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# Effect of Coexisting *KRAS* and *TP53* Mutations in Patients Treated With Chemotherapy for Non–small-cell Lung Cancer

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## Abstract

**The LACE-Bio group found adjuvant chemotherapy to be deleterious in non–small-cell lung cancer with coexisting *KRAS/TP53* mutations. We analyzed 218 patients with non–small-cell lung cancer (28 with coexisting *KRAS/TP53* mutations, 77 with *TP53* mutations, 37 with *KRAS* mutations, and 76 with no *KRAS/TP53* mutations) who received chemotherapy. There was no difference in disease-free or progression-free survival between the 4 groups. Overall survival was longer in the no *KRAS/TP53* group.**

**Background:** *KRAS* and *TP53* are common mutations in non–small-cell lung cancer (NSCLC). The Lung Adjuvant Cisplatin Evaluation Biological Program group found adjuvant chemotherapy to be deleterious in patients with coexisting *KRAS/TP53* mutations. **Patients and Methods:** To validate these results, patients with NSCLC tested for *KRAS* and *TP53* mutations and receiving chemotherapy for any stage NSCLC were selected. Mutation status was analyzed using next generation sequencing (Illumina) or multiplex recurrent mutation detection (MassARRAY, Agena Biosciences) assays, and was correlated with clinical and demographic data. Disease-free (DFS) or progression-free survival (PFS) was the main endpoint, and overall survival (OS) was the secondary endpoint. **Results:** Among 218 patients, 28 had coexisting *KRAS/TP53* mutations, 77 *TP53*, 37 *KRAS*, 76 had neither *KRAS* nor *TP53* mutation (WT/WT). There was no DFS/PFS difference for the *KRAS/TP53* group versus all others among 99 patients who received adjuvant chemotherapy (hazard ratio [HR], 1.22; 95% confidence interval [CI], 0.61-2.44; $P = .57$), 27 stage III patients who received chemoradiation (HR, 0.87; 95% CI, 0.32-2.38; $P = .8$), and 63 patients who received palliative chemotherapy (HR, 0.68; 95% CI, 0.31-1.48; $P = .33$). OS was longer in the WT/WT group compared with any other group (*KRAS*: HR, 1.87; 95% CI, 1.02-3.43; $P = .043$; *TP53*: HR, 2.17; 95% CI, 1.3-3.61; $P = .0028$; *KRAS/TP53*: HR, 2.06; 95% CI, 1.09-3.88; $P = .026$). No OS difference was seen for *KRAS/TP53* compared with the other groups (HR, 1.26; 95% CI, 0.75-2.13; $P = .38$). **Conclusions:** There was no significant difference in DFS/PFS between the 4 groups. However, OS was longer for patients with *TP53* and *KRAS* wild-type NSCLC who received chemotherapy for any stage compared with patients with *KRAS*, *TP53* mutation, or double mutant tumors.

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## Introduction

Lung cancer is one of the most frequently diagnosed cancers.[1] Although chemotherapy remains a key component of treatment for all stages of non–small-cell lung cancer (NSCLC), there is a need to improve treatment as the median overall survival (OS) of these patients remains poor, especially for advanced stage disease,

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where most patients survive less than 1 year.[2] Standard chemotherapy also has a high rate of grade 3 to 5 toxicities, and 15% to 27% of patients receiving platinum-based combination chemotherapy discontinue treatment because of chemotherapy-related adverse events.[2]

Over the past decade, NSCLC molecular profiling and identification of targetable driver oncogenic mutations such as *EGFR* mutations or *ALK/ROS1* rearrangements have revolutionized advanced NSCLC treatment. Targeted therapies such as erlotinib,[3] gefitinib,[4] afatinib,[5] or crizotinib[6,7] are more effective than standard chemotherapy for patients with *EGFR*- or *ALK/ROS1*-positive tumors. However, for early stage or locally advanced disease as well as for *EGFR* and *ALK/ROS1* wild-type advanced disease, the role of chemotherapy remains dominant.

*KRAS* and *TP53* are the most frequently mutated genes in lung adenocarcinoma,[8] yet there is no approved therapy targeting either of these mutations. However, because of their high prevalence and easy identification, *KRAS* and *TP53* predictive and prognostic effects have been studied extensively.[9,10]

*KRAS* mutations were first described as a negative prognostic factor in NSCLC.[9] However, the prognostic significance of *KRAS* mutation remains unclear, and several meta-analyses and literature reviews have reported conflicting data.[11,12] Moreover, although previous studies have suggested that *KRAS* mutations were associated with resistance to standard chemotherapy and EGFR tyrosine kinase inhibitors (EGFR-TKIs),[13] current data do not support the clinical utility of *KRAS* mutation testing to exclude patients for these treatments.[14,15] Similarly, some *TP53* mutations such as nondisruptive mutations were described as prognostic factors of shorter survival in advanced NSCLC.[16] However, there are a large number of different *TP53* mutations,[17] and the impact of each mutation on survival in lung cancer is heterogeneous.[18] *TP53* mutation status has not been found to be useful to predict chemotherapy response in solid tumors.[19]

Until recently, the prognostic/predictive value of concomitant *KRAS* and *TP53* mutations has not been demonstrated. The Lung Adjuvant Cisplatin Evaluation Biological Program (LACE-Bio) group studied the prognostic and predictive value of *KRAS* and *TP53* mutations in patients with early stage NSCLC from 4 randomized phase III trials of adjuvant chemotherapy (ACT) versus observation. *TP53* mutations had no prognostic effect.[20] Similarly, there was no prognostic effect of *KRAS* mutations, and especially *KRAS* codon 12 (hazard ratio [HR], 1.04; 95% confidence interval [CI], 0.77-1.40) or codon 13 (HR, 1.01; 95% CI, 0.47-2.17) mutations.[21] However, ACT was deleterious for patients with codon 13 mutations (HR, 5.78; 95% CI, 2.06-16.2; $P < .001$; interaction $P = .002$).[21] Recently, Shepherd et al showed that the patients with tumors harboring concomitant *KRAS* and *TP53* mutations had a worse outcome when treated with ACT compared with those with wild-type tumors (HR 2.76; 95% CI, 1.62-4.68; $P = .0002$).[22]

In an attempt to validate these results and to generalize them to patients with advanced disease, we studied the clinical outcomes of patients tested for *KRAS* and *TP53* mutations and treated with chemotherapy for any stage NSCLC at the Princess Margaret Cancer Centre.

## Material and Methods

### *Patient Population and Data Collection*

In this retrospective study, patients older than 18 years of age treated with chemotherapy for any stage NSCLC at Princess Margaret Cancer Centre up to March 2015 and tested for both *KRAS* and *TP53* mutations were included.

Following institutional research ethics board approval, patients were identified through the lung cancer translational research database and other institutional molecular profiling studies[23] to identify those whose tumors had been profiled using assays described in the Tumor Molecular Profiling section (below). Clinical data were retrieved from Princess Margaret Cancer Centre electronic medical records. The collected data included baseline patient and tumor demographic characteristics, disease stage, histology and molecular findings, local treatments, and systemic therapies. Clinical outcomes after chemotherapy also were retrieved from the electronic medical record and included disease-free survival (DFS) for adjuvant chemotherapy, progression-free survival (PFS) and objective response rate (ORR) for palliative chemotherapy, and overall survival (OS) for all evaluable patients.

The primary objective of the study was to identify a difference in DFS or PFS after chemotherapy between 4 groups of patients identified according to *KRAS* and *TP53* mutation status: double wild-type tumors (WT/WT), *KRAS*-mutant and *TP53* wild-type tumors (*KRAS*), *KRAS* wild-type and *TP53*-mutant tumors (*TP53*), and double mutant tumors (*KRAS/TP53*). The secondary objective was to identify differences in OS or ORR among the 4 groups.

### *Tumor Molecular Profiling*

Tumor tissue biopsies or surgical resection specimens were macro-dissected from 10 to 15 slides, and tissues deparaffinized using xylene followed with proteinase K treatment.[23] Genomic tumor DNA was extracted using the QIAmicro DNA extraction kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. Tumor DNA was tested for somatic mutations using one of the following: (1) a custom mass spectrometry-based recurrent mutation detection assay including 279 mutations within 23 genes (MassARRAY, Agena Biosciences, San Diego, CA); or (2) a targeted 48-gene next generation sequencing panel (TruSeq Cancer Amplicon Panel, Mi-Seq platform, Illumina, San Diego, CA) containing 212 amplicons withing 48 genes, with a minimum read depth of 500×.[24] As the custom MassARRAY assay did not include *TP53*, patients tested with this assay were also tested for *TP53* mutations using bi-directional Sanger sequencing of *TP53* coding exons and 2 base pairs of intronic flanking regions.[25] Molecular profiling data captured included the type of somatic mutations and the mutant allele fraction.

### *Statistical Analysis*

Patient characteristics were summarized using median and range for continuous variables and percentages for categorical variables. Differences between the 4 groups (WT/WT, *KRAS*, *TP53*, and *KRAS/TP53*) with respect to the clinical covariates were assessed utilizing the Kruskal-Wallis test or the Fisher exact test, as

**Table 1** Baseline Patient and Tumor Characteristics by Mutation Subgroup

| Demographic | WT/WT[a] (n = 76), n (%) | *KRAS* Mutant (n = 37), n (%) | *TP53* Mutant (n = 77), n (%) | *KRAS/TP53* Mutant (n = 28), n (%) | *P* |
|---|---|---|---|---|---|
| Median age, y | 62 | 64 | 59 | 61 | .067 |
| Range | (32-89) | (37-78) | (34-78) | (38-82) | |
| Gender | | | | | |
| Female | 36 (47) | 22 (59) | 42 (55) | 19 (68) | .27 |
| Male | 40 (53) | 15 (41) | 35 (45) | 9 (32) | |
| Ethnicity | | | | | |
| Caucasian | 46 (60) | 32 (86) | 50 (65) | 27 (96) | .0079 |
| Asian | 20 (26) | 5 (14) | 19 (25) | 1 (4) | |
| Other | 10 (14) | 0 (0) | 8 (10) | 0 (0) | |
| Family history of cancer | | | | | .088 |
| No | 32 (42) | 22 (60) | 42 (55) | 10 (35) | |
| Yes | 30 (40) | 12 (32) | 25 (32) | 17 (61) | |
| Missing data | 14 (18) | 3 (8) | 10 (13) | 1 (4) | |
| Smoking history | | | | | |
| Never smoker | 30 (39) | 3 (8) | 26 (34) | 1 (4) | < .0001 |
| Smoker | 46 (61) | 34 (92) | 51 (66) | 27 (96) | |
| Histology | | | | | |
| Adenocarcinoma | 52 (68) | 34 (92) | 57 (74) | 27 (96) | .0084 |
| Non-adenocarcinoma | 24 (32) | 3 (8) | 20 (26) | 1 (4) | |
| Stage at diagnosis | | | | | |
| I/II | 25 (33) | 15 (41) | 34 (44) | 11 (39) | .62 |
| III | 23 (30) | 12 (32) | 25 (33) | 7 (25) | |
| IV | 28 (37) | 10 (27) | 18 (23) | 10 (36) | |

Abbreviations: *KRAS* = group of patients with NSCLC harboring *KRAS* mutation but no *TP53* mutation; *KRAS/TP53* = group of patients with NSCLC harboring concomitant *KRAS* and *TP53* mutations; NSCLC = non–small-cell lung cancer; *TP53* = group of patients with NSCLC harboring *TP53* mutation but no *KRAS* mutation; WT/WT = wild type/wild type: group of patients with NSCLC harboring no *KRAS* or *TP53* mutation.
[a]In the WT/WT group, 23 patients had an EGFR-mutant tumor and 3 patients had an ALK-rearranged tumor.

appropriate. The outcomes of OS, DFS, and PFS were calculated to the date of death, disease relapse, or progression, respectively. For the whole group, the starting date was the diagnosis date; for the 99 with ACT, it was the first date of treatment; and for the 116 with palliative chemotherapy, it was the date of first chemotherapy. DFS, PFS, and OS were calculated utilizing the Kaplan-Meier method, and the 4 groups were compared using the Wald test within Cox proportional hazards models. ORR was assessed by the investigators using RECIST (Response Evaluation Criteria In Solid Tumors) version 1.1.

## Results

### *Baseline Characteristics*

We identified 218 patients treated with standard chemotherapy for any stage NSCLC whose tumor was assessed for both *TP53* and *KRAS* mutations. Among these patients, 76 had *TP53* and *KRAS* wild-type tumors (WT/WT), 37 had *KRAS*-mutated tumors (*KRAS*), 77 had *TP53*-mutated tumors, and 28 had *KRAS* and *TP53* co-mutated tumors (double mutant tumors, *KRAS/TP53*) (Table 1). In the WT/WT group, 23 patients had an *EGFR*-mutant tumor and 3 had an *ALK* rearrangement. There was no significant difference among the groups in median age ($P = .067$), gender ($P = .27$), or stage at diagnosis ($P = .62$). There were more Caucasian patients with *KRAS*-mutant tumors (*KRAS* and *KRAS/TP53*) than in the other groups (86% for *KRAS* and 96% for *KRAS/TP53* vs. 70% for WT/WT and 72% for *TP53*; $P = .0019$). There were more smokers with *KRAS*-mutant tumors (92% *KRAS* and 96% *KRAS/TP53*) than in the other groups (61% WT/WT and 66% *TP53*; $P < .0001$). Histology was significantly different among the groups ($P = .0084$). Furthermore, patients with double mutant tumors had a higher rate of family history of cancer (61% vs. 40% for WT/WT, 32% for *KRAS*, and 32% for *TP53*; $P = .038$).

### *Survival and Response Analyses*

Results for the entire population of 218 patients receiving chemotherapy for any stage NSCLC are summarized in Table 2. In univariate analyses, no significant PFS difference was found when comparing the WT/WT group with any of the other groups with at least 1 mutation (*KRAS*: HR, 1.18; 95% CI, 0.79-1.76; $P = .41$; *TP53*: HR, 1.05; 95% CI, 0.76-1.45; $P = .78$; *KRAS/TP53*: HR, 1.33; 95% CI, 0.85-2.06; $P = .21$) (Figure 1A). In multivariate analyses adjusted for other variables (age, gender, tobacco smoking, and histology), the differences also were not significant when comparing the WT/WT group with the other groups (*KRAS*: HR, 0.95; 95% CI, 0.61-1.49; $P = .83$; *TP53*: HR, 0.94; 95% CI, 0.66-1.33; $P = .73$; *KRAS/TP53*: HR, 1.10; 95% CI, 0.66-1.81; $P = .72$) (Table 2). On univariate analysis, OS was significantly longer in the WT/WT group compared with the other groups with

**Table 2** Progression-free Survival and Overall Survival by Subgroup

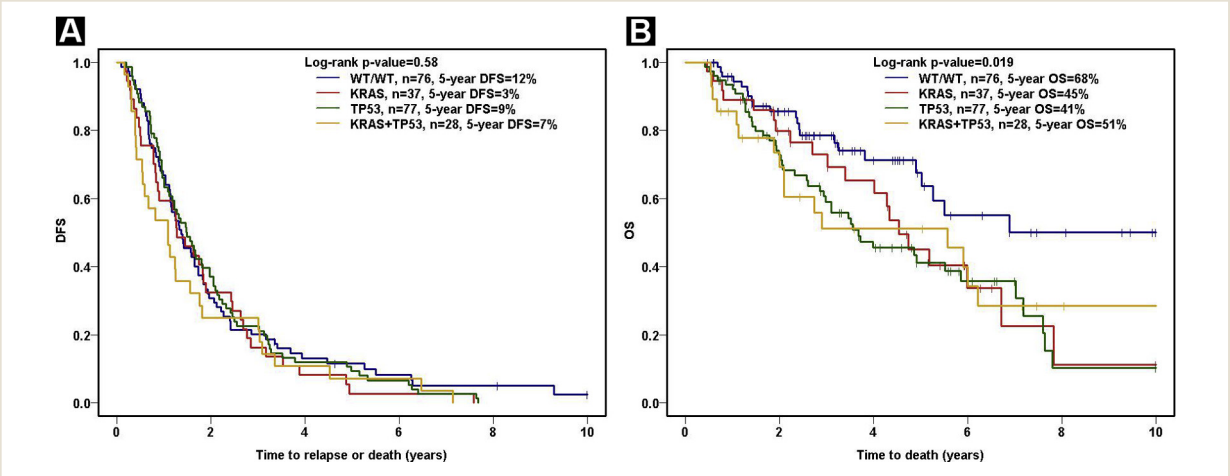
| Subgroup | OS | | | PFS | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *P* Value | HR | 95% CI | *P* Value |
| Age | 1.01 | 0.99-1.03 | .15 | 1.01 | 0.99-1.02 | .31 |
| Gender | 0.99 | 0.65-1.5 | .95 | 1.03 | 0.77-1.37 | .86 |
| Men versus women | | | | | | |
| Histology | 0.83 | 0.49-1.4 | .49 | 1.39 | 0.96-2.02 | .081 |
| ADK versus non-ADK | | | | | | |
| Stage | 2.02 | 1.33-3.04 | .00087 | 1.76 | 1.32-2.33 | .00011 |
| III/IV versus I/II | | | | | | |
| Smoking | | | | | | |
| Ever versus never | 0.91 | 0.55-1.5 | .71 | 1.25 | 0.88-1.79 | .21 |
| Genetic markers | | | | | | |
| KRAS mutant versus WT/WT | 1.99 | 1.02-3.89 | .045 | 0.95 | 0.61-1.49 | .83 |
| TP53 mutant versus WT/WT | 2.33 | 1.36-3.97 | .0019 | 0.94 | 0.66-1.33 | .73 |
| KRAS/TP53 mutant versus WT/WT | 2.43 | 1.16-5.08 | .019 | 1.10 | 0.66-1.81 | .72 |

Abbreviations: ADK = adenocarcinoma; CI = confidence interval; HR = hazard ratio; *KRAS* = group of patients with NSCLC harboring *KRAS* mutation but no *TP53* mutation; *KRAS/TP53* = group of patients with NSCLC harboring concomitant *KRAS* and *TP53* mutations; NSCLC = non–small-cell lung cancer; OS = overall survival; PFS = progression-free survival; Ref = reference value; *TP53* = group of patients with NSCLC harboring *TP53* mutation but no *KRAS* mutation; WT/WT = group of patients with NSCLC harboring no *KRAS* or *TP53* mutation.

at least 1 mutation (*KRAS*: HR, 1.87; 95% CI, 1.02-3.43; $P = .043$; *TP53*: HR, 2.17; 95% CI, 1.3-3.61; $P = .0028$; *KRAS/TP53*: HR, 2.06; 95% CI, 1.09-3.88; $P = .026$) (Figure 1B). Furthermore, these results were confirmed in multivariate analyses when the WT/WT group was compared with the other groups (*KRAS*: HR, 1.99; 95% CI, 1.02-3.89; $P = .045$; *TP53*: HR, 2.33; 95% CI, 1.36-3.97; $P = .0019$; *KRAS/TP53*: HR, 2.43; 95% CI, 1.16-5.08; $P = .019$) (Table 2). OS was similar for the 3 mutation groups, but inferior to the 76-patient subgroup with WT/WT tumors ($P = .019$) (Figure 1B).

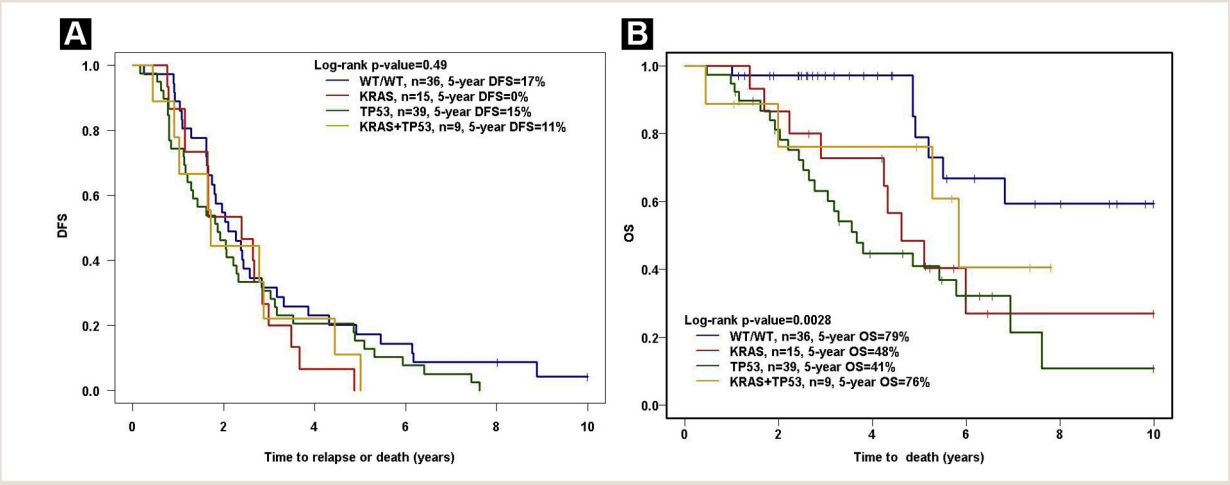
*Survival Analyses in the Surgically Resected ACT Subgroup.* A total of 99 patients received ACT for early-stage disease and were assessed for DFS. Although there was a trend to shorter DFS in patients whose tumors harbored any mutation (Figure 2A), the differences were not significant when compared with the WT/WT group (*KRAS*: HR, 1.48; 95% CI, 0.79-2.77; $P = .22$; *TP53*: HR, 1.33; 95% CI, 0.83-2.13; $P = .23$; *KRAS/TP53*: HR, 1.48; 95% CI, 0.70-3.13; $P = .30$). OS also favored patients with WT/WT tumors ($P = .0028$), with no differences among the 3 mutation subgroups (Figure 2B). Differences were significant when comparing the WT/

**Figure 1** Disease- or Progression-free Survival (A) and Overall Survival (B) in the Entire Population




Abbreviations: DFS = disease-free survival; KRAS = patients with *KRAS*-mutant tumors; KRAS/TP53 = patients with *KRAS* and *TP53* double mutant tumors; OS = overall survival; TP53 = patients with *TP53*-mutant tumors; WT/WT = patients with *TP53* and *KRAS* wild-type tumors.

**Figure 2** Disease-free Survival (A) and Overall Survival (B) After Adjuvant Chemotherapy




Abbreviations: DFS = disease-free survival; KRAS = patients with *KRAS*-mutant tumors; KRAS/TP53 = patients with *KRAS* and *TP53* double mutant tumors; OS = overall survival; TP53 = patients with *TP53*-mutant tumors; WT/WT = patients with *TP53* and *KRAS* wild-type tumors.

WT group with the *KRAS* or *TP53* groups but not with the *KRAS/TP53* group (*KRAS*: HR, 3.41; 95% CI, 1.26-9.19; $P = .016$; *TP53*: HR, 4.35; 95% CI, 1.87-10.14; $P = .00067$; *KRAS/TP53*: HR, 2.12; 95% CI, 0.62-7.27; $P = .23$).

*Response and Survival Analyses in the Advanced Chemotherapy Subgroup.* A total of 116 patients received either palliative chemotherapy or chemo-radiation therapy for advanced disease. In this population, the ORR was 41% for the WT/WT group, 38% for the *KRAS* group, 43% for the *TP53* group, and 56% for the *KRAS/TP53* group. No difference was found when comparing ORR in the WT/WT group with ORR in the *KRAS/TP53* group ($P = .39$).

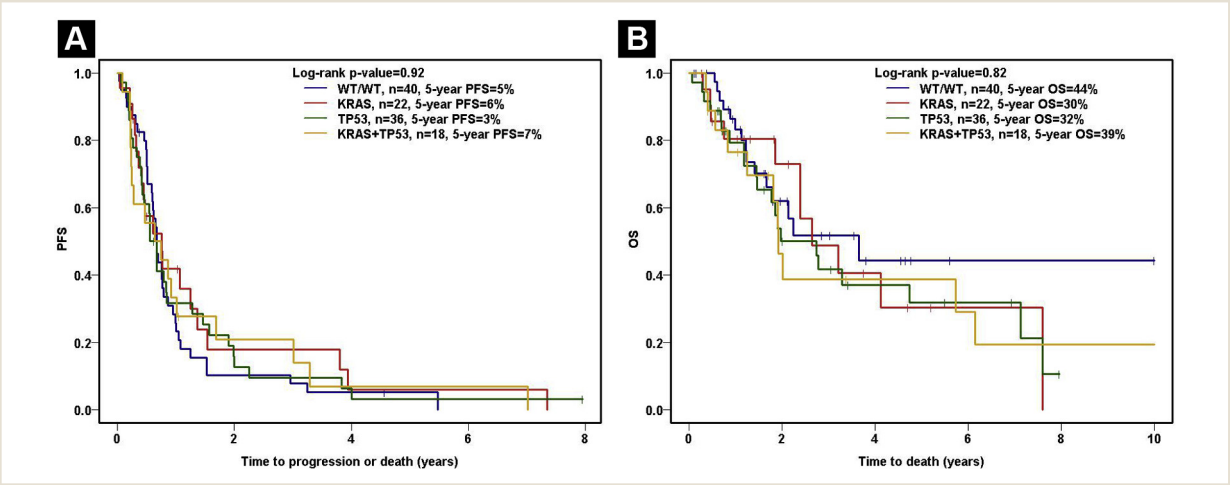
No significant difference in PFS was found when comparing the WT/WT group with the other groups (*KRAS*: HR, 0.82; 95% CI, 0.47-1.43; $P = .48$; *TP53*: HR, 0.92; 95% CI, 0.57-1.47; $P = .71$; *KRAS/TP53*: HR, 0.91; 95% CI, 0.51-1.62; $P = .75$). Patients in the *KRAS/TP53* group did not experience a significantly shorter PFS in comparison with all other mutation subgroups (HR, 0.99; 95% CI, 0.59-1.66; $P = .96$) (Figure 3A). Similar results were observed for OS when comparing the WT/WT group with the other groups (*KRAS*: HR, 1.19; 95% CI, 0.54-2.59; $P = .67$; *TP53*: HR, 1.36; 95% CI, 0.7-2.66; $P = .36$; *KRAS/TP53*: HR, 1.32; 95% CI, 0.6-2.9; $P = .49$) (Figure 3B).

Fourteen patients with advanced disease survived longer than 5 years, including 6 patients with *TP53*-mutant tumors, 4 patients with double mutant tumors, 2 patients with *KRAS*-mutant tumors, and 2 patients with double wild-type tumors.

## Discussion

NSCLC molecular profiling has revolutionized treatment selection with the identification of predictive biomarkers and matched targeted therapies. Although *KRAS* and *TP53* mutations are the most frequent somatic mutations found in large series of NSCLC genomic analyses[26] to date, attempts to target *KRAS*[27] or *TP53* have not been successful.[28] Although EGFR-TKIs should be prescribed for tumors harboring *TP53* and *EGFR* co-mutations, cytotoxic chemotherapy and immunotherapy remain the standard of care for patients with *KRAS*- and/or *TP53*-mutant NSCLC. Subgroup analysis of phase III trials comparing immune check-point inhibitors (ICIs) with chemotherapy suggest *KRAS* mutations to be predictive of a better efficacy of ICIs over chemotherapy. In patients with *KRAS*-mutant tumors, OS was 17.2 months with atezolizumab and 10.0 months with docetaxel (HR, 0.71; 95% CI, 0.38-1.35), whereas in patients with *KRAS* wild-type tumors, OS was 13.8 months with atezolizumab and 11.3 months with docetaxel (HR, 0.83; 95% CI, 0.58-1.18).[29] In the same way, the difference between nivolumab and docetaxel was higher in the subgroup of patients with *KRAS*-mutant tumors (HR, 0.52; 95% CI, 0.29-0.95) than in patients with *KRAS* wild-type tumors (HR, 0.98; 95% CI, 0.66-1.48).[30] However, there is no data regarding the predictive effect of *KRAS* and *TP53* co-mutations on ICI efficacy, and the prognostic and predictive effect of *KRAS* and *TP53* mutations for NSCLC patients treated with cytotoxic chemotherapy is still unclear. The LACE-Bio group studied the impact of mutation status on clinical outcomes of platinum-based ACT for patients with NSCLC enrolled in 4 phase III clinical trials. Patients with double mutant tumors (with *KRAS* and *TP53* co-mutations) had a worse outcome with ACT compared with the double wild-type subgroup (HR, 2.76; 95% CI, 1.62-4.68; $P = .0002$).[31] This observation, however, required confirmation, as only 49 patients fell into this subset. More recently, a case series of 330 patients with *KRAS*-mutant NSCLC found no correlation between the *KRAS/TP53* co-mutation and survival.[32] Here, we report the results of a validation cohort of 218 patients treated with chemotherapy for any stage NSCLC. This study was not restricted to ACT and also included patients receiving palliative chemotherapy for advanced disease. Furthermore, as this study was not restricted to patients enrolled in clinical trials but also included patients ineligible for clinical trials, it

**Figure 3** Progression-free Survival (A) and Overall Survival (B) After Chemotherapy for Advanced Disease




Abbreviations: DFS = disease-free survival; KRAS = patients with *KRAS*-mutant tumors; KRAS/TP53 = patients with *KRAS* and *TP53* double mutant tumors; OS = overall survival; TP53 = patients with *TP53*-mutant tumors; WT/WT = patients with *TP53* and *KRAS* wild-type tumors.

may be more representative of real-life outcomes after chemotherapy.

The presence of *KRAS* and *TP53* co-mutations is frequent, especially in smokers or former smokers, for whom *KRAS* mutation is found in approximately one-third of cases and *TP53* mutation is found in almost one-half.[33,34] *TP53* is a tumor suppressor gene with multiple different mutation subtypes, and, as it is not actionable, *TP53* mutations were not always tested in the early panels of NSCLC molecular profiling,[35] and so the incidence of co-mutation of *TP53* with other driver mutations may have been underestimated in early mutation profiling studies. In preclinical studies, however, the p53/p14 pathway has been shown to be inactivated in 69.2% of *KRAS*-mutant lung adenocarcinomas,[36] highlighting the need to better investigate the association between *KRAS* and *TP53*. In the same way, *KRAS* mutation testing is not part of the routine biomarker panel for NSCLC recommended by national and international guidelines.[37,38] Furthermore, even when both *KRAS* and *TP53* mutations are tested, the frequency of concomitant *KRAS* and *TP53* co-mutations is not always reported.[39] For all these reasons, the interaction of *KRAS* and *TP53* mutations in lung cancer has been studied poorly, and its effect on chemo-sensitivity or chemo-resistance is not known.

The association of *KRAS* and *TP53* mutations has been studied in other types of cancer such as glioblastoma,[40] ovarian cancer,[41] and colorectal cancer.[42] In ovarian cancer, *KRAS* and *TP53* mutations were associated with specific histologic subtypes and specific aggressiveness patterns.[41] Moreover, in colorectal carcinomas, *KRAS* codon 13 mutations were shown to be associated with a lower response rate to adjuvant chemo-radiation therapy.[43] This prognostic effect of *KRAS* codon 13 mutations may be related to a higher incidence of *TP53* mutations in this population.[43]

The mechanisms of *KRAS* and *TP53* co-mutation interaction and effect on chemo-resistance or chemo-sensitivity have been studied in preclinical models. Wörmann et al showed that loss of p53 function or *TP53* mutation activated JAK2-STAT3 signaling and promoted modifications of tumor stroma, tumor growth, and resistance to gemcitabine chemotherapy in mice with *KRAS* G12D-mutated pancreatic tumors.[44] Furthermore, sensitivity of human colon cancer cell lines to cucurbitacin-induced apoptosis was shown to depend on *KRAS* and *TP53* mutation status, and that anti-tumorigenic activity was reduced in *KRAS*- and *TP53*-mutant cell lines.[45] Luu et al also showed a reduced sensitivity to chemo-radiation therapy in *TP53*-mutant HCT116 colorectal cancer cells. This chemo-resistance was increased in double mutant cells with both *TP53* and *KRAS* mutations.[46] Finally, Yang et al showed that this chemo-resistance observed in *TP53*- and *KRAS*-mutant cancer cells may be owing to the activation of the NFKB signaling pathway by *KRAS* and *TP53* mutations in lung cancer cell lines.[47]

As all these clinical and preclinical studies suggest an interaction between *KRAS* and *TP53* mutations resulting in chemo-resistance, it was important to assess the clinical outcomes of patients with NSCLC receiving chemotherapy. In this study, we found longer OS in the group of patients with no *KRAS* or *TP53* mutation (WT/WT) compared with patients whose tumor harbored any mutation in the entire population of patients receiving chemotherapy for any stage NSCLC. Subgroup analysis of patients with ACT found a trend to longer DFS and OS in the WT/WT group compared with patients with *KRAS*-mutant, *TP53*-mutant, or double mutant tumors. However, there was no PFS or OS difference when comparing the 4 groups in the subgroup of patients receiving chemotherapy for advanced stage NSCLC. Moreover, mutation status was not predictive for response to chemotherapy. The results presented by the LACE-Bio group among patients randomized in the ACT arm of 4 phase III clinical trials showed that patients with double mutant tumors (n = 17) had a shorter OS than patients with double wild-type tumors (n = 109).[31] Although we found the same results in the entire population of patients receiving chemotherapy for any stage NSCLC, we found only a trend to longer survival of

patients with WT/WT tumors in the subgroup of patients receiving ACT. In addition, the LACE-Bio group found double *KRAS*/*TP53* mutations to be predictive of shorter survival in the ACT arms compared with control arms. In this study, there was no control arm and all patients received chemotherapy.

The main limitation of this work is the lack of power, because we only had 28 patients with concomitant *KRAS* and *TP53* mutations among 218 patients studied. The population studied is also heterogeneous because we chose to study ACT, chemo-radiation therapy, and palliative chemotherapy. For this reason, and because there was no control group without chemotherapy, we did not observe the same predictive effect of coexisting *KRAS* and *TP53* mutations described by the LACE-Bio group. Further, the population was different from the one studied by the LACE-Bio group as we enrolled a higher population of women and Asians,[48] resulting in 26 patients in the WT/WT group having *EGFR* mutations or *ALK* rearrangements. This also may have influenced the survival results in our study.

In conclusion, patients with *TP53* and *KRAS* double wild-type NSCLC receiving chemotherapy had better outcomes than patients with either or both mutations. In the future, further exploration of chemo-sensitivity according to *KRAS* and *TP53* mutation status in larger populations has to be performed. Furthermore, next generation sequencing techniques including a large panel of somatic mutations in many genes are being developed as a standard of care for NSCLC routine molecular profiling. These techniques will provide new information regarding the influence of other mutations on chemo-sensitivity or chemo-resistance of patients with *KRAS*- and *TP53*-mutant NSCLC.

### *Clinical Practice Points*

- *KRAS* and *TP53* mutations are common in NSCLC. The prognostic and predictive role of each mutation has been investigated, but there are few data about the prognostic and predictive role of *KRAS* and *TP53* co-mutations. The LACE-Bio group found an association between *KRAS and TP53* co-mutations and poor outcomes after adjuvant chemotherapy and these results had to be validated.
- When comparing 4 groups of patients (*KRAS*-mutant, *TP53*-mutant, double wild-type, and double mutant) receiving chemotherapy for any stage NSCLC, no significant difference was found in DFS or PFS. However, OS was longer for patients with double wild-type tumors. This validation cohort did not confirm the predictive effect of *KRAS*/*TP53* co-mutations on chemotherapy. However, as it was not restricted to patients enrolled in clinical trials but also included patients ineligible for clinical trials, it may be more representative of real-life outcomes after chemotherapy.
- Further studies using next generation sequencing with large panel of genes are needed to assess the influence of other mutations on chemo-sensitivity or chemo-resistance of patients with *KRAS*- and *TP53*-mutant NSCLC.

## Disclosure

The authors have stated that they have no conflicts of interest.

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