Original Article

Evaluation of Clinical and Genetic Determinants of Treatment OutCome In EGFR Mutation Positive Advanced Lung Adenocarcinoma

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Abstract

Background: The aim of this research was to evaluate clinical and low-cost genetic determinants of treatment outcome in *EGFR* mutation positive advanced lung adenocarcinoma patients.

Material and Methods: *EGFR* mutation testing and *EGFR* 181946C>T genotyping were performed in 101 advanced lung adenocarcinoma patients using qRT-PCR and PCR-RFLP, respectively. Progression-free survival was defined as the time from the start of TKI therapy to date of progression, and overall survival as the time from diagnosis to death from any cause. Pain level was evaluated using a Numerical Rating Scale and the Verbal Descriptor Scale. Statistical significance was considered for $P < .05$.

Results: Patients were treated with EGFR-TKIs for a period of 1–39months (median 9), with a median PFS of 12.0 months (10.4-13.6, CI 95%), and a median OS of 19.0 months (15.1-22.7, CI 95%). The presence of pain was significantly correlated with the existence of bone ($P < .001$) and adrenal glands metastases ($P = .029$). Genetic factors did not have a direct impact on pain management but had a significant effect on the response to TKIs leading to pain alleviation.

Conclusions: *EGFR* mutation subtype and the *EGFR* 181946 C>T SNP had a significant effect on the response to TKI inducing an indirect anti-dolorous effect.

Keywords

EGFR, lung cancer, pain, tyrosine kinase inhibitors

Introduction

Lung cancer (LC) prevention measures are not sufficiently effective even in economically developed countries. Specific clinical symptoms are absent in early stages of lung cancer and uptake of screening is inadequate even in countries which perform them, often not including individuals without smoking history.[1,2] Thus, around 75% of LC cases are diagnosed in advanced stages when the currative success rate is very low due to high tumor burden and the presence of cancer-related pain is higher.[3] As a temporary stop/slowing down of LC screening programs and diagnostic procedures worldwide

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during the COVID-19 pandemic has been reported,[4,5] concerns have been raised that lung cancer-related pain can origin from direct effects of the tumor, its regional or distant spread, as well as from anti-cancer treatment.[6] Pharmacotherapy is the standard approach for the treatment of cancer pain, but a combination of pharmacological and non-pharmacological measures should be applied when needed.[7-9] Bone metastases represent one of the most common sites of metastatic spread of LC and they are usually associated with shorter survival.[10] Radiation therapy (RT) remains the golden standard of palliative management of painful bone metastases and it is performed to relieve pain, prevent pathologic fractures, spinal cord compression as well as to maintain or improve patients' quality of life.[11] Optimal medical analgesic therapy is essential alongside RT.[12] An individual approach to each patient is necessary, as well as constant monitoring by a multidisciplinary team which includes a pain management specialist.

Serbia was among the highest ranked countries in the world in 2018 when age-standardized rate of lung cancer occurrence was taken into account,[13] mostly due to the combined effect of very low tobacco cost and the lack of a structured national lung cancer screening program. As a higher percentage of Serbian lung cancer patients are diagnosed in advanced stages, around 20–30% exhibit chest pain at diagnosis, and around 6–13% bone pain.[14] Recently, we and others have made investigator-initiated efforts to explore genetic risk factors for LC in Serbia, in an effort to decipher the mechanisms driving its carcinogenesis, reduce the national mortality rate, and lower the number of patients who are diagnosed with advance stage lung cancer.[15-18] Advancement in diagnosis and treatment have also been introduced, but the accessibility of new molecular techniques and drugs still needs improvement.[19,20] Although many discrepancies are present between various countries in availability of innovative drugs, *EGFR* mutation testing is regularly applied as companion diagnostics for tyrosine kinase inhibitors (TKIs) in advanced stages of lung cancer.[21] In Serbia, *EGFR* mutation testing of lung cancer patients was initiated in 2011 from tumor tissue and in 2016 from liquid biopsy.[19,22] Patients with confirmed *EGFR* mutations are treated with first- and second-generation EGFR-TKIs until progression, at which point they are tested again for the presence of a resistant *EGFR* T790 M mutation which renders them candidates for third generation EGFR-TKIs.[23] All symptoms of the disease, including the pain level, as well as adverse effects and patient performance status are evaluated at diagnosis and closely monitored and managed during this entire TKI treatment course, contributing to the overall patients' quality of life.

The aim of this research was to detect the possible clinical and low-cost genetic determinants of treatment outcome in *EGFR* mutation positive advanced lung adenocarcinoma patients treated at our Institute, in an effort to provide data from the Balkan area, which is usually underrepresented in larger meta-analyses.

Methods

Patient Samples

This retrospective study included 101 patients, with inclusion criteria of a minimum age of 18years and clinically and histologically confirmed primary advanced lung adenocarcinoma according to the current WHO classification (stage IIIB/IV, ECOG performance status 0, 1, or 2).[24] According to data that LC risk slowly diminishes and is reduced to 50% after 30years of cessation of active smoking[14] the subjects were divided into 2 groups, non-smoker, that is, never smoker or ex-smoker ≥30years, and smoker, that is, current smoker or ex-smoker <30years, and further into ex-smoker and smoker <15 and ≥15years. The procedures used in this study were approved by the Ethics Committee of the Institute for Oncology and Radiology of Serbia (No. 5665-01) and were in accordance with the Helsinki Declaration of 1975, as revised in 2000. All patients had a minimum age of 18years and signed an informed consent.

Pain Level Assesment and Management

Pain level was evaluated using 2 approaches—a Numerical Rating Scale (NRS) and the Verbal Descriptor Scale (VDS), at baseline and continuously during TKI treatment. For the purpose of this study, a second time-point (after baseline) of official recording of pain level was the time of the first computed tomography (CT) evaluation of the efficacy of TKI treatment, which was after 3months of treatment initiation.[25] Numeric rating scale involved patients' self-reported pain rating from 0 to 10, with 0 representing no pain and 10 representing the worst pain. Mild category of pain had NRS values from 1 to 3, moderate pain had NRS values from 4 to 6, and severe pain had NRS values from 7 to 10. In patients who had difficulty responding to NRS, the VDS was used that included several descriptive phrases that referred to different levels of pain severity or intensity (e.g., "no pain," "mild pain," "moderate pain," "severe pain," and "the worst pain imaginable"). Pain management was achieved using acetaminophens, non-steroidal anti-inflammatory, and weak and strong opioids[7] (Supplementary Table 1). Single 8-Gy dose radiation therapy and 2 long-course regimens including 20 Gy in 5 fractions and 30 Gy in 10 fractions were used in patients with painful bone metastases as previously described.[26,27]

EGFR Mutation Testing and TKI Treatment

Formalin-fixed and paraffin-embedded (FFPE) tumor samples were obtained by biopsy/resection and used for routine *EGFR* (LRG_304, NM_005228.5) mutation testing using the Cobas® *EGFR* Mutation Test v2 on Cobas® 4800 (Roche Diagnostics). Patients with sensitizing *EGFR* mutations were treated with *EGFR* TKIs in the first line until progression or unacceptable toxicity. Progression-free survival (PFS) was

defined as the time from the start of TKI therapy to date of progression, and overall survival (OS) as the time from diagnosis to death from any cause. Primary resistance to TKIs was defined as the absence of any response, that is, progression of disease within the first 3months of therapy.

EGFR 181946C>T Genotyping

EGFR 181946C>T (rs2293347, *NM_005228.5(EGFR): c.2982 C>T* polymorphism was analyzed from FFPE derived DNA using a standard PCR-RFLP approach.[28] A previously sequenced heterozygote sample was used as a control to assure adequate genotyping.

Statistical Analysis

Descriptive methods of statistical analysis (frequencies, percentage, mean, median, and standard deviation/SD/and range) were used to summarize the sample data. The associations between the patients' and tumor characteristics were analyzed using Pearson Chi-Square, Fisher's exact, t-test, and Mann-Whitney tests and the analyses were performed IBM SPSS Statistics 22 (SPSS Inc, Chicago, IL, USA). Survival analysis was performed using a standard Kaplan–Meier product limit method for graphical presentation; median with corresponding 95% confidence interval (95%CI) was used for description and the Log-rank test for the analysis of difference. Descriptive analyses included genotype and allelic frequencies, and their distribution between groups was tested by Fisher's exact test; logistic regression was used to calculate the odds ratio (OR) and the 95% confidence interval (CI) to estimate the strength of associations. Two-sided P values <.05 were considered to indicate statistical significance.

Results

In the period from 2011 to 2020, 5650 *EGFR* mutation analyses were performed, and 10% of *EGFR* mutated samples were detected,[18] which is in good accordance with literature data for the Caucasian population.[29]

Patients were treated with first-line EGFR-TKIs for a period of 1–39months (median 9months). Median PFS in the whole group was 12.0months (10.4-13.6, CI 95%), with 33% patients still alive at the time of data analysis. Patients who presented with pain at diagnosis did not have a significantly lower PFS compared to those without pain (11.0 (6.8-15.2 95%CI) vs 11.0 (6.3-15.7 95%CI), P = .153) (Figure 1A). Median OS in the whole group was 19.0 months (15.1-22.7, CI 95%). Median OS for patients who presented with pain at diagnosis was 19.0 months (16.7-21.3, 95%CI) and 17.0months (9.2-24.8, 95%CI) for patients without pain, and the difference was not statistically significant (P = .906) (Figure 1B). Primary resistance to TKIs was detected in 15% of patients, and it was not correlated with the presence of pain at diagnosis (P =.904). Smoking status had no significant effect on the occurrence of primary resistance to TKIs (<30 vs ≥30years, P = .752; <15 vs ≥15years, P = .173). AEs were detected in 72% of TKI-treated patients with 97% of them presenting toxicity of a maximum grade 2. The presence of pain at diagnosis was not correlated with higher probability of AEs (P = .557). Smoking status had no significant effect on the occurrence of AEs (<30 vs ≥30years, P = .918; <15 vs ≥15years, P = .240).

At diagnosis, pain was detected in 41 (41%) patients, and it was significantly correlated with the presence of metastases (P < .001) (Table 1). Upon analysis of the presence of various metastatic sites, it was found that pain was significantly more common in patients with bone and adrenal gland metastases (Table 2). *EGFR* mutation type was not associated with the

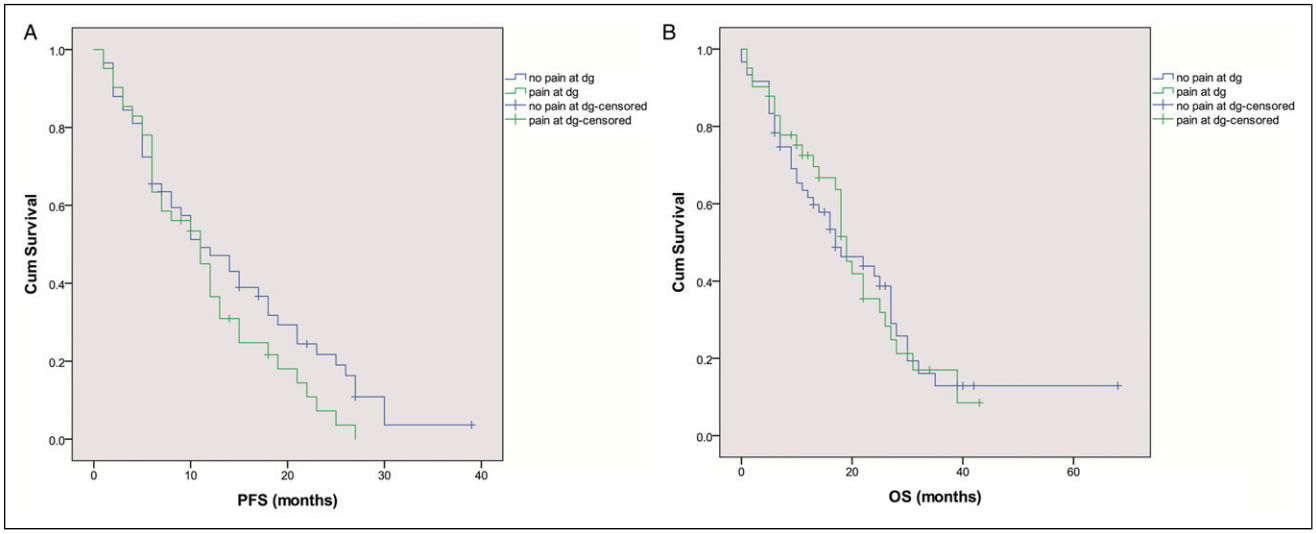

Figure 1. (a) Progression free survival (PFS) and (b) overal survival (OS) curves of patients with and without pain at diagnosis.

Table 1. The Presence of Pain in *EGFR* Mutated Patients at Diagnosis.

Characteristics at Diagnosis		No Pain N (%)	With Pain N (%)	*P* value
Age (mean)		63	60	.109
Gender	Male	18 (30)	17 (41.5)	.234
	Female	42 (70)	24 (58.5)	
Performance status (PS)	ECOG 0	5 (8.3)	0 (0)	.080
	ECOG 1	48 (80)	33 (80.5)	
	ECOG 2	4 (6.7)	5 (12.2)	
	ECOG 3	2 (3.3)	3 (7.3)	
	ECOG 4	1 (1.7)	0 (0)	
Smoking status	Non-smoker	30 (53.6)	15 (39.5)	.179
	Smoker	26 (46.4)	23 (60.5)	
TNM staging	IIIB	8 (13.3)	2 (4.9)	.195
	IV	52 (86.7)	39 (95.1)	
Presence of metastases	Without mets	8 (13.3)	2 (4.9)	**<.001**
	M1a	29 (48.3)	9 (22.0)	
	M1b	14 (23.3)	14 (34.1)	
	M1c	9 (15)	16 (39)	

M1a - tumor in contralateral lung or pleural/pericardial nodule/malignant effusion. M1b – single extrathoracic metastasis including single non-regional lymph node, M1c – multiple extrathoracic metastases in one or more organs. Statistically significant values are presented in bold.

Table 2. Localisation of metastases associated with the presence of pain at baseline.

Metastatic Site		No Pain N (%)	With Pain N (%)	*P* value
Liver	No liver mets	46 (76.7)	35 (85.4)	.281
	Liver mets	14 (23.3)	6 (14.6)	
Bone	No bone mets	55 (91.7)	19 (46.3)	**<.001**
	Bone mets	5 (8.3)	22 (53.7)	
Non-regional lymph nodes (ln)	No ln mets	58 (96.7)	38 (92.7)	.393
	Ln mets	2 (3.3)	3 (7.3)	
Pericard	No pericard mets	53 (88.3)	36 (87.8)	1.000
	Pericard mets	7 (11.7)	5 (12.2)	
Brain	No brain mets	53 (88.3)	38 (92.7)	.736
	Brain mets	7 (11.7)	3 (7.3)	
Lung	No lung mets	34 (56.7)	24 (58.5)	.852
	Lung mets	26 (43.3)	17 (41.5)	
Adrenal gland	No adr.gl.mets	58 (96.7)	34 (82.9)	**.029**
	adr.gl. Mets	2 (3.3)	7 (17.1)	
Malignant pleural effusion	No pleural eff	33 (55)	21 (51.2)	.708
	Pleural eff	27 (45)	20 (48.8)	

Statistically significant values are presented in bold.

presence of pain at baseline when most common *EGFR* mutations (exon 19 deletion, L858 R point mutation in exon 21) and rare mutations (L861Q, G719X, exon 20 insertion, S768I, double mutants) were compared ($P = .351$). The frequency of the *EGFR* 181946T allele was .25 in the whole group, that is, .23 in the group of patients that exhibited pain at diagnosis and .26 in the group without pain (Figure 2). The *EGFR* 181946 T allele was not associated with the presence of pain at baseline in both the dominant (CC vs CT/TT) [$P = .657$; OR (95%CI) = 1.31 (.58-2.98)], and the recessive models (CC/CT vs TT) [$P = .846$; OR (95%CI) = .87 (.21-3.47)].

Patients with pain at baseline were treated with analgesics (and radiation in 21 patients with bone metastases) (Supplemental Table 1 and 2) and good pain management was achieved. At the second time-point of official recording of pain level (3 months after the initiation of TKI treatment and the first CT evaluation of its efficacy), 20% of patients manifested pain which was statistically lower compared to baseline ($P = .011$), and there was a statistically significant improvement of the pain level ($P < .001$) (Table 3).

Of the 41 patients that presented with pain at diagnosis, 20 patients had a worsening of pain after having achieved good

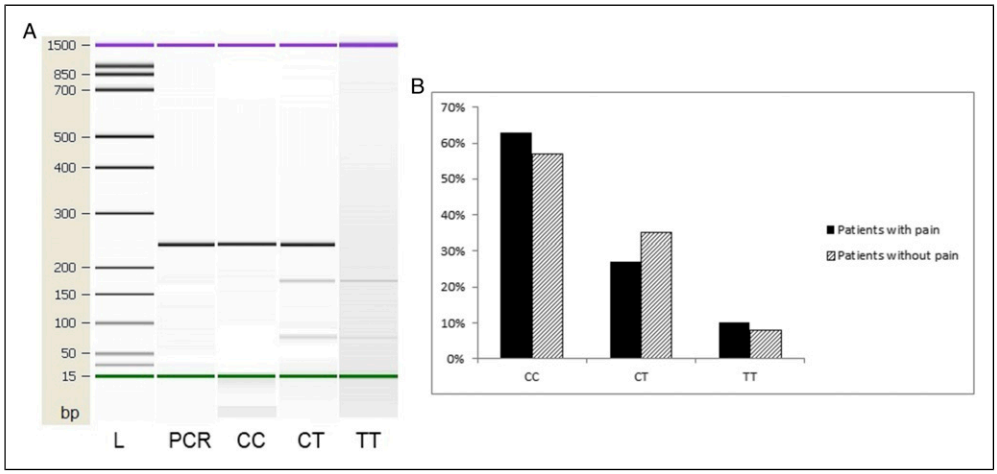

Figure 2. (a) PCR and RFLP results and (b) genotype distribution of *EGFR* 181946 CT polymorphic variants in patients with and without pain at diagnosis. Column 1: 244 bp PCR product. Column 2: CC (wild type), Column 3: CT (heterozygot, Column 4: TT (recessive homozygote). L – High-sensitivity DNA ladder (Agilent Technologies). 1500 bp upper and 15 bp lower marker are present in each column.

Table 3. Pain intensity according to the Numerical Rating Scale (NRS) and the Verbal Descriptor Scale (VDS).

Pain Intensity	Baseline (N, %)	After 3 months (N, %)
1–3 (NRS) or mild (VDS)	13 (31.7)	17 (65.4)
4–7 (NRS) or moderate (VDS)	22 (53.7)	7 (26.9)
8–10 (NRS) or severe (VDS)	6 (14.6)	2 (7.7)
Total	41	26

pain control initially, probably due to disease progression (14 cases of progression of the primary lesion in the first three months). Additional 6 patients who did not present with pain at diagnosis developed pain, again mostly due to disease progression despite TKI treatment. The remaining 21 patients achieved optimal pain control throughout the treatment course, although 17 of them eventually progressed (6 cases of a new lesion and 11 cases of progression of the primary lesion). There was no significant difference in mPFS of patients who had a worsening of pain after having achieved good pain control initially and those who achieved optimal pain control throughout the treatment course (P = .779).

EGFR mutation subtype (Figure 3A) and the type of the *EGFR* 181946 CT polymorphic variant (Figure 3B) did not have a significantly impact on pain management (P = .837), but had a significant effect on response to TKI treatment (Figure 3C). Patients with most frequent mutations (ex19del and L858 R) had a median PFS of 10 months, while patients with rare mutations (L861Q, G719X, exon 20 insertion, S768I, and double mutants) had a median PFS of 5months (P = .016). Similarly, the presence of the *EGFR* 181946 T allele influenced the response to TKIs, as carriers of the wild type CC genotype had a median PFS of 11months, CT carriers of 8 months, and teratesla carriers of 6months.

Discussion

Underlying genetic variations in the *EGFR* gene (mutations and small nucletide polymorphisms) are known to alter protein function and affect the biology of the tumor, inducing a different symptom presentation in patients and altering the therapeutic efficacy of EGFR-TKIs.[30-32] We analyzed the effect of the most common *EGFR* mutations in exons 18-21 and the polymorphism rs2293347 (181946G>A) on pain occurrence and combined analgesic/TKI treatment efficacy. *EGFR* mutation subtype and the type of the *EGFR* 181946 CT polymorphic variant did not have a direct impact on pain occurrence and management, but had a significant effect on response to TKI indirectly affecting patients' symptoms. Thus, the presence of specific *EGFR* genetic changes induces a different response to TKIs which ultimately led to a decrease in the disease burden and general pain alleviation during treatment.

Cancer-related pain is very common in lung cancer patients as majority of cases are diagnosed in advanced stages especially in countries like Serbia without appropriate lung screening programs and smoking cessation measures. As early palliative care and symptom management have proven effects on the improvement of the quality of life and longer survival they are an essential part of lung cancer patients' care.[33] Although other countries have performed multicenter prospective studies,[34] no detailed studies that evaluate the factors associated with pain in *EGFR* mutated advanced lung adenocarcinoma patients treated with EGFR-TKIs have been performed in our region so far.

Most commonly described causes of pain in patients with advanced LC are related to skeletal and adrenal metastases and pancoast tumors.[35] In our study, the presence of pain at diagnosis was significantly correlated with the presence of metastases, especially in the bones and adrenal glands, which

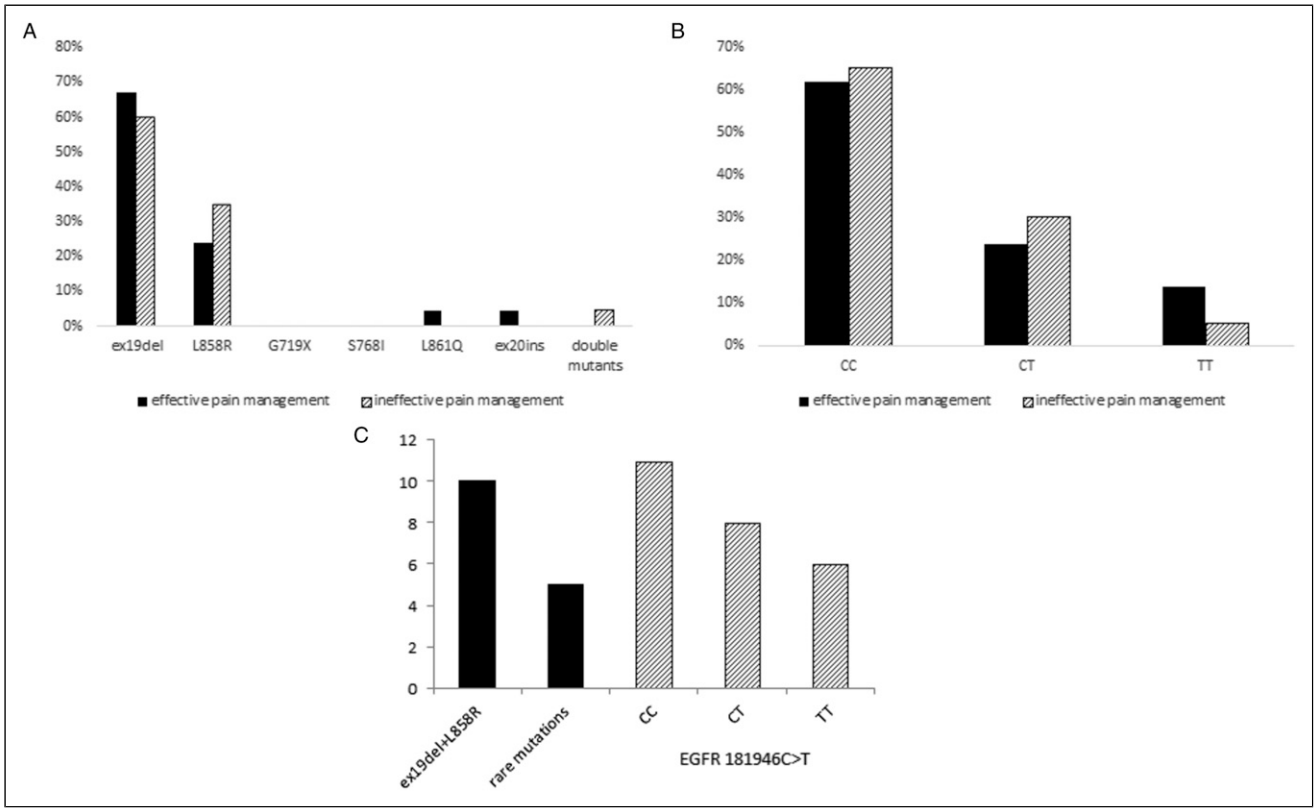

Figure 3. The effect of (a) *EGFR* mutation types and (b) *EGFR* 181946 CT polymorphic variants on pain management and (c) response to TKI treatment.

is in accordance with previously published data.[10,36] The World Health Organization (WHO) recommends use of Three-Step Analgesic Ladder model for adequate pain management in cancer patients.[37] Paracetamol is the mainstay of the first two steps of the WHO Analgesic Ladder in many countries as well as in our study.[38] Taking into account the well-known side effects of long-term use of NSAIDs they were not used regularly in our study except as a single intermittent therapy or in combination with paracetamol for mild pain relief. Since some authors suggested replacing weak opioids with low doses of oral morphine thereby eliminating step 2 of the Ladder, the cornerstone of analgesic therapy in our study was oral morphine.[39] Nevertheless, 10% of our patients had adequate pain relief control with tramadol as a mild opioid of step 2 in maximum used dose 200 mg per day. Oxycodone or hydromorphone, in both immediate-release and slow-release formulations for oral administration are effective alternatives to oral morphine.[40,41] In this study, oral methadone was used only in a small set of patients (2%) with severe pain resistant to previous pharmacotherapy. Transdermal fentanyl was used as an efficient option for patients with stable opioid requirements, as well as for patients that had problems with oral administration of drugs or patients. Adjuvant analgesics that were used in this study were pregabaline, gabapentin, and dexamethasone. Overall, good pain management was achieved, with a significant decrease of patients who still had pain during treatment, and with a statistically lower pain level. In patients who developed a new lesion or had progression of the primary tumor during TKI treatment, we also observed worsening of pain despite having previously achieved good pain control.

Radiotherapy has an important role in bone pain management, treatment of metastatic spinal cord compression and palliative treatment of pathological bone fractures that cannot be surgically stabilized.[11,42] Various palliative radiotherapy regimens are recommended for this indication, but an 8 Gy single dose should be considered as a regimen of choice for patients with painful bone metastases according to newest ESMO Clinical Practice Guideline for management of pain in adult cancer patients.[38] In this study, the most commonly used regimens for palliative antidolorous radiation therapy were short-course 8 Gy single fraction as well as two long-course regimens including 20 Gy in 5 fractions and 30 Gy in 10 fractions.[27] Although the presence of painful bone metastases is usually associated with shorter survival,[43] in our study no difference in PFS or OS was observed in patients who presented with bone metastases-related pain at diagnosis.

Conclusions: *EGFR* mutation subtype and the *EGFR* 181946 C>T SNP had a significant effect on the response to TKI inducing an indirect anti-dolorous effect and might be useful for accessible, low-cost prediction of treatment

outcome in advanced NSCLC, especially in low-income countries.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics Approval

The procedures used in this study were approved by the Ethics Committee of the Institute for Oncology and Radiology of Serbia and were in accordance with the Helsinki Declaration of 1964 and its later amendments or comparable ethical standards. All patients signed an informed consent.

Data Availability

Data The data that support the findings of this study are not publicly available due to ethics restrictions, and can be obtained from the corresponding author upon reasonable request.

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Supplemental Material

Supplemental material for this article is available online.

References

1. Kerpel-Fronius A, Tammemägi M, Cavic M, et al. Screening for lung cancer in individuals who never smoked: an international association for the study of lung cancer early detection and screening committee report. *J Thorac Oncol*. 2022;17(1):56-66. doi:10.1016/j.jtho.2021.07.031
2. Kerpel-Fronius A, Tammemägi MC, Tammemägi MC, et al. Lung cancer screening in persons who never smoked has to be evaluated—a response to letter to the editor. *J Thorac Oncol*. 2022;17(2):e20-e21. doi:10.1016/J.JTHO.2021.12.009
3. Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol Off J Eur Soc Med Oncol*. 2018;29(suppl 4):iv192-iv237. doi:10.1093/annonc/mdy275
4. Huber RM, Cavic M, Kerpel-Fronius A, et al. Lung cancer screening considerations during respiratory infection outbreaks, epidemics or pandemics: an international association for the study of lung cancer early detection and screening committee report. *J Thorac Oncol*. 2022;17(2):228-238. doi:10.1016/J.JTHO.2021.11.008
5. Cavic M, Krivokuca A, Boljevic I, et al. Exploring the real-world effect of the SARS-CoV-2 pandemic on the molecular diagnostics for cancer patients and high-risk individuals. *Expert Rev Mol Diagn*. 2021;21(1):101-107. doi:10.1080/14737159.2021.1860760
6. Simmons CPL, Macleod N, Laird BJA. Clinical management of pain in advanced lung cancer. *Clin Med Insights Oncol*. 2012;6:331-346. doi:10.4137/CMO.S8360
7. Swarm RA, Paice JA, Anghelescu DL, et al. Adult cancer pain, version 3.2019, nccn clinical practice guidelines in oncology. *J Natl Compr Cancer Netw*. 2019;17(8):977-1007. doi:10.6004/jnccn.2019.0038
8. Dans M, Smith T, Back A, et al. NCCN guidelines insights: palliative care, version 2.2017. *J Natl Compr Cancer Netw*. 2017;15(8):989-997. doi:10.6004/jnccn.2017.0132
9. Cavic M, Kovacevic T, Zaric B, et al. Lung cancer in Serbia. *J Thorac Oncol*. 2022;17:867-872. Published online 2022.
10. Kuchuk M, Kuchuk I, Sabri E, et al. The incidence and clinical impact of bone metastases in non-small cell lung cancer. *Lung Cancer*. 2015;89(2):197-202. doi:10.1016/j.lungcan.2015.04.007
11. De Felice F, Piccioli A, Musio D, Tombolini V. The role of radiation therapy in bone metastases management. *Oncotarget*. 2017;8(15):25691-25699. doi:10.18632/oncotarget.14823
12. Lutz S. The role of radiation therapy in controlling painful bone metastases. *Curr Pain Headache Rep*. 2012;16(4):300-306. doi:10.1007/s11916-012-0271-1
13. World Cancer Research Fund. Lung cancer statistics. 2018. https://www.wcrf.org/dietandcancer/cancer-trends/lung-cancer-statistics. https://www.wcrf.org/dietandcancer/cancer-trends/lung-cancer-statistics Accessed on March 3, 2018
14. Serbia M of H of the R of. *National Guidelines of Good Clinical Practice for the Diagnostics and Treatment of Lung Cancer 2012*; 2012. http://www.onk.ns.ac.rs/pdf/VodicZaDijagnostikovanjeILlecenjeKarcinomaPluca.pdf Accessed April 14, 2022.
15. Cavic M, Krivokuca A, Spasic J, et al. The influence of methylenetetrahydrofolate reductase and thymidylate synthetase gene polymorphisms on lung adenocarcinoma occurrence. *J BUON*. 2014;19(4):1024-1028. https://www.jbuon.com/archive/19-4-1024.pdf
16. Cavic M, Spasic J, Krivokuca A, et al. TP53 and DNA-repair gene polymorphisms genotyping as a low-cost lung adenocarcinoma screening tool. *J Clin Pathol*. 2019;72(1):75-80. doi:10.1136/jclinpath-2018-205553
17. Velinovic M, Jankovic R, Jovanovic D, et al. Tumor characteristics, expressions of ERCC1, Bax, p53, IGF1R, Bcl2, Bcl2/Bax and prognostic factors for overall survival in patients with lung carcinoid. *J BUON*. 2019;24(1):256-266.

18. Jokic V, Savic-Vujovic K, Spasic J, et al. Hematological parameters in EGFR-mutated advanced NSCLC patients treated with TKIs: predicting survival and toxicity. *Expert Rev Anticancer Ther*. 2021;21(6):673-679. doi:10.1080/14737140.2021.1893694
19. Cavic M, Krivokuca A, Boljevic I, et al. Pharmacogenetics in cancer therapy - 8 years of experience at the Institute for Oncology and Radiology of Serbia. *J BUON*. 2016;21(5):1287-1295.
20. Cavic M, Krivokuca A, Pavlovic M, et al. EGFR mutation testing from liquid biopsy of non-small cell lung cancer at the Institute for oncology and radiology of Serbia. *J BUON*. 2020;25(6):2635-2648. https://jbuon.com/archive/25-6-2635.pdf
21. Jankovic R, J Goncalves H, Cavic M, et al. LungCARD - Report on worldwide research and clinical practices related to lung cancer. *J BUON*. 2019;24(1):11-19.
22. Cavic M, Krivokuca A, Jankovic R, Radulovic S. Personalized therapy of malignant diseases. *Pirot Zb Arch*. 2015;40:69-79. doi:10.5937/pirotzbor1540069C
23. Jiang T, Su C, Ren S, et al. A consensus on the role of osimertinib in non-small cell lung cancer from the AME lung cancer collaborative group. *J Thorac Dis*. 2018;10(7):3909-3921. doi:10.21037/jtd.2018.07.61
24. Detterbeck FC, Boffa DJ, Kim AW, Tanoue LT. The eighth edition lung cancer stage classification. *Chest*. 2017;151(1):193-203. doi:10.1016/j.chest.2016.10.010
25. Karcioglu O, Topacoglu H, Dikme O, Dikme O. A systematic review of the pain scales in adults: which to use? *Am J Emerg Med*. 2018;36(4):707-714. doi:10.1016/j.ajem.2018.01.008
26. Howell DD, James JL, Hartsell WF, et al. Single-fraction radiotherapy versus multifraction radiotherapy for palliation of painful vertebral bone metastases-equivalent efficacy, less toxicity, more convenient: a subset analysis of Radiation Therapy Oncology Group trial 97-14. *Cancer*. 2013;119(4):888-896. doi:10.1002/cncr.27616
27. Chow R, Hoskin P, Hollenberg D, et al. Efficacy of single fraction conventional radiation therapy for painful uncomplicated bone metastases: a systematic review and meta-analysis. *Ann Palliat Med*. 2017;6(2):125-142. doi:10.21037/apm.2016.12.04
28. Choi JE, Park SH, Kim KM, et al. Polymorphisms in the epidermal growth factor receptor gene and the risk of primary lung cancer: a case-control study. *BMC Cancer*. 2007;7:199. doi:10.1186/1471-2407-7-199
29. Gahr S, Stoehr R, Geissinger E, et al. EGFR mutational status in a large series of Caucasian European NSCLC patients: data from daily practice. *Br J Cancer*. 2013;109(7):1821-1828. doi:10.1038/bjc.2013.511
30. Castellanos E, Feld E, Horn L. Driven by mutations: the predictive value of mutation subtype in EGFR-mutated non-small cell lung cancer. *J Thorac Oncol*. 2017;12(4):612-623. doi:10.1016/j.jtho.2016.12.014
31. Winther-Larsen A, Nissen PH, Jakobsen KR, et al. Genetic polymorphism in the epidermal growth factor receptor gene predicts outcome in advanced non-small cell lung cancer patients treated with erlotinib. *Lung Cancer*. 2015;90(2):314-320. doi:10.1016/j.lungcan.2015.09.003
32. Ma F, Sun T, Shi Y, et al. Polymorphisms of EGFR predict clinical outcome in advanced non-small-cell lung cancer patients treated with Gefitinib. *Lung Cancer*. 2009;66(1):114-119. doi:10.1016/j.lungcan.2008.12.025
33. Irwin KE, Greer JA, Khatib J, Temel JS, Pirl WF. Early palliative care and metastatic non-small cell lung cancer: potential mechanisms of prolonged survival. *Chron Respir Dis*. 2013;10(1):35-47. doi:10.1177/1479972312471549
34. Wei Y-F, Huang W-T, Liu T-C, et al. Factors associated with improvement in symptoms and quality of life for first-line EGFR-tyrosine kinase inhibitor treatment in patients with EGFR-mutated non-small-cell lung cancer - A multicenter prospective SMILE study. *J Cancer*. 2019;10(17):4151-4158. doi:10.7150/jca.30507
35. Marulli G, Battistella L, Mammana M, Calabrese F, Rea F. Superior sulcus tumors (Pancoast tumors). *Ann Transl Med*. 2016;4(12):239. doi:10.21037/atm.2016.06.16
36. Bazhenova L, Newton P, Mason J, et al. Adrenal metastases in lung cancer: clinical implications of a mathematical model. *J Thorac Oncol*. 2014;9(4):442-446. doi:10.1097/JTO.0000000000000133
37. World Health Organization. *Cancer Pain Relief with a Guide to Opioid Availability*. Second Edition; 1996. https://apps.who.int/medicinedocs/documents/s22085en/s22085en.pdf
38. Fallon M, Giusti R, Aielli F, et al. Management of cancer pain in adult patients: ESMO clinical practice guidelines. *Ann Oncol Off J Eur Soc Med Oncol*. 2018;29(suppl 4):iv166-iv191. doi:10.1093/annonc/mdy152
39. Bandieri E, Romero M, Ripamonti CI, et al. Randomized trial of low-dose morphine versus weak opioids in moderate cancer Pain. *J Clin Oncol*. 2016;34(5):436-442. doi:10.1200/JCO.2015.61.0733
40. Pigni A, Brunelli C, Caraceni A. The role of hydromorphone in cancer pain treatment: a systematic review. *Palliat Med*. 2011;25(5):471-477. doi:10.1177/0269216310387962
41. King SJ, Reid C, Forbes K, Hanks G. A systematic review of oxycodone in the management of cancer pain. *Palliat Med*. 2011;25(5):454-470. doi:10.1177/0269216311401948
42. Lutz S, Balboni T, Jones J, et al. Palliative radiation therapy for bone metastases: update of an ASTRO evidence-based guideline. *Pract Radiat Oncol*. 2017;7(1):4-12. doi:10.1016/j.prro.2016.08.001
43. D'Antonio C, Passaro A, Gori B, et al. Bone and brain metastasis in lung cancer: recent advances in therapeutic strategies. *Ther Adv Med Oncol*. 2014;6(3):101-114. doi:10.1177/1758834014521110