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LKB1 mutations are not associated with the efficacy of first-line and second-line chemotherapy in patients with advanced non-small-cell lung cancer (NSCLC): a post hoc analysis of the TAILOR trial

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ABSTRACT

Purpose In patients with advanced lung adenocarcinoma, the impact of *LKB1* mutations on cytotoxic chemotherapy efficacy remains poorly explored. Here, we aimed at investigating the potential impact of *LKB1* mutational status on chemotherapy efficacy in advanced non-small-cell lung cancer (NSCLC) patients enrolled in the TArceva Italian Lung Optimisation tRial (TAILOR) trial.

Methods The multicenter TAILOR trial randomised patients with *EGFR*-wild type (wt) advanced NSCLC progressing on/after previous platinum-based chemotherapy to receive docetaxel or erlotinib. Here, we evaluated the impact of *LKB1* mutational status on progression-free survival (PFS) and overall survival (OS) in patients treated with second-line docetaxel/erlotinib or during prior platinum-based chemotherapy.

Results Out of 222 patients randomised in the TAILOR trial, left-over tumour tissues were available for 188 patients, and 120 patients with evaluable *LKB1* status were included. Of them, 17 (14.17%) patients had *LKB1*-mutated tumours, while 103 (85.83%) had *LKB1*-wt disease. During second-line treatment, PFS and OS were not statistically significantly different in patients with *LKB1*-mutated when compared with *LKB1*-wt NSCLC (adjusted HR (aHR)=1.29, 95% CI 0.75 to 2.21; p=0.364 and aHR=1.41, 95% CI 0.82 to 2.44; p=0.218, respectively). Similarly, we found no significant association between *LKB1* mutations and patient PFS or OS during prior first-line platinum-based chemotherapy (aHR=1.04, 95% CI 0.55 to 1.97; p=0.910 and aHR=0.83, 95% CI 0.42 to 1.65; p=0.602, respectively).

Conclusion Among advanced NSCLC patients receiving two lines of systemic therapy, *LKB1* mutations were not associated with PFS or OS during second-line docetaxel or prior first-line platinum-based chemotherapy. While larger prospective trials are needed to confirm our findings, cytotoxic chemotherapy remains the backbone of investigational combination strategies in this patient population.

Key questions

What is already known about this subject?

► In patients with advanced lung adenocarcinomas, LKB1 mutations have been associated with poor clinical benefit from single-agent immune checkpoint inhibitors (ICIs) and new chemotherapy-ICI combinations. However, whether LKB1 mutations also result in poor clinical benefit from standard, first- or second-line chemotherapy (ie, platinum- and taxane-based chemotherapy, respectively), is currently unknown.

What does this study add?

► In a post-hoc analysis of the TAILOR trial, we found that LKB1 mutations are not associated with significantly lower PFS and OS in advanced NSCLC patients treated with second-line docetaxel or with first-line, platinum-based combination chemotherapy. This is the first study to assess the impact of LKB1 mutations on the efficacy of standard first- and second-line chemotherapy in advanced NSCLC patients in the context of a prospective randomized trial.

How might this impact on clinical practice?

► This study shows that second-line taxane-based chemotherapy and first-line, platinum-based doublet chemotherapy are not less effective in patients with LKB1-mutated NSCLC when compared to patients with LKB1-wt neoplasms. Based on results of our study, we propose that standard platinum-based chemotherapy (plus/minus ICIs) should remain the backbone therapy for the design of investigational combination treatments in patients with advanced LKB1-mutated NSCLC. Therefore, our results have implications in the context of clinical practice and for the design of experimental treatments.

INTRODUCTION

The advent of immunotherapy has revolutionised the treatment of advanced non-small-cell lung cancer (NSCLC).[1–5] When compared with standard chemotherapy, the anti-PD1 (programmed cell death protein 1) monoclonal antibodies nivolumab and pembrolizumab improved overall survival (OS) in patients with advanced NSCLC treated in the second-line setting and, in the case of tumours displaying PD-L1 (programmed death ligand 1) expression in >50% of tumour cells, also in the first-line setting.[1 6–8] More recently, first-line chemo-immunotherapy combinations significantly prolonged progression-free survival (PFS) and OS when compared with chemotherapy alone in patients with both squamous and non-squamous advanced NSCLC.[3–5] However, not all patients benefit from currently available immunotherapies and, with the exception of intratumor PD-L1 expression, no predictive factors of clinical benefit, or lack of benefit, from single-agent anti-PD1 immunotherapy have been identified yet.[8]

The liver kinase B1 (LKB1)/serine/threonine kinase 11 tumour suppressor protein regulates crucial events related to cell growth, proliferation and metabolism.[9] By phosphorylating and activating the AMP-activated kinase, LKB1 contributes to inhibit energy-consuming anabolic processes, such as protein, fatty acid and cholesterol biosynthesis, in conditions of nutrient deprivation and ATP shortage.[9–12] Conversely, LKB1 inactivation makes eukaryotic cells unable to halt anabolic processes during energy stress and metabolite depletion, thus exposing them to rapid apoptosis activation.[10 13]

LKB1 is partially or completely inactivated in 15%–30% of lung adenocarcinomas, with *LKB1* point mutations or deletions being the most common genetic inactivation mechanisms.[14–16] Of note, *LKB1* mutations indirectly determine a more immunosuppressive tumour microenvironment, thus potentially explaining the lower efficacy of immunotherapy agents in mouse models and in patients with *KRAS* and *LKB1* co-mutated advanced NSCLC when compared with patients with single *KRAS*-mutated or with *KRAS* and *TP53* co-mutated neoplasms.[17 18] Moreover, recent data showed that patients with *LKB1*-mutated non-squamous NSCLC treated with first-line chemo-immunotherapy not only have shorter PFS and OS when compared with patients with *LKB1*-wt neoplasms, but they do not seem to benefit from adding pembrolizumab to first-line platinum-based chemotherapy.[19]

At the same time, accumulating preclinical evidence indicates that *LKB1*-mutated neoplasms may be exquisitely sensitive to energetic and metabolic stress; in particular, the oxidative phosphorylation inhibitors metformin or phenformin have shown promising antitumour activity in preclinical models of *LKB1*-mutated lung adenocarcinoma.[20 21] Prospective clinical trials are ongoing to test the antitumour efficacy of therapeutic approaches aimed at targeting metabolic reprogramming in LKB1-inactive advanced lung adenocarcinoma.[22]

While LKB1 inactivation could make lung adenocarcinoma cells resistant to immunotherapy and potentially sensitive to metabolic interventions, the impact of *LKB1* mutations on the efficacy of standard cytotoxic chemotherapy in patients with advanced NSCLC remains poorly clarified. Here, we conducted a post hoc analysis to investigate the impact of *LKB1* mutations on the outcome of advanced NSCLC patients receiving second-line docetaxel/erlotinib in the context of the TArceva Italian Lung Optimisation tRial (TAILOR) trial, as well as during their prior first-line platinum-based chemotherapy.[23]

METHODS

Patient population and study objectives

The TAILOR study (registered at ClinicalTrials.gov, number NCT00637910) was a non-profit, multicenter, open label, randomised phase III trial. The study, funded by the Italian Regulatory Agency AIFA (Agenzia Italiana del Farmaco), was conducted in 52 Italian centres and enrolled patients with *EGFR*-wild type (wt), advanced NSCLC progressing after adjuvant or first-line platinum-based chemotherapy. Enrolled patients were randomised in a 1:1 ratio to receive docetaxel or erlotinib. The study enrolled 222 patients between 12 October 2007 and 13 March 2012. Results of the TAILOR trial have been previously published.[23]

The primary objective of this post hoc analysis of the TAILOR trial was to evaluate the impact of *LKB1* mutational status on the clinical outcome of patients treated with second-line docetaxel/erlotinib. Another objective of this study was to investigate the impact of *LKB1* mutations on the efficacy of prior platinum-based chemotherapy, and in particular of first-line platinum-based chemotherapy. To consider patients for this post hoc analysis, the following information had to be available: *LKB1* mutational status (mutated vs wt); type of second-line treatment after patient randomisation in the TAILOR study; type of platinum-based chemotherapy regimen before enrollment in the TAILOR trial.

Evaluation of *LKB1* mutational status

Tumour tissue specimens from patients enrolled in the TAILOR trial were prospectively collected at the time of tumour diagnosis. Tumour DNA was extracted using the QIAamp Gene Read DNA FFPE kit (Qiagen). Unstained 5 µm paraffin sections were cut and incubated overnight in a drying oven at 37°C. Manual macrodissection was performed using H&E-stained slides as a guide. To prepare a genomic library, we amplified 40 ng of DNA using a customised panel of the following 111 genes: *ABL1*, *AKT1*, *ALK*, *APC*, *ARID1A*, *ARID2*, *ATM*, *ATRX*, *BAP1*, *BAX*, *BLM*, *BRACHYURY*, *BRAF*, *CDH1*, *CDK4*, *CDKN2A*, *CSF1R*, *CTNNB1*, *DAXX*, *DDR2*, *EGFR*, *EZH2*, *FANCM*, *FBXW7*, *FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, *FHIT*, *FLT3*, *FOXA1*, *GATA3*, *GNA11*, *GNAQ*, *GNAS*, *GRM3*, *HER2*, *HER4*, *GENE*, *HNF1A*, *HRAS*, *IDH1*, *IDH2*, *IGF1R*, *IGF2R*, *JAK2*, *JAK3*, *KDM5C*, *KIT*, *KRAS*, *MED12*,

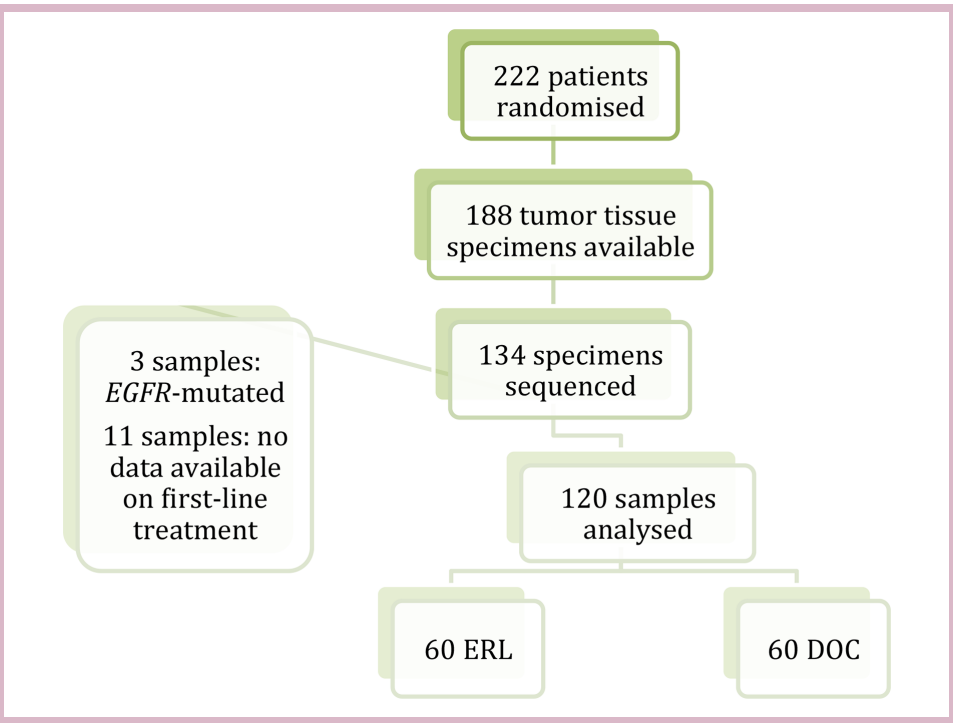

Figure 1 Study flow chart. Among 222 patients randomised in the tailor trial, 120 were finally evaluable for the current post hoc analysis. Of them, 60 patients received docetaxel and 60 received erlotinib treatment. DOC, docetaxel; ERL, erlotinib.

MEK1, *MEK4*, *MEN1*, *MET*, *MLH1*, *MLL3*, *MPL*, *MSH2*, *MSH6*, *mTOR*, *NF1*, *NOTCH1*, *NPM1*, *NRAS*, *PBRM1*, *PDGFRA*, *PDGFRB*, *PIK3CA*, *PIK3R1*, *PMS2*, *POLK*, *PPP6C*, *PRKDC*, *PTCH1*, *PTEN*, *PTPN11*, *RAC1*, *GENE*, *RAD50*, *RB1*, *RET*, *RIT1*, *RUNX1*, *RUNX3*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SETD2*, *SMAD2*, *SMAD3*, *SMAD4*, *SMARCB1*, *SMO*, *SNX31*, *SPOP*, *SRC*, *STK11 (LKB1)*, *TACC1*, *TBX3*, *TERT*, *TGFBR2*, *TNF*, *TP53*, *TR2*, *TSC1*, *TSC2*, *VEGFR1*, *VEGFR2*, *VEGFR3*, *VHL*, *WT1*. We used the Ion Ampliseq Library kit 2.0 (Thermo Fisher Scientific) according to the manufacturer's, instructions. The templates were loaded onto an Ion 316 chip and sequenced on a PGM sequencer with the Ion PGM sequencing 200 kit V.2 according to the manufacturer's instructions. The Torrent Suite Software V.3.6.2 (Thermo Fisher) was used to analyse raw data; coverage analysis was performed using the plug-in V.3.6. Each mutation in the variant list resulting from these analyses was verified in the integrative genome viewer from the Broad Institute42. We only considered mutations reported in the 'Sanger Institute Catalogue of Somatic Mutations in Cancer (COSMIC) database'41, 'Ensemble Variant Effect Predictor pipeline' and 'dbSNP (Single Nucleotide Polymorphism database) database', while silent or intronic mutations were not reported. The coverage was always more than 100X, and the reported mutations had a frequency of at least 5% in the tumour cell population. Matched normal DNA for six patients was also used. We finally considered as *LKB1*-mutated those tumours bearing *LKB1* deletions, nonsense mutations or missense mutations defined as 'probably damaging' by Polyphen tool.

Statistical analysis

The primary endpoint of this post hoc analysis was PFS, defined as the time between patient randomisation and the date of first documentation of disease progression or patient death from any cause, whichever came first. Secondary endpoints were: OS, defined as the time from randomisation to the date of patient death from any cause; PFS and OS after the initiation of first-line chemotherapy. Patients who had not died or had not undergone disease progression at the date of study cut-off were censored at the time of the last available information on their status.

PFS and OS during second-line treatment were analysed in the whole population, as well as in subgroups of patients defined on the basis of the treatment received (ie, docetaxel vs erlotinib). We also analysed PFS and OS during prior platinum-based chemotherapy in the whole patient population and in the subgroup of patients receiving platinum doublets as their first-line treatment. Survival curves were estimated with the Kaplan-Meier method, and differences between survival curves were assessed with the log-rank test. Cox proportional hazards models were used to investigate the impact of *LKB1* mutational status on PFS and OS. Univariate and multivariable analyses (adjusted for Eastern Cooperative Group- Performance Status (ECOG-PS), sex, histotype, smoking history and treatment arm) were performed for PFS and OS. Results were expressed as adjusted HRs (aHRs) with their corresponding 95% CIs. The χ^2 test was used to investigate the associations between *LKB1* mutational status and clinical or histopathological characteristics. All statistical tests were two-sided and a p value<0.05 was considered

as statistically significant. Statistical analyses were carried out using SAS V.9.4.

RESULTS

Patient population

The study flowchart is illustrated in figure 1. Out of 222 patients enrolled in the TAILOR trial, 188 had available tumour tissue specimens, and 134 samples were successfully sequenced with a customised panel of 111 genes including the *STK11/LKB1* gene. After excluding three patients due to the presence of activating *EGFR* mutations and 11 patients for the lack of knowledge about the type of first-line chemotherapy, 120 patients with evaluable tumour tissues were finally considered for this analysis; in 63 of these cases *LKB1* status was assessed in tumour tissue specimens deriving from primary tumour surgical resection, while 57 specimens evaluated for *LKB1* status derived from needle biopsies. Of 120 patients included in this post hoc analysis, 60 had been randomised to receive docetaxel and 60 to receive erlotinib as second-line treatment. Table 1 illustrates the characteristics of patients considered in this post hoc analysis. The majority of patients were males (69.2%), had an ECOG-PS of 0–1 (98.3%), were diagnosed with advanced (stage IIIb–IV) disease (64.2%), had tumours of adenocarcinoma histology (65%) and received platinum-based chemotherapy as their first-line treatment for advanced disease (66.7%). Cisplatin–gemcitabine (n=44; 36.7%) and cisplatin–pemetrexed (n=26; 21.7%) were the two most commonly used platinum combinations.

Out of 120 evaluable tumour specimens, 103 (85.83%) were wt for *LKB1*, while *LKB1* mutations were detected in 17 samples (14.17%). *LKB1* mutational status was not associated with patient sex, ECOG-PS, smoking history, tumour stage and patient age at diagnosis, tumour histology or the administration of prior adjuvant therapy, while a significant association was found between *LKB1* mutations and low tumour grade or *KRAS* mutations (table 2).

LKB1 alterations detected in tumour tissue specimens

Out of 17 *LKB1* genetic alterations detected, 15 were point mutations, five of which lead to a stop codon and a truncated protein; the remaining two alterations consisted of one microdeletion and one microinsertion (online supplementary table 1). All genetic alterations occurred at different *LKB1* gene loci, with the exception of the P324L and P324S substitutions. These data are consistent with previous findings showing that *LKB1* mutations/deletions/insertions can occur across the whole coding region of *LKB1* gene, that is, there are no hotspot mutational regions.[14–16]

Impact of *LKB1* mutations on clinical outcomes during second-line treatment

At a median follow-up of 63.22 months, 117 (97.5%) patients had undergone disease progression and 111 (92.5%) had died. PFS or OS were not significantly different between patients with *LKB1*-mutated vs *LKB1*-wt tumours (PFS: median 2.66 and 2.57 months, respectively; aHR=1.29, 95% CI 0.75 to 2.21; p=0.364; OS: median 4.41 and 6.78 months, respectively; aHR=1.41, 95% CI 0.82 to 2.44; p=0.218) (figure 2A,B). Among patients treated with second-line docetaxel, we found no statistically significant PFS or OS differences between patients with *LKB1*-mutated and *LKB1*-wt tumours (PFS: aHR=0.98; 95% CI 0.41 to 2.36; p=0.964; OS: aHR=1.38, 95% CI 0.57 to 3.34; p=0.47) (figure 2C,D). On the other hand, among erlotinib-treated patients, we observed worse clinical outcomes in patients with *LKB1*-mutated tumours when compared with patients with *LKB1*-wt neoplasms, but this effect was not statistically significant (PFS: aHR=1.63, 95% CI 0.78 to 3.38; p=0.192; OS: aHR=1.66, 95% CI 0.80 to 3.45; p=0.171) (online supplementary figure 1A,B).

Impact of *LKB1* mutations on clinical outcomes during first-line chemotherapy

In the same patient population, we also assessed the impact of *LKB1* status on the efficacy of prior platinum-based chemotherapy. At a median follow-up of 91.18 months, 120 (100%) patients had undergone tumour progression and 111 (92.5%) patients had died. Median PFS in the whole population was 6.61 months. Median PFS was 5.39 and 6.84 months in patients with *LKB1*-mutated and *LKB1*-wt tumours, respectively, with no statistically significant PFS differences (aHR=1.03, 95% CI 0.60 to 1.75; p=0.918) (figure 3A). Similarly, we observed no significant OS differences between patients with *LKB1*-mutated and *LKB1*-wt tumours (median 10.0 and 17.4 months, respectively; aHR=1.31; 95% CI 0.75 to 2.28; p=0.339) (figure 3B). Also when we limited our analysis to patients who received platinum-based chemotherapy as their first-line treatment for advanced disease, patients with *LKB1*-mutated and *LKB1*-wt tumours had not statistically significantly different PFS (median 5.31 and 5.44 months, respectively; aHR=1.04; 95% CI 0.55 to 1.97; p=0.910) or OS (median 9.88 and 12.66 months, respectively; aHR=0.83; 95% CI 0.42 to 1.65; p=0.602). Kaplan-Meier curves for PFS and OS in this subgroup are depicted in figure 3C,D.

DISCUSSION

LKB1 activation status is emerging as a crucial prognostic and predictive factor in patients with advanced NSCLC.[17] Recent studies conducted in large patient populations showed significantly worse PFS and OS in patients with *LKB1*-mutated when compared with *LKB1*-wt advanced NSCLC irrespective of first-line systemic treatment (ie, chemotherapy or immunotherapy).[24 25] *LKB1* mutations/deletions have been also found to specifically confer resistance to anti-PD1 monoclonal antibodies in mouse models of lung adenocarcinomas, and *KRAS* and *LKB1* co-mutations were associated with worse PFS and OS in advanced lung adenocarcinoma patients treated with single-agent

Table 1 Patients' characteristics

	LKB1-wt n=103	*LKB1-mut* n=17	Overall n=120
Sex			
Male	70 (68.0)	13 (76.5)	83 (69.2)
Female	33 (32.0)	4 (23.5)	37 (30.8)
ECOG-PS			
0	54 (52.4)	10 (58.8)	64 (53.3)
1	47 (45.6)	7 (41.2)	54 (45.0)
2	2 (1.9)	0 (0.0)	2 (1.7)
Smoking			
Never/ex	72 (69.9)	11 (64.7)	83 (69.2)
Current	31 (30.1)	6 (35.3)	37 (30.8)
Stage			
I, II, IIIA	39 (37.9)	4 (23.5)	43 (35.8)
IIIB, IV	64 (62.1)	13 (76.5)	77 (64.2)
Grade			
G1: well differentiated	1 (1.4)	4 (40.0)	5 (6.1)
G2: moderately differentiated	30 (41.7)	3 (30.0)	33 (40.2)
G3: poorly differentiated	41 (56.9)	3 (30.0)	44 (53.7)
Missing	31	7	38
Histology			
Other	8 (7.8)	0 (0.0)	8 (6.7)
Squamous +NOS	31 (30.1)	3 (17.6)	34 (28.3)
Adenocarcinoma	64 (62.1)	14 (82.4)	78 (65.0)
Type of line			
Other	1 (1.0)	0 (0.0)	1 (0.8)
Cisplatin	1 (1.0)	0 (0.0)	1 (0.8)
Cisplatin/gemcitabine/bevacizumab	1 (1.0)	0 (0.0)	1 (0.8)
Carboplatin/gemcitabine	13 (12.6)	5 (29.4)	18 (15.0)
Cisplatin/gemcitabine	40 (38.8)	4 (23.5)	44 (36.7)
Vinorelbine	1 (1.0)	0 (0.0)	1 (0.8)
Carboplatin/vinorelbine	8 (7.8)	0 (0.0)	8 (6.7)
Cisplatin/vinorelbine	11 (10.7)	2 (11.8)	13 (10.8)
Carboplatin/pemetrexed	6 (5.8)	0 (0.0)	6 (5.0)
Cisplatin/pemetrexed	21 (20.4)	5 (29.4)	26 (21.7)
Unknown	0 (0.0)	1 (5.9)	1 (0.8)
Setting of prior platinum-based chemotherapy			
I line	68 (66.0)	12 (70.6)	80 (66.7)
Adjuvant	35 (34.0)	5 (29.4)	40 (33.3)
KRAS status			
Wt	72 (69.9)	6 (35.3)	78 (65.0)
Mutated	31 (30.1)	11 (64.7)	42 (35.0)

_mut, mutated; NOS, not otherwise specified; wt, wild type.

immunotherapy.[17] In addition, recent data showed that first-line chemo-immunotherapy, which represents the standard-of-care treatment for patients with *EGFR*-wt, *ALK* and *ROS1* not-translocated advanced lung adenocarcinoma with lower than 50% PD-L1 expression, is not associated with superior clinical outcomes when compared with platinum chemotherapy alone in patients with *LKB1*-mutated neoplasms.[19]

However, the impact of *LKB1* mutations on the efficacy of chemotherapy, and in particular of first-line

Table 2 Association between *LKB1* status and patient/tumour characteristics

	LKB1 status	
	χ^2 test	P value
Sex	0.4913	0.4833
ECOG-PS	0.3554	0.5511
Smoking history	0.1833	0.6686
Stage	1.2932	0.2555
Grade	10.0373	**0.0015**
Histology	3.0109	0.2219
Prior platinum-based adjuvant therapy	0.1359	0.7124
Age (t-test, p value)	−0.31	0.7595
KRAS status	7.6824	**0.0056**

platinum-based chemotherapy or second-line taxanes, remains poorly investigated.

In this post hoc analysis of the TAILOR trial,[23] we did not find a statistically significant association between *LKB1* mutational status and PFS or OS in advanced NSCLC patients receiving second-line docetaxel or prior first-line platinum-based chemotherapy. On the other hand, patients with *LKB1*-mutated tumours treated with erlotinib had worse PFS and OS when compared with patients with *LKB1*-wt disease (although the observed differences did not reach statistical significance). Since erlotinib is not effective against *EGFR*-wt NSCLC,[23] this result indicates that *EGFR*-wt *LKB1*-mutated advanced NSCLC displays more aggressive clinical behaviour when compared with *EGFR*-wt *LKB1*-wt advanced NSCLC in the absence of an active antitumour treatment. Conversely, *LKB1* status may be not associated with worse patient prognosis when an active treatment, such as platinum or taxane-based chemotherapy, is administered. Together, these results point to a prognostic rather than predictive role of *LKB1* mutations in advanced NSCLC patients receiving first-line or second-line cytotoxic chemotherapy.

Of note, median PFS and OS data with second-line docetaxel or first-line platinum-based chemotherapy in patients enrolled in the TAILOR trial are consistent with survival data of large phase III trials,[6 7 23 26 27] as well as with recent retrospective analyses conducted in *LKB1*-mutated NSCLC patients treated with first-line platinum-based chemotherapy plus/minus immune checkpoint inhibitors (ICIs).[19] These data support the reliability of our post hoc analysis.

The fact that this study showed no significant differences, in terms of PFS or OS, between patients receiving first-line or second-line cytotoxic chemotherapy is potentially relevant from a clinical point of view. Indeed, patients with *LKB1*-mutated advanced NSCLC have otherwise limited therapeutic options due to poor/absent efficacy of currently available molecular targeted

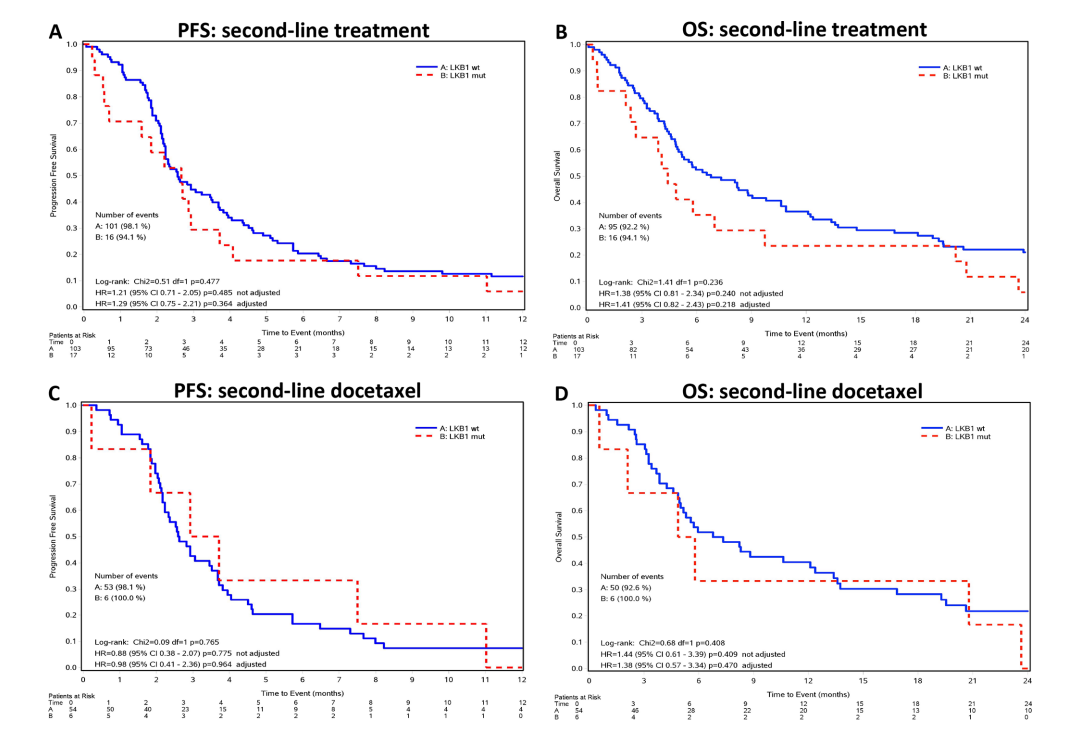

Figure 2 Impact of *LKB1* mutations on clinical outcomes during second-line treatment. Kaplan-Meier curves of PFS (A, C) and OS (B, D) during second-line treatment (docetaxel or erlotinib) (A, B) or second-line docetaxel (C, D) according to *LKB1* status. *LKB1*-wt: blue continuous curve; *LKB1*-mutated: red dashed curve. For each comparison the p value of the log-rank test, as well as the non-adjusted HRs and aHRs and 95% CIs, are reported. aHR, adjusted HR; OS, overall survival; PFS, progression-free survival; wt, wild type.

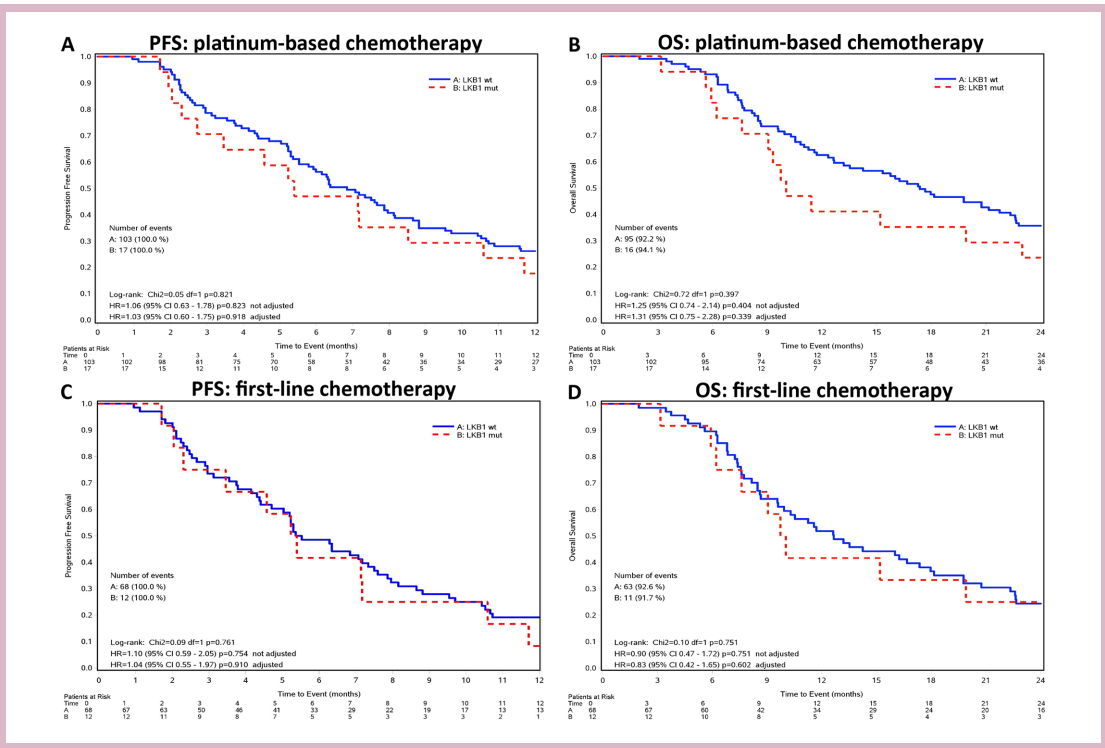

Figure 3 Impact of *LKB1* mutations on clinical outcomes during platinum-based chemotherapy. Kaplan-Meier curves of PFS (A, C) and OS (B, D) during prior platinum-based chemotherapy (A, B) and first-line platinum-based chemotherapy (C, D) according to *LKB1* status. *LKB1*-wt: blue continuous curve; *LKB1*-mutated: red dashed curve. For each comparison the p value of the log-rank test, as well as the non-adjusted HRs and aHRs and 95% CIs, are reported. aHR, adjusted HR; OS, overall survival; PFS, progression-free survival; wt, wild type.

therapies or immunotherapy strategies. Consistent with our findings, preclinical experiments showed that LKB1 inactivation is associated with impaired cancer cells' ability to repair chemotherapy-induced DNA damage and oxidative stress and, consequently, with tumour cell sensitivity to DNA-damaging agents, including platinum-based chemotherapy.[28–30] These in vitro experiments, along with findings of our post hoc analysis, indicate that the recently observed worse PFS and OS in patients with *LKB1*-mutated NSCLC[24 25] might be more indicative of a negative prognostic impact of these alterations rather than of a lack of efficacy of cytotoxic chemotherapy.

The percentage of *LKB1*-mutated tumours in our post hoc analysis was lower than previously reported in large NSCLC series.[14–16] One hypothesis to explain this discrepancy is that patients with *LKB1*-mutated NSCLC may have lower chances to receive further systemic treatment after adjuvant/first-line platinum-based chemotherapy as a result of higher disease aggressiveness and more common occurrence of precocious death events. If this hypothesis was correct, relatively more patients with *LKB1*-mutated tumours may have undergone fast deterioration of their clinical conditions or death before being enrolled in the TAILOR study, and this post hoc analysis might have included a selected subgroup of patients with *LKB1*-mutated disease characterised by relatively more favourable prognosis. However, the following data are in contrast with this hypothesis: (1) we found a trend towards lower PFS and OS in patients with *LKB1*-mutated neoplasms receiving an ineffective second-line treatment (ie, erlotinib), thus supporting the clinical aggressiveness of *LKB1*-mutated tumours included in our analysis; (2) in the subgroup of 78 lung adenocarcinoma specimens, 14 (17.9%) were *LKB1*-mutated; this percentage is in line with previously published studies. Together, these data tend to exclude the hypothesis that a negative selection of more aggressive *LKB1*-mutated neoplasms had occurred before patient enrollment in the TAILOR trial.[14–16]

Since LKB1 can be also regulated epigenetically, some works have evaluated LKB1 status by assessing its levels through immunohistochemistry (IHC).[31 32] These studies found a much higher frequency of tumours with low/absent LKB1 expression when compared with the percentage of *LKB1*-mutated neoplasms reported in previous studies. Although the most reliable method to assess the functional state of LKB1 has not been clarified yet, next generation sequencing (NGS) analysis is more objective when compared with IHC, and provides all-or-none results. Future studies should perform matched tissue evaluations of *LKB1* mutational status (by NGS) and protein expression (by IHC) to understand to which extent results of these analyses overlap, and to identify the best method to determine LKB1 functional status. Although most of the published studies (including our post hoc analysis) were based on NGS evaluations of *LKB1* status, it is reasonable to speculate that tumours

lacking LKB1 expression at IHC also have inactive LKB1. Therefore, IHC data could be integrated with NGS results to expand the subgroup of LKB1-inactive adenocarcinomas.[22]

The strengths of our study consist of the facts that we analysed a controlled population of patients enrolled in a randomised phase III trial, and we found similar results when considering second-line docetaxel or first-line platinum chemotherapy. The main limitation consists of the low absolute and relative number of patients with *LKB1*-mutated tumours. In particular, given the number of observed events and the prevalence of *LKB1*-mutated tumours, our post hoc analysis was only powered to detect an HR equal to or higher than 2 in patients with *LKB1*-mutated versus *LKB1*-wt tumours. Another limitation consists of the fact that patients included in the TAILOR study represent a selected population of advanced NSCLC patients who had sufficiently good clinical conditions to receive two subsequent lines of chemotherapy. Therefore, we should be cautious when interpreting findings of this study, since they are more realistically applicable to the subgroup of advanced NSCLC patients being able to receive a second-line therapy after first-line chemotherapy. Finally, only slightly more than half of the patients enrolled in the TAILOR trial had evaluable *LKB1* mutational status in left-over tumour tissues, and were included in this post hoc analysis.

Owing to results of our current study, as well as to poor efficacy of single-agent ICIs or ICI-chemotherapy combinations in patients with advanced *LKB1*-mutated lung adenocarcinoma, platinum-based chemotherapy can be still considered a valid first-line therapeutic option for these patients, and should be used as the backbone of experimental combination treatments in this clinical setting.[20–22] To exploit the metabolic vulnerabilities conferred by LKB1 inactivation, we are conducting the randomised, phase II FAME (Exploiting FAsting-mimicking Diet and MEtformin to Improve the Efficacy of Platinum–pemetrexed Chemotherapy in Advanced LKB1-inactivated Lung Adenocarcinoma) trial (NCT03709147) to investigate the efficacy of combining metformin, plus/minus cyclic caloric restriction, with first-line platinum–pemetrexed chemotherapy.[22] Similarly, docetaxel or other cytotoxic agents remain valid second-line therapeutic options for the treatment of patients *LKB1*-mutated advanced lung adenocarcinoma progressing on first-line platinum-based chemotherapy.

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Contributors CV and MCG designed the study. MG collected data. ER performed statistical analyses, with the help of MM, and VT, CV, MG, ER and MCG wrote the manuscript. GF, ACB, CB, LR, SI, MB and MCG took part in the TAILOR trial, including patient enrollment and biological analyses in tumour tissue specimens. All the authors contributed to editing the manuscript and approved the final version of the paper.

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