size, the lack of patient follow-up, and the used therapy protocol. Further studies with larger samples and clinical data are required.

5. Conclusions

In conclusion, we found that the p.L858R usually defined as the "classic" \( EGFR \) mutation is rare in Tunisian patients in contrast with previous reports. Inversely, the p.L861Q is predominant and identified in 35% of cases. Interestingly, the p.L861X patients showed a good response to erlotinib compared to those who were treated with only chemotherapy. Those findings are of a great importance for clinicians to better manage Tunisian NSCLC patients. However, a study on a much bigger cohort is needed to validate these results.

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List of Abbreviations

| Abbreviation | Description                           |
|--------------|---------------------------------------|
| EGFR         | Epithelial Growth Factor Receptor     |
| TKI          | Tyrosine Kinase Inhibitors            |
| NSCLC        | Non-Small-Cell Lung Carcinoma         |
| FFPE         | Formalin-Fixed Paraffin-Embedded      |
| ErbB2        | Erb-B2 Receptor Tyrosine Kinase 2     |
| MAPK         | Mitogen-Activated Protein Kinases     |
| WHO          | World Health Organization             |
| PA           | pack years                            |
| IHC          | Immunohistochemistry                  |
| Wt           | Wild type                             |
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