Review Article

Emerging insights into *STK11*, *KEAP1* and *KRAS* mutations: implications for immunotherapy in patients with advanced non-small cell lung cancer

Magdalena Knetki-Wróblewska[1]^, Kamila Wojas-Krawczyk[2]^, Paweł Krawczyk[2]^, Maciej Krzakowski[1]^

[1]Lung Cancer and Chest Tumours Department, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland; [2]Pneumonology, Oncology and Allergology Department, Medical University in Lublin, Lublin, Poland

Contributions: (I) Conception and design: K Wojas-Krawczyk, M Knetki-Wróblewska; (II) Administrative support: P Krawczyk, M Krzakowski; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: K Wojas-Krawczyk, M Knetki-Wróblewska, P Krawczyk; (V) Data analysis and interpretation: K Wojas-Krawczyk, M Knetki-Wróblewska, P Krawczyk; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Prof. Kamila Wojas-Krawczyk, MSc. Pneumonology, Oncology and Allergology Department, Medical University in Lublin, Jaczewskiego 8, 20-954 Lublin, Poland. Email: kamilawojas@wp.pl.

Abstract: Immune checkpoint inhibitors (ICIs) have become an established treatment option for patients with advanced non-small cell lung cancer (NSCLC). However, the efficacy of single-agent immunotherapy as well as in combination with chemotherapy seems to be dependent on the presence of molecular abnormalities in some genes—serine/threonine kinase 11 (*STK11*), Kelch-like ECH-associated protein 1 (*KEAP1*) and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) among them. The *KEAP1* gene is a critical regulator of the cellular response to oxidative stress and electrophilic stress, thus playing a pivotal role in maintaining cellular homeostasis. The *STK11* gene encodes a serine/threonine kinase (STK11) involved the regulation of cell growth, polarity, motility, differentiation and cell metabolism. The *STK11* gene mutations are often associated with an immunologically "cold" tumour microenvironment. The co-occurrence of *STK11* or *KEAP1* abnormalities with the *KRAS* mutation changes the composition of the tumour microenvironment as compared when presented alone. The current data, based on retrospective analyses of clinical trials, indicate that the co-existence of *STK11* and *KEAP1* genes mutations with the *KRAS* gene mutations have negative impact on the prognosis, regardless of treatment methods, in patients with advanced NSCLC. However, this group of patients should not be omitted because they constitute a significant percentage of advanced NSCLC patients. Immunotherapy focused on two ICIs [anti-programmed death 1 (PD-1)/anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4)] combined with chemotherapy, may be more effective than immunotherapy or chemotherapy alone in this group of patients. Confirmation of this thesis can be found in the results of available clinical studies. Here, we summarize the theoretical justification as well as the results of clinical trials for combining immunotherapy in patients with *STK11*-, *KEAP1*- and *KRAS*-mutated genes. There is certainly a need to create a prospective clinical trial to assess the effectiveness of combined immunotherapy in the discussed group of patients.

Keywords: Kelch-like ECH-associated protein 1 (*KEAP1*); serine/threonine kinase 11 (*STK11*); Kirsten rat sarcoma viral oncogene homolog (*KRAS*); immune checkpoint inhibitors (ICIs)

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^ ORCID: Magdalena Knetki-Wróblewska, 0000-0002-7603-099X; Kamila Wojas-Krawczyk, 0000-0001-9947-5169; Paweł Krawczyk, 0000-0001-8400-4452; Maciej Krzakowski, 0000-0003-3324-0900.

Introduction

Immune checkpoint inhibitors (ICIs) have become an established therapeutic option for patients with advanced non-small cell lung cancer (NSCLC). In patients with wild-type status of oncogenic driver genes, programmed death 1/programmed death ligand 1 (PD-1/PD-L1) axis inhibitors can be administered, either as monotherapy or in combination with chemotherapy. The primary objective of immunotherapy is to restore the antitumor immune response, which is typically suppressed by tumors. However, despite the significant efficacy of ICIs, their success is not universal, and not all patients, even those exhibiting high PD-L1 expression, obtain benefit from this treatment (1). The resistance to ICIs can be categorized into primary (intrinsic) resistance and acquired (secondary) resistance (2). Primary resistance in NSCLC patients occurs when the tumor microenvironment (TME) or the patient's immune system is not conducive to an effective immune response (2). These challenging cases often present with non-driver or non-actionable mutations, characterized as tumor-intrinsic genetic abnormalities that influence the TME, thereby affecting disease progression, therapeutic outcomes, and patient survival. Acquired resistance develops during treatment and may arise through various adaptive mechanisms that allow tumors to evade immune detection and destruction (2). Among the various defense mechanisms activated by cancer cells during disease progression, particular attention is currently focused on abnormalities in the Kelch-like ECH-associated protein 1 (*KEAP1*), serine/threonine kinase 11 (*STK11*), and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) genes (3-5). Furthermore, the analysis of genetic abnormalities in these genes at the time of diagnosis may provide predictive information regarding the success of frontline immunotherapy. Understanding the impact of these gene mutations on the functionality of cancer cells and the TME is crucial for improving the efficacy of ICIs (6).

The aim of this manuscript is to summaries data related to the effectiveness of immunotherapy alone and in combination with chemotherapy and immunotherapy in different cohorts of patients depending on the presence of genetic abnormalities in the genes discussed above.

KEAP1, *STK11* and *KRAS*: key mechanism in tumour promotion and suppression

The *KEAP1* gene is a critical regulator of the cellular response to oxidative stress and electrophilic stress, thus playing a pivotal role in maintaining cellular homeostasis (3,7,8). Cell response to reactive oxygen species (ROS) oxidative stress is controlled by multiple cellular regulatory factors and could be divided into the following steps (3,7,8). First, the cellular protein that acts as an ROS sensor detects the presence of danger signals and transduces them into the gene expression machinery. The next step is to activate the expression of a set of stress-responsive genes involved in cellular protection (7). At the molecular level, nuclear factor erythroid 2-related factor 2 (*NRF2*) is a master regulator of the antioxidant response. Controls the expression of genes that encode antioxidant proteins and enzymes, such as glutathione S-transferase (GST), NAD(P)H dehydrogenase 1, and heme oxygenase, which help neutralize ROS and reduce oxidative stress (4,7). *KEAP1* acts as a negative regulator of *NRF2*. Under normal conditions, *KEAP1* binds to *NRF2* and promotes its ubiquitination and subsequent degradation by the proteasome, preventing excessive accumulation of *NRF2* in cells. When cells experience oxidative stress, modifications in *KEAP1* lead to the release of *NRF2*. Free *NRF2* is translocated to the nucleus, where it binds to antioxidant response elements (ARE) in DNA, initiating the transcription of various cytoprotective genes involved in detoxification, antioxidant defense, and repair processes (4,7). Loss of function in the *KEAP1* gene can lead to uncontrolled activation of *NRF2*, which, while initially protective, can paradoxically contribute to cancer progression. Hyperactivation of *NRF2* in cancer cells can contribute to chemoresistance (very efficient and rapid removal of free oxygen radical induced by chemotherapy) and tumour progression. Elevated *NRF2* activity supports cancer cell survival by providing enhanced antioxidant capacities, allowing them to thrive under the high oxidative stress conditions characteristic of the tumour microenvironment (4,7,8).

The *KEAP1* gene is altered in 3.43% of all cancers with lung adenocarcinoma, squamous-cell lung carcinoma, colon adenocarcinoma, and breast invasive ductal carcinoma having the highest prevalence of alterations (9-11). The most common abnormalities in *KEAP1* are point mutations [2.74%, e.g., substitution c.1085G>A (p.Arg362Gln) and c.959G>T (p.Arg320Leu)], *KEAP1* amplification (0.29%) and homozygous or heterozygous loss of *KEAP1* (0.09%) (9-11). According to the data from the American Association for Cancer Research (AACR) Project GENIE Consortium, *KEAP1* is altered in 11% of NSCLC patients. The mutations are mostly located in an *NRF2*-binding

region (11). The impact of *KEAP1* mutations on the TME is profound and highly significant, influencing various immune and metabolic pathways. Firstly, these mutations are associated with a higher tumour mutations burden (TMB), resulting in a greater number of neoantigens and, consequently, greater immunogenicity of *KEAP1*-mutated cancer cells (12). Secondly, the highest percentage of cancer cells expressed PD-L1 was found in cancer tissue with abnormalities of *KEAP1*. These two are significant factors indicating the increased possibilities of the benefits of ICIs. Lastly, the *KEAP1-NRF2* activation pathway can lead to the expression of anti-inflammatory cytokines and suppression of pro-inflammatory signaling. Furthermore, *KEAP1-NRF2* signaling influences cell metabolism, promoting a shift toward anabolic processes, including increased glucose uptake and utilisation, enhancing the energy supply for tumour growth. In summary, these conditions create a TME that could potentially enhance the effectiveness of ICIs (12-14).

The status of *KEAP1* and *NRF2* is increasingly recognized as an essential factor in the prognosis of cancer and the outcome of therapy (12-14). Assessing *KEAP1* mutations can help predict the aggressiveness of certain cancers and their likely response to treatment with ICIs. However, the results of clinical trials involving ICIs in patients with *KEAP1* gene mutations need to be clarified and confirmed in a large group of patients, including those with other abnormalities.

The *STK11* gene, also known as liver kinase B1 (LKB1), encodes a serine/threonine kinase (STK11). This protein is involved in the regulation of cell growth, polarity, motility, differentiation and cell metabolism (9,15,16). *STK11* gene is altered in 3.04% of all cancers, mainly observed in lung adenocarcinoma, breast invasive ductal carcinoma, NSCLC, and colon adenocarcinoma. The most common alterations in *STK11* are various point mutations (1.76%), homozygous or heterozygous loss of *STK11* (0.31%), as well as *STK11* amplification (0.11%) (9-11).

STK11 germline inactivating mutations cause Peutz-Jeghers syndrome, but somatic inactivation by point mutation and frequent deletion in the short arm of chromosome 19 (19p13) occurs in approximately 30% of NSCLC (10,11,17). Inactivating *STK11* mutations are more common in male and smoker tumours and in poorly differentiated adenocarcinomas. *STK11* inactivation appears to be particularly prevalent in NSCLC, but it is rare in small cell lung cancer (SCLC). It makes *STK11* the third most frequently mutated gene in lung adenocarcinoma after *p53* and *RAS* (9-11).

STK11 is an important regulator of cell metabolism and energy sensing and is a tumour suppressor kinase. Its functions involve activation of AMP kinase (AMPK) and AMPK-related family members (5,15,16). Therefore, loss of *STK11* increases the use and synthesis of serine S-adenosyl methionine (SAM), a substrate for multiple epigenetic silencing enzymes, including DNA methyltransferase 1 (DNMT1), that can affect the expression of genes that affect immune recognition [including the DNA sensor such as the stimulator of interferon genes (STING)-related protein] and guard uncontrolled tumour growth (14-16,18). Therefore, *STK11* mutations will affect the progression and development of cancer cells and inhibit the immune system in the tumour microenvironment.

Firstly, *STK11* mutations are often associated with an immunologically "cold" tumour microenvironment (14-16). This means that tumours with *STK11* mutations could be characterized by low or no PD-L1 expression, low T cell infiltration densities, high levels of granulocyte colony-stimulating factor and interleukin-8 (IL-8) family cytokines, high density of neutrophil-like cells, and production of myeloid cell-recruiting chemokines such as interleukin-6 (IL-6). This lack of immune cell infiltration can make tumours less responsive to immunotherapies, such as checkpoint inhibitors, which rely on the presence of immune cells to attack cancer. There is often an increased presence of regulatory T cells (Tregs), which suppress the immune response and support tumour growth (14-16).

Secondly, the STK11 protein is a crucial regulator of cellular energy homeostasis through the AMPK pathway (14-16,18,19). Mutations can disrupt normal metabolic processes, leading to altered glucose and lipid metabolism within the tumour microenvironment. Changes in metabolic products and cytokine secretion also inhibit the recruitment and activity of cytotoxic T cells that support the formation of 'cold' TME. Furthermore, metabolic irregularities result in hypoxic TME, further promoting angiogenesis and tumour progression. Cancer-associated fibroblasts (CAFs) in tumours with *STK11* mutations are often more activated, contributing to extracellular matrix remodeling and promoting a more invasive tumour phenotype (16,18,19). Based on observational genomic studies in the real world, it could be summarized that *STK11* mutations had a negative prognostic value in patients with metastatic NSCLCs, and treatment of these patients involves a multifaceted approach due to the complexity of the tumour microenvironment and the associated therapeutic resistance (6,14,20).

Considering the effectiveness of immunotherapy, it seems extremely important to indicate *KRAS* gene abnormalities. *STK11* mutations, as well as *KEAP1*, often correlate with *KRAS* activation and result in the promotion of cell growth (6,21). *KRAS* is a crucial gene in cell biology, playing a significant role in several fundamental cellular processes (22). It encodes a protein that is part of the RAS family of GTPases, which acts as molecular switches within cells (6,14,21,22). The primary functions of *KRAS* in cell biology included: (I) regulation of signal transduction from cell surface receptors to the nucleus, promoting gene expression involved in cell growth, differentiation, and survival; (II) by activating the MAPK/ERK pathway, *KRAS* promotes cell proliferation crucial for normal cell growth and tissue development; (III) *KRAS* also interacts with the phosphoinositide 3-kinase (PI3K)/AKT pathway, which is involved in promoting cell survival and inhibiting apoptosis (21,22). Furthermore, *KRAS* is involved in cytoskeletal organization, migration, and cell metabolism. Genetic abnormalities in the *KRAS* gene are involved in oncogenic transformation. Mutations in *KRAS* gene are among the most common oncogenic alterations in human cancers. These mutations typically lock KRAS in its active GTP-bound state, leading to uncontrolled cell proliferation and survival, contributing to tumorigenesis and triggering cell proliferation (6,14,21,22). *KRAS* is the most frequent mutated oncogene in human cancer, with a prevalence of 25–30% in all human cancer cases. *KRAS* mutations are detected in 30–40% of patients with lung adenocarcinoma, while only 4–5% of patients with squamous-cell carcinoma. *KRAS* substitutions occur mainly in one of five hotspots in codons 12, 13, 61, 117, and 146, where the substitution of p.Gly12Cys (glycine-to-cysteine) accounts for 50% of all mutations in codon 12 (9-11). In historical observational studies, the risk of death in patients with advanced NSCLC was more significant in the presence of *KRAS* gene mutations. In the era before immunotherapy and molecularly targeted therapies, patients with *KRAS* gene mutations compared to patients without these mutations had a mortality risk of 1.47 compared to patients without these alterations (21).

The third most common mutation in *KRAS* gene is the substitution of p.Gly12Asp in codon 12 (23,24). Substitution p.Gly12Asp is observed approximately in 15% of patients with *KRAS* mutation (23,24). Moreover, *STK11* and *KEAP1* mutations co-occurred with p.Gly12Asp mutation the least common of all other mutations in the *KRAS* gene; 14.2% and <5% of patients with p.Gly12Asp substitution have also mutations in *STK11* or *KEAP1* genes (9-11). The presence of p.Gly12Asp mutation in tumor cells causes a state of immunosuppression in the tumor environment (25). The expressions of PD-L1, PD-1, TIGIT, TIM-3, and LAG-3 were up regulated on immune cells in lung cancer mouse model with p.Gly12Asp substitution (25). At the same time, infiltration of Treg lymphocytes increased. MRTX1133 is a novel non-covalent KRAS inhibitor, which showed significant preclinical antitumor activity in tumor cells with p.Gly12Asp substitution, especially pancreatic ductal adenocarcinoma (26). Treatment with MRTX1133 strongly inhibited tumor progression, reduced the percentage of Tregs and downregulated the expression of PD-1 on CD4- and CD8-positive T cells infiltrating lung cancers. MRTX1133 is being tested in clinical trials in patients with solid tumors with the p.Gly12Asp mutation in the *KRAS* gene (25,26).

Activation of the immune system is observed more frequently in patients with NSCLC with *KRAS* mutations because they are more common in smokers. Almost 90–95% of the *KRAS* gene mutations occur in current smokers or patients with a smoking history, predominantly women; 90% of patients with *KRAS* mutations have high or moderate tumour infiltration by CD8 positive T cells compared to 70% of patients without *KRAS* mutations. A higher tumour mutation burden (TMB) was detected as well as higher expression of PD-L1 in tumour cells (TC) in patients with *KRAS* mutation than in patients with *KRAS* wild type. This could inhibit the natural antitumor immune response in the tumour microenvironment (21,22).

Furthermore, *KRAS* mutations, unlike other oncogene mutations, often coexist with other abnormalities. Approximately 25% of patients mutated in *KRAS* also have a *STK11* gene mutation, and another 25% of patients have a *KEAP1* gene mutation.

It is speculated that the co-occurrence of *STK11* or *KEAP1* abnormalities with the *KRAS* mutation changes the composition of the tumour microenvironment as compared when presented alone (*Table 1*) (6,14). Interestingly, *STK11* alterations occur more frequently in tumours with low expression of PD-L1. The coexistence of mutations in the *KRAS* gene and mutations in tumour suppressor genes (especially in the *STK11* gene) may have an adverse effect on the course of the disease and the activity of the immune system (20,27,28). These could have a huge impact on the effectiveness of treatment in these patients. Data from the literature had shown that the presence of dual *STK11* and *KRAS* mutations has been associated with a trend towards

Table 1 Comparison of the impact of *KRAS*, *STK11* and *KEAP1* gene mutations on the tumour microenvironment composition [based on (14)]

Co-occurrence of *KRAS* mutation with *STK11* wild-type or *KRAS* mutation with *KEAP1* wild-type	Co-occurrence of *KRAS* mutation with *STK11* mutation	Co-occurrence of *KRAS* mutation with *KEAP1* mutation
• Infiltration of tumour tissue by: CD4-positive T memory cells and CD19-positive B cells	• Downregulation of MHC II class expression—inhibited antigen presentation and antigen presenting cells' function	• Downregulation of the chemokine and their receptors (e.g., CCL2, CXCL6, CCR1, CCR6, CCR7) expression involved in monocytes and dendritic cells recruitment
• Enriched in CD8-positive naïve and CD8-positive memory cells	• Inhibited T cell activation, leukocyte' migration and degranulation	• Downregulation of inflammatory protein expression and their receptors (e.g., type I interferon, STING, TLR4, TLR7)
• Presence of antigen presenting cells (including M1 and M2 macrophages)	• Downregulation of the chemokine and their receptors (e.g., CXCL14, CCL23, CX3CR1, CCR6) expression	• Presence of neutrophils and myeloid-derived supressor cells including mesenchymal stem cells
• High expression of immune score genes (including genes for inflammasome activation, e.g., NLRP3, IL-1β, IL-18)	• Presence of neutrophils and myeloid-derived suppressor cells	• Elevated metabolic stress via NRF2 activation, creating a hypoxic environment and increasing lactate production, which further impairs immune cell function
• Upregulation of the chemokine expression	• Metabolic reprogramming, e.g., altered AMPK and mTOR signaling that promote tumor survival and may create a hypoxic or nutrient-deprived microenvironment hostile to immune cells	Potentially sensitivity to combined immunotherapy
Potentially sensitivity to single-agent immunotherapy	Potentially sensitivity to combined immunotherapy	

STK11, serine/threonine kinase 11; *KEAP1*, Kelch-like ECH-associated protein 1; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; NLRP3, NOD-like receptor family, pyrin domain-containing 3; IL, interleukin; MHC, major histocompatibility complex; STING, stimulator of interferon genes; AMPK, AMP kinase; mTOR, mammalian target of rapamycin.

poorer survival outcomes in NSCLC in response to immunotherapy and chemotherapy (6,14).

Here, we analyze the effectiveness of immunotherapy alone and in combination with chemotherapy and immunotherapy in different cohorts of patients depending on the presence of genetic abnormalities in the genes discussed above.

First clinical data on effectiveness of immunotherapy in STK11-, KEAP1- and KRAS-mutated NSCLC patients

The first studies on immunotherapy effectiveness in patients with NSCLC with mutations of the *STK11*, *KEAP1* and *KRAS* genes concern the second line of treatment.

Skoulidis *et al.* examined 174 patients from the Stand Up to Cancer (SU2C) dataset (from three cancer centres) (29). The patients were diagnosed with lung adenocarcinoma and mutations in the *KRAS* gene. Most patients received monotherapy with the PD-1 inhibitor in second-line treatment (146 patients were treated with nivolumab and 19 with pembrolizumab). Coexistence of mutations in the *KRAS* and *STK11* genes was found in 31% of the patients, 32% had the coexistence of mutations in the *KRAS* and *TP53* genes, and 37% had only a mutation in the *KRAS* gene. Patients with commutations in the *KRAS* and *STK11* genes were mainly resistant to anti-PD-1 antibodies with an overall response rate (ORR) of 7.4%. In contrast, the remaining patients were more sensitive to PD-1 inhibitors with ORR of 35.7% in the group with commutations of the *KRAS* and *TP53* genes, as well as 28.6% in the group with only *KRAS* mutations. The risk of progression was significantly higher in the group with mutations in the *KRAS* and *STK11* genes compared to patients with mutations in the *KRAS* and *TP53* genes [hazard ratio (HR) =1.77] and with only mutations in the *KRAS* gene (HR =1.98). On the contrary, patients with *KRAS* and *TP53* mutations and only *KRAS* mutations had similar progression-free survival (PFS). The median overall survival

(OS) (mOS) was 6.4 months in patients with comutations in the *KRAS* and *STK11* genes compared to 16.0 months in patients with comutations in the *KRAS* and *TP53* genes and 16.1 months in patients with *KRAS* mutations. The risk of death was significantly higher in patients with *STK11* mutations compared to the remaining patients (HR =1.99). Furthermore, patients with STK11 protein deficiency (lack of STK11 protein due to the lack of STK11 expression or STK11 inactivation) had a significantly higher risk of progression (HR =1.80) and death (HR =2.03) compared to those with STK11-proficient tumours. It is important to note that abnormal *STK11* activity is not always associated with the presence of mutations in the *STK11* gene (29).

The authors also analysed the impact of the cooccurrence of *STK11* and *TP53* genetic alterations in 44 patients with *KRAS* mutations from the CheckMate-057 clinical trial. Next-generation sequencing was performed using whole exome sequencing technology in patients. Twenty-four patients received nivolumab (anti-PD-1 antibody) and 20 patients were treated with docetaxel. Six patients with mutations in the *KRAS* and *STK11* genes were refractory to nivolumab therapy. However, 4 of 7 patients (57.1%) with mutations in the *TP53* and *KRAS* genes and 2 of 11 patients (18.2%) with only a mutation in the *KRAS* gene responded to ICIs therapy. Although ORR did not differ significantly between the three subgroups in the docetaxel arm. The small number of patients within the subgroups resulted in the inability to determine whether *STK11* mutations are a prognostic or predictive factor for treatment results in the CheckMate-057 data set. Median values of PFS and OS could also not be reliably determined (29).

The analysis mentioned above was deepened by Ricciuti *et al.* The authors identified 1,261 patients with advanced adenocarcinoma treated with immunotherapy in four cancer centers: Dana-Farber Cancer Institute, Massachusetts General Hospital, Memorial Sloan Kettering Cancer Centre, and MD Anderson Cancer (14). Among these patients, 873 (69.2%) received second-line immunotherapy. Mutations in the *KRAS* gene were detected in 536 (42.5%) patients. Cooccurring mutations in the *KRAS* and *STK11* genes, the *KRAS* and *KEAP1* genes, as well as *STK11 and KEAP1* were found—respectively—in 10.9% (138 of 1,261), 8.4% (101 of 1,202), and 9.4% (113 of 1,202); 147 patients (11.6%) had mutations in the *STK11* gene, and 118 patients (9.8%) had mutations in the *KEAP1* gene without coexistence with mutations in the *KRAS* gene. Gene alterations were determined using the Next Generation OncoPanel (DFCI) and MSK-IMPACT (MSKCC) sequencing platforms (14).

A harmful effect of *STK11* mutations on immunotherapy results was observed only in patients with mutations in the *KRAS* gene. These patients with *STK11* mutations had significantly lower median PFS (mPFS) (2 *vs.* 4.8 months) and mOS (6.2 *vs.* 17.3 months) and higher risk of progression (HR =1.46) and death (HR =1.73) in multivariate analysis compared to patients with *KRAS* gene mutations and wild type *STK11* gene (wt). The *STK11* mutation was associated with significantly worse ORR in patients with *KRAS* mutations but not in *KRAS* wt patients. ORR was 11.6% in the first group of patients and 23.8% in the second group of patients. However, in the absence of *STK11* mutations, ORR was higher in the group of patients with a mutation in the *KRAS* gene than in patients with wild-type *KRAS* (32.4% *vs* 19.1%). *STK11* mutations were associated with significantly worse clinical outcomes in patients mutated with *KRAS* regardless of PD-L1 expression status and type of *KRAS* substitution (p.Gly12Cys, p.Gly12Val and p.Gly12Asp) (14).

Similarly, *KEAP1* mutations were also associated with decreased survival in ICIs-treated adenocarcinoma patients, but only in those patients with *KRAS* gene mutations. In multivariate models, patients with commutations in the *KEAP1* and *KRAS* genes had a higher risk of progression (HR =2.15) and death (HR =2.44) compared to patients with only mutations in the *KRAS* gene. The mPFS and mOS were 1.8 *vs.* 4.6 months, as well as 4.8 and 18.4 months, respectively. ORR in the group of patients with commutations in the *KEAP* and *KRAS* gene was 17.8%, and in the group of patients with only mutations in the *KEAP1* gene it was 26.1%. ORR was similar in patients with a mutation in the *KRAS* gene but without a mutation in the *STK11* gene (29.3%) and in patients with mutations in the *STK11* gene but without a mutation in the *KRAS* gene (26.1%). *KEAP1* loss was associated with worse immunotherapy results in patients with PD-L1 expression in <50% of TC and did not depend on genetic variants in the *KRAS* gene. The impact of concurrent *STK11* and *KEAP1* mutations on ORR, mPFS, and mOS was found in patients with *KRAS* mutations, suggesting an additive effect of *STK11* and *KEAP1* comutations on immunotherapy outcomes (14).

The authors also explored the impact of *STK11* and *KEAP1* mutations on chemotherapy efficacy in The Cancer Genome Atlas (TCGA) cohort. In this group of patients with adenocarcinoma, they found that *STK11* and *KEAP1* mutations were associated with significantly shorter PFS among patients with *KRAS* mutants, but not in patients with

the wild-type *KRAS* gene, suggesting that loss of *STK11* or *KEAP1* may be a poor prognostic factor (14).

Effectiveness of first-line immunotherapy or combined two ICIs in STK11-, KEAP1- and KRAS-mutated NSCLC patients

Mok *et al.* evaluated the clinical utility of TMB mutations, as well as *STK11*, *KEAP1*, and *KRAS* mutations, as biomarkers for pembrolizumab monotherapy (anti-PD-1 antibody) (30). The authors analyzed 793 patients from the KEYNOTE-042 trial who were treated with pembrolizumab or platinum-based chemotherapy. Patients with advanced NSCLC had expression of PD-L1 in 1% of chemotherapy and mutational status was examined with the next generation sequencing (NGS) technique in the whole exon sequencing (WES) option. *STK11* mutations were detected in 7.7% of the patients (33 of 429), *KEAP1* mutations—in 14.9% of the patients (64 of 429) and *KRAS* mutations—in 22.9% of the patients (69 of 301). Twelve patients (2.8%) had both *STK11* and *KEAP1* mutations. In the *STK11*- and *KEAP1*-assessable population, 29.8% were patients with SCC. In patients with *STK11* mutations, the expression of PD-L1 was lower and the TMB was higher than in patients with wild-type *STK11*. PD-L1 expression was similar in patients with and without *KEAP1* mutations. However, TMB was higher in patients with *KEAP1* mutations than in patients with wild-type *KEAP1* (wt). In patients with *KRAS* mutations, both PD-L1 expression and TMB were higher than in patients without these mutations (30).

An improvement in OS was observed for pembrolizumab *vs.* chemotherapy regardless of the presence of mutations in the genes analyzed. The reduction in death risk was the following: 63% for *STK11*-mutant patients (HR =0.37), 17% for *STK11* wt patients (HR =0.83), 25% for *KEAP1*-mutant patients (HR =0.75), 22% for *KEAP1* wt patients (HR =0.78), 58% for *KRAS*-mutant patients (HR =0.42) and 14% for patients of *KRAS* wt (HR =0.86). A high reduction in the risk of death occurred in patients using pembrolizumab compared to chemotherapy in patients with the p.Gly12Cys mutation in the *KRAS* gene (HR =0.28). The ORR for pembrolizumab *vs.* chemotherapy was 31.3% *vs.* 5.9% in patients with the *STK11* mutation and 29.4% *vs.* 23.6% for wild-type *STK11* patients. The ORR was 35.5% for pembrolizumab *vs.* 18.2% for chemotherapy in patients with *KEAP1* mutant and 28.6% *vs.* 22.9% for *KEAP1* wild-type patients. ORR in the pembrolizumab and chemotherapy groups was 56.7% and 18.0% in patients with *KRAS* mutant, 66.7% and 23.5% in patients with p.Gly12Cys mutation, as well as 29.1% and 21.0% in patients with *KRAS* wild-type (30).

In the phase 3 MYSTIC study, 1,003 patients with NSCLC were treated with first-line monotherapy with durvalumab (anti-PD-L1 antibody) or durvalumab in combination with tremelimumab [anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) antibody] or chemotherapy (31). Blood TMB and mutations in the *STK11*, *KEAP1*, and *ARID1A* genes were evaluated. Circulatory tumour DNA (ctDNA) was profiled from baseline blood samples using the GuardantOMNI platform. High TMB has been associated with improved OS and PFS with first-line durvalumab or durvalumab with tremelimumab *vs.* platinum-based chemotherapy. However, specific gene mutations have been associated with resistance (*STK11* and *KEAP1*) or sensitization (*ARID1A*) to anti-PD-1 monotherapy. In non-squamous NSCLC patients, the mutation frequencies were the following: 19% for *STK11* mutations, 20% for *KEAP1* mutations, and 11% for *ARID1A*. However, in squamous-cell tumours, the frequencies were lower and reached 7%, 13%, and 15%, respectively. Patients with *STK11* mutations or *KEAP1* mutations had a shorter mOS than patients without these mutations. In *STK11* or *KEAP1* mutated patients *vs.* non-mutated *STK11* patients, mOS was as follows: 10.3 *vs* 13.3 months in patients treated with durvalumab; 4.4 *vs.* 11.3 months in patients treated with durvalumab with tremelimumab and 6.7 *vs.* 13.1 months in patients receiving chemotherapy. Patients without *KEAP1* mutations also had a higher mOS, which in each study arm was as follows 14.6, 11.3, 13.3 months. Furthermore, patients with *ARID1A* mutations receiving durvalumab with tremelimumab had a higher mOS than patients without *ARID1A* mutations (23.2 *vs.* 9.8 months) (31).

In the CheckMate-227 Part 1 clinical trial, first-line nivolumab in combination with ipilimumab demonstrated a long-term and durable clinical benefit *vs.* chemotherapy in patients with NSCLC (32). In non-squamous NSCLC patients, the FoundationOne CDx TM assay was used to identify *KRAS*, *STK11*, and *KEAP1* mutations. Nivolumab with ipilimumab improved OS compared to chemotherapy in patients with or without *KRAS*, *STK11*, or *KEAP1* mutations; however, the subgroups with mutations were small [78 patients (16.4%) with *STK11* and 38 patients (8%) with *KEAP1* mutations]. The reduction in death risk by immunotherapy compared to chemotherapy was the following: 21% (HR =0.79) for patients with *KRAS* mutations (mOS 17.5 *vs.* 15.7 months), 27% (HR =0.73) for patients without *KRAS*

mutations (mOS 20.6 *vs.* 17.9 months), 22% (HR =0.78) for patients with *STK11* mutations (mOS 10.8 *vs.* 11.2 months), 25% (HR =0.75) for patients without *STK11* mutations (mOS 21.2 *vs.* 8.5 months), 69% (HR =0.31) for patients with *KEAP1* mutations (mOS 24.4 *vs.* 8.9 months), and 20% (HR =0.80) for patients without *KEAP1* mutations (mOS 20.1 *vs.* 16.7 months) (32).

The presence of *STK11* mutations seems to be a poor prognostic factor regardless of the type of treatment in NSCLC patients. The non-randomised controlled phase 2. The TELMA clinical trial included patients with NSCLC with high TMB (over 10 mutations/megabase) and without alterations in the genes *EGFR*, *ALK*, *STK11*, *MDM2*, or *ROS1*. This single-arm study used first-line treatment with atezolizumab (anti-PD-L1 antibody) and bevacizumab [anti-vascular endothelial growth factor (VEGF) antibody]. The results confirm that strict qualification for treatment based on the exclusion of poor predictive genetic factors can improve the effectiveness of immunotherapy. The 12-month PFS rate was 51.3% and the 12-month OS rate was 72.0%. The mPFS was 13.0 months, and the mOS was not reached (33).

Effectiveness of immunotherapy combined with chemotherapy in patients with STK11, KEAP1 and KRAS mutated NSCLC

Data from clinical trials of immunochemotherapy in advanced NSCLC are based on exploratory analyses of subgroups, mainly patients diagnosed with non-squamous NSCLC.

The multi-arm CheckMate-227 trial also included a Part 2 that evaluated the value of combining nivolumab with chemotherapy regardless of PD-L1 expression (34). Pathogenic variants in the *STK11* and *KEAP1* genes were found in 23% and 8% of patients, respectively. A trend toward prolonged OS with nivolumab plus chemotherapy (*vs.* chemotherapy) was observed in the overall population analysed regardless of gene status: mOS 18.8 *vs.* 15.6 months, HR =0.86, 95.62% confidence interval (CI): 0.69–1.08, P=0.18. At the same time, the benefit of immunochemotherapy was similar in the *STK11* mutant *vs. STK11* wt and *KEAP1* mutant *vs. KEAP1* wild-type subgroups. However, the small size of the subgroups limits the interpretation of the results (34).

An exploratory molecular profile analysis was also performed in patients treated in the KEYNOTE-407 and KEYNOTE-189 trials (35). The prevalence of high mutations of tissue tumour mutational burden (tTMB) and *STK11*, *KEAP1*, and *KRAS* was assessed. Of the 289 patients evaluated in KEYNOTE-189, 18.7% had *STK11* mutations. Pathogenic variants *KEAP1* and *STK11* were found to be more common in patients with lower expression of PD-L1 (additional differences were observed depending on TMB levels). In the *STK11* mutant group, lower percentages of objective responses to immunochemotherapy (30.6% *vs.* 48.8%), while in the *STK11* mutant group, the benefit of immunochemotherapy in terms of PFS was limited HR for PFS 0.81 (95% CI: 0.44–1.47) and HR for OS 0.75 (95% CI: 0.37–1.50). Similar results were observed in patients with *KEAP1* mutations. It should be noted that the subgroups analysed were small, which makes reliable conclusions difficult. In the population of the KEYNOTE-407 study, only 2% of the patients had a mutation in the *STK11* gene, so no comparative analysis was performed (35).

A publication presenting the results of the CM9LA trial, updated after 3 years, confirmed the superiority of nivolumab and ipilimumab in combination with chemotherapy *vs.* chemotherapy alone in the total population analysed; mOS was 15.8 and 11 months, respectively (HR =0.74; 95% CI: 0.62–0.87) (36). Comutation status was known in 313 patients diagnosed with non-squamous NSCLC—in this group, pathogenic variants in the *KRAS*, *TP53*, *STK11* and *KEAP1* genes were detected in 39%, 60%, 27%, and 10% of patients, respectively (30). There was a trend toward prolonged OS after immunochemotherapy in the groups of patients with pathogenic variants, but the differences were not statistically significant. The small size of the subgroups limits the value of these observations (36).

An updated analysis of the POSEIDON trial, which evaluated the value of combining chemotherapy (C) with durvalumab +/– tremelimumab (DT + C), showed that patients in the three-drug arm benefited from treatment in terms of OS compared to chemotherapy (HR =0.75; 95% CI: 0.63–0.88); a trend toward longer OS was also observed in the DT + C arm (HR =0.84; 95% CI: 0.71–0.99) (37). The greatest clinical benefit was observed in patients with non-squamous NSCLC: mOS was 17.2 months for the triplet arm compared to 13 months for chemotherapy (HR =0.68; 95% CI: 0.55–0.85). The DT + C regimen was shown to be the most effective (relative to chemotherapy) in both *STK11* mutant (found in 87 patients)—HR =0.62 (95% CI: 0.34–1.12) and *STK11* wt—HR =0.70 (95% CI: 0.55–0.89) (38). Similar observations were made for patients with *KEAP1* mutation (n=51)—HR =0.43 (95% CI: 0.16–1.25) and *KEAP1* wild-type—HR =0.75 (95% CI: 0.63–0.89). No data

on the efficacy of treatment were presented in patients with the coexistence of two pathogenic variants, of which the coexistence of mutations in the *KRAS* gene and *p53* is the most significant (38).

Among the 920 patients in the IMpower50 study population, 24.5% of the patients had pathogenic *KRAS* mutations, 14.5% had the *STK11* mutant, and 15.5% had tumours of the *KEAP1* mutant. At the same time, it was found that in the *KRAS* mutant patient group, commutations were present in 44.9% of patients (39). A multiagent regimen that includes platinum-based chemotherapy, atezolizumab, and bevacizumab provided clinical benefit in patients with *KRA*S mutants regardless of commutation status (39).

A summary of the presented clinical data is presented in *Table 2*.

The real-world data analysis of immunotherapy' effectiveness in STK11-, KEAP1- and KRAS-mutated NSCLC patients

Sun *et al.* presented an analysis of a group of 2,593 patients treated daily—patients with non-squamous NSCLC predominated (74.3%), 62.6% of patients received immunochemotherapy based on a single ICI (40). In the whole population analysed, 37.9% had mutations in the *KRAS* gene—differences were observed in the frequency of abnormalities in the *KRAS* gene determined by PD-L1 expression (most frequent in the group of patients with PD-1 expression 50–45%). At the same time, it was found that in the group of patients with PD-L1 0% with mutations in the *KRAS* gene, the presence of *KEAP1/STK11* commutation was found more frequently, in a total of 60% of the patients, while in the group of patients with high expression of PD-L1, only 18% of the patients were found. The presence of mutations in the *KRAS* gene (against the *KRAS* wt variant) was a negative prognostic factor only in the group of patients without PD-L1 expression in TC (for OS—HR =1.46, 95% CI: 1.18–1.8, P=0.001; for PFS—HR =1.23, 95% CI: 1.01–1.49, P=0.035). However, regardless of PD-L1 expression, the prognosis was particularly unfavourable for patients in whom *KEAP1/STK11* commutations coexisted with mutations in the *KRAS* gene. The risk of death increased for patients with PD-L1 expression in TC for 0%, 1–49% and >50% of TC—HR =2.73 (P<0.001), HR =2.64 (P<0.001) and HR =2.35 (P=0.008), respectively (40).

In an analysis presented by Arbour *et al.* of a group of 330 patients with advanced and recurrent NSCLC with confirmed *KRAS* gene mutations, the most common coexisting mutations were in the genes *TP53* (42%), *STK11* (29%) and *KEAP1/NFE2L2* (27%) (41). In a subgroup analysis of patients (n=86) receiving immunotherapy (nivolumab or pembrolizumab in second-line treatment), the coexistence of abnormalities in the *KEAP1/NFE2L2* gene appeared to be a negative predictor of OS—HR =1.96 (95% CI: 1.33–2.92, P<0.001). No such association was confirmed for the other commutations (42). Similar, in an analysis of 377 patients treated with pembrolizumab in combination with chemotherapy, the presence of the *STK11/LGB1* mutation (n=102) correlated with significantly shorter PFS (mPFS 4.8 *vs.* 7.2 months, HR =1.5; 95% CI: 1.1 to 2.0; P=0.0063) and OS (mOS 10.6 *vs.* 16.7 months, HR =1.58; 95% CI: 1.09 to 2.27; P=0.0083). *KRAS* gene variants were not assessed (42).

Julian *et al.* presented an analysis of a group of 2,715 patients, 445 of whom had the p.Gly12Cys variant in the *KRAS* gene (43). The variant p.Gly12Cys variant of the *KRAS* gene was correlated with a trend toward longer OS, both in patients treated with immunotherapy in the first and second lines. The co-occurrence of *STK11* and *KEAP1* mutations was associated with significantly shorter OS in both *KRAS* wt and *KRAS* mutant patients. In patients with *KRAS* wt receiving first-line immunotherapy and immunochemotherapy (n=512), a significantly shorter mOS was observed in patients with the *STK11/KEAP1* mutant variant, which comprised 10% of the analysed group, compared to the median *STK11* wt/*KEAP1* wt of 5.8 *vs.* 10.1 months (HR =1.81; 95% CI: 1.44–2.26; P<0.001). Nonsignificant differences were also observed between *STK11* mutant/*KEAP1* wild-type patients *vs. STK11* wild-type/*KEAP1* wild-type patients (7.5 *vs.* 10.1 months, HR =1.27; 95% CI: 1.00–1.62; P=0.05) and *STK11* mutatnt/*KEAP1* wild-type patients *vs. STK11* wild-type/*KEAP1* wild-type patients (8.8 *vs.* 10.1 months, HR =1.21; 95% CI: 1.00–1.48; P=0.06). Similar observations were made in the *KRAS* p.Gly12Cys population: *STK11* mutant/*KEAP1* mutant patients had the least favorable prognosis (19.4% of *KRAS* p.Gly12Cys patients) compared to *STK11* wild-type/*KEAP1* wild-type, the mOS was 6.4 *vs.* 15.0 months (HR =1.93; 95% CI: 1.35–2.75; P<0.001). The authors did not provide detailed information on treatment regimens, although given the analysis based on daily practice and patients treated between 2009 and 2020, it is reasonable to assume that most of the immunochemotherapy cases were based on pembrolizumab (43). In contrast, Papillon-Cavanagh *et al.* presented the results of an analysis of 574

Table 2 The summary of the most important clinical trials on the efficacy of immunotherapy in non-small cell lung cancer patients with mutations in the *STK11*, *KEAP1* and *KRAS* genes

Study	Number of pts	Treatment	Line of treatment	Gene variants	ORR (%)	mPFS (months)	mOS (months)
Skoulidis (29)	174	Nivolumab, pembrolizumab (SU2C study)	2nd	*STK11 mt/KRAS mt*: 54 pts (31%)	7.4	1.8	6.4
				p53 mt/KRAS mt: 56 pts (32%)	35.7	3.0	16
				KRAS mt: 63 pts (37%)	28.6	2.7	16.1
Ricciuti (14)	1,261	Different schemes of immunotherapy mostly in 2nd line (873 pts, 69.2%)	1st and 2nd	*KRAS mt*: 536 pts (42.5%)	32.1	5.5	20.8
				STK11 mt/KRAS mt: 138 pts	11.6	2.3	12.6
				STK11 wt/KRAS mt: 101 pts	32.4	1.9	7.9
				STK11 mt/KRAS wt: 122 pts	23.8	2.5	13.0
				STK11 wt/KRAS wt: 603 pts	19.1	2.8	12.4
				KEAP1 mt/KRAS mt: 101 pts	17.8	1.8	4.8
				KEAP1 wt/KRAS mt: 376 pts	29.3	4.6	18.4
				KEAP1 mt/KRAS wt: 130 pts	26.1	3.4	13.0
				KEAP1 wt/KRAS wt: 595 pts	18.5	2.7	12.4
Mok (30)	793	Pembrolizumab *vs*. chemotherapy (KEYNOTE-042 study)	1st	*STK11 mt*: 33 pts	31.3 *vs*. 5.9	4.8 *vs*. 5.1 (HR =0.75; 95% CI: 0.36–1.57)	18.1 *vs*. 7.6 (HR =0.37; 95% CI: 0.16–0.86)
				KEAP1: 64 pts	35.5 *vs*. 18.2	6.1 *vs*. 5.7 (HR =0.67; 95% CI: 0.38–1.17)	17 *vs*. 8.9 (HR =0.75; 95% CI: 0.42–1.35)
				KRAS mt: 69 pts	56.7 *vs*. 18	12.3 *vs*. 6.2 (HR =0.51; 95% CI: 0.29–0.87)	28.4 *vs*. 11 (HR =0.37; 95% CI: 0.22–0.81)
Borghaei (34)	256	Nivolumab plus chemotherapy *vs*. chemotherapy	1st	*STK11 mt*: 60 pts (23%)	No data	No data	19.8 *vs*. 15 (HR =0.64; 95% CI: 0.36–1.12)
				KEAP1 mt: 21 pts (8%)			19.8 *vs*. 8 (HR =0.28; 95% CI: 0.11–0.76)
				*KRAS m*t: 94 pts (37%)			16.2 *vs*. 17.2 (HR =0.90; 95% CI: 0.57–1.42)
Johnson (38)	1,013	Tremelimumab plus durvalumab plus chemotherapy or durvalumab plus chemotherapy *vs*. Chemotherapy (POSEIDON study)	1st	*STK11 mt*: 87 pts	No data	No data	15 *vs*. 6.9 *vs*. 10.7 (HR =0.62, 95% CI: 0.34–1.12; HR =1.06, 95% CI: 0.61–1.89)
				KEAP1 mt: 51 pts			13.7 *vs*. 8.1 *vs*. 8.7 (HR =0.43, 95% CI: 0.16–1.25; HR =0.77, 95% CI: 0.31–2.15)
				KRAS mt: 182 pts			25.7 *vs*. 12.7 *vs*. 10.4 (HR =0.55, 95% CI: 0.36–0.85; HR =0.78, 95% CI: 0.52–1.16)

STK11, serine/threonine kinase 11; *KEAP1*, Kelch-like ECH-associated protein 1; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; pts, patients; ORR, overall response rate; mPFS, median progression-free survival; mOS, median overall survival; mt, mutation in selected gene; wt, wild-type of selected gene; HR, hazard ratio; CI, confidence interval.

patients who received immunotherapy in the first-line setting (20). Pathogenic variants of *STK11/KEAP1* were found in 20% of patients, more frequently in patients with low expression of tumour PD-L1. The *STK11/KEAP1* mutant profile was found in 13% of patients receiving immunotherapy. The authors did not observe a statistically significant predictive value of molecular abnormalities in the *STK11/KEAP1* genes for PFS and OS, although a less favourable outcome was observed in patients with the *STK11* mutant patients (HR =0.88; 95% CI: 0.43–1.81; P=0.06). Furthermore, pathogenic variants also appeared to be negative prognostic factors in the chemotherapy group.

Conclusions

STK11 and *KEAP1* gene mutations have an impact on the prognosis of patients treated for advanced NSCLC, especially if they coexist with mutations in the *KRAS* gene. It is worth noting that patients with this molecular profile are significantly more likely to have low PD-L1 expression—in group of patients with no PD-L1 (0%) expression, 27% of *KRAS* mutant patients had comutations in both genes, while in a group of PD-L1 expression over 50%—only 4% (41). The presented results indicate also the prognostic, but not predictive, role of mutations in the *STK11* and *KEAP1* genes with reduction of the effectiveness of all systemic treatment methods in patients with advanced NSCLC. However, immunotherapy as monotherapy or as a combination of two ICIs in patients with PD-L1 expression or high TMB appears to be more effective than chemotherapy alone, as shown by the results of the KEYNOTE-042, CheckMate-227, and MYSTIC trials. Ongoing prospective clinical trials, for example TRITON, taking into account the molecular profile of patients eligible for ICIs, may clarify the importance of pathogenic variants in the *STK11* and *KEAP1* genes (44-46).

In conclusion, it is important to underscore that the molecular profiling of patients diagnosed with advanced NSCLC should encompass not only the identification of targetable mutations but also the detection of pathogenic variants in the *STK11*, *KEAP1*, and *KRAS* genes. This comprehensive approach has the potential to significantly enhance the stratification of patients for immunotherapy, thereby improving clinical outcomes.

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Footnote

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