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## **Clinical impact of *STK11* mutation in advanced-stage non-small-cell lung cancer**

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## **Abstract**

*Background:* Mutations in *STK11/LKB1* gene present a negative impact on tumor immune microenvironment, especially with concomitant activating *KRAS* mutation. These recent data may explain a decreased response to immunotherapy treatment in *STK11* mutant non-small cell lung cancer (NSCLC).

*Objective:* The primary objective is to evaluate, in a real-life setting, overall survival (OS) in NSCLC patients according to the presence of *STK11* mutation. The secondary objective is to assess time to treatment failure (TTF) for the first line of chemotherapy or immunotherapy.

*Methods:* This observational multicentric study was conducted in Nouvelle-Aquitaine (France), for 24 months. Clinical, histopathological and imagery data were collected in each center while NGS analysis was performed in Bordeaux Hospital University. Patient's data were longitudinally followed from NSCLC diagnosis date to the occurrence of censoring events (therapeutic failure or death, as applicable) or until study end date.

*Results:* Median OS from the first drug administration was significantly longer for *STK11*<sup>wt</sup> patients compared to *STK11*<sup>mut</sup> patients (16.2 months [11 - nr] vs. 4.7 months [2.5 - 9.4]; Log-rank test  $P < 0.001$ ). Presence of *STK11* mutation was significantly associated with shortened OS (RR = 2.26 [1.35 - 3.79],  $P = 0.002$ ). First-line TTF was significantly shorter in *STK11*<sup>mut</sup> population and the presence of the mutation was significantly associated with an increase in treatment failures (RR = 1.87 [1.21 - 2.89],  $P = 0.005$ ). The type of treatment (chemotherapy, immunotherapy) does not influence the amplitude of reduced TTF in *STK11*<sup>mut</sup> patients.

*Conclusion:* The presence of *STK11* mutation is associated to poor prognosis in NSCLC.

**Key words:** NSCLC; *STK11*; overall survival; TTF; Immunotherapy; *KRAS*

**Word count:** 2762

## **Abbreviations used**

ALK: anaplastic lymphoma kinase

CCL9: chemokine (C-C motif) ligand 9

CTLA-4: cytotoxic T-lymphocyte-associated protein 4

ECOG PS: Eastern Cooperative Oncology Group Performance Scale

EGFR: epidermal growth factor receptor

ICI: immune checkpoint inhibitors

IL-23: interleukin 23

KRAS: kirsten ras proto-oncogene, GTPase

LKB1: liver kinase B1

NGS: Next Generation Sequencing

NK cells: natural killer cells

NSCLC: non–small-cell lung cancer

ORR: objective response rate

OS: overall survival

PCR: polymerase chain reaction

PD-1: programmed cell death protein 1

PD-L1: programmed cell death-ligand 1

ROS1: c-ros oncogene 1

STK11: serine/threonine kinase 11

TMB: tumor mutational burden

TKI: tyrosine kinase inhibitor

TP53: tumor protein p53

TTF: time to treatment failure

## Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide and is responsible for 1.76 million deaths per year [1]. In Non-Small Cell Lung Cancer (NSCLC), the identification of oncogenic driver alterations and the understanding of immune checkpoint protein's role lead to the development of targeted-drug therapies and immunotherapy, respectively. While these treatments has provided clinical benefits, the tumors inevitably develop drug resistance. For example, only about 20% of patients have a durable response to immune checkpoint inhibitors (ICI) as monotherapy in advanced or metastatic NSCLC [2,3]. Expression of *programmed death-ligand 1* (PD-L1) on the membrane of tumour is associated with an increased objective response rate (ORR) to PD-1/PD-L1 inhibitors [2–9], although this biomarker is neither sensitive nor specific [2,3,6,7,10–13]. A higher burden of non-synonymous somatic mutations (tumor mutation burden, TMB), is also correlated with increased likelihood of clinical benefit [8,14–16], but its detection is not currently used in routine clinical practice [17]. Thus, the mechanisms of resistance to immunotherapy in NSCLC remains largely unknown and no single biomarker is able to predict responders from non-responders [18].

Recent studies suggest that concomitant genomic alterations in *STK11* (Serine threonine kinase 11), *KRAS* (Kirsten ras proto-oncogene) and *TP53* (tumor protein p53) genes define NSCLC subgroups with distinct immune profiles and possibly different clinical responses to immunotherapy [19,20]. *STK11* gene is a tumour suppressor gene encoding the serine threonine kinase LKB1 (Liver kinase B1) that activate AMPK signalling pathway [21–23]. *STK11* mutations are found in many types of cancer such as NSCLC [24,25] and up to 30% of NSCLC patients present LKB1 inactivation [26]. *KRAS* gene is also a common pro-oncogenic factor in NSCLC, found in approximately 30% of tumors [27,28].

*STK11* inactivation in mutated *KRAS* mouse pulmonary adenocarcinoma cell lines confers resistance to anti-PD1 and anti-CTLA4 monoclonal antibodies, decreases PD1 tumor expression and T-cell tumor infiltration [29]. In human pulmonary adenocarcinomas, concomitant *KRAS* and *STK11* mutations lead to a reduction in the T-cell-related transcriptional signature [30]. Indeed, *STK11<sup>mut</sup>/KRAS<sup>mut</sup>* lung tumors overproduce CCL9 and IL-23 cytokines inducing macrophages chemotactism and lower recruitment of T lymphocytes, NK cells and type 1 conventional dendritic cells (critical activator of antitumor

immune response) [31]. Such an immunosuppressive mechanism might explain a potential reduction of ICI treatment efficacy [31–33].

To date, few clinical studies have investigated the clinical consequences of *STK11* inactivating mutation in NSCLC, with divergent results [34–37]. In a recent review, the authors pointed out that further studies are needed to understand whether a single *STK11/LKB1* mutation, either associated with *KEAP1* and/or *KRAS* mutations, represents independent negative prognostic factors rather than predictive biomarker for treatment with ICI [38].

The main objective of this study is to evaluate, in real-life treatment situations, overall survival (OS) in NSCLC patients according to the presence of *STK11* mutation. The secondary objective is to assess, in the same study population, the time to treatment failure (TTF) for the first line of chemotherapy or immunotherapy treatment.

## Methods

### *Study design*

This observational study was conducted in several centers (Bordeaux University Hospital, Bagatelle Hospital, Bordeaux Nord Aquitaine and Saint Augustin clinics), located in Nouvelle-Aquitaine (France), for 24 months, from June 1<sup>st</sup>, 2018 to June 1<sup>st</sup>, 2020. Clinical and imagery data were collected in each center while genetic analysis was performed in a centralized laboratory (Department of Tumor Biology, Bordeaux University Hospital). Patient's data were longitudinally followed from NSCLC diagnosis date to the occurrence of censoring events (therapeutic failure or death, as applicable) or until study end date. Outcome measures were collected in an anonymised and protected database, located on Center for Clinical Investigation (CIC1401 Inserm, Bordeaux University Hospital), using Epi Info software (version 7.2.3.1, CDC, Atlanta, GA, USA). All investigations have been conducted according to the principles expressed in the Declaration of Helsinki. The research protocol was approved by the IRB/IEC of Bordeaux Hospital University (France) before the start of the study.

### *Participants*

Subjects aged more than 18 years were eligible for enrolment if they had a histopathological diagnosis of NSCLC according to the WHO classification of tumours [39]. Main inclusion criteria were stage of locally advanced (not eligible for local treatment) or metastatic disease [40]. Patients received chemotherapy and/or immunotherapy as a first-line treatment. Main exclusion criterion was presence of a druggable genetic alteration (mutation or fusion) in *EGFR*, *ALK* or *ROS1* genes resulting in tyrosine kinase inhibitor (TKI) therapy. Details of inclusion/exclusion criteria and prohibited/authorised concomitant medications are provided in the online data supplement.

### *Gene mutation analysis*

Somatic mutations within 15 genes of interest (*ALK*, *BRAF*, *EGFR*, *ERBB2*, *ERBB4*, *FGFR3*, *KIT*, *KRAS*, *MAP2K1*, *MET*, *NRAS*, *PDGFRA*, *PIK3CA*, *POLE*, *STK11*) were identified using targeted next-generation sequencing (NGS) between June 1<sup>st</sup>, 2018 and December 1<sup>st</sup>, 2019. The mutational status were determined by NGS, using a custom Ion Ampliseq panel (Thermo Fisher Scientific, Life Technologies, Les Ullis, France). Libraries were amplified by emulsion PCR (polymerase chain reaction), enriched using automatic system Ion Chef automaton

(Thermo Fisher Scientific) and then sequenced on 530 chips (ThermoFisher Scientific) using an Ion Torrent S5 sequencer (ThermoFisher Scientific) with a high average sequencing depth (greater than 300X). Torrent Suite™ version 5.0 software (ThermoFisher Scientific) was used to perform data analysis. Reads were mapped to the human hg19 reference genome. Data processing, alignment and mutation calling were performed using the Torrent Suite™. The Variant Caller detected point mutations with a variant frequency  $\geq 2\%$  for Single Nucleotide Variation (SNV) and  $\geq 5\%$  for short insertion/deletion (INDEL). VCF files generated by Variant Caller were annotated by the ANNOVAR [41].

### *Outcome Measures*

The OS (primary endpoint) is defined as the time from the first administration of chemotherapy or immunotherapy to the patient's death, whatever its cause. The TTF (secondary endpoint) is defined as the interval from first-line treatment initiation to treatment failure related to death, disease progression, drug toxicity, addition of a new cancer therapy such as radiation therapy. A complete list of collected data is available in the online data supplement.

### *Statistical Analysis*

Study endpoints were estimated based on the presence of *STK11* mutation, using Kaplan-Meier method and compared by Log-rank test. Prognostic factors were first determined using univariate Cox proportional risk models and then evaluated using time-dependent Cox models. Time-dependent variables are: First-line chemotherapy, first-line immunotherapy, second-line chemotherapy, second-line immunotherapy, period between first and second treatment and period following second-line treatment. For all models, a sensitivity analysis was performed, considering the chemo-immunotherapy combination in chemotherapy class and not in immunotherapy class, as in the main analysis. *Post hoc* analyses were carried out for all models with 4 variable categories: *STK11*<sup>wt</sup>/*KRAS*<sup>wt</sup> (Wild-type *STK11* and *KRAS* gene), *STK11*<sup>wt</sup>/*KRAS*<sup>mut</sup> (Wild-type *STK11* and Mutated *KRAS* gene), *STK11*<sup>mut</sup>/*KRAS*<sup>wt</sup> (Mutated *STK11* and Wild-type *KRAS* gene) and *STK11*<sup>mut</sup>/*KRAS*<sup>mut</sup> (Mutated *STK11* and *KRAS* gene). In each model and for each factor tested, the relative risk (RR), 95% confidence interval and the associated *P* value were presented. Imputation methods were not conducted for missing values, which are also presented. Qualitative variables were described in numbers and percentages. Quantitative variables were described in numbers, mean, standard deviation, median, range between 1<sup>st</sup> (q1) and 3<sup>rd</sup> (q3) quartiles. Comparisons between groups were

performed by means of two-sided independent t tests for variables with a parametric distribution, Chi-square or Fisher's exact test for comparison of proportions and the Mann-Whitney U test for comparison of nonparametric variables. The statistical analysis was performed using SAS software (SAS Institute, version 9.4, Cary, NC, USA).

## Results

### *Study population*

531 patients affected with NSCLC were identified in our NGS database. According to inclusion/non inclusion criteria, 159 patients were included in the study population for analysis (Figure 1).

Patients characteristics according to *STK11* mutational status are summarised in Table 1 and supplementary Table 1. In the studied population, patients, with advanced disease by definition, have higher ECOG score as compared to baseline population (score 2: 24.5% vs. 15.3%) and an increased frequency of metastases at diagnosis (81.8% vs. 51.1%). No significant differences were found for PD-L1 receptor exposure rate between the 2 populations.

Data of NGS analysis are shown in Supplementary Table 2. In the baseline population, 459 patients (86.4%) were *STK11*<sup>wt</sup>, while 72 (13.6%) were *STK11*<sup>mut</sup> (one patient had mutations in 2 exons). In the studied population, 111 patients (69.8 %) had at least one mutation and 48 patients (30.2 %) had no genetic mutation. In the study population, there was 122 (76.7%) *STK11*<sup>wt</sup> and 37 (23.3%) *STK11*<sup>mut</sup>. The median time of follow-up was 8.5 months (interval q1-q3: 3 – 13.6).

Median age at diagnosis of *STK11*<sup>mut</sup> patients was 62.7 years (range q1-q3: 53.3 - 69.7), comparable to *STK11*<sup>wt</sup> patients. ECOG score, proportion of metastatic patients at the time of diagnosis, and histological characteristics were not different between the two groups, despite the absence of squamous cell tumours in *STK11*<sup>mut</sup> patients. PD-L1 receptor expression on cancer cells was higher in *STK11*<sup>wt</sup> ( $\geq 50\%$  in 37.7% vs. 21.6%), while the presence of *KRAS* mutation was statistically higher in *STK11*<sup>mut</sup> (64.8% vs. 40.2%;  $P = 0.008$ ).

In *STK11*<sup>mut</sup> group, 31 patients (83.8%) received first-line chemotherapy and 6 (16.2%) immunotherapy. Among *STK11*<sup>wt</sup> patients, 68% received chemotherapy, 26.2% immunotherapy and 5.8% a combination of chemotherapy and immunotherapy. Following first-line therapy failure, 17 patients (46%) *STK11*<sup>mut</sup> and 62 (50.8%) *STK11*<sup>wt</sup> received a second-line treatment. Patient characteristics, according to mutations in *STK11* and *KRAS* genes, are shown in Supplementary Table 3.

### *Overall survival*

The median OS was 12.1 months (CI: 8.8 – 18, interval q1-q3: 3.34 – nr) in the studied population. Seventy-nine patients died (49.7%), among which 53 in *STK11*<sup>wt</sup> group (43.4%) and 26 in *STK11*<sup>mut</sup> group (70.3%) (Figure 2). Median OS was significantly shorter in *STK11*<sup>mut</sup> (4.7 months, 95% CI: 2.5 - 9.4 vs 16.2 months, 95% CI: 11 - nr;  $P < 0.001$ ) (Figure 2A). Similar results were obtained for co-mutations *STK11* and *KRAS* ( $P = 0.0139$ ), (Figure 3A).

In Cox multivariate time-dependent model (Table 2), *STK11* gene mutation was significantly associated with a reduced OS (RR = 2.26 [1.35 - 3.79],  $p = 0.002$ ). In *STK11*<sup>mut</sup>, higher age at diagnosis of NSCLC was associated with a longer survival (RR = 0.93 [0.90 - 0.93],  $P = 0.026$ ). *Post-hoc* analysis on the impact of double mutation *STK11* and *KRAS* showed comparable results, which is in favor of the predominant effect of *STK11* mutation on NSCLC prognosis (Supplementary Table 4).

#### *Time to Treatment Failure*

The median duration of first-line treatment was 3.2 months (q1-q3 interval: 1.4 - 8.2; mean: 5.3 months) in the studied population. In *STK11*<sup>wt</sup> group, 98 therapeutic failures (80.3%) and 24 censures (19.7%) were observed, compared to 33 failures (89.2%) and 4 censures (10.8%) in *STK11*<sup>mut</sup> group. Kaplan-Meier analysis showed that *STK11*<sup>wt</sup> had a significantly longer TTF (median: 3.9 months; 95% CI: 3.1 - 5.9 vs. median: 2.2 months; 95% CI: 1.2 - 3.5;  $P = 0.031$ ) (Figure 2B).

Multivariate analysis, adjusted to age at diagnosis, sex, smoking exposure, *KRAS* mutation, metastatic status at diagnosis, ECOG PS score at diagnosis and type of treatment, confirmed that *STK11* mutation was associated with an increased risk of first-line therapy failure (RR = 1.87 [1.21 - 2.89],  $P = 0.005$ ), (Table 3). Although the number of patients receiving ICI was low, immunotherapy treatment or the combination of immuno-chemotherapy might be a protective factor against failure of first-line treatment in the studied population (RR = 0.37 [0.23 – 0.59],  $P < 0.001$ ) and in *STK11*<sup>wt</sup> group, conversely to *STK11*<sup>mut</sup> group (Table 3).

*Post-hoc* analysis showed that reduced time to treatment failure is mainly dependent on *STK11* mutation rather than *STK11*-*KRAS* double mutation (Figure 3B and Supplementary Table 5).

## Discussion

This study demonstrated that the presence of *STK11* mutation is associated to reduction in OS and TTF in advanced-stage NSCLC. This is, to our knowledge, the first study that evaluates the clinical impact of this gene mutation in a real-life cohort.

To date, the role of *STK11* mutation in NSCLC prognosis remains a matter of debate. In early-stage NSCLC, the reduction in STK11 protein expression has been described as an independent negative prognostic factor for survival [34,35]. By contrast, tumour specimens analysis from 221 surgically resected stage I and II NSCLC demonstrated no difference in survival between *STK11*<sup>mut</sup> tumors (n=23; 10.4%) and *STK11*<sup>wt</sup> tumors (n=198; 89.6%,  $P = 0.17$ ) [36]. Similar pattern was found among 302 patients with advanced NSCLC treated with chemotherapy [37]. However, a retrospective study conducted on 2276 patients with NSCLC showed that *STK11* mutations were independently associated to poor prognosis whatever the type of treatment and regardless of the presence of *KRAS* mutations [42]. Moreover, preliminary results from the Keynote 042 trial reinforced the hypothesis that *STK11* mutation is a negative prognostic factor in NSCLC [43], which is consistent with the results obtained in our study on overall survival (primary outcome).

This observational study was not designed to compare the efficacy of chemotherapy and immunotherapy among patients with or without *STK11* mutation. Analysis of TTF (secondary outcome) provides useful information but should be interpreted with caution. First-line immunotherapy was a protective factor compared to first-line chemotherapy in the whole studied population. A similar trend was observed in *STK11*<sup>wt</sup> group, although patients had higher level of PD-L1 expression. In *STK11*<sup>mut</sup> group, TTF is reduced whatever the first-line of treatment administered. Thus, response to treatment is altered in *STK11*<sup>mut</sup> patients either treated with chemotherapy or ICI, which is probably related to the consequences of STK11 mutation on NSCLC pathogenesis and prognostic rather than hypothetical mechanisms of resistance to drugs.

Although this study was conducted in real-life conditions, we paid a special attention to the representativeness of study participants. For example, no *STK11*<sup>mut</sup> patient have been diagnosed with squamous cell histotype of NSCLC. It should be noted that the proportion of patients affected with squamous cell carcinoma is low, around 8%, in *STK11*<sup>mut</sup> NSCLC ([37]), and that patients with an epidermoid histotype represented only 2.8% of subjects in our database. In *STK11*<sup>mut</sup>, higher age at diagnosis was associated with a longer survival, as

younger patients were more likely to have aggressive diseases, rather than older patients. The proportion of *STK11*<sup>mut</sup> patients in our cohort (23.3%) is consistent with those in the most recent studies on this topic [30,44]. However, we did not detect a significant correlation between the presence of *STK11* mutation, histotype ( $P = 0.8$ ) and smoking exposure ( $P = 0.2$ ), which was found by other authors [36,45–47]. Lastly, PD-L1 expression in the overall cohort was similar to previous study [18], although we report lower levels in *STK11*<sup>mut</sup> patients.

In a *post-hoc* analysis, the effect of concomitant presence of *STK11* and *KRAS* mutations was assessed, mainly due to the abundant literature in this field of interest [19,20,44,48]. Briefly, the presence of *KRAS* mutation is not associated to a clinically important difference between groups. This finding strengthens the idea that the main results observed in our study are related to the presence of *STK11* mutation. Further studies are needed to determine if co-occurring genomic alterations in *STK11* and *KRAS* induce therapeutic vulnerabilities [38]. In addition, genomic position of *STK11* mutation, particularly the exon including the mutation, has been described as an independent prognostic factor in NSCLC [44]. Nonetheless, we paid particular attention to avoid multiple subgroups analysis. For example, the number of *STK11*<sup>mut</sup> subjects was too low to compare OS and TTF among mutations subgroups (Supplementary Table 2). Although the NGS panel used for this study revealed the presence of mutations in the 15 genes described above, it remains possible that other mutations may have influenced our clinical endpoints, notably *KEAP1* and *TP53*, respectively involved in immunoresistance mechanisms and sensitivity to PD-1 blockade. Moreover, the impact of mutations of interest cannot be assessed among each chemotherapy and immunotherapy drug. Indeed, all *STK11*<sup>mut</sup> patients who received first-line immunotherapy were treated with pembrolizumab, while nivolumab (n=4), pembrolizumab (n=2) and atezolizumab (n=6) were only administered as second-line therapy. Although this study is observational, all patients cases were discussed in a unique standardized multidisciplinary meeting and related therapeutic decisions were made according to international recommendations. Moreover, somatic mutations analysis were performed at the same Molecular Genetics Laboratory at in Bordeaux University Hospital.

## **Conclusion**

The presence of STK11 mutation results in significant clinical impact in advanced-stage NSCLC. Despite its limitations, this real-life study confirms that STK11 mutation is a negative prognostic factor in NSCLC rather than a predictive marker of treatment response. Indeed, whatever the first line systemic therapy (chemotherapy or immunotherapy), STK11 mutation is also associated to reduced TTF. The study results suggest that additional prospective studies are needed to define the best treatment strategy for those patients at higher risk of morbidity and mortality. Further developments in immunotherapy and therapies targeting STK11 are research priorities to improve clinical outcomes for NSCLC patients.

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## Tables

**Table 1**

|                              | Study population<br>(n = 159) | <i>STK11</i> <sup>mut</sup><br>(n = 37) | <i>STK11</i> <sup>wt</sup><br>(n = 122) | <i>P</i><br>values |
|------------------------------|-------------------------------|---|---|--------------------|
| Age at diagnosis             |                               |   |   |                    |
| Median                       | 62.9                          | 62.7                                    | 63.5                                    |                    |
| Interval q1 – q3             | 53.3 - 70.7                   | 53.3 – 69.7                             | 53.4 – 70.9                             |                    |
| Sex                          |                               |   |   | 0.69               |
| Men                          | 99 (62.3)                     | 22 (59.5)                               | 77 (63.1)                               |                    |
| Women                        | 60 (37.7)                     | 15 (40.5)                               | 45 (36.9)                               |                    |
| Histology                    |                               |   |   | 0.57               |
| Adenocarcinoma               | 130 (81.7)                    | 30 (81.1)                               | 100 (82)                                |                    |
| Epidermoid                   | 3 (1.9)                       | 0                                       | 3 (2.5)                                 |                    |
| Other                        | 26 (16.4)                     | 7 (18.9)                                | 19 (15.5)                               |                    |
| ECOG PS score                |                               |   |   | 0.64               |
| 0                            | 29 (18.3)                     | 5 (13.5)                                | 24 (19.7)                               |                    |
| 1                            | 83 (52.2)                     | 20 (54.1)                               | 63 (51.6)                               |                    |
| 2                            | 39 (24.5)                     | 11 (29.7)                               | 28 (23)                                 |                    |
| 3                            | 8 (5)                         | 1 (2.7)                                 | 7 (5.7)                                 |                    |
| Metastasis at diagnosis      |                               |   |   | 0.27               |
| Yes                          | 130 (81.8)                    | 28 (75.7)                               | 102 (83.6)                              |                    |
| No                           | 29 (18.2)                     | 9 (24.3)                                | 20 (16.4)                               |                    |
| PDL1                         |                               |   |   | 0.14               |
| < 1 %                        | 56 (35.2)                     | 17 (46)                                 | 39 (32)                                 |                    |
| 1 – 49 %                     | 47 (29.6)                     | 12 (32.4)                               | 35 (28.7)                               |                    |
| ≥ 50 %                       | 54 (34)                       | 8 (21.6)                                | 46 (37.7)                               |                    |
| Missing                      | 2 (1.2)                       | 0                                       | 2 (1.6)                                 |                    |
| <i>KRAS</i>                  |                               |   |   | < 0.01             |
| Muted                        | 73 (45.9)                     | 24 (64.8)                               | 49 (40.2)                               |                    |
| wt                           | 86 (54.1)                     | 13 (35.2)                               | 73 (59.8)                               |                    |
| Smoking status               |                               |   |   | 0.22               |
| Current smoker               | 80 (50.3)                     | 18 (41.9)                               | 62 (50.8)                               |                    |
| Ex-smoker                    | 67 (42.1)                     | 19 (55.8)                               | 48 (39.4)                               |                    |
| Non-smoker                   | 7 (4.4)                       | 0                                       | 7 (5.7)                                 |                    |
| Missing                      | 5 (3.2)                       | 0                                       | 5 (4.1)                                 |                    |
| First-line treatment         |                               |   |   | 0.11               |
| Chemotherapy                 | 114 (71.7)                    | 31 (83.8)                               | 83 (68)                                 |                    |
| Immunotherapy                | 38 (23.9)                     | 6 (16.2)                                | 32 (26.2)                               |                    |
| Combined chemo-immunotherapy | 7 (4.4)                       | 0                                       | 7 (5.8)                                 |                    |
| Second-line treatment        |                               |   |   | 0.76               |
| Yes                          | 79 (49.7)                     | 17 (46)                                 | 62 (50.8)                               |                    |
| Chemotherapy                 | 21 (26.6)                     | 5 (29.4)                                | 16 (25.8)                               |                    |
| Immunotherapy                | 58 (73.4)                     | 12 (70.6)                               | 46 (74.2)                               |                    |
| No                           | 80 (50.3)                     | 20 (54)                                 | 60 (49.2)                               |                    |
| Dead                         | 37 (46.3)                     | 14 (70)                                 | 23 (38.4)                               |                    |
| Censored                     | 43 (53.7)                     | 6 (30)                                  | 37 (61.6)                               |                    |
| Death on 2 Jun 2020          |                               |   |   | < 0.01             |
| Yes                          | 79 (49.7)                     | 26 (70.3)                               | 53 (43.4)                               |                    |
| Censored                     | 80 (50.3)                     | 11 (29.7)                               | 69 (56.6)                               |                    |

Characteristics of the study population.

Data are presented as n (%), except age at diagnosis. ECOG PS, Eastern Cooperative Oncology Group Performance Scale; *KRAS*: Kirsten Rat Sarcoma virus; mut: Mutant; PDL1, Programmed cell death ligand 1; *STK11*, Serine/threonine kinase 11; wt: Wild-Type. *P* values were calculated with the use of a two-sided

independent t-test for variables with a parametric distribution and Fisher's exact test for comparison of proportions and the Mann-Whitney U test for comparison of nonparametric variables.

**Table 2**

|  | Study population<br>(n = 154) |                 | <i>STK11</i> <sup>wt</sup><br>(n = 117) |                 | <i>STK11</i> <sup>mut</sup><br>(n = 37) |                 |
|--|-------------------------------|-----------------|---|-----------------|---|-----------------|
|  | RR [95% IC]                   | <i>P</i> values | RR [95% IC]                             | <i>P</i> values | RR [95% IC]                             | <i>P</i> values |
| <i>STK11</i> <sup>mut</sup> vs. <i>STK11</i> <sup>wt</sup> | 2.26 [1.35 – 3.79]            | 0.002           | -                                       | -               | -                                       | -               |
| Ex-smoker vs. current smoker                               | 0.59 [0.34 – 1.04]            | 0.067           | 0.41 [0.20 – 0.82]                      | 0.012           | 1.29 [0.450 – 3.680]                    | 0.639           |
| Non-smoker vs. current smoker                              | 0.39 [0.11 – 1.31]            | 0.127           | 0.20 [0.05 – 0.76]                      | 0.019           | -                                       | -               |
| Women vs. men  | 1.34 [0.81 – 2.20]            | 0.255           | 1.78 [0.93 – 3.39]                      | 0.081           | 1.24 [0.492 – 3.100]                    | 0.653           |
| Age at diagnosis (per year)                                | 1.01 [0.99 – 1.03]            | 0.501           | 1.03 [1.00 – 1.07]                      | 0.035           | 0.93 [0.895 – 0.933]                    | 0.026           |
| <i>KRAS</i> <sup>mut</sup> vs. <i>KRAS</i> <sup>wt</sup>   | 1.06 [0.65 – 1.73]            | 0.815           | 0.88 [0.47 – 1.63]                      | 0.675           | 2.46 [0.868 – 6.987]                    | 0.090           |
| Metastasis at diagnosis                                    | 3.48 [1.54 – 7.86]            | 0.003           | 4.16 [1.24 – 13.89]                     | 0.021           | 3.15 [0.832 – 11.909]                   | 0.091           |
| ECOG PS 2-3 scores vs. ECOG PS 0-1 scores                  | 4.73 [2.79 – 8.00]            | < 0.001         | 4.53 [2.35 – 8.75]                      | < 0.001         | 5.82 [2.101 – 16.132]                   | < 0.001         |
| First-line chemotherapy                                    | 2.10 [0.86 – 5.16]            | 0.106           | 1.80 [0.51 – 6.31]                      | 0.361           | 3.03 [0.728 – 12.581]                   | 0.128           |
| First-line immunotherapy                                   | 0.82 [0.25 – 2.68]            | 0.738           | 0.54 [0.11 – 2.72]                      | 0.452           | 1.93 [0.270 – 13.853]                   | 0.511           |
| Second-line chemotherapy                                   | 2.41 [0.52 – 11.25]           | 0.265           | 3.15 [0.43 – 23.33]                     | 0.261           | 6.03 [0.372 – 97.574]                   | 0.206           |
| Second-line immunotherapy                                  | 6.47 [2.03 – 20.58]           | 0.002           | 5.07 [1.01 – 25.45]                     | 0.048           | 13.96 [1.966 – 99.180]                  | 0.008           |
| Period between first and second treatment                  | 8.36 [2.73 – 25.61]           | < 0.001         | 7.27 [1.58 – 33.34]                     | 0.011           | 26.68 [3.062 – 232.58]                  | 0.003           |
| Period following second-line treatment                     | 23.33 [7.09 – 76.80]          | < 0.001         | 25.80 [5.07 – 131.34]                   | < 0.001         | 116.26 [7.560 – 1787.6]                 | < 0.001         |

Time-dependent Cox analysis of the overall survival in the study population and according to *STK11* status.

ECOG PS, Eastern Cooperative Oncology Group Performance Scale; KRAS: Kirsten Rat Sarcoma virus; mut: Mutant; PDL1, Programmed cell death ligand 1; *STK11*, Serine/threonine kinase 11; wt: Wild-Type.

Prognostic factors were first determined using univariate Cox proportional risk models and then evaluated using time-dependent Cox models.

**Table 3**

|  | Study population<br>(n = 154) |                 | <i>STK11</i> <sup>wt</sup><br>(n = 117) |                 | <i>STK11</i> <sup>mut</sup><br>(n = 37) |                 |
|--|-------------------------------|-----------------|---|-----------------|---|-----------------|
|  | RR [95% IC]                   | <i>P</i> values | RR [95% IC]                             | <i>P</i> values | RR [95% IC]                             | <i>P</i> values |
| <i>STK11</i> <sup>mut</sup> vs. <i>STK11</i> <sup>wt</sup> | 1.87 [1.21 - 2.89]            | 0.005           | -                                       | -               | -                                       | -               |
| Ex-smoker vs. current smoker                               | 0.96 [0.63 - 1.49]            | 0.870           | 0.75 [0.45 - 1.28]                      | 0.292           | 1.46 [0.61 - 3.51]                      | 0.402           |
| Non-smoker vs. current smoker                              | 1.29 [0.56 - 3.01]            | 0.549           | 1.15 [0.47 - 2.80]                      | 0.760           | -                                       | -               |
| Women vs. men  | 1.28 [0.85 - 1.92]            | 0.249           | 1.21 [0.71 - 2.05]                      | 0.485           | 1.35 [0.59 - 3.11]                      | 0.474           |
| Age at diagnosis (per year)                                | 1.00 [0.98 - 1.02]            | 0.723           | 1.00 [0.98 - 1.02]                      | 0.723           | 1.00 [0.98 - 1.02]                      | 0.723           |
| <i>KRAS</i> <sup>mut</sup> vs. <i>KRAS</i> <sup>wt</sup>   | 0.95 [0.65 - 1.39]            | 0.790           | 1.00 [0.64 - 1.57]                      | 0.990           | 1.22 [0.53 - 2.82]                      | 0.641           |
| Metastasis at diagnosis                                    | 3.11 [1.83 - 5.29]            | < 0.001         | 2.72 [1.40 - 5.32]                      | 0.003           | 4.19 [1.55 - 11.31]                     | 0.005           |
| ECOG PS 2-3 scores vs. ECOG PS 0-1 scores                  | 2.54 [1.69 - 3.81]            | < 0.001         | 2.13 [1.31 - 3.45]                      | 0.002           | 4.11 [1.69 - 9.97]                      | 0.002           |
| First-line immunotherapy vs. first-line chemotherapy       | 0.37 [0.23 - 0.59]            | < 0.001         | 0.35 [0.20 - 0.60]                      | < 0.001         | 0.46 [0.15 - 1.41]                      | 0.174           |

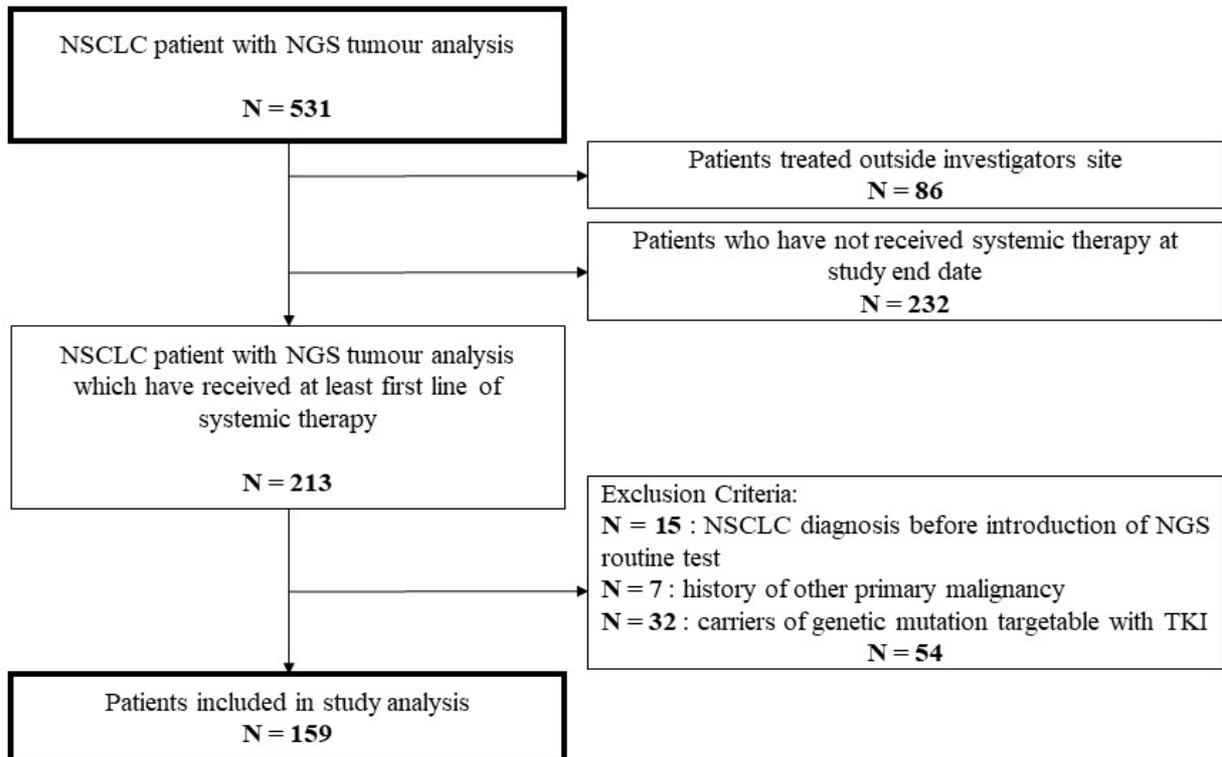
Cox analysis of the time to treatment failure in the study population and according to *STK11* status.

ECOG PS, Eastern Cooperative Oncology Group Performance Scale; KRAS: Kirsten Rat Sarcoma virus; mut: Mutant; PDL1, Programmed cell death ligand 1; *STK11*, Serine/threonine kinase 11; wt: Wild-Type.

Prognostic factors were first determined using univariate Cox proportional risk models and then evaluated using Cox models.

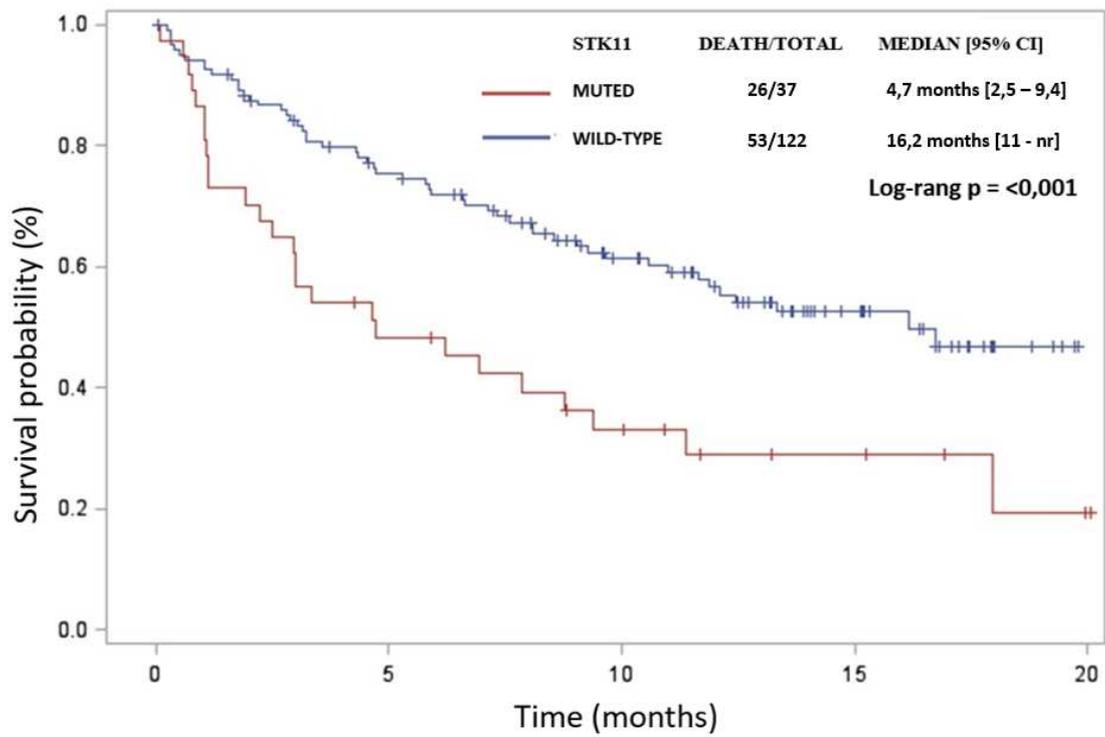
## Figures

Figure 1

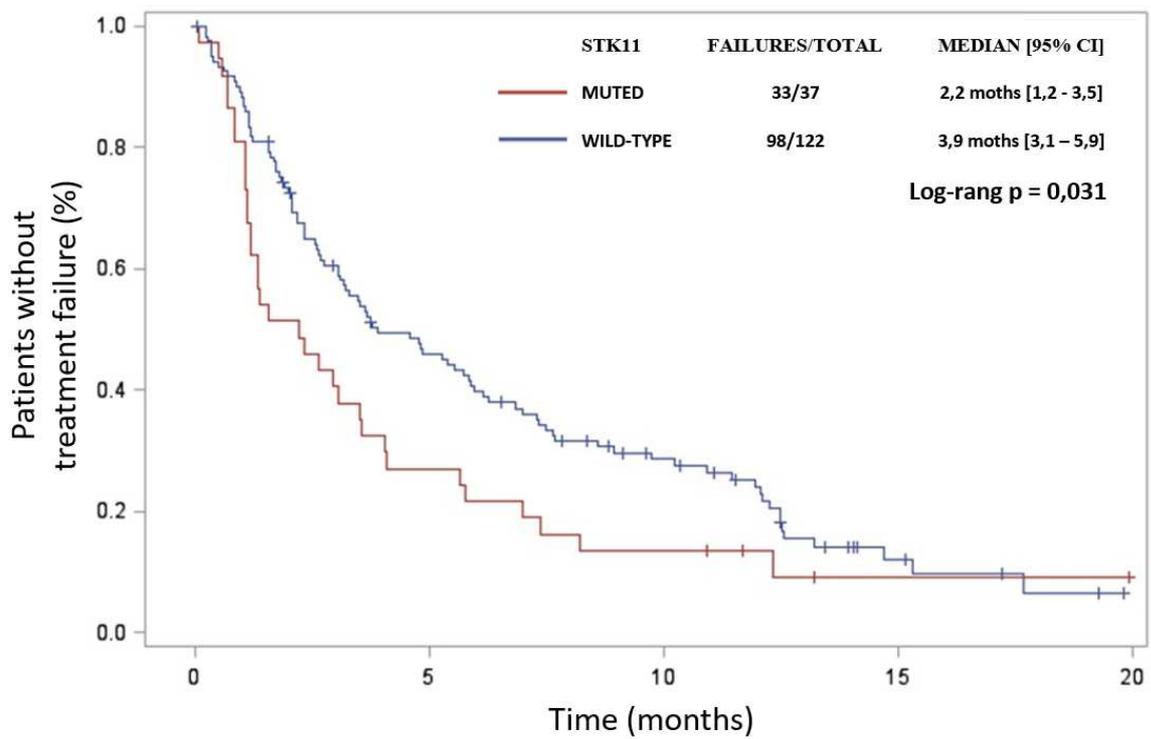


**Figure 2**

**A**

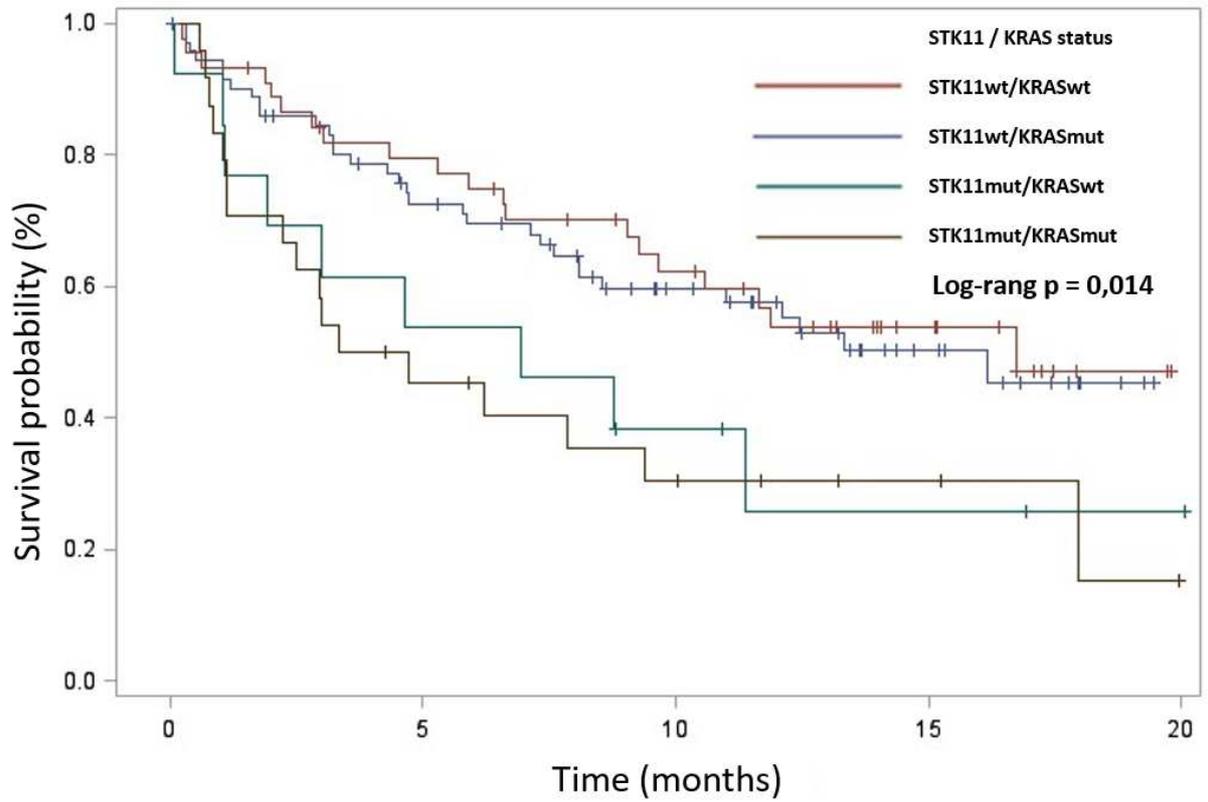


**B**

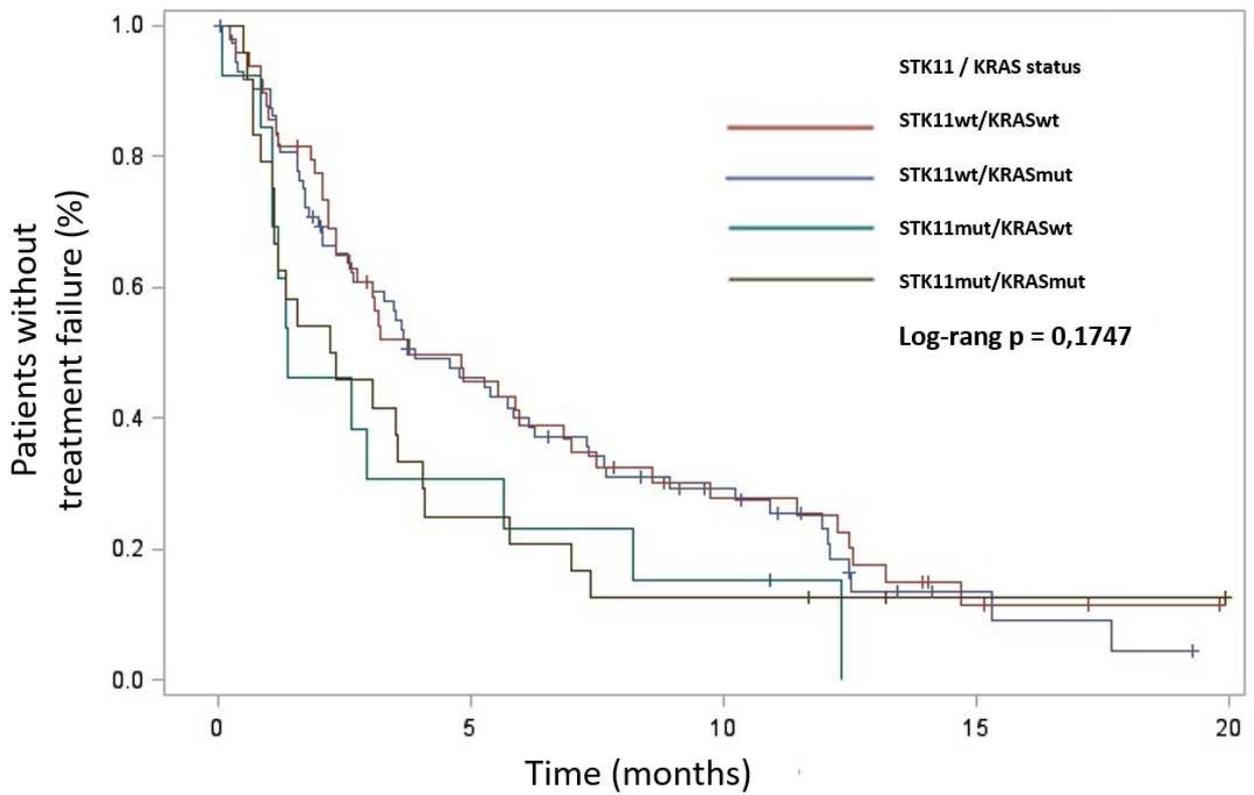


**Figure 3**

**A**



**B**



## Figure legends

### Figure 1

Flowchart describing recruitment of patients.

NGS, Next Generation Sequencing; NSCLC, non-small-cell lung cancer; TKI, tyrosine kinase inhibitor.

232 patients did not received systemic therapy for the following reasons: Early stage of NSCLC, patients treated with thoracic radiotherapy only and palliative care.

### Figure 2

Kaplan-Meier analysis of the overall survival (A) and the time to treatment failure (B) according to *STK11* status.

Blue curve: *STK11*<sup>wt</sup>. Red curve: *STK11*<sup>mut</sup>.

CI, Confidence interval; STK11, Serine/threonine kinase 11.

*P* values were calculated using Log-rank test.

### Figure 3

Kaplan-Meier analysis of the overall survival (A) and the time to treatment failure (B) according to *STK11* and *KRAS* status.

Red curve: *STK11*<sup>wt</sup> / *KRAS*<sup>wt</sup>. Blue curve: *STK11*<sup>wt</sup> / *KRAS*<sup>mut</sup>. Green curve: *STK11*<sup>mut</sup> / *KRAS*<sup>wt</sup>. Brown curve: *STK11*<sup>mut</sup> / *KRAS*<sup>mut</sup>.

*KRAS*: Kirsten Rat Sarcoma virus; mut: Mutant; STK11, Serine/threonine kinase 11; wt: Wild-Type.

*P* values were calculated using Log-rank test.