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KRAS in stage IV non-small cell lung cancer

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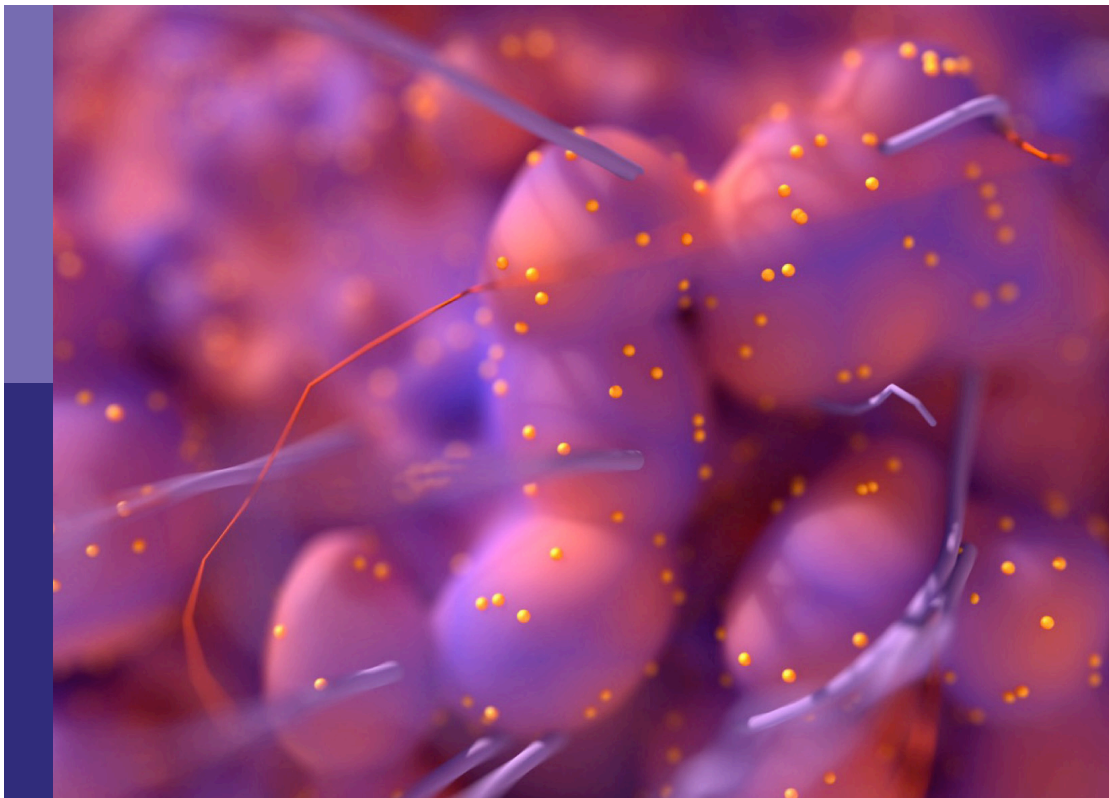

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KRAS in stage IV non-small cell lung cancer

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Editorial: *KRAS* in stage IV non-small cell lung cancer

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Editorial on the Research Topic
KRAS in stage IV non-small cell lung cancer

Despite the continuous development of drugs targeting actionable genomic alterations (AGA's), lung cancer remains the leading cause of cancer related death worldwide (1–3). In European populations, the most common AGA in stage IV non-small cell lung cancer (NSCLC), adenocarcinoma type, is the Kirsten Rat Sarcoma virus oncogene (*KRAS*) mutation (4, 5). *KRAS* was long thought to be "undruggable", but for one of the *KRAS* subtypes, the G12C mutation, targeted therapy has become available which has significantly changed the therapeutic landscape for *KRAS* G12C mutated NSCLC. However, immunotherapy with immune checkpoint inhibitors (ICI), either alone or in combination with chemotherapy, is still the current first line of treatment for all *KRAS* subtypes including G12C (6, 7).

In the second line and beyond, G12C inhibitors such as sotorasib and adagrasib have been approved for patients with *KRAS* G12C mutated NSCLC. These are small molecules that bind into a specific groove in the G12C molecule to prevent downstream signalling and cell survival. Sotorasib and adagrasib both showed overall response rates of around 40% in the CodeBreaK 100 and KRYSTAL-1 phase II studies respectively (8, 9).

In the phase III CodeBreaK 200 trial, where sotorasib was compared to docetaxel, patients receiving sotorasib had a significant longer median progression free survival (PFS) compared to those treated with docetaxel (5.6 months vs. 4.5 months). However, overall survival (OS) was not different among treatment groups (10). In the KRYSTAL-12 phase III trial comparing adagrasib to docetaxel, adagrasib also showed a significantly improved median PFS (5.5 months for adagrasib vs. 3.8 months for docetaxel), but OS data has not been reported yet (11). In both phase III-trials, all patients were previously treated with platinum-based chemotherapy and ICI.

Nevertheless, despite these recent developments, optimizing the treatment strategy of *KRAS* mutated NSCLC remains subject of interest as the outcomes with sotorasib and adagrasib are still below those achieved with targeted therapies for several other AGA's. The

combined research efforts in this Research Topic in Frontiers of Oncology "*KRAS* in stage IV non-small cell lung cancer" cover multiple aspects of important challenges in treating *KRAS* mutated NSCLC.

The prognostic role of *KRAS* has been a subject of debate both in the past when only chemotherapy was available, and also in the current immunotherapy era (12, 13). Peng et al. explored the survival of 112 patients with *KRAS* mutated NSCLC in a Chinese study. Although there were no differences in PFS, patients treated with an ICI-based regiment (± chemotherapy) had a significantly better OS than those treated with chemotherapy alone. This effect was also reported in separate *KRAS* G12C and non-G12C cohorts as well as in a subgroup harbouring a *KRAS*/*TP53* co-mutation. This Chinese single centre study may have some limitations since it did not provide any information on the PD-L1 status, yet it offered an interesting insight into the survival of patients with a *KRAS* mutation in a non-Western cohort.

Notario et al. also aims to describe the clinical outcomes of 103 patients harbouring a *KRAS* mutation treated with ICI, mostly in first or second line, either as monotherapy or in combination with chemotherapy in a Spanish monocentric cohort. In this study, PD-L1 expression was higher among patients with the G12C subtype. Better OS and PFS were observed in patients with high PD-L1 expressing tumours, regardless of *KRAS* subtype mutation.

Although the focus in this Research Topic is on patients with stage IV disease, Eklund et al. published work from a different perspective. They describe the impact of the *KRAS* mutational status in patients with stage I-II NSCLC treated in a Swedish centre. The vast majority of these patients received surgical resection of their tumour. Of interest, although *KRAS* mutational status did not have a significant impact on OS, the authors reported a shorter OS in patients with a *KRAS* mutation: the mean (median not reached) OS was 63 months for patients with a *KRAS* mutation versus 74 months for patients without. The G12C mutation patients had a similar prognosis compared to those with non-G12C *KRAS* mutations. Since there were only 113 patients with a *KRAS* mutation in this study, larger studies are needed to establish the prognostic role of *KRAS* in this patient category.

KRAS is a heterogeneous disease, where multiple co-mutations can co-occur and may affect clinical outcomes. On this ground, Frille et al. advocate for extensive predictive testing including broad panels for mutation analysis to better estimate the prognosis and treatment options for patients with advanced NSCLC. They present the survival of more than 4000 patients with different mutations: *KRAS*, *STK11*, *KEAP1* and *TP53*, either alone or in complex combinations. Patients with a *KRAS*-only mutation, or with a combination of *KRAS* + *STK11* had the longest OS. The *TP53*-comutation showed a negative influence on *KRAS* mutated NSCLC, as in this group the OS was significantly reduced by more than 30%.

The narrative review of Sreter et al. offers a detailed overview regarding the molecular basis, the role of co-mutations and an overview of clinical evidence for *KRAS* inhibition with sotorasib and adagrasib. Moreover, they offer a review of literature of intracranial responses with these two G12C-inhibitors, and they propose mechanisms of acquired resistance to G12C-inhibitors and future strategies to overcome them.

Chour et al. provide a review in which they summarize the *KRAS* pathway and the mechanism of sotorasib and adagrasib, that both bind to the inactive GDP-bound state of *KRAS*. The also discuss mechanism of resistance to these G12C-inhibitors, either primary resistance, i.e. new co-occurring mutations that prevent the binding of inhibitors, or acquired resistance, for example gain-of-function mutations in other oncogenes and thereby bypassing *KRAS*.

Current guidelines advise testing for PD-L1 status and molecular testing for AGA in patients with metastatic NSCLC (6). However, obtaining histology or cytology samples can be challenging. Cai et al. preformed a systematic review and meta-analysis to investigate the diagnostic accuracy of *KRAS* detection in plasma samples. Plasma NGS could be a suitable alternative when tissue samples are not available as it detects *KRAS* with high accuracy.

Since G12C inhibitors are now available in the second line treatment setting and beyond, managing toxicity and optimal dosing of sotorasib remains a challenge. Shigaki et al. explored the possible relation between blood sotorasib levels and therapeutic outcomes and adverse event in five patients treated with sotorasib but found no association. Nevertheless, this is an interesting concept for further evaluation in a larger number of patients and it could possibly offer insights into more personalized dosing strategies in the future.

The combined efforts of these studies published in this Research Topic have contributed in decreasing the knowledge gap on how to optimize treatment strategies for patients with *KRAS* mutated NSCLC. The treatment landscape is anticipated to change further with the development of inhibitors of other *KRAS* mutational subtypes and pan-*KRAS* inhibitors (14). Ongoing research in the acquired resistance to G12-inhibitors and their administration in combination with other types of therapy could further change the treatment landscape, but additional research is needed in these areas.

Author contributions

AN: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. TB: Methodology, Supervision, Writing – review & editing. GH: Methodology, Supervision, Writing – review & editing. LH: Methodology, Supervision, Writing – review & editing. WvG: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Efficacy of immunotherapy in *KRAS*-mutant advanced NSCLC: A real-world study in a Chinese population

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Background: Immunotherapy has improved the clinical outcomes of patients with advanced non-small cell lung cancer (NSCLC). However, in patients with Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations, the superior efficacy of immunotherapy has not been elucidated and especially in real-world practice. Our study aimed to use real-world data to assess the efficacy of immunotherapy in *KRAS*-mutant NSCLC in a Chinese cohort.

Methods: In this retrospective cohort study, we extracted the clinical, molecular, and pathologic data from the electronic health records of patients with advanced *KRAS*-mutant NSCLC at Shandong Cancer Hospital between January 2018 and May 2022. Furthermore, we evaluated the progression-free survival (PFS) and overall survival (OS) of the included patients.

Results: Between January 2018 and November 2020, 793 patients were identified with stage IIIB-IV NSCLC and a total of 122 patients with *KRAS* mutations were included in the analysis. The majority of patients were diagnosed with stage IV (82.0%) adenocarcinoma (93.4%), along with a history of smoking (57.4%). Of these, 42% of patients received anti-PD-(L)1 with or without chemotherapy (Immunotherapy-based regimens), while 58.2% of patients received chemotherapy (Chemotherapy-based regimens). The median overall survival (mOS) in this cohort was 22.9 months (95% CI: 14.1–31.7), while the median-progression-free survival (mPFS) was 9.4 months (95% CI: 6.6–12.1). Patients receiving immunotherapy-based regimens displayed better mOS than those receiving chemotherapy-based regimens (45.2 vs. 11.3 months; P=1.81E-05), with no statistical difference observed in the mPFS (10.5 vs. 8.2 months; P=0.706). Patients receiving immunotherapy-based regimens either in the first line (P=0.00038, P=0.010, respectively) or second-line setting (P=0.010, P=0.026, respectively) showed benefits in both PFS and OS. Subgroup analysis indicated that in patients having *KRAS* G12C or non-*KRAS* G12C mutant types, immunotherapy showed benefits of better OS (P=0.0037, P=0.020, respectively) than chemotherapy. Moreover, in advanced NSCLCs patients with or without *KRAS*/

TP53 co-mutation the immunotherapy-based regimen achieved longer OS and PFS than chemotherapy-based regimens.

Conclusions: In the Chinese population of patients with *KRAS*-mutant advanced NSCLC, immunotherapy-based regimens achieved longer OS than chemotherapy-based regimens, which was independent of first or second-line setting, as well as *KRAS* mutational subtypes.

KEYWORDS

NSCLC, *KRAS*, immunotherapy, co-mutation, *KRAS*-mutant subtypes

1 Introduction

Non-small cell lung cancer (NSCLC) remains one of the major causes of cancer-related deaths in China and worldwide (1). The most common oncogenic driver in NSCLC is the mutation of Kirsten rat sarcoma viral oncogene homolog (*KRAS*), exhibiting approximately 20–30% prevalence among Western countries and 10–15% among Asian countries (2). *KRAS* mutant NSCLC is considered a heterogeneous disease regarding *KRAS* mutant subtypes, co-mutations (3), and immunogenic profiles (4). Biological heterogeneity is suggested to play a role in the vulnerability to therapy, tumor microenvironment, and immune modulatory effects. For instance, patients with *KRAS/TP53* co-mutations were reported to be sensitive to immunotherapy (Objective Response Rate[ORR]: 35.7%), while patients with *KRAS/STK11* displayed poorer outcomes upon treatment with immunotherapy (ORR: 7.4%) (5). However, a retrospective study showed that *KRAS*-mutant NSCLC might benefit from chemo-immunotherapy (6). *KRAS* has long been considered 'undruggable' (7), and the management of *KRAS*-addicted lung cancer is considered the same as that of non-oncogene-addicted cancer (8). Furthermore, limited treatment options and high heterogeneity may increase the difficulties of managing advanced *KRAS*-mutant patients.

Research on optimal management of *KRAS*-mutant NSCLC is still in progress. However, a breakthrough was achieved in the treatment landscape when the US Food and Drug Administration (FDA) approved direct *KRAS* G12C inhibitor Sotorasib for advanced or metastatic NSCLC adult patients having *KRAS* G12C local mutation, with patients receiving one prior systemic therapy. Immunotherapy is considered promising cancer therapy. Although most oncogene-addicted tumors, including *EGFR*-or *ALK*-driven lung cancer, do not respond to immunotherapy (9), even at >50% of PD-L1 expression. However, this is not the case in *KRAS* mutant NSCLC. The response rate to immunotherapy in such patients is shown to be at least the same or even better than that of *KRAS*-wild type patients (10–13). Few studies have also confirmed the superior efficacy of immunotherapy over chemotherapy in the *KRAS*-mutant NSCLC population. For instance, in one meta-analysis including three clinical trials, Kim et al. showed the superior efficacy of immunotherapy over chemotherapy in *KRAS*-mutant patients in the second-line setting (14). Similarly, a recent meta–analysis including six randomized controlled trials with 386 *KRAS*-mutant NSCLC patients suggested that anti-PD-(L)1 with or without chemotherapy displayed a significant association with prolonged OS (HR=0.59, 95%CI: 0.49–0.72; P<0.00001) and PFS (HR=0.58, 95%CI:0.43–0.78; *P*=0.0003) compared to chemotherapy alone (15).

However, since these findings were from the subgroup analysis of clinical studies, validating them in a real-world setting was necessary. Therefore, we conducted a real-world study in a Chinese population to verify the efficacy of immunotherapy with or without chemotherapy in *KRAS*-mutated advanced NSCLC patients.

2 Methods

2.1 Study design and data source

The data for this retrospective observational cohort analysis was extracted from the electronic health records of patients at Shandong First Medical University Cancer Hospital and Shandong Cancer Hospital. The study was approved by the Ethics Committee of Shandong First Medical University Cancer Hospital and Shandong Cancer Hospital. Between January 2018 and November 2020, the patient records with stage IIIB-IV NSCLC were included in the study. The cohort used in this study was based on 793 patients. The above-mentioned clinical information mainly included baseline characteristics (sex, age, smoking status, histological subtype, ECOG PS, and tumor stage), *KRAS* mutation status, and treatment history of the patients. Furthermore, the patients were followed up from the date of diagnosis till the date of death due to all causes or up to the latest available follow-up.

2.2 Cohort selection

Initially, patients included in the cohort met the following inclusion criteria: Age 18 years or older; diagnosed with stage IIIB to stage IV NSCLC with evidence of mutation in *KRAS*; receiving treatments from diagnosis to the end of follow-up. The exclusion criteria included records with no adequate information of pathological diagnosis, evidence of mutation in *EGFR* or *ALK* gene arrangement and *ROS1* translocation, and records of EGFR TKIs treatment. The chemotherapy-based regimen was defined as the non-addition of anti-PD(L) 1 in the management of patients during the period of treatment.

2.3 Therapeutic regimens

Of the 51 immunotherapy-based patients, 6 received ICI monotherapy and 45 received ICI combination therapy with the following regimens: monotherapy: sintilimab, pembrolizumab, tislelizumab, and camrelizumab; combination therapy: sintilimab plus pemetrexed/platinum-based, sintilimab plus nab-paclitaxel/platinum-based, sintilimab plus docetaxel, pembrolizumab plus pemetrexed/platinum-based, tislelizumab plus pemetrexed/platinum-based, atelelizumab plus nab-paclitaxel/platinum-based, atelelizumab combined with bevacizumab and paclitaxel and platinum-based, toripalizumab combined with pemetrexed/platinum-based. Of the 71 patients treated with chemotherapy received the following conventional chemotherapy regimens: pemetrexed plus carboplatin or cisplatin, paclitaxel plus carboplatin or cisplatin, docetaxel plus carboplatin or cisplatin, gemcitabine plus carboplatin or cisplatin, bevacizumab combined with pemetrexed/platinum-based or paclitaxel/platinum-based.

Among the 122 patients, 24 patients were treated with first-line immunotherapy, 98 patients were treated with first-line chemotherapy, 21 patients were treated with second-line immunotherapy, and 26 patients were treated with second-line chemotherapy.

2.4 Study endpoints

The primary endpoint was OS, which was defined as the period starting from the diagnosis till death or the date of the last follow-up. The secondary endpoint was real-world progression-free survival (rwPFS), defined as the time from diagnosis until objective tumor progression or death, whichever occurs first. Our study used a clinician-anchored approach supported by radiology data. Based on the radiology scan and pathologic confirmation *via* tissue biopsy or through clinical assessment, the clinician-recorded assessment was used to determine disease progression. Patients with missing information regarding the date of the last clinical note and progression were excluded from the rwPFS analysis.

2.5 Molecular profiling

Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used to identify *KRAS* mutation status. Genomic alterations were detected in patient samples using targeted sequencing panels (BerryOncology, Beijing), including a 456-gene (BerryOncology, Beijing) and a 36-gene test panel (BerryOncology, Beijing).

2.6 Statistical analysis

Standard descriptive statistics were used to compare the cohort characteristics between the chemotherapy- and immunotherapy-based regimen groups. The Fisher's exact test and Mann-Whitney Wilcoxon test were used to compare the differences among variables of both groups, which included age, gender, smoking history, clinical stage, *KRAS* mutation subtype, *KRAS* gene co-mutation, distant metastasis, and the presence or absence of radiotherapy. Kaplan–Meier analysis was performed to estimate the survival rate, while the log-rank test was performed to test the differences in survival distribution among the subgroups. Moreover, the Cox proportional hazard regression model was used for univariate analyses. All statistical analyses were performed using the SPSS version 23.0, IBM software. The difference was considered statistically significant if the *P*-value was less than 0.05.

3 Results

3.1 Clinical characteristics

Of 632 patients with available gene test results, *KRAS* mutation was identified in a total of 142 advanced NSCLC patients. Among them, 20 patients did not receive any treatment at our hospital. Hence, we finally included 122 patients in our retrospective analysis, as shown in Figure 1, whose detailed clinical characteristics are summarized in Table 1. The cohort comprised 100 (82.0%) males and 22 (18.0%) females having an average age of 62 years. The major histological subtype included adenocarcinoma (n=114, 93.4%). Of these, 100 (82.0%) patients had recurrent or stage IV disease at the time of diagnosis. Additionally, 57.4% of the patients had a history of smoking. Finally, all patients were treated based on their clinical staging status. Our results showed no significant differences in clinical characteristics, except for anti-angiogenesis therapy (P=0.03)

3.2 Immunotherapy-based regimens improved the survival outcomes of *KRAS*-mutant advanced NSCLC patients in both first-line and second-line settings

Our study showed the median overall survival (mOS) of *KRAS*-mutant advanced NSCLC patients as 22.9 months (95% CI: 14.07–31.67) and the median progression-free survival (mPFS) as 9.4 months (95% CI: 6.60–12.14) (Figures 2A, B). While 51 (41.8%) patients received immunotherapy-based regimens, 71 (58.2%) received chemotherapy-based regimens (Table 1). Patients receiving immunotherapy-based regimens displayed significantly longer mOS compared to patients receiving chemotherapy-based regimens (45.2 vs. 11.3 months; P=1.81E-5), with no significant difference observed in the mPFS (10.5 vs. 8.2 months; P=0.706) (Figures 2C, D). Additionally, immunotherapy from both first-and second-line treatments showed survival benefits. Patients receiving immunotherapy-based regimens as the first line of treatment displayed better mOS and mPFS than those receiving chemotherapy-based regimens (mOS: 33.5 vs. 16.1 months; P=0.010, mPFS: 32.2 vs. 6.9 months; P=0.00038) (Figures 3A, B). Similarly, the patients receiving immunotherapy as the second line of treatment also displayed significant improvement in the mOS and mPFS compared to those receiving chemotherapy (mOS: NR vs. 9.23 months; P=0.026, mPFS: 10.8 vs. 5.5 months; P=0.010) (Figures 3C, D).

3.3 Efficacy of immunotherapy in *KRAS* G12C and *KRAS* non-G12C subgroups

Since specific *KRAS* mutational subtypes may exert different effects on treatment response and survival, we aimed to characterize the

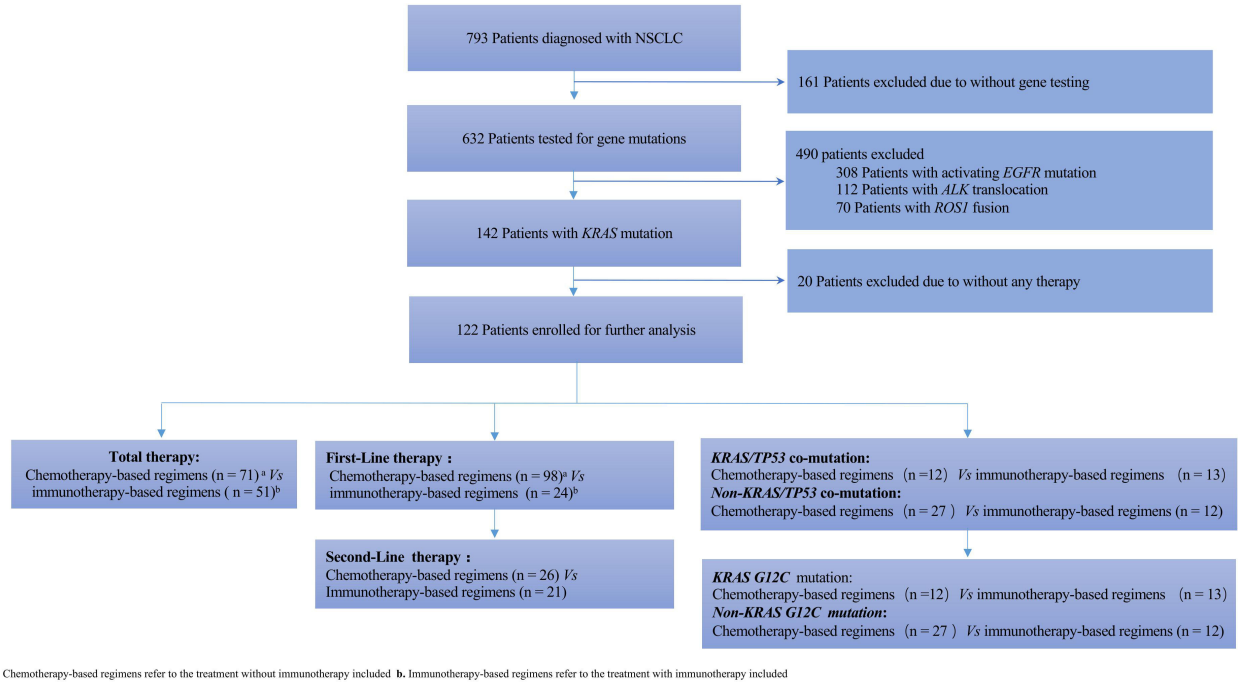

FIGURE 1
Flow chart depicting patient selection. NSCLC, non-small cell lung cancer.

effects of *KRAS* mutation subtypes on the OS and treatment response of these patients. With the available information on mutation revealed by molecular characterization, we stratified the patients into *KRAS* G12C and *KRAS* non-G12C subgroups. Genomic profiles of 64 *KRAS* mutant patients were analyzed using next-generation sequencing (Berryoncology, Beijing), which detected two major mutation subtypes, including G12C (20.5%) and non-G12C (32.0%). The G12C status was unknown for 47.5% of the patients. Among the four different categories of *KRAS*-mutant NSCLCs, significant differences were observed in both mOS and mPFS (mOS: log-rank test, *P*=0.00020; mPFS: log-rank test, *P*=0.026) (Figures 4A, B). Further analysis revealed that *KRAS* G12C and non-G12C subtype patients treated with immunotherapy-based regimens showed significantly better mOS compared to the same patients receiving chemotherapy-based regimens (G12C group HR=0.23,95%CI:0.08-0.67, *P*=0.0074; mOS: 25.2 vs. 9.1 months, *P*=0.0037; non-G12C group HR=0.13,95% CI:0.02-0.99, *P*=0.049; mOS: NR vs. 25.7 months, *P*=0.020). However, significant difference for PFS was observed in G12C group but not in non-G12C group (G12C group HR=0.38,95%CI:0.14-0.99, *P*=0.047; mPFS: 12.1 vs. 5.0 months, *P*=0.039; non-G12C group HR=0.73,95% CI:0.3-1.75, *P*=0.48; mPFS: 14.8 vs. 10.3 months, *P*=0.48).

3.4 The impact of concurrent pathogenic mutations *KRAS/TP53* on the efficacy of immunotherapy

Several studies (16–18) have indicated that under immunotherapy, the co-mutation status of advanced *KRAS*-mutant type exerts an impact on the patient's clinical outcomes. Based on the co-mutation status, we used the NGS results of 64 patients for survival analysis. The identified co-mutations included *TP53* (20.3%), *PIK3CA* (1.6%), and *STK11* (0.8%). Kaplan-Meier curves based on *TP53* mutation status and treatment group showed a significant difference in mOS (*P*=0.035) (Figure 5A) but not in mPFS (*P* = 0.41) (Figure 5B). Further analysis suggested that *KRAS/TP53* co-mutation group and non- *KRAS/TP53* mutation group patients treated with immunotherapy-based regimens showed significantly better mOS compared to the same patients receiving chemotherapy-based regimens (*KRAS/TP53* co-mutation group HR=0.32, 95%CI:0.1-0.98, *P*=0.047; mOS:33.5 vs. 11.8 months, *P*=0.036; non-*KRAS/TP53* co-mutation group HR=0.23,95%CI:0.05-0.99, *P*=0.049; mOS: NA vs. 16 months, *P*=0.031). However, no significant difference was observed in the mPFS (*KRAS/TP53* co-mutation group HR=0.78,95%CI:0.31-1.96, *P*=0.59; mPFS:12.5 vs.10.0 months, *P*=0.59; non- *KRAS/TP53* co-mutation group HR=0.49,95% CI:0.2-1.23, *P*=0.13; mPFS: 16.9 vs. 6.7 months, *P*=0.12).

4 Discussion

KRAS-mutant NSCLC is a genetically heterogeneous disease with distinct biology and therapeutic vulnerabilities. An effective choice of treatment for this disease is immunotherapy. However, further investigation, especially in real-world settings, may be required to verify the efficacy of immunotherapy in *KRAS*-mutant NSCLC patients. Therefore, we retrospectively studied 122 advanced NSCLC patients with *KRAS* mutations for their prognosis and obtained the mOS at 22.9 months (Figure 2B). This result was similar to a previous study, where mOS was 28 months (19). Furthermore, the mOS was 25.8 months in the study of El Osta., et al, which was similar to our study (20). In our study, patients receiving immunotherapy-based regimes displayed a significantly

TABLE 1 The characteristics of KRAS-mutant NSCLC patient.

Characteristics	All N = 122 (%)	Immunotherapy-based regimens N =51 (%)	Chemotherapy-based regimens N = 71 (%)	*P-value*
Gender				0.35
Male	100 (82.0)	44 (86.3)	56 (78.9)	
Female	22 (18.0)	7 (13.7)	15 (21.1)	
Age				0.55
<60	38 (31.1)	14 (27.5)	24 (33.8)	
≥60	84 (68.9)	37 (72.5)	47 (66.2)	
Smoking history	–	–	–	0.50
Smoker	70 (57.4)	27 (52.9)	43 (60.6)	
Never smoked	52 (42.6)	24 (47.1)	28 (39.4)	
Histological subtype				0.72
Adenocarcinoma	114 (93.4)	48 (94.1)	66 (93.0)	
Squamous	3 (2.5)	2 (3.9)	1 (1.4)	
Adenosquamous	3 (2.5)	1 (2.0)	2 (2.8)	
Other	2 (1.6)	0 (0)	2 (2.8)	
ECOG PS				0.21
0~1	103 (84.4)	46 (90.2)	57 (80.3)	
2	19 (15.6)	5 (9.8)	14 (19.7)	
Staging				0.64
IIIB/IIIC	22 (18.0)	8 (15.7)	14 (19.7)	
IV	100 (82.0)	43 (84.3)	57 (80.3)	
KRAS mutant				0.20
G12C	25 (20.5)	13(25.5)	12 (16.9)	–
Non-G12C	39 (32.0)	12 (23.5)	27 (38.0)	
Unknown	58 (47.5)	26 (51.0)	32 (45.1)	
Co-mutations				0.24
KRAS/TP53	25 (20.5)	12 (23.5)	13 (18.3)	
NonKRAS/TP53	39 (32.0)	12 (23.5)	27 (38.0)	
Unknown	58 (47.5)	27 (53.0)	31 (43.7)	
Metastatic sites				0.15
Brain	30 (24.6)	17 (33.3)	13 (18.3)	
Liver	5 (4.1)	0 (0)	5 (7.1)	
Bone	27 (22.1)	10 (19.6)	17 (23.9)	
Other sites	35 (28.7)	13 (25.5)	22 (31.0)	
None	25 (20.5)	11(21.6)	14(19.7)	
Radiotherapy				0.14
Yes	66(54.1)	32 (62.7)	34 (47.9)	
No	56 (45.9)	19 (37.3)	37 (52.1)	
Anti-angiogenesis therapy				
Yes	57 (46.7)	30 (58.8)	27 (38.0)	0.03
No	65 (53.3)	21 (41.2)	44 (62.0)	

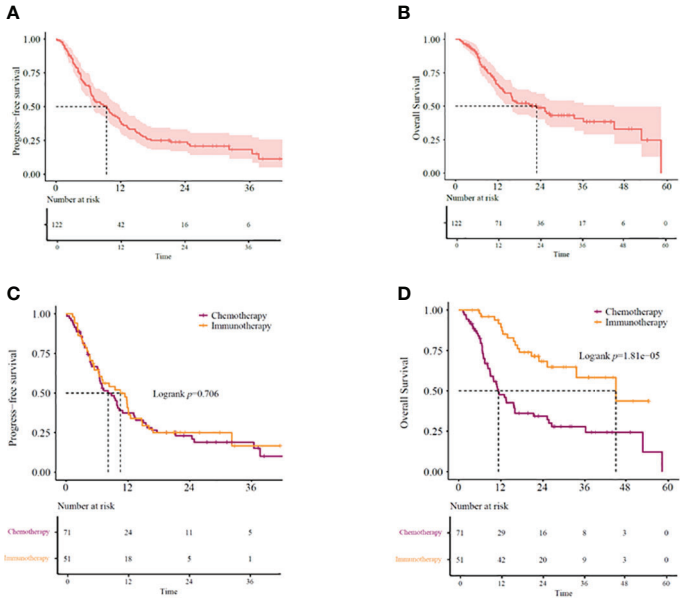

FIGURE 2
(A) PFS and **(B)** OS in *KRAS*-mutant advanced NSCLC patients. **(C)** PFS and **(D)** OS in *KRAS*-mutant advanced NSCLC patients receiving immunotherapy- or chemotherapy-based regimens. OS, overall survival; PFS, progression-free survival.

longer OS than those receiving chemotherapy-based regimens (45.2 vs. 11.3 months, *P*=0.001) (Figure 2D). Moreover, the survival benefits were independent of whether it was the first-line setting or second-line setting, which was also consistent with the subgroup analysis results of previous clinical trials (15). In addition, outcomes of the KEYNOTE189 shows that patients receiving immunotherapy plus chemotherapy have longer mPFS than those receiving chemotherapy (9 vs. 5 months, HR=0.47,95%CI [0.29-0.77]) in *KRAS* -mutated lung cancer (21). In the 2022 ASCO meeting, data scientists from the FDA conducted a large retrospective analysis, including 555 metastatic NSCLC patients with *KRAS* mutations. Their analysis concluded that the chemo-immune checkpoint inhibitor combination produced the greatest survival benefit compared to the treatment with immune checkpoint inhibitors

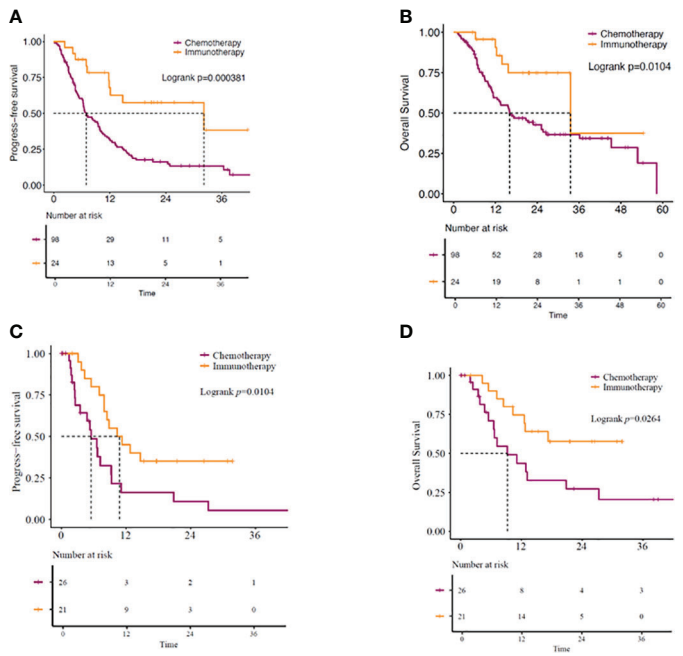

FIGURE 3
(A) PFS and **(B)** OS in *KRAS*-mutant advanced NSCLC patients. Patients receiving immunotherapy- or chemotherapy-based regimens as first-line of treatment. **(C)** PFS and **(D)** OS in *KRAS*-mutant advanced NSCLC patients receiving immunotherapy- or chemotherapy-based regimens as second-line of treatment. OS, overall survival; PFS, progression-free survival.

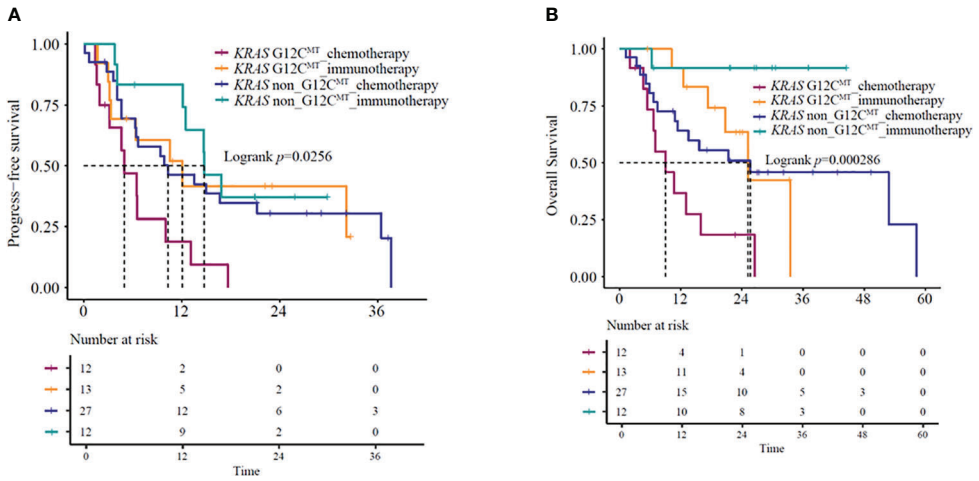

FIGURE 4
(A) PFS and **(B)** OS in *KRAS* G12C-mutant and *KRAS* non-G12C mutant NSCLC patients receiving immunotherapy- or chemotherapy-based regimens. OS, overall survival; PFS, progression-free survival.

(ICIs) or chemotherapy alone and hence, should be given to such patients upfront (22). Specifically, chemo-ICIs as the first line of treatment were linked to a response rate of 46%, while ICI alone generated a response rate of 37%, indicating that chemo-immunotherapy may be the optimal management option for the advanced *KRAS*-mutant NSCLC patients both in white and Asian populations.

The enhanced survival benefits in this study can be explained using several biological rationales. *KRAS* mutations in NSCLC were associated with tobacco smoking, a high tumor mutational burden (TMB), and an inflammatory tumor microenvironment, along with high T-cell infiltration (23). Importantly, compared to the wild-type counterparts, *KRAS*-mutant tumors showed higher expression of PD-L1, with the median PD-L1 tumor proportion scores ranging between 30–60% and 5–35% in patients with and without *KRAS* mutations, respectively (21, 24). One study suggested that the activation of the *KRAS*-signaling pathway resulted in the inhibition of tristetraprolin activity, which is important for the stabilization of PD-L1-mRNA and, thus, its synthesis (25). Another study showed that *KRAS* mutations were correlated to an inflammatory tumor microenvironment and tumor immunogenicity, which benefitted the response to ICIs (23). Since *KRAS*-mutated NSCLC is typically smoking-related lung cancer, with more than 90% of patients having a history of smoking, it is more likely that such patients will respond to ICI treatment.

Notably, the patients treated with a combination of anti-PD(L)1 and chemotherapy (immunotherapy-based regimens) showed an mOS of 45 months, which was longer than most previous studies (21, 26). This may be because the Eastern Cooperative Oncology Group performance score (ECOG PS) of the patients was between 1 and 2. The value of ECOG PS was 0~1 in 84.4% of patients and 2 in 15.6% of patients. Multiple retrospective cohort studies across different tumor types have suggested that patients with ECOG PS ≥2 showed worse response rates, faster progression, and shorter OS

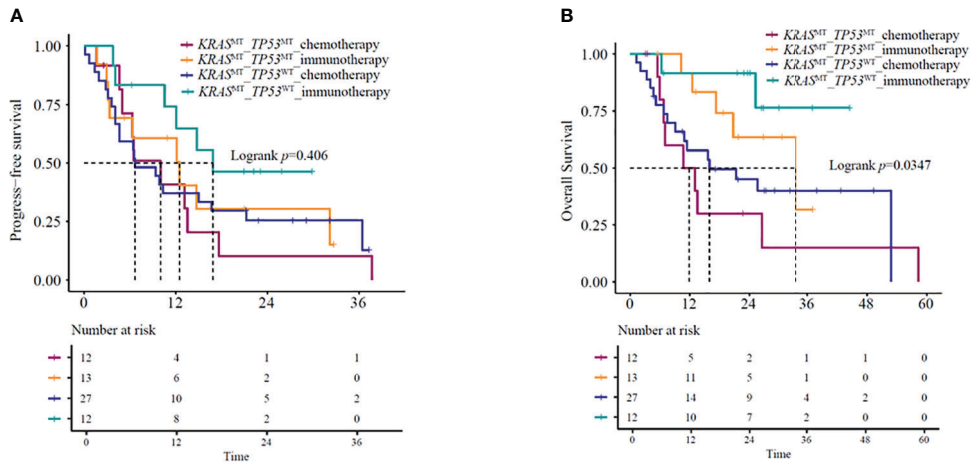

FIGURE 5
(A) PFS and **(B)** OS in *KRAS/TP53* co-mutation or *KRAS* mutant/*TP53* wild-type patients receiving immunotherapy- or chemotherapy-based regimens. OS, overall survival; PFS, progression-free survival.

(27–29). Additionally, a recent study showed that mOS of advanced NSCLC patients with good performance status was 30 months (95% CI 16.6–42.3), but in patients with poor performance status, it was only 4 months (95% CI 3.2–8.1) (30), which was similar to our results.

No significant difference was observed in PFS between immunotherapy and chemotherapy. Studies suggested no correlation between the mOS and mPFS (31, 32) in immunotherapy-related clinical trials. Moreover, in randomized clinical trials of PD-1 inhibitors, the effect of treatment was higher on OS than on PFS (31), which was consistent with our results. This suggested that PFS may not be able to capture the benefits of immune checkpoint inhibitors. PD-1 inhibitors have residual efficacy for a longer duration, and even after the discontinuation of treatment, these drugs could affect OS more than PFS. Therefore, the RECIST criteria may not be completely suitable to measure the immunotherapy response.

Previous studies demonstrated that *KRAS* G12C mutations and *TP53* co-mutations were correlated to benefits obtained from anti-PD-1/PD-L1 immunotherapy (18). Similar results were also found in this study, where patients with *KRAS*-G12C mutation receiving immunotherapy with or without chemotherapy achieved more survival benefits than chemotherapy alone. This indicated the significant role of immunotherapy in the clinical management of these patients. The combination strategy may abolish the adverse OS impact of the *KRAS* G12C mutant. A preclinical study suggested that *KRAS* G12C inhibition can swiftly change the tumor's immune-suppressive microenvironment to the one that allows effective anti-tumor immunity (33). In addition, a phase 2 trial results of sotorasib for lung cancers with *KRAS* G12C mutation showed that the mPFS was 6.8 months (95% CI, 5.1 to 8.2) and the mOS was 12.5 months (95% CI, 10.0 to could not be evaluated) (34).Due to a higher level of PD-L1 expression, T cell infiltration, and tumor immunogenicity, the *KRAS/TP53* co-mutation in NSCLC exhibited sensitivity to anti-PD-1/PD-L1 immunotherapy (17). In our study, the advanced NSCLCs patients with or without *KRAS/TP53* co-mutation benefitted more from the immunotherapy-based regimens than chemotherapy-based regimens in mOS ($KRAS^{MT}TP53^{WT}$ mOS: P=0.36; $KRAS^{MT}TP53^{WT}$ mOS: P=0.049). Furthermore, no significant differences were observed in mPFS between immunotherapy receiving $KRAS^{MT}TP53^{MT}$ and $KRAS^{MT}TP53^{WT}$ patients ($KRAS^{MT}TP53^{MT}$ mPFS: P=0.59; $KRAS^{MT}TP53^{WT}$ mPFS: P=0.12), which may be due to the small size of our study sample. Hence, this aspect may require further investigation.

4.1 Limitations

The first limitation of our study was the insufficient characterization of the genomic profiles of the patients, with ARMS-PCR being applied to only nearly half of the patients. Also, performing survival analyses in subgroups based on *KRAS*-mutation and co-mutation status was challenging. Second, since heterogeneous patients with various levels of PD-L1 expression and TMB status, *KRAS* mutation status may have affected the survival outcomes of ICIs differently as per the expression level of PD-L1. For example, patients with high PD-L1 levels receiving immunotherapy as the first line of treatment may have fared as well as those who received chemo-immunotherapy (35). Also, these levels were only available in a small proportion of patients. Hence, whether the superior efficacy of ICIs observed in this study was independent of TMB status and/or PD-L1 expression remains unknown. Thirdly, our study was a single-center study and not fully representative of the broader population of cancer patients in China, which in some cases, may limit the generalizability of the obtained data. Therefore, to make informed clinical decisions, further studies may be needed to provide sufficient evidence.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Shandong First Medical University Cancer Hospital and Shandong Cancer Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZHW and XH designed the research and revised the article. LXP and JG analyzed the data and wrote the manuscript. ZZL analyzed the data. LK, YH, NT, JGZ and MLW collected the data. XHH and YGP revised the article. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author YGP, XHH, and ZZL were employed by Berry Oncology Corporation.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Detection of *KRAS* mutation using plasma samples in non-small-cell lung cancer: a systematic review and meta-analysis

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Background: The aim of this study was to investigate the diagnostic accuracy of KRAS mutation detection using plasma sample of patients with non-small cell lung cancer (NSCLC).

Methods: Databases of Pubmed, Embase, Cochrane Library, and Web of Science were searched for studies detecting KRAS mutation in paired tissue and plasma samples of patients with NSCLC. Data were extracted from each eligible study and analyzed using MetaDiSc and STATA.

Results: After database searching and screening of the studies with pre-defined criteria, 43 eligible studies were identified and relevant data were extracted. After pooling the accuracy data from 3341 patients, the pooled sensitivity, specificity and diagnostic odds ratio were 71%, 94%, and 59.28, respectively. Area under curve of summary receiver operating characteristic curve was 0.8883. Subgroup analysis revealed that next-generation sequencing outperformed PCR-based techniques in detecting *KRAS* mutation using plasma sample of patients with NSCLC, with sensitivity, specificity, and diagnostic odds ratio of 73%, 94%, and 82.60, respectively.

Conclusion: Compared to paired tumor tissue sample, plasma sample showed overall good performance in detecting KRAS mutation in patients with NSCLC, which could serve as good surrogate when tissue samples are not available.

KEYWORDS

KRAS, plasma, non-small cell lung cancer, diagnostic accuracy, meta-analysis

1 Introduction

Lung cancer is a leading cause of cancer-related death worldwide (1). As its most prevalent subtype, non-small cell lung cancer (NSCLC) represents approximately 85% of lung cancer cases (2). Treatments of NSCLC include surgery, radiotherapy, chemotherapy, immunotherapy, and targeted therapy in tumors harboring certain oncogenetic variations, e.g., anti-epidermal growth factor receptor (EGFR) therapy (2).

Kirsten rat sarcoma viral oncogene homologue (*KRAS*) is the most frequently mutated oncogene in many types of cancer (3), with an overall prevalence of 27.5% in NSCLC (4). Mutation of *KRAS* gene is associated with resistance to anti-EGFR therapies (5–7). In addition, although KRAS was thought to be an "undruggable" target, it has become "druggable" after the successful approval of KRAS (G12C) inhibitor (Sotorasib) for the treatment of *KRAS* G12C-mutated metastatic NSCLC (8). Due to these important roles of *KRAS* mutation in targeted therapies, accurate detection of *KRAS* gene mutations, especially G12C, is crucial for the success of anti-EGFR therapies and KRAS inhibitors.

The detection of *KRAS* mutations in tumors is usually performed using tumor tissue samples, e.g., formalin-fixed paraffin-embedded (FFPE) tumor tissue samples. However, tissue samples are sometimes not available, or may not reflect the real-time mutation status of tumor due to the existence of cancer evolution (9). Research efforts were therefore made to find possible surrogates for tumor tissue samples, which are mainly cell-free DNA (cfDNA)-containing samples, such as plasma, urine, saliva, feces, exhaled breath condensate, and etc (10, 11). Before their clinical application, however, those surrogate sample types needs to be validated for their accuracy performance in detecting *KRAS* mutations. Many such studies have been conducted. A recently-published systemic review and meta-analysis by Palmieri (12) summarized the results of 40 relevant studies and reported an overall adequate accuracy of cfDNA-containing samples. This meta-analysis by Palmieri focused on cfDNA, and involved studies using plasma, urine, or sputum samples. However, cfDNA levels in the three sample types are quite different, which could potentially influence accuracy performance. In addition, compared to urine or sputum samples which could be highly concentrated or diluted, cfDNA levels in plasma samples are considered to be more stable and therefore had potentially better stability in accuracy performance. Considering these advantages, we chose to focus on plasma, and aimed to better understand the accuracy performance of plasma sample in *KRAS* mutation detection in NSCLC, including potential impact of patient characteristics.

2 Materials and methods

2.1 Literature searching and selection of publication

Literature search was performed by BY and JZ in June 2022. Online literature databases (Pubmed, Embase, Cochrane Library, and Web of Science) were searched using keywords: "KRAS", "plasma", and "NSCLC". Alternative spelling or abbreviations were also included in the literature search, e.g., non-small-cell lung cancer, non-small-cell lung carcinoma, NSCLCs, NSCLC's, plasmas, and plasma's (please see detailed searching strategy in Supplementary Material). Searching results were exported from each database. Duplicated literatures were then identified by matching titles, names of first author, or identification numbers (e.g., Pubmed ID) of literatures from different databases. After removing the duplicated literatures, the abstracts of the searching results were firstly screened to exclude irrelevant literatures. The full texts of the rest literatures were then downloaded and screened for eligible studies. The criteria used for the two screening steps were as follows. Inclusion criteria: all original studies testing *KRAS* mutation in paired plasma and tumor tissue samples of NSCLC. Exclusion criteria: 1) not a human study; 2) missing plasma or tumor tissue samples; 3) plasma and tumor tissue samples were not paired; 4) not testing *KRAS* mutation in either plasma or tissue samples; 5) lacking *KRAS* wild-type or *KRAS* mutated samples; 6) not an original study; 7) un-interpretable data; 8) not NSCLC samples. Accuracy data were then extracted from the *KRAS* mutation testing results of paired plasma and tumor tissue samples in the eligible studies, including numbers of true positive, false positive, false negative, and true negative. In addition, characteristics of patients or techniques were also extracted, including region and population of studies, tumor stage, and techniques used to test *KRAS* mutation in plasma and in tissue samples. All the eligible studies were evaluated by quality assessment of diagnostic accuracy studies 2 (QUADAS-2) (13). Any disagreement between the two investigators (BY and JZ) were solved by a third investigator (PC). PRISMA 2009 Checklist is included in Supplementary Material.

2.2 Statistical analysis

Statistical analysis was performed using Meta-DiSc 1.4 (14) and STATA 12.0 (STATA Corp.). Sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and area under curve (AUC) of summary receiver operating characteristic (SROC) curve were pooled from the accuracy data extracted from the eligible studies. During the pooling, random effects model was used when significant heterogeneity was observed ($I^2 \geq 50\%$ and $P < 0.05$), and fixed effects model was used when no significant heterogeneity was observed (14). In case of significant heterogeneity, threshold analysis and meta-regression were performed to find its possible sources. Deek's funnel plot asymmetry test was performed to find potential publication bias in the eligible studies. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Search results

As shown in Figure 1, a total of 622 publications were identified after the literature search (Pubmed: 114; Embase: 333; Cochrane

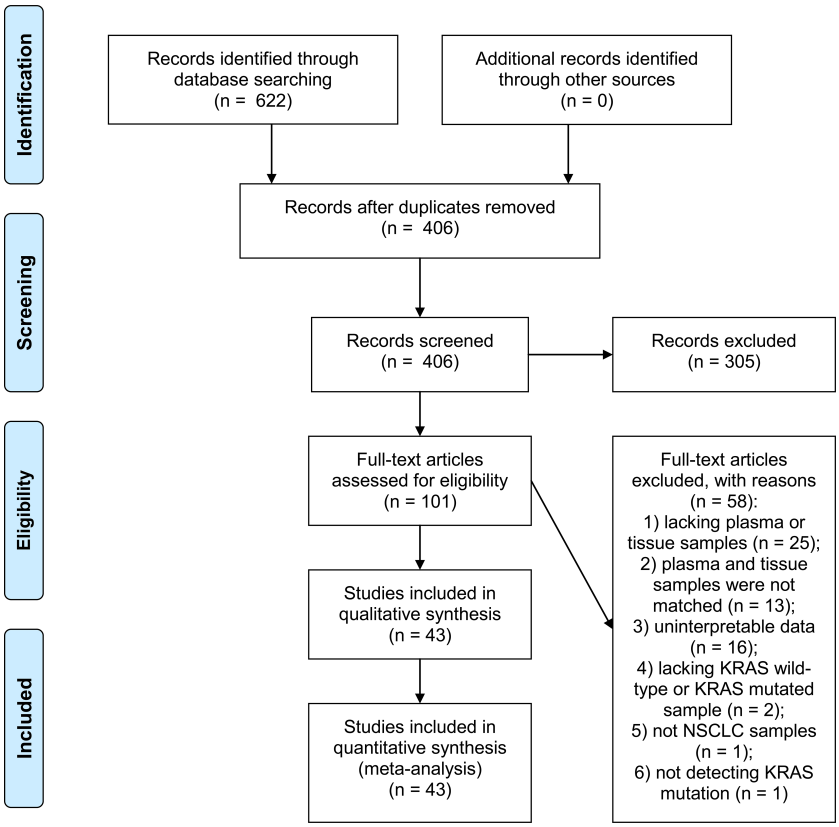

FIGURE 1
PRISMA 2009 flow diagram.

Library: 29; Web of Science: 146). After removing 216 duplicated literatures, titles and abstracts of the rest 406 publications were screened, and 305 irrelevant studies were excluded. Full text of the rest 101 publications were downloaded and carefully evaluated for their eligibility, and another 58 publications were further excluded. From the 43 eligible studies, accuracy data and other relevant information were extracted.

3.2 Review of eligible publications

Twenty-nine of the 43 eligible studies (Table 1) used next-generation sequencing (NGS) to detect *KRAS* mutation in plasma samples. In the rest 14 studies, 12 studies used PCR-based techniques, 1 study used pyrosequencing, and 1 study used MassARRAY.

3.2.1 NGS

In the eligible studies using NGS, sensitivities ranged from 25% to 100%, and specificities and concordance rates were relatively higher, ranging from 64% to 100% and from 52.63% to 100%, respectively.

Twelve studies used customized NGS panels, in which 5 studies used amplicon-based targeted sequencing (15–19). In the study by Yin (15), *KRAS* mutation detected in tumor tissue samples were all detected in paired plasma samples, resulting in 100% sensitivity. The specificity and concordance rate were 99.24% and 99.32%, respectively. Similarly, study by Narayan (17) showed perfect matching (100% concordance rate) of *KRAS* mutation results between plasma and tissue samples. However, study by Paweletz (16) and by Couraud (18) showed much lower sensitivity (54.55% and 75%, respectively), although high specificity (100%) was observed. In the study by Wang Z (19), circulating single-molecule amplification and resequencing technology (cSMART) showed sensitivity of 58.82%, specificity of 100%, and concordance rate of 93.20%. The large variations in the sensitivity of *KRAS* mutation detection in plasma samples may be due to the small number of patients included in these studies.

The rest 7 studies used hybridization-based targeted sequencing (20–26). A customized panel from xGen (Integrated DNA Technologies) showed perfect match between plasma and tumor tissue results (100% concordance rate) (20). Studies by Yao (21) and Pritchett (22) used a hybridization-based target enrichment method from Agilent Technologies (SureSelect). The two studies showed similar concordance rates (91.16% and 97.44%). Studies by Liu (23), Li BT (24), Chen Y (25), and Lin (26) also used hybridization-based capture methods to enrich customized gene panels for NGS sequencing of plasma samples. The concordance rates of those studies were all high, ranging from 93.02% to 96.92%.

Besides customized NGS panels, several commercial NGS panels were also used, such as AmpliSeq panels, Oncomine panels, AmoyDx Essential NGS panel, 56G Oncology Panel,

TABLE 1 Summary of studies detecting *KRAS* mutation in paired plasma and tissue samples from NSCLC patients.

Author, year	Sample size	Detection method (plasma)	Detection method (tissue)	Region	Tumor stage	Race
Yin J et al., 2021 (15)	147	NGS (customized panel)	NGS (customized panel)	Asia	I-IV	Asian
Paweletz CP et al., 2016 (16)	48	NGS (customized panel)	not specified	America	III-IV	Caucasian
Narayan A et al., 2012 (17)	21	NGS (customized panel)	Sanger sequencing/clinical lab	America	I-IV	Caucasian
Couraud S et al., 2014 (18)	68	NGS (customized panel)	NGS (customized panel)	Europe	I-IV	Caucasian
Wang Z et al., 2017 (19)	103	NGS (cSMART)	ARMS-PCR	Asia	III-IV	Asian
Tran LS et al., 2019 (20)	40	NGS (Ultra-deep sequencing)	NGS (Ultra-deep sequencing)	Asia	III-IV	Asian
Yao Y et al., 2017 (21)	39	NGS (Agilent SureSelect)	NGS (Agilent SureSelect)	Asia	III-IV	Asian
Pritchett MA et al., 2019 (22)	147	NGS (Agilent SureSelect)	NGS (Agilent SureSelect)	America	III-IV	Caucasian
Liu L et al., 2018 (23)	65	NGS (customized panel)	NGS (customized panel)	Asia	III-IV	Asian
Li BT et al., 2019 (24)	110	NGS (customized panel)	NGS (customized panel)	America	IV	Caucasian
Chen Y et al., 2019 (25)	43	NGS (customized panel)	NGS (customized panel)	Asia	I-IV	Asian
Lin X et al., 2019 (26)	21	NGS (customized panel)	NGS (customized panel)	Asia	III-IV	Asian
Chen KZ et al., 2016 (27)	58	NGS (AmpliSeq Cancer Panel)	NGS (AmpliSeq Cancer Panel)	Asia	I-II	Asian
Xu S et al., 2016 (28)	42	NGS (AmpliSeq Cancer Panel)	NGS (AmpliSeq Cancer Panel)	Asia	III-IV	Asian
Pécuchet N et al., 2016 (29)	107	NGS (AmpliSeq Colon and Lung Cancer Research Panel v2)	NGS (AmpliSeq Colon and Lung Cancer Research Panel v2)	Europe	III-IV	Caucasian
Pasquale R et al., 2020 (30)	107	NGS (Oncomine Lung cfDNA assay)	NGS (Oncomine Solid Tumor DNA)	Europe	not disclosed	Caucasian
Mehta A et al., 2021 (31)	21	NGS (Oncomine Lung Cell-Free Total Nucleic Acid Assay)	NGS (Tag sequencing)	Asia	III-IV	Asian
Papadopoulou E et al., 2019 (32)	36	NGS (Oncomine Lung Cell-Free Total Nucleic Acid Assay)	NGS (AmpliSeq Colon and Lung Cancer Research Panel v2)	Europe	not disclosed	Caucasian
Nicolazzo C et al., 2021 (33)	38	NGS (Oncomine Lung Cell-Free Total Nucleic Acid Assay)	NGS (AmpliSeq Colon and Lung Cancer Research Panel v2)	Europe	not disclosed	Caucasian
Ma Y et al., 2020 (34)	28	NGS (AmoyDx Essential NGS panel)	NGS (AmoyDx Essential NGS panel)	Asia	I-IV	Asian

(Continued)

TABLE 1 Continued

Author, year	Sample size	Detection method (plasma)	Detection method (tissue)	Region	Tumor stage	Race
Garcia J et al., 2018 (35)	20	NGS (56G Oncology Panel Kit, Swift Biosciences)	NGS (customized AmqliSeq panel)	Europe	not disclosed	Caucasian
Remon J et al., 2019 (36)	88	NGS (InVisionSeq Lung, NeoGenomics)	Sanger sequencing or allele-specific technique	Europe	III-IV	Caucasian
Bauml JM et al., 2022 (37)	189	NGS (Guardant360)	PCR (therascreen KRAS RGQ PCR Kit)	America	I-IV	Caucasian
Thompson JC et al., 2016 (38)	50	NGS (Guardant360)	NGS (Illumina TruSeq Amplicon - Cancer Panel, or Penn Precision Panel)	America	II-IV	Caucasian
Leighl NB et al., 2019 (39)	282	NGS (Guardant360)	Standard of care (NGS, PCR, FISH and/or IHC, Sanger sequencing	America	III-IV	Caucasian
Lam VK et al., 2021 (40)	76	NGS (Guardant360)	not specified	America	III-IV	Caucasian
Qvick A et al., 2021 (41)	52	NGS (AVENIO ctDNA Surveillance kit)	NGS (AmpliSeq Colon and Lung Cancer Research Panel v2, or AVENIO FFPE Surveillance kit (sufficient sample), or qPCR and FISH (insufficient samples)	Europe	I-IV	Caucasian
Jiao XD et al., 2021 (42)	185	NGS (LungPlasma panel)	NGS (OncoScreen Plus panel)	Asia	III-IV	Asian
Guo N et al., 2016 (43)	41	NGS (SV-CA50-ctDNA panel, San Valley Biotech Inc.)	NGS (SV-CA50-ctDNA panel, San Valley Biotech Inc.)	Asia	I-IV	Asian
Michaelidou K et al., 2020 (44)	96	ddPCR	Sanger sequencing	Europe	III-IV	Caucasian
Oxnard GR et al., 2014 (45)	31	ddPCR	Central lab	America	III-IV	Caucasian
Sacher AG et al., 2016 (46)	87	ddPCR	not specified	America	III-IV	Caucasian
Mellert H et al., 2017 (47)	100	ddPCR	not specified	America	III-IV	Caucasian
Cho MS et al., 2020 (48)	36	PCR-based multiplex assay (PANAmutyper)	PCR-based multiplex assay (PNAmutyper)	Asia	I-IV	Asian
Han JY et al., 2016 (49)	135	PCR-based multiplex assay (PANAmutyper)	PCR-based direct DNA sequencing	Asia	III-IV	Asian
Wang S et al., 2010 (50)	273	PCR-RFLP	Direct sequencing	Asia	I-IV	Asian
Gautschi O et al., 2007 (51)	9	PCR-RFLP	Sanger sequencing	Europe	I-IV	Caucasian
Zhang H et al., 2013 (52)	86	Multiplex PCR (SurPlex MEL, SurExam Biotech, Inc)	Multiplex PCR (SurPlex-xTAG70plex, SurExam Biotech, Inc)	Asia	III-IV	Asian
Punnoose EA et al., 2012 (53)	18	Multiplex PCR (customized primers) + TaqMan assay or DxS kit	not specified	USA & Australia	not disclosed	Caucasian

(Continued)

TABLE 1 Continued

Author, year	Sample size	Detection method (plasma)	Detection method (tissue)	Region	Tumor stage	Race
Mack PC et al., 2009 (54)	49	ARMS	ARMS	America	III-IV	Caucasian
Campos CDM et al., 2018 (55)	3	solid phase extraction + PCR/LDR	PCR/LDR	America	III-IV	Caucasian
Kulasinghe A et al., 2021 (56)	103	MassARRAY (UltraSEEK lung panel, Agena Biosciences)	not specified	Australia	I-IV	Caucasian
Li XQ et al., 2014 (57)	43	pyrosequencing	pyrosequencing	Asia	III-IV	Asian

NGS, next generation sequencing; PCR, polymerase chain reaction; PCR-RFLP, PCR-restriction fragment length polymorphism; ddPCR, digital droplet PCR; ARMS, Amplification Refractory Mutation System.

InVisionSeq Lung, Guardant360, AVENIO ctDNA Surveillance kit, LungPlasma panel, and SV-CA50-ctDNA panel. AmpliSeq Cancer Panel (Thermo Fisher Scientific) was used in two studies (27, 28). However, the results varied greatly between them. Sensitivity, specificity, and concordance rate were 60%, 96.23%, and 93.10% in Chen KZ's study (27), and 100%, 83.33%, and 85.71% in Xu's study (28). AmpliSeq Colon and Lung Cancer Research Panel v2 showed sensitivity of 62.96%, specificity of 100%, and concordance rate of 90.65% (29). Oncomine Lung cfDNA Assay (Thermo Fisher Scientific) showed sensitivity, specificity, and concordance rate of 61.54%, 93.83%, and 85.98%, respectively (30). Oncomine Lung Cell-Free Total Nucleic Acid Assay (Thermo Fisher Scientific) was used in three studies, and accuracy results varied greatly: sensitivity from 30.77% to 81.82%, specificity from 64% and 100%, and concordance rate from 52.63% to 94.44% (31–33). AmoyDx Essential NGS panel (Amoy Diagnostics) was used in a 28-patient cohort, and the sensitivity, specificity, and concordance rate were 66.67%, 96%, and 92.86%, respectively (34). Studies by Garcia (35) and Remon (36) also used amplicon-based targeted sequencing techniques, including 56G Oncology Panel (Swift Biosciences), InVisionSeq Lung (NeoGenomics), respectively. Results showed sensitivity of 64.29% and 88%, specificity of 83.33% and 88.89%, and concordance rate of 70% and 88.64%.

Four studies validated the accuracy of Guardant360 in detecting *KRAS* mutation in plasma samples (37–40). Sensitivity ranged from 66.67% to 87.50%. Specificity ranged from of 74.81% to 100%, and concordance rate ranged from 75.89% to 98%. AVENIO ctDNA Surveillance kit (Roche) is also a commercial panel using hybridization-based target enrichment. A study using AVENIO ctDNA Surveillance kit showed sensitivity of 72.73%, specificity of 100%, and concordance rate of 94.23% (41).

In the rest two studies using commercial NGS panels, detailed target enrichment method was not disclosed. Studies by Jiao (42) used LungPlasma NGS panel (Burning Rock Biotech), and sensitivity, specificity, and concordance rate were 68.97%, 99.36%, and 94.59%. Guo (43) used SV-CA50-ctDNA panel (San Valley Biotech), and results showed 50% sensitivity, 97.44% specificity, and 95.12% concordance rate.

3.2.2 PCR-based techniques

A total of 4 studies used digital droplet PCR (ddPCR) to detect *KRAS* mutation in plasma samples (44–47). Although ddPCR is a sensitive technique which could detect genetic mutations as low as 0.01%, the results of these studies did not show high accuracy of ddPCR in plasma-based *KRAS* mutation detection. Sensitivity ranged from 51.43% to 87.88%, and specificity ranged from 88.52% to 100%, resulting in concordance rates from 75% to 96%.

Other than ddPCR, several PCR-based techniques were also used to detect *KRAS* mutation in plasma samples, such as PANAmutyper, PCR-restriction fragment length polymorphism (PCR-RFLP), multiplex PCR, Amplification Refractory Mutation System (ARMS), and PCR/ligase detection reaction (LDR) technique. Overall, those PCR-based techniques were mostly used in early studies, which showed sensitivity ranging from 33.33% to 100%, specificity from 50% to 100%, and concordance rate from 55.56% to 100%.

PANAmutyper is a multiplex PCR method which increases sensitivity through suppressing amplification of wild-type DNA using specific peptide nucleic acids (PNA) (48). In the two studies using PANAmutyper, the sensitivity was 33.33% and 50%, and specificity was 100% and 89.43%, resulting in concordance rates of 88.89% and 85.93%, respectively (48, 49).

In the two studies using PCR-RFLP, accuracy results varied greatly. In Wang S's study (50), the sensitivity, specificity, and concordance rate were 76.67%, 95.06%, and 93.04%, respectively. In the study of Gautschi (51), these numbers were 50%, 66.67%, and 55.56%, respectively.

Multiplex PCR was used in two studies. Study by Zhang (52) used SurExam MEL (SurExam Biotech), a typical commercial multiplex PCR, to detect *KRAS* mutation in plasma samples, and sensitivity, specificity, and concordance rate were 33.33%, 98.80%, and 96.51%. In the study by Punnoose (53), the *KRAS* mutation results of plasma samples matched perfectly with tissue samples (100% concordance rate).

An early study by Mack (54) used KRAS Scorpion-ARMS test kit (DxS Ltd), and results showed 50% sensitivity, 100% specificity, and 97.96% concordance rate.

Campos (55) and colleagues developed a microfluidic solid-phase extraction device to extract cfDNA, which were then analyzed using PCR/LDR technique. Only 3 NSCLC samples were tested in the study, and the results showed 100% sensitivity, 50% specificity, and 66.67% concordance rate.

3.2.3 MassARRAY and pyrosequencing

UltraSEEK lung panel (Agena Biosciences), a commercial MassARRAY panel, was used in a 103-patient cohort, and sensitivity, specificity, and concordance rate were 62.96%, 92.11%, and 84.47%, respectively (56). Pyrosequencing was used in an early study (57), and sensitivity and specificity were 75% and 100%, respectively, resulting in a concordance rate of 97.67%.

In all, the 43 eligible studies compared *KRAS* mutation status in paired plasma and tissue samples from 3341 NSCLC patients. Thirty-nine of the 43 eligible studies (39/43) showed high specificity (≥ 80%), and 37 studies showed high concordance rate (≥ 80%). However, high sensitivity (≥ 80%) was only observed in 14 out of 43 studies.

3.3 Quality assessment of eligible studies

Quality assessment of eligible studies was performed using QUADAS-2. As shown in Table 2, the 43 eligible studies showed overall good quality, with high risk observed in only 2 studies (both in flow and timing). In the assessment of risk of bias, percentage of low risk ranged from 46.51% (n = 20, Index test) to 69.77% (n = 30, both patient selection and reference standard). In the application concerns, no high risk was observed, and percentage of low risk ranged from 83.72% (n = 36, reference standard) to 86.05% (n = 37, both patient selection and index test).

TABLE 2 QUADAS-2 assessment of eligible studies.

Author, year	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Yin J et al., 2021 (15)	low	unclear	low	low	low	low	low
Paweletz CP et al., 2016 (16)	low	low	low	low	low	low	low
Narayan A et al., 2012 (17)	low	unclear	low	unclear	unclear	low	low
Couraud S et al., 2014 (18)	low	unclear	unclear	unclear	low	low	unclear
Wang Z et al., 2017 (19)	low	low	low	unclear	low	unclear	unclear
Tran LS et al., 2019 (20)	low	unclear	unclear	low	low	low	low
Yao Y et al., 2017 (21)	unclear	unclear	low	low	low	low	low
Pritchett MA et al., 2019 (22)	low	unclear	low	unclear	low	low	low
Liu L et al., 2018 (23)	low	unclear	unclear	low	low	low	low
Li BT et al., 2019 (24)	low	low	low	unclear	low	low	low
Chen Y et al., 2019 (25)	low	unclear	low	unclear	unclear	low	low
Lin X et al., 2019 (26)	unclear	low	low	unclear	low	low	low
Chen KZ et al., 2016 (27)	unclear	unclear	low	low	low	unclear	low
Xu S et al., 2016 (28)	low	low	low	low	low	low	low
Pécuchet N et al., 2016 (29)	low	low	low	high	low	unclear	unclear
Pasquale R et al., 2020 (30)	low	low	low	low	low	low	low
Mehta A et al., 2021 (31)	unclear	unclear	low	unclear	low	low	low
Papadopoulou E et al., 2019 (32)	low	unclear	low	unclear	low	low	low
Nicolazzo C et al., 2021 (33)	unclear	unclear	low	unclear	low	low	low
Ma Y et al., 2020 (34)	unclear	low	unclear	unclear	low	low	low
Garcia J et al., 2018 (35)	low	unclear	low	low	low	unclear	unclear
Remon J et al., 2019 (36)	low	low	unclear	unclear	unclear	low	low
Bauml JM et al., 2022 (37)	low	unclear	low	low	low	low	low
Thompson JC et al., 2016 (38)	low	low	low	low	low	low	unclear

(Continued)

TABLE 2 Continued

Author, year	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Leighl NB et al., 2019 (39)	low	low	low	unclear	low	unclear	unclear
Lam VK et al., 2021 (40)	unclear	unclear	unclear	unclear	unclear	low	low
Qvick A et al., 2021 (41)	unclear	low	unclear	unclear	low	low	low
Jiao XD et al., 2021 (42)	low	unclear	low	low	low	unclear	low
Guo N et al., 2016 (43)	low	low	low	low	low	low	low
Michaelidou K et al., 2020 (44)	low	unclear	low	low	low	low	low
Oxnard GR et al., 2014 (45)	unclear	unclear	unclear	unclear	low	low	low
Sacher AG et al., 2016 (46)	low	low	low	low	low	low	low
Mellert H et al., 2017 (47)	unclear	unclear	unclear	unclear	low	low	low
Cho MS et al., 2020 (48)	low	low	low	unclear	low	low	low
Han JY et al., 2016 (49)	low	unclear	low	low	low	low	unclear
Wang S et al., 2010 (50)	low	low	low	low	low	low	low
Gautschi O et al., 2007 (51)	low	low	unclear	unclear	low	low	low
Zhang H et al., 2013 (52)	low	low	low	low	low	low	low
Punnoose EA et al., 2012 (53)	unclear	unclear	unclear	high	unclear	low	low
Mack PC et al., 2009 (54)	unclear	low	unclear	low	low	low	low
Campos CDM et al., 2018 (55)	unclear	unclear	low	unclear	unclear	low	low
Kulasinghe A et al., 2021 (56)	low	unclear	low	low	low	low	low
Li XQ et al., 2014 (57)	low	low	unclear	low	low	low	low

low, low risk; unclear, unclear risk; high, high risk.

3.4 Meta-analysis

From the 43 eligible studies, we pooled the *KRAS* mutation detection results from paired plasma and tissue samples of 3341 patients with NSCLC. The overall sensitivity and specificity were 0.71 [95% confidence interval (CI): 0.68-0.75] and 0.94 (95%CI: 0.93-0.95), respectively. The pooled DOR was 59.28 (95%CI: 34.37-102.25), and AUC of SROC curve was 0.8883. Please see Table 3 and Figure 2 for details.

Since significant heterogeneity ($I^2 \geq 50\%$ and $P < 0.05$) was observed, we further analyzed its possible sources. Analysis of diagnostic threshold showed no significant threshold effect (spearman correlation coefficient = 0.058, $P = 0.714$). Meta-regression revealed that inter-study heterogeneity was associated with techniques used for plasma sample ($P = 0.0388$), but not with techniques used for tissue sample ($P = 0.1280$), region of study ($P = 0.3299$), tumor stage ($P = 0.3049$), or race of patients ($P = 0.7798$).

Subgroup analysis was then performed on different techniques used for plasma sample. The 43 eligible studies were grouped into three subgroups: NGS, PCR-based techniques, and other techniques. Meta-analysis was performed in each subgroup except other techniques due to limited number (only two) of studies in that subgroup. As shown in Table 3, compared to PCR-based techniques, NGS showed overall better accuracy: sensitivity of 0.73 (95%CI: 0.69-0.77), specificity of 0.94 (95%CI: 0.93-0.95), DOR of 82.60 (95%CI: 40.62-167.96), and AUC of SROC curve of 0.9162. After further dividing the group of PCR-based techniques into two subgroups (ddPCR and other PCR-based techniques), ddPCR showed higher sensitivity [0.68 (95%CI: 0.59-0.77)], specificity [0.97 (95%CI: 0.93-0.99)], and DOR [85.60 (95%CI: 6.80-1978.05)], but much lower AUC of SROC curve (0.2741).

Subgroup analysis was also performed on the region of studies, including Asia, America, Australia, and Europe. Australia was excluded from the subgroup analysis due to limited number of studies in the subgroup. In the other three subgroups, studies performed in America showed overall best accuracy, with pooled sensitivity of 0.76 (95%CI: 0.71-0.81), specificity of 0.92 (95%CI: 0.90-0.94), DOR of 111.35 (95%CI: 56.05-221.20), and AUC of SROC curve of 0.9272.

Twenty-four of the 43 eligible studies used late-stage (stage III and IV) NSCLC samples, and 13 studies used NSCLC samples of any stage (stage I to IV). As shown in Table 3, pooled accuracy results of the two subgroups (stage III-IV *versus* stage I-IV) did not differ much from each other. However, this result should be treated carefully because although early-stage NSCLC samples were involved, majority of the samples were still late-stage in stage I-IV

TABLE 3 Meta-analysis results.

	No. of studies	Sensitivity	Specificity	PLR	NLR	DOR	AUC of SROC curve
Overall	43	0.71(0.68-0.75)	0.94(0.93-0.95)	16.27(10.08-26.25)	0.36(0.30-0.43)	59.28(34.37-102.25)	0.8883
Techniques used for plasma sample							
NGS	29	0.73(0.69-0.77)	0.94(0.93-0.95)	20.99(10.68-41.23)	0.33(0.26-0.41)	82.60(40.62-167.96)	0.9162
PCR-based techniques	12	0.66(0.59-0.74)	0.95(0.94-0.97)	9.88(4.60-21.19)	0.42(0.31-0.58)	31.58(11.88-83.95)	0.7888
ddPCR	4	0.68(0.59-0.77)	0.97(0.93-0.99)	26.46(2.68-261.05)	0.33(0.18-0.59)	85.60(6.80-1078.05)	0.2741
Other PCR-based techniques	8	0.63(0.50-0.75)	0.95(0.93-0.97)	7.61(3.16-18.31)	0.40(0.29-0.55)	22.01(11.18-43.33)	0.8147
Region							
Asia	18	0.71(0.63-0.78)	0.97(0.95-0.98)	18.00(9.96-32.53)	0.32(0.25-0.40)	63.84(38.95-104.65)	0.9381
America	13	0.76(0.71-0.81)	0.92(0.90-0.94)	31.28(5.36-182.47)	0.25(0.20-0.30)	111.35(56.05-221.20)	0.9272
Europe	10	0.63(0.56-0.71)	0.93(0.91-0.95)	7.42(3.17-17.41)	0.43(0.29-0.62)	22.62(6.69-76.49)	0.7013
Tumor stage							
I-IV	13	0.71(0.65-0.77)	0.97(0.96-0.98)	22.11(13.39-36.52)	0.39(0.28-0.54)	64.59(34.43-121.17)	0.9273
III-IV	24	0.73(0.69-0.78)	0.93(0.92-0.94)	18.68(9.26-37.69)	0.29(0.25-0.34)	54.70(36.59-81.75)	0.9086
Race of patients							
Asian	18	0.71(0.63-0.78)	0.97(0.95-0.98)	18.00(9.96-32.53)	0.32(0.25-0.40)	63.84(38.95-104.65)	0.9381
Caucasian	25	0.72(0.68-0.75)	0.92(0.91-0.94)	14.85(7.39-29.84)	0.34(0.27-0.42)	53.73(24.95-115.69)	0.8445

PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under curve; SROC, summary receiver operating characteristic; PCR, polymerase chain reaction; NGS, next generation sequencing; ddPCR, digital droplet PCR.

subgroup. The rest 6 studies were not involved in the subgroup analysis, including 1 study using early-stage (I and II) NSCLS samples only, and 5 studies which did not disclose the tumor stage of samples.

Majority of the 43 eligible studies were conducted using samples from Caucasian patients, and the rest studies used samples of Asian patients. Between the two subgroups, pooled accuracy data were similar (see Table 3).

Publication bias was evaluated using Deek's funnel plot (Figure 3). The results indicated no significant publication bias ($P = 0.097$).

4 Discussion

Before anti-EGFR therapies are given to NSCLC patients, it is important to determine whether the tumor carries *KRAS* mutation since it may lead to resistance to anti-EGFR therapies. Moreover, determination of *KRAS* mutation status is also required before the usage of KRAS (G12C) inhibitor, e.g., Sotorasib. Tumor tissue samples are the "gold standard" in the determination of *KRAS* mutation. However, tumor tissue samples are sometimes not available, and cfDNA-containing samples (e.g., plasma, urine, saliva, etc.) have been intensively investigated as surrogates for tissue samples. A recently-published systemic review and meta-analysis by Palmieri summarized the performance of cfDNA-containing samples in detecting *KRAS* mutation in NSCLC (12). Due to the higher and more stable levels of cfDNA in plasma compared to other cfDNA-containing sample types, we focused solely on plasma in this systemic review and meta-analysis, and investigated its accuracy in determining tumor *KRAS* mutation status in NSCLC.

In order to investigate the accuracy of *KRAS* mutation detection using plasma samples, several previous studies compared *KRAS* mutation results in paired plasma and tissue samples from patients with NSCLC. After database searching and screening, we identified 43 eligible studies. After pooling the *KRAS* mutation status from 3341 patients with NSCLC, the results showed overall moderate sensitivity (0.71) and high specificity (0.94). Other important

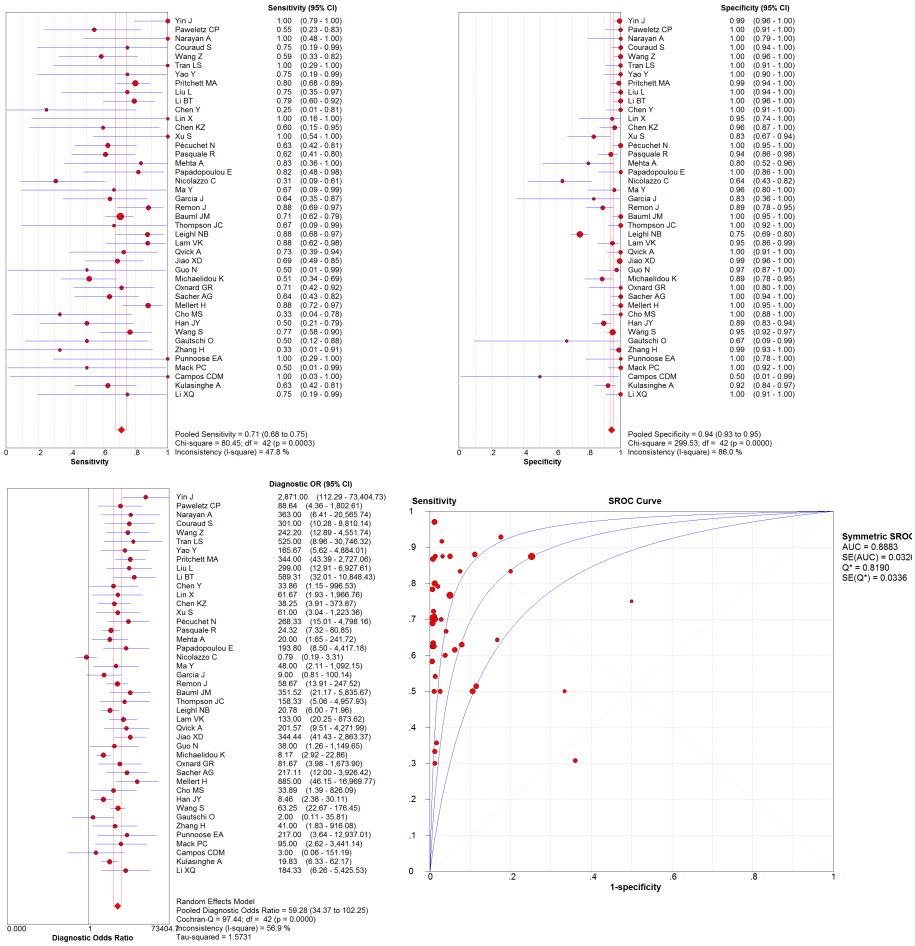

FIGURE 2
Pooled sensitivity, specificity, DOR, and SROC curve of eligible studies.

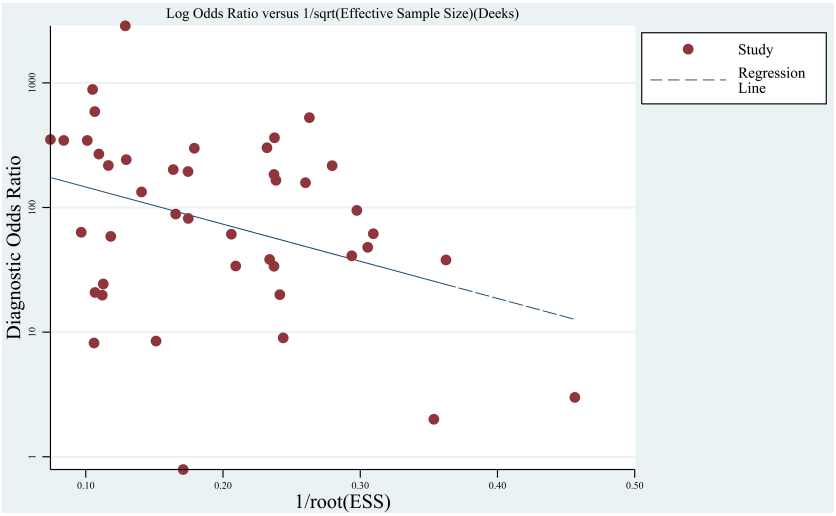

FIGURE 3
Deek's funnel plot.

indicators of diagnostic accuracy, DOR and AUC of SROC curve, were also high (59.28 and 0.8883, respectively). Although with moderate sensitivity, these results indicated overall high accuracy of plasma samples in detecting *KRAS* mutation. In the systemic review and meta-analysis by Palmieri (12), the pooled sensitivity and specificity were 0.71 and 0.93, respectively, and DOR was 35.24, which were similar to the findings of our study.

Since significant inter-study heterogeneity was observed during the pooling ($I^2 \geq 50\%$ and $P < 0.05$), we investigated its possible sources. Analysis of diagnostic threshold did not indicate significant threshold effect. Meta-regression revealed significant association between inter-study heterogeneity and techniques used for plasma sample. This is different from Palmieri's study, in which detection method did not contribute to heterogeneity (12). No significant association was shown between heterogeneity and other covariates (techniques used for tissue sample, region of study, tumor stage, and race of patients).

Different from Palmieri's study, we further conducted subgroup analysis. Subgroup analysis on technique used for plasma sample was firstly performed. After pooling the accuracy results, we found that NGS outperformed PCR-based techniques in many accuracy parameters, including sensitivity (0.73), DOR (82.60), and AUC of SROC curve (0.9162). We further divided the group of PCR-based techniques into two groups: ddPCR and other PCR-based techniques. Compared to NGS, ddPCR showed similar sensitivity (0.68), specificity (0.97), and DOR (85.60), except for surprisingly low AUC of SROC curve (0.2741) which was possibly due to the limited number of studies in this subgroup (Table 3).

We also performed subgroup analysis on region of study. Studies performed in Asia showed the highest AUC of SROC curve (0.9381). Studies performed in America showed the highest sensitivity (0.76) and DOR (111.35), and similar AUC of SROC curve with Asia (0.9272), indicating overall the highest accuracy of the studies from America.

Late-stage tumors was reported to be associated with significantly higher fraction of circulating tumor DNA (ctDNA) in cfDNA (58), which may indicate potentially better performance of genetic testing using these samples. In the 43 eligible studies, involvement of early-stage samples did not significantly influence the accuracy results. However, this result should be treated with care because numbers of early-stage samples were much smaller than late-stage samples in a large proportion of these studies. Race of patients also did not show significant impact on the accuracy results. The performance of *KRAS* mutation testing using plasma was similar between Asian and Caucasian patients. Significant publication bias was not observed using Deek's funnel plot asymmetry test.

In summary, results of this systemic review and meta-analysis indicated overall high accuracy of plasma samples in predicting *KRAS* mutation results of paired NSCLC tumor tissue samples. Plasma could serve as surrogates when tissue samples are not available, although it may miss a small proportion of patients carrying *KRAS* mutation considering its moderate sensitivity. Among different techniques, NGS showed the best accuracy. Although majority of accuracy results were comparable to NGS, ddPCR suffered from its low AUC of SROC curve. Therefore, NGS is recommended in the detection of *KRAS* mutations in plasma samples of patients with NSCLC, especially when multiple genetic variations are tested considering the high-throughput of the technology. Limitation of this study may be the small number of studies in the ddPCR subgroup and limited numbers of early-stage tumor samples used in some studies, which must be treated carefully. In addition, although different techniques are generally thought to have similar performance in tumor samples considering the high abundance of DNA, it may still cause potential bias. Large prospective studies are required to further validate the results of this study.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

PC, BY, and DY contributed to conception and design of the study. BY and JZ organized the database. PY performed the statistical analysis. PC wrote the first draft of the manuscript. BY, JZ, and PY wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1207892/full#supplementary-material

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Characterization of a cohort of metastatic lung cancer patients harboring *KRAS* mutations treated with immunotherapy: differences according to *KRAS G12C* vs. *non-G12C*

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Approximately 20% of lung adenocarcinomas harbor activating mutations at *KRAS*, an oncogene with the ability to alter the tumor immune microenvironment. In this retrospective study, we examined 103 patients with *KRAS*-mutant lung adenocarcinoma who were treated with immunotherapy-based regimens and we evaluated the clinical outcomes according to PD-L1 expression and the type of *KRAS* mutation. Among all patients included, 47% carried *KRAS G12C* mutation whereas 53% harbored *KRAS* non-G12C mutations. PD-L1 status was available for 77% of cases, with higher expression among KRAS G12C tumors (p = 0.01). Better overall survival and progression-free survival were observed in high PD-L1 expression tumors, regardless of *KRAS* mutation type. The heterogeneous nature of KRAS-mutant tumors and the presence of other co-mutations may contribute to different outcomes to immunotherapy-based strategies.

KEYWORDS

non-small cell lung cancer, lung adenocarcinoma, KRAS, PD-L1, immunotherapy

Highlights

- *KRAS G12C* mut LuADs are significantly associated with high PD-L1 expression.
- Better clinical outcomes are associated with high PD-L1 expression, regardless of *KRAS* mut type.
- A subset of long-term responders (LTR) to IT-based regimens were enriched with *KRAS G12C* mut and high PD-L1 expression.

Introduction

Lung adenocarcinoma (LuAD) harbors a significant number of targetable oncogenic mutations among lung cancer. The most common oncogenic mutations are found in *KRAS*, occurring in 20%–25% of the cases. These mutations primarily affect codons 12 (85%) and 13 (10%), found in exon 2, and codon 61 (5%), found in exon 3. The *KRAS G12C* mutation, resulting in a change from glycine to cysteine, prevails in 43% of the cases and is associated with tobacco exposure. In contrast, non-smokers, commonly exhibit G12D mutations, a change from glycine to aspartic acid, and G12V mutations, a change from glycine to valine (1).

Currently, the standard first-line treatment for patients with *KRAS G12C* LuADs involves IT-based regimens, either combined or not with platinum-based chemotherapy (ChT) according to PD-L1 expression levels. Mazieres et al., in a retrospective cohort, demonstrated that *KRAS*-driven tumors express higher rates of PD-L1 and present higher tumor mutational burden compared with other oncogenic alterations, suggesting that it might predict better responses to IT (2). However, the study did not evaluate the existing differences based on the type of *KRAS* mutation. On the other hand, most phase III pivotal trials with IT did not stratify by *KRAS* status, and the efficacy of IT according to *KRAS* mutation subtype remains to be determined (3).

Recently, the emergence of novel allosteric inhibitors of KRAS G12C is expected to change the paradigm of treatment approach for these tumors. Phase I/II trials with sotorasib and adagrasib presented an overall response rate (ORR) of 32% and 45%, respectively, along with an acceptable toxicity. As a result, these inhibitors have received the approval of the Food and Drug Administration (FDA) for the treatment of patients with *KRAS G12C* mutations after progression to initial therapy (4, 5). To further enhance the outcomes, novel drugs such as BI-3406 which disrupts the interaction of SOS1-KRAS, as well as TNO155, which inhibits SHP2, a protein that integrates growth and differentiation signals from receptor tyrosine kinases into the RAS/MAPK cascade, are being evaluated. These drugs in combination with sotorasib or adagrasib are being studied to improve treatment outcomes (5). Additionally, there have been promising findings from preclinical models combining PD-1 inhibitors with KRAS G12C-specific inhibitors. These combinations are being addressed in clinical trials (NCT04613596).

As a result, targeting KRAS beyond KRAS G12C inhibitors has emerged as a significant and rapidly evolving area of research. This refers not only to the development of novel therapeutic strategies targeting *KRAS* but also to the immunoregulatory role of *KRAS* and the effectiveness of current immunotherapies in *KRAS*-driven tumors, which has not been directly addressed in the literature. Of note, *KRAS G12C*-mutant tumors are commonly associated with tobacco exposure and exhibit higher tumor mutational burden, which might predict better responses to IT (6). We hypothesized that those patients might present better clinical outcomes to IT-based therapies.

In this work, we aim to study the clinical outcomes of existing immunotherapies based on the type of *KRAS* mutation and PD-L1 expression levels. We will examine a cohort of patients with metastatic *KRAS*-mutant tumors treated with IT-based regimens in our daily practice.

Methods

Study population

A medical record search was used to identify patients treated at the Catalan Institute of Oncology (ICO)-Badalona with a primary tumor diagnosis of NSCLC harboring *KRAS* mut and treated with IT-based regimens for metastatic disease, from June 2013 to June 2020. Clinical data were retrospectively collected, and patient consent forms were obtained with the approval of the local Institutional Review Board (PI-19-275).

Molecular analysis and PD-L1 expression

The *KRAS* mutation status was determined by analyzing the primary tumor. The tumor tissue samples were tested by KRAS Idylla Mutation Test (Biocartis), a real-time PCR test designed for the identification of mutations in codons 12, 13, and 61; in the most recent cases (2020–2021), they were tested by the NGS panels: Oncomine Solid Tumour, Oncomine Focus Assay, or Oncomine Comprehensive Assay (Thermo Fisher) which includes 22, 52, and 164 genes, respectively, involved in lung cancer pathogenesis. The PD-L1 status in tumor cells was determined by immunohistochemistry (IHC) assay (Ventana clone SP263), and it was categorized as follows: negative <1%, low 1%–49%, and high 50%–100% expression.

Statistical analysis

Clinical characteristics, *KRAS* mutation type, and line of IT treatment were collected for all patients. We classified patients into two groups based on the *KRAS* mutation type: G12C or non-G12C. Baseline characteristics were compared using the chi-square and Fisher's exact test for categorical data. Survival Kaplan–Meier model was used to estimate survival, and medians were compared between groups using the log-rank test. Progression-free survival

(PFS) to IT was calculated from the time of IT initiation to date of disease's progression or death, whichever occurred first. Overall survival (OS) was calculated from the time from starting IT treatment to date of death or last follow-up. The assessment of best overall response (ORR) to IT was performed according to RECIST 1.1 criteria, and response rates were compared between groups using the chi-square test. We defined a subset of long-term responders (LTR) to IT, defined as those patients who did not progress within 24 months after IT treatment initiation.

Results

Clinical and molecular characteristics according to *KRAS* mut type

We identified 103 patients with metastatic non-small cell lung cancer harboring *KRAS* mutations from June 2013 to June 2020, n = 47 *KRAS G12C*, n = 52 *KRAS non-G12C*, n = 4 unknown. PD-L1 was available in 78 cases (77%). Clinical and molecular characteristics according to *KRAS* mut type are shown in Table 1. The distribution of *KRAS* mutations in our non-G12C sub-cohort (n = 52) was as follows: G12V (n = 16), G12A (n = 12), G12D (n = 8), G13C (n = 5), and the frequency of the rest of mutations (G12S, G12F, G13D, Q61H) were below 5.

Clinical outcomes in *KRAS* mutant patients treated with IT

All patients included in the study were treated with IT for advanced disease: 54% in the first line, 36% in the second line, and 10% in the third or further lines. Overall, 19 patients (20%) were treated within clinical trials. Treatment schedules included different IT-based regimens at that period for the first line: ChT-IT (30%) with platinum-based doublet; IT–IT (15%); and/or IT alone (55%), the anti-PD(L)-1-based regimen being the most prevalent one (Table 1).

PD-L1 status was available in 77% of cases: 39% high, 19% low, and 19% negative. High PD-L1 (≥50%) was predominantly found in *KRAS G12C* vs. *non-G12C* (64% vs. 36%, p = 0.01). However, no statistically significant differences were observed in the overall response rate (ORR) to IT according to *KRAS* mut type: 49% of patients with KRAS G12C obtained partial or complete response compared with 42% in the non-G12C group, p value = 0.2 (Figure 1).

After a median follow-up of 26.5 months (m), the mPFS of the entire cohort was 13.3 m (95% CI 5.6-20.9) and the mOS was 17.9m (95% CI 15.5–20.3). Significant differences were observed in mPFS to IT according to PD-L1 expression, regardless of the line of treatment they received the IT: 23.1 m (95% CI 18.1–28.1) in PDL1 ≥50% vs. 10.1 m (2.5–17.6) in PDL1 <50% (p-value 0.045). However, we could not demonstrate significant differences in mPFS to IT according to *KRAS G12C* vs. no-*G12C*: 10.1 m (2.2–18) vs. 13.3 m (2.4–24.3), p = 0.612, respectively (Figure 2). No significant differences in median overall survival (mOS) were neither observed in *G12C* vs. *non-G12C*: 17.9 m (16.6–19.2) vs. 20.6 m (12.6–28.5), p = 0.39.

In addition, in the most recent cases available for NGS (n = 30), we could determine *KRAS* mutant allele frequency, which in our cohort varies from 7.1% to 84.9%, with a mean value of 40.15%. Notably, no statistically significant differences were observed in terms of PFS or OS when employing the mean as a threshold (data details are not presented).

Finally, we identified a subset of LTR to IT (n = 17, 16%). Although not significant, they were enriched with *KRAS G12C* mutations (64%, p-value = 0.09) and high PDL1 expression (57%, p-value = 0.1) compared with the non-LTR, with no significant clinical differences.

Discussion

In our cohort, we observed that tumors harboring *KRAS G12C* mutations were significantly associated with higher expression of PD-L1, as compared with *KRAS non-G12C*. No significant differences were observed according to the smoking habit or clinical characteristics. We also observed that patients with high PDL1 expression presented better mPFS to IT-based regimens compared with low PD-L1, regardless of the line they received the IT. However, we did not observe significant differences in mPFS to IT according to *KRAS* mutation type, despite the tendency of KRAS G12C to present better ORR to IT as compared with *KRAS non-G12C*.

Several phase III trials evaluating the efficacy of IT in NSCLC did not stratify by *KRAS* status, and only *post-hoc* analyses have been performed on that subset. Results remain controversial. While IT alone given as a first-line therapy seems to favor *KRAS*-mutant tumors compared with *KRAS*-wild type, no differences were observed when IT is given in further lines of treatment (3, 7) Another study from real-world data published by Frost et al. from a multicenter and retrospective study evaluated the efficacy of first-line pembrolizumab in 119 patients with *KRAS*-mutant LuADs with high PD-L1 expression (≥50%). Co-mutations in *TP53* were also evaluated. Patients with *KRAS G12C/TP53* had significantly higher ORR (100% vs. 27.3%; p = 0.003) and longer mPFS (33.3 vs. 2.8 months; HR, 0.18; 95% CI: 0.06–0.53; p = 0.002) than tumors with *KRAS non-G12C/TP53* mutations suggesting that *KRAS G12C* present better outcomes to immune-based therapies depending also on the co-mutation partners (8).

We also observed that the benefit of using IT was maintained for a subset of patients for at least ≥24 months after initiating IT. These patients, known as long-term responders (LTR), constitute 16% of our cohort population and were predominantly *KRAS G12C* and enriched with high PDL1 expression, although no significant differences were observed. The available literature lacks substantial

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TABLE 1 Patient characteristics by KRAS mutation (*KRAS G12C* vs. *non-G12C*).

	KRAS G12C n = 47	KRAS non-G12C n = 52	p-value
Median age at diagnosis	63	61	NA
Gender			
Male Female	32 (32%) 15 (15%)	41 (41%) 11 (11%)	p = 0.224
Smoking status			
Current or former Never	46 (46%) 1 (1%)	50 (51%) 2 (2%)	p = 0.883
Performance status			
ECOG 0-1 ECOG 2-3	45 (46%) 1 (2%)	48 (91%) 5 (9%)	p = 0.142
PD-L1	*n=36*	*n=40*	
Negative Low High	6 (8%) 5 (6%) 25 (33%)	13 (17%) 13 (17%) 14 (18%)	p = 0.011
Line of treatment with IT	n = 47	n = 52	
First line Second line ≥Third line	30 (30%) 11 (11%) 6 (6%)	25 (25%) 24 (24%) 3 (3%)	p = 0.049
First-line IT, schedule of treatment	n = 30	n = 24	
IT monotherapy Combination with ChT-IT IT combination	19 (34%) 6 (11%) 5 (9%)	11 (20%) 10 (18%) 3 (5%)	NA
Percentage of retreatments with IT	3 (6%)	5 (10%)	NA

IT, immunotherapy; ChT, chemotherapy; ns, not significant; NA, not applicable.

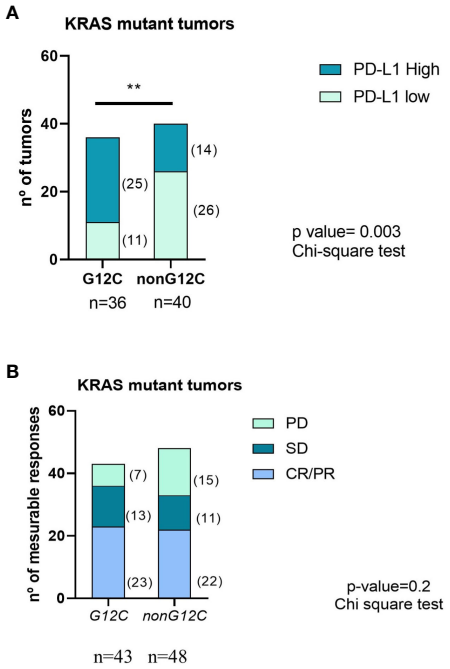

FIGURE 1
PD-L1 status **(A)** and ORR to IT **(B)** according to KRAS mutation type. PD, Progressive disease; SD, Stable disease; CR/PR, Complete/Partial response. ** means statistically significant with p-value <0.005 using chi-square test.

information regarding tumor and patient's characteristics of the LTR, although a few authors have suggested a potential association with adenocarcinoma histopathology and high PD-L1 expression (9).

One of the limitations of our study is the heterogeneity of the IT-based regimens that patients have received, which impairs to reach definitive conclusions. Only 16 patients received ChT-IT for the first-line setting (30%), which nowadays is the standard of care for tumors with PD-L1 <50%, regardless of the *KRAS* mutation status. Another caveat is the lack of the NGS profile for most of the patients included in the study, which was only performed in the most recent cases (2020–2021) due to diagnostic protocols in our daily clinical practice. Currently, next-generation sequencing (NGS) is the gold standard for molecular diagnosis in lung cancer since it provides a broad genetic information that helps to determine the therapeutic options. Optimizing novel panels including a wide range of genes related with carcinogenesis are becoming the standard of care. However, despite the advantages of the NGS technology, access to NGS panels varies broadly among the different areas and health systems worldwide. Co-mutations such as *STK11*, *KEAP1*, or *TP53* are emerging as predictive markers of response to IT, particularly in those patients with *KRAS G12C* mutations (10). In addition, in contrast to KRAS Idylla real-time PCR, NGS panels allow us to identify the KRAS mutant allele fraction, although in our cohort, additional subanalysis stratifying by *KRAS* mutant allele fraction did not allow to elucidate relevant differences in clinical outcomes. It is becoming essential to assess the genetic profile to predict different outcomes when testing different therapeutic strategies.

Another relevant topic to be addressed is the predictive value to PD-(L)1 blockade among KRAS non-G12C mutations. In this current work comprising more than 2,000 *KRAS* mutant LC patients, Ricciuti et al. show that *KRAS G12D* mutant patients harbor distinct clinical, genomic, and immunologic features and present worse clinical outcomes to PD-(L)1 blockade. Owing to the limited size of our subcohort, definitive conclusions referring to this aspect could not be reached. These inquiries continue to be of considerable interest and merit in-depth exploration within more extensive patients cohorts (11). Finally, in four cases, the *KRAS* mutation subtype was unknown because we could not access this piece of information. Those patients were remitted from other hospitals, and this constitutes another caveat of the retrospective nature of our study.

On the other hand, the strength of this study is the sample size from a multidisciplinary oncologic institution, and the long-term follow-up for all the patients included, which will help to elucidate the role of current IT in *KRAS*-mutant LuAD patients and the

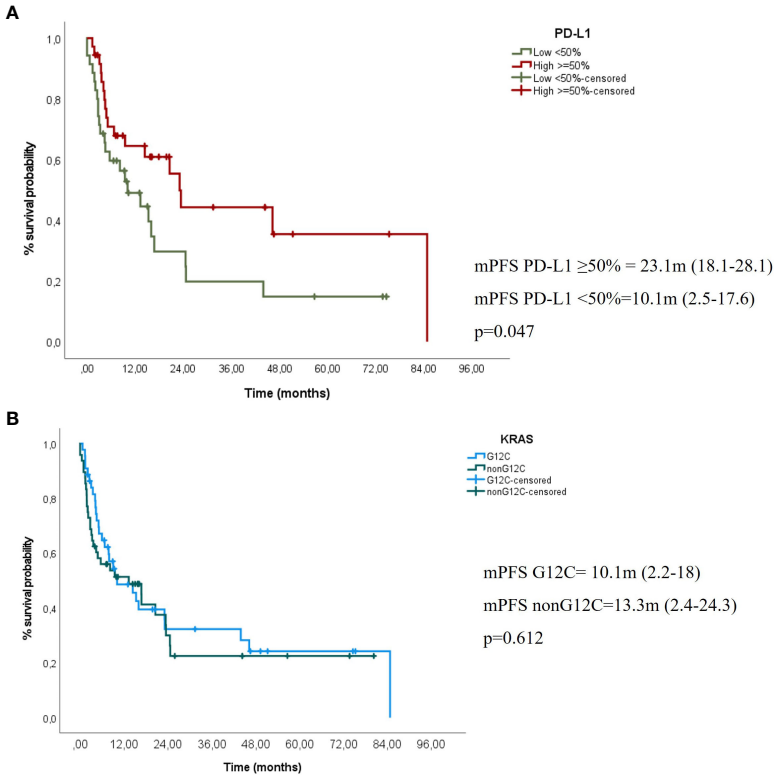

FIGURE 2
(A) mPFS to IT according to PD-L1 expression (high ≥50% vs. negative/low <50% **(B)** mPFS to IT according to *KRAS* mutation type (KRAS G12C vs. non-G12C).

impact on OS, including a subset of LTR, in the era of the incorporation of KRAS G12C-specific inhibitors.

Future directions in the therapeutic landscape will focus on how to integrate IT with KRAS G12C inhibitors or panKRAS inhibitors. Recently the first data of the Codebreak 100/101 study, evaluating the combination of anti-PD(L)1 pembrolizumab or atezolizumab with sotorasib in *KRAS G12C*-mutant patients, showed promising results and represents a novel potential strategy. However, the balance between efficacy and toxicity with the combination, particularly grade 3–4 hepatotoxicity, remains crucial in this setting (NCT03600883, NCT04185883) (12).

In conclusion, despite that no significant differences were observed in IT-based regimens in lung cancer patients according to the type of *KRAS* mutation (G12C vs. non-G12C), efforts to find novel predictive biomarkers in addition to PD-L1 for *KRAS* mutant patients will help to tailor treatment in this specific population and offer them rationally designed therapeutic strategies combining both IT-based regimens with targeted therapy.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Germans Trias i Pujol Institutional Review Board (PI-19-275). The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from a by- product of routine care or industry. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

LN: Conceptualization; Data curation; Formal analysis; Writing: original draft; review & editing. MCu: Formal analysis; Writing: original draft; review & editing. GC: review & editing. CS: Data curation; Formal analysis, review & editing. EC: Conceptualization; review & editing. AM-M: Data curation; Formal analysis, review & editing. AH: review & editing. MD: review & editing. TM: review & editing. MS-C: review & editing. MCo: review & editing. J-LM: review & editing. AE: Data curation; Formal analysis; review & editing. MS: Conceptualization; Data curation; Formal analysis; Writing: original draft; review & editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

TM reports Consulting/Advisory Role fees by Roche, Bristol Myers, Boehringer, and Astra Zeneca; Research Funding Grant by Kyowa Kirin and Janssen, all of them unrelated with the current work. MS reports a sponsored research agreement with Merck Serono and Roche Farma outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Case Report: Case series: association between blood concentration and side effects of sotorasib

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Introduction: Sotorasib is a crucial therapeutic agent for patients with non-small cell lung cancer (NSCLC) harboring the KRAS p.G12C mutation. Despite its efficacy, the relationship between blood sotorasib concentrations and side effects remains largely unexplored.

Methods: This study enrolled five patients with KRAS p.G12C-positive NSCLC treated with sotorasib (LUMAKRAS® Tablets, Amgen, Japan) between July 2022 and February 2023 at Asahikawa Medical University Hospital. Blood sotorasib levels were monitored, and their association with adverse events was examined, with no adjustments made to drug dosages based on these levels.

Results: Variable blood sotorasib levels were observed among the participants. Notably, one patient developed interstitial pneumonitis, although a definitive attribution to sotorasib was uncertain due to prior pembrolizumab treatment. The study revealed no consistent association between blood sotorasib levels and adverse events or therapeutic outcomes, with some patients experiencing severe side effects at higher concentrations, while others did not.

Conclusion: Preliminary findings suggested that monitoring blood sotorasib levels may aid in anticipating adverse events in this small cohort. However, future studies with larger sample sizes and extended follow-up periods are required to validate these initial observations. Such studies could potentially offer insights into personalized dosing strategies, thereby mitigating adverse effects and enhance patient care for individuals with KRAS p.G12C-positive NSCLC.

KEYWORDS

blood level, Kirsten rat sarcoma viral oncogene homologue, patients, side effect, sotorasib

1 Introduction

Activating mutations in the Kirsten rat sarcoma viral oncogene homologue (*KRAS*) are frequently reported in human cancers (1). Among *KRAS* mutations, the p.G12C mutation occurs in 13–15% of patients with non-small cell lung cancer (NSCLC) (2–4). Sotorasib (AMG510) is a small molecule that irreversibly and selectively inhibits *KRAS* p.G12C tumors (5).

Sotorasib has been reported to significantly increase progression-free survival in patients with advanced NSCLC harboring the *KRAS* p.G12C mutation who had been previously treated with other anticancer drugs. While most adverse events associated with sotorasib were tolerable, 56 of 171 (33%) patients experienced grade 3 or worse adverse events that required drug discontinuation or dose reduction (6). As adverse events are evaluated based on the Common Terminology Criteria for Adverse Events (CTCAE), nausea and gastrointestinal symptoms (which are frequently observed with sotorasib) may not be accurately reflected because they are based on subjective findings, unlike objective findings, such as blood toxicity. Therefore, early detection and management of these adverse events are important for the optimal use of sotorasib.

Although previous studies have indicated an association between blood afatinib maleate levels and treatment-related adverse events in NSCLCs exhibiting epidermal growth factor receptor (*EGFR*) mutations (7, 8), the association between sotorasib blood concentration and treatment-related adverse events in patients with NSCLC with the *KRAS* p.G12C mutation has not been previously reported. This study investigated the association between sotorasib blood concentration and related side effects in five patients with NSCLC harboring the *KRAS* p.G12C mutation.

2 Case description

2.1 Patients

This study included patients who received sotorasib (LUMAKRAS® Tablets, Amgen, Japan) for the treatment of NSCLC at Asahikawa Medical University Hospital between July 2022 and February 2023. All five patients were diagnosed with KRAS p.G12C-positive NSCLC using the Therascreen polymerase chain reaction kit (QIAGEN, Hilden, Germany).

2.2 Sotorasib administration

Sotorasib was administered at 960 mg/day. When grade ≥3 side effects were observed, sotorasib administration was discontinued. When the side effect severity decreased to grade 1, the dose was reduced by half, and sotorasib administration was restarted. Administration was discontinued in cases of pneumonitis. The minimum sotorasib dose was 240 mg/day.

2.3 Evaluation of sotorasib efficacy and side effects

The chief physician evaluated sotorasib effectiveness based on the Response Evaluation in Solid Tumors (Japan Oncology Group version) criteria. Sotorasib-related side effects were assessed according to CTCAE version 5.0.

2.4 Determination of blood sotorasib (AMG 510) levels

Blood samples were collected at least 24 h after the last dose of sotorasib. Blood was collected and centrifuged for the immediate preparation of serum, which was stored at -80°C until analysis. Serum AMG 510 levels were measured using a liquid chromatography-tandem mass spectrometer (API 3200 LC-MS/MS system, Framingham, MA, USA), according to a previous report (9). Chromatographic separation of AMG 510 and internal standard (IS) was achieved on an L-column3 C18 (Chemicals Evaluation and Research Institute, Tokyo, Japan) column (50 × 2.1 mm, 3 μm) maintained at 40°C using a mobile phase containing 0.2% formic acid and acetonitrile (25:75, v/v). The flow rate was 0.65 mL/min, and the injection volume was 10 μL. Mass spectrometry was performed using positive electrospray ionization for quantification of AMG 510 and IS. Detection was via multiple reaction monitoring. Mass transitions (precursor ion-product ion) m/z 561.3–133.9 and 566.3–98.1 were monitored for AMG 510 and IS, respectively. The lower limit of quantification was set at 50 ng/mL, and the final value was corrected with a calibration curve.

2.5 Patient characteristics

The patient characteristics are presented in Table 1. There were three and two female and male patients, respectively (mean age, 79.6 years). The mean height, weight, body surface area, and body mass index were 153.9 cm, 51.6 kg, 1.47 m^2, and 21.9 kg/m^2, respectively. All patients received sotorasib as a second-line therapy. The median follow-up duration was 7.0 months. Sotorasib administration resulted in a partial response in four patients and stable disease in one patient with an overall response rate of 80%. A total of 11 samples were collected from the five patients, and the blood sotorasib levels were analyzed. The values after correction by the calibration curve were defined as negative when <100 ng/mL and positive when ≥100 ng/mL. The data obtained below the lower limit of quantification were treated as 50 ng/mL. The mean and 95% confidence interval (CI) for each group were as follows: mean ± standard error of the mean (SEM) for the positive group was 2085 ± 1460, n=3; mean ± SEM for the negative group was 66.64 ± 8.147, n=8 (95% CI, 192.2–3845) (Figure 1). Patients 1, 2, and 3 exhibited blood sotorasib levels <100 ng/mL. Patients 1 and 2 did not experience side effects that necessitated sotorasib discontinuation (Figure S1, 2). Although Patient 3 developed drug-induced

TABLE 1 Patient background characteristics.

Case	1	2	3	4	5
Age (years)	84	82	76	78	78
Sex	Female	Female	Male	Male	Female
Height (cm)	144.2	154.2	161.5	162.8	147
Weight (kg)	56.5	40	66.9	44.9	49.8
BSA (m^2)	1.46	1.329	1.71	1.45	1.41
BMI (kg/m^2)	27.2	16.8	25.6	16.9	23
Performance status	0	0	0	3	0
Histology	Adeno	Adeno	Adeno	Sarcomatoid carcinoma	Adeno
KRAS mutation	G12C	G12C, G13C	G12C	G12C	G12C
PD-L1 (22C3)	<1%	5%	20%	<1%	<1%
Clinical stage	IVA	IVB	IIIC	IVB	IVB
Metastasis	Pulmonary	Pulmonary bone	–	Brain, bone, gastric, adrenal gland, skin	Bone
Treatment line	2	2	2	2	2
First-line treatment	PEM	PEM	CBDCA+PEM+Pemb	CBDCA+PTX+Pemb	CBDCA+PEM
Sotorasib administration period (days)	211	210	277	On-going	134
PFS (months)	6.9	4	9	2	4.6
OS (months)	6.9	4	9	2.5	5.4
eGFR ($mL/min/1.73\ m^2$)	68	54.6	47.1	95.4	35.2
Alb (IU/L)	3.8	3.7	4.1	3.1	3.5
Best overall response	PR	PR	PR	SD	PR
Side effects	none	none	interstitial pneumonitis (suspicion of pembrolizumab) Grade 3	none	nausea/vomiting Grade 2 fatigue Grade 4

Adeno, adenocarcinoma; Alb, albumin; BMI, body mass index; BSA, body surface area; CBDCA, carboplatin; eGFR, estimated glomerular filtration rate; KRAS, Kirsten rat sarcoma viral oncogene homologue; OS, overall survival; PD-L1, programmed death-ligand 1; PEM, pemetrexed; Pemb, pembrolizumab; PFS, progression-free survival; PR, partial response; PTX, paclitaxel; SD, stable disease.

interstitial pneumonitis and required the discontinuation of sotorasib, this side effect could not be conclusively attributed to sotorasib due to prior pembrolizumab therapy. Despite sotorasib levels being below the detection sensitivity in blood samples taken after discontinuation, pembrolizumab (up to 3.504 μg/m) was detected in Patient 3's samples, even though it had been approximately 6 months since the last dose (Figure S3). Patient 4 exhibited a high blood level of 565.5 ng/ml of sotorasib in a blood draw 4 weeks after initiation, with no apparent side effects observed during the observation period (Figure S4).

2.6 Treatment course in patient 5

Patient 5 was a 78-year-old woman who presented with pneumonia that did not respond to antibiotics. Computed tomography (CT) revealed consolidation of the right lung; a biopsy was performed, and the diagnosis was invasive mucinous adenocarcinoma (cT4N0M0 c Stage IIIA harboring the *KRAS* p.G12C mutation and programmed death-ligand 1 [22C3] positivity at <1%). The patient was initially treated with carboplatin and pemetrexed. After three treatment cycles, renal dysfunction occurred, and chemotherapy was discontinued. Nine weeks after the last dose of chemotherapy, CT revealed an enlarged right lung lobe cancer, new metastatic mediastinal lymph nodes, and bone metastases. Four months after the initiation of the first treatment, second-line sotorasib therapy (960 mg once daily) was initiated. Eleven days after the initiation of oral medication, a chest radiograph showed tumor regression in the right lung, indicating a partial response (Figure 2). The blood sotorasib level on day 68 after administration was 656 ng/mL. On day 103 of sotorasib administration, the patient experienced grade 2 nausea; therefore, the dose was subsequently reduced to 480 mg

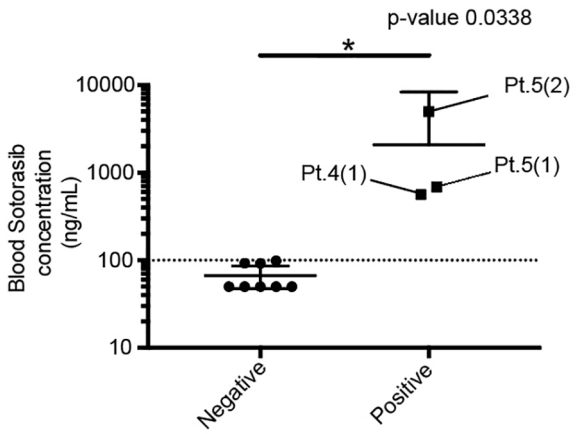

FIGURE 1
Comparison of the blood sotorasib concentration between the positive and negative groups. Each of the three patients in the positive group was assigned a patient number (Pt.). The numbers in parentheses represent the sequence of blood samples taken from the patients. *P < 0.05.

once daily. Despite dose reduction, blood sotorasib levels were as high as 5055 ng/mL 3 weeks later. She concurrently took multiple oral medications during the period of treatment with sotorasib, but no medications were identified as suspects for elevating her blood levels.

Sotorasib administration was terminated 4 weeks after dose reduction owing to disease progression in the re-enlarged mediastinal lymph nodes. The blood sotorasib level at 10 days after discontinuation was <50 ng/mL. At 4.5 months after sotorasib treatment, the patient was hospitalized for pneumonia and severe dehydration. Despite therapeutic intervention after admission, the patient's general condition gradually deteriorated, and the patient died 5.3 months after sotorasib treatment. The total duration of sotorasib treatment was 134 days. A pathological autopsy was performed, and the results confirmed death due to advanced lung cancer.

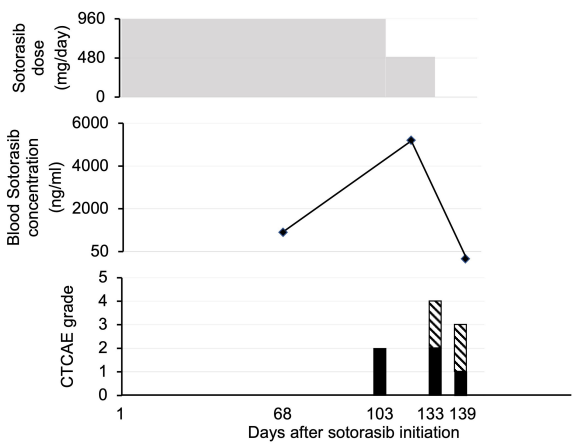

FIGURE 2
Patient 5. Course of sotorasib administration, blood sotorasib levels, and associated adverse events. In the side effect graph, the black bar represents nausea/vomiting, while the hatched pattern indicates fatigue. CTCAE, Common Terminology Criteria for Adverse Events.

3 Discussion

To our knowledge, no prior studies have examined the association between the blood sotorasib levels and side effects in patients with *KRAS* p.G12C-positive NSCLC in a clinical setting in Japan. Notably, drug dosages were not adjusted based on blood sotorasib levels as there is currently no evidence to support this approach.

We observed variable blood sotorasib levels among the five patients. The approved starting dose of sotorasib was 960 mg once daily. The pharmacokinetic profile of this regimen was as follows: the maximum plasma concentration was 7500 ng/mL (coefficient of variation: 98.3%), the median time to maximum plasma concentration was 2.0 (range: 0.3–6.0) h, and the mean (± standard deviation) elimination half-life was 5.5 ± 1.8 h (10). Patients 4 and 5 exhibited blood sotorasib levels >500 ng/mL 24 h after the dose. While Patient 5 experienced grade 2 nausea and fatigue, which led to sotorasib discontinuation, Patient 4 did not report any severe adverse events. In the CodeBreaK100 trial, treatment-related side effects led to dose modification of sotorasib (dose interruption, reduction, or both) in 22.2% of the patients and therapy discontinuation in 7.1% (11). We identified the potential utility of measuring blood sotorasib levels (particularly blood trough levels) after administration, which might serve as a predictor of side effects. Further studies are needed to validate this hypothesis and examine whether dose reduction or discontinuation is warranted in cases with a significant elevation in blood sotorasib levels. In addition, it will be important in future studies to ascertain the appropriate timing for the initial blood draw when monitoring potential side effects.

The study limitations include the single-center design, small sample size, and short follow-up duration. Consequently, further investigations with larger patient cohorts and extended follow-up durations are necessary to validate our findings and expand the potential clinical applications of blood sotorasib level monitoring.

In conclusion, the assessment of blood sotorasib concentrations is beneficial for monitoring adverse events as they may serve as a predictive marker for the emergence of side effects. If this hypothesis is valid, it would be advisable to consider dose reduction or discontinuation in cases where a significant elevation in blood sotorasib levels is observed.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study protocol was approved by the institutional review board of Asahikawa Medical University on July 11, 2022 (approval number: 22031) and conducted in accordance with the principles of the Declaration of Helsinki. All patients provided informed consent for blood collection and the use of their clinical data for analysis and publication. Written informed consent was obtained from the patients/participants for the publication of this case report.

Author contributions

RS: Data curation, Visualization, Writing – original draft. RY: Conceptualization, Methodology, Supervision, Visualization, Writing – review & editing. AY: Investigation, Writing – review & editing. KaN: Investigation, Writing – review & editing. TN: Investigation, Writing – review & editing. HY: Investigation, Writing – review & editing. KiN: Investigation, Resources, Writing – review & editing. TT: Investigation, Resources, Writing – review & editing. RK: Investigation, Writing – review & editing. YU: Investigation, Writing – review & editing. CM: Investigation, Resources, Writing – review & editing. YM: Investigation, Supervision, Writing – review & editing. HS: Formal Analysis, Methodology, Writing – review & editing. KI: Formal Analysis, Methodology, Writing – review & editing. YH: Formal Analysis, Methodology, Writing – review & editing. MF: Formal Analysis, Methodology, Supervision, Writing – review & editing. TS: Conceptualization, Project administration, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1269991/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Patient 1. Course of sotorasib administration, blood sotorasib levels, and associated adverse events. Patient 1 was transferred to another hospital 14 days after the initiation of sotorasib treatment; hence, we were unable to track their subsequent clinical progress.

SUPPLEMENTARY FIGURE 2
Patient 2. Course of sotorasib administration, blood sotorasib levels, and associated adverse events. It has been over a year since the initiation of sotorasib; however, Patient 2 did not experience any adverse events.

SUPPLEMENTARY FIGURE 3
Patient 3. The course of sotorasib dose, blood sotorasib levels, and associated adverse event. In the side effect graph, shaded bars indicate interstitial pneumonitis.

SUPPLEMENTARY FIGURE 4
Patient 4. Course of sotorasib administration, blood sotorasib levels, and associated adverse events. Patient 4 did not experience any adverse events.

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TP53 co-mutations in advanced lung adenocarcinoma: comparative bioinformatic analyses suggest ambivalent character on overall survival alongside *KRAS*, *STK11* and *KEAP1* mutations

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Background: Recently, we could show that the co-mutations of *KRAS* + *KEAP1*, *STK11* + *KEAP1* and *KRAS* + *STK11* + *KEAP1* lead to a significantly shorter median overall survival (mOS) in patients with lung cancer across treatments by analyzing multiple dataset. *TP53*, a tumor suppressor gene, plays a crucial role in regulating cell cycle progression. Its mutations occur in approximately 40-50% of non-small lung cancer (NSCLC). Co-occurrence of all four mentioned mutations has been a matter of debate for years. The aim of this study was to assess the distribution of these four mutations and the influence of the different co-mutational patterns on survival.

Methods: We present a comparative bioinformatic analysis and refer to data of 4,109 patients with lung adenocarcinoma (LUAD).

Results: Most of the mutations within the LUAD belong to *TP53*-only (29.0%), quadruple-negative (25.9%) and *KRAS*-only (13.4%). Whereas TP53-mutations seem to have protective effects in the context of further *KEAP1*- and *KRAS* + *KEAP1*-alterations (improved mOS), their role seems contrary if acquired in an already existing combination of mutations as *KRAS* + *STK11*, *KRAS* + *STK11* + *KEAP1* and *STK11* + *KEAP1*. *TP53* co-mutations had a negative influence on *KRAS*-only mutated LUAD (mOS reduced significantly by more than 30%).

Discussion: These data underline the need for complex mutational testing to estimate prognosis more accurately in patients with advanced LUAD.

KEYWORDS

NSCLC, lung adenocarcinoma, *KRAS*, *STK11*, *KEAP1*, *TP53*, co-mutations, survival

1 Introduction

Lung Cancer is the leading cause of cancer death worldwide, with non-small lung cancer (NSCLC) representing the largest group. Lung adenocarcinoma (LUAD) belongs to the most common and best studied histological subgroups (1). Besides the common treatment strategies consisting of surgery, radiation and chemotherapy, the development and approval of targeted therapies and immune checkpoint inhibitors (ICI) explicitly improved therapy options and patients' outcome within the past decade. However, treatment responses still vary in a wide range even for the personalized treatment options available (2). Therefore, amongst others, one major need is to acknowledge the significance of genetic co-alterations and their influence on therapy responses.

KRAS (Kirsten rat sarcoma viral oncogene homolog) plays a key role in the development and progression of various cancers, including NSCLC. Mutations occur in about 25% of cases, leading to the constitutive activation of *KRAS* signaling pathways, promoting uncontrolled cell growth and survival (3). *KRAS*-altered NSCLC frequently show co-mutations within the genes Kelch-like ECH-associated protein 1 (*KEAP1*) and serine/threonine kinase 11 (*STK11*), also known as liver kinase B1 (*LKB1*) (4). Recently, we could show, by analyzing multiple datasets, that the co-mutations of *KRAS* + *KEAP1*, *STK11* + *KEAP1* and *KRAS* + *STK11* + *KEAP1* lead to a significantly shorter median overall survival (mOS) across treatments. In contrast, patients with tumors harboring only *KRAS* mutations or being negative for all above-mentioned alterations show a significantly improved mOS in a multivariate analysis. Furthermore, triple co-mutated primary tumors showed a significantly increased frequency of distant metastases to bone and adrenal glands (5). Thus, analysis of the complex mutational network seems inevitable in the clinical routine setting.

TP53 (tumor protein p53), a tumor suppressor gene, plays a crucial role in regulating cell cycle progression, DNA repair, as well as apoptosis and is one of the most common alterations among all cancers, and among LUAD in particular (6). Its mutations occur in approximately 40-50% of NSCLC cases, leading mainly to a loss of function, allowing cells to evade normal regulatory mechanisms and promoting tumorigenesis (7).

Co-occurrence of all four mentioned mutations has been a matter of debate for years: Aredo et al. (8) described concurrent pathogenic mutations of *KRAS* with *TP53* (39%), *STK11* (12%) and *KEAP1* (8%), discussing distinct molecular subtypes (study with a total of 186 patients). Furthermore, they could show that combined KRAS *G12C* and *TP53* mutations predict benefit from immunotherapy.

Frost et al. focused on 119 patients with lung adenocarcinoma receiving pembrolizumab monotherapy as first-line palliative treatment. Here, rates for *KRAS*, *TP53* and combined mutations were 52.1%, 47.1% and 21.9%, respectively. Whereas, *TP53* mutations alone had no impact on response and survival, a subgroup (12 patients) with *KRAS G12C* + *TP53* co-mutations defined long-term responders to immunotherapy (9).

Recently, Proulx-Rocray et al. described 100 patients with known *KRAS* status. They postulated that *KRAS* mutation in NSCLC might be associated with a favorable response to ICI therapy in the absence of a concurrent mutation in the *STK11* and/or *KEAP1* tumor suppressor genes (10). A survival advantage associated with *TP53* mutation in NSCLC treated with ICIs has been reported in current literature (9, 11–13).

However, the above-mentioned studies only encompass a low number of patients. Furthermore, besides ICI, "classical" chemotherapy still presents the main cornerstone of therapy. Therefore, we here present a comprehensive bioinformatic analysis encompassing two datasets retrieved from cBioPortal and tested the influence of *TP53* co-mutations depending on the *KRAS*, *STK11* and *KEAP1* status.

The aim of this study was to assess the distribution of the four mutations KRAS, STK11, KEAP1, and *TP53* as single mutations as well as co-mutations in patients with advanced LUAD in a large dataset. Furthermore, we want to study the influence of the different co-mutational combinations on survival.

2 Materials and methods

For this study, the following two datasets from the MSK institute were retrieved from cBioPortal (14, 15): the "MSK-IMPACT Clinical Sequencing Cohort (MSKCC, Nat Med 2017)" (16) and the "MSK MetTropism (MSK, Cell 2021)" dataset (17). To create one dataset across treatments the two datasets were merged into one "MSK across treatments" dataset (N = 4,855 NSCLC patients, 4,109 LUAD patients, 542 lung squamous cell carcinoma (LSCC) patients). Therapy details were not annotated. Due to the more recent data, data from the "MSK MetTropism (MSK, Cell 2021)" were preferred over the "MSK-IMPACT Clinical Sequencing Cohort (MSKCC, Nat Med 2017)". All data comprised patients with advanced tumor stages (mainly stage IV). Analyses were performed on LUAD data.

In total, 16 combinatory groups of patients were established based on the four genes *KRAS*, *STK11*, *KEAP1*, and *TP53* (Table 1, Figures 1, 2): quadruple negative, *KRAS*-only, *STK11*-only, *KEAP1*-only, *TP53*-only, *KRAS* + *TP53*, *STK11* + *TP53*, *KEAP1* + *TP53*, *KRAS* + *STK11*, *KRAS* + *STK11* + *TP53*, *KRAS* + *KEAP1*, *KRAS* + *KEAP1* + *TP53*, *STK11* + *KEAP1*, *STK11* + *KEAP1* + *TP53*, *KRAS* + *STK11* + *KEAP1*, *KRAS* + *STK11* + *KEAP1* + *TP53*.

Statistical analyses were performed in Python (v.3.9.). All p-values were corrected for multiple testing using false discovery rates (q-value) and q-values < 0.05 were defined as significant. The Kaplan-Meier method was performed to calculate OS curves and medians. Pairwise differences were calculated by log-rank tests.

3 Results

3.1 Demographics and incidences of (co-) mutations in NSCLC and LUAD

The total dataset consisted of 4,855 NSCLC patients (male: 41.7%; female: 58.3%). Thereby, 84.6% (N = 4,109) were lung adenocarcinoma, 11.2% (N = 542) lung squamous cell carcinoma, and 4.2% (N = 204) other histologic types of lung cancer: e.g.

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TABLE 1 Distribution of co-mutations in the genes of KRAS, STK11, KEAP1, and *TP53*, and overall survival in patients with LUAD.

(Co-) mutations	LUAD				
	N	%	mOS (months)	lower 95% CI (months)	upper 95% CI (months)
Total	**4,109**	**100**			
TP53-only	1,193	29.0	30.0	26.88	33.96
Quadruple-negative	1,062	25.9	64.0	59.64	82.56
KRAS-only	552	13.4	56.5	46.8	76.08
KRAS + *TP53*	384	9.4	38.3	30.72	49.44
KEAP1 + *TP53*	123	3.0	52.2	27.84	NR
KRAS + *STK11* + *KEAP1*	172	4.2	12.4	8.88	16.08
KRAS + *STK11*	139	3.4	53.0	35.64	83.64
STK11 + *TP53*	84	2.0	36.8	23.4	NR
STK11 + *KEAP1* + *TP53*	79	1.9	14.8	9.36	19.56
STK11 + *KEAP1*	79	1.9	25.1	13.8	35.28
STK11-only	60	1.5	32.3	24.36	50.52
KRAS + *STK11* + *TP53*	45	1.1	23.0	15.12	54.36
KRAS + *KEAP1*	44	1.1	16.1	6.6	22.8
KRAS + *KEAP1* + *TP53*	35	0.9	NR	13.8	NR
KRAS + *STK11* + *KEAP1* + *TP53*	33	0.8	8.6	3.96	15.12
KEAP1-only	25	0.6	21.1	3.96	41.64

(Co-)mutations listed in rows are sorted according to their frequency. Quadruple negative signifies that within the tumor, no mutations in the genes of *KRAS*, *STK11*, *KEAP1*, and *TP53* were found. CI, confidence interval; LUAD, lung adenocarcinoma, mOS, median overall survival; N, number of patients, NR, not reached.

adenosquamous carcinoma, sarcomatoid lung cancer, lung neuroendocrine tumors (large cell neuroendocrine carcinoma, carcinoids), or not otherwise specified NSCLC. For the following analyses we concentrated on lung adenocarcinoma data. Thereby, we found the following mutation frequencies: 45.6% *TP53*, 34.17% *KRAS*, 16.82% *STK11*, 14.35% *KEAP1*. Further, *KRAS* mutations showed the subsequent distribution of point mutations: G12C 40.17%, G12V 17.32%, G12D 13.76%, G12A 7.59%, Q61H 4.26%, G13C 3.62%.

TP53-only mutation (29%; N = 1193), the absence of the four mutations (quadruple-negative: 25.9%; N = 1,062) and *KRAS*-only (13.4%; N = 552) were the most prevalent (co-)mutational patterns. The least prevalent (co-)mutational patterns were *KEAP1*-only (0.6%; N = 25) and the quadruple mutation (*KRAS* + *STK11* + *KEAP1* + *TP53*; 0.8%; N = 33). Full data are shown in Table 1 and Figure 1.

While mutations in *KRAS*, *STK11* and *KEAP1* significantly co-occurred among themselves ($q < 0.05$), there was neither a significant co-occurrence nor a significant mutual exclusivity between mutations in one of the three genes and *TP53* mutations.

3.2 *TP53* mutations influence overall survival for better or worse depending on co-mutations

Kaplan-Meier curves were calculated for all 16 mutation groups and are shown in Figure 2. Quadruple negative (mOS = 64 months), *KRAS*-only (56.5 months) and *KRAS* + *STK11* (mOS = 53 months) mutated patients had the longest mOS, while patients mutated in *KRAS* + *STK11* + *KEAP1* + *TP53* (mOS = 8.6 months), *KRAS* + *STK11* + *KEAP1* (mOS = 12.4 months) and *STK11* + *KEAP1* + *TP53* (mOS = 14.8 months) showed the shortest mOS (Figure 2, Table 1). To determine the influence of *TP53* co-mutations on *KRAS*-, *STK11*- and/or *KEAP1*-mutated LUAD, pairwise tests were performed (Figure 3).

Co-mutations in *TP53* led to significantly reduced mOS in LUAD patients harboring only a *KRAS* mutation (mOS: 56.5 vs. 38.3 months; $p = 0.0026$; $q = 0.021$; Figure 3A) or a co-mutation in *KRAS* + *STK11* (mOS: 53.0 vs. 23.0 months; $p = 0.032$; $q = 0.085$; Figure 3E), albeit, significance for the latter does not remain after correcting for multiple testing. The well-known negative impact of the KRAS + STK11 + KEAP1 (mOS: 12.4 months) and *STK11* + *KEAP1* mutation co-occurrence (mOS: 25.1 months) worsened mOS by trend through an add-on *TP53* mutation (mOS: 8.6 or 14.8 months, respectively; Figures 3G, H); however, not statistically significant.

In contrast, concurrent *TP53* mutations to *KEAP1*-only and to *KRAS* + *KEAP1* mutations showed an opposite effect and led to an improved mOS: 21.1 months for *KEAP1*-only improved to 52.2 months for *KEAP1* + *TP53* ($p = 0.03$; $q = 0.085$; Figure 3C) and 16.1 months for *KRAS* + *KEAP1* improved to a mOS which did not reach the median for *KRAS* + KEAP1 + TP53 ($p = 0.053$; $q = 0.1$; Figure 3F).

When considering only *KRAS* mutations harboring the G12C alteration, the add-on *TP53* mutation still led to reduced mOS (85.7 vs. 36.5 months; $p = 0.02$; $q = 0.08$), albeit without significance after correcting for multiple testing, while for *KRAS* (G12C) + KEAP1, the *TP53* co-mutation still did not significantly change mOS (20 months vs. NR; p-value = 0.25; $q = 0.5$) (Table 2). The occurrence of *TP53* co-mutation in *KRAS* (G12C) + STK11 altered LUAD did not lead to a reduced mOS anymore (49 vs. 54 months; $p = 0.87$; $q = 0.87$). This is also true for *KRAS* G12C + *STK11* + *KEAP1* (18.7 vs. 8.6 months; $p = 0.48$; $q = 0.64$). These results must be interpreted with caution due to partly small group sizes ($N < 20$; Table 2).

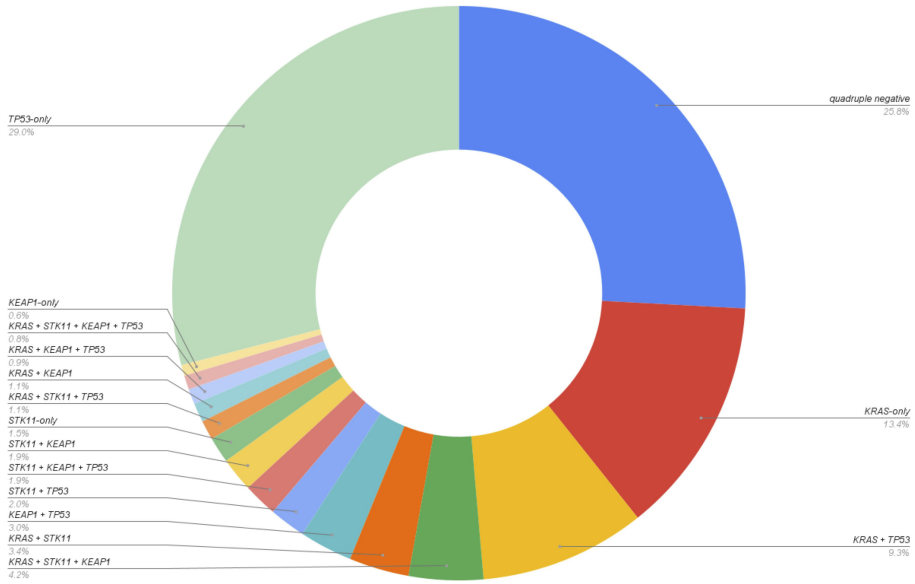

FIGURE 1
Distribution of mutation groups in the LUAD dataset (N=4,109).

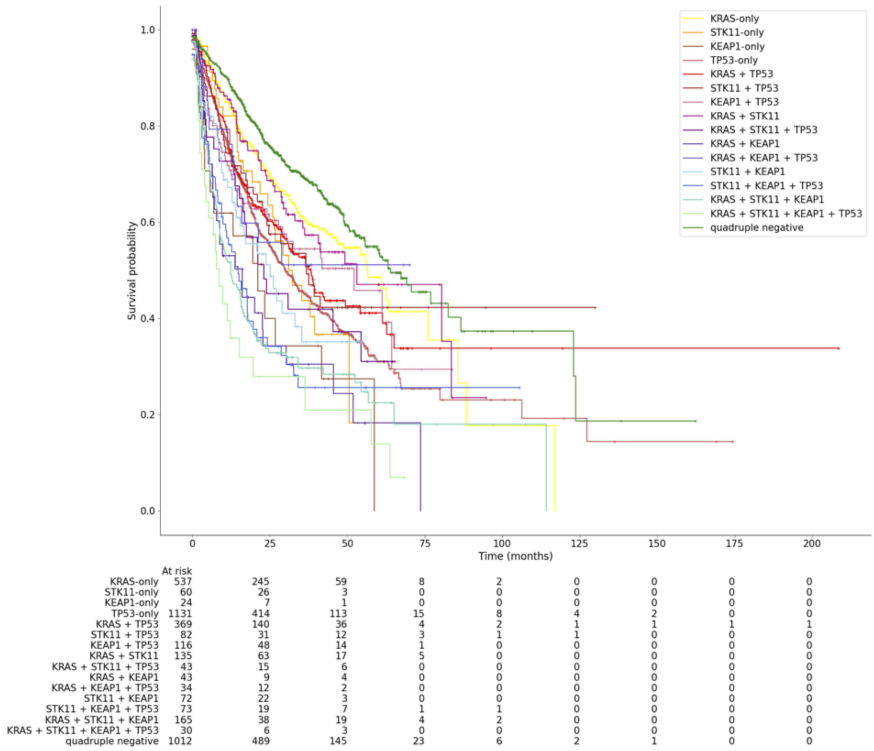

FIGURE 2
Kaplan Meier curve showing overall survival of LUAD patients among all mutation groups.

4 Discussion

Here, we presented a comparative bioinformatic analysis referring to data of 4,109 patients with the aim of analyzing the influence of *TP53* mutations in *KRAS*, *STK11* and *KEAP1* (co-) mutated LUAD on the patients' overall survival. By employing this database approach, we were able to show that *TP53* mutations had an influence on mOS for better or worse depending on the concurrent mutational pattern.

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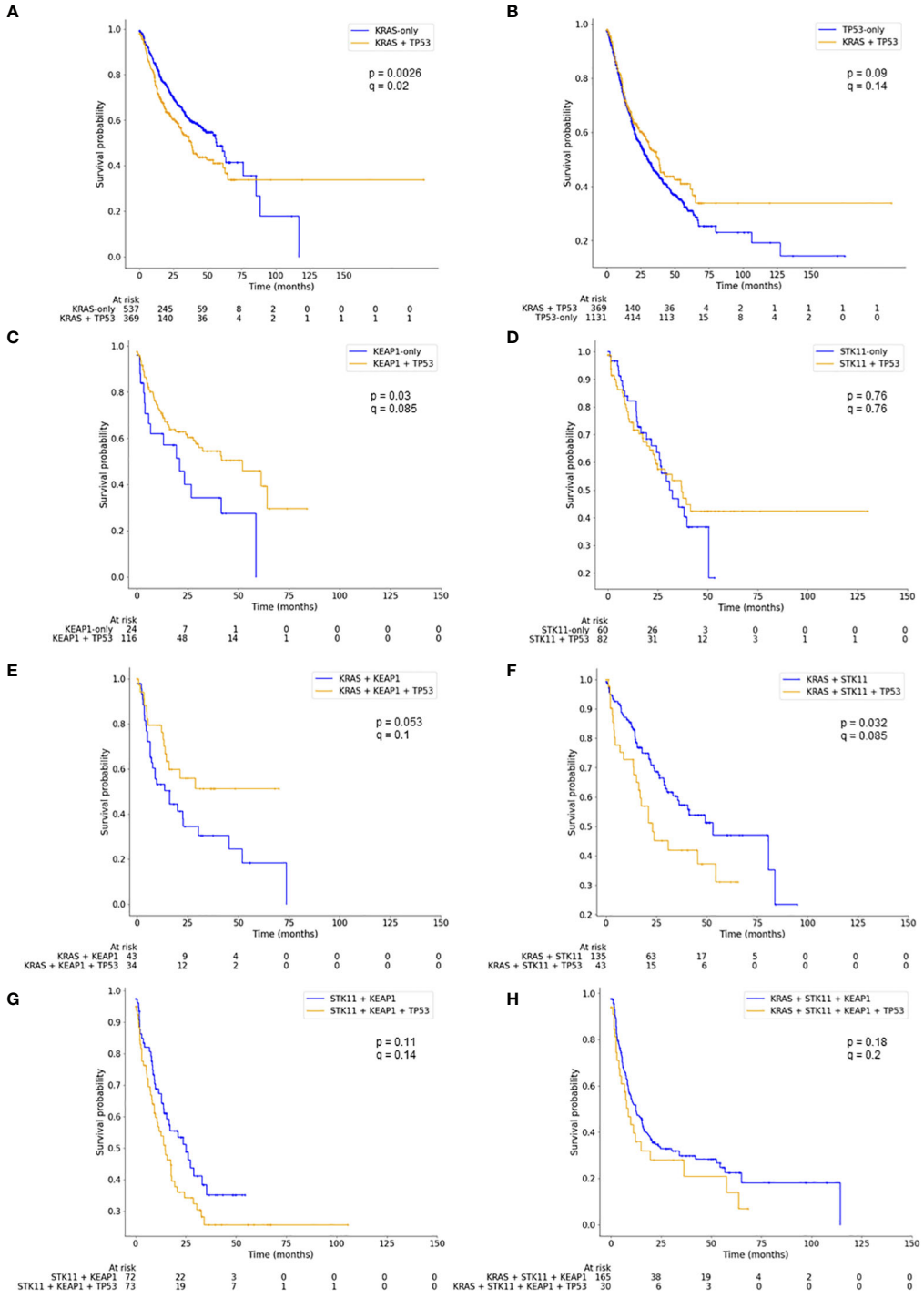

FIGURE 3
Kaplan Meier curves showing pairwise comparison of mutation groups with (blue) and without (yellow) additional *TP53* mutation. Log-rank tests were performed (p-values) and p-values corrected for multiple testing using false discovery rates (q-values). Panel A compares *KRAS*-only with *KRAS+TP53*, panel B *TP53*-only with *KRAS-TP53*, panel C *KEAP1*-only with *KEAP1+TP53*, panel D *STK11*-only with *STK11+TP53*, panel E *KRAS+KEAP1* with *KRAS+KEAP1+TP53*, panel F *KRAS+STK11* with *KRAS+STK11+TP53*, panel G *STK11+KEAP1* with *STK11+KEAP1+TP53*, and panel H *KRAS+STK11+KEAP1* with *KRAS+STK11+KEAP1+TP53*.

Therapy and prognosis of NSCLC has changed in the last 15 years as several treatable targets have been detected within the concept of so-called personalized therapies. In daily practice, these targets encompass testing for rearrangements (*ALK*, *ROS*, *RET*, *NTRK*, *MET*) and mutations (*KRAS*, *EGFR*, *BRAF*, *ERBB2*) (18–20). For a long time *KRAS*-mutations were called "untreatable targets". Since early 2022 a specific (second-line) therapy for *KRAS* G12C has been available in Europe. However, the majority of NSCLC do not

TABLE 2 Log-rank test comparing overall survival of *KRAS* G12C mutation groups with and without *TP53* co-mutation.

Group 1			Group 2 (with *TP53* mutation)			Statistics		
Mutations	N	mOS (months)	Mutations	N	mOS (months)	p-value	Reject	FDR p-value
KRAS G12C only	207	85.7	*KRAS* G12C + *TP53*	157	36.5	0.02	false	0.08
KRAS G12C + *KEAP1*	14	20.0	*KRAS* G12C + *KEAP1* + *TP53*	16	NR	0.25	false	0.50
KRAS G12C + *STK11*	71	49.0	*KRAS* G12C + *STK11* + *TP53*	17	54	0.87	false	0.87
KRAS G12C + *STK11* + *KEAP1*	72	18.7	*KRAS* G12C + *STK11* + *KEAP1* + *TP53*	12	8.6	0.48	false	0.64

FDR, false discovery rate; mOS, median overall survival; N, number of patients; NR, not reached.

harbor the above-mentioned mutations and thus do not qualify for these treatment options. Thus, therapy is still based on different chemotherapy protocols with or without ICIs.

In our study, the mutation frequencies of the four genes (*TP53*: 45.6%, *KRAS*: 34.17%, *STK11*: 16.82%, *KEAP1*: 14.35%) correspond to the generally described values in LUAD (6). Further, as already shown (5), *KRAS*-only and *KRAS* + *STK11* mutated patients have the longest mOS (56.5 and 53 months). This seems also true for the quadruple negative group as presented here (64 months). Nevertheless, it must be noticed that the overall survival of quadruple negative patients might be biased due to further common mutations or genetic rearrangements in genes like *EGFR*, *ALK* or *ROS1* and their already approved targeted therapies. However, the comparable long survival times of these groups, especially for *KRAS*-only, further underlines the fact that the time has passed to describe *KRAS* as a prognostically unfavorable factor. Moreover, it seems more appropriate to analyze the complex surrounding mutational landscape, as patients mutated in *KRAS* + *STK11* + *KEAP1* + *TP53*, *KRAS* + *STK11* + *KEAP1* and *STK11* + *KEAP1* + *TP53* show the shortest overall survival. So far, several studies described *STK11* and *KEAP1* alone or co-mutated with *KRAS* having a negative impact on OS, response to ICI-therapy (4, 5, 21–26) and also across treatment classes independent of immunotherapy (4, 5, 24, 27). However, especially *KEAP1* mutations seemed to be the driving factor being the only one significant in a multivariate model (4). This is also reflected in the current analyses of the CodeBreak 100 clinical trial. Here, *KEAP1* mutations also appear to be a negative prognostic marker for sotorasib (28).

Therefore, it is particularly interesting that this role seems only true if *TP53*-mutations are absent, as patients with the combination of KRAS + KEAP1 + TP53 or KEAP1 + TP53 co-mutations show an improved mOS in comparison to *KEAP1*-only and *KRAS* + *KEAP1* mutated patients. Thus, somewhat surprisingly, in this setting *TP53* mutations seem to have a protective effect as long as *STK11* is not co-mutated. So far, survival advantage of *TP53* mutations could be shown under therapy with ICI (9, 11–13), however not across treatments. Regardless, it must be noticed that these studies did not include *KEAP1* co-mutations into their analyses.

Vice versa, *TP53* will have a negative influence on KRAS-*only* mutated LUAD. Here, the patients' mOS was reduced significantly more than 30% (from 56.5 to 38.3 months, see Table 1, Figure 3A). The negative influence of *TP53* mutations were also visible for the following co-mutations: *KRAS* + *STK11*, *KRAS* + *STK11* + *KEAP1* and *STK11* + *KEAP1* (Figure 3). Therefore, the potential positive or negative impact of the altered tumor suppressor p53 seems to depend on the surrounding mutational network. This effect had already been described by Saleh et al. and Scalera et al., pointing out that molecular stratification of both alterations should be implemented for localized and advanced-stage NSCLC to optimize and modify clinical decision-making (29, 30), even though both studies did not include *KRAS* and/or *STK11* in their investigations.

For *KRAS* G12C, sotorasib, a targeted therapy, is approved and has shown that its efficacy is influenced by the co-mutations of *STK11* and *KEAP1*. While the co-mutation with *STK11* leads to a slightly improved efficacy, *KEAP1* and the co-mutation with both genes result in a reduced response (31). Therefore, we performed our analyses in the context of *KRAS* G12C. Comparable results were observed with a reduced overall survival when *KRAS* G12C (mOS = 85.7 month) is co-mutated with *TP53* (mOS = 36.5; p = 0.02; q = 0.08). The benefits of *TP53* co-mutations shown under ICI were subsequently not apparent across treatments (8, 9). The reported tendency was not shown in the context of KRAS *G12C* + STK11 + TP53. Here again, a somewhat protective effect might be discussed for *TP53* co-mutations in *KRAS* G12C + *KEAP1* mutated patients, albeit group sizes are small and results not significant. This underlines the importance of the now available G12C-targeted therapy and the need for more druggable options in *KRAS*-mutated LUAD and should be kept in mind when interpreting the results of the recently published phase III CodeBreaK 200 trial (32) and the still ongoing phase III CodeBreaK 202 trial (NCT05920356) evaluating sotorasib for the second-line or first-line treatment, respectively.

Finally, we here present a bioinformatic analysis of merged data sets. This might be a limitation at first sight, as we did not refer to our own data. However, the sample sizes of the molecular subgroups, as summarized here, are too small within the single studies to obtain group sizes sufficient for robust statistical results. Thus, integrating database approaches, as presented here, are needed to draw first preliminary conclusions and to generate new hypotheses. These hypotheses must be then tested in future multi-center investigations. This study is further limited by the given annotations. Analyzing the mutation groups in correlation with further clinical variables like age, sex, smoking history, and in particular different treatment patterns is an

important task for future studies. Another point of interest is the assessment of progression-free survival in addition to the overall survival. Nevertheless, our study gives important insights into the mutual influence of co-mutations and provides a starting point for future research approaches.

To conclude, the more mutations are analyzed to a greater extent, the greater will be the complexity of the mutational network of lung cancer and cancer in general. In the daily clinical routine setting, referring to panel-based sequencing (as e.g. suggested by the national network of genomic medicine/nNGM) seems mandatory and focusing on different combinations of mutations can help define different prognostic groups and might be the starting point for new treatment strategies.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

AF: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. HW: Writing – review & editing. MS: Methodology, Writing – review & editing. HB: Funding acquisition, Supervision, Writing – review & editing. ML: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Corrigendum: *TP53* co-mutations in advanced lung adenocarcinoma: comparative bioinformatic analyses suggest ambivalent character on overall survival alongside *KRAS*, *STK11* and *KEAP1* mutations

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NSCLC, lung adenocarcinoma, *KRAS*, *STK11*, *KEAP1*, *TP53*, co-mutations, survival

A Corrigendum on

TP53 co-mutations in advanced lung adenocarcinoma: comparative bioinformatic analyses suggest ambivalent character on overall survival alongside *KRAS*, *STK11* and *KEAP1* mutations

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In the published article, there was a mistake in the **Funding** statement. The original text of the **Funding** statement was unspecific, since financial support was received for the publication costs only.

The first two sentences of the original **Funding** statement read:

"The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This publication was funded by the Open Access Publishing Fund of Leipzig University, supported by the German Research Foundation (DFG)."

The correct **Funding** statement appears below.

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In the published article, there was an error in the **Abstract** section.

The words "in patients with lung cancer" were added after the term "(mOS)".

Abstract, *Background*. This sentence previously stated:

"Recently, we could show that the co-mutations of *KRAS* + *KEAP1*, *STK11* + *KEAP1* and *KRAS* + *STK11* + *KEAP1* lead to a significantly shorter median overall survival (mOS) across treatments by analyzing multiple datasets."

The corrected sentence appears below:

"Recently, we could show that the co-mutations of *KRAS* + *KEAP1*, *STK11* + *KEAP1* and *KRAS* + *STK11* + *KEAP1* lead to a significantly shorter median overall survival (mOS) in patients with lung cancer across treatments by analyzing multiple datasets."

In the published article, there was an error in the **Abstract** section.

The word "*STK1*" was replaced with "*STK11*" at the end of the sentence.

Abstract, *Results*. This sentence previously stated:

"Whereas TP53-mutations seem to have protective effects in the context of further *KEAP1*- and *KRAS* + *KEAP1*-alterations (improved mOS), their role seems contrary if acquired in an already existing combination of mutations as *KRAS* + *STK11*, *KRAS* + *STK11* + *KEAP1* and *STK1* + *KEAP1*."

The corrected sentence appears below:

"Whereas TP53-mutations seem to have protective effects in the context of further *KEAP1*- and *KRAS* + *KEAP1*-alterations (improved mOS), their role seems contrary if acquired in an already existing combination of mutations as *KRAS* + *STK11*, *KRAS* + *STK11* + *KEAP1* and *STK11* + *KEAP1*."

In the published article, there was an error in the **Abstract** section. The word "co-mutationshad" was replaced with "co-mutations had" in the last sentence of the results section.

Abstract, *Results*. This sentence previously stated:

"TP53 co-mutationshad a negative influence on KRAS- only mutated LUAD (mOS reduced significantly by more than 30%)."

The corrected sentence appears below:

"TP53 co-mutations had a negative influence on KRAS- only mutated LUAD (mOS reduced significantly by more than 30%)."

In the published article, there was an error in the **Introduction** section.

The word "KRAS" was italicized.

1 Introduction, Paragraph 5. This sentence previously stated:

"Whereas, *TP53* mutations alone had no impact on response and survival, a subgroup (12 patients) with KRAS *G12C* + *TP53* co-mutations defined long-term responders to immunotherapy (9)."

The corrected sentence appears below:

"Whereas, *TP53* mutations alone had no impact on response and survival, a subgroup (12 patients) with *KRAS G12C* + *TP53* co-mutations defined long-term responders to immunotherapy (9)."

In the published article, there was an error in the **Material and methods** section.

The word "compromise" was replaced with "comprised".

2 Materials and methods, Paragraph 1. This sentence previously stated:

"All data compromise patients with advanced tumor stages (mainly stage IV)."

The corrected sentence appears below:

"All data comprised patients with advanced tumor stages (mainly stage IV)."

In the published article, there was an error in the **Results** section.

The symbol ">" was replaced with "<".

3 Results, *3.1 Demographics and incidences of (co-)mutations in NSCLC and LUAD*, Paragraph 3. This sentence previously stated:

"While mutations in *KRAS*, *STK11* and *KEAP1* significantly co- occurred among themselves ($q > 0.05$), there was neither a significant co-occurrence nor a significant mutual exclusivity between mutations in one of the three genes and *TP53* mutations."

The corrected sentence appears below:

"While mutations in *KRAS*, *STK11* and *KEAP1* significantly co-occurred among themselves ($q < 0.05$), there was neither a significant co-occurrence nor a significant mutual exclusivity between mutations in one of the three genes and *TP53* mutations."

In the published article, there was an error in the **Discussion** section.

The word "mutation" was replaced with "mutational pattern".

4 Discussion, Paragraph 1. This sentence previously stated:

"By employing this database approach, we were able to show that *TP53* mutations had an influence on mOS for better or worse depending on the concurrent mutation."

The corrected sentence appears below:

"By employing this database approach, we were able to show that *TP53* mutations had an influence on mOS for better or worse depending on the concurrent mutational pattern."

The authors apologize for these errors and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Resistance to KRAS inhibition in advanced non-small cell lung cancer

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Lung cancer remains the leading cause of cancer death globally. More than 50% of new cases are diagnosed in an advanced or metastatic stage, thus contributing to the poor survival of such patients. Mutations in the *KRAS* (Kirsten rat sarcoma virus) gene occur in nearly a third of lung adenocarcinoma and have for decades been deemed an 'undruggable' target. Yet, in recent years, a growing number of small molecules, such as the GTPase inhibitors, has been investigated in clinical trials of lung cancer patients harboring *KRAS* mutations, yielding promising results with improved outcomes. Currently, there are only two approved targeted therapies (adagrasib and sotorasib) for advanced or metastatic *KRAS*-mutated NSCLC from the second-line setting onwards. In this narrative review, we will focus on *KRAS*, its molecular basis, the role of its co-mutations, clinical evidence for its inhibition, putative mutation to resistance, and future strategies to overcome resistance to KRAS inhibition.

KEYWORDS

non-small cell lung cancer, lung adenocarcinoma, *KRAS*, co-mutations, resistance to therapy

1 Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide (1). The poor survival rate of lung cancer patients is mainly due to the late stage of disease found in over half of them at the time of diagnosis (2). Therapeutic progress has been achieved in non-small cell lung cancer (NSCLC) through the introduction of immune checkpoint inhibitors (ICI) (3) and personalized treatment strategies against driver mutations within the tumor, including targeted therapy (4). These driver or oncogenic mutations are localized within kinase domains of receptor tyrosine kinases (RTKs) (5) and are not equally distributed among histologic subtypes of NSCLC (6). Most notably, lung adenocarcinoma (LUAD) harbors those driver mutations and rearrangements that can be therapeutically addressed,

such as *EGFR*, *BRAF*, *ALK*, *ROS1*, *RET*, *NTRK*, and also *KRAS* (6, 7). Mutations in the *KRAS* (Kirsten rat sarcoma virus) gene occur in approximately 29–32% of LUAD and, until recently, have been considered to be 'undruggable' for the past several decades (8–10).

In the last few years, an increasing number of small-molecule anti-cancer drugs, the so-called GTPase inhibitors as well as others, has been tested in clinical trials, generating encouraging results with improved efficacy of lung cancer treatment for *KRAS*-mutated NSCLC. Presently, sotorasib and adagrasib are the only approved targeted therapies in locally advanced or metastatic *KRAS*-mutated NSCLC patients, but just in those who have received at least one prior systemic therapy. In this narrative review, we will focus on *KRAS*, its molecular basis, the role of its co-mutations, clinical evidence for its inhibition, putative mutation to resistance, and future strategies to overcome resistance to KRAS inhibition.

2 Molecular basis of *KRAS* as an oncogenic driver in lung cancer

The RAS proto-oncogenes encode intracellular guanine nucleotide binding proteins that belong to the GTPase family harboring a catalytic domain and a hypervariable region (11). The former binds guanine nucleotides and activates signaling while the latter determines how RAS proteins are localized on the cell membrane (11). RAS GTPases control downstream signaling by switching between the active nucleotide guanosine triphosphate (GTP)-bound and inactive nucleotide guanosine diphosphate (GDP)-bound states in response to extracellular signals (11). RAS-GTP commonly activates multiple signaling cascades including the canonical RAS-RAF-MEK-ERK (= mitogen-activated protein kinase, [MAPK]), PI3K-AKT-mTOR, and RAS-like (RAL and tumor invasion and metastasis-inducing protein 1 [TIAM1-RAC1]) pathways (11, 12). The first two signaling pathways are most relevant to tumor biology since they play an essential role in cell cycle regulation, thus cell proliferation, and tumor cell survival (Figure 1).

In contrast to colorectal cancer and pancreatic adenocarcinoma, the point mutation G12C is the most prevalent genetic alteration in the *KRAS* gene of LUAD, occurring in 39% of cases, followed by the point mutations G12V (18.1%), G12D (13.8%), and G12A (7.2%) (13). However, to date, *KRAS* G12C is the only molecular target for which the two therapeutic agents, sotorasib and adagrasib, have been approved in NSCLC. Conversely, 61% of all *KRAS* point mutations in LUAD are still ineligible for targeted therapy.

3 Role of co-mutations with *KRAS*

It is well known that *KRAS* altered NSCLC frequently shows co-occurring mutations with other genes, including tumor protein 53 (*TP53*), serine/threonine kinase 11 (*STK11*), and Kelch-like ECH-associated protein 1 (*KEAP1*), also known as liver kinase B1 (*LKB1*), as well as concurrent amplifications in the *MET* and erb-b2 RTK 2

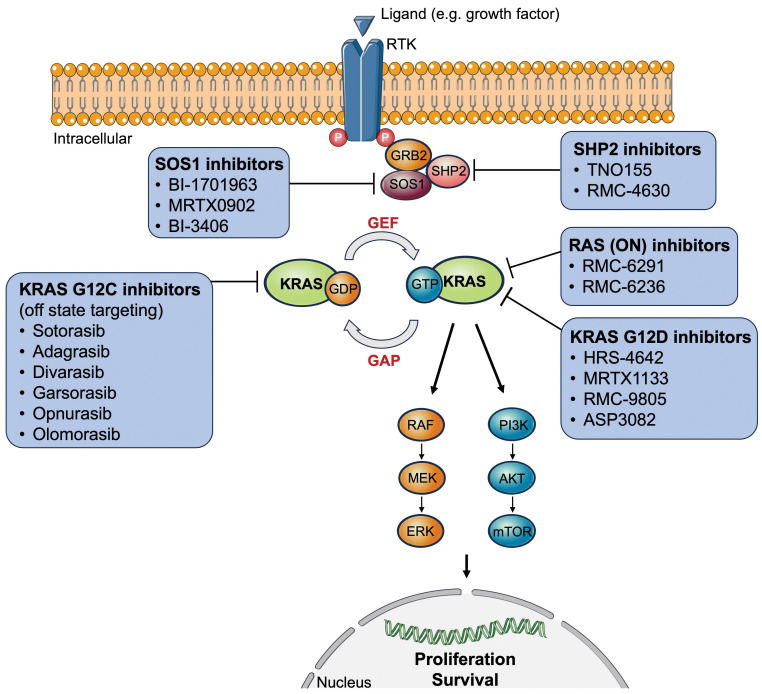

FIGURE 1
Overview of approved or clinically tested (direct/indirect) KRAS-targeted therapy inhibitors. AKT, protein kinase B; ERK, extracellular signal-regulated kinase; GAP, GTPase activating proteins; GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; GRB2, growth factor receptor-bound protein 2; GTP, guanosine triphosphate; KRAS, Kirsten rat sarcoma virus; MEK, mitogen-activated protein kinase kinase; mTOR, mechanistic target of rapamycin; P, phosphorylated tyrosine residues; PI3K, phosphoinositide 3-kinases; RAF, rapidly accelerated fibrosarcoma; RTK, receptor tyrosine kinase; SOS1, son of sevenless 1; SHP2, Src homology region 2 domain-containing phosphatase-2.

(*ERBB2*) genes (9, 14–18). Both the triple (*KRAS* + *KEAP1* + *STK11*) and quadruple (*KRAS* + *KEAP1* + *STK11* + *TP53*) co-mutations have been shown to serve as a negative prognostic and predictive factor compared to the single *KRAS* mutational status (15, 18).

Chapter 9 will further elaborate on co-mutations in the context of mechanisms of resistance to KRAS inhibition.

4 Clinical evidence for KRAS inhibition in *KRAS*-mutant NSCLC

Historically considered undruggable, *KRAS*-mutant NSCLC now has two approved targeted therapies as well as other potential therapeutic agents that are still under clinical development (10, 13, 19–22). This recent milestone in modern medicine was achieved thanks to the discovery of the allosteric regulatory site of KRAS G12C, thereby leading to the design of irreversible covalent inhibitors (23). Such small compounds bind to the switch-II binding pocket of KRAS G12C (24). Previous crystallography studies were paramount in finding molecules capable of interacting with the unique conformation of the KRAS protein (25). A major scientific breakthrough was made in 2013 with the identification of the switch-II pocket of KRAS by the Shokat Lab, resulting in the structure-based validation of direct targeting of the compound binding region of KRAS in a "mutant-specific" and selective manner (26). The stage was set for the optimization of compounds, leading to the creation of the current KRAS G12C inhibitors available for clinical use today (27). By binding specifically to the inactive GDP-bound form of the KRAS oncoprotein in its switch-II pocket, a covalent bond is created with the mutant cysteine residue of KRAS G12C, blocking the reactivation of KRAS by nucleotide exchange (from GDP to GTP) (27, 28). Hence, KRAS G12C inhibitors essentially trap KRAS G12C in an inactive KRAS-GDP state (off state), hindering a switch to the active KRAS-GTP state (on state), and, thereby, impeding oncogenic activity. This has led to improved drug efficacy and selectivity (29). Currently, sotorasib and adagrasib are recommended by the National Comprehensive Cancer Network (NCCN) guidelines as a subsequent treatment option for patients with *KRAS* G12C-mutant NSCLC in second-line or beyond, if no previous KRAS G12C-targeted therapy was given (30). Given their similar mechanism of action, it is not recommended to switch between these two therapeutic agents at the time of progression (30). Table 1 summarizes the efficacy data of KRAS G12C inhibitors from published clinical trials.

The following chapters will give an overview of direct inhibitors of *KRAS* G12C in NSCLC.

5 Sotorasib (AMG 510)

The first drug to enter clinical trials geared toward targeting mutant *KRAS*, sotorasib (previously known as AMG 510), was granted accelerated approval by the U.S. Food and Drug Administration on May 28, 2021, for adult patients with previously treated (immunotherapy and/or chemotherapy) locally advanced or metastatic NSCLC harboring the *KRAS* p.G12C mutation (38, 39). In turn, Health Canada approved this KRAS G12C inhibitor in September 2021 (23), while the European Medicine Agency followed suit in January 2022 (Amgen, 2022). These approvals were based on the results of phase 2 of the CodeBreaK 100 trial (32). Preclinical analyses of sotorasib were very promising, showing inhibition of tumor cell growth in both *in vitro* and murine models (40). Sotorasib first entered clinical trial in 2018, and the results of the phase 1 CodeBreaK 100 trial demonstrated encouraging anticancer activity of sotorasib monotherapy in the NSCLC subgroup as follows: 32.2% had an objective response (complete or partial) rate (ORR), 88.1% had disease control (objective response or stable disease), and the median progression-free survival (PFS) was 6.3 months (31). A durable clinical benefit of monotherapy with daily sotorasib

TABLE 1 Published clinical trials for KRAS G12C inhibitors.

Inhibitor	Study name, phase	Line of treatment	# of patients[2]	Control	ORR[2] (%)	PFS[2] (median months, HR)	OS[2] (median months, HR)	Ref.
Sotorasib	CodeBreaK100, Phase 1	≥2	59	None	32.2	6.3	NA	(31)
	CodeBreaK100, Phase 2	≥2	124	None	37.1	6.8	12.5	(32)
	CodeBreaK200, Phase 3	≥2	171 vs. 174	Docetaxel	28.1 vs. 13.2	5.6 vs. 4.5, 0.66 (P=0.0017)[1]	10.6 vs. 11.3, 1.01 (P=0.53)[1]	(33)
Adagrasib	KRYSTAL-1 Phase 1/2	≥2	116	None	42.9	6.5	12.6	(34) (35),
Divarasib[3]	GO42144, Phase 1	≥2	58	None	60.3	13.1	NR	(36)
Garsorasib	Phase 1	≥2	74 (all doses) 62 (RP2D)	None	40.5 38.7	8.2 7.6	NA NA	(37)

[1]One-sided P-value, [2]KRAS inhibitor versus control, [3]neither approved by FDA nor EMA, #, number; ORR, objective response rate (number of patients with complete response plus partial response); HR, hazard ratio; NA, not available; NR, not reached; PFS, progression-free survival; Ref., reference; RP2D, recommended phase 2 dose; OS, overall survival; vs., versus.

(administered orally at a dose of 960 mg) was confirmed in the phase 2 CodeBreaK 100 trial, showing a 37.1% ORR, median PFS of 6.8 months, and median overall survival (OS) of 12.5 months in *KRAS* p.G12C-mutant advanced NSCLC patients previously treated with standard therapies (Table 1) (32). The two-year pooled analysis of the CodeBreaK 100 phase 1/2 clinical trial showed that almost 25% of these previously treated advanced stage *KRAS* G12C-mutant NSCLC patients derived long-term benefit from additional sotorasib treatment, with few late-onset treatment-related toxicities (41). These results support the continuing clinical use of sotorasib both in the current therapeutic setting and in studies (ongoing and future) examining its potential role in earlier lines of therapy (41).

In the CodeBreaK 200 study, a randomized, open-label, phase 3 trial (June 2020 to April 2021) of sotorasib (n=171) versus docetaxel (n=174) in the second-line setting and beyond of advanced NSCLC patients with *KRAS* G12C mutation, sotorasib significantly increased PFS (i.e., median PFS 5.6 months [95% CI, 4.3–7.8] vs. 4.5 months [3.0–5.7]; hazard ratio 0.66 [0.51–0.86]; p=0.0017) and exhibited a better safety profile (33). In addition, sotorasib elicited a more rapid (1.4 months vs. 2.8 months) and longer response (8.36 months vs. 6.8 months) compared with docetaxel (33). Unfortunately, although PFS, ORR, and disease control rate (DCR) were improved in the sotorasib group, these results were disappointing when compared to the phase 1 and 2 CodeBreaK 100 trials that showed a longer PFS (6.3 and 6.8 months, respectively) and had a similar ORR and DCR (42).

In addition to sotorasib monotherapy, ongoing clinical studies are also investigating sotorasib-based combinations for the possible treatment of pretreated *KRAS* G12C-mutant NSCLC (20). The single-arm, phase-2 SCARLET study enrolled 30 patients with chemotherapy-naïve, advanced non-squamous, *KRAS* G12C-mutant NSCLC between October 2021 and July 2022 (43). Results from this clinical trial were recently presented in June 2023 at the American Society for Clinical Oncology (ASCO) Annual Meeting, and showed a favorable ORR (88.9%; 80% CI, 78.5–94.8%) (n=27) and tolerability (n=29) for sotorasib plus platinum-doublet chemotherapy (carboplatin/pemetrexed). The PFS and OS rates at 6 months were 61.2% and 87.0%, respectively; median PFS was not reached given the shorter follow-up period (median 4.2 months).

Most recently, exciting positive data from the study arm of sotorasib in combination with carboplatin and pemetrexed for *KRAS* G12C-mutant advanced NSCLC in the ongoing, phase 1b, CodeBreaK 101, global clinical trial have further endorsed the approach to repositioning sotorasib with novel therapeutic combinations into earlier lines of therapy within the treatment paradigm (44). These highly anticipated results, based on a median follow-up of 3 months, were presented at the 2023 International Association for the Study of Lung Cancer (IASLC) World Conference on Lung Cancer (2023) on September 10th, 2023, in Singapore. Patients (n=20) treated in the frontline (i.e., first-line) setting experienced a better ORR and DCR than their counterparts (n=13) treated in the second-line setting (ORR 65% vs. 54%, respectively; and DCR 100%; 95% CI: 83.2, 100, vs. 85%; 95% CI: 54.6, 98.1, respectively). Similar ORRs were reported among patients with programmed cell death ligand-1 (PD-L1) expression less than 1% (i.e., 62% vs. 50% in the frontline vs. second-line setting, respectively). Mature PFS and OS data were unavailable. Due to the very promising results from the global CodeBreaK 101 trial, a new multicenter, randomized, open-label, phase 3 study (CodeBreaK 202) of sotorasib plus carboplatin and pemetrexed as frontline therapy of PD-L1 negative, *KRAS* G12C-mutant advanced NSCLC has been recently initiated by Amgen and is currently recruiting patients (enrollment start date: November 26, 2023; estimated study completion date: March 1, 2031) (45).

6 Adagrasib (MRTX849)

Adagrasib is the second approved, orally administered, potent, covalent KRAS G12C inhibitor that selectively and irreversibly binds the switch-II pocket of KRAS G12C (46). Adagrasib was granted accelerated approval by the FDA in December 2022 as a targeted treatment option for locally advanced or metastatic NSCLC with a *KRAS* G12C mutation (47). This decision was based on the results of the ongoing phase 1/2 KRYSTAL-1 clinical trial (Table 1) (34, 35). This multicenter single-arm study included patients with histologically confirmed unresectable or metastatic *KRAS* G12C-mutant NSCLC whose disease progressed with frontline chemotherapy and/or immunotherapy. With respect to efficacy outcome measures, 42.9% (95% confidence interval [CI], 33.5 to 52.6) of the 112 patients with measurable disease at baseline had a confirmed objective response. The median duration of response (DOR) was 8.5 months (95% CI, 6.2 to 13.8) and the median PFS was 6.5 months. Confirmed ORRs were similar across PD-L1 expression subgroups (41.7 to 46.8%). The ORRs in patients with co-mutations in *STK11*, *KEAP1*, *TP53*, and *CDKN2A* ranged from 28.6% (*KEAP1*) to 58.3% (*CDKN2A*). As of January 15, 2022 (median follow-up, 15.6 months), the median OS was 12.6 months (95% CI, 9.2 to 19.2).

Updated, longer follow-up data from the KRYSTAL-1 trial, recently presented on September 10, 2023, at the World Congress on Lung Cancer 2023 (WCLC 2023) in Singapore, confirmed durable clinical activity and benefit of adagrasib in advanced *KRAS* G12C-mutant NSCLC across patient groups, including those with CNS metastases and co-mutations (48). Gadgeel and colleagues presented favorable safety and efficacy data (ORR, DOR, PFS, and OS) from a two-year follow-up pooled analysis of the Phase 1/1b Cohort and Phase 2 Cohort A of KRYSTAL-1. As of January 1, 2023, 132 patients received adagrasib, and showed an ORR of 43.0%, with a median DOR of 12.4 months. The median PFS was 6.9 months (95% CI 5.4–8.7), and the median OS was 14.1 months (95% CI 9.2–18.7). Approximately one in three patients (31.3%) remained alive at two years. Exploratory analyses suggested heterogeneity of clinical benefit based on the presence of co-mutations, requiring further evaluation. The safety profile was consistent with previous reports. A confirmatory, multi-center, randomized Phase 3 study, KRYSTAL-12, evaluating adagrasib monotherapy versus docetaxel in patients with previously treated advanced *KRAS* G12C-mutant NSCLC, is ongoing (Table 2) (51).

It is important to mention that preliminary pharmacodynamics and mechanistic biomarker analysis on pre- and post-treatment tumor NSCLC biopsies of patients (n=3) treated with adagrasib

TABLE 2 Ongoing phase 3 trials targeting KRAS G12C.

Inhibitor	Study name, Clinical trial identifier	Combination class	Test arm	Control arm	# of patients	Line of treatment	ORR (%)	DCR (%)	Ref.
Sotorasib	CodeBreaK 202, NCT05920356	Chemotherapy	Carboplatin, pemetrexed, sotorasib	Carboplatin, pemetrexed, pembrolizumab	750	1	NA	NA	(49), no data reported so far
Adagrasib	KRYSTAL-7, NCT04613596	PD-1	Pembrolizumab (PD-1≥50%), adagrasib	Pembrolizumab (PD-1≥50%)	51	1	62.7	84.0	(50)
	KRYSTAL-12, NCT04685135		Adagrasib	Docetaxel	450	≥2	NA	NA	(51), no data reported so far
Opnurasib	KontRASt-02, NCT05132075		Opnurasib	Docetaxel	360	≥2	NA	NA	(52), no data reported so far
Olomorasib	SUNRAY-01, NCT06119581	PD-1 Chemotherapy A: PD-L1 ≥50% B: PD-L1 0–100%	A: Olomorasib, pembrolizumab B: Olomorasib, platinum, pemetrexed, pembrolizumab	A: pembrolizumab B: platinum, pemetrexed, pembrolizumab	1,016	1	NA	NA	No data reported so far

#, number; ORR, objective response rate (number of patients with complete response plus partial response); NA, not available; NR, not reached; PD-1, programmed cell death protein 1; PD-L1, programmed cell death 1 ligand 1, Ref., reference.

(phase 1/1b and 2) demonstrated down-regulation of *KRAS*/MAPK pathway genes, including *DUSP6* and *SPRY4* (53). Patients with *STK11*-co-mutations had an impressive ORR of 64%. This was a surprising finding given that *STK11* mutations typically portend a poor response and survival to immune checkpoint inhibitors in metastatic NSCLC (54). However, Riely et al. (2021) showed that treatment with adagrasib increased the expression of immune transcripts (e.g., CD4 and CD8) that are minimal at baseline, suggesting a potential immune response to therapy (53).

As noted by Cheema and colleagues (2022), data from preclinical and clinical studies have revealed that drug resistance to single-agent KRAS G12C-targeted therapy occurs quite early after treatment initiation (often within a few months) (23). This suggests that the use of KRAS G12C-targeted therapies in combination with other treatments may help overcome drug resistance observed with anti-G12C monotherapies. Updated, late-breaking data (safety and efficacy results) from the phase 2 KRYSTAL-7 study were recently presented at the European Society of Medical Oncology (ESMO) Congress 2023 in Madrid, Spain (October 20–24, 2023) (55). The results of the KRYSTAL-7 trial, with three patient cohorts stratified according to PD-L1 tumor proportion score (TPS), found that concurrent adagrasib and pembrolizumab in patients with treatment-naïve, advanced, unresectable, or metastatic NSCLC harboring *KRAS* G12C mutation demonstrated encouraging preliminary efficacy with clinically meaningful antitumor activity, especially in patients with high PD-L1 expression (TPS ≥ 50%), and a manageable safety profile (Table 2). The patients in this cohort (PD-L1 TPS ≥ 50%) had an ORR of 63% (32/51; 95% CI, 48–76) and a DCR of 84% (43/51; 95% CI, 12.6-not evaluable [NE]). This ORR for the adagrasib-pembrolizumab combination compares favorably with the ORR of pembrolizumab as a single agent (range: 39% to 45%). The median follow-up was longer for patients with PDL-1 TPS ≥ 50% versus all patients (10.1 months vs. 8.7 months, respectively). The median time to response was 1.4 months, and the median PFS was not reached (95% CI, 8.2-NE).

7 Intracranial responses with the selective KRAS-G12C inhibitors sotorasib and adagrasib

Patients with *KRAS* G12C-mutant NSCLC are prone to developing brain metastases (BMs) (56, 57). At diagnosis, BMs were detected in 27% to 42% of patients (56, 58–61). *KRAS*-mutant NSCLC patients with untreated central nervous system (CNS) metastases have poorer clinical outcomes (i.e., worse prognosis and higher CNS failure) compared to those without *KRAS* mutations (62–64). For this very important reason, the efficacy of selective G12C inhibitors in the CNS and untreated intracranial lesions remains the subject of intense active research (65). It should be noted that the initial KRYSTAL-1 and CodeBreak100 trials excluded patients with active, untreated BMs (66).

Despite their similarities as allele-specific inhibitors and covalent drugs, sotorasib and adagrasib are indeed different in many ways, reflecting the speed of drug development and their

intrinsic properties (67). Notably, with respect to BMs in *KRAS* G12C-mutant NSCLC patients, efficacy data for adagrasib have become available earlier than for sotorasib. Preclinically, adagrasib has shown CNS penetration and its efficacy on *KRAS* G12C-BM in a LU99Luc mouse model showed CNS tumor regression with dose-dependent effects (56). Clinically, it has demonstrated cerebrospinal fluid penetration and BM regression in preliminary findings from the phase 1b portion of the KRYSTAL-1 trial; a retrospective database analysis was initially performed to better understand the clinicopathological features of *KRAS* G12C-mutant NSCLC patients with BM (56). The registrational phase 2 cohort of the KRYSTAL-1 reported findings consistent with the earlier preclinical models of tumor shrinkage, demonstrating an intracranial ORR of 33.3% (11/33 patients) with one intracranial complete response and a median duration of intracranial response of 11.2 months (35). Furthermore, Negrao and colleagues (2023) recently published the first prospective data for the KRAS G12C inhibitor adagrasib in patients with NSCLC and radiologically evaluable, active, and untreated CNS metastases (57). The results of this phase 1b limited BM expansion cohort of the KRYSTAL-1 trial provided proof-of-concept for adagrasib's ability to penetrate the CNS and achieve promising intracranial activity, with a high concordance rate between intracranial and systemic activity (79%) and a low rate of CNS failure (37%). In early 2024, a case series taken from the KRYSTAL-1 CNS metastases cohort showed that most patients did not discontinue adagrasib because of CNS progression, which was consistent with the overall KRYSTAL-1 CNS metastases cohort and indicated that adagrasib may delay development of additional CNS metastases (68).

Until very recently, published CNS activity data for sotorasib remained relatively scant in comparison to adagrasib (65). Thus far, three case reports describe a remarkable intracranial response of previously untreated, active BMs (69–71). Both Koster et al. (2022) and Yeh et al. (2022) documented a rapid intracranial response in less than two months for their patients treated with sotorasib monotherapy following stereotactic body radiotherapy (SBRT) alone vs. postoperative stereotactic radiosurgery to the cranial resection cavity, respectively, and first-line systemic treatment (i.e., immunotherapy with pembrolizumab) (69, 70). Inno et al. (2023) reported the case of a long duration of intracranial response to sotorasib in the second-line setting lasting 16 months in a patient with both pretreated and untreated symptomatic BMs from *KRAS* G12C mutant NSCLC (71). The importance of exploring dose-dependent CNS response, control, and penetration of the selective inhibitor is emphasized by Lu & Husain (2023) in their case report (65). The patient showed intracranial stability for 5 months on the standard dose of second-line sotorasib monotherapy (960 mg daily), but following a reduction of the sotorasib to 480 mg daily as a result of seizures and vasogenic edema (without new BMs) developed new BMs 5 months later (65).

Clearly, further prospective clinical studies are required to fully characterize the intracranial efficacy of both sotorasib and adagrasib as currently approved therapies as well as other selective G12C inhibitors still in development, including divarasib (GDC-6036) and opnurasib (JDQ-443), among others (66).

8 Novel direct *KRAS* G12C inhibitors

In addition to sotorasib and adagrasib, several other direct KRAS G12C inhibitors, such as divarasib (GDC-6063), opnurasib (JDQ-443), garsorasib (D-1553), olomorasib (LY3537982), MK-1084, and JAB-21822 are now in clinical development as monotherapy or in combination with other treatments, as discussed in several recently published reviews (Tables 2, 3, Figure 1) (10, 13, 20–22, 82–84). A very recent review touches quite comprehensively and thoughtfully on the manifold combinatorial therapeutic strategies in RAS-driven cancers (84).

Two formerly promising, orally available, investigational, small molecules, LY3499446 and JNJ-74699157 (ARS-3248), were abruptly removed from the G12C inhibitor landscape (82, 83). The discontinuation of the initial phase 1 trial of LY3499446 was due to unexpected toxicity (20, 27). Likewise, JNJ-74699157 (ARS-3248) was investigated in a phase 1 study of patients with advanced solid tumors, including NSCLC (n=5), but enrolment was terminated at just 10 patients due to dose-limiting skeletal muscle toxicities and the lack of efficacy at the lowest administered dose (100 mg) (83, 85).

Data from preclinical and *in vitro* studies have suggested that divarasib (GDC-6063) is more potent and selective than sotorasib or adagrasib (86). In a phase 1 clinical trial, among the 60 NSCLC patients who received divarasib, a confirmed response was observed in 53.4% of patients (95% confidence interval [CI], 39.9 to 66.7), and the median PFS was 13.1 months (95% CI, 8.8 to NE), with an acceptable safety profile (mainly low-grade adverse events) (36).

Opnurasib (JDQ-443), structurally unique and currently in clinical development, has been optimized by design to overcome resistance mechanisms through novel interactions with the binding pocket (83, 87–89). A stable atropisomer with PK/PD activity *in vivo* and dose-dependent antitumor activity in mouse xenograft models, opnurasib has performed in an encouraging manner as evidenced by the early phase data reported from an ongoing Phase 1b/2 clinical trial, with a confirmed ORR of 41.7% (83, 88, 89). As a promising therapy, opnurasib is being investigated in the combination arms of the ongoing, phase 1b/2, multicenter, KontRaSt-01 study, with either TNO155 (SHP2 inhibitor) or tislelizumab (anti-PD-1 monoclonal antibody), as well as in a phase 3 trial of opnurasib monotherapy versus docetaxel (Table 2) (73, 83, 90). An update of the KontRaSt-01 was recently presented at the ASCO 2023 Congress, demonstrating promising efficacy and well-tolerated safety data (73).

Garsorasib (D-1553), a novel small molecule inhibitor that selectively targets *KRAS* G12C, is currently in phase 2 clinical trials (91). Preclinical data have already demonstrated antitumor activity of garsorasib. In the phase 1, garsorasib dose-escalation study in *KRAS* G12C-mutant NSCLC patients (n=62), partial response occurred in 24 patients (ORR, 38.7%) and stable disease in 32 patients (DCR, 90.3%) (37).

Olomorasib (LY3537982) monotherapy was tested in a phase-1 clinical trial, in which 5 treatment-naïve and 9 previously treated patients with *KRAS* G12C mutational status showed an ORR of 60% or 0%, respectively, and a DCR of 80% or 67%, respectively (72).

TABLE 3 Novel agents for KRAS inhibition.

Inhibitor	Clinical trial identifier, study name, phase	Line of treatment	Mechanism	# of patients	Control	ORR (%)	DCR (%)	PFS (median months, HR)	Ref.
KRAS G12C inhibitor									
Olomorasib	NCT04956640, Phase 1	≥1	Off state inhibitor	KRAS G12C inhibitor naïve, N = 5	None	60.0	80.0	NA	(72)
				KRAS G12C inhibitor treated, N = 9	None	0.0	67.0	NA	
Opnurasib	NCT04699188, KontRASt-01, Phase 1/2	≥2	Off state inhibitor	24	None	42.0	93.0	NA	(73)
IBI351	NCT05005234, NCT05497336, Phase 2	≥2	Off state inhibitor	116	None	46.6	90.5	8.3	(74, 75)
RMC-6291	NCT05462717, Phase 1	≥2	On state, tri-complex inhibitor	KRAS G12C inhibitor naïve (N = 7)	None	42.8	100.0	NA	(76)
				KRAS G12C inhibitor treated (N = 10)	None	50.0	100.0	NA	
MK-1084	NCT05067283, Phase 1	≥2	Unknown	Arm 1: previously treated, receiving MK-1084 monotherapy	None	19.0	NA	NA	(77)
				Arm 2: treatment-naïve, receiving MK-1084 + pembrolizumab	None	47.0	NA	NA	
Glecirasib (JAB-21822)	NCT05009329, Phase 1	≥2	Off state inhibitor	22	None	70.0	100.0	NA	(78)
KRAS G12D inhibitor									
HRS-4642	NCT05533463, Phase 1	≥2	Unknown	10	None	10.0	90.0	NA	(79)
MRTX1133	NCT05737706, Phase 1/2	≥2	Off state inhibitor	NA	None	NA	NA	NA	NA
RMC-9805	NCT06040541, Phase 1	≥2	On state tri-complex inhibitor	NA	None	NA	NA	NA	(80)
Pan/multi-RAS inhibitors (KRAS G12X)									
RMC-6236	NCT05379985, Phase 1	≥2	RAS-multi, on state, tri-complex inhibitor	11 4 with efficacy assessment	None	75.0	100.0	NA	(81)

#, number; DCR, disease control rate (number of patients with partial response or stable disease); HR, hazard ratio; NA, not available; NR, not reached; ORR, objective response rate (number of patients with complete or partial response); PFS, progression-free survival; Ref., reference; OS, overall survival.

The phase-3 SUNRAY-01 trial (NCT06119581) will assess the efficacy of olomorasib in combination with pembrolizumab or pembrolizumab with chemotherapy in 1,016 patients with locally advanced or metastatic NSCLC.

MK-1084 is being tested for *KRAS* G12C mutations as monotherapy in pretreated patients with advanced solid tumors (arm 1) and in combination with pembrolizumab in previously untreated metastatic NSCLC with PD-L1 TPS≥1% in an ongoing, phase 1, global, dose-escalation trial (arm 2) (23). The preliminary results, presented at the ESMO Congress 2023 in October 2023, showed manageable safety and preliminary antitumor activity in both arms (ORR 19% and 47% in arm 1 and 2, respectively) (77).

JAB-21822, now designated glecirasib, was tested in a first-in-human clinical trial comprising 22 patients with advanced NSCLC. The results proved quite promising showing that ORR and DCR were 70% and 100%, respectively (78). Results from future clinical trials are awaited.

9 Mechanisms of resistance to KRAS inhibition

The vast majority of advanced NSCLC will progress due to treatment resistance. Tumor cell intrinsic mechanisms are the primary drivers of resistance to radiation, cytotoxic agents, and targeted therapies (6).

Resistance mechanisms to KRAS G12C inhibition cover primary resistance and acquired resistance (92, 93).

Primary resistance or early disease progression (PFS < 3 months) to KRAS G12C inhibitors occurs in about 36% of patients who received sotorasib therapy, as shown in recently published data from the 2-year analysis of the CodeBreaK100 study in NSCLC (41). In NSCLC, co-mutations with genetic alterations in *KEAP1*, *SMARCA4* (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4), and *CDKN2A* (cyclin dependent kinase inhibitor 2A) are associated with inferior clinical outcomes to sotorasib therapy (94). Some studies have demonstrated that co-mutations in *STK11*, *KEAP1*, and *TP53* could modulate the responsiveness of patients with *KRAS* alterations to either KRAS G12C inhibitors or to immunotherapy (14–16, 18, 95). Proulx-Rocray and colleagues (2021) showed that the presence of *STK11* and/or *KEAP1* mutations was associated with a negative impact on survival when compared with wild-type NSCLC patients treated with immune check point inhibitors (96). These authors also reported that in patients harboring *KRAS* mutation, improved prognosis was observed in *STK11+KEAP1* wild-type tumors but not in *STK11 +/-KEAP1* mutant tumors. Interestingly, the presence of *KRAS* G12D is associated with diminished infiltration of CD8+ T cells in NSCLC (97). Patients harboring *KRAS* G12D mutations had worse clinical outcomes to PD-(L)1 inhibition compared to wild-type (97). The biological mechanism of resistance mediated by these mutations has yet to be explored. Co-occurring mutations that predict response to treatment might serve as markers for patient stratification and therapy intensification in randomized clinical trials (10).

In terms of allele amplification, high-level amplifications of the *KRAS* G12C allele were observed in some patients undergoing sotorasib treatment (98, 99).

Acquired resistance inevitably occurs and is responsible for disease progression after an initial benefit from targeted therapies. Principly, acquired resistance to KRAS G12C inhibitors are functionally divided into off-target and on-target mechanisms.

On-target resistance mechanisms include alterations that concern the molecular target, against which the inhibitor is directed, such as KRAS. These mechanisms comprise (92, 98, 100):

- Novel *KRAS* mutations in the switch II pocket (e.g. sotorasib: Y96c/d/s, R68S, adagrasib: H95D/Q/R);
- Acquired *KRAS* activating mutation (e.g. G12D on trans and G12W on cis, preventing inhibitor to bind);
- New production of KRAS G12C, and
- *KRAS* G12C gene amplification.

On-target resistance mechanisms were described in a recent *in vitro* study showing that secondary *KRAS* mutations (Y96D, A59T, A59S, R68M, R68M, M721, V8E, G13D, Q61L, Q99L, and H358) conferred resistance to the KRAS (G12C) inhibitors. Moreover, Y96D and Y96S secondary mutations caused resistance to both sotorasib and adagrasib, while the *KRAS* mutations G13D, R68M, A59S, and A59T were highly resistant only to sotorasib and Q99L was resistant to adagrasib but sensitive to sotorasib (101). These acquired mutations were also observed in a clinical study that included *KRAS* G12C-mutant cancer patients treated with adagrasib in monotherapy, of whom 71% were NSCLC patients (98). Furthermore, cell lines with co-mutations of *KRAS* G12C and G12V were described as acquired mechanisms of resistance to KRAS G12C inhibition *in vitro* (102). Similarly, a preclinical and clinical study from Tanaka and colleagues described two *KRAS* activating mutations (G12D, G12V) and a Y96D mutation affecting the cryptic Switch II pocket as mechanisms of resistance during adagrasib treatment (103). Interestingly, G12D-mutant cell lines are reported to have high levels of phosphorylated AKT, leading to the activation of the PI3K-AKT-mTOR pathway (102).

Off-target resistance mechanisms include alterations that comprise upstream and downstream signaling pathways of KRAS as well as histological transformation. These mechanisms comprise (92, 98, 100):

- Activating wild-type isoforms of RAS-proteins, such as NRAS and HRAS;
- Gain of function in oncogenes (e.g. downstream as in the MAPK pathway: *NRAS*, *BRAF*, *MEK1*, *RET* etc.);
- Loss of function in tumor suppressor genes (e.g. cell-cycle transition: *CDKN2A*);
- Gene amplifications, such as in *cMET;*
- Fusion of gene, such as *ALK, RET, RAF1, BRAF, FGFR3*, appear to be more common in colo-rectal cancer;
- Histological transformation (e.g. LUAD to squamous cell carcinoma).

A recent *in vitro* and *in vivo* study demonstrated that *MET* amplification in *KRAS* G12C was associated with resistance to sotorasib *in vitro* and the introduction of a MET inhibitor restored sensitivity by eliminating RAS–MEK–ERK and AKT signaling (104). Furthermore, MET copy level gain was an off-target mechanism of resistance to sotorasib in a patient with *KRAS* G12C-mutant LUAD (105). Activating mutations in *NRAS*, *BRAF*, *MAP2K1*, and *RET*; oncogenic fusions involving *ALK*, *RET*, *BRAF*, *RAF1*, and *FGFR3*; and loss-of-function mutations in tumor suppressor genes, such as *PTEN* and *NF1*, were described as acquired off-target resistance mechanisms of KRAS G12C inhibitors (19, 92, 101, 106).

Table 3 and Figure 1 give an overview of three potential agents targeting *KRAS* G12D mutations: HRS-4642, MRTX1133, and RMC-9805. Moreover, G12V mutations are shown to preferentially activate RAL signaling (102).

10 Future strategies to overcome resistance to KRAS inhibition

For NSCLC harboring a *KRAS* G12D mutation, there are several specific inhibitors undergoing testing in clinical and preclinical studies (Table 3, Figure 1). MRTX1133 is a non-covalent KRAS G12D inhibitor that showed significant preclinical antitumor activity in *KRAS* G12D-bearing tumor cells, especially pancreatic ductal adenocarcinoma (107). This compound might be a potential treatment in combination with KRAS G12C inhibitors for patients harboring co-mutations (*KRAS* G12C, G12D). Further studies are needed to clarify the role of adaptive resistance mechanisms in acquiring resistance to KRAS inhibitors.

RM-018, a tricomplex KRAS G12C active-state inhibitor, retains the ability to inhibit KRAS (G12C, Y96D) (103), thus being a promising therapy to address acquired resistance. Adaptive resistance mechanisms due to reactivation of MAPK pathway and upregulation of PI3K-AKT pathway were identified as likely resistance mechanisms and, according to *in vitro* and *in vivo* models, combination with PI3K inhibitors could overcome this resistance (108).

Several studies have uncovered the mechanisms underlying resistance to KRAS G12C inhibition and there have been pioneering efforts to overcome drug resistance using combinatorial treatments (108–111).

One approach is to target upstream effector proteins of the KRAS protein itself. For instance, the phosphatase son of sevenless homolog 1 (SOS1) is a RAS guanine nucleotide exchange factor (RasGEF), which is activated by SHP2 promoting RAS activation through GTP binding (Figure 1) (112). The combination of a novel SOS1 inhibitor (BI-3406) and trametinib exhibited potent activity against Y96D and Y96S (113). In addition, other SOS inhibitors, such as BI-1701963 and MRTX0902, are currently being tested in clinical trials (10).

SHP2 is another upstream adapter protein that is phosphorylated upon activation of RTK. Two SHP2 inhibitors are currently under clinical investigation: TNO155 and RMC-4630 (10, 13). KRAS G12C inhibitors in combination with SHP2 inhibition led to sustained RAS pathway suppression and improved efficacy *in vitro* and *in vivo* (111).

Recently, a phase 3 clinical trial showed that sotorasib in combination with panitumumab (EGFR inhibitor) resulted in longer PFS than standard treatment in metastatic colon cancer patients (114). Further studies are needed to test whether this combination could improve the outcome in lung cancer. Promising evidence has demonstrated that adagrasib plus pembrolizumab improves overall response rate in patients with newly diagnosed NSCLC harboring a *KRAS* G12C mutation, particularly in those with higher levels of PD-L1 (115).

As such, specific therapeutic combinations may help in cases of either intrinsic resistance or acquired resistance.

11 Conclusion

The *KRAS* mutation plays a major role in the development of tumor progression and resistance to treatment. Despite this, G12C point mutation (making up only 39% of all KRAS alterations) remains the only molecular target for which the two therapeutic agents, sotorasib and adagrasib, have been approved so far. The advent of novel inhibitors against *KRAS* mutations will further improve survival of lung cancer patients. Nevertheless, the co-occurrence of add-on mutations (co-mutations) and by-pass track pathways will remain challenging obstacles to overcome since they reduce treatment success. Future research efforts must be directed toward comprehensive molecular testing of lung cancer, allowing for the development of multimodal treatment strategies including immune checkpoint inhibitors, tyrosine kinase inhibitors, KRAS upstream inhibitors, and multi-kinase inhibitors against co-mutations.

Author contributions

KS: Writing – original draft, Writing – review & editing. MC: Writing – original draft, Writing – review & editing. MV: Writing – review & editing. AF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Assessing the prognostic value of *KRAS* mutation combined with tumor size in stage I-II non-small cell lung cancer: a retrospective analysis

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Background: *KRAS* mutation status is a well-established independent prognostic factor in advanced non-small cell lung cancer (NSCLC), yet its role in early-stage disease is unclear. Here, we investigate the prognostic value of combining survival data on *KRAS* mutation status and tumor size in stage I-II NSCLC.

Methods: We studied the combined impact of *KRAS* mutational status and tumor size on overall survival (OS) in patients with stage I-II NSCLC. We performed a retrospective study including 310 diagnosed patients with early (stage I-II) NSCLCs. All molecularly assessed patients diagnosed with stage I-II NSCLC between 2016–2018 in the Västra Götaland Region of western Sweden were screened in this multi-center retrospective study. The primary study outcome was overall survival.

Results: Out of 310 patients with stage I-II NSCLC, 37% harbored an activating mutation in the *KRAS* gene. Our study confirmed staging and tumor size as prognostic factors. However, *KRAS* mutational status was not found to impact OS and there was no difference in the risk of death when combining *KRAS* mutational status and primary tumor size.

Conclusions: In our patient cohort, *KRAS* mutations in combination with primary tumor size did not impact prognosis in stage I-II NSCLC.

KEYWORDS

lung cancer, *KRAS*, tumor size, stage I and II, clinical outcome

Introduction

Non-small cell lung cancer (NSCLC) is the second most common cancer worldwide with 2.1 million new cases annually and the highest mortality rate with 1.8 million deaths (1). Staging is a crucial aspect of NSCLC management, as it is one of the most important predictors of survival. The TNM staging system describes key tumor characteristics such as size, location, and whether the disease has spread to lymph nodes and/or distant organs (2–5). There are four main stages in NSCLC (stage I-IV), with stage IV having the worst prognosis. Pathological stage is considered the most important prognostic factor for resected patients, with 5-year survival rates, gradually decreasing across stages, of 83% for stage IA, 71% for IB, 57% for IIA, 49% for IIB, 36% for IIIA, and 23% for IIIB (4).

The most frequent oncogenic driver in NSCLC is the Kirsten rat sarcoma viral oncogene (*KRAS*), which is present in up to 40% of all cases, with the most common mutations being *G12C, G12V*, and *G12D* (6). *KRAS* mutations are associated with worse outcomes after chemotherapy and radiotherapy, with shorter OS in stage III and IV patients (7–14). In early-stage NSCLC, however, while several studies have shown that *KRAS* mutations negatively influence the prognosis (15–17), others have shown no significant effect (18–20). Most recently, it was reported that *KRAS G12C* mutation (but not other *KRAS* mutations or with no mutation in *KRAS*) significantly increased risk of disease recurrence in stage I surgically resected lung adenocarcinomas (21). However, while the study found this in two distinct local cohorts of IRE-LUAD (Rome, Italy) and MSK-LUAD (New York, USA), data extracted from The Cancer Genome Atlas (TCGA) showed no significant difference. Another recent study reported that while *STK11* mutation decreased survival probability in stage I lung adenocarcinoma, *KRAS* mutation showed no significant impact (22). Hence, the debate about the prognostic value of *KRAS* mutational status in early NSCLC is ongoing (23, 24). In fact, given the lack of consensus regarding its effects on prognosis, testing for *KRAS* mutations for resectable stage I and II tumors is currently not recommended in clinical guidelines (25). In addition, several inhibitors that specifically bind *KRAS-G12C* have been investigated in clinical trials, with sotorasib becoming the first treatment to gain approval for adults with stage IV NSCLC harboring a *KRAS-G12C* mutation as second-line therapy (26–30). However, treatment with sotorasib is not currently recommended for patients with early-stage NSCLC due to lack of evidence showing positive outcomes of treatment in this group.

Therefore, further investigations are warranted to identify potential subgroups in Stage I-III disease who may still have to gain from effective and well-established treatments, and to add to the pool of clinical data required to study this further. One strategy is to stratify patients according to *KRAS* mutational status together with other key prognostic factors, such as tumor size. Primary tumor size is an established prognostic factor in NSCLC, with larger tumors being associated with poorer survival (24, 31–34). The reason for this association is not yet fully understood but larger tumors may be more resistant to therapy due to having poorer blood supply, differential metabolism, and potentially a higher likelihood of micrometastatic disease compared to smaller tumors (35). Further research is needed to elucidate the underlying mechanisms. However, when considering primary tumor size, the grouping as early (I-II), advanced (III), and metastasized (IV) NSCLC can be argued to be more clinically relevant due to that stage I-II is primarily based on tumor size whereas a spread to the lymph nodes, a negative prognostic factor, is more common in stage III (3, 24).

To our knowledge, no one has investigated the combined impact of primary tumor size and *KRAS* mutational status on OS and risk of death in stage I-II NSCLC. However, in Sweden, reflex testing for targetable alterations in NSCLC, including *KRAS* mutational status, has been widely implemented since 2015 for all stages. By screening all consecutive patients diagnosed with stage I-II NSCLC and molecularly assessed between 2016–2018 in Västra Götaland, the second largest county in Sweden with a population of 1.7 million, the current retrospective cohort study provides a unique real-world dataset for assessing the impact of combining *KRAS* mutations with primary tumor size.

To summarize, primary tumor size is a key determinant of prognosis especially in the early stages of NSCLC. At the same time, the prognostic value of *KRAS* mutational status in early disease stages remains unclear. Hence patients diagnosed at an early stage are not automatically tested for *KRAS* mutations and recommended treatment with *KRAS*-targeted therapy. Here, we investigate whether there is prognostic value in combining *KRAS* mutational status with tumor size to aid in clinical stratification of potentially treatment-responsive subgroups in early-stage NSCLC.

Materials and methods

Patient population

We conducted a multi-center retrospective study screening all consecutive NSCLC patients diagnosed with stage I-II NSCLC and molecular assessment performed between 2016–2018 in Västra Götaland, Sweden (n = 354). Further inclusion criteria included the availability of tumor size from CT scanning or a pathology report as well as follow-up data. Patients were excluded if diagnosed before 2016, had no digitally accessible patient charts, no tumor measurements noted in the patient charts, or had recurrent disease (study cohort n = 310).

Patient demographics (age, sex, Eastern Cooperative Oncology Group [ECOG] performance status [PS], and smoking history), cancer stage, pathological details (histology, mutational status including *KRAS* mutational status and subtype), first-line treatment and outcome data were retrospectively collected from patient charts and the Swedish Lung Cancer Registry. Clinical staging was based on TNM staging guidelines 7th edition (4). TNM staging 8th edition released in 2017 was introduced in

Abbreviations: CT, Computed Tomography; ECOG, Eastern Cooperative Oncology Group; HR, Hazard Ratio; NSCLC, Non-Small Cell Lung Cancer; NGS, Next Generation Sequencing; PS, Performance Status; OS, Overall Survival.

Swedish guidelines in 2018, and full implementation was reached in 2019. Ethical approval was obtained from the Swedish Ethical Review Authority prior to study commencement (Dnr 2019–04771 and 2021–04987). No informed consent was required due to all data presented in a de-identified form according to the Swedish Ethical Review Authority.

Mutational status

Patients were assessed with next-generation sequencing (NGS) for mutational status on DNA from formalin-fixed paraffin-embedded (FFPE) blocks or cytological smears using the Ion AmpliSeq™ Colon and Lung Cancer Panel v2 from Thermo Fisher Scientific as part of the diagnostic workup process at the Department of Clinical Pathology at Sahlgrenska University Hospital, assessing hotspot mutations in *EGFR, BRAF, KRAS*, and *NRAS*. Until June 2017, *ALK*-fusions were assessed with immunohistochemistry (IHC), and with fluorescence *in situ* hybridization (FISH) if positive or inconclusive IHC. *ROS1* was analyzed upon request with FISH. Thereafter, *ALK, ROS1*, and *RET* fusions were assessed on RNA using the Oncomine Solid Tumor Fusion Panel from Thermo Fisher Scientific.

Tumor size

To obtain the most recent and accurate untreated primary tumor size, measurements were collected from the radiology report of computed tomography (CT) performed before a final diagnosis of NSCLC was established; this is referred as clinical staging. In patients who underwent surgical resection, the actual primary tumor size was also collected from the pathology report, also referred as pathological staging (PAD). The largest tumor diameter was collected and reported in millimeters.

Study objectives

The primary outcome of this study was OS, defined as the interval between the date of first treatment and the date of death from any cause. Patients alive or lost to follow-up at the cut-off date were censored at last contact. Median follow-up time was estimated using the reverse Kaplan-Meier method. We compared OS and risk of death stratified by *KRAS* mutational status, i.e., with no mutation in *KRAS* (wildtype, $KRAS^{WT}$), all *KRAS* mutations ($KRAS^{MUT}$), *KRAS G12C* mutations ($KRAS^{MUT\ G12C}$) and all *KRAS* mutations other than *G12C* ($KRAS^{MUT\ not\ G12C}$).

Statistical analysis

Clinical characteristics were summarized using descriptive statistics and analysis of associations between *KRAS* mutational status and clinicopathological parameters was performed using Pearson´s X^2 test or T-test. Survival was estimated using the Kaplan-Meier method, visualized at 5-year follow up. The log-rank test was used to assess significant differences in OS between $KRAS^{WT}$ and $KRAS^{MUT}$ groups. To evaluate if there was a significant difference in primary tumor size between $KRAS^{MUT}$ and $KRAS^{WT}$, the Mann-Whitney U test was used. We defined an interaction term between tumor size (largest diameter in mm) and *KRAS* mutational status to assess the combined impact on the risk of death (HR). First, the mean size of all primary tumors was calculated. Thereafter a dummy variable was calculated by subtracting the mean size from all individual measurements following multiplication with 1 if *KRAS* was mutated and 0 if *KRAS* was WT. The interaction term was included in Multivariable Cox regression analysis, also correcting for sex, age, tumor size in mm and *KRAS* mutational status. Statistical significance was set at $p < 0.05$ and no adjustments were made for multiple comparisons. Data analysis was conducted using IBM SPSS Statistics version 27 and GraphPad Prism version 9.

Results

Patients and tumor characteristics

A total of 310 patients, who were diagnosed with stage I-II NSCLC during 2016–2018 in Västra Götaland, Sweden and for whom genetic data was available, were included in this retrospective cohort study (Figure 1). In the total population, majority of patients were female (187, 60.3%), with a median age of 70 years, and most were current or former smokers (267, 86%) (Table 1). Most patients had good PS with ECOG 0–1 at diagnosis (285, 92%) and the proportion of N1 was low (18, 5.8%). NSCLC was predominantly adenocarcinoma of the lung (281, 90.6%), while squamous cell carcinoma incidence was relatively low (11, 3.5%), which was expected due to the selection of histological type for NGS assessment. Of included patients, over a third (115, 37%) had a *KRAS* mutation (Table 1). This percentage matches what has been previously reported (9), showing good representativeness of the patient group studied here. When comparing the baseline characteristics of $KRAS^{WT}$ with $KRAS^{MUT}$ patients, a greater proportion of those with $KRAS^{MUT}$ were female and current or former smokers. There were no cases of squamous cell carcinoma in the $KRAS^{MUT}$ group. The most common KRAS mutation was G12C (47%). In the total population, majority of patients underwent surgical resection (273, 88%; Table 2). Three patients did not receive any treatment and were excluded from further survival analyses. Median follow-up time was 63 months (95% CI, 59.7–68.3) and the data cut-off date was 31 October 2022.

No significant difference in survival for all patients stratified by *KRAS* mutations

When comparing OS for all (stage I-II) patients stratified by *KRAS* mutational status, no significant difference was detected with a mean OS (median not reached) of 74 months for $KRAS^{WT}$ vs 63 months for $KRAS^{MUT}$ ($p = 0.847$; Figure 2A). Further stratification of the *KRAS*

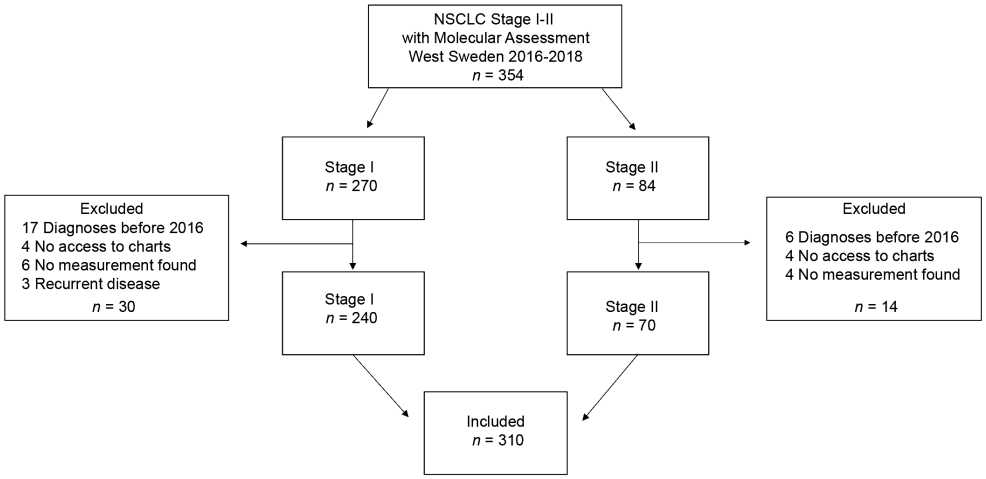

FIGURE 1
Patient selection. Flow chart showing patient selection for the study.

mutated group by the G12C mutation also did not significantly change survival: 74 months for $KRAS^{WT}$, 61 months for $KRAS^{MUT\ not\ G12C}$ and 63 months for $KRAS^{MUT\ G12C}$ ($p = 0.834$; Figure 2B).

No significant difference in survival for patients in stage I or stage II disease combined with *KRAS* mutations

There were also no significant differences according to *KRAS* mutational status in stage I (Figures 2C, D) or Stage II (Figures 2E, F). Similarly in resected patients, no significant difference was observed with a mean OS (median not reached) of 78 months for $KRAS^{WT}$ vs 65 months for $KRAS^{MUT}$ ($p = 0.856$; Supplementary Figure 1A), or between the subgroups of $KRAS^{MUT}$ ($p = 0.471$; Supplementary Figure 1B).

Next, we stratified by stage and found mean OS (median not reached) of 79 months for stage I vs 50 months for stage II (Supplementary Figure 2A). We then conducted the analysis separately according to *KRAS* mutational status. For $KRAS^{WT}$, the mean OS (median not reached) was 78 months for stage I vs 46 months for stage II (Supplementary Figure 2B), and for $KRAS^{MUT}$, 65 months for stage I vs 53 months for stage II (Supplementary Figure 2C).

No significant difference in survival for patients with TNM-stage T1, T2 or T3 disease combined with *KRAS* mutations

Next, we stratified patients using T-staging and studied OS according to *KRAS* mutational status. Among those with T1 disease, $KRAS^{WT}$ had 83 months while $KRAS^{MUT}$ had 66 months OS ($p = 0.751$; Figure 3A). Further, $KRAS^{MUT\ not\ G12C}$ patients had survival of 70 months and $KRAS^{MUT\ G12C}$ had 61 months ($p = 0.344$; Figure 3B). In the T2 group, $KRAS^{WT}$ had 53 months while $KRAS^{MUT}$ had 59 months OS ($p = 0.495$; Figure 3C). $KRAS^{MUT\ not\ G12C}$ patients

TABLE 1 Characteristics of the total cohort as well as stratified by $KRAS^{WT}$ and $KRAS^{MUT}$.

	Total	$KRAS^{WT}$	$KRAS^{MUT}$	*P*-value
	n (%)	n (%)	n (%)	
Total	310 (100)	195 (63.0)	115 (37.0)	
Age in years, median (range)	70 (35–85)	70 (35–85)	70 (48–84)	0.896
Sex				0.011
Male	123 (39.7)	88 (45.1)	35 (30.4)	
Female	187 (60.3)	107 (54.9)	80 (69.6)	
Smoking history				<0.001
Current smoker	99 (31.9)	51 (26.2)	48 (41.7)	
Former smoker	168 (54.2)	106 (54.4)	62 (53.9)	
Never smoker	43 (13.9)	38 (19.5)	5 (4.3)	
Perfomance status				0.208
ECOG 0	144 (46.5)	82 (42.1)	62 (53.9)	
ECOG 1	141 (45.5)	96 (49.2)	45 (39.1)	
ECOG 2	24 (7.7)	16 (8.2)	8 (7.0)	
ECOG 3	1 (0.3)	1 (0.5)	0	
ECOG 4	0	0	0	
Histology				0.014
Adenocarcinoma	281 (90.6)	168 (86.2)	113 (98.3)	
Squamous cell carcinoma	11 (3.5)	11(5.6)	0	
NCSLC NOS	18 (5.9)	16 (8.2)	2 (1.7)	
Mutation status				<0.001
None known	124 (40.2)	124 (63.6)	0	

(Continued)

TABLE 1 Continued

	Total	$KRAS^{WT}$	$KRAS^{MUT}$	*P*-value
	n (%)	n (%)	n (%)	
KRAS	115 (37.0)	0	115 (100)	
EGFR	54 (17.4)	54 (27.7)	0	
BRAF	6 (1.9)	6 (3.1)	0	
ALK	4 (1.3)	4 (2.1)	0	
ROS1	3 (1.0)	3 (1.5)	0	
RET	1 (0.3)	1 (0.5)	0	
Other	3 (1.0)	3 (1.5)	0	
KRAS submutation				
G12A			9 (7.8)	
G12C			54 (47.0)	
G12D			15 (13.0)	
G12V			26 (22.6)	
Other			11 (9.6)	
TNM				
T-stage				0.254
T1a	99 (31.9)	55 (28.2)	44 (38.3)	
T1b	76 (24.5)	54 (27.7)	22 (19.1)	
T1c	12 (3.9)	7 (3.6)	5 (4.3)	
T2a	67 (21.6)	46 (23.6)	21 (18.3)	
T2b	28 (9.0)	15 (7.8)	13 (11.3)	
T3	28 (9.0)	18 (9.2)	10 (8.7)	
N-stage				0.506
N0	292 (94.2)	185 (94.9)	107 (93.0)	
N1	18 (5.8)	10 (5.1)	8 (7.0)	
Stage at diagnosis				0.568
I	240 (77.4)	153 (78.5)	87 (75.7)	
II	70 (22.6)	42 (21.5)	28 (24.3)	
Measurement modality				
CT-scan (mm)	305 (98.4)	190 (97.4)	115 (100)	0.046
PAD	273 (88.0)	171 (88.7)	102 (88.7)	0.161
At last follow up 31/10–2022				0.694
Alive	206 (66.5)	128 (65.6)	78 (67.8)	
Deceased	104 (33.5)	67 (34.4)	37 (32.2)	
Survival				
Mean survival (months)	63	62	64	0.508

ECOG PS, Eastern Cooperative Oncology Group Performance Status. T, Tumor. N, Nodulus. Data are presented as n (%).

TABLE 2 Summary of first-line treatments in the total cohort as well as stratified by $KRAS^{WT}$ and $KRAS^{MUT}$.

	Total	$KRAS^{WT}$	$KRAS^{MUT}$
	n (%)	n (%)	n (%)
Total	310 (100)	195 (63.0)	115 (37.0)
Surgery	273 (88.0)	171 (87.7)	102 (88.7)
Curative chemoradiotherapy	7 (2.3)	6 (3.1)	1 (0.9)
Medical treatment	2 (0.6)	1 (0.5)	1 (0.9)
Stereotactic radiotherapy	11 (3.5)	11 (5.6)	0 (0)
Radiotherapy	14 (4,5)	3 (1,5)	11 (9.6)
No treatment	3 (1.0)	3 (1.5)	0 (0)

Data are presented as n (%).

had survival of 51 months and $KRAS^{MUT\ G12C}$ had 66 months (p = 0.389; Figure 3D). Similarly, in the T3 group, $KRAS^{WT}$ had 47 months while $KRAS^{MUT}$ had 50 months OS (p = 0.966; Figure 3E). $KRAS^{MUT\ not\ G12C}$ patients had survival of 50 months and $KRAS^{MUT\ G12C}$ had 53 months (p = 0.984; Figure 3F).

We further analyzed the impact of T stage on survival and found that it correlated as expected with mean OS of 82 months for T1, 55 months for T2, and 46 months for T3 ($p < 0.001$; Supplementary Figure 3A). The same trend was observed when separately analyzing $KRAS^{WT}$ with a mean OS (median not reached) of 83 months for T1, 53 months for T2, and 45 months for T2 ($p < 0.001$; Supplementary Figure 3B), and $KRAS^{MUT}$ with a mean OS of 65 months for T1, 58 months for T2, and 48 months for T3 ($p < 0.023$; Supplementary Figure 3C).

KRAS mutations are associated with smaller tumor size measured from CT scans, but not resection specimens

To evaluate differences between primary tumor size from CT scans at diagnosis stratified by *KRAS* mutational status, we used the Mann-Whitney U test. The test revealed that $KRAS^{MUT}$ primary tumors were significantly smaller at diagnosis, with a median size of 20 mm (n = 115) vs $KRAS^{WT}$ primary tumors with a median size of 25 mm (n = 190) (p = 0.043; Figure 4A). However, when looking at tumor size as assessed in resected specimens, there were no differences; $KRAS^{WT}$ median size 22 mm (n = 171) vs $KRAS^{MUT}$ median size 21 mm (n = 102) (p = 0.16; Figure 4B).

Larger tumor size measured from resection specimens, but not CT scans, is associated with a higher risk of death

We found that increase in primary tumor size determined from CT scans did not have a significant effect on risk of death (HR, 1.006; 95% CI, 0.922–1.021; $p > 0.5$) (Figure 4C). However, when testing the correlation between primary tumor size as assessed in

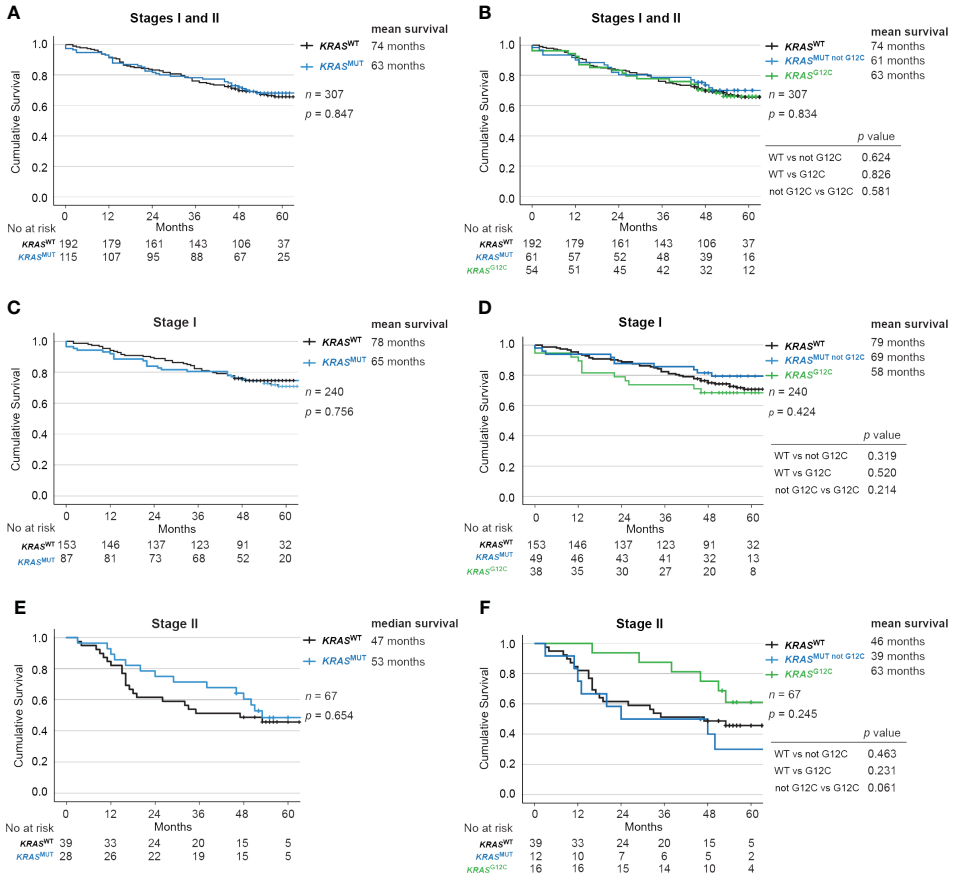

FIGURE 2
Impact of *KRAS* mutational status on overall survival in Stage I and II NSCLC. Kaplan-Meier estimates comparing overall survival between **(A, B)** all patients, **(C, D)** Stage I and **(E, F)** Stage II patients with no mutation in *KRAS* (wildtype, $KRAS^{WT}$), with all *KRAS* mutations ($KRAS^{MUT}$), only *KRAS-G12C* mutations ($KRAS^{MUT\ G12C}$) and *KRAS* mutations other than *G12C* ($KRAS^{MUT\ not\ G12C}$).

resection specimens, we found a significantly increased risk of death (HR, 1.029; 95% CI, 1.012–1.046; $p < 0.001$) (Figure 4D). The risk of death increases with 2.9% for every mm increase of size.

When analyzing stage I and stage II patients separately we found that the primary tumor size, as assessed in resection specimens, correlated to a significantly increased risk of death (HR, 1.051; 95% CI, 1.026–1.077; $p < 0.001$) (Supplementary Table 1) for stage I patients, with 5.1% for every mm increase in tumor size. However, for stage II patients, no correlation was found between tumor size and risk of death. Furthermore, the primary tumor size determined by CT did not impact the risk of death in either stage. Along these lines, we analyzed the risk of death separately for T1, T2 and T3 groups regarding tumor size from CT and resected specimens and no correlation was found (Supplementary Table 1).

The combination of *KRAS* mutational status and tumor size does not impact the risk of death

To test if the combination of tumor size and *KRAS* mutational status impacts the risk of death, we defined an interaction term including both variables. For primary tumor size from CT scans and *KRAS* mutational status, no significant difference in the risk of death was detected (HR, 1.008; 95% CI, 0.988–1.030; $p > 0.5$) (Figure 4C). Similarly, there were no significant differences for primary tumor size and *KRAS* mutational status when measured in resection specimens (HR, 1.002; 95% CI, 0.978–1.027; $p = 0.807$) (Figure 4D). Along these lines, we analyzed the risk of death separately for stage I and II, as well as for T1, T2 and T3 groups regarding the interaction term combining tumor size and *KRAS* mutational status and no correlation was found (Supplementary Table 1).

Discussion

In this study, we assessed the prognostic value of combining *KRAS* mutational status with tumor size in early-stage NSCLC. We found that combining these variables had no significant effect on overall survival or the risk of death.

In alignment with previous findings, we found in our patient cohort that later disease stage and larger primary tumor size is associated with worse survival. Interestingly, we found that these correlations are sustained independent of *KRAS* mutational status. Importantly, the established literature on how KRAS mutations affect outcomes in early-stage NSCLC is varying between worse survival

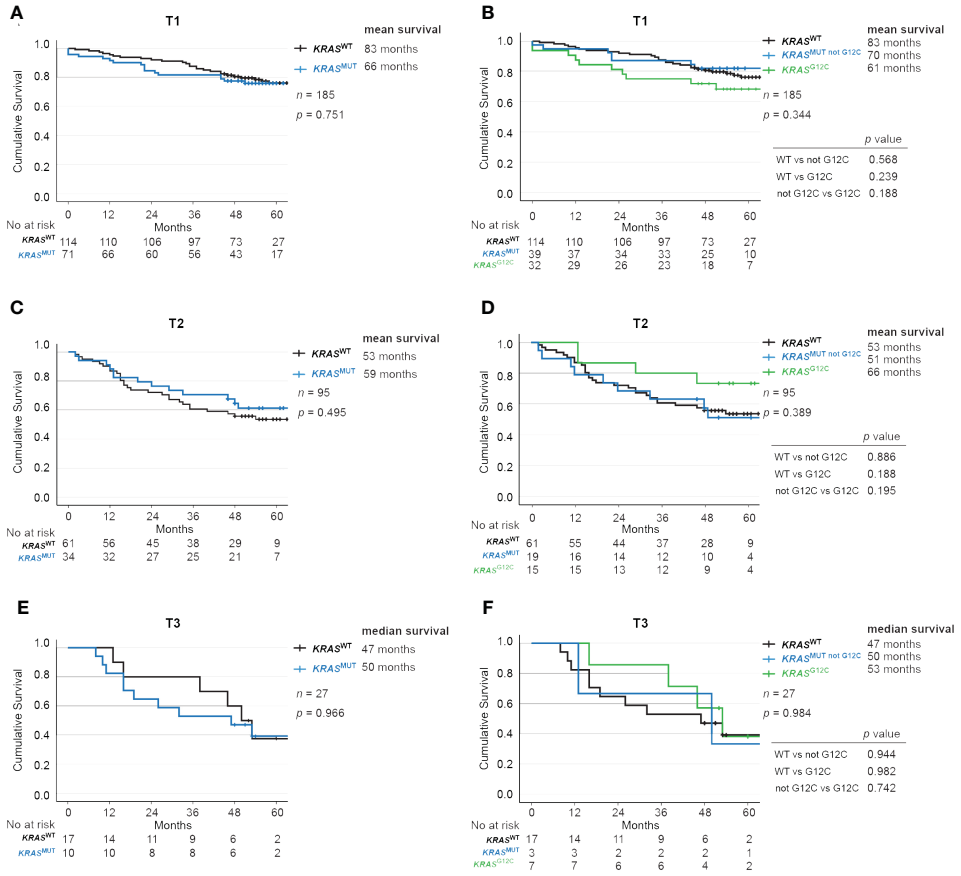

FIGURE 3
Impact of *KRAS* mutational status on overall survival across TNM-stages in NSCLC. Kaplan-Meier estimates comparing overall survival between **(A, B)** T1, **(C, D)** T2 and **(E, F)** T3 patients with no mutation in *KRAS* (wildtype, $KRAS^{WT}$), with all *KRAS* mutations ($KRAS^{MUT}$), only *KRAS-G12C* mutations ($KRAS^{MUT\ G12C}$) and *KRAS* mutations other than *G12C* ($KRAS^{MUT\ not\ G12C}$).

and no significant difference. We find that *KRAS* mutational status alone does not significantly impact OS or risk of death in patients with stage I-II NSCLC. Taken together, these findings show good representativeness of this well-defined patient cohort.

Our study included only patients with stage I-II disease due to the focus on primary tumor size and to limit the prognostic impact of local invasion and regional lymph node involvement. Only 5.8% of the patients had N1 disease that could affect the prognosis. The major portion of the patients had tumor resection and more than 90% of tumors were adenocarcinoma. During this period, patients diagnosed with squamous cell carcinoma were molecularly assessed to a lesser extent, thus our study is more representative of adenocarcinoma. Even though most tumors were classified according the TNM staging guidelines 7th edition, changes included in the 8th edition, mainly covering substages that were not analyzed in this study, do not alter our findings (4).

No significant differences were observed when comparing OS for all stage I-II patients stratified by *KRAS* mutational status. However, the mean OS was 11 months shorter for $KRAS^{MUT}$ patients. The same trend was observed when looking at resected patients with a 13-month shorter mean survival for $KRAS^{MUT}$ patients. Although trends toward a poorer prognosis were present, the $KRAS^{MUT}$ subgroup consisted of a relatively small number of patients, potentially necessitating larger cohorts to achieve statistical significance. This observation aligns with recent findings in a similar cohort of stage I LUAD with relatively small sample size in the $KRAS^{MUT}$ group (22). Within the *KRAS* mutational subgroups in our cohort, contrary to prior reports (36, 37), $KRAS^{G12C}$ mutation in stage I disease did not indicate a worse prognosis. However, as noted in another study (38), there was a tendency toward improved survival among $KRAS^{G12C}$ patients with stage II disease and T2/T3 tumors, although these differences did not reach statistical significance.

Outcome variables other than survival such as recurrence rates and progression-free survival were not examined here. In addition, there remain confounders that were not include in the analyses such as the effect of different treatment methods on survival. Further, we use the T descriptor of the TNM staging system for tumor size but the descriptor also includes invasion status and or intrapulmonary metastasis. In addition, we found that larger tumor size measured from resection specimens, but not CT scans, is associated with a higher risk of death. However, one confounder here is that non-resected patients are included in the CT group but not in the PAD group, which biases toward worse prognosis.

Going forward, much remains to be explored on the role of *KRAS* mutation in early NSCLC. In the age of precision medicine, our study contributes toward the detailed level clinical data that is

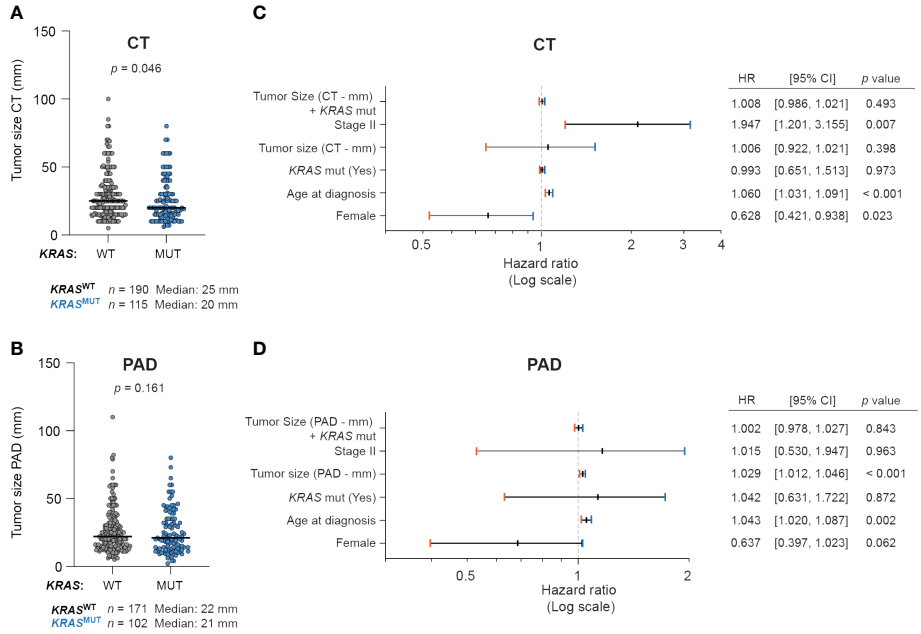

FIGURE 4
KRAS mutations are associated with smaller tumor size at diagnosis. Primary tumor size from **(A)** CT scans and **(B)** resection specimens (PAD) in patients with no mutation in *KRAS* (wildtype, $KRAS^{WT}$) or with *KRAS* mutations ($KRAS^{MUT}$). Forest plot of multivariable COX regression analysis for patients with tumor size from **(C)** CT scan and **(D)** resection specimens (PAD).

required for future pooled analysis of prognosis assessments that can help guide clinical decisions.

In conclusion, we confirm the importance of primary tumor size and stage as a prognostic factor for survival in stage I-II NSCLC. *KRAS* mutations were not found to impact OS and no difference in the risk of death was observed when combining *KRAS* mutations and primary tumor size.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Swedish Ethical Review Authority. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because all data are presented in a de-identified form according to the Swedish Ethical Review Authority, no informed consent is required.

Author contributions

EE: Methodology, Data curation, Visualization, Formal analysis, Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. AM: Writing – original draft, Formal Analysis, Data curation. CW: Formal analysis, Visualization, Writing – review & editing, Funding acquisition. SS: Writing – original draft, Writing – review & editing, Visualization. HF: Writing – review & editing, Resources, Funding acquisition, Data curation, Conceptualization. AH: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. VS: Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1396285/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Kaplan-Meier estimates of overall survival for resected Stage I-II NSCLC patients stratified by KRAS mutational status. **(A)** No mutation in *KRAS* (wildtype, $KRAS^{WT}$), with all *KRAS* mutations ($KRAS^{MUT}$). **(B)** Only *KRAS-G12C* mutations ($KRAS^{MUT\ G12C}$), *KRAS* mutations other than *G12C* ($KRAS^{MUT\ not\ G12C}$).

SUPPLEMENTARY FIGURE 2
Kaplan-Meier estimates comparing overall survival for **(A)** all patients, **(B)** patients with no mutation in *KRAS* (wildtype, $KRAS^{WT}$), and **(C)** with all *KRAS* mutations ($KRAS^{MUT}$), stratified by Stages I and II.

SUPPLEMENTARY FIGURE 3
Kaplan-Meier estimates comparing overall survival for **(A)** all patients, **(B)** patients with no mutation in *KRAS* (wildtype, $KRAS^{WT}$), and **(C)** with all *KRAS* mutations ($KRAS^{MUT}$), stratified by TNM-stages T1, T2 and T3.

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Mechanisms of resistance to KRASG12C inhibitors in KRASG12C-mutated non-small cell lung cancer

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The KRAS protein, a product of the KRAS gene (V-ki-ras2 Kirsten rat sarcoma viral oncogene homolog), functions as a small GTPase that alternates between an active GTP-bound state (KRAS(ON)) and an inactive GDP-bound state (KRAS (OFF)). The *KRAS*^{G12C} mutation results in the accumulation of KRASG12C(OFF), promoting cell cycle survival and proliferation primarily through the canonical MAPK and PI3K pathways. The *KRAS*^{G12C} mutation is found in 13% of lung adenocarcinomas. Previously considered undruggable, sotorasib and adagrasib are the first available OFF-state KRASG12C inhibitors, but treatment resistance is frequent. In this review, after briefly summarizing the KRAS pathway and the mechanism of action of OFF-state KRASG12C inhibitors, we discuss primary and acquired resistance mechanisms. Acquired resistance is the most frequent, with "on-target" mechanisms such as a new *KRAS* mutation preventing inhibitor binding; and "off-target" mechanisms leading to bypass of *KRAS* through gain-of-function mutations in other oncogenes such as *NRAS*, *BRAF*, and *RET*; or loss-of-function mutations in tumor suppressor genes such as *PTEN*. Other "off-target" mechanisms described include epithelial-to-mesenchymal transition and histological transformation. Multiple co-existing mechanisms can be found in patients, but few cases have been published. We highlight the lack of data on non-genomic resistance and the need for comprehensive clinical studies exploring histological, genomic, and non-genomic changes at resistance. This knowledge could help foster new treatment initiatives in this challenging context.

KEYWORDS

non-small cell lung cancer, KRASG12C mutation, KRASG12C inhibitor resistance, translational research, sotorasib, adagrasib

1 Introduction

The RAS (rat sarcoma viral oncogene) protein is a small guanosine triphosphate hydrolase (GTPase) that alternates between an active GTP-bound state (RAS(ON)) and an inactive GDP-bound state (RAS (OFF)). Guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) regulate the transition from RAS(ON) to RAS(OFF) and from RAS(OFF) to RAS(ON) (1). RAS(ON) promotes several important signaling pathways, primarily the RAS-RAF-MEK-ERK pathway and the RAS-PI3K-AKT-mTOR pathway, thus playing an important role in cell survival and cell cycle proliferation (2).

Growth factors can induce rapid dimerization and autophosphorylation of their receptors (GFRs). Specific tyrosine residues in noncatalytic regions of autophosphorylated GFRs can interact with the SH2 domain of the Grb2 protein. Coupled with the Son of Sevenless (Sos) protein, the Grb2-Sos complex stimulates the exchange of GDP for GTP on RAS, thus leading to RAS(ON) promotion. The Grb2-Sos complex is the primary GEF (3, 4).

Even though RAS possesses intrinsic low GTPase activity, additional proteins are needed to accelerate GTP hydrolysis. Those GAPs (such as RASA1, neurofibromin, or DAB2IP) aid, via their arginine finger, in the structural rearrangement and assembly of a catalytically competent active site, leading to nucleotide release (4, 5). Regulation of RAS signaling thus depends on a balance between GEFs and GAPs.

The RAS(ON) protein binds to RAS binding domains (RBD) located on RAS effectors, which are proteins with a strong affinity to RAS(ON). RAF (rapidly accelerated fibrosarcoma) is a critical RAS effector that triggers the RAS-RAF-MEK-ERK pathway. PI3K is another important RAS effector that activates the RAS-PI3K-AKT-mTOR pathway (4).

Four isoforms of the RAS protein are found in humans: HRAS, NRAS, KRAS4A, and KRAS4B (6), with RAS mutations detected in 19% of cancer patients (75% in *KRAS*, 17% in *NRAS*, and 7% in *HRAS*) (7).

KRAS mutations are frequent in lung adenocarcinomas, accounting for 43% of cases (8), while *NRAS* and *HRAS* mutations account collectively for approximately 1.2% of cases. Furthermore, 80% of NSCLC-*KRAS* mutations involve a glycine on position 12 substitution ($KRAS^{G12C}$, $KRAS^{G12V}$, $KRAS^{G12D}$…) and 11% involve a glycine on position 13 substitution ($KRAS^{G13C}$, $KRAS^{G13D}$, $KRAS^{G13R}$…).

The most frequent *KRAS* mutation is $KRAS^{G12C}$, present in 13% of lung adenocarcinomas (9). This glycine at position 12 substitution to cysteine in the KRASG12C protein prevents interaction with GAPs through steric blockade, resulting in reduced GTPase activity responsible for the accumulation of active KRASG12C-GTP bound protein (KRASG12C(ON)). Unlike other oncogenic mutations such as *EGFR*'s classical L858R exon 21 mutation or exon 19 deletions, *KRAS* mutations are predominantly seen in the majority of patients with a history of smoking and co-mutations are not rare (mainly *TP53*, *STK11*, and *KEAP1*). However, *KRAS* mutations are considered mutually exclusive with other NSCLC driver alterations, such as *EGFR* mutations, *EML4-ALK* fusions, or *ROS1* fusions.

Long deemed undruggable due to its lack of apparent hydrophobic pockets and its picomolar affinity for GTP/GDP, new KRASG12C inhibitors are finally under investigation in preclinical and clinical studies (10). These treatments bind covalently to an H95 residue located in an allosteric binding pocket behind switch-II, referred to as P2, near the mutated cysteine 12, in the inactive KRASG12C-GDP bound protein (KRASG12C(OFF) (Figure 1). This covalent binding in the P2 pocket induces the blocking of nucleotide exchange from GDP to GTP (10) thereby inhibiting RAS-effector interaction in $KRAS^{G12C}$ mutant cells. Despite the $KRAS^{G12C}$ mutation inducing the accumulation of KRASG12C(ON) protein, 25% of proteins in each cell remain in a GDP-bound inactive state, explaining the potential for protein inhibition (11).

Sotorasib is the first-in-class OFF-state KRASG12C inhibitor, available in France since early 2021 through an early access program. The phase III open-label randomized controlled trial (RCT) CodeBreak 200 demonstrated superior progression-free survival (PFS) over docetaxel (median PFS 5.6 months (4.3-7.8) in the sotorasib group vs 4.5 months (3.0-5.7) in the docetaxel group; 12-month PFS rate of 24.8% with sotorasib vs 10.1% with docetaxel) (12). However, the overall survival (OS) did not reach statistical significance, partly due to a decrease in sample size after protocol amendment and a 26% cross-over rate in the docetaxel group.

Adagrasib is another OFF-state KRASG12C inhibitor with results from the recently published phase I/II KRYSTAL-1 study (13) showing promising outcomes with adagrasib in the same second-line setting as sotorasib. A phase III randomized controlled trial (NCT04685135) is in progress. Additionally, studies investigating sotorasib and adagrasib (alone or combined with chemo- or immunotherapy) in the first-line and the second-line settings are ongoing (34 trials listed on clinicaltrials.gov). A summary of the main efficacy results in the second-line setting is described in Table 1.

Despite promising efficacy, resistance to OFF-state KRASG12C inhibitors occurs in virtually all patients. Notably, one-third of patients experienced early disease progression (PFS < 3 months) on sotorasib in the CodeBreaK100 study. Primary and early adaptative resistance mechanisms may drive early disease progression (14). Recent preclinical and clinical datasets suggest that resistance mechanisms to the KRASG12C inhibitors sotorasib and adagrasib may be categorized into two distinct groups: genetic and non-genetic mechanisms, which can explain early and delayed resistance at different levels.

This review aims to describe and examine emerging mechanisms of resistance to OFF-state KRASG12C inhibitors in $KRAS^{G12C}$-mutated non-small cell lung cancer (NSCLC) and to demonstrate how this body of data is shaping the therapeutical development in KRASG12C targeting.

2 Genetic mechanisms of resistance to OFF-state KRASG12C inhibitors

2.1 Genetic determinants of primary resistance

Genomic alterations were correlated with long-term benefit (PFS ≥ 12 months) versus early progression (PFS < 3 months) in

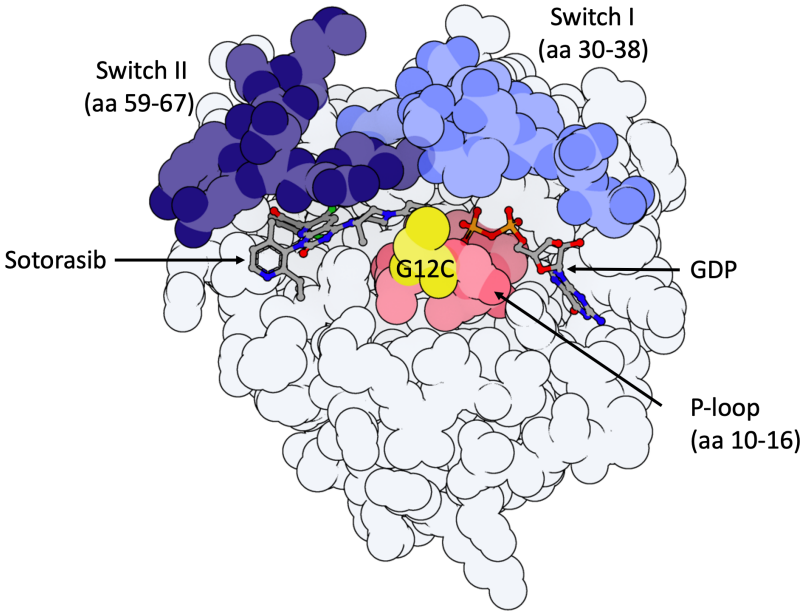

FIGURE 1
Model of KRASG12C-protein in the inactive GDP-bound state with important domains highlighted. 3D molecule was rendered with ProteinImager (http://3dproteinimaging.com) based on the crystal structure (PBD ID: 6OIM, rcsb.org/structure/6OIM). The structure of the *KRAS* gene comprises a G-domain coding region and a hypervariable region, including the conserved CAAX motif, a membrane anchor sequence (C: cysteine, A: aliphatic amino acids, X: any amino acid and residues coding for a lipid tail; not shown here). Selected structural regions of the KRAS protein G-domain are highlighted: the phosphate-binding loop (P-loop, amino acids (aa) 10 to 16) and the two switch regions (switch I (aa 30 to 38) and switch II (aa 59 to 67)). Both switch regions change conformation to make hydrogen bonds with the gamma-phosphate in GTP-bound-KRAS. Sotorasib is observed in its binding pocket (P2, behind Switch II region, near mutated Cysteine-12). The mutated Cystein-12 residue is shown in yellow. Adagrasib interacts with the P2 binding pocket in the same way as sotorasib.

the CodeBreaK100 study dataset (14). The most significant enrichment in patients with early progression was observed with mutant *KEAP1*. Aggregated with other emerging data, co-occurring mutations in *KEAP1*, *SMARCA4*, and *CDKN2A* are associated with worse clinical outcomes with sotorasib or adagrasib therapy (14–16). The biological mechanisms driving early progression in this subgroup of patients with co-occurring *KEAP1*, *SMARCA4*, and *CDKN2A* mutations are not clearly understood.

2.2 Genetic mechanisms of acquired resistance

Mechanisms of acquired resistance have been partly described following treatment with sotorasib and adagrasib. Resistance has been categorized as "on-target" or "off-target", with the majority of published data focusing on "on-target" mechanisms. Figure 2 summarizes acquired mechanisms of resistance to OFF-state KRASG12C inhibitors (11).

2.2.1 "On-target" genetic mechanisms of resistance to OFF-state KRASG12C inhibitors

"On-target" genetic mechanisms of resistance to OFF-state KRASG12C inhibitors encompass mutations of the $KRAS^{G12C}$ codon to another mutant variant on the same allele (*cis*) or a secondary KRAS mutation on the *trans-KRAS* allele. CfDNA analysis of a 67-year-old patient with $KRAS^{G12C}$-mutant NSCLC after progression on adagrasib showed a new *(trans)* $KRAS^{G12V}$ mutation (11), coexisting with the *(cis)* $KRAS^{G12C}$ mutation and probably arising from the wild-type *KRAS* allele. The persistence of a wild-type *KRAS* allele in multiple *KRAS*-mutated lung cancer cell lines was observed in preclinical studies (17). Acquired $KRAS^{G12D/R/V/W}$ mutations in other patients led to the reactivation of the KRAS downstream pathway (18). Non-$KRAS^{G12}$ mutations affecting switch II pocket and precluding drug binding, such as $KRAS^{Y96C}$, $KRAS^{R68S}$, or $KRAS^{H95D/Q/R}$, were described (18). Other KRAS mutations, like $KRAS^{G13D}$ or $KRAS^{A59S}$, induce resistance by decreasing GTP hydrolysis or promoting GDP to GTP nucleotide exchange (18).

$KRAS^{G12C}$ allele amplification or copy number gain were the only identifiable resistance mechanisms in two patients treated with adagrasib (18).

Upstream reactivation of associated proteins such as Aurora Kinase A (AURKA), a serine/threonine kinase essentially involved in mitosis and DNA repair, has been shown to facilitate effector activation by stabilizing the interaction between newly formed KRASG12C protein and RAF, thus aiding cell cycle progression in *in vitro* models (19).

In summary, the inhibition of KRASG12C(OFF) downregulates physiological negative feedback mechanisms, leading to the upregulation of GFR (19). Similar resistance mechanisms have already been described with MEK inhibitors (20) and BRAF inhibitors (21).

TABLE 1 Efficacy data from Phase II/III trials involving KRASG12C inhibitors sotorasib and adagrasib in pretreated advanced non-small cell lung cancer.

KRASG12C Inhibitor	Study	Phase	Control	Number of patients		Objective Response[a] - %, (95% CI)		Median duration of response[b], mo (95% IC)		Median PFS, mo (95% IC)		Median OS, mo (95% IC)	
				Control	KRASG12Ci	Control	KRASG12Ci	Control	KRASG12Ci	Control	KRASG12Ci	Control	KRASG12Ci
Sotorasib	CodeBreak 100	II[15]			126		37.1 (28.6-46.2)		11.1 (6.9-NE)		6.8 (5.1-8.2)		12.5 (10-NE)
	CodeBreak 200	III[13]	Docetaxel	174	174	13.2 (8.6-19.2)	28.1 (21.5-35.4)	6.8 (4.3-8.3)	8.6 (7.1-18.0)	4.5 (3.0-5.7)	5.6 (4.3-7.8)	11.3 (9.0-14.9)	10.6 (8.9-14.0)
Adagrasib	Krystal-1	II[14]			116		42.9 (33.5-52.6)		8.5 (6.2-13.8)		6.5 (4.7-8.4)		12.6 (9.2-19.2)

[a]Objective response was defined as a complete or partial response.
[b]Duration of response was evaluated based on patients with a complete or partial response. KRASG12Ci, KRASG12C inhibitor; CI, confidence interval; mo, months; NE, not evaluated.

2.2.2 "Off-target" genetic mechanisms of resistance to OFF-state KRASG12C inhibitors

"Off-target" genetic mechanisms of resistance to OFF-state KRASF12C inhibitors include:

- Amplifications or mutations of upstream RTK genes (such as *EGFR*).
- Bypass of KRASG12C through activating mutation in downstream pathway components, including *MEK*, *BRAF*, or *PI3KCA*.
- Activating mutation in *NRAS* or *HRAS*.

In an analysis of 38 patients with $KRAS^{G12C}$-mutant cancers resistant to adagrasib (18), a putative resistance mechanism was seen in only 17 patients. These mechanisms included multiple acquired bypass resistances (such as *EGFR*, *RET*, *NRAS*, *BRAF*, *PI3K*, and *PTEN* mutations), acquired gene fusions (e.g., *EML4-ALK*), or amplification (e.g., *MET*). An important limitation of this study was the limited number of tissue samples, with most analyses conducted using cfDNA sequencing. Interestingly, multiple co-resistance mechanisms were found in patients.

For example, one patient developed a $KRAS^{G12D}$ and $KRAS^{Q61H}$ mutation (an "on-target" resistance mechanism) associated with a $BRAF^{V600E}$ bypass mutation and a *CCD6-RET* fusion (an "off-target" resistance mechanism). The MET amplification was found *in vitro* to bypass the RAS pathway through the HGF/MET pathway, leading to AKT and ERK activation, but also reactivating the RAS-BRAF-MEK-ERK pathway through other RAS isoforms (22)

3 Non-genetic mechanisms of resistance to OFF-state KRASG12C inhibitors

A large majority of patients have no identifiable genetic mechanisms of resistance to OFF-state KRASG12C inhibitors, suggesting that resistance may arise from non-genetic alterations.

3.1 Upstream reactivation of growth factor receptors

Rapid adaptative resistance may be driven by GFR reactivation (19, 23) The upstream reactivation of GFRs (including multiple tyrosine kinase receptors (RTKs)) not only increases KRASG12C output but also activates wild-type KRAS and other RAS isotypes (NRAS and HRAS), which can at least partially restore MAPK signaling. This rebound mechanism, with higher concentrations of NRAS(ON) and HRAS(ON) and downstream pathway reactivation (shown by phosphorylation of downstream MAPK proteins ERK (extracellular signal-regulated kinases) and RSK (ribosomal s6 kinase)), was seen in the first 48h following KRASG12C inhibition in $KRAS^{G12C}$-mutant cell lines (19). RTKs such as EGFR, but also HER2, FGFR, and cMET were activated with different levels in different cell lines. RTK reactivation can activate

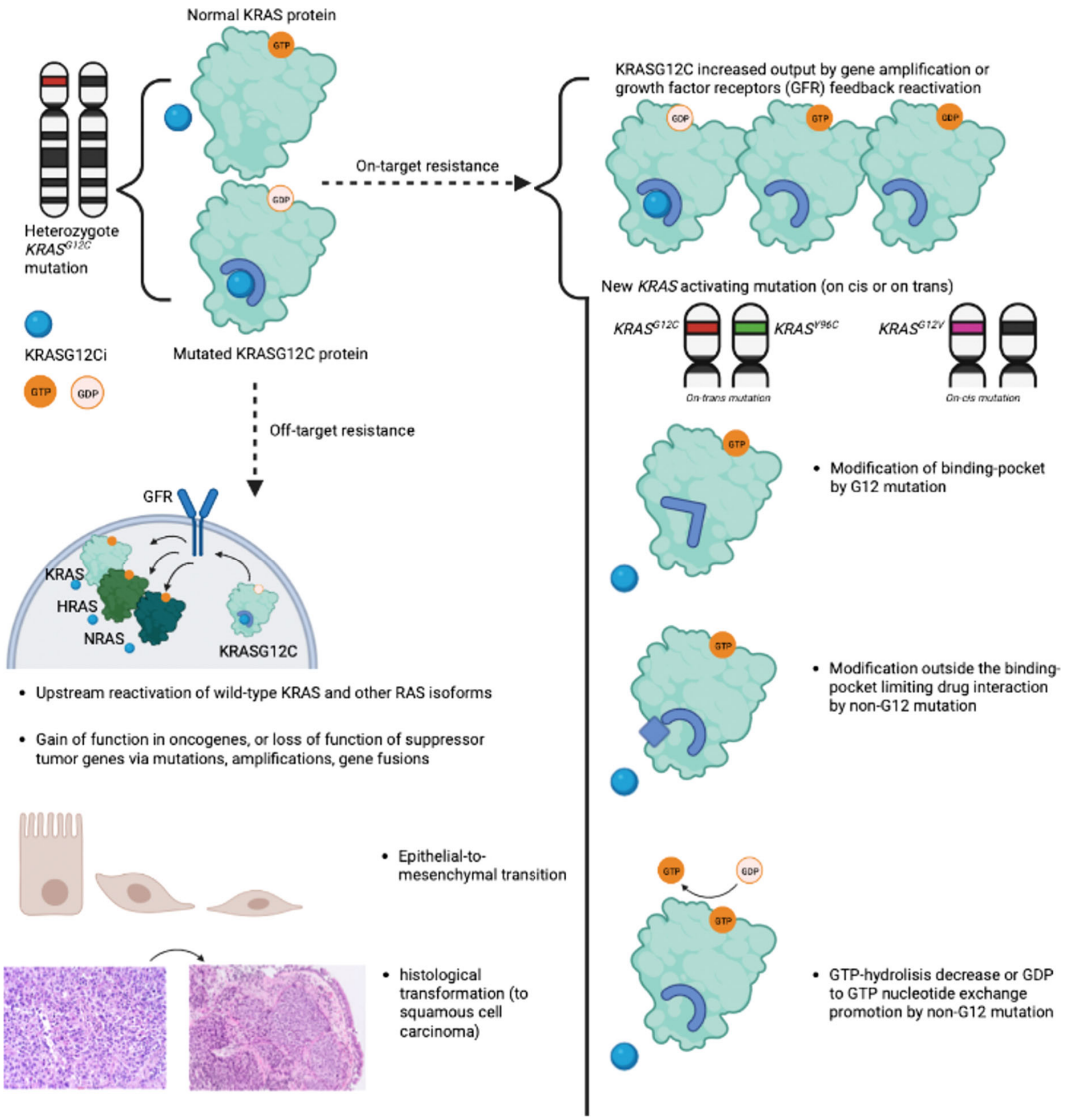

FIGURE 2
Summary of "on-target" and "off-target" resistance mechanisms to OFF-state KRASG12C inhibitors. Summary of "on-target" and "off-target" resistance mechanisms described with OFF-state KRASG12C inhibitors sotorasib and adagrasib. "On-target" resistance encompasses new *KRAS* activating mutations and increased KRASG12C output due to growth factor receptors' feedback reactivation. "Off-target" resistance mechanisms include amplification or mutations of other oncogenes, upstream reactivation of wild-type KRAS and other RAS isoforms, epithelial-to-mesenchymal transition, and adeno-to-squamous transition. The illustration was created with BioRender.com. KRASG12Ci, OFF state KRASG12C inhibitor; GFR, Growth Factor Receptor.

the downstream ERK pathway via SHP2 tyrosine phosphatase interaction with Grb2. This has been shown *in vitro* with the rapid increase of SHP2 activation in multiple *KRAS*-mutant lung, colon, and pancreatic cell lines after initial reduction following MEK inhibition (23).

3.2 Histological transdifferentiation and cell lineage plasticity

Histological transdifferentiation and cell lineage plasticity may play a role in the resistance to OFF-state KRASG12C inhibitors. Two $KRAS^{G12C}$ mutant lung adenocarcinomas treated with adagrasib showed squamous cell carcinoma histology in biopsies at progression, with no genomic alterations explaining the resistance otherwise (18). Transcriptomic and genomic analysis on pre-treatment biopsies from patients in the KRYSTAL-1 trial revealed that patients presenting a baseline high expression of squamous cell carcinoma-related genes and *STK11/LKB1* co-mutations had a shorter treatment duration with adagrasib (24). *STK11/LKB1* co-mutations are frequent in $KRAS^{G12C}$ mutant lung adenocarcinomas, and *LKB1* is a regulator of chromatin accessibility linked with cellular plasticity (25, 26). Its inactivation induced squamous transition in $KRAS^{G12D}$-mutant lung adenocarcinoma cell lines. In a preclinical study, adagrasib-resistant *KRAS/LKB1* mutant NSCLC showed enrichment in

adenosquamous transition-associated genes, including *Wnt4, Sfn, Aqp3*, and *Krt6a* (24). In another preclinical study, KRAS inhibition was associated with the transition of lung adenocarcinoma alveolar type 2 cells to alveolar type 1 (AT1) cells. AT1 cells exhibited less dependency on *KRAS* and more quiescent activity (27). Both mechanisms have been described with EGFR and ALK inhibitors in *EGFR*-mutation-positive and *ALK*-fusion-positive NSCLC models (28).

3.3 Epithelial-to-mesenchymal transition

Epithelial-to-mesenchymal transition (EMT) has been observed in KRASG12C-mutated cancer cell lines after induced resistance to sotorasib. EMT, the process by which epithelial cells acquire mesenchymal features, is associated in cancer with tumor invasion, initiation, metastasis, and resistance to therapy (29), notably with EGFR and ALK inhibitors in *EGFR*-mutation-positive and *ALK*-fusion-positive NSCLC models (30, 31). EMT can be induced by numerous biological drivers such as TGFβ, TNF-α, HIF-α, Wnt signaling, interleukins, Hedgehog, and Hippo pathways (32). Genes related to EMT were enriched in sotorasib-resistant NSCLC cell lines (33). Induction of EMT via chronic TGFβ treatment was sufficient to induce resistance to sotorasib. In this population of EMT-induced cells, rebound activation of ERK and S6 was observed. Cell growth was inhibited after the addition of a PI3K inhibitor to sotorasib, implying that KRASG12C-independent AKT activation is a cause of resistance to sotorasib in EMT-induced $KRAS^{G12C}$-mutant NSCLC cell lines. These results were confirmed in a xenograft mouse model. EMT dependence on CDK-4 (cyclin-dependent kinase 4) has also been described in *in vitro* models, with promising efficacy for CDK4 inhibitors in reducing tumor volume in a murine model with autochthonous mesenchymal-like lung cancer with a $KRAS^{G12D}$ mutation (34).

3.4 Tumor microenvironment

The tumor microenvironment (TME) associated with *KRAS*-mutant tumor cells is highly immunosuppressive (35). The TME is transiently converted to a less immunosuppressive state following RAS inhibition and may increase susceptibility to immunotherapies.

Due to a lack of data, other non-genomic resistance mechanisms to KRASG12C cannot be described to date. For example, no data about epigenetic dysregulation in this context are yet available.

4 Lessons from known mechanisms of resistance to OFF-state KRASG12C inhibitors and therapeutical perspectives

Co-mutations in key tumor suppressor genes (*KEAP1*, *SMARCA4*, and *CDKN2A*) define different subgroups of KRASG12C-mutant NSCLC with clearly different clinical outcomes with OFF-state KRASG12C inhibitors. These co-mutations may serve as biomarkers of clinical activity for OFF-state KRASG12C inhibitors, should be integrated as stratification factors in clinical trials, and may guide escalated or de-escalated treatment strategies. The key role of co-mutations in defining patient subgroups with primary resistance and the diversity of on-target mechanisms of resistance explaining early and acquired resistance to OFF-state KRASG12C inhibitors suggest that KRASG12C-mutant lung adenocarcinomas are highly genetically heterogeneous. This intra-tumoral genetic heterogeneity could partially explain the high proportion of early progressors and the low proportion of durable responders. Pan-RAS/KRAS inhibitors may prevent the emergence of acquired on-target mutations on *KRAS* and partly address the role of genetic heterogeneity in resistance to KRAS inhibition. For example, RMC-6236 is an ON-state RAS multi-selective noncovalent inhibitor of the active, GTP-bound state of both mutant and wild-type variants of RAS isoforms (36). RMC-6236 exhibited potent anticancer activity across RAS-addicted tumor models and showed early clinical activity (36, 37).

Amplifications or mutations of upstream RTK and upstream reactivation of GFR that drive RTK-driven pathway rebound can be prevented by RMC-6236 and ON/OFF-state direct inhibitors such as FMC-376 (38).

Upstream and downstream dysregulation of the RAS signaling pathway induced by OFF-state KRASG12C inhibitors offer attractive targets for combination therapies. SOS1 and SHP2 are activated by RTK and regulate the switch of RAS from the OFF state to the ON state. Inhibition of SOS1 and SHP2 activity stabilizes GDP-bound RAS in an inactive form (39–41). Several combinations including SOS1 and SHP2 inhibitors are under clinical evaluation (42).

Other combinations under clinical evaluation include anti-PD-(L)1 with sotorasib or adagrasib (43). Sotorasib induced a proinflammatory tumor microenvironment highly sensitive to immunotherapy in a preclinical study (44). The combination of sotorasib and anti-PD-1 therapy resulted in a higher response rate and more durable responses in mice compared to sotorasib monotherapy or anti-PD-1 monotherapy (44). However, this strategy with sotorasib is limited in clinical practice due to higher rates of side effects, mainly hepatotoxicity, when sotorasib is prescribed in combination with or following anti-PD(L)1 therapy (45, 46). Preliminary results from the KRYSTAL-7 phase II trial did not show a higher rate of hepatotoxicity with adagrasib and pembrolizumab, hinting at a possible non-class effect (47). Multiple ongoing studies are investigating OFF-state KRASG12C inhibitors and anti-PD(L)1 agents (43).

5 Conclusion

Several phase III trials comparing OFF-state KRASG12C inhibitors (sotorasib, adagrasib, divarasib, olomorasib, opnurasib) to standard-of-care chemotherapy and immunotherapy in NSCLC are ongoing (47–49). They will provide highly valuable data on the benefit of these drugs, the optimal sequencing strategy, and hopefully, insights into mechanisms of resistance to these drugs.

The design of these trials mainly relies on patient selection according to *KRAS*^{G12C} mutation and PD-L1 biomarkers.

The understanding of resistance mechanisms to KRASG12C inhibitors is in its early stages. It relies on data generated with OFF-state KRASG12C inhibitors, mainly sotorasib and adagrasib, but provides an essential framework for future rationally designed therapeutic development. Thus, the early emergence of RAS-MAPK signaling reactivation through acquired resistance mutations and upstream reactivation of GFR underscores the strong need for RAS signaling in *KRAS*^{G12C}-mutant cancers as well as the major role of tumor heterogeneity in resistance to OFF-state KRASG12C inhibitors. As a result, therapeutic strategies based on strong inhibition of RAS signaling (ON- or ON/OFF-state RAS inhibitors), broad inhibition of the RAS pathway (pan-RAS inhibitors), and combination strategies that target upstream or downstream of the RAS pathway are relevant and currently being evaluated in clinical trials. There is a strong biological rationale supporting KRASG12C inhibitors and immunotherapy, specifically anti-PD-(L1) agents, and combination strategies, and multiple clinical trials are evaluating the safety and clinical activity of such combinations. Overall, the clinical evaluation of drug combination strategies is the way to address the problem of primary and acquired resistance to KRASG12C inhibitors. These efforts should focus on understanding the biology driving KRASG12C-targeting clinical efficacy and selecting the most effective and relevant combination strategy and predictive biomarker of efficacy for future development, especially through phase III trials. Therapeutic platforms such as Master Protocols can effectively evaluate multiple combination strategies in KRAS^{G12C}-mutant NSCLC. ctDNA sample analysis can be a highly valuable tool for identifying early signals of efficacy and understanding mechanisms of resistance that may drive future preclinical and clinical development.

Author contributions

AC: Conceptualization, Validation, Writing – original draft, Writing – review & editing. A-CT: Conceptualization, Validation, Writing – review & editing. EB: Validation, Writing – original draft, Writing – review & editing. MD: Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

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