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Mutational landscape of lung adenocarcinoma in Czechia

Filip Ambrozkiewicz [a], Esraa Ali [a], Martina Bradova [b,c], Petr Slavík [b,c], Petr Martinek [b], Martin Svaton [d], Akseli Hemminki [e,f], Kari Hemminki [a,g,*]

[a] Biomedical Center, Faculty of Medicine, Charles University Pilsen 30605 Pilsen, Czech Republic
[b] Bioptical Laboratory Ltd, Pilsen, Czech Republic
[c] Department of Pathology, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[d] Department of Pneumology and Phtiseology, University Hospital Pilsen, Pilsen, Czech Republic
[e] Cancer Gene Therapy Group, Translational Immunology Research Program, University of Helsinki, Finland
[f] Comprehensive Cancer Center, Helsinki University Hospital, Helsinki, Finland
[g] Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 580 D-69120, Heidelberg, Germany

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ABSTRACT

Limited DNA sequencing data on lung cancer (LC) patients are available from Eastern Europe where male smoking is commonest in Europe. As sequence analysis is the prerequisite for targeted therapy we provide such data from Czechia (CZ). Additionally, we present novel sequencing data for adenocarcinoma subtypes. Panel sequencing data on 1218 adenocarcinoma patients were available from a single histology laboratory for 2016 to 2024. The highest variant frequencies were observed for *KRAS* (51.6%), *EGFR* (18.8%) and *TP53* (16.3%). Commonest types of variants were codon 12 mutations for *KRAS*, exon 21 L858R for *EGFR* and V600E *BRAF*. *EGFR* variants were more common in females (25.3% vs 11.5%). *KRAS* mutations were frequent in males (56.9% vs 46.9%), as were *PIK3CA* mutations (3.8% vs 1.9%). *KRAS* mutations were frequent in patients diagnosed below 70 years, whereas *EGFR, PIK3CA* and *MET* alterations were frequent in patients above age 70 years. As stage distinctions, high prevalence of *KRAS* mutations was found in early stage tumors (II and IIIA) and of *TP53* mutations in stages IIIB and IV. As to adenocarcinoma subtypes, lepidic type showed a high frequency of *EGFR* variants. We could show differential distribution of gene variants by sex, diagnostic age, stage and subtype of adenocarcinoma. The data are in line with current literature that targeted treatment is being applied for selected LC patients in CZ. It can be expected that LC patients benefit from therapeutic and other clinical improvements, which however do not reduce the primary importance of anti-smoking campaigns.

Introduction

Comparison of global incidence and mortality rates for lung cancer (LC) for selected countries based on the GLOBOCAN database shows the highest age-standardized rates for European countries [1]. The incidence trends from 2000 onwards were declining for men but slightly increasing for women in many countries [1]. A closer look into each European Union (EU) country in 2021 showed that male mortality rates in LC were highest in Hungary, followed by several Eastern European countries and Greece (https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Cancer_statistics_-_specific_cancers#Lung_cancer). Female mortality was also highest in Hungary, followed by Denmark and the Netherlands, but many Eastern European countries ranked below the EU average. Male LC mortality in Czech Republic (CZ) was below the EU average, and female mortality was exactly at the level of the average. The same source reported also on hospital occupancy by LC patients. According to the numbers of discharged in-patients, Austria and Germany were leading by far and exceeded the EU median, including CZ rates, three times. Curiously, the average length of in-patient stay varied completely opposite to the number of discharges; countries with low numbers of discharges kept patients longer time in hospitals.

Adenocarcinoma has increased relative to other histological types, and by 2020 adenocarcinoma had become the most common LC histology in most countries in the world for males and in all countries for females [2]. The 2015 World Health Organization (WHO) Classification of Tumors of the Lung, Pleura, Thymus and Heart introduced subclassification which can be diagnosed from small biopsies and cytology

* Corresponding author at: Biomedical Center, Faculty of Medicine, Charles University Pilsen, 30605 Pilsen, Czech Republic.
E-mail address: k.hemminki@dkfz.de (K. Hemminki).

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specimens [3]. Five major subtypes by predominant growth types include lepidic, acinar, papillary, micropapillary and solid which are characterized by clinical course and response to treatment [4]. These are distinguished by presentation and prognosis, lepidic presenting in low-grade with a good prognosis, acinar and papillary with intermediate grades and micropapillary and solid with high grade and poor prognosis [4,5]. In the 2021 WHO Thoracic Adenocarcinoma Histological Classification remained largely as in the 2015 version but generally more emphasis was devoted to molecular testing [6].

Survival in LC has been poor, 5-year survival has been around 20 % due to late stages at diagnosis; in CZ close to 60 % of LC with definite stage data were diagnosed at stage IV which is somewhat higher proportion than in some Western European countries [7,8]. Nevertheless, survival in LC has increased globally but preferentially in favor of young and early-stage patients in Europe, including CZ [7–12]. Survival improvement has been achieved through detection of earlier stages by sensitive thorax computed tomography (CT) and positron emission tomography (PET)/CT; this has resulted in increased numbers of patients amenable to surgery [7,13]. Therapies for more advanced LC have improved and for metastatic disease the treatment modalities include surgery, chemotherapy, chemoradiation, targeted therapy and most recently immunotherapy; the therapeutic choices are guided by appropriate individual tumor data (e.g., mutation profile and PD-L1 expression) for optimal treatment selection [7,13,14]. According to the 2023 European Society for Medical Oncology (ESMO) recommendations, all advanced non-small cell LC (NSCLC) cases should be tested for mutations in *EGFR* exons 18–21, *MET* exon14 skipping, *BRAFV600, KRAS G12C* and *HER2* as well as rearrangements in *ALK, ROS1, NTRK* and *RET* [15,16]. Some adenocarcinoma histologies are enriched in specific mutations, such as invasive solid mucinous type harbors frequently *KRAS* mutations, lepidic tumors *EGFR* and micropapillary and solid tumors *TP53* mutations [5,6].

Relatively little recent clinical genetics data are available from Eastern Europe which has been a high-risk area for male LC because of wide-spread smoking habits. For Poland a survey on genomic testing of LC found that in thoracic clinics *EGFR* variants and a few other genes were analyzed in NSCLC patients [17]. In Hungary, the ESMO recommendations are recognized but to what extent they are followed in practice was not documented [18]. In CZ, molecular testing in NSCLC includes variants in *EGFR, ALK, ROS1,* as well as PD-L1 analysis from tumor tissue [7]. The results are then evaluated for appropriate therapy which may include systemic therapy according to the ESMO guidelines [7]. Pricing and reimbursement for targeted therapies and immunotherapy are regulated at the national level [7]. In the present study we analyze whole-exome sequencing data on 1200 CZ adenocarcinoma patients carried out in a large private pathology laboratory, which provides complete service with any necessary immunohistochemical and molecular genetic testing for outside medical practices and hospitals.

Methods

Mutation and immunohistochemical analyses of LC samples were conducted in Biopticka laboratory, accredited by ČIA (Czech Institute for Accreditation) under ČSN EN ISO 15,189 ed. 3:2023. Biopticka obtained clinical samples and related data from the customers who were referring pathologists, clinicians, and hospitals The current mutation analysis team received anonymized mutation data from the Biopticka staff with the understanding that no reidentification was possible. The current mutation analysis team had no access to the samples that had been handled by Biopticka laboratory nor had it any data other than those received from Biopticka. The study adhered to the guidelines of the Ethics Committee of the Faculty Hospital in Pilsen which implied adoption of the principles of the Declaration of Helsinki. Informed consent is not required to anonymous samples according to the Czech law (Act. no 373/11 and its amendment Act no 202/17).

Clinical characteristics of 1218 lung adenocarcinoma patients are shown in **Supplementary Table 1**. Women slightly outnumbered men but the median age (68 years) was equal in both sexes. Sampling started in 2016 and the annual case numbers climbed to over 200 in 2020. As reference, in CZ 6162 LC were diagnosed in year 2020 [7]. Most samples were lung biopsies. Thus tumor architecture could not be systematically defined to distinguish adenocarcinoma subtypes. As a result, subtypes remain often unreported.

The standard immunohistochemistry considered p40, CK5/6, NapsinA and TTF1 with addition of CK7. Analysis of PDL1 (Dako product 22C3), ALK (Dako product OTI1A4) and ROS1 (Cell Signaling product D4D6®) was additionally performed according to the manufacturer's instructions.

For genetic analysis 10 μm thick formalin-fixed paraffin-embedded (FFPE) sections were used for DNA and RNA extraction with DNA/RNA RSC kits (automated on Maxwell RSC 48 Instrument, Promega, Madison, Wisconsin, USA). DNA and RNA was quantified using the Qubit Broad Range DNA/RNA Assay Kits (ThermoFisher Scientific). The PreSeq RNA QC Assay using iTaq Universal SYBR Green Supermix (Biorad, Hercules, CA) was performed to assess RNA quality. The FusionPlex CTL kit containing 195 targets in 36 genes or FusionPlex Lung v2 with 323 gene specific primers targeting 17 genes mutated in NSCLC were used for fusion and single nucleotide variant (SNV) detection from RNA. The VariantPlex CTL kit was used for DNA SNV analysis in 290 regions of interest across 31 genes. Library preparations were performed following Fusion Plex or Variant Plex Protocols for Illumina (ArcherDX Inc., Boulder, CO) and were quantified following the Library Quantification for Illumina Libraries protocol (KAPA, Wilmington, MA). Samples were multiplexed and sequenced on NextSeq 550 or Novaseq 6000 sequencers (Illumina, San Diego, CA) spiked with up to 20 % PhiX to ensure library nucleotide diversity. Reference genome build GRCh38/hg19 was used for alignment, and Archer Analysis software (version 7, ArcherDX Inc.) was used for variant calling and annotation with annotations from Ensembl Variant Effect Predictor (VEP) and ClinVar. Parameters for filtering strong fusions as determined by the Archer Analysis software were set to a minimum of 5 fusion supporting reads with a minimum of 3 unique start sites. For SNV and indel detection the parameters were empirically set to 5 % allelic frequency and minimum depth 40x; limits for alternate observations were set at 5 (counts of reads with alternative base, set as default in the analysis settings), unique start sites of alternate observation >3 (another metric for variant calling that counts the number of unique start positions of the reads supporting the event), 0.5 % population frequency using GnomAD database. All raw reads for filtered variants were visually inspected by two molecular biologists to exclude potential artefacts and confirmed variants were reported.

Differences in frequencies of specific mutation were assessed by the Fisher exact test; 0.05 was considered the limit of significance. Analysis was performed in GraphPad Prism v.10.

Results

Most frequent alterations (>1 % of 1218 adenocarcinoma patients) in 11 genes and rare variants 'others' are shown in Fig. 1 for all, women and men. The most common variants were *KRAS* (51.6 %), *EGFR* (18.8 %) and *TP53* (16.3 %). *KRAS* mutations were more frequent in males (56.9 % vs 46.9 %, $p = 0.0006$), as were *PIK3CA* mutations (3.8 % vs 1.9 %, $p = 0.05$). *EGFR* mutations were more common in females (25.3 % vs 11.5 %, $p < 0.0001$).

The WHO LC classifications have categorized adenocarcinomas into 5 subtypes. We were able to identify some subtypes in a limited number of samples. As significant observations, *EGFR* mutation had highest frequency in the lepidic type (48 %), whereas it's frequency in the acinar type was low (6.5 %, $p = 0.0005$) and it was not detected in the mucinous type ($p = 0.0004$) (Table 1). Frequency differences (non-significant) were noted also for *KRAS* mutations in the acinar (58.1 %) and mucinous (61.1 %) types compared to the lepidic (32.0 %) subtype.

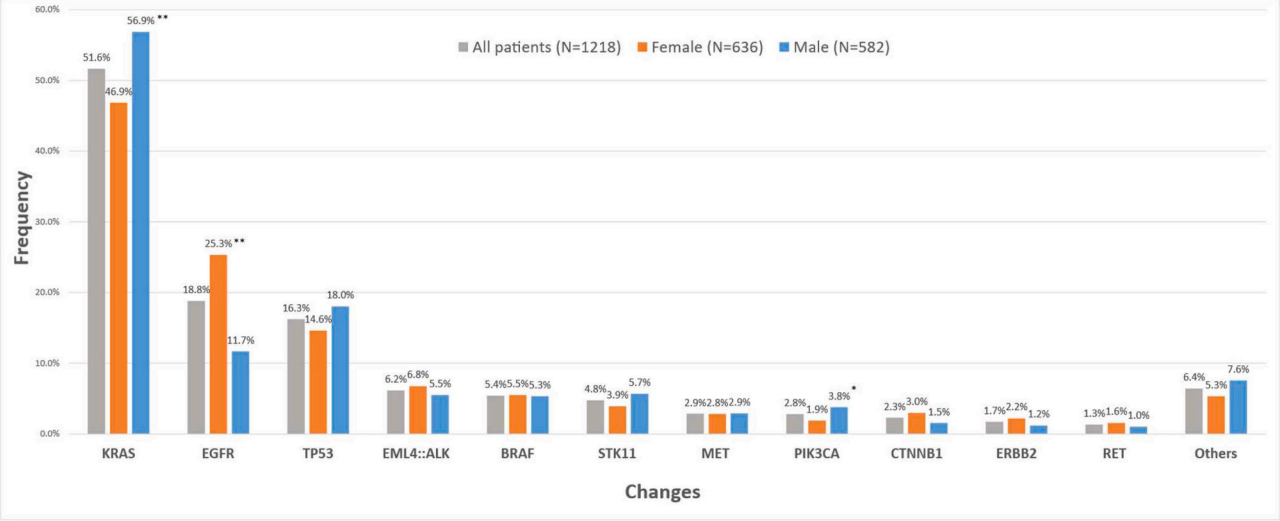

Fig. 1. Frequency of detected genetic variants in all patients and women and men. The 'others' include rare variants (<1 % frequency) listed in Methods. While most variants were SNVs, those for EGFR, STK1, MET, ERBB2 and RET included also more complex variants; for EML4::ALK only fusions were considered. Category "Others" contains variants at frequency <1 %: AKT1, IDH1, IDH2, RAF1, NTRK1, EIF1AX, DDR2, KIT, GNAS, HRAS, NRAS, FGFR2, FGFR3, PTEN, TERTp, MAP2K1, CD74::ROS1, SLC34A2::ROS1, EZR::ROS1, SPAG9::ROS1, VAMP2::NRG1, CD74::NRG1, LRP12::NRG1, CSGALNACT1::NRG1, SLC3A2::NRG1, STRN::ALK, PURA::ALK, NPM1::ALK, NR0B2::ALK,LLGL2::ALK, HIP1::ALK, GALNT2::ALK, TPM3::NTRK1, CPLX1::NTRK2, EML4::NTRK3, FGFR2::BICC1, FGFR2::TACC2, FGFR3::TACC3, CHRNA6::FGFR1.

Table 1
Frequency of genetic changes in different subtypes of lung adenocarcinoma.

Genes (change)	Adenocarcinoma (Defined subtype)					
	Acinar (N = 31)		Lepidic (N = 25)		Mucinous (N = 18)	
	N	Frequency	N	Frequency	N	Frequency
KRAS	18	58.1 %	8	32.0 %	11	61.1 %
TP53	6	19.4 %	3	12.0 %	2	11.1 %
EGFR	2	6.5 %	12	48.0 %***	0	0.0 %
BRAF	3	9.7 %	0	0.0 %	0	0.0 %
MET	3	9.7 %	1	4.0 %	0	0.0 %
STK11	2	6.5 %	2	8.0 %	2	11.1 %
RET	1	3.2 %	0	0.0 %	0	0.0 %
EML4::ALK	1	3.2 %	0	0.0 %	3	16.7 %
CTNNB1	0	0.0 %	0	0.0 %	1	5.6 %
PIK3CA	0	0.0 %	1	4.0 %	0	0.0 %
ERBB2	0	0.0 %	0	0.0 %	1	5.6 %

*** P < 0.001 against acinar and mucinous types.

Table 2
Differences in mutation frequencies according to age.

Genes (change)	AGE 30–94 median = 70			
	Age <70 (M = 597)		Age ≥70 (N = 621)	
	N	Frequency	N	Frequency
KRAS	341	57.1 %***	288	46.4 %
TP53	106	17.8 %	92	14.8 %
EGFR	89	14.9 %	140	22.5 %***
EML4::ALK	51	8.5 %***	24	3.9 %
STK11	33	5.5 %	25	4.0 %
BRAF	31	5.2 %	35	5.6 %
ERBB2	12	2.0 %	9	1.4 %
MET	10	1.7 %	25	4.0 %*
CTNNB1	9	1.5 %	19	3.1 %
PIK3CA	9	1.5 %	25	4.0 %**
RET	5	0.8 %	11	1.8 %

* P < 0.05,.
** P = 0.01,.
*** P< = 0.001.

TP53 mutations were more frequent in acinar (19.4 %) than in lepidic (12.0 %) or mucinous (11.1 %) cancers. Frequencies of other mutations were lower (Table 1).

Median diagnostic age for the cohort was 70 years with a range from 30 to 94. We categorized age according to median below and equal or above 70 years (Table 2). *KRAS* ($p < 0.0002$), EML4::ALK ($p = 0.001$) and *TP53* (non-significant) mutations were more frequent in patients below 70 years old, whereas *EGFR* ($p = 0.001$), *PIK3CA* ($p = 0.01$) and *MET* ($p = 0.02$) alterations were more frequent in patients above 70 years old, as were variants in *CTNNB1, RET, STK11* but without statistical significance.

The next step in our analysis focused on comparing mutation frequencies between stages of adenocarcinoma. Our cohort encompassed 616 patients with assigned stage, including 148 stage I patients (24 %), 69 stage II patients (11 %), 115 stage III patients (19 %) and 284 stage 4 patients (46 %) (Table 3). Variant frequencies were rather even between different stages for the majority of genes without significant differences; *BRAF* variants were most common in stage I and *TP53* mutations were most frequent in stage IV tumors. The 7th edition of the TNM classification separated stage III into A and B because of clinical significance. We could observe differences between these two in variant frequencies (Table 3). *KRAS* mutations were more frequent in patients diagnosed with stage IIIA ($p = 0.03$); *TP53* mutations were more frequent in patients diagnosed with stage IIIB ($p = 0.03$).

In the final analysis we focused on 3 oncogenes *KRAS, EGFR* and *BRAF* to assess the distribution of the specific variants in the CZ population. In the *KRAS* gene we observed 3 variants with frequency higher than 10 %, *KRAS* c.34G>*T*, *KRAS* c.35G>*T* and *KRAS* c.35G>*A*, reaching 39.4 %, 19.4 % and 14.6 %, respectively (Table 4). For *EGFR*, c.2573T>*G* accounted for 25.4 % and c.2235_2249del 17.1 %; the rest were diverse rare variants. For *BRAF* c.1799T accounted for 37.8 % and almost half of the variants were diverse rare ones. The rare variants are listed in the footnote.

Discussion

The present data on CZ adenocarcinoma may be the largest genetic study yet published from Eastern Europe. The results generated in a single pathology laboratory showed the predominance of *KRAS* mutations followed by *EGFR* variants and *TP53* and *BRAF* mutations. While

Table 3
Differences in mutation distribution according to stage.

Changes	I (N = 148)		II (N = 69)		III (N = 115) †				IV (N = 284)	
					IIIA(N = 69)		IIIB (N = 31)			
	N	Frequency	N	Frequency	N	Frequency	N	Frequency	N	Frequency
KRAS	71	48.0 %	34	49.3 %	35	50.7 %*	8	25.8 %	124	43.7 %
TP53	31	20.9 %	13	18.8 %	16	23.2 %	14	45.2 %*	89	31.3 %
EGFR (mutations, duplications, deletions)	30	20.3 %	16	23.2 %	15	21.7 %	5	16.1 %	52	18.3 %
BRAF (mutations, fusions)	12	8.1 %	4	5.8 %	4	5.8 %	0	0.0 %	11	3.9 %
STK11 (point mutations, deletions)	12	8.1 %	6	8.7 %	5	7.2 %	4	12.9 %	22	7.7 %
PIK3CA	6	4.1 %	5	7.2 %	1	1.4 %	0	0.0 %	10	3.5 %
MET (exon 14 skipping and point mutations)	6	4.1 %	2	2.9 %	3	4.3 %	0	0.0 %	7	2.5 %
EML4::ALK (fusion)	6	4.1 %	3	4.3 %	1	1.4 %	3	9.7 %	25	8.8 %
CTNNB1	2	1.4 %	0	0.0 %	1	1.4 %	1	3.2 %	9	3.2 %
RET (mutation, fusions)	2	1.4 %	0	0.0 %	2	2.9 %	0	0.0 %	4	1.4 %
ERBB2 (duplications, point changes, fusions)	1	0.7 %	1	1.4 %	1	1.4 %	2	6.5 %	3	1.1 %

† 6 patients with stage IIIC and 9 patients without assigned substage were not included in the analysis.
* $P < 0.05$ between IIIA and IIIB.

Table 4
Specific variants frequencies in KRAS, EGFR and BRAF gene.

KRAS (N = 629)			EGFR (N = 229)			BRAF (N = 66)		
Change	N	Frequency	Change	N	Frequency	Change	N	Frequency
c.34G>T	248	39.4 %	c.2573T>G	58	25.4 %	c.1799T>A	25	37.9 %
c.35G>T	122	19.4 %	c.2235_2249del	39	17.1 %	c.1742A>G	6	9.1 %
c.35G>A	92	14.6 %	c2236_2250del	19	8.3 %	c.1801A>G	4	6.1 %
c.35G>C	49	7.8 %	c.2156G>C	8	3.5 %	Others	31	47.0 %
c.37G>T	18	2.9 %	c.2240_2254del	6	2.6 %			
c.34G>A	15	2.4 %	c.2237_2255delinsT	5	2.2 %			
c.38G>A	15	2.4 %	c.2239_2251del	4	1.8 %			
c.183A>T	15	2.4 %	c.2300_2308dup	4	1.8 %			
c.183A>C	11	1.7 %	Others	86	37.7 %			
c.34_35delinsTT	9	1.4 %						
c.34G>C	8	1.3 %						
c.182A>T	7	1.1 %						
Others[1)]	20	3.2 %						

1) 'Others' and included: KRAS c.436G>A, c.64C>A, c.351A>T, c.437C>T, c.34_34delinsTT, c.34_35delinsTC, c.34_35delinsAT, c.38_39delinsTT, c.33_34delinsCT, c.180_181delinsAA; EGFR c.2438T>G, c.2573T>G, c.2447T>A, c.2360A>G, c.2369T>C, c.2504A>T, c.2320G>A, c.2126A>C, c.2582T>A, c.2155G>A, c.2497T>G, c.2573T>G, c.2543C>T, c.2125G>A, c.2369C>T, c.2369C>T, c.2303G>T, c.2273A>G, c.2228C>T, c.2303G>T, c.323G>A, c.2240_2257del, c.2127_2129delAAC, c.2237_2254del, c.2239_2248delinsC, c.2126_2128delAAA, exon19 del, c.2236_2250del, del e.2–7,c.2320_2321ins, c.2235_2254delinsAAAGCCAACAA, c.2239_2256del18, c.2239_2248delinsC, c.2156del, c.2237_2253delinsTTCCT, c.2236_2244del9, c.2214_2231dup, c.2308_2309insCTGGGG, c.2239_2258delinsCA, c.2240_2261delins, c.2240_2257del, c.2310_2311insGGT, c.2154_55delinsTA,c.2303_2311dup, c.2239_2248delinsC, c.2237_2251del, c.2335_2336delinsTT, c.2311_2319dup, c.2284–5_2290dup, c.2232_2248delinsAAAATTCC, c.2307_2308insTCCAGCGTG, c.2235_2248delinsAATTC, c.2235_2244delinsAATTCCCGTC, c.896_899delinsG, c.2303_2305delinsTCT, c.2315_2326dup, c.2314_2319dup, c.2237_2257delinsTTT, c.2296_2297ins, c.2302_2303ins, EGFR::SEPTIN14, EGFR::RAD51 and BRAF c.1406G>A, c.1786G>C, c. 1391G>T, c.1388T>G, c.1743T>G, c.1454T>G, c.1781A>G, c.1742A>G, c.1397G>T, c.1790T>G, c.1405G>A, c.1780G>C, c.1796_1799delinsG, c.1405_1406delinsTC, c.1795_1797dup, c.1442C>A, MKRN1::BRAF, CDC23::BRAF.

the frequency of variants in commonly mutated genes in lung adenocarcinoma generally includes these genes, the frequencies depend on many demographic, clinical and life style related factors [5,19–23]. Recently targeted sequencing has focused on genes with therapeutic intentions which also influence focusing on relatively early stage patients. A large German study on stage III and IV NSCLC reported on potential therapeutic targets [19]. It included some 90 % of adenocarcinomas reporting 18.1 % *EGFR*, 5.3 % *PIK3CA*, 4.3 % *BRAF* and 2.4 % *CTNNB1* variants, the frequencies of which are very close to our results on these genes [19]. A large US study on circulating-tumor DNA focusing on therapy targets in NSCLC of stage IIIB and higher reported that females were more likely to harbor variants with a known targeted therapy (*EGFR, KRAS G12C, ERBB2* ex20), while males were more likely to present with variants in *STK11* or *TP53* which are associated with poor response to therapy and worse outcomes [24]. They found also differences in variant frequency by age of onset. The applied medication included earlier mainly kinase inhibitors but more recently the molecular targets have widened and also include *KRAS* [22]. The present stage distribution was also skewed to earlier stages because of therapy considerations (46 % stage IV which was lower than for all LC in CZ) and focus on adenocarcinoma [7].

In the present adenocarcinoma cohort *EGFR* variants were more than twice as common in females compared to males while *KRAS* and *PIK3CA* mutations were more common in men which has been found also earlier [25]. Smoking is a predisposing factor of *KRAS* mutations and in CZ the prevalence of smokers has been higher among men than women [7,22]. Mutational signatures show the smoking connection for *KRAS* mutations while for *EGFR* the signature reveals origins via an endogenous mutation process of cytidine deamination, however without a clue to the female excess of these variants [26]. The common types of variants in the CZ adenocarcinoma were similar to those reported in the literature; for *KRAS* codon 12 mutations dominated and for *EGFR* exon 21 L858R and *BRAF* V600E were the commonest [19].

We noted a modest but statistically significant early onset (<70 years) preference for *KRAS,* EML4::ALK and a late onset preference for *EGFR, PIK3CA* and *MET* variants. There were additionally minor stage-specific variations in mutation frequencies: *BRAF* variants were most common in stage I while *KRAS* and *EGFR* variants were most common in stage II. Most *TP53* mutations were found in stage IV. Stage III was divided into A and B in the 7th edition of the TNM classification [27]. Stage IIIA involves more localized lymph node and tumor invasion on the same side of the chest as the primary tumor, whereas stage IIIB

includes more extensive or distant lymph node involvement, such that cancer may have spread to lymph nodes above the collarbone or to lymph nodes on the opposite side of the chest [27]. Interestingly, we could show genetic distinction between A and B: *KRAS* mutations were more frequent in patients diagnosed with stage IIIA ($p = 0.03$); *TP53* mutations were more frequent in patients diagnosed with stage IIIB ($p = 0.03$). Thus our results could show a high prevalence of *KRAS* mutations in early stage tumors (II and IIIA) and that of *TP53* mutations in stages IIIB and IV, suggests accumulation of these mutations in early and late stage adenocarcinoma, respectively. Subsequently, stage III has been further divided into IIIC.

We were also able to test mutations in the subtypes of adenocarcinoma for which literature is slowly emerging as the new WHO classification system is being adopted [3]. One obstacle in adopting this is the difficulty of accurate diagnosis from core biopsies instead of resection specimens [3]. Even if our results are quite novel for European LC, the limitation is the samples size. Nevertheless, they show feasibility of mutation analysis from core biopsies. The lepidic type is considered the most benign subtype and we found a low frequency of *KRAS* and high frequency of *EGFR* variants in this subtype, in agreement with a large US study [21].

In conclusion we analyzed genomic variants among 1218 CZ lung adenocarcinoma patients and found the highest frequency of variants in genes *KRAS, EGFR* and *TP53*. The frequencies of variants showed different distributions by sex, diagnostic age, stage and subtype of adenocarcinoma. Even if our cohort is not the only sequenced LC population in CZ, it provides evidence that targeted treatment is commonplace for selected LC patients in the country in line with a recent report [7]. Therapeutic and other clinical improvements will keep improving outcomes, but LC will remain a life-threatening disease for which non-smoking is the best prevention.

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CRediT authorship contribution statement

Filip Ambrozkiewicz: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Esraa Ali:** Writing – review & editing, Investigation, Data curation. **Martina Bradova:** Writing – review & editing, Supervision, Investigation, Data curation. **Petr Slavík:** Writing – review & editing, Validation, Data curation. **Petr Martinek:** Writing – review & editing, Formal analysis, Data curation. **Martin Svaton:** Writing – review & editing, Investigation. **Akseli Hemminki:** Writing – review & editing, Investigation, Data curation. **Kari Hemminki:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization.

Declaration of competing interest

A.H. is the shareholder in Circio Holdings ASA. A.H. is an employee and shareholder in TILT Biotherapeutics Ltd. Other authors declared no conflict of interest. Other authors have no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ctarc.2026.101095.

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