Case Report

**HRAS Q61L Mutation as a Possible Target for Non-Small Cell Lung Cancer: Case Series and Review of Literature**

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**Abstract:**

Introduction: Assessment of actionable gene mutations and oncogene fusions have made a paradigm shift in treatment strategies of non-small cell lung cancer (NSCLC). HRAS mutations involved around 0.2–0.8% of NSCLC patients, mostly on codon 61. For these patients, few data are available regarding clinical characteristics and response to therapies. Methods: Next-Generation Sequencing (NGS) done routinely at Nantes University Hospital was used to identify HRAS molecular alterations in NSCLC patients. We identified and described four HRAS p.Gln61Leu mutated patients. Literature of previously HRAS-mutant NSCLC cases was reviewed, and available data in solid tumour with the most advanced H-Ras specific inhibitor, tipifarnib, were presented. Results: Of 1614 patients diagnosed with advanced NSCLC from January 2018 to December 2020, four (0.25%) had HRAS p.Gln61Leu mutation. Three of them died during the first-line systemic therapy. Furthermore, three additional cases were identified in literature. All cases were current or former smokers, most of them had pleural or pericardial effusion at diagnosis. Conclusions: The clinical course of patients with HRAS-mutant NSCLC remains unclear. Further cases should be identified in order to clarify prognosis and response to therapies. Tipifarnib, a farnesyl transferase inhibitor, is a promising candidate to target HRAS-mutant tumours and should be explored in NSCLC patients.

**Keywords:** non-small cell lung cancer; HRAS Gln61Leu; tipifarnib; oncogenic driver

1. Introduction

Lung cancer accounted for more than 2.2 million cases and almost 1.8 million deaths worldwide in 2020 [1]. Non-small cell lung cancer (NSCLC) is the main type of lung cancer and represents 85% of all cases. The prognosis of oncogenic-addicted NSCLC has been transformed due to the development of targeted therapies, firstly inhibiting the mutant epidermal growth factor receptor (EGFR) [2,3], then ALK [4–6] and ROS1 rearrangement [7]. More recently, KRAS appeared as a promising target in NSCLC patients, due to the development of efficient targeted therapies such as sotorasib targeting the KRAS p.Gly12Cys mutation [8–10].

The RAS genes belong to a well-described family of oncogenic drivers, encoding four small GTPase proteins, K-Ras4A and K-Ras4B (which are two splice variants of the KRAS gene), H-Ras and N-Ras [9–11]. These proteins have an active form, Ras-GTP, and...
an inactive form, Ras-GDP. Transition from one to another is regulated by Ras-Guanine Nucleotide Exchange Factors (Ras-GEFs) that catalyse the exchange of GDP for GTP, and by intrinsic GTPase activity hydrolysing GTP to GDP with the assistance of Ras-GTPase Activating Proteins (Ras-GAPs). In humans, Ras proteins participate in many physiological processes related to cell growth, division, and survival [10]. Substitution mutations, mainly involving codons Gly12, Gly13, and Gln61 of the RAS genes, are found in about 20% of human cancers. They are known to cause constitutive activation of the signalling activity mainly through impairment of their intrinsic GTPase activity [12,13] and thus of the conversion of active form Ras-GTP to inactive form Ras-GDP. Ras-GTP interact with downstream effectors such as Phosphoinositide 3-Kinase (PI3K) and Mitogen Activated Protein Kinases (MAPK) pathways, that enhanced growth, proliferation, differentiation, and survival of cancer cells [10].

Based on a recent publication evaluating several leading cancer mutation databases, KRAS is by far the most commonly mutated of the three RAS genes in solid tumours (75%), followed by NRAS (17%) and HRAS (7%) [13]. HRAS is mostly altered in head and neck squamous cell carcinoma (HNSCC) (5–9%), salivary glands (15%), and bladder cancer (5–30%) [13,14]. HRAS mutations are involved around 0.2–0.8% of lung adenocarcinoma and squamous cell lung cancer patients [13]. Among HRAS mutated tumours, single-base substitutions mutations occurring most frequently on codon 12 (27–33%), 13 (25–27%), or 61 (37–40%) [13,15,16].

In this study, we reported the incidence of HRAS mutations among a large cohort of NSCLC patients, and we focused on the clinical-pathological features of four cases with HRAS p.Gln61Leu mutations. Then, we completed with literature review of previously described HRAS-mutant NSCLC cases. Last, we described available data in solid tumours (lung cancer and others) with the most advanced H-Ras specific inhibitor, tipifarnib.

2. Patients and Methods

A multicentre retrospective study was performed including all newly diagnosed NSCLC patients between 1 January 2018 and 31 December 2020 with their genomic analyses performed at Nantes University Hospital. Next-generation sequencing (NGS) was systematically performed for all newly diagnosed non-squamous NSCLC patients presenting advanced disease, and never smokers squamous NSCLC patients. Briefly, DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumours using the Maxwell RSC RNA FFPE kit (Promega, Madison, WI, USA). NGS libraries were synthesized using the QIAseq Targeted DNA Custom Panel (QIAGEN, Hilden, Germany) kit, an amplicon library construction kit based on Anchored Multiplex PCR (AMP) technology. Sequencing was performed on a MiSeq sequencer (Illumina, San Diego, CA, USA), and NGS data analysis was performed using the Biomedical Genomics Workbench (QIAGEN). Panel of tested genes is shown in the Table A1. Patients with HRAS-mutant tumours were identified, and clinical data from the medical records of HRAS p.Gln61Leu mutated patients (the most frequently observed HRAS mutation) were extracted. Other cases included in the literature were reviewed from Medline database, using the keywords “non-small cell lung cancer” and “HRAS”. This study was conducted in accordance with the local regulations, and it was approved by the local independent ethics committee.

3. Results

3.1. Cases Presentation

NGS was performed on 1614 patients during recruitment (Figure 1). Nineteen (1.18%) had HRAS mutation, 572 (35.43%) had a KRAS mutation, and 18 (1.12%) had an NRAS mutation. The mutation most frequently observed was HRAS p.Gln61Leu, in four patients (Table 1). All cases are reported in Table 2.
Figure 1. Flowchart. NGS: Next-Generation Sequencing.

Table 1. HRAS mutations type among all 19 patients.

| HRAS Mutations         | Numbers of Patients |
|------------------------|---------------------|
| p.Q61L (p.Gln61Leu; C.182A>T) | 4                   |
| p.G13V (p.Gly13Val; c.38G>T) | 2                   |
| p.E98K (p.Glu98Lys; c.292G>A) | 1                   |
| p.S89F (p.Ser89Phe; c.266C>T) | 1                   |
| p.A11P (p.Ala11Pro; c.31G>C) | 1                   |
| p.K117N (p.Lys117Asn; c.351G>T) | 1                   |
| p.R102L (p.Arg102Leu; c.305G>T) | 1                   |
| p.D107fs (p.Asp107fs; c.319del) | 1                   |
| p.V109L (p.Val109Leu; c.325G>T) | 1                   |
| p.T58I (pThr58Ile; c.173C>T)  | 1                   |
| p.T148P (pThr148Pro; c.442A>C) | 1                   |
| p.R41W (p.Arg41Trp; c.121C>T)  | 1                   |
| p.R135Q (p.Arg135Gln; c.404G>A) | 1                   |
| p.M72I (p.Met72Ile; c.216G>T)  | 1                   |
| p.E76D (p.Glu76Asp; c.228G>T)  | 1                   |

The first one was an active smoker 50-year-old female, with a past medical history of hypertension and chronic obstructive pulmonary disease. In 2015, she underwent right lobectomy and adjuvant chemotherapy (cisplatin and pemetrexed) for a pT4N0M0 lung adenocarcinoma. In January 2019, a control computed tomography (CT) scan revealed an upper left lobe mass, mediastinal adenopathies, liver metastases, and pericardial effusion. She had no symptoms at this time. Histology of pericardial effusion after drainage showed lung adenocarcinoma cells, Tumour Proportion Score (TPS) PD-L1 < 1%. NGS showed an HRAS p.Q61L mutation (variant allele fraction (VAF) 10.0%) with no other alteration. She started a first-line chemotherapy with carboplatin and pemetrexed; however, she only received three cycles of chemotherapy due to a major cachexia. A CT scan confirmed a lung, liver, and bone progression. She died four weeks later.
Table 2. Clinico-pathologic features of non-small cell lung cancer patients with HRAS p.Gln61Leu mutation reported in literature. ADC: adenocarcinoma; SCC: squamous cell carcinoma; ADSQ: adenosquamous; NOS: not otherwise specified; WBRT: whole brain radiotherapy; PFS: Progression-Free-Survival; OS: Overall Survival; NA: Not Available.

| Reference | Sex (Female/Male) | Age at Diagnosis (Years) | Smoking Status | Pathology | PD-L1 (%) | Other Alterations | Metastatic Site | Treatment | PFS (Weeks) | OS (Weeks) |
|-----------|-------------------|--------------------------|----------------|-----------|-----------|-------------------|----------------|-----------|-------------|------------|
| Current   | F                 | 50                       | Active         | ADC       | <1        | None             | Lung/Liver/Pericardial effusion | Carboplatin—Pemetrexed | 11         | 15         |
| Current   | M                 | 55                       | Former         | NOS       | <1        | KRAS p.Gly12Cys  | Locally-advanced disease | Carboplatin—Pemetrexed | 22         | 30         |
| Current   | M                 | 63                       | Active         | NOS       | 60        | TP53 p.Glu105Val | Brain/Pericardial effusion | Carboplatin—Pemetrexed—Pembrolizumab | 24         | 24         |
| Current   | F                 | 61                       | Active         | ADC       | 60        | TP53 p.Glu105Val | Pleural effusion | On treatment | On treatment |
| Cathcart-Rake E., 2014 [17] | M | 79                       | Former         | ADC       | NA        | None             | Brain/Bone/Liver | Carboplatin—Pemetrexed—Stereotactic radiosurgery | NA         | 3          |
| Zhao J., 2021 [18] | M | 58                       | Active         | ADSQ      | NA        | EGFR p.Leu858Arg and p.Thr790Met (only on pleural effusion), NRAS p.Gly12Val, LRP1B p.Arg271Leu | Pleural effusion | Carboplatin—Osimertinib | 4          |
| Long Y., 2021 [19] | M | 76                       | Active         | SCC       | 50        | NRAS p.Gly12Val | Locally-advanced disease | Pembrolizumab | 24         | NA         |

* is used for stop codon in HGSV nomenclature.
The second case was a 55-year-old male, with a past medical history of pulmonary embolism and diabetes mellitus. He was a former smoker of around 60 packs per year. Lung cancer was diagnosed in July 2019. The histology revealed a Not Otherwise Specified (NOS) carcinoma, TTF1 and P40 negative, TPS PD-L1 = 0%. NGS showed KRAS p.Gly12Cys (VAF 19.6%) and HRAS p.Gln61Leu mutations (VAF 27.9%). CT showed a right upper lobe mass with bronchus invasion, bilateral mediastinal and hilar lymph node invasion, with no extra-thoracic extension. Health status was preserved with a performance status (PS) score of 1. He initiated a first-line chemotherapy with four cycles of carboplatin and pemetrexed, followed by maintenance with pemetrexed. He was hospitalized one month after the beginning of maintenance therapy for altered general condition. CT revealed disease progression, and the patient died one month after.

The third case was an active smoker 63-year-old male, 35 packs per year. His only antecedent was hypertension. He was diagnosed in August 2020, in a context of headache, dizziness, and asthenia. CT showed three brain metastases, a 67mm right mediastino-hilar mass, multiple mediastinal adenopathies, and a pericardial effusion. Histology revealed a NOS carcinoma, TPS PD-L1 = 60%. NGS showed KRAS p.Gly12Ser (VAF 27.7%), TP53 c.784_809del (VAF 26.45%) and HRAS p.Gln61Leu mutations (VAF 29.1%). A whole brain radiotherapy of 30 Gy was performed leading to a neurological improvement. He was treated with a combination of chemotherapy plus immunotherapy (carboplatin, pemetrexed and pembrolizumab) on September 2020. He received four cycles followed by monotherapy with pembrolizumab. The first evaluation in December 2020 showed a partial response of 39%. The patient was still treated at the time of data closure in November 2021.

The fourth case was a 61-year-old female, without past medical history. She was a highly active smoker with 68 packs per year. The presence of a dysphonia led to a chest CT in September 2020 and revealed a large compressive mediastino-hilar mass, and moderate abundance of bilateral pleural effusion. A brain CT and a PET-CT showed no extra-thoracic metastases. Histology was an adenocarcinoma, TPS PD-L1 = 60%. NGS showed TP53 p.Ile195Thr (VAF 13.6%) and HRAS p.Gln61Leu mutations (VAF 28.1%). She was hospitalized for obstructive pneumopathy, atrial fibrillation, and cardiogenic failure. She died in November 2020.

3.2. Review of Literature

To our knowledge, only three other individual clinical cases of NSCLC patients with HRAS-mutant tumours have been described in literature. All cases were p.Gln61Leu mutations (Table 2). According to the TCGA database (projects TCGA-LUAD and TCGA-LUSC), HRAS mutations were found in 3/550 (0.55%) adenocarcinomas and 8/480 (1.67%) squamous cell carcinomas (data available online: https://portal.gdc.cancer.gov/ accessed on 14 April 2022). One adenocarcinoma had HRAS p.Gln61Leu mutation. The first case was from the USA, and the two others came from China. The first patient was a former smoker 79-year-old male, with a rapid progression from stage IB disease to metastatic adenocarcinoma and death [17]. His only antecedent was meningioma. The patient underwent right upper lobectomy in September 2010, for a localized pT2aN0M0 adenocarcinoma. He received four cycles of adjuvant carboplatin and pemetrexed. He relapsed 10 months after the surgery, with brain metastases, multiple bone, liver, and bilateral adrenal lesions. He underwent craniotomy for a temporal lobe mass and stereotaxic radiosurgery of additional brain lesions. The molecular biology of the brain lesion revealed a HRAS p.Gln61Leu mutation. Other mutations, such as KRAS, TP53, and 51 other genes were negative. He was unable to receive chemotherapy and died in January 2012.

The second patient was a 58-year-old male without past medical history, who showed rapid metastatic progression and passed away due to respiratory failure after 2 weeks of systemic treatment [18]. He was an active smoker. He was diagnosed with a cavitory nodule of the right lung and malignant pleural effusion. Histology suggested a poorly differentiated carcinoma. Video-assisted thoracoscopic lobectomy and lymph node dissection was performed for palliative symptoms management. Amplification Refractory Mutation
A System (ARMS)-PCR assay showed a weak signal for p.Leu858Arg and p.Thr90Met of EGFR mutation, thus he was treated with cisplatin and osimertinib. He died 15 days after.

A reassessment of the primary tumour and lymph nodes showed HRAS p.Gln61Leu and NRAS p.Gln61Lys mutations, but EGFR mutations were not detected.

A third case, although from China, was reported. It was a 76-year-old man with an antecedent of Lynch syndrome and 50-year smoking history [19]. Chest CT showed tumour masses in the upper lobe and hilar of right lung, with mediastinal lymphadenopathies, presenting no other distant metastases on PET-CT. Histology identified a squamous cell carcinoma, TPS PD-L1 = 50%. NGS showed HRAS p.Gln61Leu and TP53 p.Arg158Leu. Other genes such as LRP1B, DNMT3A, POLE, TCF7L2, or NTM were also mutated. A heterozygous germline mutation of MSH6 was detected, but immunohistochemistry suggested normal expression of MLH1, MSH2, MSH6, and PMS2 in tumour biopsy. He was treated with pembrolizumab and achieved stable disease as the best response. Disease progression finally occurred after eight cycles. At this moment, plasma circulating tumour DNA test revealed a STK11 mutation, which was not present at diagnosis. Subsequently, the patient received chemotherapy with albumin-bound paclitaxel.

4. Discussion

Among non-squamous NSCLC, while KRAS mutations account for around 30% of patients, HRAS and NRAS mutations account for less than 1% [13,20,21]. Concordant with previous studies, we found a low prevalence of 0.82% HRAS mutations among NSCLC patients (19/1614 patients). In our centre, according to national guidelines, NGS was not routinely performed in squamous cell carcinoma, excepted for exceptional never-smokers. Thus, we might have missed other HRAS-mutant tumours, since HRAS mutations were also rarely described in squamous cell carcinoma [13,22]. A dedicated study in this population should be performed.

The clinical course of a patient with HRAS-mutant NSCLC remains unclear. Indeed, we have found only three cases describing HRAS-mutant lung cancer patients, all of them being HRAS p.Q61L. Our four cases plus the three others described in literature found that all patients were active or former smokers. Pleural and/or pericardial effusion was described in four out of seven cases. Clinical presentation was mostly aggressive, with one newly diagnosed patient who died before any treatment and four others who died during first-line treatment. Two patients received immunotherapy: one was still under treatment after more than one year, and the other received eight cycles with a stable disease as the best response.

HRAS mutations have been mainly documented in HNSCC, salivary glands, pheochromocytoma and bladder cancers [10,13,14,20,21,23–29] (Figure 2). HRAS mutations were known to be oncogenic; it was demonstrated in vitro that mutant HRAS hyperactivated MAPK and mTOR pathways in various cancer cell lines including lung, bladder, and oesophageal cancer [30,31]. Thus, it might appear as a potential interesting target. Among therapeutic strategies targeting HRAS-mutant cancers, tipifarnib, a farnesyl transferase (FT) inhibitor, is one of the most promising candidates [16,32]. Considerable work on FT inhibitors have been performed since 20 years, but these treatments failed to demonstrate any benefit in unselected populations, ending the development of these treatments as pan-Ras-targeted strategy [33]. Farnesylation is a mechanism needed for RAS molecules to be integrated in plasma membranes and for its activation. Contrary to tyrosine kinase inhibitors (TKI) such as EGFR TKIs that directly inhibit enzyme, tipifarnib indirectly disrupts RAS activity through preventing appropriate intracellular localization by interfering with RAS prenylation [31]. Although all RAS isoforms are FT substrates, only H-Ras exclusively depends on farnesylation for its membrane localization. Indeed, blockade of FT is sufficient to inhibit the action of H-Ras. Due to the development of RAS mutations profiling, tipifarnib appeared as an interesting strategy to inhibit HRAS-
mutant tumours [33]. Its indirect mechanism of action is very different from covalent KRAS inhibitors (such as sotorasib and adagrasib), which act by selectively forming a covalent bond with cysteine 12 within the switch-II pocket of KRAS-G12C protein, thereby locking KRAS in the inactive state to arrest cell proliferation [35].

Figure 2. Frequencies of HRAS mutations among solid cancer types.

Importantly, 2/4 patients with HRAS mutation in our cohort also had a co-occurring KRAS mutation on codon 12. As KRAS mutations are described as strong oncogenic mutations, it remains unclear whether HRAS is a driver mutation or a passenger one and if those patients would benefit from HRAS inhibition.

Tipifarnib activity was characterized in a wide panel of HRAS-mutant and wild-type HNSCC xenograft models, and also in HNSCC patient-derived xenografts [31]. Considering the rarity of this mutation in lung cancer, extensive pre-clinical approach seems difficult to assess. However, using CRISPR-CAS 9 technology, HRAS mutation might be introduced in an appropriate lung cancer cell line in order to mimic the clinical situation. It is important to highlight that most of the activating HRAS mutations involved codon 12, 13, 61, 117, and 146. To our knowledge, other mutations have not been characterized in NSCLC. Nonetheless, tipifarnib seemed efficient regardless of HRAS hotspot mutation, as it inhibited activity on patient derived xenograft HNSCC models harbouring Q61L, G12C, G12S, G13R, and K117N mutations [31].

We resumed published clinical trials evaluating tipifarnib in solid tumours in Table 3 [36–44]. Only two were specifically conducted in lung cancer, with no selection based on HRAS status. The first clinical trial was carried out in 2003 [43]. This phase II study tested the tipifarnib in first-line setting of 44 patients with a locally-advanced NSCLC, not amenable to chemoradiotherapy. No objective responses were documented, whereas inhibition of farnesylation in vivo was consistently documented. Median time to progression was 2.7 months, and seven patients (16%) had disease stabilization for greater than 6 months. Tipifarnib was also tested in 22 patients with sensitive relapsed small-cell lung cancer in a phase II trial [42]. No significant antitumour activity was observed. Median progression-free survival was only 1.4 months.
Table 3. Clinical trials evaluating Tipifarnib in solid tumours. HNSCC: Head and Neck Squamous Cell Carcinoma; NSCLC: Non-Small Cell Lung Cancer; SCLC: Small Cell Lung Cancer; NGS: Next-Generation Sequencing; PO: Per Os; ORR: Overall Response Rate; SD: Stable Disease; PFS: Progression Free Survival; OS: Overall Survival; VAF: Variant Allele Frequency; NA: Not Applicable.

| Reference          | Phase | Tumour Site                          | Number of Patients | Setting          | Biomarker                                                                 | Tipifarnib Dose & Schedule                                                                 | Primary Endpoint                                                                 | ORR (%) (SD) | Median PFS (Month) | Median OS (Month) |
|--------------------|-------|--------------------------------------|--------------------|------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|---------------|--------------------|--------------------|
| Ho A.L., 2021 [36] | 2     | HNSCC                                | 22                 | Relapsed         | Missense HRAS mutation, VAF > 20% in blood, primary tumour tissue, recurrent or metastatic disease | 800 or 900 mg PO twice daily on days 1-7 and 15-21 of 28-day cycles                        | ORR                                                                | 50 (41)       | 5.6                | 15.4               |
| Haddad R., 2021    | 2     | HNSCC                                | NA                 | Relapsed         | R/M mfHRAS VAF > 20% (tumour tissue) detected by NGS                      | 600 mg PO with a meal twice a day for 7 days in alternating cycles (Days 1-7 and 15-21) of 28-day cycles | ORR in High VAF population                                                      | 55 (NA)      | NA                 | 15.4               |
| Hanna G.J., 2020 [35] | 2     | Salivary gland carcinoma              | 13                 | Relapsed         | Missense HRAS mutation with a VAF > 20%: p.Gln61Arg (tumour tissue)       | 900 mg PO twice daily on days 1 to 7 and days 15 to 21 of a 28-day                       | ORR                                                                | 8 (54)        | 7.0                | 18.0               |
| Lee H.W., 2020     | 2     | Urothelial carcinoma                  | 21                 | Relapsed         | Missense, nonsynonymous HRAS mutations (p.Gly13Arg, n = 7; p.Gln61Arg, n = 4; p.Gly125Cys, n = 3; p.Gly125Val, n = 2) (tumour tissue) | 900 mg PO twice daily on days 1-7 and 15-21 of 28-day                                    | 6-month PFS                                                                 | 24 (62)       | 4.7                | 6.1                |
| Jazieh K., 2019 [37] | 1     | Advanced, recurrent or metastatic solid tumours | 27                 | Relapsed         | No selection on HRAS status Tumour tissue (diagnostic)                    | 4 dose levels, ranging from tipifarnib 200 mg PO twice daily plus erlotinib 75 mg PO plus daily to tipifarnib 300 mg PO twice daily plus erlotinib 150 mg PO twice daily | Safety, tolerability, maximum tolerated dose                                       | 7.4 (37)      | NA                 | NA                 |
| Whitehead R.P., 2006 [38] | 2     | Metastatic colorectal adenocarcinoma  | 62                 | No prior chem: 33/55 Prior chem: 22/55 | No selection on HRAS status Tumour tissue (diagnostic)                    | 4 dose levels, ranging from tipifarnib 200 mg PO twice daily plus erlotinib 75 mg PO plus daily to tipifarnib 300 mg PO twice daily plus erlotinib 150 mg PO twice daily | Confirmed response probability | 2 (20)        | 1.7                | 8.1                |
| Lara Jr PN., 2005 [39] | 1     | Advanced, recurrent or metastatic malignant tumours (8) NSCLC, (8) colorectal, (5) prostate, (5) oesophageal, (1) pancreatic, (1) parotid, (1) renal | 21                 | Relapsed         | No selection on HRAS status Tumour tissue (diagnostic)                    | Starting dose was 300 mg PO twice daily with escalation by 300 mg increments over six dose levels to a maximum of 1800 mg PO twice daily, on days 1-7 and 15-21 of 28-day treatment cycles 5-week cycles at a dose of 400 mg PO twice daily for 14 consecutive days followed by 7 days off treatment | Not mentioned | 0 (32)          | NA                 | NA                 |
| Heymach J.V., 2004 [40] | 2     | SCLC                                 | 22                 | Relapsed         | Missense HRAS mutation                                                      | 400 mg PO twice daily for 14 consecutive days followed by 7 days off treatment          | ORR                                                                | 0 (5)         | 1.4                | 6.8                |
| Adjei A., 2003 [41] | 2     | NSCLC                                | 44                 | 100% No prior chemotherapy (eligibility criteria) 9/44 had radiotherapy | No selection on HRAS status Tumour tissue (diagnostic) | 300 mg PO twice daily for 21 of every 28 days                                    | ORR                                                                | 0 (54)        | 2.7                | 7.7                |
| Hahn S., 2002 [42] | 1     | NSCLC                                | 9                  | No prior therapy: 7/9 Prior chemotherapy: 2/9 | No selection on HRAS status Tumour tissue (diagnostic) | Dose-escalation study of tipifarnib Dose level, 280 mg/m2 daily PO during weeks 1, 2, 4, and 5 of radiotherapy Dose level, 560 mg/m2 daily during weeks 1, 2, 4, 5, and 7 of radiotherapy | Maximum tolerated dose, dose limiting toxicity | NA            | NA                 | NA                 |
In 2021, a phase II study enrolling 30 patients with recurrent and/or metastatic HNSCC harbouring HRAS mutation shown highly promising results. Objective response rate was 55% (11/20 patients; 95% confidence interval (CI), 31.5 to 76.9), with a median progression-free-survival of 5.6 months (95% CI, 3.6 to 16.4), and a median overall survival of 15.4 months (95% CI, 7.0 to 29.7) [36]. The most frequent adverse events were anaemia (37%) and lymphopenia (13%). The mechanistic basis of tipifarnib toxicity might be related to the inhibition of several dozen other farnesylated proteins in cells [45]. Moreover, tipifarnib was recently described as an inhibitor of the CXCL12/CXCR4 pathway, that might lead to other side effects [46]. A phase II study evaluating tipifarnib in HRAS-mutant squamous NSCLC (NCT03496766) and another phase II study of tipifarnib in HNSCC with HRAS mutations are ongoing (NCT02383927).

5. Conclusions

HRAS mutations are uncommon genomic alterations in NSCLC patients, representing less than 1% of patients. Further studies are needed to better understand clinical course and prognosis associated with these mutations. However, preliminary data seems to show an association with tabagic status, aggressive presentation, and co-occurring mutations in MAPK pathway and TP53. Interestingly, a targeted therapy against HRAS-mutant showed promising results in HNSCC patients. Whether HRAS could represent a druggable oncogene in NSCLC is still unknown and should be explored in further studies.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare they have no conflict of interest.

Appendix A

Table A1. Next-Generation Sequencing (NGS).

| Sequenced Regions          |
|---------------------------|
| AKT1 exon 3 (NM_001014451.1) |
| ALK exons 22 to 25 (NM_004804.1) |
| BRAF exons 11 and 15 (NM_014333.4) |
| CTSNII exon 3 (NM_015084.4) |
| DDR2 exons 5 to 10 and 14 to 19 (NM_00114766.2) |
| EGFR exons 18 to 21 (NM_005228.3) |
| ERBB2 (HER2) exons 19 to 22 (NM_044448.2) |
| ERBB4 exons 393 and 452 (NM_005235.2) |
| FGFR2 exons 252, 284 and 699 (NM_001414.4) |
| FGFR3 exons 6, 8 and 15 (NM_001414.4) |
| FGFR4 exons 2, 3 and 4 (NM_005443.2) |
| FGFR5 exons 100 and 132 (NM_009866.3) |
| FGFR6 exons 172 (NM_021606.3) |
| KIT exons 8, 9, 11, 13, 14, 17 and 18 (NM_002222.2) |
| KRAS exons 2, 3 and 4 (NM_005250.1) |
| MAP2K1 (MEK1) exon 2 (NM_002755.3) |
| MET exon 2, 12, and 14 (NM_006206.1) |
| MET exons 2, 12, and 14 (NM_006206.1) |
| NRAS exons 2, 3 and 4 (NM_005254.3) |
| PDGFR exons 12, 14 and 15 (NM_006218.4) |
| PIK3CA exons 10 and 21 (NM_006218.4) |
| RET exons 11 and 16 (NM_005270.6) |
| TP53 exons 2 to 13 (NM_009844.4) |

Microsatellites: BAT25, BAT26, NR21, NR22, MONO27
