Received: 19 July 2022 | Revised: 30 November 2022 | Accepted: 3 February 2023

DOI: 10.1002/cncr.34731

ORIGINAL ARTICLE

Real-world retrospective study of KRAS mutations in advanced nonsmall cell lung cancer in the era of immunotherapy

Paolo Bironzo MD, PhD[1] | Massimiliano Cani MD[1] | Francesca Jacobs MD[1] | Valerio M. Napoli MD[1] | Angela Listì PhD[2] | Francesco Passiglia MD, PhD[1] | Luisella Righi MD, PhD[2] | Massimo Di Maio MD[3] | Silvia Novello MD, PhD[1] | Giorgio V. Scagliotti MD, PhD[1]

[1]Department of Oncology, Oncology Unit, University of Torino, San Luigi Gonzaga Hospital, Orbassano, Italy

[2]Department of Oncology, Pathology Unit, University of Torino, San Luigi Gonzaga Hospital, Orbassano, Italy

[3]Department of Oncology, Oncology Unit, University of Torino, Ordine Mauriziano Hospital, Torino, Italy

Correspondence

Paolo Bironzo, Department of Oncology, San Luigi Hospital, University of Torino, Regione Gonzole 10, Orbassano (Torino) 10043, Italy.
Email: paolo.bironzo@unito.it

Funding information

Verastem Oncology, Inc.

Abstract

Background: KRAS mutation-positive (*KRAS*-positive), advanced nonsmall-cell lung cancer (NSCLC) is characterized by a poor prognosis. KRAS mutations are extremely heterogeneous from a biologic point of view, and real-world data by mutation subtype in the era of immunotherapy are still incomplete.

Methods: The objective of this study was to retrospectively analyze all consecutive patients with advanced/metastatic, *KRAS*-positive NSCLC who were diagnosed at a single academic institution since the advent of immunotherapy. The authors report on the natural history of the disease as well as the efficacy of first-line treatments in the entire cohort and by KRAS mutation subtypes as well as the presence/absence of co-mutations.

Results: From March 2016 to December 2021, the authors identified 199 consecutive patients who had *KRAS*-positive, advanced or metastatic NSCLC. The median overall survival (OS) was 10.7 months (95% confidence interval [CI], 8.5–12.9 months), and there were no differences by mutation subtype. Among 134 patients who received first-line treatment, the median OS was 12.2 months (95% CI, 8.3–16.1 months), and the median progression-free survival was 5.6 months (95% CI, 4.5–6.6 months). At multivariate analysis, only an Eastern Cooperative Oncology Group performance status of 2 was associated with significantly shorter progression-free survival and OS.

Conclusions: *KRAS*-positive, advanced NSCLC is characterized by a poor prognosis despite the introduction of immunotherapy. Survival was not associated with KRAS mutation subtype.

Plain Language Summary

This study evaluated the efficacy of systemic therapies for advanced/metastatic nonsmall cell lung cancer harboring KRAS mutations, along with the potential predictive and prognostic role of mutation subtypes.

- The authors found that advanced/metastatic, *KRAS*-positive nonsmall cell lung cancer is characterized by a poor prognosis and that first-line treatment efficacy is not related to different KRAS mutations, although a numerically shorter median progression-free survival was observed in patients who had p.G12D and p.G12A mutations.
- These results underline the need for novel treatment options in this population, such as next-generation KRAS inhibitors, which are in clinical and preclinical development.

KEYWORDS
chemotherapy, immunotherapy, KRAS, nonsmall cell lung cancer, real-world study

INTRODUCTION

Worldwide, lung cancer is one of the leading causes of cancer-related death, with an estimated 1.8 million deaths in 2020.[1] Approximately 30% of patients with nonsmall cell lung cancer (NSCLC), mostly adenocarcinoma, harbor activating gene mutations of the Kirsten rat sarcoma oncogene homologue (KRAS).[2] Most patients with *KRAS*-positive NSCLC are current or former smokers, whereas some specific mutations (e.g., KRAS p.G12D) are prevalent in never-smokers.[3] Despite conflicting data from retrospective studies, a recent meta-analysis suggests a negative prognostic role of KRAS mutations in advanced and metastatic NSCLC.[4] However, until recently, no targeted treatment has proven to be active in this population. KRAS-activating mutations are extremely heterogeneous, and it has been demonstrated that isoforms differ functionally in terms of both downstream oncogenic signals and oncogenic networks.[5–8] Recent data on an isogenic system, including the most common NSCLC KRAS-activating mutations, have shed new light on growth patterns as well as sensitivity to targeted agents of specific isoforms.[9]

This is noteworthy because one of the direct KRAS G12C inhibitors, sotorasib, was granted accelerated approval and breakthrough therapy designation by the US Food and Drug Administration and the European Medicines Agency for patients with previously treated, advanced NSCLC, showing an overall response rate (ORR) of 37.1% with a median progression-free survival (mPFS) of 6.8 months in a phase 2 trial.[10] Otherwise, adagrasib, another KRAS G12C inhibitor, has been approved by the US Food and Drug Administration but is still waiting European Medicines Agency approval, although it has demonstrated results similar to those reported for sotorasib.[10,11] Indeed, currently, the standard first-line approach in patients with advanced, KRAS mutation-positive NSCLC is based on immune checkpoint inhibitors (ICIs), with or without platinum-based chemotherapy.[12,13] Subgroup analysis of trials exploring ICIs did not show differences in survival outcomes by KRAS mutational status.[14–16] However, retrospective as well as computational data suggest that specific concurrent genomic alterations, such as those occurring in the *TP53*, *STK11/LKB1*, and *KEAP1* genes, may predict ICI efficacy.[17–21] Indeed, whereas co-occurring *TP53* mutations are associated with inflammatory tumor microenvironment (TME) as well as higher levels of neoantigens and programmed death ligand 1 (PD-L1) expression, *STK11/LKB1* and *KEAP1* mutations correlate with immunosuppressive TME and shorter survival. Preliminary data suggest an association of the latter alterations with specific KRAS isoforms, such as G13X.[9] In this retrospective, real-world study, we report the natural history of all consecutive patients with KRAS mutation-positive, advanced non-squamous NSCLC at our institution since the introduction of ICIs in clinical practice, according to KRAS isoforms and concurrent genomic alterations evaluated by routine next-generation sequencing (NGS).

MATERIALS AND METHODS

Overall study design

This study is a retrospective collection of clinical and molecular data retrieved from electronic medical records of patients with advanced, *KRAS*-positive NSCLC who were treated at San Luigi University Hospital from March 2016 (when the first ICI was approved in Italy for patients who had chemotherapy-treated, advanced NSCLC) to December 2021.

Main inclusion criteria were aged 18 years or older at the time of diagnosis; a diagnosis of *KRAS*-positive, advanced NSCLC at the time of enrolment; and availability of clinical data. Main exclusion criteria included unavailability of follow-up data and absence of KRAS mutation.

The primary objective was to evaluate the efficacy of therapeutic regimens administered for advanced or metastatic, *KRAS*-positive NSCLC and to characterize outcomes in the presence or absence of different variants. The activity and efficacy of systemic treatments were measured by investigators in terms of the ORR, the disease control rate, PFS (for regimens followed on study), and overall survival (OS). Reasons for discontinuation of each regimen were collected. Response assessment was done according to the RECIST (Response Evaluation Criteria in Solid Tumors, version 1.1) by the investigators. The secondary objective was the description of co-occurring mutations.

Molecular and immunohistochemical analyses

Tumor DNA and RNA were extracted from FFPE samples by using semiautomated purification kits Maxwell (Promega). From 2016 to 2019, NGS analysis was performed by using Oncomine Solid Tumor DNA and RNA kit assay to investigate deletions, insertions, and single or multiple nucleotide variations occurring within 22 cancer-related genes and to detect transcript rearrangements in four genes (Thermo Fisher Scientific, Inc.; www.thermofisher.com). From 2020 to 2021 we adopted Oncomine Dx Target test DNA and RNA kit assay allowing to implement the selected DNA targets region and transcript rearrangements across 46 driver gene variants (Thermo Fisher Scientific, Inc.). A full list of genes analyzed by each assay is provided in Supporting Information material (see Table S1). The library preparation was manually performed according to the manufacturer's instructions (MAN0011016, revision A.0, and MAN0006735, revision F.0, respectively). Prepared libraries were loaded in the Ion Chef System, and MAN0016854 (revision F.0) was loaded onto Ion 520-530 chips sequenced in the Ion GeneStudio S5 Prime System. The molecular data were mapped to the human genome assembly 19, and the analyses were provided by the Ion Reporter Software, version 5.14 (Thermo Fisher Scientific, Inc.). Quality assessment of sequenced reactions was performed by studying the coverage analysis, average depth, and percentage of alignment uniformity over the target regions. Finally, visual inspections of BAM file (.bam) were performed with graphic alignment programs, such as Thermo Fisher Scientific's Ion Reporter Genomics Viewer. Only pathogenic or likely pathogenic variants were included in the analyses based on the current literature.

Immunohistochemical evaluation of tumor PD-L1 expression was performed using the anti-PD-L1 antibody (clone 22C3 pharmDx kit) and the Dako Omnis platform (both from Agilent Technologies, Inc.) according to the manufacturer's recommendations. The percentage of tumor cells with PD-L1 expression (positive membrane staining) was obtained by counting at least 100 viable cells, called the *tumor proportion score*. The evaluation of PD-L1 expression followed the specific requests of the treating clinician in terms of selection of the tested cohort and timing (diagnostic biopsy or re-biopsy, according to routine clinical practice).

Statistical analysis

Quantitative data are summarized by arithmetic mean, standard deviation, median, minimum, and maximum values. Associations between the response to treatment in different KRAS mutation variants were analyzed using the Fisher exact test, χ^2 test, or logistic regression. Differences in continuous variables were assessed with the Student *t*-test or general linear models. A two-sided $p < .05$ was considered to indicate a statistically significant difference without adjustment for multiple comparisons. Survival curves were estimated using the Kaplan–Meier method and were compared using the log-rank test. The Cox proportional hazard model was used for estimating hazard ratios after adjusting for relevant variables. An exploratory analysis of associations between response to treatment according to co-occurring emergent genomic alterations identified during NGS analysis was conducted. All analyses were conducted using SPSS software (IBM Corporation).

Ethical aspects

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The prospective part of this study was approved by the local independent Ethics Committee on February 2021 (PRE-V-LUNG study; code 34/2021/U; registry number 2867). Retrospective data were retrieved from electronic medical records upon patient informed consent. Molecular analyses were performed according to clinical practice or under the aforementioned protocol.

RESULTS

Overall survival in the entire cohort

From March 2016 to December 2021, 199 consecutive patients with *KRAS*-positive, advanced or metastatic NSCLC were identified (Figure 1). The median patient age at diagnosis was 69 years (range, 38–90 years), 135 were men (67.8%), and 197 (99.0%) had clinical stage IV disease (Table 1). Eastern Cooperative Oncology Group performance status (ECOG PS) was 0 in 108 patients (54.3%), and 184 patients (92.5%) were current or former smokers. Liver and brain metastases were present at diagnosis in 7% and 17.1% of patients, respectively. In the latter group, 47% of patients received locoregional therapy (radiation therapy and/or surgical resection) at diagnosis of stage IV disease. PD-L1 expression levels were high, intermediate, and negative in 44 (22.1%), 58 (29.1%), 88 (44.2%) patients, respectively, whereas data were not available for nine patients (4.5%). Most patients were diagnosed with lung adenocarcinoma ($n = 170$; 85.4%). First-line treatment for advanced disease was administered to 134 patients (67.3%), whereas 38 patients (19.1%) received an indication for best supportive care at the time of

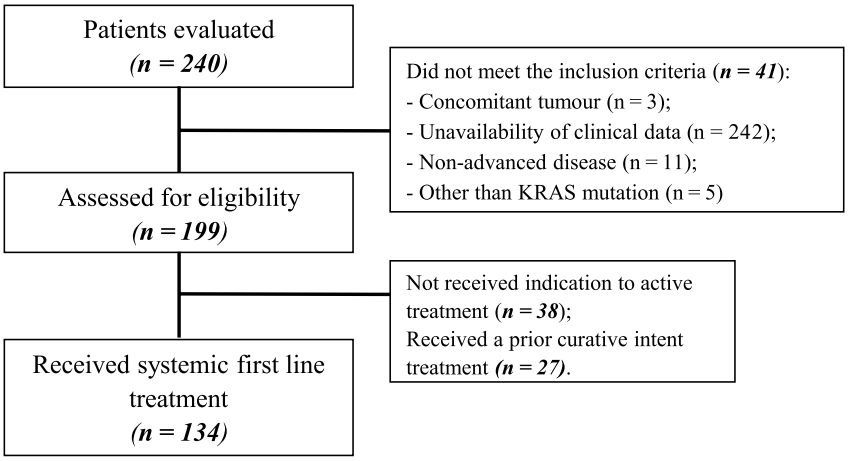

FIGURE 1 Patients evaluated and included in the study.

diagnosis. Twenty-seven patients (13.6%) who were previously treated with chemoradiation therapy or surgery were not further evaluated in our study at the progression disease because of the high heterogeneity of this subgroup (see Table S2).

KRAS mutations were evaluated either on metastatic tissue (n = 39; 19.6%) or on primary tumor, depending on the diagnostic specimen obtained at the time of diagnosis. The most prevalent KRAS mutation subtypes were p.G12C (n = 78; 39.2%), followed by p.G12V (n = 34; 17.1%), and p.G12D (n = 29; 14.6%; Table 1). Two patients harbored double KRAS mutations, one with p.G12C plus p.G13C and the other with p.G12C and p.A146T. Co-mutations other than KRAS occurred in 49 patients (24.6%; see Table S3). There were no associations between the type of KRAS mutation (dichotomized as G12C vs. others) and sex, performance status, or the number or site of metastasis. Indeed, a significant association with positive smoking history and p.G12C mutation was observed (p = .043).

After a median follow-up of 28.2 months, with 129 deaths recorded, the median OS (mOS) in the entire patient data set was 10.7 months (95% confidence interval [CI], 8.5–12.9 months; Figure 2A). In a comparison of different mutation subtypes, no significant differences were observed in terms of OS (p = .33; Figure 2B). However, a numerically lower mOS was observed in patients harboring p.G12D and p.G12A (7.1 and 5.2 months, respectively).

OS and PFS in patients who underwent first-line treatment

In total, 134 patients received first-line systemic treatment (67.3%), most for stage IV disease (n = 132; 98.5%). In this subgroup, the median age was 69 years (range, 41–85 years), 93 patients were men (69.4%), and 116 were diagnosed with lung adenocarcinoma (86.6%). ECOG PS was 0 in 70 patients (52.6%), and 129 patients (94.0%) were current or former smokers. PD-L1 expression levels were high, intermediate, or negative in 32 (23.9%), 41 (30.6%), and 60 (44.8%) patients, respectively, and were not available for one patient (0.7%). According to the mutation subtype, p.G12C mutation was identified in 52 patients (38.8%), whereas p.G12D, p.G12V, and p.G12A were identified in 24 (17.9%), 15 (11.2%), and 14 (10.4%) patients, respectively. Twenty-nine patients harbored co-mutations (21.6%). The type of first-line treatment included platinum-based chemotherapy (n = 42; 31.3%), single-agent anti–PD-(L)1 immunotherapy (n = 35; 26.1%), chemoimmunotherapy (n = 27; 20.2%), single-agent chemotherapy (n = 22; 16.4%), and experimental treatments in clinical trials (n = 8; 6%; Table 1). Second-line therapy was administered to 44 patients (56.8% received single-agent immunotherapy; 41.6% received chemotherapy with or without antiangiogenics, and 9.0% received experimental treatments). Notably, eight patients received a KRAS p.G12C inhibitor during their disease course. Figure S1 illustrates the percentage of patients who received immunotherapy with or without chemotherapy according to the line of treatment and KRAS mutation.

After a median follow-up of 25.1 months and 84 deaths, five patients (3.7%) were still receiving first-line treatment, whereas the others had discontinued treatment, mainly for disease progression (76.1%). The mOS was 12.2 months (95% CI, 8.3–16.1 months; Figure 3A). No differences in terms of OS were observed according to KRAS mutation subtypes (p = .39; Figure 3B).

PFS was documented in 102 patients, and the mPFS for first-line treatment was 5.6 months (95% CI, 4.5–6.6 months; Figure 4A). The ORR to first-line therapy was 32.0%, whereas the disease control rate was 61.1%. No differences in terms of mPFS were observed according KRAS mutation subtypes (Figure 4B). However, a numerically shorter mPFS was observed in patients with KRAS p.G12D (4.1 months; 95% CI, 2.5–5.6 months) and p.G12A (1.9 months; 95% CI, 0.7–3.1 months) mutations. No differences in terms of OS or PFS were observed by first-line treatment type, although both chemotherapy and experimental therapies in clinical trials were characterized by shorter mPFS (5.1 and 2.8 months, respectively). Because one

TABLE 1 Characteristics of patients included in the study.

	Patients with stage IV disease, No. (%)	Patients treated for advanced disease, No. (%)	No indication for active treatment, No. (%)
No. of patients	199	134	38
Sex			
Men	135 (67.8)	93 (69.4)	26 (68.4)
Women	64 (32.2)	41 (30.6)	12 (31.6)
Age at diagnosis [range], years	69 [38–90]	69 [41–85]	72 [48–90]
Smoking habit			
Yes, NOS	3 (1.5)	3 (2.2)	0 (0.0)
Current	60 (30.2)	44 (32.8)	8 (21.1)
Former	124 (62.3)	82 (61.2)	26 (68.4)
Never	7 (3.5)	4 (3.0)	2 (5.3)
Unknown	5 (2.5)	1 (0.7)	2 (5.3)
ECOG performance status at diagnosis			
0	108 (54.3)	81 (60.4)	11 (28.9)
1	72 (36.2)	48 (35.8)	14 (36.8)
2	17 (8.5)	5 (3.8)	11 (28.9)
3	1 (0.5)	0 (0.0)	1 (2.6)
Unknown	1 (0.5)	0 (0.0)	1 (2.6)
ECOG performance status at stage IV			
0	87 (43.7)	70 (52.3)	8 (21.1)
1	72 (36.2)	50 (37.3)	14 (36.8)
2	19 (9.5)	7 (5.2)	11 (28.9)
3	2 (1.0)	0 (0.0)	2 (5.3)
Unknown	19 (9.5)	7 (5.2)	3 (7.9)
No. of metastatic sites			
Unknown	20 (10.1)	8 (6.0)	2 (5.3)
1	39 (19.6)	21 (15.7)	6 (15.8)
2	72 (36.2)	54 (40.3)	14 (36.8)
3	41 (20.6)	32 (23.9)	8 (21.1)
4	17 (8.5)	12 (9.0)	5 (13.2)
5	9 (4.5)	6 (4.5)	3 (7.9)
6	1 (0.5)	1 (0.7)	0 (0.0)
Of which			
Brain	34 (17.1)	26 (19.4)	6 (15.8)
Liver	14 (7.0)	9 (6.71)	3 (7.9)
Histology			
Adenocarcinoma	170 (85.4)	116 (86.6)	30 (78.9)
Large cell carcinoma	1 (0.5)	1 (0.7)	0 (0.0)
NSCLC NOS	26 (13.1)	15 (11.2)	8 (21.1)
Squamous cell carcinoma	2 (1.0)	2 (1.5)	0 (0.0)

(Continues)

TABLE 1 (Continued)

	Patients with stage IV disease, No. (%)	Patients treated for advanced disease, No. (%)	No indication for active treatment, No. (%)
Mutation type			
p.G12C	78 (39.2)	52 (38.8)	15 (39.5)
p.G12V	34 (17.1)	15 (11.2)	11 (28.9)
p.G12D	29 (14.6)	24 (17.9)	4 (10.5)
p.G12A	19 (9.5)	14 (10.4)	3 (7.9)
Other	39 (19.6)	29 (21.6)	5 (13.2)
Co-mutation rate			
Yes	49 (24.6)	29 (21.6)	11 (28.9)
No	149 (74.9)	104 (77.6)	27 (71.1)
Unknown	1 (0.5)	1 (0.7)	0 (0.0)
PD-L1 expression			
Negative	88 (44.2)	60 (44.8)	19 (50.0)
1%–49%	58 (29.1)	41 (30.6)	9 (23.7)
≥50%	44 (22.1)	32 (23.9)	6 (15.8)
Incomplete data	9 (4.5)	1 (0.7)	4 (10.5)
First-line treatment			
Anti–PD-1/PD-L1		35 (26.1)	
Chemotherapy and anti–PD-1/PD-L1		27 (20.1)	
Chemotherapy		64 (47.8)	
Clinical trial		8 (6.0)	
Chemotherapy: Single agent versus doublet			
Single agent		22 (16.4)	
Doublet		42 (31.3)	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NOS, not otherwise specified; PD-1, programed death 1; PD-L1, programmed death ligand 1.

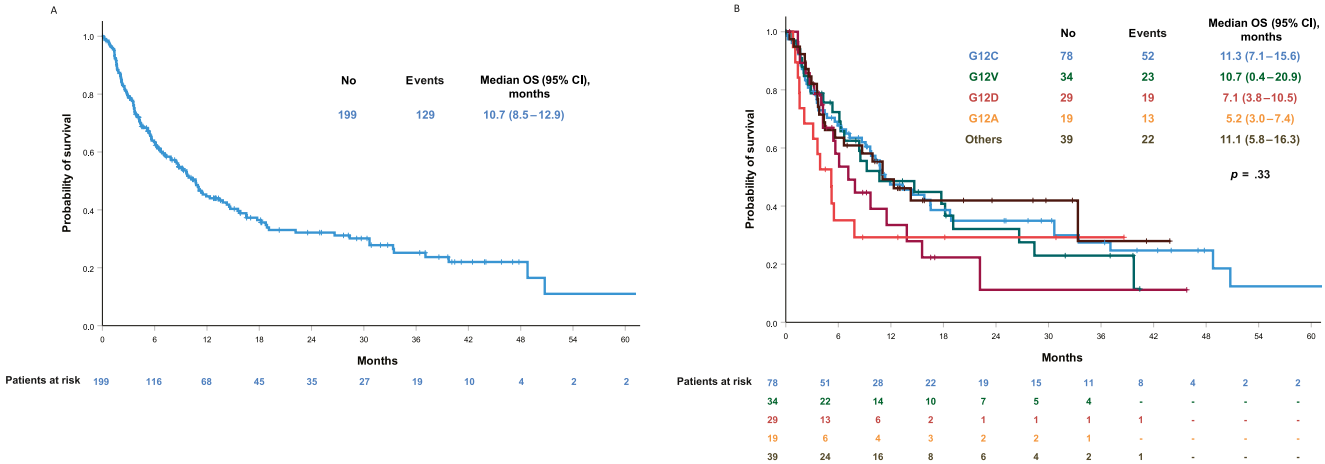

FIGURE 2 OS (A) in all patients and (B) by KRAS mutation status. CI indicates confidence interval; OS, overall survival.

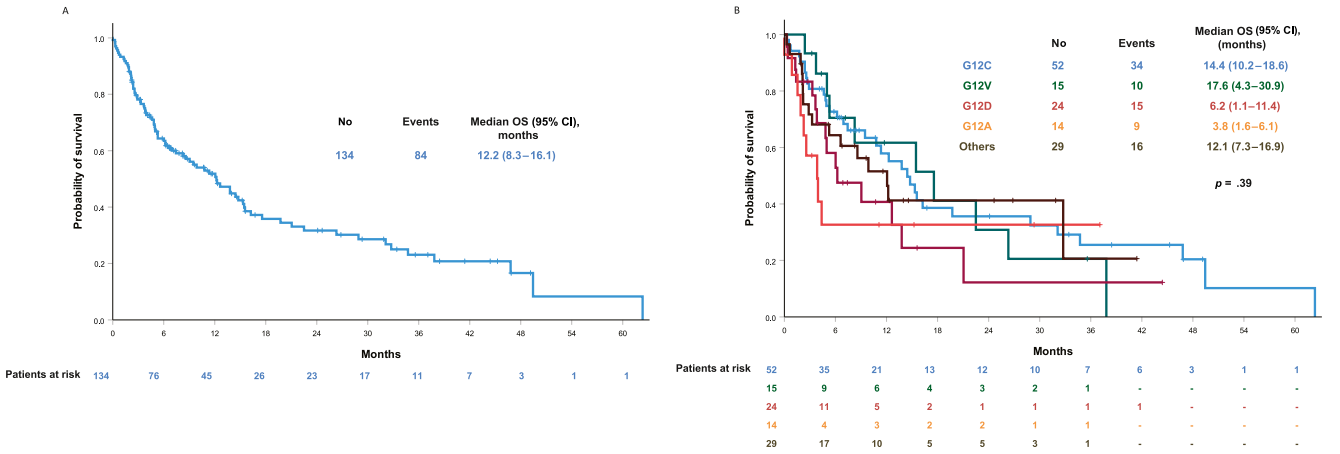

FIGURE 3 OS (A) in patients who underwent first-line therapy and (B) by KRAS mutation status. CI indicates confidence interval; OS, overall survival.

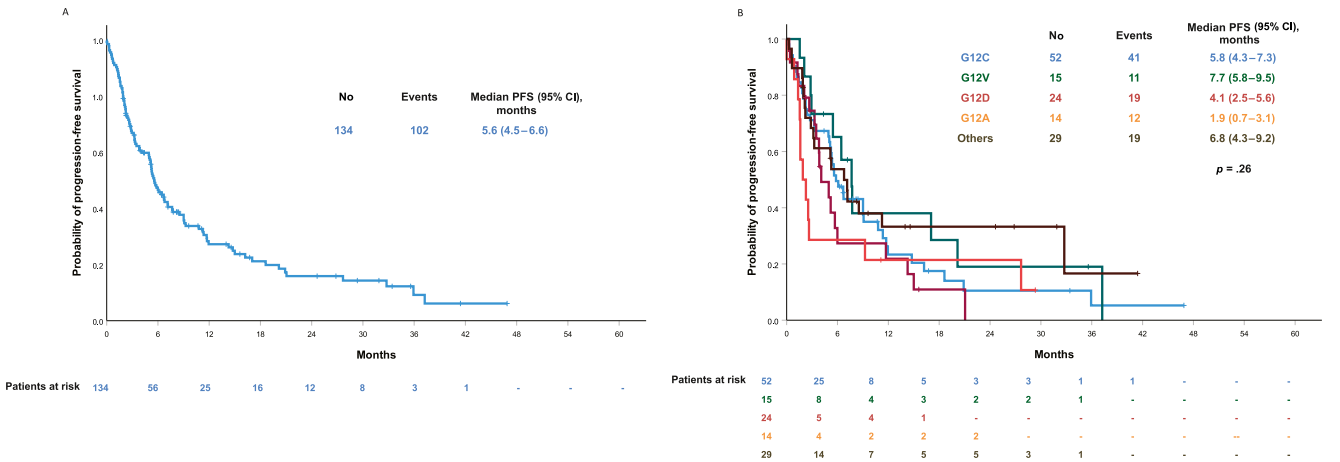

FIGURE 4 (A) PFS in patients who underwent first-line therapy and (B) by KRAS mutation status. CI indicates confidence interval; PFS, progression-free survival.

third of patients who underwent chemotherapy received single-agent treatment only, we compared PFS and OS according to the type of chemotherapy (platinum doublet vs. single agent). Although no statistically significant difference was observed for PFS ($p = .32$), there was a significant difference in terms of OS (mOS, 13.8 vs. 5.3 months; $p = .02$). More patients who underwent platinum-based chemotherapy received second-line treatment compared with those who underwent single-agent chemotherapy (54.8% vs. 27.3%). PFS and OS did not significantly differ by PD-L1 expression, although mPFS was numerically higher in patients who had high PD-L1 expression (11.0 vs. 5.0 months; see Figure S2). At multivariate analysis, only an ECOG PS of 2 was associated with significantly shorter PFS and OS, whereas no association was observed for the other variables.

PFS was also evaluated according to KRAS mutation and type of first-line systemic treatment (chemotherapy. immunotherapy, or chemoimmunotherapy). No differences in terms of mPFS were observed in patients with different KRAS mutations in any treatment group (see Figure S3).

OS in patients who did not receive first-line systemic therapy

Thirty-eight patients (19.1%) in our cohort received an indication for best supportive care at the time of diagnosis. In this subgroup, the mOS was 1.84 months (95% CI, 1.55–2.13 months), with 34 deaths recorded. Eleven patients (28.9%) received palliative radiation treatment, including eight at the bone level for antalgic purposes, whereas three patients underwent mediastinal radiotherapy.

Patients who received second-line systemic therapy

In total, 48 patients received second-line therapy. Twenty-three patients (47.92%) received anti–PD-1/PD-L1 single-agent therapy, whereas the others received chemotherapy or were enrolled in clinical trials (including two patients receiving sotorasib). Notably, in this subgroup, non–p.G12C KRAS mutations were less frequent

(20.8% and 8.3% of patients with p.G12D and p.G12A, respectively, vs.45.8% of those with p.G12C).

DISCUSSION

This retrospective series of *KRAS*-positive, advanced or metastatic NSCLC confirms the overall bad prognosis for this subgroup of patients despite the introduction of ICIs in the therapeutic landscape. Indeed, in entire cohort, the mOS was 10.7 months, and it was 12.2 months in patients who received first-line treatment, with no statistically significant difference by type of first-line treatment. Retrospective data as well as subgroup analyses of clinical trials of ICIs did not show a consistent predictive role of KRAS mutations in advanced NSCLC. In our series, the prevalence of KRAS mutation variants was similar to that reported in the literature, even when the small sample size was taken into account.[22] Moreover, there was no difference in terms of survival according to each KRAS mutation, although the numerically shorter mPFS in patients with G12D and G12A mutations warrants further investigation. This latter finding is in accordance with Lee and colleagues, who reported a shorter median duration of first-line treatment in patients with non–G12C KRAS mutations.[23] Until recently, *KRAS*-positive NSCLC has been clinically considered a unique entity, with a dismal prognosis compared with oncogene-addicted lung tumours.[24] The development of specific inhibitors against p.G12C KRAS mutation has renewed the interest of both scientists and clinicians in KRAS-mutant NSCLC.[10,11] In this regard, a recent article reviewed the effectiveness of chemotherapy, antiangiogenic therapy, targeted therapy, or immunotherapy among patients with lung cancer who had different KRAS mutant subtypes and demonstrated that the results were far from satisfactory.[24] Although some studies suggested that these patients may derive greater benefit from ICIs alone or in combination with chemotherapy,[16,25] others did not.[14,17,26,27] Moreover, a recent meta-analysis of randomized clinical trials of anti–PD-(L)1 with or without chemotherapy in advanced NSCLC pointed out a greater OS benefit in *KRAS*-positive patients compared with those who had wild-type KRAS,[28] whereas another retrospective analysis of randomized trials submitted to the US Food and Drug Administration for marketing approval showed that, among 1430 patients with known KRAS mutational status who underwent first-line therapy, both response rate and survival were similar between *KRAS*-positive patients and those with KRAS wild-type, although the mOS was numerically higher in *KRAS*-positive patients who received chemo-immunotherapy.[29] The extent to which different KRAS mutation subtypes may predict the efficacy ICIs is still unclear. A retrospective study suggested that tumors harboring G12D, G12V, and G13C mutations had significantly higher PD-L1 expression compared with tumors harboring KRAS G12C and G12A mutations, and the latter were less sensitive to ICIs.[30] It has been demonstrated that PD-L1 expression levels are both prognostic and predictive of immunotherapy efficacy in NSCLC.[31] However, the predictive value of PD-L1 is far from optimal. Our analysis in this regard did not identify any association between PD-L1 expression levels and survival. However, administered treatments were heterogeneous, based on PD-L1 expression, and related to national regulatory issues. Indeed, first-line, single-agent immunotherapy was reimbursed in Italy for patients who had high PD-L1 expression (≥50%) in May 2017, whereas the combination of chemotherapy and pembrolizumab was reimbursed for patients who had PD-L1 tumor proportion scores <50% in December 2019.

A critical consideration is related to the high degree of heterogeneity among the different subtypes of KRAS mutations leading to differences in metabolic profiles and the TME. More specifically, in KRAS tumors the TME, ranges from T-cell–depleted (*cold*) to T-cell–inflamed (*hot*).[32,33] Co-occurring genetic events are observed in approximately 30% of KRAS-mutant tumors, some associated with a hot TME (like *TP53* mutations) and others associated with a cold TME (such as *STK11/LKB1* and *KEAP1* mutations). Therefore, such profiles may define clinically relevant subtypes, as suggested by retrospective evidence of different sensitivity to ICIs depending on co-mutational status.[19,34,35] Only 21.6% of patients in our series harbored co-mutations, mainly involving *TP53*. The lower prevalence of co-mutations may be caused in part by the limited gene panel that was used, not including, for example, *KEAP1* mutations (which are reported to be present in 8.4% of cases)[20] and *TP53* mutations in cases analyzed from 2020 to 2021. However, the adoption of clinically available NGS panels reflects real-word practice.

Others obvious limitations in the current study are related to its retrospective nature, although we included all patients registered in our clinical database who had with complete clinical and pathologic information. Because of the number of included patients, data on the impact of different therapeutic approaches in relation to specific mutation isoforms should be taken with caution. The reduced mOS of patients who received only best supportive care seems to be mainly determined by their poor performance status, thus providing only limited information about the natural course of the disease in unfit and untreated patients.

In conclusion, we reported the clinical history of *KRAS*-positive, advanced NSCLC in a large cohort of consecutive patients who were treated mainly with immunotherapy. Overall, *KRAS*-positive, advanced and metastatic NSCLC is characterized by a poor prognosis; and the efficacy of first-line ICIs, both alone and in combination with chemotherapy, did not differ between patients who had different isoforms of KRAS mutations, even when analyzed by the type of first-line treatment, although a shorter mPFS, which did not reach statistical significance, was observed in patients with p.G12D and p.G12A mutations.

Because of the rising translational and clinical interest around the next generation of KRAS inhibitors, it will be critical to continuously collect prospective data about the clinical implications of the different isoforms, the role of co-mutations, and the role of intrinsic and acquired resistance to specific KRAS inhibitors.

AUTHOR CONTRIBUTIONS

Paolo Bironzo: Conceptualization, formal analysis, visualization, supervision, methodology, writing–original draft, and writing–review/editing. **Massimiliano Cani:** Data curation, formal analysis,

visualization, writing–original draft, and writing–review/editing. **Francesca Jacobs:** Data curation, writing–original draft, and writing–review/editing. **Valerio M. Napoli:** Data curation, writing–original draft, and writing–review/editing. Angela **Listì:** Data curation, writing–original draft, and writing–review/editing. **Francesco Passiglia:** Conceptualization, writing–original draft, and writing–review/editing. **Luisella Righi:** Conceptualization, writing–original draft, and writing–review/editing. **Massimo Di Maio:** Formal analysis, methodology, validation, writing–original draft, and writing–review/editing. **Silvia Novello:** Conceptualization, writing–original draft, and writing–review/editing. **Giorgio V. Scagliotti:** Conceptualization, methodology, supervision, writing–original draft, and writing–review/editing. All authors approved the submitted version of the article and agreed to be accountable for all aspects of the work.

ACKNOWLEDGMENTS

This study was funded by Verastem Oncology Inc.

Open Access Funding provided by Universita degli Studi di Torino within the CRUI-CARE Agreement.

CONFLICT OF INTEREST STATEMENT

Paolo Bironzo reports personal fees from AstraZeneca, Bristol Myers Squibb, F. Hoffman La-Roche, Janssen Biotech, and Takeda Oncology; institutional research grants from Pfizer Canada Inc.; and travel expenses from Amgen and Daiichi Sankyo outside the submitted work. **Francesco Passiglia** reports personal fees from Amgen, AstraZeneca, BeiGene Switzerland GmbH, Janssen Biotech, Merck Sharpe & Dohme, Sanofi, and ThermoFisher Scientific outside the submitted work. **Massimo Di Maio** reports honoraria from Amgen, AstraZeneca, Boehringer Ingelheim, H. Hoffman-La Roche, Janssen Biotech, Merck Sharpe & Dohme, Novartis, and Pfizer; personal fees from Takeda Oncology for consultancy or participation to advisory boards; direct research funding from Tesaro/GlaxoSmithKline; and institutional funding for work in clinical trials/contracted research from BeiGene Switzerland GmbH, Exelixis, F. Hoffman-La Roche, Merck Sharpe & Dohme, and Pfizer outside the submitted work. **Silvia Novello** reports grants/contracts from Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lilly & Company, F. Hoffman-La Roche, Merck Sharpe & Dohme, Pfizer Canada Inc., and Takeda Oncology; and personal fees (as speaker or advisor) from AMG, AstraZeneca, BeiGene Switzerland GmbH, Boehringer Ingelheim, Bristol Myers Squibb, Eli Lilly & Company, F. Hoffman-La Roche, Merck Sharpe & Dohme, Novartis, Pfizer, Sanofi, and Takeda Oncology outside the submitted work. **Giorgio V. Scagliotti** reports honoraria, research funding, and personal fees from AstraZeneca, Bayer, BeiGene Switzerland GmbH, F. Hoffman-La Roche, Merck Sharpe & Dohme, Eli Lilly & Company, Johnson & Johnson, Pfizer, Takeda Oncology, Tesaro, and Verastem Inc. outside the submitted work. The remaining authors made no disclosures.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article (and its Supporting Information files).

ORCID

Massimiliano Cani https://orcid.org/0000-0001-9608-4840
Massimo Di Maio https://orcid.org/0000-0001-8906-3785

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209-249. doi:10.3322/caac.21660
2. Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS: mission possible? *Nat Rev Drug Discov*. 2014;13(11):828-851. doi:10.1038/nrd4389
3. Dogan S, Shen R, Ang DC, et al. Molecular epidemiology of EGFR and KRAS mutations in 3,026 lung adenocarcinomas: higher susceptibility of women to smoking-related KRAS-mutant cancers. *Clin Cancer Res*. 2012;18(22):6169-6177. doi:10.1158/1078-0432.ccr-11-3265
4. Goulding RE, Chenoweth M, Carter GC, et al. KRAS mutation as a prognostic factor and predictive factor in advanced/metastatic non-small cell lung cancer: a systematic literature review and meta-analysis. *Cancer Treat Res Commun*. 2020;24:100200. doi:10.1016/j.ctarc.2020.100200
5. Cook JH, Melloni GEM, Gulhan DC, Park PJ, Haigis KM. The origins and genetic interactions of KRAS mutations are allele- and tissue-specific. *Nat Commun*. 2021;12(1):1808. doi:10.1038/s41467-021-22125-z
6. Hood FE, Klinger B, Newlaczyl AU, et al. Isoform-specific Ras signaling is growth factor dependent. *Mol Biol Cell*. 2019;30(9):1108-1117. doi:10.1091/mbc.e18-10-0676
7. Stolze B, Reinhart S, Bulllinger L, Frohling S, Scholl C. Comparative analysis of KRAS codon 12, 13, 18, 61, and 117 mutations using human MCF10A isogenic cell lines. *Sci Rep*. 2015;5(1):8535. doi:10.1038/srep08535
8. Brubaker DK, Paulo JA, Sheth S, et al. Proteogenomic network analysis of context-specific KRAS signaling in mouse-to-human cross-species translation. *Cell Syst*. 2019;9(3):258-270.e6. doi:10.1016/j.cels.2019.07.006
9. Ricciuti B, Son J, Okoro JJ, et al. Comparative analysis and isoform-specific therapeutic vulnerabilities of KRAS mutations in non-small cell lung cancer. *Clin Cancer Res*. 2022;28(8):1640-1650. doi:10.1158/1078-0432.ccr-21-2719
10. Skoulidis F, Li BT, Dy GK, et al. Sotorasib for lung cancers with KRAS p.G12C mutation. *N Engl J Med*. 2021;384(25):2371-2381. doi:10.1056/nejmoa2103695
11. Ou SI, Janne PA, Leal TA, et al. First-in-human phase I/IB dose-finding study of adagrasib (MRTX849) in patients with advanced KRAS(G12C) solid tumors (KRYSTAL-1). *J Clin Oncol*. 2022;40(23):2530-2538. doi:10.1200/JCO.21.02752
12. Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2018;29:iv192-iv237. doi:10.1093/annonc/mdy275
13. Hanna NH, Schneider BJ, Temin S, et al. Therapy for stage IV non-small-cell lung cancer without driver alterations: ASCO and OH (CCO) joint guideline update. *J Clin Oncol*. 2020;38(14):1608-1632. doi:10.1200/jco.19.03022
14. Passiglia F, Cappuzzo F, Alabiso O, et al. Efficacy of nivolumab in pre-treated non-small-cell lung cancer patients harbouring KRAS mutations. *Br J Cancer*. 2019;120(1):57-62. doi:10.1038/s41416-018-0234-3
15. Lee CK, Man J, Lord S, et al. Clinical and molecular characteristics associated with survival among patients treated with checkpoint inhibitors for advanced non-small cell lung carcinoma: a systematic

review and meta-analysis. *JAMA Oncol*. 2018;4(2):210-216. doi:10.1001/jamaoncol.2017.4427
16. Herbst RS, Lopes G, Kowalski DM, et al. LBA4-Association of KRAS mutational status with response to pembrolizumab monotherapy given as first-line therapy for PD-L1-positive advanced non-squamous NSCLC in KEYNOTE-042 [abstract]. *Ann Oncol*. 2019;30 (suppl 11):xi63-xi64. doi:10.1093/annonc/mdz453.001
17. Gadgeel S, Rodriguez-Abreu D, Felip E, et al. LBA5-KRAS mutational status and efficacy in KEYNOTE-189: pembrolizumab (pembro) plus chemotherapy (chemo) vs placebo plus chemo as first-line therapy for metastatic non-squamous NSCLC [abstract]. *Ann Oncol*. 2019;30 (suppl 11):xi64-xi65. doi:10.1093/annonc/mdz453.002
18. Gu M, Xu T, Chang P. KRAS/LKB1 and KRAS/TP53 co-mutations create divergent immune signatures in lung adenocarcinomas. *Ther Adv Med Oncol*. 2021;13:17588359211006950. doi:10.1177/17588359211006950
19. Skoulidis F, Byers LA, Diao L, 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(8):860-877. doi:10.1158/2159-8290.cd-14-1236
20. Ricciuti B, Arbour KC, Lin JJ, et al. Diminished efficacy of programmed death-(ligand)1 inhibition in STK11- and KEAP1-mutant lung adenocarcinoma is affected by KRAS mutation status. *J Thorac Oncol*. 2022;17(3):399-410. doi:10.1016/j.jtho.2021.10.013
21. Padda SK, Aredo JV, Vali S, et al. Computational biological modeling identifies PD-(L)1 immunotherapy sensitivity among molecular subgroups of KRAS-mutated non-small-cell lung cancer. *JCO Precis Oncol*. 2021;5:153-162. doi:10.1200/po.20.00172
22. Ferrer I, Zugazagoitia J, Herbertz S, John W, Paz-Ares L, Schmid-Bindert G. KRAS-mutant non-small cell lung cancer: from biology to therapy. *Lung Cancer*. 2018;124:53-64. doi:10.1016/j.lungcan.2018.07.013
23. Lee J, Tan AC, Zhou S, et al. Clinical characteristics and outcomes in advanced KRAS-mutated NSCLC: a multicenter collaboration in Asia (ATORG-005). *JTO Clin Res Rep*. 2022;3(1):100261. doi:10.1016/j.jtocrr.2021.100261
24. Shen M, Qi R, Ren J, Lv D, Yang H. Characterization with KRAS mutant is a critical determinant in immunotherapy and other multiple therapies for non-small cell lung cancer. *Front Oncol*. 2021;11:780655. doi:10.3389/fonc.2021.780655
25. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*. 2015;373(17):1627-1639. doi:10.1056/nejmoa1507643
26. Noordhof AL, Damhuis RAM, Hendriks LEL, et al. Prognostic impact of KRAS mutation status for patients with stage IV adenocarcinoma of the lung treated with first-line pembrolizumab monotherapy. *Lung Cancer*. 2021;155:163-169. doi:10.1016/j.lungcan.2021.04.001
27. West H, Cappuzzo F, Reck M, et al. 1265P-IMpower150: a post hoc analysis of efficacy outcomes in patients with KRAS, STK11 and KEAP1 mutations [abstract]. *Ann Oncol*. 2020;31(suppl 4):S817-S818. doi:10.1016/j.annonc.2020.08.1579
28. Landre T, Justeau G, Assie JB, et al. Anti-PD-(L)1 for KRAS-mutant advanced non-small-cell lung cancers: a meta-analysis of randomized-controlled trials. *Cancer Immunol Immunother*. 2022;71(3):719-726. doi:10.1007/s00262-021-03031-1
29. Nakajima EC, Ren Y, Vallejo JJ, et al. Outcomes of first-line immune checkpoint inhibitors with or without chemotherapy according to KRAS mutational status and PD-L1 expression in patients with advanced NSCLC: FDA pooled analysis [abstract]. *J Clin Oncol*. 2022;40(16 suppl):9001. doi:10.1200/jco.2022.40.16_suppl.9001
30. Jeanson A, Tomasini P, Souquet-Bressand M, et al. Efficacy of immune checkpoint inhibitors in KRAS-mutant non-small cell lung cancer (NSCLC). *J Thorac Oncol*. 2019;14(6):1095-1101. doi:10.1016/j.jtho.2019.01.011
31. Lantuejoul S, Sound-Tsao M, Cooper WA, et al. PD-L1 testing for lung cancer in 2019: perspective from the IASLC Pathology Committee. *J Thorac Oncol*. 2020;15(4):499-519. doi:10.1016/j.jtho.2019.12.107
32. Assoun S, Theou-Anton N, Nguenang M, et al. Association of TP53 mutations with response and longer survival under immune checkpoint inhibitors in advanced non-small-cell lung cancer. *Lung Cancer*. 2019;132:65-71. doi:10.1016/j.lungcan.2019.04.005
33. Dong ZY, Zhong WZ, Zhang XC, et al. Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. *Clin Cancer Res*. 2017;23(12):3012-3024. doi:10.1158/1078-0432.ccr-16-2554
34. Koyama S, Akbay EA, Li YY, 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(5):999-1008. doi:10.1158/0008-5472.can-15-1439
35. Frost N, Kollmeier J, Vollbrecht C, et al. KRAS(G12C)/TP53 co-mutations identify long-term responders to first line palliative treatment with pembrolizumab monotherapy in PD-L1 high (≥50%) lung adenocarcinoma. *Transl Lung Cancer Res*. 2021;10(2):737-752. doi:10.21037/tlcr-20-958

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bironzo P, Cani M, Jacobs F, et al. Real-world retrospective study of KRAS mutations in advanced nonsmall cell lung cancer in the era of immunotherapy. *Cancer*. 2023;1-10. doi:10.1002/cncr.34731