



UNIVERSITÀ DI PARMA

ARCHIVIO DELLA RICERCA

University of Parma Research Repository

Modulating Tumor Microenvironment: A Review on STK11 Immune Properties and Predictive vs Prognostic Role for Non-small-cell Lung Cancer Immunotherapy

This is the peer reviewed version of the following article:

Original

Modulating Tumor Microenvironment: A Review on STK11 Immune Properties and Predictive vs Prognostic Role for Non-small-cell Lung Cancer Immunotherapy / Mazzaschi, G.; Leonetti, A.; Minari, R.; Gnetti, L.; Quaini, F.; Tiseo, M.; Facchinetti, F.. - In: CURRENT TREATMENT OPTIONS IN ONCOLOGY. - ISSN 1527-2729. - 22:11(2021), p. 96.96. [10.1007/s11864-021-00891-8]

Availability:

This version is available at: 11381/2901106 since: 2022-01-09T15:34:02Z

Publisher:

Springer

Published

DOI:10.1007/s11864-021-00891-8

Terms of use:

Anyone can freely access the full text of works made available as "Open Access". Works made available

Publisher copyright

note finali coverpage

(Article begins on next page)

Modulating tumor microenvironment: A Review on STK11 immune properties and predictive vs prognostic role for non-small cell lung cancer immunotherapy

Giulia Mazzaschi MD¹, Alessandro Leonetti MD¹, Roberta Minari PhD¹, Letizia Gnetti MD², Federico Quaini MD³, Marcello Tiseo MD, PhD^{1,3}, Francesco Facchinetti MD, MSc^{4*}

¹ Medical Oncology Unit, University Hospital of Parma, Via Gramsci 14, 43126 Parma, Italy.

² Pathology Unit, University Hospital of Parma, Via Gramsci 14, 43126 Parma, Italy.

³ Department of Medicine & Surgery, University of Parma, Via Gramsci 14, 43126 Parma, Italy.

⁴ Université Paris-Saclay, Institut Gustave Roussy, Inserm, Biomarqueurs Prédicatifs et Nouvelles Stratégies Thérapeutiques en Oncologie, 114 Rue Edouard Vaillant, 94800 Villejuif, France.

*** Corresponding Author:**

Dr. Francesco Facchinetti, MD MSc

Université Paris-Saclay, Institut Gustave Roussy, Inserm, Biomarqueurs Prédicatifs et Nouvelles Stratégies Thérapeutiques en Oncologie

114 Rue Edouard Vaillant, 94800 Villejuif, France

Francesco.Facchinetti@gustaveroussy.fr

Short title

STK11 and immunotherapy in lung cancer

Opinion statement

The quest for immunotherapy (IT) biomarkers is an element of highest clinical interest in both solid and hematologic tumors. In non-small cell lung cancer (NSCLC) patients, besides PD-L1 expression evaluation with its intrinsic limitations, tissue and circulating parameters, likely portraying the tumor and its stromal/immune counterparts, have been proposed as potential predictors of IT responsiveness. *STK11* mutations have been globally labeled as markers of IT resistance. After a thorough literature review, *STK11* mutations condition the prognosis of NSCLC patients receiving ICI-containing regimens, implying a [relevant](#) biological and clinical [significance](#).

ha eliminato: higher

On the other hand, waiting for prospective and solid data, the putative negative predictive value of *STK11* inactivation towards IT is sustained by less evidence. The physiologic regulation of multiple cellular pathways performed by *STK11* likely explains the multifaceted modifications in tumor cells, stroma and tumor immune microenvironment (TIME) observed in *STK11* mutant lung cancer, particularly explored in the molecular subgroup of *KRAS* co-mutation. IT approaches available thus far in NSCLC, mainly represented by anti-PD-1/PD-L1 inhibitors, are not promising in the case of *STK11* inactivation. Perceptive strategies aimed at modulating the TIME, regardless of *STK11* status or specifically addressed to *STK11*-mutated cases, will hopefully provide valid therapeutic options to be adopted in the clinical practice.

Keywords: Prognostic factor; Predictive factor; PD-1; PD-L1; NSCLC; LKB1.

1. Introduction

The immunotherapy (IT) revolution in non-small cell lung cancer (NSCLC) has left unanswered fundamental questions on our actual understanding of the immune control of cancer [1,2]. Major drawbacks reside in the still limited population of patients responsive to IT and the partial success of combinatory approaches aimed at improving survival outcomes [3]. Thus, the identification of predictive biomarkers represents a priority in the actual strategies to optimize IT efficacy [4,5]. Ideal candidates to predict IT response should reflect the tumor - host interaction and its evolutionary changes following treatment. In this scenario the tumor immune microenvironment (TIME) and mutational status are pillars to guide comprehensive evaluation of NSCLC patients suitable for IT approach.

The characterization of TIME has been the object of intense investigations, mainly involving the assessment of PD-L1 status, tumor infiltrating lymphocytes (TILs) density and phenotype, and activating (e.g. interferon- γ , IFN- γ) or inhibitory (e.g. CD38⁺, transforming growth factor- β) signaling pathways [6–8]. Specifically, cancer classification into "hot" (T cell-inflamed, PD-L1^{high}, CD8^{rich}, IFN- γ signature) and "cold" (immune-excluded, characterized by PD-L1^{low}, immune suppressive phenotypes and TGF- β signature; immune-desert, with low CD8⁺ infiltration) tumors has demonstrated prognostic and predictive potential [9,10] (**Figure 1**). Since cancer immunoediting and immune response rely on the cross talk between tumor and its microenvironment not only at the organ level, but also involving the peripheral circulation that provides immune cells, cytokines, growth factors and chemokines, great efforts have been addressed to circulating parameters [11] (**Figure 1**).

The notion that specific genetic mutations encompassing oncogenic drivers such as *EGFR* and *ALK* or the interferon (IFN)- γ - related signaling pathways [12] might impact on IT effectiveness has been consolidated in the last decades [13]. Reproducible and consistent evidence, mostly obtained in *KRAS*-mutant NSCLC, have suggested serine/threonine kinase 11 (*STK11*), coding for the liver kinase B1 (*LKB1*) protein, as a critical factor implicated in anti-tumor immune response, ultimately affecting the proneness to respond to IT [14].

While the biological heterogeneity and therapeutic responsiveness of *KRAS*-mutant (*KRASmut*) NSCLC represents a formidable challenge, it has been widely demonstrated that the coexistence of *STK11* mutations (*STK11mut*) confers an intrinsic resistance to IT [14,15]. [STK11 is a tumor suppressor gene whose mutations can be identified in nearly 15% of lung adenocarcinoma and in up to 30% of *KRASmut* tumors](#), [16]. Acting as a tumor suppressor gene, *STK11mut* with functional significance inevitably lead to gene inactivation which, in addition to promote autonomous cell growth, conditions metabolic alterations and an immunosuppressive TIME [16,17].

ha eliminato:

ha formattato: Tipo di carattere: Non Corsivo

ha eliminato: *STK11mut* are present in nearly 15% of lung adenocarcinoma and their incidence reaches 30% in *KRAS*-mutated tumors

ha formattato: Tipo di carattere: Corsivo

Thus, NSCLC carrying *STK11mut* may represent a suitable model to assess the contribution of both tumor intrinsic and immune microenvironmental variables to the negative outcome of IT. Negative predictive biomarkers for IT benefit may well play a similar or even superior role compared to positive one not only in sparing unnecessary treatment but also in the identification of defective pathways and their potential therapeutic targeting. By dissecting the multifaceted involvement of STK11 on cancer biology and immune evasion, the attempt of the present review is to shed light on the still controversial issue of compelling biomarkers predictive of the response to immunotherapy.

2. How to define STK11 deficiency

Located on the short arm of chromosome 19 (19p13.3), *STK11* gene spans 23Kb and is comprised of nine coding exons and one non-coding exon. The STK11 protein consists of 433 amino acids with an N-terminal domain containing a nuclear localization signal and a phosphorylation site with unknown function at Serine 31, a central kinase domain (residues 44-309), and a C-terminal domain.

Germline *STK11* mutations are responsible for the Peutz-Jeghers syndrome, an autosomal-dominant hamartomatous polyposis syndrome [18]. In sporadic cancers, a wide range of somatic *STK11* genomic alterations were observed (single nucleotide variation, indels, hypermethylation of the promoter and homozygous deletions of LKB1) making challenging the development of a single assay capable of detecting all such alterations [19–21]. Hence, STK11 deficiency could be assessed with different screening strategy, mainly represented by next generation sequencing (NGS) technology for assessing *STK11* variants or immunohistochemical (IHC) approach to study LKB1 expression.

Both tissue and liquid biopsies have been used as testing material for detection of *STK11mut* [22–25]; the choice of NGS targeted panel must be carefully considered mostly for co-occurrence mutation analysis. In fact, in many daily routine practice NGS panel, genes of interest like *KEAP1* and/or *SMRCA4* are absent [26,27]. Usually, classification of mutations was categorized as disease associated on the basis of the designation in the NGS report (*i.e.* disease associated *versus* variant of unknown significance, VUS) querying the reference database *e.g.* cBioPortal, COSMIC and TCGA-Biportal [22]. Currently, more than 400 unique mutations have been described for the *STK11* gene, among which ~70% promote the truncation of the protein and the other 30% represent missense mutations [28].

A correlation between the position of the *STK11mut* and their behavior as tumor suppressor *versus* oncogene was also observed. Pecuchet et al. described that disruptive mutations (nonsense,

frameshift and splice mutations) tended to be over-represented in exons 1 and 2 [29], data confirmed also in a dataset from *STK11* NGS across a variety of cancers in cBioPortal [30]. Truncating mutations could favor the use of alternative ATG initiation codon and the expression of a short isoform lacking the 124 N-terminal amino acid described as a putative oncogenic Δ N isoform [29].

On the contrary, no direct correlation between mutational status and loss of expression by IHC was observed making NGS technology a not exhaustive strategy to define LKB1 status [25,31,32]. For example, Skoulidis et al reported 17.3% of *STK11* wild-type tumors with the absence of LKB1 protein expression by IHC, thus confirming that genetic (*i.e.* mutational) LKB1 assessment might be insufficient to predict LKB1 functional status [25].

Studies on protein expression could be performed with different STK11/LKB1 rabbit monoclonal antibody: different clones were tested such as D60C5F10 (Cell Signaling Technology) [24] or Ley 37D/G6 (Abcam) [32]. The expression could be evaluated grading the cytoplasmic staining intensity, ranging from no discernible (0) to strong cytoplasmic staining (3+). Quantitative IHC for STK11/LKB1 can capture STK11/LKB1-deficient tumors in the absence of *STK11/LKB1* genomic alterations, representing a potentially reliable, simple, and cost-effective method to evaluate LKB1 loss.

Other approaches are emerging as useful tool to investigate alternative mechanisms responsible for LKB1 loss of function: studying the RNA level could highlight the effect mediated by epigenetic inactivation [33] or homozygous and intragenic deletions [20,34]. Chen et al. developed a NanoString-based assay, validated in multiple datasets and subsequently tested in a cohort of 150 lung adenocarcinoma patients, demonstrating high superior overall performance of STK11 signature studied by NanoString with respect to IHC and mutation status [31].

The unequivocal definition of LKB1 deficiency is actually an open issue: the presence of inactivating *STK11* mutations or the lack of LKB1 protein expression at IHC analysis are often discordant. Thus, an integrative analysis of LKB1 genetic alteration is timely and important to provide a better estimate of the incidence of this important tumor suppressor gene.

Thus far, both preclinical and clinical evidence regarding the impact of STK11 status on the immune context and response to IT in lung malignancies have been mainly documented through the analysis of *STK11mut*, while the evaluation of LKB1 expression being less represented across studies.

3. Biological implications of STK11 in NSCLC

3.1. Implication of *STK11* and its genomic loss on cancer growth and metabolism

Under physiologic conditions, STK11 acts a master upstream kinase, directly phosphorylating and activating AMP-activated protein kinase (AMPK) and 12 related kinases with crucial roles in cell processes, such as metabolic regulation, DNA integrity, proliferation, polarity establishment and control of spatial orientation of the cellular structures, angiogenesis, and interaction with tissue milieu [35] (**Figure 1**). STK11-AMPK pathway serves as a cell metabolic checkpoint arresting cell growth in conditions of low intracellular ATP levels [35,36]. In addition, under exogeneous activation, STK11-AMPK signaling is able to induce p53 activity and interfere with cyclins/cyclin-dependent kinase interplay, thus blocking G1/S transition [37]. Moreover, whereas DNA damage occurs, this complex is able to localize into the nucleoplasm and preserves cells from genomic instability [38].

Based on these physiologic actions, STK11 has been described as tumor suppressor and experimental and clinical observations support the tumorigenic potential of its mutations. Evidence of the impact of *STK11* *mut* on cancer growth, aggressiveness, metabolism and angiogenesis [28] have been comprehensively reviewed in several reports [16,35]. Moreover, STK11 loss of function alters the regulation of PAK1 [39], FAK and CDC42 [40] thereby leading to epithelial-to-mesenchymal transition (EMT) and [metastases](#) [41,42].

ha eliminato: metastatization

Importantly, the metabolic derangement produced by STK11 defects is translated in a redox imbalance. As a consequence of increased energetic and metabolic stress, STK11-deficient NSCLC cells generate elevated levels of reactive oxygen species (ROS) [43] (**Figure 1**). As the extent and type of ROS modulate basic biological processes in cancer cells including mutation, proliferation, DNA damage, autophagy and apoptosis [44], this phenomenon has several implications on the mechanisms responsible for the pathogenicity of STK11 loss of function. Moreover, the hypothesis has been advanced that this metabolic driven property may be linked to the close interaction of STK11 with signaling molecules instrumented to tightly control intracellular displaced DNA or chromatin fragments and their impact on immune surveillance (see section 3.3).

Several other downstream regulated genes are also fundamental to decipher the multiple pathways involved STK11 onco-suppressive nature. Recently, a striking similarity in histologic and gene expression features of STK11- and salt-induced kinases (SIKs)-deficient tumors suggests a common tumor suppressive trait [45]. Moreover, *STK11* *mut* human adenocarcinoma cell lines and primary tumors display at high rate a SIK-deficient signature, thus opening new scenarios in

deciphering alternative mechanistic insights and potential therapeutic implications in this complex subset of NSCLC patients [45].

3.2. *STK11 and tumor immune microenvironment*

In the era of immunotherapy, the complex interplay between STK11 and TIME, has been the object of several preclinical and clinical investigations in both non-neoplastic diseases and cancer. Linking cellular bioenergetics with cell proliferation and metabolism, STK11 impacts on both tumor biology (see section 3.1) and T cell modulation [46], thereby encompassing multiple steps to shape TIME (**Figure 1**). In well-designed preclinical studies T cell selective knock out of STK11 reduced the progenitor pool of T effector cells [46,47]. Accordingly, transgenic STK11 ablation in mice prevented TCR- β -selected thymocyte differentiation and expansion via CD98 alterations, thus blunting the proliferative response of peripheral T cells [48].

Additional evidence on the involvement of STK11 in T effector and suppressor cell balance have been provided by its critical role in sustaining T regulatory cells (Tregs) function through the stabilization of FOXP3 [49]. In contrast, conditional knockout of *STK11* gene in dendritic cells (DCs) leads to excessive Treg cell proliferation involving IKK/I κ B α -independent activation of NF- κ B/OX40L pathway [50]. Moreover, knock-out of *STK11* in mice results in increased levels of pro-inflammatory cytokines and enzymes in bone marrow-derived macrophages upon lipopolysaccharide (LPS)-induced NF- κ B activation [51].

In the last few years, the close interplay between *STK11mut* cancer cells and TIME has been deeply investigated in the context of NSCLC. Not surprisingly, also in reason of the physiologic implication of STK11 in phenotypic and functional integrity of the immune system, multiple TIME compartments are affected by defective mutation of the gene.

Inactivating *STK11* aberrations, mainly present in *KRASmut* adenocarcinoma, have arisen as major determinants of cold NSCLC TIME, dominated by low CD3⁺, CD4⁺ and CD8⁺ TILs, high Tumor Associated Neutrophils (TANs) and decreased levels of PD-L1 in spite of a high/intermediate TMB[16]. The negative effect exerted by STK11 on PD-L1 expression has been repeatedly reported [52–54] and appears to overcome PD-L1 mRNA stabilization promoted by *KRAS* [55].

In depth proteogenomic analysis of a series of nearly 100 lung adenocarcinomas revealed that, among the genomic landscape, the most significant impairment in immune activation was present in *STK11mut* tumors [56]. At transcriptional and post-transcriptional levels, severe reductions in DCs, NK T cells, and macrophages were coupled with a defined neutrophil degranulation signature.

Intriguingly, this functional defect was independent from the abundance of neutrophils. Importantly, when these downregulated immune features were entered in a deep-learning-based predictive algorithm, a highly accurate prediction of *STK11* mutational status from histopathological slides was attained. The predominant immunosuppressive TIME of *STK11mut* tumors is also supported by a retrospective analysis of 282 NSCLC documenting a reduced intratumor DC density [57]. Findings from a microarray-based gene expression analysis on a cohort of more than 400 lung adenocarcinoma showed that only *STK11mut* were associated with significantly lower immune surveillance signature characterized by NF- κ B activity and T-cell receptor α - β chain expression [58].

On both mouse models and cell lines, STK11 loss resulted in an altered cytokine milieu with increased expression of proinflammatory CXCL7, G-CSF, IL-1 β and IL-6 fostering neutrophils recruitment and T-effector cell suppression [59]. Compared to *KRASmut* tumors, *KRASmut/STK11mut* tumors displayed higher infiltration of TANs with enhanced expression of arginase 1 (ARG1) and IL-10, which exerted a negative effect on T lymphocytes balance leading to Tregs expansion and T-effector cells exhaustion. Moreover, STK11 inactivation conditioned lower levels of tissue PD-L1 expression in *KRASmut/STK11mut* tumors and in cultured murine and human cells lines [59].

Genetically engineered mouse models exploring the progenitor-specific etiologies of lung cancer histotypes, in addition to highlight the oncogenic boost of STK11, have shown the prominent ability of immune escape in tumors generated in mutant mice by downregulation of the antigen presenting machinery and TAN infiltration [60].

The critical impact of STK11 loss of function on lung cancer immune microenvironment is generally ascribed to epigenetic repression of stimulator of interferon genes (STING), causing insensitivity to cytosolic double-stranded DNA (ds-DNA) accumulation [61] (see section 3.3). An additional molecular mechanism contributing to the inert immune phenotype associated to *STK11mut* may reside in the oncogenic synergism between KRAS and MYC [62,63]. This co-operation induces high levels of CCL9 and IL-23, epithelial-derived signaling molecules involved in stromal reprogramming, thus triggering the recruitment of macrophages and the exclusion of adaptive and innate immune response by T and B lymphocytes and NK cells, respectively.

Consistently, the negative impact of STK11 aberrations on cancer immune background has been recently supported by the observation that both early-stage and advanced NSCLC harboring *STK11mut* display a lower expression of PD-L1 coupled with poor immune cell infiltration [54,64].

Thus, in keeping with its central role in shaping tumor immunobiology and immune contexture, *STK11* genomic alteration endows the tumor with multiple paths to escape the immune system, significantly affecting patient outcome and response to IT.

The complexity of *STK11*-driven immune features prompts the development of novel synergistic and highly personalized therapeutic approaches.

ha eliminato: s, in addition to enlighten the actual limited IT effectiveness,

ha eliminato: able to target specific NSCLC subtypes

3.3. Defective *STK11* Desensitizes the *STING* pathway to Promote Tumor Immune Evasion

Under normal conditions, the physiologic activation of AMPK by *STK11* is not only involved in metabolic processes mainly converging to mammalian target of rapamycin complex 1 (mTORC1) inhibition, but also results in enhancement of *STING* [65] (**Figure 1**). The relevant role of *STING* in innate immunity resides in its ability to sense, through cyclic GMP–AMP synthase (c-GAS), cytosolic genomic dsDNA (gDNA) and, as more recently documented, also mitochondrial dsDNA (mtDNA) [66]. The immune signaling cascade triggered by *STING* activation ultimately results in increased expression of cytotoxic type 1 IFNs and T cell recruiting chemokines as well as PD-L1 [61]. This tightly regulated mechanism of immune surveillance is disrupted in *KRASmut/STK11mut* NSCLC and represents the molecular underpinning of the evidence that *STK11* aberrations confer an unfavorable TIME and condition an impaired immune response [65,67]. Specifically, *STK11* loss of function is transduced in downregulation of AMPK-*STING* pathway and refractoriness to cytoplasmic dsDNA sensing which is further aggravated by the unrestrained inhibitory pressure of AMPK on epigenetic silencing enzymes (DNA (cytosine-5)-methyltransferase 1 [DNMT1], Enhancer of zeste homolog 2 [EZH2]) [61]. Strong supporting data on the clinical implication of these in vitro observations have been provided by the analysis of *STING* expression on a large series of NSCLC [68]. Indeed, *STK11mut* cases had the lowest levels of *STING* and immune gene expression, pointing to the derangement driven by genetic defects of this kinase in orchestrating the innate and IT induced immune response against cancer.

As deceptive *STING* plays a central role in the immunosuppressive trait of *STK11* mutated lung cancer, several preclinical and clinical (NCT04096638, NCT03843359 and NCT04420884) attempts have been proposed to overcome IT resistance by local [69] or systemic cyclic dinucleotide (CDN) *STING* agonists. Acceptable safety and efficacy were obtained by MK-1454, a locally delivered CDN *STING* agonist, when combined with pembrolizumab in a phase I trial (NCT03010176). Preliminary evidence of *STING* activation by this approach were documented by increased serum levels of *STING*-associated cytokines [70]. Due to the limited applicability of

ha eliminato: associated

intratumor injection in advanced tumors, more recently, an oral formulation of two non-CDN STING agonists has been explored, showing promising experimental results in terms of anti-tumor immunity and sensitization to IT [71,72]. With the potential of changing the actual therapeutic landscape, optimization of these approaches together with the adoption of patient- and disease-specific selective measures may open new venues in the overall management of cancer.

3.4. *STK11 and tumor stromal-vascular network*

In solid malignancies the contribution of stromal compartments typically exceeds that of neoplastic cells in the structural composition of the tumor mass. This intricate network of fibroblasts, mesenchymal cells, blood and lymphatic endothelial cells embedded in extracellular matrix physically and functionally sustains the multiple players regulating cancer fate. Compelling evidence of the relevance of STK11 in shaping tumor stroma arises from its interplay with TGF- β pathway and extracellular matrix (ECM) remodeling (**Figure 1**).

In addition to a direct biochemical interaction with TGF- β [73], in the context of gastrointestinal tumors, STK11 plays a crucial role in epithelial-stromal cross talk dictating multistep tumor formation. Specifically, the engagement of mesenchymal STK11 with TGF- β signaling in epithelial cells represents a determinant factor for the development of gastrointestinal polyps in humans and mice [74]. STK11 deficiency has also been shown to affect stromal TGF- β expression thus altering tumor suppression [75]. To the best of our knowledge, no such mechanisms have been shown to be operative in *STK11mut* lung cancer.

Lysyl oxidase (LOX) is a fundamental enzyme promoting collagen and elastin stabilization and cross-linking. NanoString-based quantitative mRNA/miRNA readouts in human lung adenocarcinoma, have shown LOX as a STK11 downstream regulated gene [76]. Defective STK11 increases LOX activity resulting in remarkable collagen deposition and formation of fibrotic foci in lung adenocarcinoma [77] which can be reverted by pharmacological inhibition of LOX enzymatic activity [78]. The alteration of ECM homeostasis with STK11 mutations is associated with phenotypic plasticity [78] and greater cancer cell proliferation and invasion [77]. Moreover, enhanced LOX activity was detected in serum from advanced lung cancer patients, strongly correlating with poor clinical outcome. Of note, upregulation of LOX expression by *STK11mut* also involves mTOR-HIF-1 α pathway further underlining the role of STK11 in tumor angiogenesis, metastases, and glycolytic metabolism. The close link between STK11 and angiogenesis has been originally

ha eliminato: tization

documented by the midgestation lethal phenotype associated with vascular abnormalities in transgenic mice carrying homozygous inactivating mutation of *STK11* [79].

More recently, the suppressive role of STK11 on angiogenesis was found to be mediated by its interaction with the angiogenic receptor neuropilin-1 (NRP-1) [80]. Indeed, STK11 loss of function in cancer cells from NSCLC patients unleashes NRP-1 trafficking and fosters its aberrant expression. Consistently, an inverse correlation between STK11 and NRP-1 expression was present in tumor samples at gene and protein levels [80].

From a therapeutic perspective, only one clinical observation is available regarding the impact of *STK11mut* on the effectiveness of anti-angiogenic agents. NSCLC patients with negative/weak STK11 status did not significantly benefit from the addition of bevacizumab to standard chemotherapy. Conversely, among intermediate/high STK11 cases, significantly fewer deaths were observed in those receiving bevacizumab [81]. Although the exact contribution of *STK11* alterations to the sensitivity to antiangiogenic drugs is uncovered, mechanistic insight has been provided in STK11-deficient PDX by the evidence of reduced AMPK activation and increased tumor necrosis as a result of bevacizumab administration [81].

4. Clinical implications of STK11 in NSCLC

STK11mut have been recently proposed as an important regulator of resistance to immune checkpoint inhibitors (ICI) in NSCLC (**Table 1**).

Table 1. Impact of *STK11* mutations upon immunotherapy activity and efficacy in NSCLC patients.

| Cohorts of pts | N of pts | Clinical setting | Treatment | ORR | mPFS (months) | mOS (months) | Ref |
|--|-------------|---|---------------------------|-----------------------------------|---|---------------------------|---------|
| <i>STK11mut</i> <i>STK11wt</i> | 6 17 | Stage III – maintenance after CCRT (Sq+NonSq) | Pembrolizumab/durvalumab | - | 11.3 17.5 (p = 0.174) | - | [82] |
| <i>MDACC cohort</i> <i>STK11mut</i> <i>STK11wt</i> | 11 55 | Stage IV, PD-L1 \geq 1% (NonSq) | Anti-PD-1/PD-L1 | 0% 34.5% (p = 0.026) | 1.7 19.3 (p = 0.0012) | 11.1 26.5 (p < 0.0001) | [25] |
| <i>STK11mut</i> <i>STK11wt</i> | 31 96 | Stage IIIB-IV | ICI | - | 5.6 6.3 (p = 0.35) | 8.6 12.1 (p = 0.035) | [83] |
| <i>STK11mut</i> <i>STK11wt</i> | 6 26 | Stage IV – 2 nd -line (NonSq) | Nivolumab | - | 1.2 4.8 (p = 0.07) | 11.4 13.8 (p = 0.5) | [84] |
| <i>STK11mut</i> <i>STK11wt</i> | 15 104 | Stage IV (NonSq) | Durvalumab | 6% 16% (X ² = 0.05) | - | 4.9 14.2 (p = 0.008) | [85,86] |
| <i>STK11mut</i> <i>STK11wt</i> | 6 57 | Stage IV - \geq 3 rd -line (NonSq) | Durvalumab | 0% 25% | - | - | [85,87] |
| <i>STK11mut</i> <i>STK11wt</i> | 23 97 | Stage IV - \geq 3 rd -line (NonSq) | Durvalumab + tremelimumab | 4% 25% (X ² = 0.02) | - | 6.7 15.6 (p = 0.001) | [85,88] |
| <i>STK11mut</i> <i>STK11wt</i> | 1310 | Nonsq | ICI | - | 2.5 3.1 (p = 0.01) | - | [89] |
| <i>STK11mut</i> <i>STK11wt</i> | 117 332 | Stage IV – 1 st -line (NonSq) | CPP | 32.6% 44.7% (p = 0.049) | 4.8 6.9 (p = 0.0012) HR in PD-L1+ 1.73, p=0.016 | 10.6 16.7 (p = 0.0083) | [90,91] |
| <i>STK11mut</i> <i>STK11wt</i> | 137 122 | | CP | - | 3.7 5.6 (p = 0.052) HR in PD-L1+ 1.00, p=0.99 | - | |
| <i>STK11mut</i> <i>STK11wt</i> | 102 120 | | CPP CP | - | 4.8 4.3 (p = 0.75) | 10.6 10.3 (p = 0.79) | |
| <i>STK11mut</i> <i>STK11wt</i> | 40 230 | Stage IV – 1 st -line (Sq+NonSq) | ICI | 41.2% 44.5% | 4.0 4.8 (p = 0.4) | 11.2 17.7 (p = 0.1) | [92] |
| <i>STK11mut</i> <i>STK11wt</i> | 111 559 | Stage IV – 2 nd -line (Sq+NonSq) | ICI | 24.7% 34.0% | 2.2 3.0 (p = 0.0002) | 6.3 12.0 (p = 0.0002) | |
| <i>STK11mut</i> <i>STK11wt</i> | 288 1849 | Stage IV – 1 st -line (Sq+NonSq) | Chemotherapy | 59.3% 65.7% | 4.5 5.8 (p < 0.0001) | 11.2 17.8 (p < 0.0001) | |
| <i>STK11mut</i> <i>STK11wt</i> | 83 780 | Stage IV – 2 nd -line (Sq+NonSq) | Chemotherapy | 33.3% 39.1% | 4.0 4.3 (p = 0.7) | 11.5 13.2 (p = 0.7) | |
| 6 ICI cohorts <i>STK11mut</i> <i>STK11wt</i> | 807 | Stage IV (Non-sq) | ICI | RR 0.71, p = 0.251 | HR 1.54, p = 0.002 | HR 1.57, p = 0.003 | [93] |

| | | | | | | | |
|--|-----------|---|---------------------------|--------------|---|-------------------------|------|
| 1 CT cohort <i>STK11mut</i> <i>STK11wt</i> | 244 | | Docetaxel | - | - | HR 1.82, p = 0.006 | |
| <i>STK11mut</i> <i>STK11wt</i> | 32 272 | Stage IV – 2 nd /3 rd line POPLAR and OAK trials (NonSq) | Atezolizumab | - | - | 7.3 15.6 (p = 0.004) | [94] |
| <i>STK11mut</i> <i>STK11wt</i> | 28 266 | | Docetaxel | - | - | 4.8 10.2 (p = 0.001) | |
| <i>STK11mut</i> <i>STK11wt</i> | 55 257 | Stage IV (Sq+NonSq) | Durvalumab | 16.7 25.2 | - | 10.3 13.3 | [95] |
| <i>STK11mut</i> <i>STK11wt</i> | 51 271 | | Durvalumab + tremelimumab | 21.6 23.6 | - | 4.4 11.3 | |
| <i>STK11mut</i> <i>STK11wt</i> | 41 268 | | Chemotherapy | 12.2 33.6 | - | 6.7 13.1 | |

Abbreviations: CCRT, concurrent chemo-radiotherapy; CP, platinum-pemetrexed; CPP, pembrolizumab plus platinum-pemetrexed; ICI, immune checkpoint inhibitor; n, number; NonSq, non-squamous; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; pts, patients; Sq, squamous.

Skoulidis and collaborators firstly determined *STK11mut* as a negative predictive factor of response to immunotherapy in the clinical setting [25]. The authors found that the presence of *STK11mut* was associated with inferior clinical outcomes to PD-1 blockade in multiple independent cohorts of *KRASmut* NSCLC, including those treated with nivolumab in the CheckMate 057 phase III study [25]. In addition, they examined the impact of *STK11mut* on clinical response to anti-PD-1/PD-L1 in 66 PD-L1 positive non-squamous (NonSq) NSCLC patients, regardless of *KRAS* mutational status and PD-L1 expression levels. Impressively, none of the patients in the *STK11mut* group responded to ICI compared to 34.5% of the *STK11* wild-type (*STK11wt*) group ($p = 0.026$), despite including PD-L1 high expressing tumors. *STK11mut* patients had a dramatically shorter median progression-free survival (mPFS) and overall survival (mOS) with PD-1 axis blockade (PFS Hazard Ratio [HR] 4.76, $p = 0.00012$; OS HR 14.3, $p < 0.0001$). This effect was observed across both the PD-L1 high ($\geq 50\%$) and PD-L1 low (PD-L1 $< 50\%$) groups [25].

The negative influence of *STK11mut* on ICI response was further confirmed by Biton et al [84]. Among different genetic alterations which constituted a tumor immune profile (e.g. *EGFRmut*, *TP53mut*), *STK11mut* alone was associated to shorter mPFS in a small cohort of 32 NonSq NSCLC patients treated with second-line nivolumab compared to the counterpart [84]. Concordantly, in a larger cohort of 1310 ICI treated individuals, mPFS was 2.5 months in *STK11mut* patients compared to 3.1 months in *STK11wt* group ($p = 0.01$) [89]. Jure-Kunkel et al also confirmed the negative impact of somatic *STK11mut* versus *STK1wt* in advanced Non-Sq NSCLC patients enrolled in two independent trials evaluating durvalumab monotherapy (CP1108/ATLANTIC), and in a durvalumab plus tremelimumab trial (D4190C00006), both in terms of shorter OS and significantly reduced overall response rate (ORR) [85]. Similarly, in exploratory analysis from the phase I/II MYSTIC trial evaluating durvalumab +/- tremelimumab in treatment-naïve NSCLC patients, *STK11mut* patients had a significantly shorter OS compared to the *wt* cohort [95]. Other research groups supported *STK11mut* as marker of poor response to ICI in NSCLC [83], even in the maintenance setting after concurrent chemoradiotherapy for stage III disease [82]. Moreover, [in different studies](#) *STK11mut* has been associated with hyperprogression to ICI, [in terms of an](#) accelerated tumor growth with worsening clinical status [96,97].

Of interest, *STK11mut* also defined a group of patients with apparently inferior clinical outcomes to the chemo-immunotherapy combination with platinum-pemetrexed plus pembrolizumab in the first-line setting [90]. Interestingly, in this study, patients who harbored *STK11mut* did not benefit from the addition of pembrolizumab to chemotherapy, both in terms of mPFS (4.8 versus 4.3 months, HR 1.13, 95% CI 0.83-1.54, $p = 0.75$) and mOS (10.6 versus 10.3 months, HR 1.03, 95% CI 0.71 to 1.49, $p = 0.79$) compared to platinum-pemetrexed alone [90]. Nonetheless, a recent report on

ha eliminato: in different studies

ha eliminato: intended

ha eliminato: s

patients enrolled in KEYNOTE-189 documented a better outcomes with pembrolizumab plus chemotherapy regardless of the presence of STK11 or KEAP1 mutation [98].

The notion that *STK11mut* may confer innate resistance to ICI in NSCLC was mainly drawn from retrospective studies that lacked the chemotherapy control group. In this regard, different reports explored the significance of *STK11mut* in both ICI and chemotherapy cohorts [92,93,95]. When focusing on chemotherapy, patients with *STK11mut* achieved worse outcomes to different chemotherapeutic regimens than *STK11wt* patients, across different treatment lines, to a similar extent for ICI [92,93,95]. In a large real-world cohort of 2137 advanced NSCLC patients treated with frontline chemotherapy (65.6% platinum-based), mPFS and mOS were shorter in *STK11mut* patients with regard to *STK11wt* (mPFS 4.5 versus 5.8 months, HR 1.4 [95% CI, 1.2-1.6], $p < 0.0001$; mOS 11.2 versus 17.8 months, HR 1.4 [95% CI, 1.2-1.6], $p < 0.0001$), with the same detrimental effect seen in ICI cohort [92]. When comparing anti-PD-1/PD-L1 efficacy to platinum-based chemotherapy in a treatment naïve cohort of 2276 patients, there was no association between *STK11mut* and both mPFS and OS (mPFS anti-PD-1/PD-L1 versus chemo, HR 1.05, 95% CI 0.76-1.44; mOS anti-PD-1/PD-L1 versus chemo, HR 1.13, 95% CI 0.76-1.67) [99]. Pooling data from POPLAR and OAK trials (randomizing pretreated patients to receive either atezolizumab or docetaxel), *STK11* status confirmed its prognostic role in non-squamous histology. Compared to *STK11wt* group indeed, median estimations of OS in *STK11mut* were halved in both immunotherapy and chemotherapy arms[94]. Moreover, in a recent post-hoc analysis of patients enrolled in IMpower150 study focused on *KRASmut* patients, *STK11mut* and/or *KEAP1mut* cohort achieved numerically shorter mPFS and mOS than the *wt* group, regardless of the treatment arm [100]. These results might remodulate the value of *STK11mut* as prognostic rather than predictive factor. However, further prospective research is needed to confirm these findings.

Other retrospective studies tried to assess whether *STK11mut* negative impact on ICI efficacy was either independent or conditioned from *KRAS* mutational status (**Table 2**). Shire et collaborators found that outcomes of *KRASmut/STK11mut* patients were similar to those in patients with *STK11mut* only, suggesting no additional deterioration in the double mutants [92]. On the contrary, other reports demonstrated that co-mutations in *STK11* and *KRAS* were associated with worse mPFS and mOS than *STK11mut* only patients treated with different regimens, including ICIs [83,101]. Still, a single-center retrospective study did not show significant differences in mPFS between *STK11mut/KRASmut* and *STK11mut* only NSCLC patients, but oppositely, – among ICI-treated patients – double mutants appeared to have a better prognosis (mOS 20.7 versus 13.6 months, $p = 0.049$) [102]. Lastly, in a retrospective analysis which included 1261 patients treated with ICIs, *STK11mut* was found to confer resistance to ICI in *KRASmut* but not *KRASwt* NSCLC patients [103].

ha eliminato: contraddittoriamente

Of note, the recent clinical development of KRAS G12C inhibitors (namely sotorasib and adagrasib opens new scenarios in the treatment of *KRASmut* NSCLC. Albeit limited to early evidence, *STK11* status does not affect negatively affect KRAS G12C inhibition, and *STK11* mutations have been associated to higher response rates to adagrasib [104,105].

The reshaping of TIME by KRAS G12C inhibitors has been documented in preclinical models, and the potential impact of *STK11* deficiency represents a topic of crucial interest, also in view of potential treatment combinations including KRAS targeted agents and immunotherapy [106].

ha eliminato: - Li BT, Skoulidis F, Falchook G, et al. CodeBreak 100: Registrational Phase 2 Trial of Sotorasib in *KRAS p.G12C* Mutated Non-small Cell Lung Cancer. Presented at: International Association for the Study of Lung Cancer 2020 World Conference on Lung Cancer; January 28-31, 2021; virtual. Abstract PS01.07[¶]
- Riely G, Ou SI, Rybkin I, et al. KRYSTAL-1: Activity and Preliminary Pharmacodynamic (PD) Analysis of Adagrasib (MRTX849) in Patients (Pts) With Advanced Non-Small- Cell Lung Cancer (NSCLC) Harboring *KRASG12C* Mutation. *J Thoracic Oncol.* 2021;16(suppl 4):990_PR. <https://bit.ly/3rvczEd>.

ha eliminato: [The clinical *KRAS(G12C)* inhibitor AMG 510 drives anti-tumour immunity. [¶]
Canon J, Rex K, Saiki AY, Mohr C, Cooke K, Bagal D, Gaida K, Holt T, Knutson CG, Koppada N, Lanman BA, Werner J, Rapaport AS, San Miguel T, Ortiz R, Osgood T, Sun JR, Zhu X, McCarter JD, Volak LP, Houk BE, Fakhri MG, O'Neil BH, Price TJ, Falchook GS, Desai J, Kuo J, Govindan R, Hong DS, Ouyang W, Henary H, Arvedson T, Cee VJ, Lipford JR. [¶]
Nature. 2019 Nov;575(7781):217-223. doi: 10.1038/s41586-019-1694-1.J. ...

Table 2. Impact of *STK11* mutations upon immunotherapy activity and efficacy in NSCLC patients according to *KRAS* status.

| Cohorts of pts | N of pts | Clinical setting | Treatment | ORR | mPFS (months) | mOS (months) | Ref | |
|--|----------|---|---|---------------------|--------------------------------|---------------------|-------|-------|
| <i>STK11</i> mut | 18 | Stage IV – 1 st -line (Sq+NonSq) | Different treatments (51 platinum-doublet; 6 targeted-therapy; 5 ICI) | - | 5.1 (p = 0.048) | 16.1 (p < 0.001) | [22] | |
| <i>STK11/KRAS</i> mut | 19 | | | | 2.4 | 7.1 | | |
| <i>STK11/TP53</i> mut | 18 | | | | 4.3 (p = 0.043) | 28.3 (p < 0.001) | | |
| <i>STK11/KRAS/TP53</i> mut | 7 | | | | 13.0 (p = 0.03) | 22.0 (p = 0.025) | | |
| SKT11mut | 37 | Stage IV | Different treatments | - | No differences | 11.9 (p = 0.028) | [102] | |
| <i>STK11/KRAS</i> mut | 36 | | | | | 20.3 | | |
| <i>STK11-KEAP1</i> wt | 2276 | Stage IV – 1 st -line (NonSq) | Anti PD-1/PD-L1 | - | - | - | [99] | |
| <i>STK11-KEAP1</i> mut | | | | | HR 0.8 [0.48-1.47] | HR 0.88 [0.43-1.81] | | |
| <i>STK11</i> mut | | | | | HR 1.33 [0.93-1.9] | HR 1.43 [0.91-2.26] | | |
| <i>KEAP1</i> mut | | | | | HR 1.71 [1.2-2.45] | HR 1.71 [1.04-2.81] | | |
| <i>STK11-KEAP1</i> wt | | Platinum chemotherapy | - | - | - | | | |
| <i>STK11-KEAP1</i> mut | | | | HR 1.07 [0.73-1.57] | HR 0.96 [0.61-1.52] | | | |
| <i>STK11</i> mut | | | | HR 1.32 [1.04-1.68] | HR 1.19 [0.89-1.6] | | | |
| <i>KEAP1</i> mut | | | | HR 1.53 [1.22-1.93] | HR 1.49 [1.14-1.95] | | | |
| <i>STK11</i> wt/ <i>KEAP1</i> wt | 74 | Stage IV – 1 st -line (NonSq) | CPP | 44.6% | - | - | [91] | |
| <i>STK11</i> mut/ <i>KEAP1</i> wt | 24 | | | | | | | 33.3% |
| <i>STK11</i> wt/ <i>KEAP1</i> mut | 14 | | | | | | | 28.6% |
| <i>STK11</i> mut/ <i>KEAP1</i> mut | 27 | | | | | | | 7.4% |
| <i>STK11</i> wt/ <i>KEAP1</i> wt, PD-L1+ | - | Stage IV (NonSq) | ICI | - | HR 0.36 [0.2-0.65], p = 0.0008 | - | [107] | |
| <i>STK11</i> mut and/or <i>KEAP1</i> mut, PD-L1+ | - | | | | HR 0.99 [0.59-1.69], p = 0.84 | - | | |
| <i>DFCI/MGH</i> cohort | 620 | Stage IV (NonSq) | ICI | 34.9% | 6.4 | 19.8 | [107] | |
| <i>KRAS</i> mut/ <i>STK11</i> wt | 189 | | | | 12.7% | 1.8 | | 8.0 |
| <i>KRAS</i> mut/ <i>STK11</i> mut | 55 | | | | 20.6% | 2.9 | | 11.2 |
| <i>KRAS</i> wt/ <i>STK11</i> wt | 320 | | | | 25.0% | 3.2 | | 14.4 |
| <i>KRAS</i> wt/ <i>STK11</i> mut | 56 | | | | 30.1% | 3.6 | | 16.9 |
| <i>MSKCC/MDACC</i> cohort | 641 | | | | 10.8% | 2.0 | | 5.9 |
| <i>KRAS</i> mut/ <i>STK11</i> wt | 209 | | | | 17.3% | 2.7 | | 14.1 |
| <i>KRAS</i> mut/ <i>STK11</i> mut | 83 | | | | 22.7% | 2.3 | | 9.1 |
| <i>KRAS</i> wt/ <i>STK11</i> wt | 283 | | | | 32.4% | 4.8 | | 17.3 |
| <i>KRAS</i> wt/ <i>STK11</i> mut | 66 | | | | 11.6% | 2.0 | | 6.2 |
| <i>Combined</i> cohort | 1261 | | | | 19.1% | 2.8 | | 12.4 |
| <i>KRAS</i> mut/ <i>STK11</i> wt | 398 | | | | 23.7% | 2.5 | | 13.0 |
| <i>KRAS</i> mut/ <i>STK11</i> mut | 138 | | | | | | | |
| <i>KRAS</i> wt/ <i>STK11</i> wt | 603 | | | | | | | |
| <i>KRAS</i> wt/ <i>STK11</i> mut | 122 | | | | | | | |
| <i>Group 1</i> | 166 | Stage IV – 1 st -line (Sq+NonSq) | ICI | | | | [92] | |

ha formattato: Inglese (Regno Unito)

| | | | | | | | |
|---|-------------------|---|--------------------|------------------------------------|--|--|-----------|
| <i>KRASmut/STK11mut</i> <i>KRASwt/STK11wt</i> | | | | - | 4.1 4.4 | 10.0 16.3 | |
| <i>Group 2</i> <i>KRASmut/STK11mut</i> <i>KRASwt/STK11wt</i> | 427 | Stage IV – 2 nd -line (Sq+NonSq) | ICI | - | 2.2 2.8 (p < 0.005) | 6.9 12.0 (p < 0.005) | |
| <i>Group 3</i> <i>KRASmut/STK11mut</i> <i>KRASwt/STK11wt</i> | 1493 | Stage IV – 1 st -line (Sq+NonSq) | Chemotherapy | - | 4.5 5.9 (p < 0.005) | 11.7 18.2 (p < 0.005) | |
| <i>Group 4</i> <i>KRASmut/STK11mut</i> <i>KRASwt/STK11wt</i> | 650 | Stage IV – 2 nd -line (Sq+NonSq) | Chemotherapy | - | 4.4 4.3 | 11.3 13.2 | |
| <i>SU2C cohort</i> <i>KRASmut/STK11mut (KL)</i> <i>KRASmut/TP53mut (KP)</i> <i>KRASmut/STK11wt/TP53wt (K-only)</i> | 54 56 64 | Stage IV (NonSq) | ICI | 7.4% 35.7% 28.6% (p < 0.001) | 1.8 3.0 2.7 (p = 0.0018) | 6.4 16.0 16.1 (p = 0.0045) | [25,108] |
| <i>KRASmut/STK11mut</i> <i>KRASwt/STK11wt</i> | 54 120 | Stage IV – 2 nd -line (NonSq) | Nivolumab | - | 1.8 2.7 (p < 0.001) | 6.4 16.0 (p = 0.0015) | |
| <i>CM-057 cohort</i> <i>KRASmut/STK11mut (KL)</i> <i>KRASmut/TP53mut (KP)</i> <i>KRASmut/STK11wt/TP53wt (K-only)</i> | 6 7 11 | Stage IV – 2 nd -line (NonSq) | Nivolumab | 0% 57.1% 18.2% (p = 0.047) | 2.0 5.1 2.1 (p = 0.62) | - | |
| <i>KRASmut/STK11mut</i> <i>KRASwt/STK11wt</i> | 3 17 | | | - | 4.2 5.5 (p = 0.22) | - | |
| <i>STK11mut/KRASmut</i> <i>STK11mut/KRASwt</i> | 14 63 | Stage IIIB-IV | ICI | - | 3.0 5.1 (p = 0.56) | 5.3 11.4 (p = 0.13) | [83] |
| <i>KRASmut</i> | 80 74 71 | Stage IV – 1 st -line (NonSq) | ABCP ACP BCP | - | 8.1 (HR 0.42) 4.8 (HR 0.80) 5.8 | 19.8 (0.50) 11.7 (HR 0.63) 9.8 | [100,109] |
| <i>KRASwt</i> | 235 234 226 | | ABCP ACP BCP | - | 8.4 (HR 0.65) 6.8 (HR 0.82) 7.0 | 18.9 (HR 0.98) 19.5 (HR 0.90) 18.2 | |
| <i>KRASmut/STK11mut and/or KEAP1mut</i> | 34 38 29 | | ABCP ACP BCP | - | 6.0 (HR 0.49) 3.2 (HR 0.88) 3.4 | 11.1 (HR 0.60) 7.9 (HR 0.87) 8.7 | |
| <i>KRASmut/STK11wt/KEAP1wt</i> | 46 36 42 | | ABCP ACP BCP | - | 15.2 (HR 0.36) 7.4 (HR 0.64) 6.9 | 26.2 (HR 0.43) 21.0 (HR 0.43) 10.7 | |

Abbreviations: ABCP, atezolizumab, bevacizumab, carboplatin, paclitaxel; ACP, atezolizumab, carboplatin, paclitaxel; BCP, bevacizumab, carboplatin, paclitaxel; CCRT, concurrent chemotherapy; CP, platinum-pemetrexed; CPP, pembrolizumab plus platinum-pemetrexed; ICI, immune checkpoint inhibitor; n, number; NonSq, non-squamous; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; pts, patients; Sq, squamous.

5. Summary

Unveiling the contribution of molecular underpinnings to cancer outcomes and therapeutic response is critical to reach the goal of personalized medicine in the current immunotherapy-driven scenario of NSCLC treatment. Through its principal substrate AMPK, STK11 acts as master kinase playing a crucial role in basic cellular processes, such as metabolism, DNA integrity, proliferation, cell polarity and angiogenesis.

ha eliminato: to

STK11 loss of function is translated in defective networking within the tissue milieu and promotes cancer growth, aggressiveness, epithelial-to-mesenchymal transition (EMT) and metastases. Moreover, *STK11* mutations significantly affect immune cell function and tumor-host immune homeostasis, leading to a cold, non-T cell-inflamed, tumor immune microenvironment (TIME). The mechanistic underpinnings of *STK11*-mediated immune suppressive TIME, featured by low effector cells and PD-L1 levels together with increased tumour-associated neutrophils and altered tumour cytokine/chemokine composition, mostly reside in epigenetic silencing of stimulator of interferon genes (STING).

ha eliminato: tization

Hence, it is conceivable to understand how and to which extent *STK11* genomic aberrations, fostering the tumor immune escape processes, critically impact on patient outcome and response to (immuno)therapy, harbouring a predictive and/or prognostic role that needs to be deciphered. With the support of a thorough literature review, *STK11* mutations harbors a prognostic rather than predictive role in NSCLC patients treated with ICIs. In STK11-deficient lung tumors, the development of novel potential treatment strategies aims at harnessing the immune system to turn immune-cold tumors in immune-reactive diseases, eventually improving outcomes of NSCLC patients.

Figure legend

Figure 1. Schematic representation of *STK11*_{mut}-driven inhibitory and activating molecular pathways.

The lack of activation of the key substrate AMPK resulting from STK11 deficiency is translated in multiple deranged immunomodulatory and stromal signaling consisting of:

-impaired immune response: through indirect SAM- or direct EZH2 and DNMT1-mediated epigenetic silencing of STING.

-immunosuppressive TIME: through dampening STING-triggered production of cytokines, as IFN β , and chemokines (CXCL10, CCL5 and CCL3) which in an autocrine or paracrine fashion can activate DCs for antigen presentation, including MHC, and cross-priming of anti-tumor T cells. These changes in cytokines and chemokines milieu also result in suppressed PD-L1 expression and reduced recruitment of T and B lymphocytes, DCs and TAM. Conversely, STK11 defective mutations increases the expression of proinflammatory cytokines/chemokines (IL-1 β , IL-6, G-CSF, CXCL7, CXCL3 and CXCL5) promoting the recruitment of ARG-1 secreting TAN and expansion of Tregs.

-angiogenesis and extracellular matrix (ECM) remodelling: disruption of the canonical STK11/AMPK/ TSC 1-2/mTOR/raptor pathway, in addition to alter the metabolic status, activates HIF1 α -mediated angiogenic boost, through VEGF and Ang2, and collagen cross-linking through upregulation of LOX. Unleashing of NRP1 and activation of NOX1 are two additional pro-angiogenic routes taking place from deregulation of STK11/AMPK axis.

AMPK: Adenosine mono phosphate Activated Protein Kinase; SAM: S-adenosyl methionine; EXH2: Enhancer of zeste homolog 2; DNMT1: DNA (cytosine-5)-methyltransferase 1; IFN β : Interferon β ; DCs: Dendritic Cells; MHC: Major Histocompatibility Complex; PD-L1: Programmed Death Ligand-1; TAM: Tumor Associated Macrophages; ARG-1: Arginase-1; G-CSF: Granulocyte-Colony Stimulating Factor; TAN: Tumor associated Neutrophil; Treg: T regulatory cell; TSC 1-2: tuberous sclerosis complex 1-2; mTOR: mammalian target of rapamycin; HIF1 α : Hypoxia Inducible Factor α ; VEGF: Vascular Endothelial Growth Factor; Ang2: Angiopoietin 2; LOX: Lysyl Oxidase; NRP1: Neuropilin-1; NOX1: NADPH oxidase 1.

► activation; ◄ inhibition; •••• missed upon *STK11*_{mut}; — induced by *STK11*_{mut}

References

- [1] Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science* (80-) 2018;359:1350–5. <https://doi.org/10.1126/science.aar4060>.
- [2] Pan C, Liu H, Robins E, Song W, Liu D, Li Z, et al. Next-generation immuno-oncology agents: Current momentum shifts in cancer immunotherapy. *J Hematol Oncol* 2020;13:1–15. <https://doi.org/10.1186/s13045-020-00862-w>.
- [3] Seliger B. Combinatorial Approaches with Checkpoint Inhibitors to Enhance Anti-tumor Immunity. *Front Immunol* 2019;10:1–10. <https://doi.org/10.3389/fimmu.2019.00999>.
- [4] de Miguel M, Calvo E. Clinical Challenges of Immune Checkpoint Inhibitors. *Cancer Cell* 2020;38:326–33. <https://doi.org/10.1016/j.ccell.2020.07.004>.
- [5] Bai R, Lv Z, Xu D, Cui J. Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors. *Biomark Res* 2020;8:34. <https://doi.org/10.1186/s40364-020-00209-0>.
- [6] Altorki NK, Markowitz GJ, Gao D, Port JL, Saxena A, Stiles B, et al. The lung microenvironment: an important regulator of tumour growth and metastasis. *Nat Rev Cancer* 2019;19:9–31. <https://doi.org/10.1038/s41568-018-0081-9>.*
- [7] Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med* 2018;24:541–50. <https://doi.org/10.1038/s41591-018-0014-x>.*

**These two reviews provides a comprehensive overview on the prognostic and predictive relavance of TIME.*

- [8] Mazzaschi G, Madeddu D, Falco A, Bocchialini G, Goldoni M, Sogni F, et al. Low PD-1 expression in cytotoxic CD8 β tumor-Infiltrating lymphocytes confers an immune-privileged tissue microenvironment in NSCLC with a prognostic and predictive value. *Clin Cancer Res* 2018;24:407–19. <https://doi.org/10.1158/1078-0432.CCR-17-2156>.
- [9] Gajewski TF. Next Hurdle in Cancer Immunorapy: Overcoming Non-T-Cell-Inflamed Tumor Microenvironment. *Semin Oncol* 2015;42:663–71. <https://doi.org/10.1053/j.seminoncol.2015.05.011>.
- [10] Trujillo JA, Sweis RF, Bao R, Luke JJ. T cell–inflamed versus Non-T cell–inflamed tumors: a conceptual framework for cancer immunotherapy drug development and combination therapy selection. *Cancer Immunol Res* 2018;6:990–1000. <https://doi.org/10.1158/2326->

6066.CIR-18-0277.

- [11] Nixon AB, Schalper KA, Jacobs I, Potluri S, Wang IM, Fleener C. Peripheral immune-based biomarkers in cancer immunotherapy: can we realize their predictive potential? *J Immunother Cancer* 2019;7:1–14. <https://doi.org/10.1186/s40425-019-0799-2>.
- [12] Jorgovanovic D, Song M, Wang L, Zhang Y. Roles of IFN- γ in tumor progression and regression: A review. *Biomark Res* 2020;8:1–16. <https://doi.org/10.1186/s40364-020-00228-x>.
- [13] Rizvi H, Sanchez-Vega F, La K, Chatila W, Jonsson P, Halpenny D, et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. *J Clin Oncol* 2018;36:633–41. <https://doi.org/10.1200/JCO.2017.75.3384>.*

**This study identifies specific genetic assets critical for the response to ICI in a large cohort of advanced NSCLC.*

- [14] Skoulidis F, Byers LA, Diao L, Papadimitrakopoulou VA, Tong P, Izzo J, et al. Co-occurring genomic alterations define major subsets of KRAS-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities. *Cancer Discov* 2015;5:861–78. <https://doi.org/10.1158/2159-8290.CD-14-1236>.
- [15] Camidge DR, Doebele RC, Kerr KM. Comparing and contrasting predictive biomarkers for immunotherapy and targeted therapy of NSCLC. *Nat Rev Clin Oncol* 2019;16:341–55. <https://doi.org/10.1038/s41571-019-0173-9>.
- [16] Skoulidis F, Heymach J V. Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. *Nat Rev Cancer* 2019;19:495–509. <https://doi.org/10.1038/s41568-019-0179-8>.**

***This review on the significance of co-mutations in NSCLC describes the distinctive immunobiological features STK11mut/ KRAS mut lung adenocarcinoma with therapeutic implications*

- [17] Ji H, Ramsey MR, Hayes DN, Fan C, McNamara K, Kozlowski P, et al. LKB1 modulates lung cancer differentiation and metastasis. *Nature* 2007;448:807–10. <https://doi.org/10.1038/nature06030>.

- [18] Aretz S, Stienen D, Uhlhaas S, Loff S, Back W, Pagenstecher C, et al. High proportion of large genomic STK11 deletions in Peutz-Jeghers syndrome. *Hum Mutat* 2005;26:513–9. <https://doi.org/10.1002/humu.20253>.
- [19] Sanchez-Cespedes M. A role for LKB1 gene in human cancer beyond the Peutz-Jeghers syndrome. *Oncogene* 2007;26:7825–32. <https://doi.org/10.1038/sj.onc.1210594>.
- [20] Gill RK, Yang SH, Meerzaman D, Mechanic LE, Bowman ED, Jeon HS, et al. Frequent homozygous deletion of the LKB1/STK11 gene in non-small cell lung cancer. *Oncogene* 2011;30:3784–91. <https://doi.org/10.1038/onc.2011.98>.
- [21] Fang R, Zheng C, Sun Y, Han X, Gao B, Li C, et al. Integrative genomic analysis reveals a high frequency of LKB1 genetic alteration in Chinese lung Adenocarcinomas. *J Thorac Oncol* 2014;9:254–8. <https://doi.org/10.1097/JTO.000000000000056>.
- [22] Bange E, Marmarelis ME, Hwang W-T, Yang Y-X, Thompson JC, Rosenbaum J, et al. Impact of KRAS and TP53 Co-Mutations on Outcomes After First-Line Systemic Therapy Among Patients With STK11 -Mutated Advanced Non-Small-Cell Lung Cancer . *JCO Precis Oncol* 2019;3:1–11. <https://doi.org/10.1200/po.18.00326>.*
- * *This study documents how KRAS and P53 co-occurring mutations differentially affect the response to first line treatment in NSCLC harboring STK11 mutations.*
- [23] Nadal E, Heeke S, Benzaquen J, Vilariño N, Navarro A, Azuara D, et al. Two Patients With Advanced-Stage Lung Adenocarcinoma With Radiologic Complete Response to Nivolumab Treatment Harboring an *STK11 / LKB1* Mutation. *JCO Precis Oncol* 2020:1239–45. <https://doi.org/10.1200/PO.20.00174>.
- [24] Hasegawa T, Yanagitani N, Ninomiya H, Sakamoto H, Tozuka T, Yoshida H, et al. Association between the efficacy of pembrolizumab and Low STK11/LKB1 expression in high-PD-L1-expressing non-small-cell lung cancer. *In Vivo (Brooklyn)* 2020;34:2997–3003. <https://doi.org/10.21873/invivo.12131>.
- [25] Skoulidis F, Goldberg ME, Greenawalt DM, Hellmann MD, Awad MM, Gainor JF, et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. *Cancer Discov* 2018;8:822–35. <https://doi.org/10.1158/2159-8290.CD-18-0099>**

** *This represents a well documented evidence of the negative impact of STK11 mutation on the response to ICI in lung adenocarcinoma.*

- [26] Malapelle U, Pisapia P, Rocco D, Smeraglio R, Spirito M di, Bellevicine C, et al. Next generation sequencing techniques in liquid biopsy: Focus on non-small cell lung cancer patients. *Transl Lung Cancer Res* 2016;5:505–10. <https://doi.org/10.21037/tlcr.2016.10.08>.
- [27] Heeke S, Hofman V, Long-Mira E, Lespinet V, Lalvée S, Bordone O, et al. Use of the ion PGM and the genereader NGS systems in daily routine practice for advanced lung adenocarcinoma patients: A practical point of view reporting a comparative study and assessment of 90 patients. *Cancers (Basel)* 2018;10. <https://doi.org/10.3390/cancers10040088>.
- [28] Granado-Martínez P, Garcia-Ortega S, González-Sánchez E, McGrail K, Selgas R, Grueso J, et al. STK11 (LKB1) missense somatic mutant isoforms promote tumor growth, motility and inflammation. *Commun Biol* 2020;3. <https://doi.org/10.1038/s42003-020-1092-0>.
- [29] Pécuchet N, Laurent-Puig P, Mansuet-Lupo A, Legras A, Alifano M, Pallier K, et al. Different prognostic impact of STK11 mutations in nonsquamous non-small-cell lung cancer. *Oncotarget* 2017;8:23831–40. <https://doi.org/10.18632/oncotarget.6379>.
- [30] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 2013;6. <https://doi.org/10.1126/scisignal.2004088>.
- [31] Chen L, Engel BE, Welsh EA, Yoder SJ, Brantley SG, Chen DT, et al. A sensitive nano string-based assay to score STK11 (LKB1) pathway disruption in lung adenocarcinoma. *J Thorac Oncol* 2016;11:838–49. <https://doi.org/10.1016/j.jtho.2016.02.009>.
- [32] Calles A, Sholl LM, Rodig SJ, Pelton AK, Hornick JL, Butaney M, et al. Immunohistochemical Loss of LKB1 Is a biomarker for more aggressive biology in KRAS-mutant lung Adenocarcinoma. *Clin Cancer Res* 2015;21:2851–60. <https://doi.org/10.1158/1078-0432.CCR-14-3112>.
- [33] Esteller M, Avizienyte E, Corn PG, Lothe RA, Baylin SB, Aaltonen LA, et al. Epigenetic inactivation of LKB1 in primary tumors associated with the Peutz-Jeghers syndrome. *Oncogene* 2000;19:164–8. <https://doi.org/10.1038/sj.onc.1203227>.
- [34] Matsumoto S, Iwakawa R, Takahashi K, Kohno T, Nakanishi Y, Matsuno Y, et al. Prevalence and specificity of LKB1 genetic alterations in lung cancers. *Oncogene* 2007;26:5911–8. <https://doi.org/10.1038/sj.onc.1210418>.
- [35] Shackelford DB, Shaw RJ. The LKB1-AMPK pathway: Metabolism and growth control in

tumour suppression. *Nat Rev Cancer* 2009;9:563–75. <https://doi.org/10.1038/nrc2676>.

- [36] Shaw RJ, Kosmatka M, Bardeesy N, Hurley RL, Witters LA, DePinho RA, et al. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc Natl Acad Sci U S A* 2004;101:3329–35. <https://doi.org/10.1073/pnas.0308061100>.
- [37] Liang X, Wang P, Gao Q, Tao X. Exogenous activation of LKB1/AMPK signaling induces G1 arrest in cells with endogenous LKB1 expression. *Mol Med Rep* 2014;9:1019–24. <https://doi.org/10.3892/mmr.2014.1916>.
- [38] Wang YS, Chen J, Cui F, Wang H, Wang S, Hang W, et al. LKB1 is a DNA damage response protein that regulates cellular sensitivity to PARP inhibitors. *Oncotarget* 2016;7:73389–401. <https://doi.org/10.18632/oncotarget.12334>.
- [39] Lizcano JM, Göransson O, Toth R, Deak M, Morrice NA, Boudeau J, et al. LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J* 2004;23:833–43. <https://doi.org/10.1038/sj.emboj.7600110>.
- [40] Zhang S, Schafer-Hales K, Khuri FR, Zhou W, Vertino PM, Marcus AI. The tumor suppressor LKB1 regulates lung cancer cell polarity by mediating cdc42 recruitment and activity. *Cancer Res* 2008;68:740–8. <https://doi.org/10.1158/0008-5472.CAN-07-2989>.
- [41] Roy BC, Kohno T, Iwakawa R, Moriguchi T, Kiyono T, Morishita K, et al. Involvement of LKB1 in epithelial-mesenchymal transition (EMT) of human lung cancer cells. *Lung Cancer* 2010;70:136–45. <https://doi.org/10.1016/j.lungcan.2010.02.004>.
- [42] Lin R, Elf S, Shan C, Kang HB, Ji Q, Zhou L, et al. 6-Phosphogluconate dehydrogenase links oxidative PPP, lipogenesis and tumour growth by inhibiting LKB1-AMPK signalling. *Nat Cell Biol* 2015;17:1484–96. <https://doi.org/10.1038/ncb3255>.
- [43] Li F, Han X, Li F, Wang R, Wang H, Gao Y, et al. LKB1 Inactivation Elicits a Redox Imbalance to Modulate Non-small Cell Lung Cancer Plasticity and Therapeutic Response. *Cancer Cell* 2015;27:698–711. <https://doi.org/10.1016/j.ccell.2015.04.001>.
- [44] Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? *Free Radic Biol Med* 2010;49:1603–16. <https://doi.org/10.1016/j.freeradbiomed.2010.09.006>.
- [45] Murray CW, Brady JJ, Tsai MK, Li C, Winters IP, Tang R, et al. An lkb1–sik axis

suppresses lung tumor growth and controls differentiation. *Cancer Discov* 2019;9:1590–605. <https://doi.org/10.1158/2159-8290.CD-18-1237>.

- [46] MacIver NJ, Blagih J, Saucillo DC, Tonelli L, Griss T, Rathmell JC, et al. The Liver Kinase B1 Is a Central Regulator of T Cell Development, Activation, and Metabolism. *J Immunol* 2011;187:4187–98. <https://doi.org/10.4049/jimmunol.1100367>.
- [47] Windt G, Pearce E. Metabolic switching and fuel choice during T-cell differentiation and memory development. *Immunol Rev* 2014;27:1–19. <https://doi.org/10.1111/j.1600-065X.2012.01150.x>. Metabolic.
- [48] Tamás P, Macintyre A, Finlay D, Clarke R, Feijoo-Carnero C, Ashworth A, et al. LKB1 is essential for the proliferation of T-cell progenitors and mature peripheral T cells. *Eur J Immunol* 2010;40:242–53. <https://doi.org/10.1002/eji.200939677>.
- [49] Wu D, Luo Y, Guo W, Niu Q, Xue T, Yang F, et al. Lkb1 maintains T reg cell lineage identity. *Nat Commun* 2017;8. <https://doi.org/10.1038/ncomms15876>.
- [50] Chen S, Fang L, Guo W, Zhou Y, Yu G, Li W, et al. Control of T reg cell homeostasis and immune equilibrium by Lkb1 in dendritic cells. *Nat Commun* 2018;9. <https://doi.org/10.1038/s41467-018-07545-8>.
- [51] Liu Z, Zhang W, Zhang M, Zhu H, Moriasi C, Zou MH. Liver kinase B1 suppresses lipopolysaccharide-induced nuclear factor κ B (NF- κ B) activation in macrophages. *J Biol Chem* 2015;290:2312–20. <https://doi.org/10.1074/jbc.M114.616441>.
- [52] Michels S, Heydt C, van Veggel B, Deschler-Baier B, Pardo N, Monkhorst K, et al. Genomic Profiling Identifies Outcome-Relevant Mechanisms of Innate and Acquired Resistance to Third-Generation Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Therapy in Lung Cancer. *JCO Precis Oncol* 2019;1–14. <https://doi.org/10.1200/po.18.00210>.
- [53] Schoenfeld AJ, Rizvi H, Bandlamudi C, Sauter JL, Travis WD, Rekhtman N, et al. Clinical and molecular correlates of PD-L1 expression in patients with lung adenocarcinomas. *Ann Oncol* 2020;31:599–608. <https://doi.org/10.1016/j.annonc.2020.01.065>.*

* *This is an updated article on the debated issue of the significance of PD-L1 expression and its correlation with clinical and molecular profiles in lung adenocarcinoma patients.*

- [54] Wang H, Shan Q, Guo J, Han X, Zhao C, Li H, et al. PDL1 high expression without TP53, KEAP1 and EPHA5 mutations could better predict survival for patients with NSCLC

receiving atezolizumab. Lung Cancer 2020. <https://doi.org/10.1016/j.lungcan.2020.11.006>.

- [55] Coelho MA, de Carné Trécesson S, Rana S, Zecchin D, Moore C, Molina-Arcas M, et al. Oncogenic RAS Signaling Promotes Tumor Immuno-resistance by Stabilizing PD-L1 mRNA. *Immunity* 2017;47:1083-1099.e6. <https://doi.org/10.1016/j.immuni.2017.11.016>.
- [56] Gillette MA, Satpathy S, Cao S, Dhanasekaran SM, Vasaikar S V., Krug K, et al. Proteogenomic Characterization Reveals Therapeutic Vulnerabilities in Lung Adenocarcinoma. *Cell* 2020;182:200-225.e35. <https://doi.org/10.1016/j.cell.2020.06.013>.**
- ** *This investigation, undertaken by advanced biomolecular approaches on a multigenomic scale, pinpoints the immunosuppressive feature associated with STK11 mutation.*
- [57] Mansuet-Lupo A, Alifano M, Cuchet NP, Biton JR, Becht E, Goc J, et al. Intratumoral immune cell densities are associated with lung adenocarcinoma gene alterations. *Am J Respir Crit Care Med* 2016;194:1403–12. <https://doi.org/10.1164/rccm.201510-2031OC>.
- [58] Schabath MB, Welsh EA, Fulp WJ, Chen L, Teer JK, Thompson ZJ, et al. Differential association of STK11 and TP53 with KRAS mutation-associated gene expression, proliferation and immune surveillance in lung adenocarcinoma. *Oncogene* 2016;35:3209–16. <https://doi.org/10.1038/onc.2015.375>.
- [59] Koyama S, Akbay EA, Li YY, Aref AR, Skoulidis F, Herter-Sprie GS, et al. STK11/LKB1 deficiency promotes neutrophil recruitment and proinflammatory cytokine production to suppress T-cell activity in the lung tumor microenvironment. *Cancer Res* 2016;76:999–1008. <https://doi.org/10.1158/0008-5472.CAN-15-1439>.
- [60] Nagaraj AS, Lahtela J, Hemmes A, Pellinen T, Blom S, Devlin JR, et al. Cell of Origin Links Histotype Spectrum to Immune Microenvironment Diversity in Non-small-Cell Lung Cancer Driven by Mutant Kras and Loss of Lkb1. *Cell Rep* 2017;18:673–84. <https://doi.org/10.1016/j.celrep.2016.12.059>.
- [61] Kitajima S, Ivanova E, Guo S, Yoshida R, Campisi M, Sundararaman SK, et al. Suppression of STING associated with lkb1 loss in KRAS-driven lung cancer. *Cancer Discov* 2019;9:34–45. <https://doi.org/10.1158/2159-8290.CD-18-0689>.*
- * *A well-documented evidence on the strong interplay between STK11 and STING pathways and its impact on patient immune response.*
- [62] Partanen JI, Nieminen AI, Mäkelä TP, Klefstrom J. Suppression of oncogenic properties of

c-Myc by LKB1-controlled epithelial organization. *Proc Natl Acad Sci U S A* 2007;104:14694–9. <https://doi.org/10.1073/pnas.0704677104>.

- [63] Kortlever RM, Sodir NM, Wilson CH, Burkhart DL, Pellegrinet L, Brown Swigart L, et al. Myc Cooperates with Ras by Programming Inflammation and Immune Suppression. *Cell* 2017;171:1301-1315.e14. <https://doi.org/10.1016/j.cell.2017.11.013>.
- [64] Kadara H, Choi M, Zhang J, Parra ER, Rodriguez-Canales J, Gaffney SG, et al. Whole-exome sequencing and immune profiling of early-stage lung adenocarcinoma with fully annotated clinical follow-up. *Ann Oncol* 2017;28:75–82. <https://doi.org/10.1093/annonc/mdw436>.
- [65] Corte CM Della, Byers LA. Evading the STING: LKB1 loss leads to STING silencing and immune escape in KRAS-mutant lung cancers. *Cancer Discov* 2019;9:16–8. <https://doi.org/10.1158/2159-8290.CD-18-1286>.
- [66] Barber GN. STING: Infection, inflammation and cancer. *Nat Rev Immunol* 2015;15:760–70. <https://doi.org/10.1038/nri3921>.
- [67] Hamarshah S, Groß O, Brummer T, Zeiser R. Immune modulatory effects of oncogenic KRAS in cancer. *Nat Commun* 2020;11. <https://doi.org/10.1038/s41467-020-19288-6>.
- [68] Della Corte CM, Sen T, Gay CM, Ramkumar K, Diao L, Cardnell RJ, et al. STING Pathway Expression Identifies NSCLC With an Immune-Responsive Phenotype. *J Thorac Oncol* 2020;15:777–91. <https://doi.org/10.1016/j.jtho.2020.01.009>.
- [69] Corrales L, Glickman LH, McWhirter SM, Kanne DB, Sivick KE, Katibah GE, et al. Direct Activation of STING in the Tumor Microenvironment Leads to Potent and Systemic Tumor Regression and Immunity. *Cell Rep* 2015;11:1018–30. <https://doi.org/10.1016/j.celrep.2015.04.031>.
- [70] Harrington KJ, Brody J, Ingham M, Strauss J, Cemerski S, Wang M, et al. Preliminary results of the first-in-human (FIH) study of MK-1454, an agonist of stimulator of interferon genes (STING), as monotherapy or in combination with pembrolizumab (pembro) in patients with advanced solid tumors or lymphomas. *Ann Oncol* 2018;29:viii712. <https://doi.org/10.1093/annonc/mdy424.015>.
- [71] Pan BS, Perera SA, Piesvaux JA, Presland JP, Schroeder GK, Cumming JN, et al. An orally available non-nucleotide STING agonist with antitumor activity. *Science (80-)* 2020;369. <https://doi.org/10.1126/science.aba6098>.

- [72] Chin EN, Yu C, Vartabedian VF, Jia Y, Kumar M, Gamo AM, et al. Antitumor activity of a systemic STING-activating non-nucleotide cGAMP mimetic. *Science* (80-) 2020;369:993–9. <https://doi.org/10.1126/science.abb4255>.
- [73] Morén A, Raja E, Heldin CH, Moustakas A. Negative regulation of TGF β signaling by the kinase LKB1 and the scaffolding protein LIP1. *J Biol Chem* 2011;286:341–53. <https://doi.org/10.1074/jbc.M110.190660>.
- [74] Katajisto P, Vaahromeri K, Ekman N, Ventelä E, Ristimäki A, Bardeesy N, et al. LKB1 signaling in mesenchymal cells required for suppression of gastrointestinal polyposis. *Nat Genet* 2008;40:455–9. <https://doi.org/10.1038/ng.98>.
- [75] Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, et al. TGF- β Signaling in Fibroblasts Modulates the Oncogenic Potential of Adjacent Epithelia. *Science* (80-) 2004;303:848–51. <https://doi.org/10.1126/science.1090922>.
- [76] Boldrini L, Giordano M, Lucchi M, Melfi F, Fontanini G. Expression profiling and microRNA regulation of the LKB1 pathway in young and aged lung adenocarcinoma patients. *Biomed Reports* 2018;9:198–205. <https://doi.org/10.3892/br.2018.1122>.
- [77] Gao Y, Xiao Q, Ma HM, Li L, Liu J, Feng Y, et al. LKB1 inhibits lung cancer progression through lysyl oxidase and extracellular matrix remodeling. *Proc Natl Acad Sci U S A* 2010;107:18892–7. <https://doi.org/10.1073/pnas.1004952107>.
- [78] Han X, Li F, Fang Z, Gao Y, Li F, Fang R, et al. Transdifferentiation of lung adenocarcinoma in mice with *Lkb1* deficiency to squamous cell carcinoma. *Nat Commun* 2014;5. <https://doi.org/10.1038/ncomms4261>.
- [79] Ylikorkala A, Rossi DJ, Korsisaari N, Luukko K, Alitalo K, Henkemeyer M, et al. Vascular abnormalities and deregulation of VEGF in *Lkb1*-deficient mice. *Science* (80-) 2001;293:1323–6. <https://doi.org/10.1126/science.1062074>.
- [80] Okon IS, Coughlan KA, Zhang C, Moriasi C, Ding Y, Song P, et al. Protein kinase LKB1 promotes RAB7-mediated neuropilin-1 degradation to inhibit angiogenesis. *J Clin Invest* 2014;124:4590–602. <https://doi.org/10.1172/JCI75371>.
- [81] Bonanno L, Paoli A De, Zulato E, Esposito G, Calabrese F, Favaretto A, et al. LKB1 Expression Correlates with Increased Survival in Patients with Advanced Non-Small Cell Lung Cancer Treated with Chemotherapy and Bevacizumab. *Clin Cancer Res* 2017;23:3316–24. <https://doi.org/10.1158/1078-0432.CCR-16-2410>.

- [82] An J, Yan M, Yu N, Chennamadhavuni A, Furqan M, Kruser T, et al. Outcomes of patients with stage III non-small cell lung cancer (NSCLC) that harbor a STK11 mutation. . *J Clin Oncol* 2020;38:9033–9033. https://doi.org/10.1200/jco.2020.38.15_suppl.9033.
- [83] Uba R, Raez LE, Dumais K, Gentile F, Powery HW, Domingo GC, et al. Serine/threonine kinase 11 (STK11) mutations and immunotherapy resistance in patients with non-small cell lung cancer. *J Clin Oncol* 2020;38:e15055–e15055. https://doi.org/10.1200/jco.2020.38.15_suppl.e15055.
- [84] Biton J, Mansuet-Lupo A, Pécuchet N, Alifano M, Ouakrim H, Arrondeau J, et al. TP53, STK11, and EGFR mutations predict tumor immune profile and the response to anti-PD-1 in lung adenocarcinoma. *Clin Cancer Res* 2018;24:5710–23. <https://doi.org/10.1158/1078-0432.CCR-18-0163>.
- [85] Jure-Kunkel M, Wu S, Xiao F, Abdullah SE, Gao G, Englert JM, et al. Somatic STK11/LKB1 mutations to confer resistance to immune checkpoint inhibitors as monotherapy or in combination in advanced NSCLC. . *J Clin Oncol* 2018;36:3028–3028. https://doi.org/10.1200/jco.2018.36.15_suppl.3028.
- [86] A Phase 1/2 Study to Evaluate MEDI4736 - Full Text View - ClinicalTrials.gov n.d.
- [87] Garassino MC, Cho BC, Kim JH, Mazières J, Vansteenkiste J, Lena H, et al. Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (ATLANTIC): an open-label, single-arm, phase 2 study. *Lancet Oncol* 2018;19:521–36. [https://doi.org/10.1016/S1470-2045\(18\)30144-X](https://doi.org/10.1016/S1470-2045(18)30144-X).
- [88] Planchard D, Reinmuth N, Orlov S, Fischer JR, Sugawara S, Mandziuk S, et al. ARCTIC: durvalumab with or without tremelimumab as third-line or later treatment of metastatic non-small-cell lung cancer. *Ann Oncol* 2020;31:609–18. <https://doi.org/10.1016/j.annonc.2020.02.006>.
- [89] Murugesan K, Li G, Kaushik G, Singal G, Miller VA, Frampton GM, et al. Identification of genomic markers of sensitivity and resistance to checkpoint inhibitors in non-small cell lung cancer in a real world clinico-genomic database. *Ann Oncol* 2018;29:viii509–10. <https://doi.org/10.1093/annonc/mdy292.033>.
- [90] Skoulidis F, Arbour KC, Hellmann MD, Patil PD, Marmarelis ME, Awad MM, et al. Association of STK11/LKB1 genomic alterations with lack of benefit from the addition of pembrolizumab to platinum doublet chemotherapy in non-squamous non-small cell lung

cancer. *J Clin Oncol* 2019;37:102–102. https://doi.org/10.1200/jco.2019.37.15_suppl.102.

- [91] Skoulidis F, Arbour K, Hellmann M, Patil P, Marmarelis M, Owen D, et al. MA11.11 STK11/LKB1 Genomic Alterations Are Associated with Inferior Clinical Outcomes with Chemo-Immunotherapy in Non-Squamous NSCLC. *J Thorac Oncol* 2019;14:S294–5. <https://doi.org/10.1016/j.jtho.2019.08.591>.
- [92] Shire NJ, Klein AB, Golozar A, Collins JM, Fraeman KH, Nordstrom BL, et al. STK11 (LKB1) mutations in metastatic NSCLC: Prognostic value in the real world. *PLoS One* 2020;15:1–14. <https://doi.org/10.1371/journal.pone.0238358>.
- [93] Zhao H, Qi N, Chen D, Li D, Fu Y, Xu Y, et al. STK11/LKB1 revisited: A prognostic rather than predictive biomarker for immune checkpoint inhibitor in EGFR/ALK WT nonsquamous non-small cell lung cancer (NSCLC). *J Clin Oncol* 2020;38:e21548–e21548. https://doi.org/10.1200/jco.2020.38.15_suppl.e21548.
- [94] Shang X, Li Z, Sun J, Zhao C, Lin J, Wang H. Survival analysis for non-squamous NSCLC patients harbored STK11 or KEAP1 mutation receiving atezolizumab. *Lung Cancer* 2021;0. <https://doi.org/10.1016/j.lungcan.2021.02.010>.
- [95] Rizvi N, Cho BC, Reinmuth N, Lee KH, Luft A, Ahn M, et al. OA04.07 Mutations Associated with Sensitivity or Resistance to Immunotherapy in mNSCLC: Analysis from the MYSTIC Trial. *J Thorac Oncol* 2019;14:S217. <https://doi.org/10.1016/j.jtho.2019.08.428>.
- [96] Tadesse E ale, Heslin K, Hendawi M, Hirsch J, Idyro C, Thompson MA. Molecular alterations with hyperprogression in lung cancer patients treated with immune checkpoint inhibitors in a large health system. *J Clin Oncol* 2020;38:e15082–e15082. https://doi.org/10.1200/jco.2020.38.15_suppl.e15082.
- [97] Fricke J, Mambetsariev I, Pharaon R, Subbiah S, Rajurkar S, Salgia R. Hyperprogression on immunotherapy with complete response to chemotherapy in a NSCLC patient with high PD-L1 and STK11: A case report. *Medicine (Baltimore)* 2020;99:e22323. <https://doi.org/10.1097/MD.00000000000022323>.
- [98] Gadgeel SM, Rodriguez-Abreu D, Felip E, Esteban E, Speranza G, Reck M, et al. Abstract LB-397: Pembrolizumab plus pemetrexed and platinum vs placebo plus pemetrexed and platinum as first-line therapy for metastatic nonsquamous NSCLC: analysis of KEYNOTE-189 by STK11 and KEAP1 status. *Cancer Res.*, vol. 80, American Association for Cancer Research (AACR); 2020, p. LB-397-LB-397. <https://doi.org/10.1158/1538-7445.am2020-lb->

- [99] Papillon-Cavanagh S, Doshi P, Dobrin R, Szustakowski J, Walsh AM. STK11 and KEAP1 mutations as prognostic biomarkers in an observational real-world lung adenocarcinoma cohort. *ESMO Open* 2020;5. <https://doi.org/10.1136/esmoopen-2020-000706>.
- [100] West H, Cappuzzo F, Reck M, Mok T, Jotte RM, Nishio M, et al. 1265P IMpower150: A post hoc analysis of efficacy outcomes in patients with KRAS, STK11 and KEAP1 mutations. *Ann Oncol* 2020;31:S817–8. <https://doi.org/10.1016/j.annonc.2020.08.1579>.
- [101] Bange E, Marmarelis ME, Hwang W-T, Yang Y-X, Thompson JC, Rosenbaum J, et al. Impact of KRAS and TP53 Co-Mutations on Outcomes After First-Line Systemic Therapy Among Patients With STK11 -Mutated Advanced Non-Small-Cell Lung Cancer . *JCO Precis Oncol* 2019;9:1–11. <https://doi.org/10.1200/po.18.00326>.
- [102] Basher F, Saravia D, Fanfan D, Cotta JA, Lopes G. Impact of STK11 and KRAS co-mutations on outcomes with immunotherapy in non-small cell lung cancer. *J Clin Oncol* 2020;38:e15135–e15135. https://doi.org/10.1200/jco.2020.38.15_suppl.e15135.
- [103] Ricciuti B, Arbour KC, Lin JJ, Vokes N, Vajdi Hoojghan A, Li YY, et al. Effect of STK11 mutations on efficacy of PD-1 inhibition in non-small cell lung cancer (NSCLC) and dependence on KRAS mutation status. *J Clin Oncol* 2020;38:e15113–e15113. https://doi.org/10.1200/jco.2020.38.15_suppl.e15113.
- [104] Li B, Skoulidis F, Falchook G, Sacher A, Velcheti V, Dy G, et al. PS01.07 Registrational Phase 2 Trial of Sotorasib in KRAS p.G12C Mutant NSCLC: First Disclosure of the Codebreak 100 Primary Analysis. *J Thorac Oncol* 2021;16:S61. <https://doi.org/10.1016/j.jtho.2021.01.321>.
- [105] Riely GJ, Ou S-HI, Rybkin I, Spira A, Papadopoulos K, Sabari JK, et al. 99O_PR KRYSTAL-1: Activity and preliminary pharmacodynamic (PD) analysis of adagrasib (MRTX849) in patients (Pts) with advanced non-small cell lung cancer (NSCLC) harboring KRASG12C mutation. *J Thorac Oncol* 2021;16:S751–2. [https://doi.org/10.1016/s1556-0864\(21\)01941-9](https://doi.org/10.1016/s1556-0864(21)01941-9).
- [106] Canon J, Rex K, Saiki AY, Mohr C, Cooke K, Bagal D, et al. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. *Nature* 2019;575:217–23. <https://doi.org/10.1038/s41586-019-1694-1>.
- [107] Ricciuti B, Arbour KC, Lin JJ, Vajdi Hoojghan A, Vokes N, Hong L, et al. Effect of STK11

mutations on efficacy of PD-1 inhibition in non-small cell lung cancer (NSCLC) and dependence on KRAS mutation status. Present IASLC 2020 WCLC World Conf Lung Cancer 2021.

- [108] Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* 2015;373:1627–39. <https://doi.org/10.1056/NEJMoa1507643>.
- [109] Reck M, Mok TSK, Nishio M, Jotte RM, Cappuzzo F, Orlandi F, et al. Atezolizumab plus bevacizumab and chemotherapy in non-small-cell lung cancer (IMpower150): key subgroup analyses of patients with EGFR mutations or baseline liver metastases in a randomised, open-label phase 3 trial. *Lancet Respir Med* 2019;7:387–401. [https://doi.org/10.1016/S2213-2600\(19\)30084-0](https://doi.org/10.1016/S2213-2600(19)30084-0).