Value of KRAS as prognostic or predictive marker in NSCLC: results from the TAILOR trial

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Background: The prognostic and predictive role of KRAS mutations in advanced nonsmall-cell lung cancer (NSCLC) is still unclear. TAILOR prospectively assessed the prognostic and predictive value of KRAS mutations in NSCLC patients treated with erlotinib or docetaxel in second line.

Patients and methods: NSCLC patients from 52 Italian hospitals were genotyped for KRAS and EGFR mutational status in two independent laboratories. Wild-type EGFR patients (N = 218) received first-line platinum-based chemotherapy and were randomly allocated at progression to erlotinib or docetaxel. Overall survival (OS) according to KRAS mutational status was the primary end point.

Results: KRAS mutations were present in 23% of TAILOR randomized cases. The presence of a KRAS mutation did not adversely affect progression-free (PFS) or overall (OS) survival [hazard ratio (HR) PFS = 1.01, 95% confidence interval (CI) 0.71–1.41, P = 0.977; OS = 1.24, 95% CI 0.87–1.77, P = 0.233], nor influenced treatment outcome (test for interaction: OS P = 0.965; PFS P = 0.417). Patients randomized to docetaxel treatment experienced longer survival independently from the KRAS mutational status of their tumors (HR: mutated KRAS 0.81, 95% CI 0.45–1.47; wild-type KRAS 0.79, 95% CI 0.57–1.10).

Conclusion: In TAILOR, KRAS was neither prognostic nor predictive of benefit for either docetaxel or erlotinib. Docetaxel remains superior independently from KRAS status for second-line treatment in EGFR wild-type advanced NSCLC patients.

Clinical trial registration: NCT00637910.

Key words: NSCLC, KRAS, docetaxel, erlotinib

introduction

KRAS is the most frequently mutated oncogene in nonsmall-cell lung cancer (NSCLC) known to be involved in multiple processes during lung carcinogenesis, tumor growth and metastatic spread [1]. In the majority of cases, KRAS alterations are missense mutations in exon 2, codon 12 and 13, but rarer alleles are also present [2]. Although the oncogene was discovered in 1982, the role of KRAS mutations as either a prognostic or predictive factor in NSCLC is still contentious due to the fact that very few prospective studies have been completed using KRAS status as a stratifier [3, 4].

An individual patient data meta-analysis of four large adjuvant trials showed that KRAS mutational status was not prognostic either for progression-free survival (PFS) or overall survival (OS) in early-stage disease. In the same analysis, a significant negative interaction was found between codon-13 mutations and chemotherapy [5]. In contrast, in metastatic disease, KRAS was shown to have a moderate negative impact on survival by a pooled data meta-analysis of 53 trials assessing KRAS mutations by different techniques with non-comparable limits of sensibility [6]. In the latter, no data were available for PFS and response rate (RR). Curiously, two further pooled data
meta-analyses supported KRAS as negative predictor for EGFR tyrosine kinase inhibitors (TKI) treatment but only in regards of RR [7, 8]. It has been suggested that the inconsistency of these contradictory findings might reflect prognostic and predictive differences within the range of KRAS mutations [9, 10], adding to the inherent biases due to the high percentage of patients with unknown KRAS status, in most cases as high as 80% [11], due in turn to the retrospective and unplanned nature of the analyses so far conducted. For this reason, within the TAILOR trial [12, 13], we planned an analysis specifically aimed at evaluating KRAS mutational status as a prognostic marker in second-line treatment of advanced NSCLC, and a predictive stratifier for efficacy to erlotinib or docetaxel.

patients and methods

study design and patients
TAILOR was a not-for-profit multicenter, open label, randomized trial, funded by the Italian Regulatory Agency conducted in 52 Italian hospitals, comparing erlotinib versus docetaxel in second-line NSCLC patients failing front-line platinum-based chemotherapy. Tumor samples were centrally reclassified according to the 2004 WHO classification. Suitable samples were genotyped in parallel by investigators in two independent laboratories using two different techniques. Patients were considered former smokers if they smoked more than 100 cigarettes in their life. All patients who were eligible for participation provided written informed consent with all applicable governing regulations before undergoing any study procedure. Further details can be found in [13].

procedures
Treatment consisted of erlotinib 150 mg given orally every day, or docetaxel given i.v., at either 75 mg/m² every 21 days, or 35 mg/m² on days 1, 8, and 15, every 28 days. At progression, treatment crossing was not permitted. Because of the nature of the interventions, patients were not masked to assigned treatment, but investigators who did tumor genotyping were masked to treatment allocation while investigators who gave treatment and assessed outcomes were masked to KRAS mutational status.

statistical analysis
The trial was initially designed to assess the different effects of docetaxel and erlotinib according to KRAS 12–13 mutation and both EGFR protein expression and amplification, as suggested by the literature in 2007. At the first planned interim analysis, the independent data and safety Monitoring Committee did a preplanned masked efficacy analysis which suggested, in conjunction with other data [14], that EGFR expression and EGFR amplification were irrelevant. Therefore, the primary objective of the trial became the comparison of efficacy between erlotinib and docetaxel and the sample size was recalculated accordingly. As a result, the power to test the interaction between treatments and KRAS status was greatly reduced and the current analysis was replanned only for exploratory purposes. That notwithstanding, the number of events (187/218 patients) and the mutational rate of KRAS in 23.4% of patients and amplified in 218/218 (218.9%) in the docetaxel arm and 26/109 (23.8%) in the erlotinib arm (supplementary Figure S1, available at Annals of Oncology online). The baseline characteristics of the randomized patients according to KRAS mutational status are reported in supplementary Table S1, available at Annals of Oncology online. The mutated KRAS subgroup presents, as expected, a higher percentage of adenocarcinoma histology and smokers compared with wild-type KRAS patients. Remaining characteristics were well balanced between the two groups. KRAS mutational status by isoforms according to histology and smoking habit is reported in supplementary Table S2, available at Annals of Oncology online. The most frequent isoform was G12C accounting for 43% of all mutations followed by G12D (20%) and G12V (18%). G13 mutation isoforms (G13C and G13D) were seen in 7.9% (N = 4) of all mutated cases and in 9.1% of the smokers patients.

At a median follow-up of 33 months (interquartile range 25–33), 211 patients had disease progression or death and 187 died.

prognostic role of KRAS
Survival by KRAS status is reported in Figure 1A. There was no significant difference between the two groups. After accounting for treatment and imbalances in histology types and smoking habits, the hazard ratio (HR) for OS was 1.24 [95% confidence interval (CI) 0.87–1.77, P = 0.232] with median values of 5.7 versus 6.8 months (95% CI 3.8–9.7 and 5.2–8.7), respectively, in the mutated and in the wild-type KRAS group. Within the mutated KRAS group, the four patients harboring G13 mutations tended to have a longer survival (7.3, 13.6, 13.8, and 19.3 months). In Figure 1B are reported the curves for PFS. The multivariate analysis showed a nonsignificant HR for PFS of 1.01 (95% CI 0.71–1.41, P = 0.977). The median PFS was 2.7 months (95% CI 2.4–3.7) in the mutated KRAS group and 2.4 months (95% CI 2.2–2.9) in the wild-type KRAS group. Again, the four patients harboring G13 mutations displayed longer PFS (2.5, 6.9, 7.5, and 12.3 months). Detailed results on univariate and multivariate analyses for OS and PFS are reported in Table 1.
Figure 1. Kaplan–Meier curves by KRAS status. Overall survival (A); progression-free survival (B).

A: KRAS wild-type
B: KRAS mutated

Overall survival

Number of events
A: 139 (83.2%)
B: 47 (92.2%)

Log-rank: \( \chi^2 = 2.19 \), \( dt = 1 \), \( P = 0.139 \)
HR = 1.29 (95% CI 0.92–1.79) \( P = 0.139 \) not adjusted
HR = 1.24 (95% CI 0.87–1.77) \( P = 0.233 \) adjusted

Patients at risk

<table>
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Progression-free survival

Number of events
A: 161 (96.4%)
B: 50 (98.0%)

Log-rank: \( \chi^2 = 0.01 \), \( dt = 1 \), \( P = 0.929 \)
HR = 1.01 (95% CI 0.74–1.39) \( P = 0.930 \) not adjusted
HR = 1.01 (95% CI 0.71–1.41) \( P = 0.977 \) adjusted

Patients at risk

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</table>
predictive role of KRAS

Within the KRAS subgroup, the HR for death was 0.81, nonsignificantly favoring docetaxel (95% CI 0.45–1.47, \( P = 0.492 \)). Median survivals for docetaxel and erlotinib were 7.0 and 5.5 months, respectively. Survival in patients with wild-type KRAS tumors also nonsignificantly favored docetaxel (HR = 0.79, 95% CI 0.57–1.10, \( P = 0.167 \)), with medians of 8.3 (95% CI 6.3–12.5) and 5.3 (95% CI 4.2–7.8) months in the docetaxel and erlotinib group, respectively. Survival curves by KRAS status and treatment arm are reported in supplementary Figure S2A, available at *Annals of Oncology* online (curves by treatment of KRAS mutated and wild-type patients are reported in supplementary Figure S3, available at *Annals of Oncology* online). The PFS analysis also showed a trend favoring docetaxel which was statistically significant in wild-type (HR = 0.68, 95% CI 0.50–0.93, \( P = 0.016 \)), but not KRAS mutated patients (HR = 0.89, 95% CI 0.51–1.57, \( P = 0.694 \)). PFS curves by treatment and KRAS mutational status are shown in supplementary Figure S2B, available at *Annals of Oncology* online (curves by treatment of KRAS mutated and wild-type are reported in supplementary Figure S3, available at *Annals of Oncology* online).

The relative strength of treatment effects on survival and PFS for the overall population and the two KRAS subgroups, is shown in the forest plot on Figure 2. The test for interaction was not statistically significant at both the univariate (OS \( P = 0.992 \), PFS \( P = 0.460 \)) and multivariate analysis (OS \( P = 0.965 \), PFS \( P = 0.417 \)), with point estimates clearly favoring the docetaxel arm in all subgroups.

The results obtained by comparing OS and PFS between wild-type KRAS group and the different KRAS mutations

### Table 1. Prognostic role of KRAS

<table>
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<tr>
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<td>HR 95% CI</td>
<td>( P )</td>
<td>HR 95% CI</td>
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<tr>
<td>KRAS (mut versus wt)</td>
<td>1.01 0.74 1.39</td>
<td>0.93 0.71 1.41</td>
<td>0.977</td>
<td>1.01 0.71 1.41</td>
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<td>Gender (F versus M)</td>
<td>0.82 0.61 1.10</td>
<td>0.189 0.94 0.67 1.31</td>
<td>0.701</td>
<td>0.69 0.50 0.95</td>
</tr>
<tr>
<td>Histology (sq versus adeno)</td>
<td>1.21 0.89 1.66</td>
<td>0.229 1.11 0.79 1.55</td>
<td>0.559</td>
<td>1.12 0.80 1.58</td>
</tr>
<tr>
<td>Histology (other versus adeno)</td>
<td>1.17 0.66 2.06</td>
<td>0.595 1.11 0.62 1.98</td>
<td>0.736</td>
<td>0.69 0.37 1.29</td>
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<tr>
<td>Smoke (ex, current versus non)</td>
<td>1.30 0.94 1.81</td>
<td>0.119 1.06 0.73 1.56</td>
<td>0.756</td>
<td>1.27 0.89 1.81</td>
</tr>
<tr>
<td>ECOG-PS (2 versus 0.1)</td>
<td>3.22 1.91 5.41</td>
<td>(&lt;0.0001) 3.04 1.78 5.21</td>
<td>(&lt;0.0001) 1.80</td>
<td>0.94 0.53 1.61</td>
</tr>
<tr>
<td>Best response (CR/PR versus adj)</td>
<td>1.04 0.61 1.78</td>
<td>0.888 0.93 0.54 1.61</td>
<td>0.800</td>
<td>0.94 0.53 1.61</td>
</tr>
<tr>
<td>Best response (SD versus adj)</td>
<td>1.20 0.69 2.08</td>
<td>0.530 1.18 0.67 2.09</td>
<td>0.559</td>
<td>0.98 0.54 1.78</td>
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<tr>
<td>Best response (PD versus adj)</td>
<td>1.67 0.96 2.91</td>
<td>0.069 1.49 0.85 2.61</td>
<td>0.167</td>
<td>1.50 0.83 2.71</td>
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</tbody>
</table>

#### Figure 2.

Forest plot on treatment effect by KRAS status.

mut, mutated; wt, wild-type; F, female; M, male; sq, squamous; adeno, adenocarcinoma; ex, ex-smoker; current, current smoker; non, nonsmoker; CR, complete response; PR, partial response; adj, adjuvant; SD, stable disease; PD, progression disease; doc, docetaxel; erl, erlotinib.
(G12C, G12D, and G12V) groups are reported in supplementary Figure S4A and B, available at *Annals of Oncology* online, respectively.

RR was 4.5% in the mutated KRAS compared with 10.5% in the wild-type KRAS group. In the mutated KRAS population, 2/20 patients (10%) experienced objective response to docetaxel and none to erlotinib (N = 24). In the wild-type population, 13/76 patients (17.1%) achieved objective response with docetaxel compared with 3/76 (3.9%) with erlotinib (supplementary Table S3, available at *Annals of Oncology* online). The test for interaction was not significant (P = 0.476).

**discussion**

TAILOR is the only prospective randomized trial in advanced wild-type *EGFR* NSCLC patients where the determination of KRAS mutational status was mandatory before randomization to chemotherapy with docetaxel or target therapy with the small molecule erlotinib. Since KRAS discovery in 1982, several retrospective analyses and meta-analyses, involving more than 8000 patients, have been published on the prognostic and predictive values of mutated KRAS in second-line metastatic NSCLC. Two meta-analyses have reported a moderately negative prognostic role for KRAS on survival of NSCLC patients with advanced disease [7, 8].

In our study, we are unable to confirm mutated KRAS as a negative prognostic marker in advanced NSCLC. Because of limited power, the analysis could have missed a small prognostic effect, which in any case would not be clinically relevant. On the other hand, a more biologically plausible reason for this discrepancy lies in the fact that our study was conducted solely in *EGFR* wild-type patients. All published analyses suggesting KRAS mutation as a negative prognostic marker, instead, have been conducted in unselected advanced NSCLC populations [4]. In Caucasian patients, the prevalence of *EGFR* mutation is ~10%, and mutated *EGFR* is a known positive prognostic factor [15]. *EGFR* and KRAS mutations are almost mutually exclusive [3]. In a prognostic analysis focusing on KRAS but conducted in a NSCLC population not genotyped for *EGFR*, the expected 10% quota of better prognosis patients harboring the *EGFR* mutation will automatically segregate in the KRAS wild-type subgroup, carrying to the KRAS wild-type status a ‘best prognosis burden’ in reality ascribable to the *EGFR* mutation. Indeed, in the Interest trial randomizing gefitinib to docetaxel in unselected NSCLC patients, a subgroup analysis by *EGFR* mutation status showed a median survival of 15.3 versus 6.2 months for *EGFR* mutated and wild-type patients, respectively [16]. In our study, the median survival for wild-type patients was 6.8 months. We have simulated what would have been the impact of including a 10% of *EGFR* mutated cases in our analysis to establish the prognostic value of KRAS. As a result of this simulation, we have estimated that our median in the ‘unselected’ wild-type KRAS population (i.e. comprising 90% of *EGFR* wild-type and 10% of *EGFR* mutated patients) would have increased the median survival of the wild-type KRAS subgroup from 6.8 to 8.9 months, with a corresponding increase in the HR from 1.32 to 1.45, likely rendering statistically significant, while biologically mistaken, the negative prognostic role of KRAS mutation.

Our analysis also failed to recognize KRAS as significant negative predictor for erlotinib efficacy. Again and interestingly, the only other trial which analyzed the impact of KRAS mutational status within a randomized comparison between an *EGFR* TKI (gefitinib) and chemotherapy (docetaxel) also failed to find mutated KRAS as a negative predictor of response [17]. In 2007, when TAILOR started, *EGFR* and KRAS were the only potential biomarkers known for NSCLC. Today, what was once considered the *EGFR* wild-type ‘space’, has been populated by a basketful of other mutually exclusive actionable targets, including gene rearrangements in *ALK*, *ROS1*, *RET*, and *NTRK1*, mutations in *HER2* and *BRAF*, and amplification of *MET*. All these genotypes account for at least 40% of the previous *EGFR* wild-type patients [18]. Each of these subpopulations carries its own prognostic significance and while their matching selective inhibitors have been proven active [19], their specific responsiveness to *EGFR* TKIs is unknown. Dissecting a true potential predictive role for KRAS in the *EGFR* wild-type population of TAILOR thus requires extensive genotyping of the non-KRAS mutated subgroup, which is underway. In the meanwhile, and with a pragmatic intent, our results confirm the superiority of docetaxel over erlotinib independently from KRAS, essentially showing an even more favorable trend precisely in the double wild-type subgroup (*EGFR* and KRAS wild-type). Indeed, docetaxel should have done worse rather than better in this subgroup if KRAS was a strong negative predictor for anti-*EGFR* inhibition, as in colon cancer [20]. Our findings thus once more support the notion that, in the absence of an identified true target, a ‘one fits all’ approach is still to be preferable. This strategy is also supported by the results of other recently published trials [21, 22].

Albeit our results show that KRAS is not a critical prognostic marker or a predictive indicator for tailoring treatments in clinical practice, that is not to say that patients should not be tested for KRAS within the framework of clinical trials or for the purpose of referral. Fortunately, the therapeutic option for wild-type *EGFR*/KRAS patients is not solely limited to chemotherapy. As already said, due to the plethora of new genetic-driven insights guiding the use of matched targeted agents, a host of new target drugs is already, or will be soon, available for many NSCLC patients with specific genomic determinants [23]. Since these genomic alterations are usually mutually exclusive, the value of testing for KRAS mutational status, in addition to *EGFR* status, in clinical settings where tumor genotyping at point of care is not yet available on a routine basis, is to identify double wild-type patients for referral to cancer centers with extensive multiplex genotyping programs.

It remains that patients harboring KRAS mutations are ~20% of NSCLC, pointing to a vast yet unmet clinical need. Currently, there are no KRAS inhibitors in the clinic and targeting KRAS down-stream effectors such as with MEK inhibitors is under extensive investigation [24]. The recent discovery of a selective allosteric inhibitor of G12C mutant KRAS, however, opens the possibility in the future to target at least specific mutant KRAS populations, and is particularly welcomed in NSCLC where the G12C is the most prevalent mutation [25]. In preparation for the clinical development of mutation-specific RAS inhibitors, the biological consequences of the different G12 or G13 substitutions should be dissected. Although the number of patients in our study did not allow to infer on the prognostic or predictive
role of the different KRAS substitutions, recent preclinical evidence from our group clearly indicate that not all the KRAS mutations are born equal and should probably be considered as separated identities [9, 26]. In conclusion, our results suggest that knowledge on KRAS mutational status in EGFR wild-type NSCLC patients, not otherwise genotyped, should not be determinate including patients treatment, least of all toward the use of anti-EGFR inhibitors, nor does carries a particularly informative prognostic value for patients and relatives.

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disclosure
The authors have declared no conflicts of interest.

references