Scientific Reports

https://doi.org/10.1038/s41598-026-48898-1

Article in Press

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Received: 19 February 2026

Accepted: 10 April 2026

Published online: 25 May 2026

Cite this article as: Giménez-Capitán A., Olmo-Gónzalez D., Martínez-Pérez E. *et al.* Expression of *LIFR* in tumor and *SHOC2*, *YAP1* in plasma mRNA as potential biomarkers in *KRAS* ^{G12C} NSCLC. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-48898-1

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Expression of *LIFR* in tumor and *SHOC2*, *YAP1* in plasma mRNA as potential biomarkers in *KRAS* ^{G12C} NSCLC

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ABSTRACT

Background

Advanced non–small cell lung cancer (NSCLC) harboring *KRAS* ^{G12C} mutations can be treated with selective inhibitors, however progression-free survival remains limited. Resistance has been associated with co-mutations in *TP53*, *STK11*, and *KEAP1*, persistent plasma *KRAS* ^{G12C}, and activation of the MRAS–SHOC2–PP1C pathway with downstream YAP1 signaling. We evaluated the expression of MRAS–SHOC2–PP1C and YAP1-related genes in *KRAS* ^{G12C} mutant NSCLC.

Methods

Messenger RNA levels of twenty genes were quantified using nCounter in tumor samples from 98 NSCLC patients, including *KRAS* ^{G12C} (n=23), *KRAS* $^{non\text{-}G12C}$ (n=24), and *KRAS* $^{wild\text{-}type}$ (n=51) cases. Longitudinal plasma samples from ten *KRAS* ^{G12C} patients treated with selective inhibitors were analyzed at baseline, day 3, week 6, and week 12.

Results

Eight genes (*NFE2L2, NRAS, KRAS, ENO1, SHOC2, VCP1, LIFR*, and *MRAS*) were differentially expressed in *KRAS* ^{G12C} compared with *KRAS* wild-type tumors ($p<0.05$). *LIFR*, *KRAS*, and *MET* were differentially expressed in *KRAS* $^{non\text{-}G12C}$ tumors. Among 30 *KRAS*-mutant patients, high *LIFR* expression was associated with improved survival. In plasma, twelve genes were consistently detected, and *SHOC2, YAP1, LZTR1, RGS3*, and *ZDHHC7* were significantly upregulated at week 6.

Conclusions

Low tumor *LIFR* expression was associated with poorer survival, supporting its potential as a prognostic biomarker and highlighting the feasibility of plasma-based gene expression monitoring.

1. Introduction

KRAS is often mutated in lung adenocarcinoma (Rosell and Karachaliou, 2016), most commonly as a glycine-to-cysteine substitution at position 12 (G12C). GDP- and/or GTP-bound *KRAS* ^{G12C} inhibitors have been extensively developed (Ebright et al., 2025), including adagrasib, sotorasib, divarasib, olomorasib and fulzerasib, among others. Common co-mutations in TP53, serine-threonine Kinase 11 (STK11), and Kelch-like ECH-associated protein 1 (KEAP1), along with less frequent mutations, diminish the clinical efficacy (shorter progression-free survival and overall survival) of *KRAS* ^{G12C} inhibitors. In previously treated *KRAS* ^{G12C}–mutated lung cancer patients receiving sotorasib, median progression-free survival was 7.75 months with TP53 mutations and 5.72 months with KEAP1 co-mutations, with corresponding median overall survival of 16.00 and 16.62 months, respectively (Skoulidis et al., 2025). Mechanisms of resistance to single-agent *KRAS* ^{G12C} inhibitors include increased *KRAS* mRNA and *KRAS* protein resynthesis. In *KRAS* ^{G12C} mutant cells lines, treatment with *KRAS* ^{G12C} inhibitors initially suppressed extracellular signal-regulated kinase (ERK), followed by a rebound within 72 hours (Xue et al., 2020). The MRAS-SHOC2-protein phosphatase 1 catalytic subunit (PP1C) complex activates rapidly accelerating fibrosarcoma (RAF)-ERK pathway signaling (Sieburth et al., 1998; Simanshu et al., 2017). Genetic inhibition of the leucine-rich repeat scaffolding protein *SHOC2* suppresses tumor growth in a subset of *KRAS*-mutant NSCLC and inhibits tumor progression in murine *KRAS*-driven lung cancer models (Jones et al., 2019). Interestingly, *SHOC2* deletion selectively sensitizes *KRAS* and *EGFR*-mutant NSCLC to MEK inhibitors. *SHOC2* deletion prevents MEK-induced RAF dimerization, permitting stronger and more durable ERK suppression (Jones et al., 2019). *SHOC2* expression levels influence sensitivity to EGFR tyrosine kinase inhibitors in *EGFR*-mutant NSCLC and to *EGFR* inhibitors in NSCLC, mediated through the SHOC2–MRAS–PP1C and SHOC2–Scribble signaling pathways (Terai et al., 2021). Scribble is deregulated in NSCLC and cooperates with oncogenic *KRAS* in the murine lung, as Scribble loss synergizes with *KRAS* ^{G12D}, resulting in more aggressive lung tumors (Elsum et al., 2014). Scribble directly binds *SHOC2* and prevents its interaction with

PP1C (Kwon and Hahn, 2021). Recent studies have shown that the SHOC2–MRAS–PP1C complex is formed only when MRAS is in an active state and dependent on SHOC2 functioning as a scaffolding protein. It is posited that while MRAS is GDP-bound, PP1C and SHOC2 exist in a bound and unbound equilibrium in the cytoplasm (Bonsor et al., 2022; Hauseman et al., 2022; Liau et al., 2022). After receptor tyrosine kinase stimulation and MRAS-GTP activation, the SHOC2-PP1C complex binds with MRAS at the plasma membrane to produce stable complex formation, leading to dephosphorylation of RAF serine 259 and allowing MAPK signaling (Kwon et al., 2022). It was previously reported that MRAS–SHOC2 signaling modulates E-cadherin turnover (Kota et al., 2019). Interestingly, *KRAS* ^{G12C} inhibitors sotorasib or adagrasib induced MRAS expression, forming complexes with SHOC2 and relocalizing Scribble in *KRAS* ^{G12C} mutated NSCLC cell lines, leading to yes-associated protein 1 (YAP1) activation, which in turn activated MRAS (Adachi et al., 2023). Palmitoylation of the Scribble protein is regulated by the zinc-finger Asp-His-His-Cys (ZDHHC) domain-containing protein acyltransferase ZDHHC7 (Santoni et al., 2020), and ZDHHC7 protein expression was reduced after sotorasib treatment in *KRAS* ^{G12C} mutated NSCLC cell lines (NCI-H358 and LU65) (Adachi et al., 2023). Mislocalization of Scribble with relocalization of E-cadherin, leading to YAP1 activation and transcriptional upregulation of *MRAS*, was confirmed by quantitative PCR (Adachi et al., 2023). Further evidence has shown that mislocalized Scribble interacts with the non-receptor tyrosine kinase YES1, promoting nuclear *YAP1* and *YAP1* tyrosine 357 phosphorylation (Zhao et al., 2021). Recently, Scribble loss increased *YAP1* activation in mouse pancreatic ductal adenocarcinoma organoids, and clinically, Scribble expression was decreased in human pancreatic ductal adenocarcinomas and associated with poorer patient outcomes (Bermejo-Rodriguez et al., 2024). These findings stress the importance of *SHOC2* and *PP1C*, which form complexes with *MRAS* and H-, K-, and N-RAS proteins (Bonsor and Simanshu, 2024).

Earlier studies reported that expression of leukemia inhibitory factor receptor (LIFR) in breast cancer cells maintains Scribble cell polarity, thereby promoting cytoplasmic sequestration and degradation of *YAP1* (Chen et al., 2012). In nearly 1000 breast cancers, *LIFR* expression status correlated with metastasis-free and

overall survival outcomes (Chen et al., 2012). *LIFR* was later shown to be diminished in pancreatic cancer tissues and to induce E-cadherin expression (Ma et al., 2016). Low *LIFR* expression is associated with shorter survival in pancreatic and *KRAS-mutant* lung adenocarcinomas (Liu et al., 2021b). Intriguingly, the MRAS–SHOC2–PP1C complex is inhibited by the HECT, UBA, and WWE domain–containing E3 ligase 1 (*HUWE1*) via the ubiquitin–proteasome system; however, its resetting is regulated by other proteins, including the adenosine triphosphatase associated with diverse cellular activities (AAA-ATPase) valosin-containing protein (*VCP1*), also known as p97. *VCP1* recognizes and binds polyubiquitinated *SHOC2*, resulting in *HUWE1* dissociation from the complex and subsequent MAPK reactivation (Kwon and Hahn, 2021). Furthermore, we were also interested in examining regulator of G protein signaling 3 (RGS3). Higher RGS3 expression was associated with lower mutant KRAS output in lung cancers harboring G12C or any *KRAS* mutation, and RGS3-null cells had an attenuated response to G12C inhibitors (Li et al., 2021a). Nuclear factor erythroid 2-related factor 2 (NFE2L2) reprograms cell metabolism by regulating the expression of genes and enzymes involved in various metabolic pathways, including glycolysis (e.g., enolase 1) (He et al., 2020). High expression levels of enolase 1 (*ENO1*) were associated with poor survival in NSCLC patients, and *ENO1* interacts with hepatic growth factor receptor and activates Wnt signaling (Li et al., 2021b). Enhanced *MET* expression and signaling has been shown to be essential for anchorage-independent growth of *KRAS*-mutant NSCLC (Fujita-Sato et al., 2015; Ma et al., 2005). No less important, leucine zipper-like transcriptional regulator (LZTR1), a regulator of RAS GTPase proteins, mediates proteasomal degradation of RAS proteins such as NRAS, KRAS, HRAS, MRAS, and others. Finally, elevated levels of fatty acid synthase (FASN) have been related to shorter overall survival in *KRAS*-mutated NSCLC (Gouw et al., 2017; Liu et al., 2021b).

In this study, we surmise that assessing transcripts of the twenty genes above could reveal differences between *KRAS* ^{G12C} mutated NSCLC, *KRAS* $^{non\text{-}G12C}$ mutated NSCLC, and KRAS wild-type (*KRAS* ^{WT}) NSCLC tumor tissues. We also sought to determine whether transcript expression levels could serve as a means of monitoring response to *KRAS* ^{G12C} inhibitors through serial transcript analysis

in plasma mRNA from *KRAS* ^{G12C} mutated NSCLC patients. We hypothesized that monitoring plasma transcripts could, to some degree, mirror the principal adaptive response pathways, such as *SHOC2-MRAS-PP1C*, alongside changes in Scribble, *HUWE1*, *VCP1*, *ZDHHC7*, *YES*, and *YAP1* expression. Additionally, we examined *LIFR*, E-*cadherin*, *MET*, *ENO1*, *NFE2L2*, *LZTR1*, *RGS3*, and *NRAS* and *HRAS* (Rosell et al., 2024a).

2. Materials and Methods

2.1 Patients and sample collection

A prospective, multicenter, observational study was conducted at the Dr. Rosell Oncological Institutes and Pangaea Oncology, Laboratory of Oncology in the Dexeus University Hospital, Barcelona, Spain between November 2023 and October 2025 **(Supplementary Table 1)**. The study was carried out in accordance with the principles of the Declaration of Helsinki, under a protocol approved by the institutional review board of Quirón Hospital (internal code 2023/93-ONC-DEX; act no. 21/2023). Written informed consent was obtained from all patients, and samples were de-identified to ensure patient confidentiality.

A total of 106 formalin-fixed paraffin-embedded (FFPE) lung cancer samples obtained at diagnosis, along with one FFPE sample collected at disease progression, were included in the study. Clinical data, histology and programmed death-ligand 1 (PD-L1) tumor proportion score (TPS) from tumor tissue were retrieved from the clinical reports available in the electronic patient records and data was corroborated with the oncologist physician. All tumor samples were reviewed by in-house pathologists, and only those with a tumor cellularity of ≥20% were accepted for analysis, ending with 98 FFPE samples.

During the same period, as part of the approved protocol, peripheral blood samples were collected from a subset of patients harboring *KRAS* ^{G12C} mutations (N = 10) at multiple time points: prior to initiation of *KRAS* ^{G12C} inhibitor treatment, on day 3 after the first dose, at weeks 6 and 12, and at the time of disease progression **(Supplementary Figure 1)**. Whole blood was drawn into sterile 10-

mL BD Vacutainer® K2E tubes (BD, Plymouth, UK) following standardized venipuncture procedures to minimize hemolysis. Samples were processed within 24 hours of collection, and visual inspection for hemolysis was performed before further handling. Blood was centrifuged twice at 500 × g for 10 minutes, and the plasma supernatant was carefully transferred to a new sterile tube using a Pasteur pipette and recentrifuged under the same conditions to minimize cellular contamination. Circulating cell-free DNA (cfDNA) and RNA (cfRNA) were immediately isolated using standardized extraction protocols. RNA and DNA concentration were measured using a Qubit fluorometer, and only samples meeting predefined quality and quantity criteria were included in downstream analyses. All procedures were conducted consistently across samples to ensure reproducibility and reduce technical variability.

2.2 DNA/cfDNA Extraction, NGS and Real-Time Quantitative PCR (qPCR) Analysis

FFPE biopsies with a minimum area of 4 mm^2 with at least 10% tumor infiltration were required for NGS analysis. DNA from samples larger than 20 mm^2 was purified using the QIAamp DNA FFPE Advanced kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. DNA from samples with less than 20 mm^2 was not purified being directly extracted by macrodissection in a proteinase K (QIAGEN) solution. DNA concentration was measured by Qubit™ (Thermo Fisher Scientific, Waltham, MA). DNA-based next-generation sequencing (NGS) was performed using a custom-designed panel (Qiagen®) following the manufacturer's instructions. The panel is designed to enrich specific target regions for mutations and copy number variations (CNVs) in 30 genes frequently altered in solid cancer tumors (*ALK, ARID1A, BRAF, CDK4, CDK6, EGFR, ERBB2, ERBB4, FAT1, FGFR1, FGFR2, FGFR3, IDH1, IDH2, KEAP1, KIT, KRAS, MET, MYC, NFE2L2, NRAS, PDGFRA, PIK3CA, POLD1, POLE, RET, ROS1, SETD2, STK11, TP53*, including *MET* exon 14 skipping mutations). Up to 83.75 ng of DNA was used as a template to generate libraries. Libraries were quantified using a QIAxcel Advanced System and Qubit dsDNA HS Assay kit, diluted to 4 nM. Pooled libraries (3.5-5 pM) were sequenced using MiSeq Illumina Platform according to manufacturer's instructions.

cfDNA from plasma samples was isolated from 4 mL of plasma using the QIAsymphony DSP Virus/Pathogen Midi Kit in a QIAsymphony robot (Qiagen), following the manufacturer's DNA instructions. Final elution volume was 50 µL in all cases. DNA concentration was estimated by Qubit™. For NGS analysis, purified DNA (16.75 µL, ~ 15-84 ng) was used as a template to generate libraries for sequencing using a DNA custom-designed panel (Qiagen®) according to manufacturer's instructions. The panel is designed to enrich specific target regions in 19 selected genes frequently altered in solid cancer tumors (*ALK, BRAF, CDK4, CDK6, EGFR, ERBB2, ERBB4, FGFR1, IDH1, IDH2, KIT, KRAS, MET, NRAS, PDGFRA, PIK3CA, ROS1, STK11, TP53*), including *MET* exon 14 skipping mutations. Libraries were quantified using a QIAxcel Advanced System and Qubit dsDNA HS Assay kit, diluted to 4 mM. Pooled libraries (3.5-5 pM) were sequenced using Illumina Platform according to manufacturer's instructions.

Additionally, in ten cases, longitudinal follow-up of *KRAS* ^{G12C} mutation in plasma samples was performed using a Real-Time Quantitative PCR (qPCR) with TaqMan™ assay to evaluate changes in the detection of *KRAS* ^{G12C} mutation throughout the course of the disease. Methods previously described in (Mayo de Las Casas et al., 2019; Mayo-de-Las-Casas et al., 2020; Villatoro et al., 2019). **(Supplementary Figure 1).**

2.3 RNA/cfRNA Extraction and Gene Expression Analysis

For FFPE tissue samples, RNA isolation was performed using the High Pure RNA Isolation Kit (Roche Diagnostics). The purity and concentration of the RNA extracts were determined using a Qubit™ fluorometer. A total of 260 ng/µL of RNA was then hybridized with a custom-designed mixture of biotinylated capture tags and fluorescent labeled reporter probes (Elements Chemistry). All hybridization, capture, clean-up, and digital data acquisition processes were conducted using the nCounter Prep Station and Digital Analyzer (NanoString Technologies), following the manufacturer's instructions. The hybridization was carried out using two custom-designed panels. On one hand, a panel targeting 20 transcripts for human gene expression analysis (*MET, KRAS, MRAS, SHOC2, PP1C, HUWE1, VCP1, LIFR, SCRIBBLE, ZDHHC7, YES, YAP1, E-CADHERIN,*

FASN, LZTR1, NFE2L2, ENO1, RGS3, NRAS and HRAS), as well as one transcript from the *Fusobacterium nucleatum* associated bacterium (*F. nucleatum*) and three housekeeping genes (*GAPDH, MRPL9,* and *PSMC4*), was used. On the other hand, a custom panel targeting transcripts for fusions detection (*ALK, RET, ROS1, NTRK1-3, FGFR1-3, NRG1*) and *METΔex14* splicing variant (Reguart et al., 2017) was employed.

For cfRNA isolation, 1.2 mL of plasma was processed using the QIAsymphony DSP Virus/Pathogen Midi Kit on a QIAsymphony automated instrument (Qiagen), following the manufacturer's RNA protocol. RNA was eluted in a final volume of 50 µL for all samples. RNA concentration was quantified using a Qubit™ fluorometer. A total of 5 ng/µL of RNA was reverse-transcribed into complementary DNA (cDNA) using the nCounter® Low RNA Input Amplification Kit (NanoString Technologies, Seattle, WA), which includes proprietary reverse transcription primers and enzyme mixes. The resulting cDNA was subjected to a 14-cycle preamplification step using the same kit and a custom-designed primer pool, performed on a Veriti™ thermal cycler (Applied Biosystems) according to the manufacturer's instructions. Subsequent processing steps, including hybridization with the previously described 20-gene custom panel, post-hybridization processing, and data acquisition using the nCounter® Digital Analyzer, were performed in accordance with the manufacturer's guidelines. **(Supplementary Figure 1).**

2.4 Data normalization and statistical analysis

For the NGS analysis, sequence alignment was performed against the GRCh38 (hg38) reference genome using the CLC Genomics Workbench software (Qiagen®), with a minimum coverage of 100X for FFPE samples and 200X for liquid biopsy samples. Screening , classification and variant analysis is performed with QIAGEN Clinical Insight Interpret One (QCI-One) software (Qiagen) in all cases.

For the nCounter analysis, raw data were processed using the nSolver software (NanoString, version 4.0) and R software (version 4.1.2) with package NanoStringNorm.

In tissue samples, to minimize technical assay variability, background correction was applied to the target gene counts of each sample by subtracting the mean plus two standard deviation (SD) of the negative control probes. Subsequently, target gene counts were normalized by dividing each count by the geometric mean of the housekeeping genes within each sample. Normalized counts were subsequently log_2-transformed and used for downstream differential expression analyses performed in R. Samples were considered non-valuable if the geometric mean of housekeeping gene counts was below the cut off of 300. The fold change (FC) for each gene was calculated by comparing the mean normalized counts in the *KRAS* ^{G12C} mutant cohort to those in the *KRAS* ^{WT} or *KRAS* $^{non\text{-}G12C}$ groups. Student's t-test was used to calculate p-values for gene expression analysis. Genes showing a statistically significant difference between groups (cut off, *p-value* < 0.05, determined by Student's *t*-test) and/or $|Log_2(FC)|$ greater than 2, were classified as differentially expressed. Volcano plots were generated to represent the $Log_2(FC)$ on the X-axis and the $-Log_{10}$(p-value) on the Y-axis for each gene. The Student's *t*-test p-values were not adjusted for false discovery rate (FDR), as the analysis was limited to a predefined panel of 20 genes selected based on prior biological knowledge and specific hypotheses, rather than a large-scale exploratory approach.

In plasma samples measurements were performed in duplicate to ensure technical reliability. To minimize technical assay variability, background correction was applied to the target gene counts of each sample by subtracting the mean of the negative control probes. Subsequently, target gene counts were normalized by dividing each count by the geometric mean of the housekeeping genes within each sample. Samples were considered non-valuable if the geometric mean of housekeeping gene counts was below the cut off of 300.

Finally, correlation analyses were performed using the Spearman Rank Correlation test, while comparisons between groups were conducted using the Wilcoxon Signed-Rank test. Overall survival by gene expression differences were evaluated using the Log-Rank test.

3. Results

Demographic and clinicopathological characteristics of the study population are summarized in **(Table 1)**. The cohort comprised an equal distribution of males and females (50% each), with ages at diagnosis ranging from 37 to 85 years (mean age: 64 years). Most patients (76.54%) were former or current smokers. According to pathology reports, the majority of tumors were adenocarcinomas (89.79%), and most patients presented with advanced disease (stage IIIB–IV; n = 61, 62.24%). The most frequent metastatic site was the brain (14.28%), followed by the adrenal glands (12.7%) and bone (11.11%). Regarding PD-L1 status, most patients exhibited negative or low expression levels. Concerning *KRAS* mutant status, 23.47% (n = 23) of cases harbored a *KRAS*^{G12C} mutation, whereas 24.49% (n = 24) carried *KRAS*$^{non\text{-}G12C}$ mutations, the most frequent being *KRAS* ^{G12A} (n = 6, 25%), *KRAS* ^{G12V} (n = 5, 20.8%), and *KRAS* ^{G13C} (n = 4, 16.6%). The remaining 52.04% (n = 51) were *KRAS* ^{WT}.

3.1 Alterations detection by NGS in FFPE tissue samples

For all 98 FFPE samples, successful NGS results were obtained. The most frequently altered gene was *TP53*, followed by *STK11*, *EGFR*, and *KEAP1*. In the *KRAS* ^{WT} cohort, *TP53* alterations were identified in 31 samples, *EGFR* alterations in 9 samples, and *STK11* alterations in 5 samples. Within the *KRAS* ^{G12C} subgroup, the most common co-occurring mutations involved *KEAP1*, *TP53*, and *STK11*, detected in 7, 5, and 5 samples, respectively; two patients harbored concomitant mutations. *ARID1A* mutations were observed in two samples. In the *KRAS* $^{non\text{-}G12C}$ subgroup, *TP53* was the most prevalent co-mutation (n = 12), followed by *STK11* (n = 5) and *KEAP1* (n = 2), with one sample exhibiting concomitant mutations. **(Fig. 1)**.

3.2 Gene expression profiling in FFPE tissue samples

From the total of 98 FFPE tumor samples, satisfactory results were obtained for the nCounter gene expression analysis. Individual gene expression analyses

were first performed for those genes for which at least two groups contained more than 15% valid samples. To identify differentially expressed genes in *KRAS* ^{G12C} and *KRAS* $^{non\text{-}G12C}$ mutant samples, average expression levels were compared to those of *KRAS* ^{WT} samples. Hierarchical clustering was generated using the 98 samples, stratifying them by mutation type (*KRAS* ^{G12C}, *KRAS* $^{non\text{-}G12C}$, or *KRAS* ^{WT}), to identify and visually represent genes with differential expression across KRAS mutation subtypes **(Fig. 2A)**.

Genes showing a statistically significant difference between groups (*p-value* < 0.05, determined by Student's *t*-test) and/or $\left|\text{Log}_2(\text{FC})\right|$ greater than 2, were classified as differentially expressed. In *KRAS* ^{G12C} mutant samples, seven genes showed statistically significant differences in expression compared to *KRAS* ^{WT} samples. These genes, ranked by highest fold change or p-value < 0.05, were *LIFR*, *NRAS*, *KRAS*, *SHOC2*, *ENO1*, *VCP1*, and *MRAS* **(Fig. 2B; Supplementary Table 2)**. In *KRAS* $^{non\text{-}G12C}$ mutant samples, three genes were identified as significantly differentially expressed compared to *KRAS* ^{WT}. These genes, ranked by highest fold change or p-value < 0.05, were *LIFR*, *KRAS*, and *MET* **(Fig. 2C; Supplementary Table 3)**. When comparing *KRAS* ^{G12C} to *KRAS* $^{non\text{-}G12C}$ mutants, *SHOC2* and *NFE2L2* were the only genes showing statistically significant expression differences **(Fig. 2D; Supplementary Table 4)**.

Next, gene expression correlation in *KRAS*-mutant samples were performed with Spearman Rank Correlation test **(Table 3)**. Correlations were considered significant when p-value < 0.05 and Spearman's rho (ρ) ≥ 0.70. A strong correlation was observed between *MRAS* and *RGS3* expression levels (ρ = 0.77, *p* = 0.00000042), regardless of *KRAS* mutation subtype. In samples with *KRAS*^{G12C} mutations, significant correlations were detected between *RGS3* and *LZTR1*, *VCP1*, and *ZDHHC7*. Additionally, *ZDHHC7* correlated with *LZTR1* and *VCP1*, and *VCP1* correlated with *MRAS*. In *KRAS* $^{non\text{-}G12C}$ samples, *VCP1* expression correlated with *NFE2L2*, *HUWE1*, and *LIFR*; *NFE2L2* correlated with *SHOC2*; *HUWE1* correlated with *LIFR*; and *MRAS* correlated with *LZTR1*. No significant correlations were observed among the other genes analyzed.

Finally, the impact of the twenty biomarkers on survival was also evaluated in the 30 *KRAS*-mutant NSCLC patients included in our study. Five genes *RGS3*, *KRAS*, *LIFR*, *E-CADHERIN* and *ENO1*, showed a statistically significant

association between high mRNA expression levels and overall survival (OS). The Kaplan–Meier curves shown in **(Fig. 2E-I)** illustrate these effects. Patients with high mRNA expression of *RGS3* (p = 0.0043; hazard ratio (HR) = 0.22; 95% CI: 0.07–0.68, *KRAS* (p = 0.0052; HR = 0.23; 95% CI: 0.07–0.71), *LIFR* (p = 0.0089; HR = 0.26; 95% CI: 0.09–0.77), *E-CADHERIN* (p = 0.011; HR = 0.26; 95% confidence interval (CI): 0.09–0.78) and *ENO1* (p = 0.047; HR = 0.36; 95% CI: 0.13–1.03) exhibited longer median OS, with no overlap in median survival compared with patients with low mRNA expression, whose median OS was approximately 18 months for all five genes. No significant differences in OS were observed for the remaining genes when comparing high versus low tumor mRNA expression.

Kaplan–Meier analyses were also performed by stratifying patients according to their *KRAS* mutational status (*KRAS* ^{G12C} vs. *KRAS* $^{non\text{-}G12C}$). Among *KRAS* ^{G12C} mutant patients, high mRNA expression levels were significantly associated with improved OS for *KRAS* (p = 0.0054; HR = 0; 95% CI: 0–inf) and *SCRIBBLE* (p = 0.0054; HR = 0; 95% CI: 0–inf) (**Fig. S2A-B**;). In *KRAS* $^{non\text{-}G12C}$ mutant patients, high mRNA expression was significantly associated with longer OS for *E-CADHERIN* (p = 0.036; HR = 0.26; 95% CI: 0.07–1.00), *NRAS* (p = 0.031; HR = 0.27; 95% CI: 0.07–0.95), *VCP1* (p = 0.033; HR = 0.25; 95% CI: 0.06–0.98), and *LIFR* (p = 0.033; HR = 0.25; 95% CI: 0.06–0.98) **(Fig. S2C-F;)**.

3.3 Following up in serial analysis of plasma samples

The clinical and pathological characteristics of the 10 patients with serial blood sampling are summarized in **Table 2**. Blood samples were collected prior to initiation of *KRAS* ^{G12C} inhibitor therapy, on day 3 after the first dose, at weeks 6 and 12, and at the time of disease progression. Consistently collected samples were available up to week 6 of treatment. Three patients experienced disease progression at week 12, two discontinued treatments due to toxicity, and one patient died before disease progression. All samples underwent NGS or TaqMan and nCounter gene expression analysis.

Regarding mutational status, *KRAS* ^{G12C} was detected at baseline in cfDNA from seven patients. The variant allele frequency (VAF) of *KRAS* ^{G12C} was monitored

across all collected plasma samples. Among these seven patients, the mutation became undetectable after 6 weeks of treatment in four cases. In the remaining three patients, *KRAS* ^{G12C} VAF decreased at week 6 but subsequently increased at the time of disease progression, which occurred in all cases by week 12. The other three patients, who had undetectable *KRAS* ^{G12C} at baseline, also showed no detectable mutation at the time of disease progression **(Table 4)**.

For gene expression analyses, data obtained from baseline through week 6 of treatment were evaluated. Of the 20 genes included in the panel, 12 genes (*ENO1, LZTR1, NRAS, PPP1C, RGS3, SHOC2, YAP1, YES, FASN, VCP1, MET*, and *ZDHHC7*) were consistently available for analysis across all samples. Comparisons between baseline and day 3 were performed using the Wilcoxon Signed-Rank test, and no statistically significant differences in gene expression were observed**.** In contrast, comparisons between baseline and week 6 revealed statistically significant changes in the expression of five genes: *FASN* (p = 0.04), *ZDHHC7* (p = 0.002), *RGS3* (p = 0.03), *YAP1* (p = 0.02), and *SHOC2* (p = 0.04) **(Supplementary Table 5)**.

When gene expression at day 3 was compared with week 6, four of these five genes remained significantly different (*FASN* (p = 0.04), *ZDHHC7* (p = 0.03), *RGS3* (p = 0.05), and *YAP1* (p = 0.04)), and an additional significant difference was observed for *YES* (p = 0.01) **(Supplementary Table 6)**.

3.4 Analysis of *KRAS* ^{G12C} mutation and gene expression in paired baseline and progression sample

A clinical case in which an initial biopsy, a re-biopsy, and serial liquid biopsies were available for analysis will be described in detail. The case corresponds to a patient diagnosed in August 2022 with stage IVB lung adenocarcinoma and brain metastases, harboring a *KRAS* ^{G12C} mutation (VAF 66%), *KEAP1* co-mutation (VAF 70%) and *FGFR3* amplification in tumor tissue **(Fig. 3A)**.

Regarding gene expression profiling, all targeted genes except *MET* were detectable in tumor tissue biopsies. At re-biopsy, several genes were upregulated compared with the initial biopsy, including *E-CADHERIN, HUWE1, KRAS, LIFR, RGS3, LZTR1, MRAS, NRAS, NFE2L2, PP1c, SCRIBBLE, SHOC2, VCP1,*

YAP1, and *YES*. In contrast, *ENO1, FASN, HRAS*, and *ZDHCC7* were downregulated at re-biopsy (**Fig. 3B**).

A similar trend was observed in plasma samples. Comparison of baseline and progression samples revealed increased expression of *LZTR1, PP1c, RGS3, SHOC2, YAP1*, and *YES*. Conversely, *ENO1*, *NRAS*, and *VCP1* showed decreased expression. Notably, *FASN*, *MET*, and *ZDHCC7* exhibited higher expression levels in plasma compared with the tissue re-biopsy (**Fig. 3C**).

4. Discussion

The primary aim of the study was to look at the plasma mRNA levels of KRAS-mutant lung cancer patients to determine whether some transcripts could be a readout of the protein changes reported in KRAS cell lines of rapid adaptive resistance to *KRAS* ^{G12C} inhibitors *(Xue et al. Nature 2020).* ERK reactivation, by MRAS and *YAP1* protein phosphorylation (Adachi et al., 2023), could be measured at the mRNA level and, hence, monitored in *KRAS* ^{G12C} mutated NSCLC patients treated with *KRAS* ^{G12C} inhibitors using liquid biopsies. Plasma RNA would be collected at multiple time points— before treatment, at 3 days, 6 weeks, 12 weeks and at disease progression—to quantify the expression of cardinal genes involved in activating the MRAS–SHOC2–PP1C complex, as well as other fundamental genes involved in coordinating the adaptive resistance processes observed in *KRAS* mutated NSCLC cells (Adachi et al., 2023). It was surmised that nCounter assays could provide information on the mRNA expression levels of the MRAS-SHOC2-PP1C complex and on the influence of *HUWE1* and *VCP1* mRNA in further deactivating or activating the trimeric complex. In addition, the governance of Scribble over *SHOC2* (Kwon and Hahn, 2021) made it tempting to examine the potential involvement of *LIFR* mRNA in controlling Scribble mRNA as well (Chen et al., 2012; Piccolo, 2012).

We were prompted to examine, in NSCLC tissue samples, the differential expressions of the genes described above, plus others related to the biology of *KRAS* mutated NSCLC, using nCounter. These were compared with tumor

samples from NSCLC patients that were either *KRAS* ^{wt} or *KRAS* $^{non\text{-}G12C}$ mutated. Eight genes surface as differentially expressed in tumor tissue of *KRAS* ^{G12C} in contrast with tumor tissue of *KRAS* ^{WT} NSCLC patients (**Fig. 2B and Supplementary Table 2).** *LIFR* mRNA expression was significantly lower in *KRAS* ^{G12C} mutated samples. Other identifiable genes ranked by significant differential mRNA expression compared with *KRAS* ^{WT} tumor tissues included *NRAS, SHOC2, ENO1, VCP1* and *MRAS*. In *KRAS* $^{non\text{-}G12C}$ mutated tumor samples, *LIFR, KRAS* and *MET* were significantly differentially expressed compared with *KRAS* ^{WT} tumor samples (**Fig. 2C, Supplementary Table 3**). We previously reported that the MET inhibitor tepotinib, in combination with omeprazole, caused tumor growth inhibition across a broad range of *KRAS*-mutated cell lines, as well as in a xenograft mouse model (Rosell et al., 2024b). The third comparison between *KRAS* ^{G12C} and *KRAS* $^{non–G12C}$ tumor samples resulted in significant mRNA differences, primarily in *SHOC2* and *NFE2L2*. (**Fig. 2D and Supplementary Table 4**).

When looking at overall survival in patients with *KRAS* ^{G12C} and *KRAS* $^{non\text{-}G12C}$ mutations, low *LIFR* mRNA levels were associated with significantly worse survival. (Hazard ratio=0.26, P= of 0.0089) (**Fig. 2G**). Other genes influenced the overall survival, such as *E-cadherin, RGS3, KRAS* and *ENO1* mRNA (**Fig.2 F,H,E, and I**). Another notable finding was that *KRAS* ^{G12C} mutated NSCLC patients with high Scribble mRNA expression exhibited markedly better survival (**Fig. S2**; Hazard ratio=0, P=0.0054). While survival outcomes for *LIFR*, *Scribble, and E-cadherin* mRNA were as expected, it was unexpectedly observed that high *VCP1* mRNA expression was associated with improved overall survival in *KRAS* $^{non\text{-}G12C}$ mutated NSCLC patients (**Fig S2E**; hazard ratio=00.25, P=0033). VCP1(p97), a AAA-ATPase chaperone, governs cellular proteolysis through endoplasmic reticulum-associated degradation and ubiquitin-proteasome system coordination. It has been shown to promote degradation of casein kinase 1 alpha (Nguyen et al., 2017) and potentially of inositol-requiring enzyme 1 alpha, a mechanism implicated in resistance to sotorasib in *KRAS* ^{G12C} cell lines (Lv et al., 2023).

Low *LIFR* destabilizes Scribble and can lead to *YAP1* nuclear localization (Chen et al., 2012; Piccolo, 2012). According to TCGA datasets in *KRAS*-mutant lung adenocarcinoma, low *LIFR* expression (24 cases) was associated with significantly worse survival than high *LIFR* expression *(24 cases)* (p=0.0230) (Liu et al., 2021a). The finding in our study that that *LIFR* significantly affects overall survival in both *KRAS* ^{G12C} and *KRAS* $^{non\text{-}G12C}$ patients is particularly relevant, as histone deacetylase inhibitors (entinostat, panobinostat, romidepsin, and vorinostat) have been shown to increase *LIFR* mRNA levels in breast cancer cells, as well as LIFR protein levels measured by Western blotting (Clements et al., 2021). Further interest stems from the fact that *HDAC3* was identified to play a critical role in *KRAS*-mutated NSCLC and *HDAC3* becomes hyperactivated in lung cancer cells with *KRAS* and *STK11* co-mutations. Resistance to the MEK inhibitor trametinib was reversed in *KRAS* and *STK11* co-mutated cancer cells with entinostat (HDAC1/3 inhibitor) (Eichner et al., 2023). Currently, the co-existence of *STK11* and other co-mutations accompanying *KRAS* ^{G12C} mutated NSCC patients is broadly recognized *(Gregorc et al., 2026; Skoulidis et al., 2025).* Our results pave the way for developing toolkit assays that incorporate nCounter mRNA analysis to gain mechanistic insights into the rewiring of cancer cells in patients. *LIFR* mRNA emerges as a promising biomarker to be assessed, although it was not detectable in the plasma of *KRAS* ^{G12C} patients.

It is compelling to track the activation of *SHOC2*, and *YAP1* mRNA in plasma following treatment with *KRAS* ^{G12C} inhibitors in *KRAS* ^{G12C} mutated NSCLC, albeit recognizing the limitations posed by protein-level and post-translational modifications. In this study we were unable to detect *MRAS* mRNA in the plasma. A protocol was approved by our institution in 2023 and blood samples of 10 *KRAS* ^{G12C} mutated NSCLC patients were collected before therapy, three days after treatment, at 6 weeks, 12 weeks, and at the time of tumor progression. Of the twenty genes examined by Nano string, twelve were consistently detected across all time points: *SHOC2, PP1C, ZDHHC7, YAP1, YES, VCP1, RGS3, LZTR1, NRAS, MET, ENO1* and *FAS* mRNA. Among the numerous changes in expression levels, it is notable that at six weeks *SHOC2* and *YAP1* mRNA were increased, as well as *FASN*, *ZDHHC7*, and *RGS3* mRNA (**Supplementary Table 5**); the significance of the latter two remains unknown. In the original report, *KRAS*

^{G12C} inhibitors (sotorasib or adagrasib) were shown to cause mislocalization of *Scribble* via *ZDHHC7*, contributing to adaptive resistance, and overexpression of *ZDHHC7* prevented *Scribble* mislocalization following sotorasib treatment. However, *ZDHHC7* mRNA levels in our study did not show a consistent trend after sotorasib treatment, suggesting that *ZDHHC7* expression is regulated primarily at the protein level (Adachi et al., 2023). Therefore, post-translational modifications that promote aberrant activation of bypass signaling pathways represent an inherent limitation of mRNA-based analyses. Of further interest is the specific contribution of other *ZDHHC* palmitoyl acyltransferase-mediated palmitoylation, such as DHHC20 (Kharbanda et al., 2020). Although the G protein signaling regulator RGS3 has been reported to increase sensitivity to G12C inhibitors (Li et al., 2021a), it is also involved in epithelial-mesenchymal transition and activation of the TGF-β signaling pathway (Shi et al., 2012; Wang et al., 2025). From a clinical perspective, the significance of *ZDHHC7* mRNA may be limited. *RGS3* mRNA, however, warrants further investigation as a potential early indicator of underlying tumor progression, even while the patient appears radiographically stable or responding, which could inform the development of combinatorial approaches, such as with TGF-β inhibitors. The major interest lies in the clear significance of the observed increases in *SHOC2* and *YAP1* mRNA, which mirror *SHOC2* and *YAP1* protein phosphorylation in *KRAS* ^{G12C} cell lines (Adachi et al., 2023; Bonsor and Simanshu, 2024; Jones et al., 2019). *YAP1*, transcriptional coactivator with PDZ-binding motif (TAZ), and TEA domain family members (TEADs) mediate resistance to chemotherapy, immunotherapy, and *KRAS* ^{G12C} inhibitors (Hagenbeek et al., 2023). We noted that high expression of *YAP1* mRNA predicted worse progression-free survival in EGFR-mutated NSCLC patients (Chaib et al., 2017). *YAP1-TEAD* inhibitors are currently being developed to avert resistance to *KRAS* ^{G12C} inhibitors (Chapeau et al., 2024; Edwards et al., 2023).

This study has several limitations that should be considered when interpreting the results. First, the sample size in both tissue and plasma cohorts was relatively modest, which may limit statistical power for certain analyses. Second, gene expression analysis was restricted to a predefined panel of 20 genes selected based on prior biological knowledge and specific hypotheses, rather than an exploratory large-scale or genome-wide approach.

Additionally, *KRAS* $^{non\text{-}G12C}$ mutations were grouped together due to sample size constraints, despite their potentially distinct biological behaviors, and exploratory subgroup analyses should therefore be interpreted with caution. Furthermore, although the observed changes in *SHOC2* and *YAP1* mRNA are consistent with known resistance mechanisms, transcript levels do not necessarily reflect protein activity or pathway activation, and these findings should be considered hypothesis-generating. Despite these limitations, the study provides valuable preliminary insights and identifies trends that may inform and guide future investigations in larger, independent cohorts.

Our results cannot fully satisfy all principles underlying plasma-based analysis. However, although *MRAS* was not expressed, the detection of *SHOC2* and *YAP1* in the longitudinal assessment of blood samples can suggest that tracking gene expression in liquid biopsies may help identify novel biomarkers for predicting responses to *KRAS* ^{G12C}–based combinatorial therapies. A further nCounter assessment is planned in a multicenter Phase 2 trial of adagrasib in patients with *KRAS* ^{G12C} mutated NSCLC (ADEPPT)(*Naidoo et al., 2026*), in which blood samples have been collected at baseline and time points of 6, 12, 18, and 24 weeks, as well as at the time of progression. This study could serve to identify some of the genes that are expressed in plasma samples such as *SHOC2*, *YAP1* and *RGS3* mRNA; others, including *YES1* and *PP1C* mRNA, showed a similar increasing trend in both tumor re-biopsy and plasma samples from the patient in whom re-biopsy was obtained.

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Table 1. Demographic and clinical characteristics of patients included in the study.

Parameter	***KRAS*** ^{G12C} ***(N=23),(23.47%)***	***KRAS*** $^{non\text{-}G12C}$ ***(N=24),(24.49%)***	***KRAS*** ^{WT} **(N=51),(52.04%)**	**Total (N=98) (%)**
Demographics				
Age (years)				
Median	64	62	65	64
Range	(46-85)	(37-82)	(39-85)	37-85
Sex assigned at birth (N)				
Male	11	12	30	53 (54.08%)
Female	12	11	20	43 (43.87%)
missing	0	1	1	2 (2.04%)
Smoking history (N)				
Never	0	3	8	11 (11.22%)
Former	17	16	24	57 (58.16%)
Current	4	4	10	18 (18.36%)
missing	2	1	9	12 (12.24%)
Clinical and tumor characteristics				
Histology type (N)				
Adenocarcinoma	20	24	44	88 (89.8%)
Squamous Cell	0	0	5	5 (5.1%)
Others	3	0	2	5 (5.1%)
Stage (N)				
(I-IIIA)	6	7	16	29 (29.59%)
(IIIB-IV)	16	15	32	63 (64.28%)
missing	1	2	3	6 (6.12%)
Site of metastases (IIIB-IV)				
Brain	2	4	3	9 (14.28%)
Adrenal gland	2	4	2	8 (12.7%)
Bone	1	3	3	7 (11.11%)
Breast	0	1	0	1 (1.58%)
Liver	1	1	1	3 (4.76%)
***KRAS* mutation**				
KRAS: p.G12C	23 (100%)	0	0	23 (23.47%)
KRAS: p.G12A	0	6 (25%)	0	6 (6.12%)
KRAS: p.G12V	0	5 (20.8%)	0	5 (5.10%)
KRAS: p.G13C	0	4 (16.6%)	0	4 (4.08%)
KRAS: p.G12D	0	2 (8.3%)	0	2 (2.04%)
KRAS: p.G12F	0	2 (8.3%)	0	2 (2.04%)

KRAS: Q61L	0	2 (8.3%)	0	2 (2.04%)
KRAS: G61H	0	1 (4.2%)	0	1 (1.02%)
KRAS : p.A146T	0	1 (4.2%)	0	1 (1.02%)
KRAS:p.G13D	0	1 (4.2%)	0	1 (1.02%)
PD-L1 TPS (N)				
<1%	7	8	20	35 (35.71%)
1–49%	7	4	11	22 (22.45%)
≥50%	4	6	9	19 (19.38%)
missing	5	6	11	22 (22.45%)

Table 2. Clinical and demographic characteristics of patients with serial plasma follow-up.

Parameter	*(N=10),*
Demographics	
Age (years)	
Median	64
Range	(46-85)
Sex assigned at birth (N)	
Male	4
Female	6
Smoking history (N)	
Former	9
Current	1
Clinical and tumor characteristics	
Histology type (N)	
Adenocarcinoma	10
Stage (N)	
(I-IIIA)	2
(IIIB-IV)	8
KRAS ^{G12C} **status in plasma baseline (N)**	
Detected	7
Non-Detected	3
Co-mutations detected in plasma baseline (N)	
KEAP1	5
TP53	4
STK11	2
SETD2	1
MYC (amplification)	1
KRAS ^{G12C} **inhibitor (N)**	
Sotorasib	3
Adagrasib	4
Divarasib	1
Olomorasib	2

Table 3. Statistically significant gene correlations in *KRAS*, *KRAS* ^{G12C} and *KRAS* $^{non\text{-}G12C}$ mutant in tissue samples.

rho: Spearman's rank-correlation coefficient; Selection by p-value <0.05 and $|\text{rho}| \geq 0.7$

		***KRAS* mutant**		***KRAS* G12C**		***KRAS* non-G12C**	
GENE	**GENE**	**p-value**	**rho**	**p-value**	**rho**	**p-value**	**rho**
MRAS	***RGS3***	**0.00000042**	**0.77**	**0.000034**	**0.76**	**0.0000082**	**0.79**
RGS3	***LZTR1***	**0.00000054**	**0.70**	**0.00033**	**0.70**	0.00080	0.65
VCP1	***RGS3***	**0.00000065**	**0.70**	**0.000052**	**0.75**	0.0029	0.59
VCP1	***NFE2L2***	**0.00000031**	**0.70**	0.0011	0.65	**0.000053**	**0.74**
HUWE1	***LIFR***	**0.00000056**	**0.70**	0.00067	0.65	**0.00014**	**0.71**
MRAS	***LZTR1***	**0.0000014**	**0.70**	0.0030	0.59	**0.00023**	**0.70**
ZDHHC7	***RGS3***	0.00019	0.52	**0.000011**	**0.79**	0.15	0.30
ZDHHC7	***LZTR1***	0.000094	0.54	**0.00018**	**0.72**	0.14	0.31
VCP1	***ZDHHC7***	0.036	0.31	**0.00034**	**0.70**	0.82	-0.047
VCP1	***MRAS***	0.00017	0.52	**0.00035**	**0.70**	0.05	0.39
NFE2L2	***SHOC2***	4.14567E-06	0.61	0.11	0.34	**0.0000022**	**0.82**
VCP1	***HUWE1***	2.88249E-05	0.57	0.050	0.41	**0.00011**	**0.72**
VCP1	***LIFR***	0.012	0.36	0.44	0.17	**0.00024**	**0.70**

Table 4. *KRAS* G12C variant allele frequency in serial plasma samples during follow-up.

	Baseline VAF%	Day 3 VAF%	6 Weeks VAF%	12 Weeks VAF%	Progression Disease VAF%	co-alterations VAF% at baseline	Comments
Sample 1	5.5%	6.5%	ND	ND	60.1%		
Sample 2	1.12%	0.07%	ND	ND	ND		
Sample 3	1.57%	0.28%	ND	ND	---		Exitus before PD
Sample 4	0.36%	1.42%	ND	ND	---		
Sample 5	6.04%	9.75%	3.93%	---	49.8%	*KEAP1*: p.Arg601Leu (27%) *TP53*: p.Gly154Val (25%) *STK11*: p.Trp239 (26%) *MYC*: Amplification CNV:8.67	PD at 12 weeks
Sample 6	0.13%	0.13%	0.39%	---	2.62%		PD at 12 weeks
Sample 7	0.12%	0.37%	0.21%	---	3.38%		PD at 12 weeks
Sample 8	ND	ND	ND	ND	ND		
Sample 9	ND	ND	ND	ND	ND		
Sample 10	ND	ND	ND	ND	ND		

VAF: Variant allele frequency: ND: not detected; PD: Progression disease

Abbreviations

ALK, anaplastic lymphoma kinase (Ki-1),anaplastic lymphoma receptor tyrosine kinase; ARID1A, actin dependent regulator of chromatin, subfamily f, member 1;

BRAF, v-raf murine sarcoma viral oncogene homolog B; CDK4, CDK6, EGFR, epidermal growth factor receptor (avian erythroblastic leukemia viral (v-erb-b) oncogene homolog; ERBB2, v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2; ERBB4, Erb-B2 Receptor Tyrosine Kinase 4; FAT1, FAT Atypical Cadherin 1; FGFR1, Fibroblast Growth Factor Receptor 1; FGFR2, Fibroblast Growth Factor Receptor 2; FGFR3, Fibroblast Growth Factor Receptor 3; IDH1, Isocitrate Dehydrogenase (NADP(+)) 1; IDH2, Isocitrate Dehydrogenase (NADP(+)) 2; KEAP1, Kelch Like ECH Associated Protein 1; KIT, KIT Proto-Oncogene, Receptor Tyrosine Kinase ; KRAS, KRAS Proto-Oncogene, GTPase; MET, MET Proto-Oncogene, Receptor Tyrosine Kinase ; MYC, MYC Proto-Oncogene, BHLH Transcription Factor; NFE2L2, NFE2 Like BZIP Transcription Factor 2; NRAS, NRAS Proto-Oncogene, GTPase; PDGFRA, Platelet Derived Growth Factor Receptor Alpha; PIK3CA, Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha ; POLD1, DNA Polymerase Delta 1, Catalytic Subunit; POLE, DNA Polymerase Epsilon, Catalytic Subunit; RET, Ret Proto-Oncogene; ROS1, ROS Proto-Oncogene 1, Receptor Tyrosine Kinase; SETD2, SET Domain Containing 2, Histone Lysine Methyltransferase; STK11, Serine/Threonine Kinase 11; TP53, Tumor Protein P53; MET, MET Proto-Oncogene, Receptor Tyrosine Kinase; SHOC2, SHOC2 Leucine Rich Repeat Scaffold Protein; PP1C, Protein Phosphatase 1 Catalytic Subunit Gamma; HUWE1, HECT, UBA And WWE Domain Containing E3 Ubiquitin Protein Ligase 1; VCP1, valosing containing protein 1; LIFR, LIF Receptor Subunit Alpha; SCRIBBLE, Scribble Planar Cell Polarity Protein; ZDHHC7, ZDHHC Palmitoyltransferase 7; YES1, YES Proto-Oncogene 1, Src Family Tyrosine Kinase; YAP, Yes1 Associated Transcriptional Regulator; E-CADHERIN, Cadherin 1; FASN, Fatty Acid Synthase ; LZTR1, Leucine Zipper Like Post Translational Regulator 1; NFE2L2, Nuclear Factor Erythroid 2-Like 2; ENO1, Enolase 1; RGS3, Regulator Of G Protein Signaling 3.

Figure Legends

Figure 1. Heatmap representing the results of the mutations, fusions, amplifications and PD-L1 expression detected by NGS, nCounter or IHC in tissue biopsies. *NGS: next-generation sequencing; IHC: immunohistochemistry.*

Figure 2. Differential expression analysis of all 20 human transcripts included in the panel. **(A)** Hierarchical clustering of patients based on KRAS mutational status on the 20 mRNA transcripts that were found to be expressed at significantly different levels between *KRAS* ^{wt}, *KRAS* ^{G12C} and *KRAS* $^{non\text{-}G12C}$ patients. **(B)** Volcano Plot representing the Log_2(FC) and nominal-Log_{10} (p-values) of all transcripts included in the panel for *KRAS* ^{G12C} vs *KRAS* ^{WT} samples. **(C)** Volcano Plot representing the Log_2(FC) and nominal-Log10 (p-values) of all transcripts included in the panel for *KRAS* $^{non\text{-}G12C}$ vs *KRAS* ^{WT} samples. **(D)** Volcano Plot representing the Log_2(FC) and nominal-Log10 (p-values) of all transcripts included in the panel for *KRAS* ^{G12C} vs *KRAS* $^{non\text{-}G12C}$ samples. **(E-I)** Kaplan–Meier curves were used to evaluate overall survival in 30 patients with *KRAS*-mutant NSCLC. High mRNA expression levels of RGS3, KRAS, LIFR, *E-cadherin*, and *ENO1* were significantly associated with overall survival.

Figure 3. (A) Evolution of the allelic fractions of *KRAS* ^{G12C} mutation in plasma from a 62-year-old woman, heavy smoker, diagnosed in 2024 with stage IV non–small cell lung cancer with leptomeningeal and spinal metastases. The patient-initiated treatment with adagrasib on July 31, 2024, and disease progression was documented on January 31, 2025. **(B)** Gene expression profiling of tissue samples obtained from the initial biopsy and subsequent rebiopsy. **(C)** Gene expression profiling of plasma samples at baseline and at disease progression during treatment with *KRAS* ^{G12C} inhibitors.