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EGFR MUTATIONS (DEL19/L858R) IN LUNG ADENOCARCINOMA IN PATIENTS OF THE YAKUT REPUBLICAN ONCOLOGICAL DISPENSARY

The frequency of Del 19 and L858R mutations of the EGFR gene was studied among patients of the Yakut Republican Oncology Dispensary with lung adenocarcinoma (n=177). Mutations were found 3.8 times more commonly in female population than in male. Mutations were detected 2.8 times more regularly among patients with stages I and II of the disease in compare with stages III and IV. The mutation frequency was 3.4 times over in the Sakha (Yakut) ethnic group patients as opposed to Russian ethnic group. In addition the mutation frequency was 9.6 times higher in the Sakha (Yakut) ethnic group male patients as opposed to Russian ethnic group. It was shown that the overall 36-month survival of patients with a positive status of EGFR mutations increases by 2.5 times (from 29.3% to 74.1%). Moreover in patients with stages I and II of the disease, survival rate increases by 1.4 times, in patients over 65 years old in 2.9 times, in female patients in 2.4 times.

Keywords: non-small cell lung cancer, adenocarcinoma, EGFR gene mutations, survival.

Introduction. Lung cancer is one of the most common types of malignant neoplasms (MN) in the world [10]. In the structure of mortality from MN in Russia, it is 16.8%, in the Republic of Sakha (Yakutia) it reaches 18.5% [3]. Lung cancer is characterized by late detection, rapid and aggressive course and high mortality [16, 18]. A regional feature of the nosology is that among the female population of Yakutia, the primary incidence rates of MN of the trachea, bronchi, lung (C33, 34) are 1.9 times higher than the Russian average [1]. The most common form (80-85%) is non-small cell lung cancer (NSCLC) [16]. Molecular genetic studies have shown that in 60-70% of cases (30-45% of which are adenocarcinomas), EGFR gene hyperexpression is detected in tumor cells due to the presence of activating mutations [13, 15]. This discovery was a key moment in the development of an effective strategy for the treatment of NSCLC and led to the emergence of a new molecular indicator of lung tumor sensitivity to low-molecular-weight EGFR tyrosine kinase inhibitors (TKIs).

Currently, the study of EGFR gene mutations in tumors is standard in the diagnosis of patients with NSCLC and is included in Clinical Guidelines for determining indications for targeted therapy. For patients with locally advanced or metastatic NSCLC with EGFR gene mutations in exons 19 or 21, EGFR tyrosine kinase inhibitors are recommended as first-line therapy: gefitinib, erlotinib, afatinib, or osimertinib [5]. Since TKIs have become common in the treatment of NSCLC in Russia, information on the incidence of EGFR mutations is important. It is known that the incidence of EGFR mutations in patients with lung adenocarcinoma may depend on race, smoking status, and gender of the patient [9, 11, 14]. Thus, EGFR mutations are found in 35-62% of NSCLC cases in East Asians and only in 10% of NSCLC cases in Europeans and North Americans [12]. In Russia, EGFR gene mutations are found in 13-29% of patients with NSCLC adenocarcinoma [4, 7]. There are no data on the frequency of EGFR oncogene mutations in patients with NSCLC in the Republic of Sakha (Yakutia), where the majority of the population are indigenous people - "Sakha (Yakuts)", who have Turkic-Mongolian (Asian) origin with high genetic homogeneity [17, 19]. However, these data are important in determining indications for targeted therapy, prognosis of treatment and planning, as well as in obtaining new information in oncological studies of the frequency of driver mutations in Russia. The aim of this work is to study the frequency of Del 19 and L858R mutations of the EGFR gene and to assess the prognostic significance of its diagnosis in patients of the Yakut Republican Oncology Dispensary with lung adenocarcinoma.

Materials and research methods. The study included patients whose tumor material was tested for mutations in the EGFR gene (Del 19 and L858R) and who received treatment at the Yakutsk Republican Oncology Dispensary (YROD) from

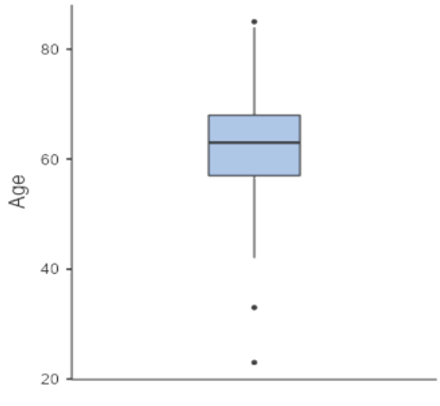

Fig. 1. Average age of examined patients (years)

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2018 to 2020. Data for a longer period of time were not available due to the start of using research data in the dispensary, with appropriate therapy, since 2018. The study included 177 patients with a verified diagnosis of non-small cell lung cancer, with a histological tumor type of adenocarcinoma. The clinical part of the study was carried out at the Department of Antitumor Drug Therapy, and studies for the presence of mutations were carried out in the molecular biology laboratory of the dispensary. Patients with a positive status of EGFR gene mutations received therapeutic targeted therapy using first- and third-generation tyrosine kinase inhibitors (TKIs) (erlotinib, gefitinib, osimertinib). Patients in whom EGFR mutations were not detected received other types of therapy prescribed individually according to treatment regimens, according to Clinical Guidelines [5]. DNA was isolated from tumor cells of paraffin block sections containing formalin-fixed postoperative or biopsy material. DNA isolation was performed after deparaffinization, using QIAmp DNA FFPE Tissue Kit reagent kits (Qiagen, Germany) in accordance with the protocol, according to the principle of proteinase treatment with rehydration and sorption on a membrane, followed by elution. To determine mutations in exons 19 and 21 of the EGFR gene (Del 19 and L858R), DNA amplification with Real-Time detection was performed on BioRad CFX 96 devices (BioRad, USA).

Information on smoking status was not available for most patients, and therefore was not taken into account in the study.

The study included 177 patients aged 42 to 83 years. The average age of patients was 62.8 ± 9.1 years (Fig. 1).

Considering the influence of age on the development of clinical changes, patients were divided into two age groups: under 65 years and 65 years and older. The age category "65 years and older" is distinguished in clinical studies due to the progressive increase in biological changes in the body [2, 6, 8]. Splitting into smaller age groups did not form a representative sample. For the same reason, when dividing patients by disease stages, 2 combined groups were identified: stages I, II and stages III, IV. The majority of the examined patients (68.9%) were men, women - 31.1% (Table 1). The proportion of patients under 65 years of age was 57.1%, 65 years and older - 42.9%. By disease stage, the predominant part (71.8%) were patients with stages III, IV, and 28.2% - with stages I, II of the disease. When analyzing by ethnicity, 2 main groups were identified:

Distribution of the identified mutations in groups by gender, age, stage of the disease, ethnicity of patients

Group	Observed, abs., (%)	EGFR mutations detected, abs., (%)		
		Deletions (del 19)	Spot Replacement (L858R)	Total
Total patients	177 (100.0)	13 (7.3)	14 (7.9)	27 (15.2)
Sex				
male	122 (68.9)	3 (2.5)	7 (5.7)	10 (8.2)
female	55 (31.1)	10 (18.2)	7 (12.7)	17 (30.9)
Age				
up to 65 years		9 (8.9)	6 (5.9)	15 (14.8)
over 65 years old		4 (5.3)	8 (10.5)	12 (15.8)
Stage of the disease				
I, II	50 (28.2)	8 (16.0)	6 (12.0)	14 (28.0)
III, IV	127 (71.8)	5 (3.9)	8 (6.3)	13 (10.2)
Ethnic group				
Russians	74 (41.8)	4 (5.4)	1 (1.4)	5 (6.8)
Sakha (Yakuts)	92 (52.0)	9 (10.9)	11 (11.9)	21 (22.8)
other	11 (6.2)	0	1 (9.1)	1 (9.1)
Male				
Russians	59 (48.4)	1 (1.7)	0	1 (1.7)
Sakha (Yakuts)	55 (45.1)	2 (3.6)	7 (12.7)	9 (16.3)
Female				
Russians	15 (27.3)	3 (20.0)	1 (6.7)	4 (26.7)
Sakha (Yakuts)	37 (67.3)	7 (18.9)	5 (13.5)	12 (32.4)

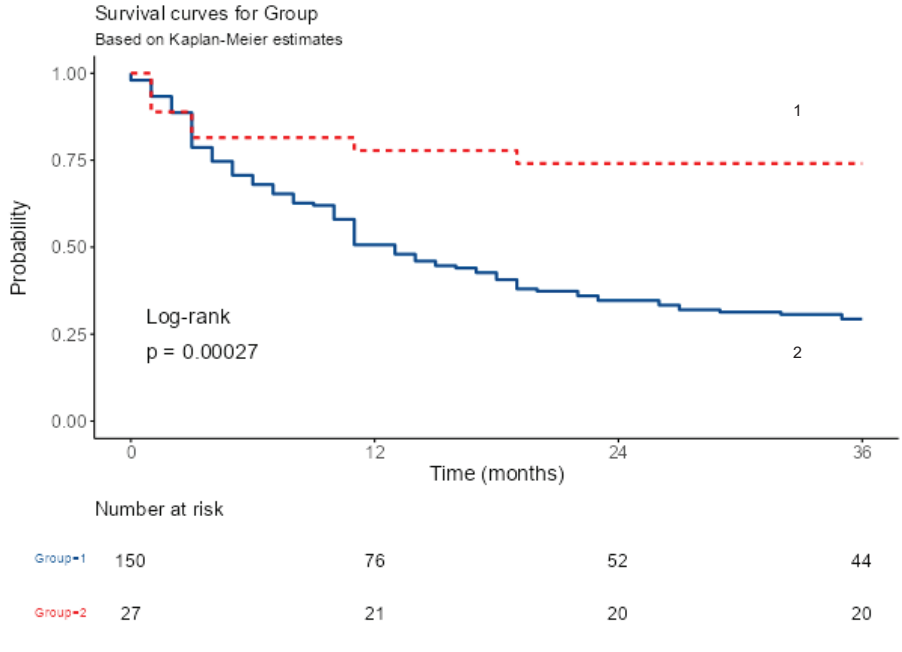
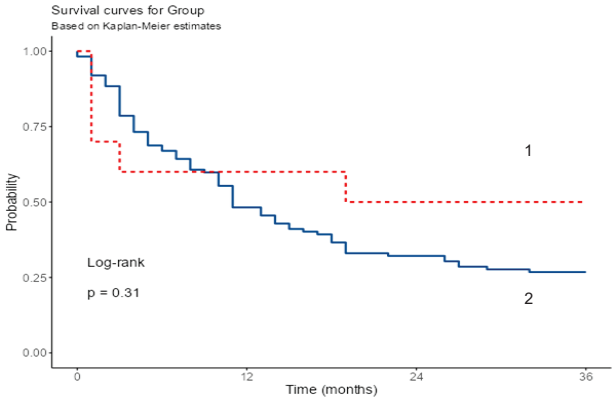
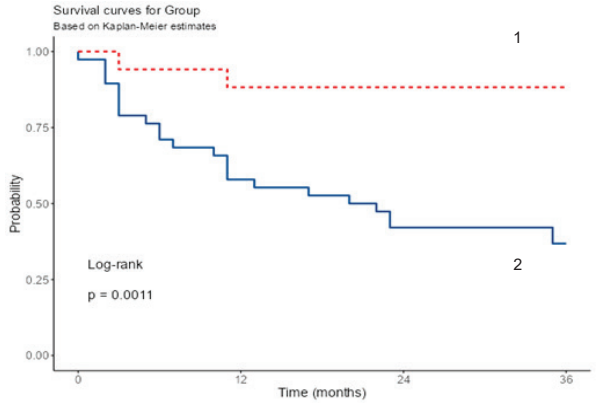


Fig. 2. Overall survival of patients (Kaplan-Meier curve) depending on the EGFR mutation status: 1 – yes; 2 – no

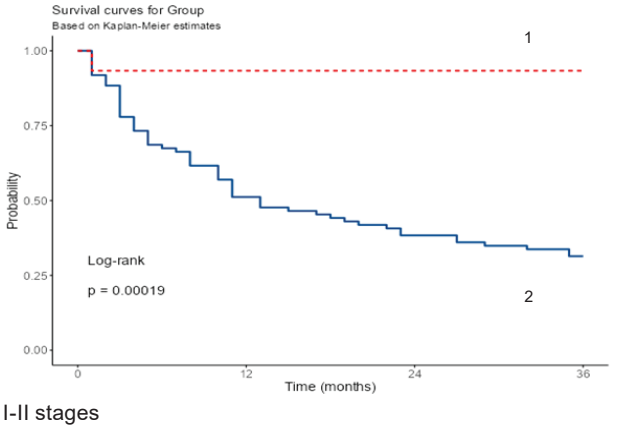
men



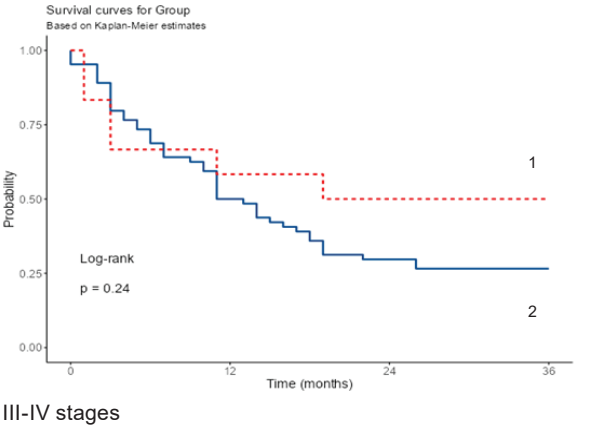
women



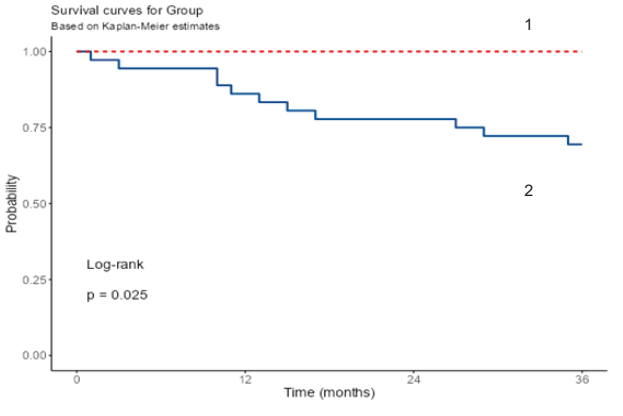
up to 65 years



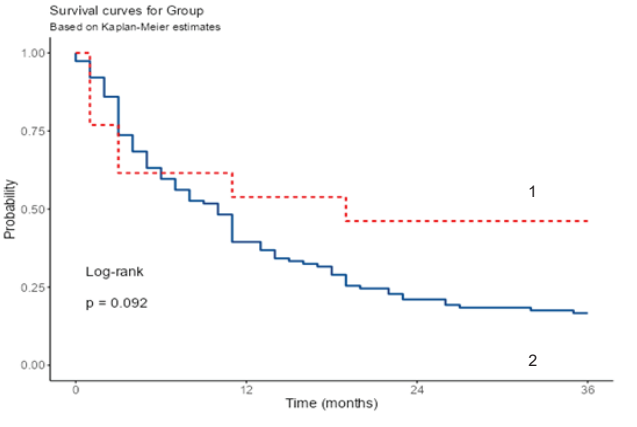
65 years and older



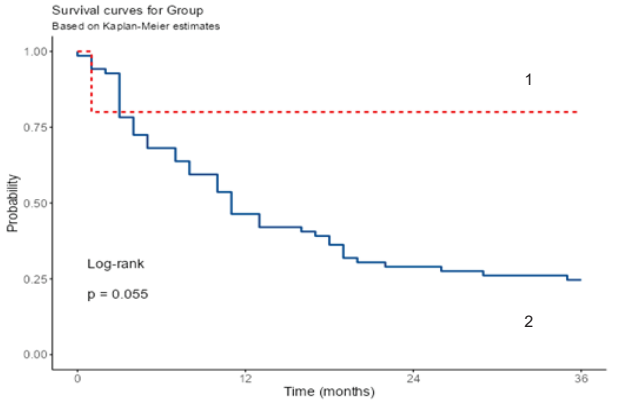
I-II stages



III-IV stages



Russians



Sakha (Yakuts)

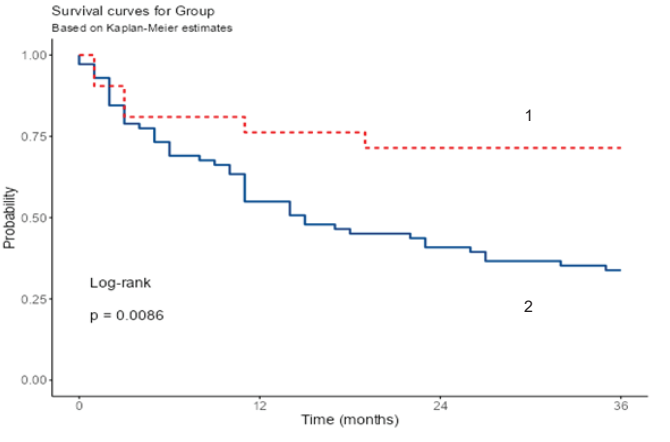


Fig. 3. Subgroup analysis of overall survival of patients depending on the EGFR mutation status: 1 – yes; 2 – no

"Sakha (Yakuts)" 92 examined - 52.0% of patients and "Russians" (41.8%) - 74 patients. The results of analyses of patients of other nationalities (n=11): Armenians (1), Georgians (1), Kyrgyz (1), Tatars (4), Ukrainians (1), Evenks (2) and Evens (1), the total share of which was 6.2% of all examined, were not used in the analysis by ethnicity due to the non-representativeness of the sample. Combining nationalities into groups by racial classification was not carried out for the same reason, as well as to exclude controversial issues of ethnogenesis. The data obtained during the study were subjected to statistical analysis using the free software development environment RStudio and the statistical package Jamovi (χ2 criterion, Fisher's exact criterion, survival according to the Kaplan-Meier method, median survival, overall survival, log-rank criterion).

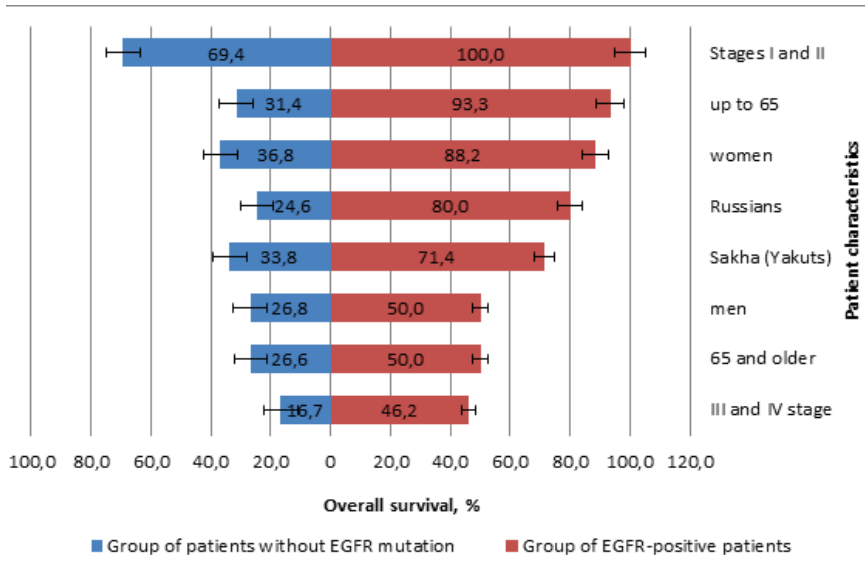


Fig. 4. 3-year survival, subgroup analysis (observation time – 36 months)

Results and discussion. *Mutation frequency.* Data on the presence and types of mutations were analyzed according to the patients' gender, age, nationality, and stage of the disease (Table 1). The total frequency of mutations in exons 19 and 21 of the EGFR gene in NSCLC (adenocarcinoma) in the total sample of patients was 15.2% (27/177). The obtained value corresponds to the frequency range of these mutations in the Russian population (13–29%) [4, 7]. Moreover, the studied mutations were distributed evenly: deletions in exon 19 were detected in 13 patients (7.3%), and L858R substitution in exon 21 was detected in 14 patients (7.9%). Mutations were significantly more common in women (30.9%, 17/55) than in men (8.2%, 10/122), (p <0.001). An assessment of the distribution of mutation rates by age group of patients did not reveal any significant differences: in the "under 65" group, mutations were detected in 14.8% of patients (15/101), in the "65 years and older" group – in 15.8% of patients (12/76), (p = 0.864). The differences in the frequency of Del 19 and L858R mutations did not reveal any significant differences.

In patients with stages I and II of the disease, mutations were detected 2.8 times more often (28.0%, 14/50) than in patients with stages III and IV (10.2%, 13/127), (p = 0.003). The differences in the frequency of Del 19 and L858R mutations did not reveal any significant differences. In patients of the "Sakha (Yakut)" group, the incidence of mutations was 22.8% (21/92), which is 3.4 times higher than in the "Russian" group (6.8%, 5/74), (p = 0.005). It should be noted that the obtained frequency for the "Sakha (Yakut)" group is significantly lower than for residents of East Asian countries (35-62%), and is comparable with the average value in the Russian population (13-29%). In the "Russian" group, the value obtained was also twice lower than expected (6.8%). Here it can be noted that in the "Russian" group, deletions (del 19) were more common - 5.4% and point substitutions (L858R) were less common - 1.4%, while in the "Sakha (Yakut)" group they were distributed approximately equally (10.9/11.9%). For men of the "Sakha (Yakut)" ethnic group, the incidence of EGFR gene mutations was 16.3% (9/55), which is 9.6 times higher than in men of the "Russian" group (1.7%, 1/59), (p = 0.006). In women of the "Sakha (Yakut)" and "Russian" ethnic groups, mutations were detected in 32.4% (12/37) and 26.7% (4/15), respectively, the differences are not significant (p = 0.683). Thus, in contrast to men, in women, no reliable significant differences in the frequency of the studied EGFR mutations were found in the "Sakha (Yakut)" and "Russian" groups.

Survival assessment. Overall survival in the entire patient sample after 36 months of follow-up was 36.2% (64/177), with a median survival of 16 months. In patients with NSCLC with a positive EGFR mutation status, 36-month survival is 2.5 times higher (Fig. 2). Thus, in treated patients with the wild type EGFR (wt), overall survival was 29.3% (44/150), with the mutant type (mt) of the gene - 74.1% (20/27), respectively. Differences in survival between patients with EGFR-wt and patients with mt-EGFR NSCLC are statistically significant (p = 0.0003). The median survival for patients without mutations was 13 months, in patients with the EGFR-mutant type, the median was more than 36 months, the event corresponding to the median value did not occur. The obtained data confirm the fact that the presence of EGFR gene mutation is a reliably significant positive prognostic factor for increased survival.

Gender. In the patient groups by gender, survival in men and women differed (Figs. 3 and 4). Thus, in men with the wild type of EGFR, the overall survival was 26.8% (30/112), with the mutant type of the gene 50.0% (5/10), the differences were insignificant (p = 0.31). The median survival, in this case, was 11 and 19 months, respectively. In women, in the presence of mutations, the overall survival significantly increased by 2.4 times. Thus, with a negative status of the EGFR gene, survival was 36.8% (14/38), with a positive one - 88.2% (15/17), (p = 0.001). The median survival in the absence of mutations is 11 months. In patients with the mutant type of the EGFR gene, the event corresponding to the median value of three-year overall survival did not occur.

Age. In the cohort of patients divided by age, survival also differed. In the group of patients under 65 years old, three-year overall survival, in the presence of mutations, was significantly 3 times higher. Thus, in the group of patients without mutations, it was 31.4% (27/86), in patients with mutation - 93.3% (14/15), the differences are significant (p = 0.0002). The median survival in the absence of mutations is 13 months. In patients under 65 years old with a mutant type of the EGFR gene, the event corresponding to the median value of three-year overall survival did not occur. In the group of patients 65 years and older, EGFR-wt status corresponded to survival of 26.6% (17/64), EG-

FR-mt - 50.0% (6/12), the differences are insignificant (p = 0.24). The median survival is 12 and 19 months, respectively.

Disease stages. Patient survival also differed depending on the disease stage. Thus, in the group with stages I and II, in the absence of mutations, the survival rate was 69.4% (25/36), with the mutant type, all 14 patients were alive at the time of observation (p = 0.025). In patients with stages I and II of the disease with both types of the EGFR gene, the event corresponding to the median value of three-year overall survival did not occur. In the group of patients with stages III and IV of the disease, survival is lower. In patients without mutations, survival is 16.7% (19/114), with a mutation it is 2.8 times higher - 46.2% (6/13), the differences are insignificant (p = 0.09). The median survival was 10 and 19 months, respectively.

Nationality. Patient survival did not show significant differences depending on ethnicity; the presence of the mutation increased survival in both national groups. Thus, in the "Russian" group, survival increased by 3.2 times from 24.6% (17/69) to 80% (4/5), (p=0.055) and by 2.1 times in the "Sakha (Yakut)" group, from 33.8% (24/84) to 71.4% (15/21), (p=0.009). The median survival in the absence of mutations was 11 and 15 months, respectively. With the mutant type of the gene, the event corresponding to the median value of three-year overall survival did not occur in both national groups.

As a result of the subgroup analysis of overall survival data, a number of prognostic factors for the survival of patients with NSCLC adenocarcinoma were constructed (Fig. 4). In all studied patient groups, the presence of EGFR mutations resulted in increased overall survival. Taking into account statistical significance, the following can be distinguished, arranged in descending order of significance: early stage of the disease (I and II), patient age under 65, female gender. In patients without EGFR mutations, the early stage of the disease (stages I and II) is reliably significant as a positive prognostic factor.

Conclusion. The total frequency of Del 19 and L858R mutations of the EGFR gene in the total sample of examined patients (15.2%) generally corresponds to the frequency range of these mutations in the Russian population (13-29%). In women, mutations were significantly 3.8 times more common (30.9%) than in men (8.2%). In patients with stages I and II of the disease, mutations were detected 2.8 times more often (28.0%) than in patients with stages III and IV (10.2%). In patients in the "Sakha (Yakut)" group, the frequency of mutations is 3.4 times higher than in the "Russians" group (22.8/6.8%). In the "Russian" group, deletions (del 19) were more common - 5.4% and point substitutions (L858R) were less common - 1.4%, while in the "Sakha (Yakut)" group they were distributed approximately equally. In "Sakha (Yakut)" men, the incidence of EGFR gene mutations was 9.6 times higher than in men in the "Russian" group (16.3/1.7%). In women in the "Sakha (Yakut)" and "Russian" ethnic groups, the frequency of mutations did not differ significantly. Evaluation by age groups of patients did not show significant differences in the frequency of EGFR gene mutations.

With a positive status of EGFR mutations in patients with NSCLC adenocarcinoma, 36-month survival increases by 2.5 times from 29.3% to 74.1%. Subgroup analysis allowed us to additionally identify stages I and II of the disease, age up to 65 years and female gender as positive prognostic factors, in which patient survival is higher by 1.4/2.9/2.4 times, respectively.

Data on the frequency of EGFR gene mutations in patients with NSCLC adenocarcinoma in the Yakutsk Republican Oncology Dispensary were obtained for the first time and may be important for filling the "blank spots" of the frequency map of driver oncomutations in Russia and the world. Analysis of the results of the work showed that the presence of EGFR gene mutation is a significant positive prognostic factor for diagnosis, and the data obtained are important in determining treatment tactics and planning medical care in oncology.

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