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Next Generation Sequencing Analysis and its Benefit for Targeted Therapy of Lung Adenocarcinoma

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Abstract. *Background/Aim: Targeted therapy has become increasingly important in treating lung adenocarcinoma, the most common subtype of lung cancer. Next-generation sequencing (NGS) enables precise identification of specific genetic alterations in individual tumor tissues, thereby guiding targeted therapy selection. This study aimed to analyze mutations present in adenocarcinoma tissues using NGS, assess the benefit of targeted therapy and evaluate the progress in availability of targeted therapies over last five years. Patients and Methods: The study included 237 lung adenocarcinoma patients treated between 2018-2020. The Archer FusionPlex CTL panel was used for NGS analysis. Results: Gene variants covered by the panel were detected in 57% patients and fusion genes in 5.9% patients. At the time of the study, 34 patients (14.3% of patients) were identified with a targetable variant. Twenty-five patients with EGFR variants, 8 patients with EML4-ALK fusion and one patient with CD74-ROS1 fusion received targeted therapy. Prognosis of patients at advanced stages with EGFR variants treated by tyrosine kinase inhibitors and patients with EML4-ALK fusion treated by alectinib was significantly favorable compared to patients without any targetable variant treated by chemotherapy (p=0.0172, p=0.0096, respectively). Based on treatment guidelines applicable in May 2023, the number of patients who could profit from targeted therapy would be 64 (27.0% of patients), this is an increase by 88% in comparison to recommendations valid in 2018-2020. Conclusion: As lung adenocarcinoma patients significantly benefit from targeted therapy, the assessment of mutational profiles using NGS could become a crucial approach in the routine management of oncological patients.*

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Key Words: NGS, lung adenocarcinoma, targeted therapy, prognosis.

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Lung cancer is a leading cause of cancer deaths worldwide (1). Based on histology, lung cancer is divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Adenocarcinoma is the most common subtype of NSCLC and represents about 40% of all lung cancers (2).

Additionally to surgery and radiotherapy, current treatment of lung cancer based on administration of pharmaceutical agents shows the increasing importance of targeted therapy and immunotherapy (3). The aforementioned goes hand in hand with the technically and financially available possibility of molecular characterization of tumor tissue and body fluids containing molecules originating from tumor cells, dominantly DNA and RNA (4).

A significant contribution in this direction is the use of targeted next-generation sequencing (NGS) enabling for feasible analysis of selected oncogene/tumor suppressor gene variants for the prediction of targeted treatment response.

Our study investigated a cohort of 237 patients treated in 2018-2020 at the University Hospital in Pilsen, Czech Republic, for lung adenocarcinoma, in whom the tumor tissue was analyzed by NGS panel detecting variants of 36 cancer related genes. The distribution of genetic alterations as well as the impact of targeted therapies available at that time on patient survival were assessed. Concurrently, we evaluated the percentage of patients who could potentially benefit from currently available treatments based on the 2022 guidelines.

Treatment of patients with lung adenocarcinoma enrolled to this study was based on recommendations valid in 2018-2020, which are set out in the Blue Book of the Czech Oncological Society (5). These clinical practice guidelines are based on European Society for Medical Oncology (ESMO) guidelines (6), the National Comprehensive Cancer Network (NCCN) guidelines (7) and include also updates based on recent clinical trials (8). For stages IIIb, IIIc and IV in patients with a proven activation mutation of *EGFR*, EGFR tyrosine kinase inhibitors (gefitinib, erlotinib, afatinib) were indicated. Alectinib was indicated in patients with anaplastic lymphoma kinase (ALK) fusion-positive tumors, crizotinib was administered in the treatment of ROS1 fusion-positive tumors (5).

Recently, newly approved TKIs have been incorporated into the treatment recommendations. This applies to new TKIs for mutations for which no treatment has been available until now, but also to new TKIs more effective for mutations whose oncoproteins could already be inhibited (9). The results of the clinical trials showed the benefit of TKI administration in the adjuvant treatment of low stages of lung cancer. This has been reflected in treatment recommendations (9). Osimertinib is indicated after complete resection of stage IB, II and IIIB tumors with presence of *EGFR* exon 19 deletion or exon 21 substitution mutation L858R (8). The decision to indicate targeted treatment or immuno(chemo)therapy is absolutely dependent on the histological typing of the tumor and the subsequent determination of predictive markers at the DNA, RNA and protein level. Predictive testing currently includes specific testing according to valid algorithms for evaluating the mutational status of the *EGFR* gene, chromosomal translocations affecting the *ALK* gene, *ROS1* or complex molecular testing using the NGS method (9).

Although the analysis of tumor tissue is irreplaceable, the benefit of liquid biopsy is now convincingly demonstrated. Therefore, it can be expected that in cases where tissue analysis is not feasible, a liquid biopsy approach could be used, especially circulating tumor DNA (ctDNA) analysis by sensitive methods like digital PCR (dPCR) or NGS (4). In case the ctDNA variant is detected in the blood stream, then the treatment schedule is definitely given. If no particular ctDNA variant is detected, the situation is more unclear. This may be the result of the variant either not being present or just not being detected. It can be expected that tissue-based and body-fluid (blood)-based approaches will be combined in the future, regarding their advantages and disadvantages.

Knowledge of the spectrum of the variants present in the tumor tissue, their frequency and influence on the prognosis is a prerequisite for the appropriate application of NGS with the aim to decide on targeted therapy. The benefit of detecting all potentially targetable variants using NGS is the increase of the number of patients who can profit from administration of targeted therapy. Evaluating this benefit is what this article deals with.

Table I. *Characteristics of patients included in the study.*

Variables	Number of patients	%
Sex		
Male	134	56.5
Female	103	43.5
Age		
<50	9	3.8
50-60	38	16.0
60-70	101	42.6
70-80	73	30.8
>80	16	6.8
Smoking status		
Nonsmoker	49	20.7
Ex-smoker	78	32.9
Smoker	110	46.4
Performance status (ECOG)		
0	11	4.6
1	166	70.1
2	34	14.3
3	26	11.0
Histology of NSCLC		
Adenocarcinoma	237	100.0
Stage		
Ia	32	13.5
Ib	13	5.5
IIa	7	3.0
IIb	9	3.8
IIIa	27	11.4
IIIb	6	2.5
IIIc	6	2.5
IVa	48	20.2
IVb	89	37.6
Surgery		
Yes	72	30.4
No	165	69.6
Radiotherapy		
Yes	28	11.8
No	209	88.2

ECOG: Eastern Cooperative Oncology Group - performance status scale; NSCLC: non-small cell lung cancer.

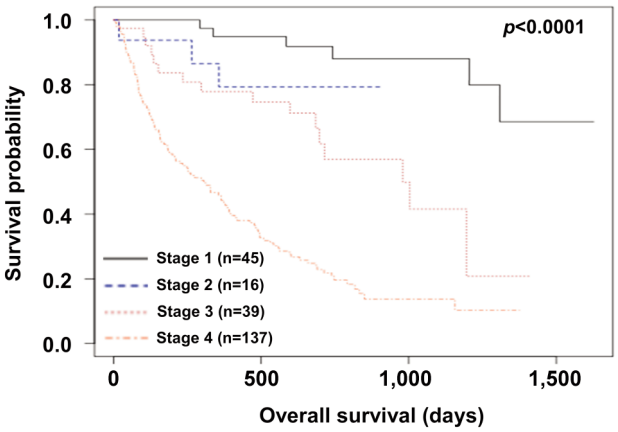

Figure 1. *Kaplan-Meier curves comparing overall survival of all lung adenocarcinoma patients included in the study according to the stage of disease. The treatment was based on the recommendations of the Czech Society for Oncology at the time of enrollment to the study (2018-2020).*

Patients and Methods

Patients. Inclusion of patients with adenocarcinoma histological subtype of non-small cell lung carcinoma (NSCLC) in this prospective study was performed in the period of 2018-2020. The study was approved by the Ethics Committee of the Faculty Hospital in Pilsen (No. 113/19). All patients provided their informed consent for inclusion in the study. The detailed characteristics of the group of 237 patients are provided in Table I. In all patients, the treatment management was based on the recommendations of the Czech Society for Oncology at the time of enrollment to the study (5). For stages IIIb, IIIc and IV in patients with a proven activation mutation of *EGFR*, EGFR tyrosine kinase inhibitors (gefitinib, erlotinib, afatinib) were indicated in the first line of treatment. Concomitant administration of chemotherapy with tyrosine kinase inhibitors (TKIs) targeting intracellular domain of epidermal growth factor receptor (EGFR) was not appropriate in most cases. Alectinib was indicated as monotherapy for the first-line treatment in patients with anaplastic lymphoma kinase (ALK) fusion-positive tumors. Crizotinib was administered in the first-line treatment of ROS1 fusion-positive tumors (5). Clinicopathological characteristics were obtained based on the authorized access to the Medicalc information system of the Faculty Hospital in Pilsen. Medium follow-up was 466 days. Overall survival (OS) was defined as the time from diagnosis to death caused by the disease. The relationship between patient stage of disease and OS is presented in Figure 1. The curves in the graph are consistent with the classification of patients into individual stages.

Analysis of gene variants and chromosomal rearrangements. Formalin-fixed, paraffin-embedded (FFPE) tissue samples verified by pathologist following macrodissection of tumor tissue were used for RNA isolation using the RNeasy FFPE Kit (Qiagen, Hilden, Germany). Subsequent NGS analysis was performed using the Archer FusionPlex Comprehensive Thyroid and Lung (CTL) panel (ArcherDX, Boulder, CO, USA) according to the manufacturer's protocol. The MiSeq instrument (Illumina, San Diego, CA, USA) was used for NGS analysis. Sequencing data were processed using the Archer Analysis 6 Software.

Table II. *Summary of gene changes detected by NGS in primary lung adenocarcinomas.*

	Number of patients	%
All patients	237	100.0
Gene variants	135	57.0
Fusion genes	14	5.9
No detected gene changes	88	37.1

NGS: Next-generation sequencing.

Statistical analysis. The statistical analysis was performed using SAS 9.4 software (SAS, Institute Inc., Cary, NC, USA). The Kaplan-Meier survival distribution functions for OS were generated based on the presence or absence of the evaluated DNA variant.

Results

Gene variants detected by NGS. It was found that about two thirds of lung adenocarcinoma tissue samples contained gene changes using the NGS panel Archer FusionPlex Comprehensive Thyroid and Lung (CTL). The overview of detected gene changes is shown in Table II. The most prevalent mutations detected were those in *KRAS* oncogene (32.5% of analyzed samples) followed by *EGFR* (14.8% of samples). All detected variants and fusion genes are presented in Table III.

Decision of targeted therapy based on detected gene variants. From targetable mutations, 34 patients with the variants in *EGFR* and *EML4-ALK* and *CD74-ROS1* fusions were treated by targeted therapy at the time of study. Patients with identified EGFR variants (exon 19 del, exon 21 L813R, exon 21 L858R, exon 21 L861Q, exon 20 T790M, exon 2-7 del) were treated by afatinib, gefitinib or erlotinib. Patients with EML4-ALK and CD74-ROS1 gene fusion were treated by alectinib and crizotinib, respectively. The details on treatment of patients with particular genetic alterations by TKIs are presented in Table IV.

Benefit of targeted therapy. The significant benefit of targeted therapy on OS for patients with *EGFR* variants treated by TKI compared to those treated by standard chemotherapy with no gene changes detected is shown in Figure 2. We also recorded favorable OS in patients with *EML4-ALK* fusion treated by TKI in comparison to those with no gene changes detected by NGS treated by standard chemotherapy (Figure 3).

Prognostic significance of KRAS G12C variant. From the spectrum of mutations of the *KRAS* oncogene, it is worth mentioning the most frequent variant G12C. The survival

Table III. *Detailed list of detected gene changes including distribution of variants in the study group.*

Gene	Variant/Gene fusion	N	% of all patients in the study (237)	% of patients with detected variant/gene fusion (149)
KRAS	G12C (29), G12V (15), G12D (12), G12A (10), G12S (3), Q61L (3), G13C (2), Q61H (2), G12F (1)	77	32.5	51.7
EGFR	exon 19 del (19), exon 21 L858R (5), exon 21 L813R (4), exon 21 L861Q (1), exon 20 dup (1), exon 20 T70M (1), exon 2-7 del (1), exon 18 del (1), exon 20 ins (1), exon 2 ins (1)	35	14.8	23.5
BRAF	V600E (4), L597R (1), K601E (1), G469S (1)	7	3	4.7
ERBB2	dup (3), V812I (1)	4	1.7	2.7
MET	exon 14 del (4)	4	1.7	2.7
CTNNB1	D32Y (1), S37C (1), S37F (1)	3	1.3	2.0
NRAS	Q61L (1), Q61K (1)	2	0.8	1.3
PIK3CA	H1047R (1)	1	0.4	0.7
RAF1	S257L (1)	1	0.4	0.7
DDR2	R714Q (1)	1	0.4	0.7
Gene fusion	*EML4-ALK*	10	4.2	6.7
	NR0B2-ALK	1	0.4	0.7
	KIF5B-RET	1	0.4	0.7
	CD74-ROS1	1	0.4	0.7
	CDC23-BRAF	1	0.4	0.7

KRAS: Kirsten rat sarcoma; *EGFR*: epidermal growth factor receptor; *BRAF*: V-Raf murine sarcoma viral oncogene homolog B; *ERBB2*: Erb-B2 receptor tyrosine kinase 2; *MET*: tyrosine-protein kinase Met; *CTNNB1*: catenin beta 1; *NRAS*: neuroblastoma RAS viral oncogene homolog; *PIK3CA*: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *RAF1*: V-Raf-1 murine leukemia viral oncogene homolog 1; *DDR2*: discoidin domain receptor tyrosine kinase 2; *ALK*: anaplastic lymphoma receptor tyrosine kinase; *RET*: rearranged during transfection proto-oncogene; ROS1: C-Ros oncogene 1.

Table IV. *Patients treated with targeted therapy at the time of the study (2018-2020).*

Gene	Variant/Gene fusion	Treatment (inhibitor)	N	% of all patients in the study (237)	% of patients with detected variant/gene fusion (149)
EGFR	exon 19 del	Afatinib	7	3.0	4.7
		Gefitinib	7	3.0	4.7
		Erlotinib	1	0.4	0.7
	exon 21 L813R	Afatinib	4	1.7	2.7
	exon 21 L858R	Afatinib	1	0.4	0.7
		Gefitinib	2	0.8	1.3
	exon 21 L861Q	Gefitinib	1	0.4	0.7
	exon 20 T790M	Afatinib	1	0.4	0.7
	exon 2-7 del	Erlotinib	1	0.4	0.7
Gene fusion	*EML4-ALK*	Alectinib	8	3.4	5.4
	CD74-ROS1	Crizotinib	1	0.4	0.7
Total			34	14.3	23.0

EGFR: Epidermal growth factor receptor; *EML4*: EMAP Like 4; *ALK*: anaplastic lymphoma receptor tyrosine kinase; *ROS1*: C-Ros oncogene 1.

graph of patients without targeted therapy with *KRAS* gene variants shows a worse prognosis for patients with the G12C mutation compared with other detected KRAS variants of codon 12 (Figure 4).

Advancements in targeted therapy options. Looking at the spectrum of detected variants from the point of view of currently available treatment (9), the number of patients who could profit from targeted therapy increases approximately two times (Table V). Table V includes patients with detected variants of advanced stages. The substantial progress is the introduction of sotorasib in targeted therapy of patients with detected *KRAS* G12C variant. This is important due to very high prevalence of this variant in lung adenocarcinoma.

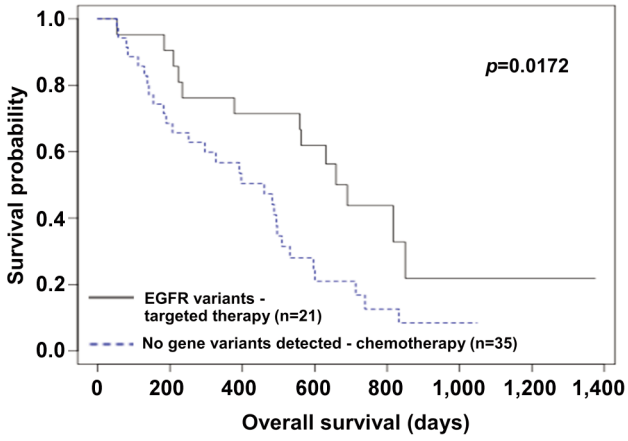

Figure 2. *Patients with advanced stages (stage 3c, 4a, 4b) of lung adenocarcinoma. Kaplan-Meier curves comparing overall survival of patients with EGFR variants treated by TKI (n=21) versus those with no gene changes, detected by NGS, treated by standard chemotherapy (n=35). EGFR: Epidermal growth factor receptor.*

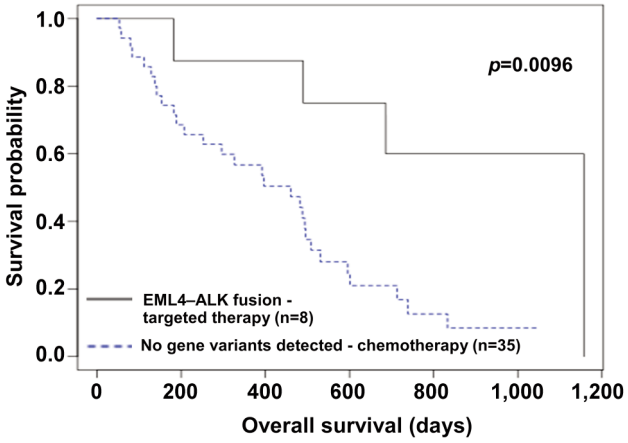

Figure 3. *Patients with advanced stages (stage 3c, 4a, 4b) of lung adenocarcinoma. Kaplan-Meier curves comparing overall survival of patients with EML4-ALK fusion treated TKI (n=8) versus those with no gene changes detected by NGS treated by standard chemotherapy (n=35). EML4: EMAP Like 4; ALK: anaplastic lymphoma receptor tyrosine kinase.*

Discussion

The future of oncology therapeutics for lung cancer will most likely use two main directions. The first direction is the blockade of aberrantly activated pathways by next-generation drugs having specific target effects, and the second is immunotherapy including personalized treatment tailored to specific patients, *e.g.*, adoptive cell therapy (ACT) (3, 10). However, it is known that several tumors with identified known driver genes (except *KRAS*) respond poorly to immunotherapy (11). Therefore, targeted therapy and immunotherapy complement each other appropriately in the treatment of oncological patients.

Apart from the extent of disease (clinical stage), a key necessity for the most effective treatment of patients is the detailed knowledge of the molecular changes from which the tumor phenotype arises. It will allow precise application of targeted therapy. The above mentioned is a result of two achievements of the last ten years which are currently clashing together. Firstly, affordability and ease of implementation of sequencing (12) and secondly, results of applied research in the direction of blocking signaling pathways involved in cancer pathogenesis (13, 14). We must not forget results of the basic research on immune processes, which are applied in the form of immunotherapy (15). Routine introduction of NGS methods allowing complex analysis of the genetic changes in tumor tissue, feasible both from the point of view of detection of all potentially expected mutations, and from the economic point of view, is a prerequisite for this approach (16).

NGS analysis of tumor tissue has become irreplaceable for appropriate use of the most modern treatment options. The

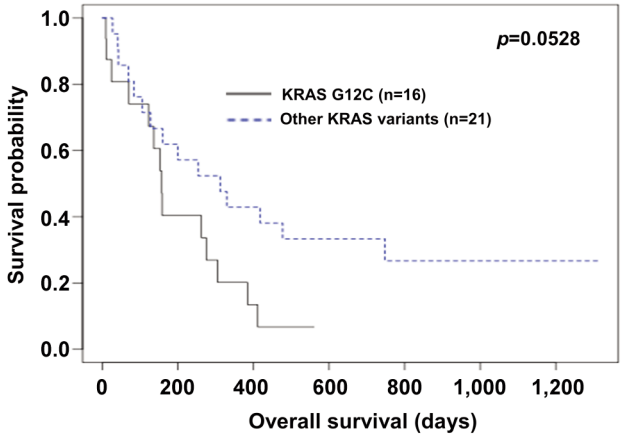

Figure 4. *Patients with advanced stages (stage 3c, 4a, 4b) of lung adenocarcinoma. Kaplan-Meier curves comparing overall survival of patients with KRAS G12C variant (n=16) versus those with other detected KRAS variants of codon 12 (G12A, G12D, G12F, G12S, G12V) (n=18). KRAS: Kirsten rat sarcoma.*

question is whether the aforementioned approach is acceptable from the economic point of view for repeated analyses of body fluids (liquid biopsy). The application of the method of detection of individual variants by the dPCR or real-time PCR methods seems to be also suitable for the detection of ctDNA in body fluids. For liquid biopsy, approach of detection particular variants linked to available targeted therapy could be based on the knowledge of previous results of NGS tissue analysis. In the case when

Table V. *Patients in the study who could profit from targeted therapy according to the current guidelines (2023).*

Gene	Variant/Gene fusion	Treatment (inhibitor)	N	% of all patients in the study (237)	% of patients with detected variant/gene fusion (149)
KRAS	G12C	Sotorasib	22	9.3	14.8
EGFR	exon 19 del	Osimertinib	15	6.3	10.1
	exon 18 del	Afatinib	1	0.4	0.7
	exon 20 ins	Amivantamab	1	0.4	0.7
	exon 21 L858R	Osimertinib	4	1.7	2.7
	exon 21 L813R	Afatinib	4	1.7	2.7
	exon 21 L861Q	Gefitinib	1	0.4	0.7
	exon 20 T790M	Osimertinib	1	0.4	0.7
BRAF	V600E	Dabrafenib+Trametinib	3	1.3	2.0
MET	exon 14 del	Capmatinib/Tepotinib	3	1.3	2.0
Gene fusion	*EML4-ALK*	Alectinib/Brigatinib/Lorlatinib	8	3.4	5.4
	CD74-ROS1	Entrectinib	1	0.4	0.7
Total			64	27.0	43.2

KRAS: Kirsten rat sarcoma; *EGFR*: epidermal growth factor receptor; *BRAF*: V-Raf murine sarcoma viral oncogene homolog B; *MET*: tyrosine-protein kinase Met; *EML4*: EMAP Like 4; *ALK*: anaplastic lymphoma receptor tyrosine kinase; *ROS1*: C-Ros oncogene 1.

tissue is not available, the most frequent variant for prediction of target therapy could be tested (17).

In the group of lung adenocarcinoma patients, in the tumor tissue samples we have detected gene variants covered by the Archer FusionPlex CTL (ACTL) panel in 57% patients and fusion genes in 6.2% patients. When comparing our results with those of other studies, it must be taken into account the fact that the same sets of analyzed genes are not always used. A typical example can be the inclusion or non-inclusion of the TP53 gene in such a panel. It should be mentioned that although it is a very frequently mutated tumor suppressor in NSCLC, it does not belong to the category of clinically targetable genes. In the study by Claerhout *et al*., among 106 cases in the diagnostic cohort of NSCLC patients, in FFPE tissue it was detected by NGS 17 *KRAS* variants (*i.e*., 16% of patients), 10 *EGFR* variants (9.4% of patients) and 6 *BRAF* variants (5.7% of patients) (18). Another study (Kim *et al*.) including the 391 patients with lung adenocarcinoma, identified mutated *EGFR* gene in 130 patients (33.2%), *KRAS* in 48 patients (12.3%), and *BRAF* in 2% patients (19). Comparison of studies and obtaining average values of the distribution of individual mutations can be complicated in such studies by the varying degree of preselection of patients, but in principle it is possible to say that the *EGFR*, *KRAS* (G12C), and *BRAF* genes are most often mutated targetable ones.

At the time of treatment of these patients (2018-2020), it was possible to administer targeted therapy to 34 patients (14.3% of all patients) according to the then valid recommendations for treatment (5). Evaluating the benefit of targeted treatment on patient survival, survival analysis Kaplan-Meier graph clearly showed significant benefit of targeted therapy by TKIs for patients with *EGFR* variants treated by TKI in comparison with those treated by standard chemotherapy with no gene changes detected by NGS (Figure 2). Similarly, there is a benefit of targeted therapy by TKIs for patients with *EML4-ALK* fusion compared to those with no gene changes detected by NGS treated by standard chemotherapy (Figure 3).

The possibility of drug intervention in case of identification of mutation of oncogenes in lung cancer tissue has been progressively increasing in recent years. With this, an improvement in prognosis can be expected. In the presence (2023), according to current recommendations for treatment (9), the number of patients from our study group who could on basis of mutational profile profit from targeted therapy would be 64 (27.0% of all patients), this is approximately increase by 88% in five years.

Over the past decades, there has been an ongoing effort by biomedical research to identify inhibitors targeting variants of the *KRAS* oncogene (20, 21). The survival graph of patients without targeted therapy with *KRAS* gene variants shows a worse prognosis for patients with the G12C mutation (Figure 4). Targeted TKI treatment is currently available for patients with this particular mutation. Inhibition of aberrantly activated pathways due to G12C codon mutation is available *via* the low-molecular-weight inhibitor sotorasib. In our group of patients, this drug would be indicated to 22 patients (28.6% of patients with any *KRAS* mutation). As already stated, it can be assumed that the prognosis of these patients would be more favorable at present.

A similar situation can be expected for other cancer-related genes. Therefore, methods capable of sequencing a large number of oncogenes as well as tumor suppressor genes expand the number of lung cancer patients who can be directed to targeted treatment instead of standard chemotherapy. In

addition, data produced by such methods also open the field for the development of new drugs inhibiting relevant oncogenes or reactivating damaged tumor suppressors.

Conclusion

NGS analysis provides precise information about the specific mutations present in an individual's tumor tissue. Using NGS upfront is one of the best ways to identify in a timely and economically feasible manner patients who can profit from targeted therapy. The most commonly observed genetic alterations in lung adenocarcinoma are those in KRAS and EGFR. Our results showed that therapy targeting EGFR variants and EML4-ALK fusion contributes to prolonging the lives of patients with advanced stages of lung adenocarcinoma. Due to the availability of NGS analysis and progress in therapies targeting other genetic alterations over the last five years, targeted therapy is available for approximately two times more patients. In conclusion, with ongoing advancements in targeted therapies and the expanding repertoire of actionable genetic alterations, the future holds immense potential for further improving patient outcomes through NGS-guided precision oncology.

Data Availability

The data underlying the findings of the study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The Authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Study conception and design: MP, MS, and JP; patient enrollment and samples characterization: MS, MB, and PM; laboratory analysis: TV, KH, SBM, TK, JW, and BV; data analysis and interpretation: VK, VB, JP, and MP; drafting the manuscript: VK, MP, and JP; editing and revising the manuscript: all Authors.

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References

1 Siegel R, Miller K, Fuchs H, Jemal A: Cancer statistics, 2022. CA: CA Cancer J Clin 72(1): 7-33, 2022. DOI: 10.3322/caac.21708

2 Zappa C, Mousa S: Non-small cell lung cancer: current treatment and future advances. Transl Lung Cancer Res 5(3): 288-300, 2016. DOI: 10.21037/tlcr.2016.06.07

3 Hirsch F, Scagliotti G, Mulshine J, Kwon R, Curran W, Wu Y, Paz-Ares L: Lung cancer: current therapies and new targeted treatments. Lancet 389(10066): 299-311, 2017. DOI: 10.1016/S0140-6736(16)30958-8

4 Pesta M, Shetti D, Kulda V, Knizkova T, Houfkova K, Sharif Bagheri M, Svaton M, Polivka J: Applications of liquid biopsies in non-small-cell lung cancer. Diagnostics 12(8): 1799, 2022. DOI: 10.3390/diagnostics12081799

5 Planchard D, Popat S, Kerr K, Novello S, Smit E, Faivre-Finn C, Mok T, Reck M, Van Schil P, Hellmann M, Peters S: Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 29: iv192-iv237, 2018. DOI: 10.1093/annonc/mdy275

6 Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman J, Chirieac LR, D'Amico TA, DeCamp MM, Dilling TJ, Dobelbower M, Doebele RC, Govindan R, Gubens MA, Hennon M, Horn L, Komaki R, Lackner RP, Lanuti M, Leal TA, Leisch LJ, Lilenbaum R, Lin J, Loo BW, Martins R, Otterson GA, Reckamp K, Riely GJ, Schild SE, Shapiro TA, Stevenson J, Swanson SJ, Tauer K, Yang SC, Gregory K, Hughes M: Non-small cell lung cancer, version 5.2017, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw 15: 504–535, 2017. DOI: 10.6004/jnccn.2017.0050

7 Herbst R, Tsuboi M, John T, Grohé C, Majem M, Goldman J, Kim S, Marmol D, Rukazenkov Y, Wu Y: Osimertinib as adjuvant therapy in patients (pts) with stage IB-IIIA EGFR mutation positive (EGFRm) NSCLC after complete tumor resection: ADAURA. J Clin Oncol 38(18_suppl): LBA5-LBA5, 2020. DOI: 10.1200/JCO.2020.38.18_suppl.LBA5

8 Vyzula R: Modrá kniha České onkologické společnosti. Brno, Masaryk Memorial Cancer Institute, 2018.

9 Kiss I: Modrá kniha České onkologické společnosti. Brno, Masaryk Memorial Cancer Institute, 2022.

10 Rosenberg S, Restifo N: Adoptive cell transfer as personalized immunotherapy for human cancer. Science 348(6230): 62-68, 2015. DOI: 10.1126/science.aaa4967

11 Vokes N, Pan K, Le X: Efficacy of immunotherapy in oncogene-driven non-small-cell lung cancer. Ther Adv Med Oncol 15: 175883592311614, 2023. DOI: 10.1177/17588359231161409

12 Zheng Y, Vioix H, Liu F, Singh B, Sharma S, Sharda D: Diagnostic and economic value of biomarker testing for targetable mutations in non-small-cell lung cancer: a literature review. Future Oncol 18(4): 505-518, 2022. DOI: 10.2217/fon-2021-1040

13 Xiao Y, Liu P, Wei J, Zhang X, Guo J, Lin Y: Recent progress in targeted therapy for non-small cell lung cancer. Front Pharmacol 14: 1125547, 2023. DOI: 10.3389/fphar.2023.1125547

14 Yun J, Nakagawa R, Tham K: KRAS-targeted therapy in the treatment of non-small cell lung cancer. J Oncol Pharm Pract 29(2): 422-430, 2023. DOI: 10.1177/10781552221118848

15 Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N: Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci 99(19): 12293-12297, 2002. DOI: 10.1073/pnas.192461099

16 Willard N, Sholl L, Aisner D: Panel sequencing for targeted therapy selection in solid tumors. Clin Lab Med 42(3): 309-323, 2022. DOI: 10.1016/j.cll.2022.04.004

17 Pellini B, Szymanski J, Chin R, Jones P, Chaudhuri A: Liquid biopsies using circulating tumor DNA in non-small cell lung cancer. Thorac Surg Clin 30(2): 165-177, 2020. DOI: 10.1016/j.thorsurg.2020.01.005

18 Claerhout S, Lehnert S, Vander Borght S, Spans L, Dooms C, Wauters E, Vansteenkiste J, Weynand B, Deraedt K, Bourgain C, Vanden Bempt I: Targeted RNA sequencing for upfront analysis of actionable driver alterations in non-small cell lung cancer. Lung Cancer 166: 242-249, 2022. DOI: 10.1016/j.lungcan.2022.02.013

19 Kim J, Yoon S, Lee D, Jang S, Chun S, Kim S: Real-world utility of next-generation sequencing for targeted gene analysis and its application to treatment in lung adenocarcinoma. Cancer Med 10(10): 3197-3204, 2021. DOI: 10.1002/cam4.3874

20 Cascetta P, Marinello A, Lazzari C, Gregorc V, Planchard D, Bianco R, Normanno N, Morabito A: KRAS in NSCLC: State of the art and future perspectives. Cancers 14(21): 5430, 2022. DOI: 10.3390/cancers14215430

21 Hofmann M, Gerlach D, Misale S, Petronczki M, Kraut N: Expanding the reach of precision oncology by drugging all KRAS mutants. Cancer Discov 12(4): 924-937, 2022. DOI: 10.1158/2159-8290.CD-21-1331

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