**NF1 mutations identify molecular and clinical subtypes of lung adenocarcinomas**

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**Abstract**
The tumor suppressor gene neurofibromin 1 (NF1) is a major regulator of the RAS-MAPK pathway. NF1 mutations occur in lung cancer but were not extensively explored. We hypothesized that NF1-mutated tumors could define a specific population with a distinct clinical and molecular profile. We performed NF1 sequencing using next generation sequencing (NGS) in 154 lung adenocarcinoma surgical specimens with known KRAS, EGFR, TP53, BRAF, HER2, and PIK3CA status, to evaluate the molecular and clinical specificities of NF1-mutated lung cancers. Clinical data were retrospectively collected, and their associations with molecular profiles assessed. In this series, 24 tumors were NF1 mutated (17.5%) and 11 were NF1 deleted (8%). There was no mutation hotspot. NF1 mutations were rarely associated with other...
RAS-MAPK pathway mutations. Most of patients with NF1 alterations were males (74.3%) and smokers (74.3%). Overall survival and disease-free survival were statistically better in patients with NF1 alterations (N = 34) than in patients with KRAS mutations (N = 30) in univariate analysis. Our results confirm that NF1 is frequently mutated and represents a distinct molecular and clinical subtype of lung adenocarcinoma.

**KEYWORDS**
lung adenocarcinoma, molecular subtype, next generation sequencing, NF1, RAS-MAPK pathway

1 | INTRODUCTION

Neurofibromin 1 (NF1) is a major tumor suppressor gene located on chromosome 17q11.2. NF1 encodes a RAS (rat sarcoma)-GAP (GTPase activating protein) known as neurofibromin. Neurofibromin facilitates the transit of RAS to their inactive state and functions as an inhibitor of the RAS-mitogen-activated protein kinase (MAPK) pathway.1 The RAS-MAPK pathway has major implications in cancer biology, and drives cell differentiation, proliferation, and survival.2

Germline dominant loss-of-function mutations of the NF1 gene cause the common inherited tumor predisposition syndrome neurofibromatosis type 1 (NF1; Online Mendelian Inheritance in Man database 162200). Tumor genome sequencing has resulted in the identification of somatic NF1 mutations in various non-NF1–associated sporadic cancers, including melanoma,3,4 lung cancer,5 glioblastoma,6 ovarian cancers,3 breast cancer, and acute myeloid leukemia.8 More than 1500 mutations in the NF1 gene have been reported in the Human Gene Mutation Database (HGMD), most of which are obvious loss-of-function alleles. The identification of NF1 mutations remains challenging owing to the large size and structure of the gene, the presence of numerous pseudogenes, and the different types of mutations that can occur. Moreover, it is not yet known if biallelic or monoallelic loss of NF1 contributes to tumor progression in sporadic cancers. Preclinical and clinical data suggested that treatment with MAP2K (MEK) inhibitor or in combination with mTOR inhibitors could be efficient to treat NF1-associated tumors.9,10

Lung adenocarcinoma is the most common form of lung cancer and has an average 5-year survival rate of 15%, mainly because of late-stage detection and a paucity of late-stage treatments. Somatic activating mutations in the RAS-MAPK pathway genes KRAS, EGFR, and BRAF were, respectively, identified in 30%, 14%, and 4% of lung adenocarcinoma with mutual exclusion in Caucasian population.7 Targeted therapies have been developed alone or in combination, allowing an increased in survival in patients with metastatic lung adenocarcinomas, especially in case of EGFR mutations. Adenocarcinomas in never-smokers frequently contain mutations within the EGFR tyrosine kinase domain; those patients who often respond to tyrosine kinase inhibitor drugs (TKIs) usually develop drug resistance. Conversely, KRAS mutations are more common in ever-smokers (former and current) and are associated with resistance to EGFR-TKIs. Drug combinations including MEK inhibitors are currently under evaluation for KRAS-mutated non–small-cell lung carcinoma.11,12

Although NF1 is a major regulator of RAS-MAPK pathway, only few clinical studies have described the pattern of NF1 somatic mutations in lung adenocarcinoma.5,13,14 Using a targeted next generation sequencing (NGS) approach, we analyzed a large cohort of resected lung adenocarcinomas to characterize NF1 mutations, and we evaluated the molecular and clinical specificities of NF1-mutated lung cancers.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

A total of 154 frozen tumor samples of primary lung adenocarcinoma were analyzed from 154 patients who underwent surgery between January 2001 and December 2006 in the Department of Thoracic Surgery of European Georges Pompidou Hospital (AP-HP, Paris, France). Clinical and survival data were retrospectively collected. Patient age, gender, date of surgery, stage at diagnosis and at surgery (pTNM), history of smoking, treatment before surgery, date of relapse, and date of death were collected. Overall survival (OS) was calculated from the date of surgery to the date of death or last follow-up. Disease-free survival (DFS) was calculated from the date of surgery to the date of relapse or last follow up.

Samples were collected with appropriate consents that were reviewed and approved by regulatory and ethical authorities (CCP Île-de-France II n°2008-136). Experienced pathologists histologically confirmed all cases, according to the 2004 World Health Organization (WHO) classification of lung neoplasms. Tumor and adjacent lung parenchyma were snap frozen in liquid nitrogen at the time of surgery and stored at −80°C. The Qiamp extraction kit (Qiagen) was used
for subsequent extraction after proteinase K digestion. Most samples were previously analyzed for the following genes using Sanger sequencing as previously described by Blons et al\(^{15}\): EGF\(R\) (exons 18-21), TP\(S\) (exons 4-10), KR\(A\)S (exon 2), BRA\(F\) (exons 11 and 15), HER\(2\) (exons 18-23), PIK3\(C\)A (exons 10 and 21), and STK\(11\) (exons 1-9).\(^{15}\)

### 2.2 NF1 sequencing

The coding sequence of the NF1 gene was analyzed using a targeted NGS approach, as previously described.\(^{16}\) Experiments were performed on the NGS platform of the Cochin Hospital (AP-HP, Paris, France). The targeted region included the entire NF1-coding exons, intron boundaries (25 bp), and the 5' and 3' untranslated regions (UTRs). Next generation sequencing library preparation used the Ion AmpliSeq Library Kit 2.0 according to the manufacturer's instructions. The template-positive ion sphere particles were loaded on Ion 318 chips and sequenced with an Ion PGM sequencer (Thermo Fisher Scientific).

Sequence alignment and extraction of SNPs and short insertions/deletions (indels) were performed using the Variant Caller plugin on the Ion Torrent Browser and DNA sequences visualized using the Integrated Genomics Viewer (IGV, v2.3) from Broad Institute. Major calling parameters were as follows: minimum allele frequency (MAF) ≥ 5% and minimum sequencing depth ≥200X for both SNPs and short indels. Variant annotation was performed using the PolyPhen-2,\(^{17}\) Mutation Taster,\(^{18}\) and SIFT\(^{19}\) software that provide in silico prediction of impact of an amino acid substitution. The creation of a new splice site was evaluated using the Human Splicing Finder V.2.4.1 (HSF) software.\(^{20}\)

The assessment of variants implication was performed based on population databases (dbSNP and ExAC),\(^{21}\) mutation databases (HGMD), and predictions software. The criteria used for classifying missense variants as pathogenic were as follows: (a) MAF ≤ 0.1% in population databases, (b) in silico prediction with a “possibly damaging” or a “probably damaging” impact of the non-synonymous variant on the structure and function of the protein, (c) in silico prediction of splicing alteration, and (d) report of the mutation by other groups or in mutations databases, as previously described.\(^{22}\)

Identification of copy number alterations (CNAs) was performed using sequencing depth analysis. Read numbers for each separated NF1 amplicon were normalized by dividing each amplicon read numbers by the total of amplicon read numbers of the control gene (SPRED1) from the same sample, as previously described.\(^{16}\) Normalized read numbers obtained for each amplicon of a sample were then divided by the average normalized read numbers of control samples for the corresponding amplicon. Copy number ratios of <0.7 and >1.3 were considered deleted and duplicated, respectively.

### 2.3 Statistical analysis

Associations between categorical variables were analyzed by a chi-square test and between continuous variables by a Wilcoxon signed-rank or t test. Survival was assessed by the Kaplan-Meier method, and differences were analyzed by the log-rank test. Variables significantly associated with OS (P-value <0.05) on univariate analysis were included in multivariate analysis using a Cox proportional hazard regression model.

### 2.4 Ethics

The research was conducted according to the recommendations outlined in the Helsinki declaration. This study was approved by the institutional review board (CPP Ile de France II, 2008-136).

### 3 RESULTS

#### 3.1 NF1 sequencing

For a typical run of 24 samples, ~468 megabases (Mb) were generated, corresponding to 3.5 × 10^6 reads. The mean read length was 141 bp. On average, for every sample, 99% of high quality sequencing reads (98% of bases) were mapped to the reference genome. This resulted in an evenly distributed mean sequencing depth for NF1 of 656X. A good uniformity between samples and between amplicons was obtained. NF1 was sequenced in 154 samples: of these, 17 samples were excluded from the subsequent analysis because the mean depth was <200X.

Pathogenic NF1 point mutations were identified in 24 of 137 (17.5%) samples: 6 non-sense mutations, 13 missense mutations, 5 frameshift mutations, and 4 splice site mutations. Among them, 4 (16%) were compound heterozygous mutations. There was no mutation hotspot (Figure 1). The variant allele frequency (VAF) ranged from 5% to 91% and 3 of the 24 (8%) mutations had a VAF > 55% suggesting loss of heterozygosity (Table S1). In 11 of the 137 (8%) samples, NF1 deletion was suggested by unbalanced copy number ratios using sequencing depth analysis (Figure 2).

#### 3.2 Correlation with molecular characteristics

Among the 137 analyzed samples: EGF\(R\), KR\(A\)S, and PIK3\(C\)A mutations were, respectively, found in 15 (10.9%), 30 (21.9%), and 2 (1.5%) samples (Figure 2). No BRAF mutation was found in the 86 tested samples. In addition, mutations were found in TP\(S\): 48/136 (35.3%); STK\(11\): 9/135 (6.7%); and HER\(2\): 2/122 (1.6%). Samples with NF1 mutations samples were exclusive of KR\(A\)S or EGF\(R\).
in 19 of the 24 cases (79%). Among the 24 samples with NF1 point mutations, co-mutations were found in TP53: 8 (33.3%); KRAS: 4 (16.7%); EGFR: 1 (4.2%); PIK3CA: 1 (4.2%); and HER2: 1 (4.2%; Figure 2, Tables S1-S3). There was no NF1 and STK11 co-mutation. Among the 11 samples with NF1 deletions, co-mutations were found in EGFR 3 (27.3%); KRAS: 3 (27.3%); and TP53: 4 (36.4%). No mutation in STK11, PIK3CA, or HER2 was found in NF1-deleted samples. Among the four samples with biallelic NF1 alterations, only one had a KRAS co-mutation (Tables S1, S2, and S4). NF1 mutations and deletions were not statistically associated with KRAS mutations ($P = 0.44$ and $P = 0.64$, respectively) or EGFR mutations ($P = 0.22$ and $P = 0.06$, respectively).

### 3.3 Correlation with clinical characteristics

The cohort was mainly constituted of early lung adenocarcinoma stages: 70.1% of patients were with stage I or II disease (Table 1). Patients with NF1 alterations were more frequently men (74.3% vs 62% in the whole cohort), with significant enrichment in the NF1 mutations subgroup ($P = 0.04$; Table S5). Past or current smoking status was reported for 104/137 (75.9%) patients in the whole cohort, 25/30 (83.3%) in the KRAS mutated group, 3/15 (20.0%) in the EGFR-mutated group, 48/77 (62.3%) in the TP53-mutated group, and 31/35 (82.9%) in the NF1 alterations group. The proportion of patients with early stage disease (stages I and II) was not different between the genotype subgroups: 97/137 (70.1%) in the whole cohort, 21/30 (70%) in the KRAS-mutated group, 8/15 (53.3%) in the EGFR-mutated group, 31/48 (64.6%) in the TP53-mutated group, and 27/35 (77.1%) in the NF1 alterations group. The

### TABLE 1 Clinical characteristics of the cohort

| Gender  | Count (%) |
|---------|-----------|
| Female  | 52 (38%)  |
| Male    | 85 (62%)  |

| Mean age (y) | 61.4 (32.8-85.2) |

| Tobacco | Count (%) |
|---------|-----------|
| Yes     | 104 (75.9%) |
| No      | 21 (15.3%)  |
| Unknown | 12 (8.8%)   |

| Stage | Count (%) |
|-------|-----------|
| I     | 73 (53.3%) |
| II    | 23 (16.8%) |
| III   | 28 (20.4%) |
| IV    | 13 (9.5%)  |

Chemotherapy or radiochemotherapy before surgery

| Yes | Count (%) |
|-----|-----------|
| 9   | 6.6%      |

| No  | Count (%) |
|-----|-----------|
| 128 | 93.4%     |

*For each patient, the stage was established thanks to surgical samples. Nine patients received chemotherapy or chemoradiotherapy before surgery. For these nine samples, the stage was established after receiving systemic treatments. Pathological stage is detailed in the Table S2.
TABLE 2  Clinical characteristics according to molecular profile

|                      | All population (N = 137) | NF1 alterations (N = 35) | P* | KRAS mutations (N = 30) | P** | EGFR mutations (N = 15) | P*** |
|----------------------|--------------------------|--------------------------|----|-------------------------|-----|-------------------------|------|
| Gender               |                          |                          |    |                         |     |                         |      |
| Female               | 52 (38%)                 | 9 (25.7%)                | 0.12 | 12 (40%)                | 0.83 | 9 (60%)                 | 0.07 |
| Male                 | 85 (62%)                 | 26 (74.3%)               |     | 18 (60%)                | 0.60 | 6 (40%)                 |      |
| Mean age             | 61.4 (32.8-85.2)         | 61.3 (46.1-76.4)         | 0.92 | 59.7 (41.9-81.7)        | 0.32 | 63.2 (50.3-84)          | 0.48 |
| Tobacco              |                          |                          |    |                         |     |                         |      |
| Yes                  | 104 (75.9%)              | 26 (74.3%)               | 0.79 | 5 (83.3%)               | 0.15 | 7 (46.7%)               | <0.0001 |
| No                   | 21 (15.3%)               | 6 (17.1%)                |     | 2 (6.7%)                | 0.79 | 3 (20%)                 |      |
| Unknown              | 12 (8.8%)                | 3 (8.6%)                 |     | 2 (6.7%)                | 0.79 | 7 (46.7%)               |      |
| Stage                |                          |                          |    |                         |     |                         |      |
| I-II                 | 96 (70.1%)               | 27 (77.1%)               | 0.29 | 21 (70%)                | 0.99 | 8 (53.3%)               | 0.13 |
| III-IV               | 41 (29.9%)               | 8 (22.9%)                |     | 9 (30%)                 | 0.53 | 7 (46.7%)               |      |
| Chemotherapy or radiochemotherapy before surgery | | | | | | |
| Yes                  | 9 (6.6%)                 | 5 (14.3%)                | 0.04 | 3 (10%)                 | 0.53 | 1 (6.7%)                | 0.91 |
| No                   | 128 (93.4%)              | 30 (85.7%)               |     | 27 (90%)                | 0.53 | 14 (93.4%)              |      |

*Statistical analysis between patients with NF1 alterations (N = 35) and patients without NF1 alteration (N = 102).
**Statistical analysis between patients with KRAS mutations (N = 30) and patients without KRAS mutation (N = 107).
***Statistical analysis between patients with EGFR mutations (N = 15) and patients without EGFR mutation (N = 122).

Bold values mean that the result is statistically significant.

Proportion of patients with NF1 alterations was not statistically different according to disease stage (P = 0.29); among patients with early stage disease (N = 96), 28.8% (27/96) had NF1 alterations; among patients with advanced stage disease (N = 41), 19.5% (8/41) had NF1 alterations (Table 2).

3.4 | Univariate analyses for DFS and OS according to molecular subgroups

No statistical difference in DFS and OS was found between patients with NF1 alterations (point mutation or deletions) or without NF1 alterations (Figure 3A). Disease-free survival was 57.1 months in patients without NF1 alteration vs 43.1 months in the NF1 alterations group (P = 0.3), 92.6 months in the EGFR group (P = 0.5), and 25.7 months in the KRAS mutations group (P = 0.01). Disease-free survival in the NF1 alterations group was statistically better than in the KRAS mutations group (P = 0.004).

Overall survival was 60.7 months in the NF1 alterations group, 36.1 months in the KRAS mutations group, 100.6 months in the EGFR mutations group, and 75.2 months in the entire cohort (Figure 3B). There was no statistical OS difference between patients with or without NF1 point mutations (P = 0.42), and between patients with or without NF1 deletion (P = 0.52). There was a trend for an increased OS in NF1-deleted vs NF1-mutated patients: 119.1 vs 58.2 months, respectively (P = 0.06). Overall survival was significantly shorter in patients with KRAS mutations vs patients without KRAS mutations (P = 0.009) and vs patients with NF1 mutations (P = 0.004). The median OS in NF1- and KRAS-co-mutated patients (52.1 months) was significantly decreased vs NF1-mutated patients without KRAS mutations (81 months; P = 0.03). No statistically significant difference was found in KRAS-mutated patients without NF1 mutations (27.9 months; P = 0.59).

3.5 | Multivariate analysis

In multivariate analysis (including age, tumor stage, KRAS mutations, and NF1 alterations), KRAS mutations were strongly independently associated with a poor prognosis (P = 0.004, Hazard ratio, HR = 2.5), as well as KRAS and NF1 co-mutations (P = 0.04, HR = 2.3) and advanced tumor stage (P < 0.0001, HR = 2.9; Table 3). NF1-altered/KRAS wild-type (WT) patients did not show a worse OS compared with NF1 WT/KRAS WT patients.

In multivariate analyses separating NF1 mutations and NF1 deletions, NF1 mutations or deletions were not associated with prognosis. KRAS mutations were independently associated with poor prognosis regardless of NF1 alteration status.

3.6 | Impact of neoadjuvant treatment

In the whole cohort, only 9 of the 137 (6.6%) patients received neoadjuvant chemotherapy or radiochemotherapy. Among them, 5 (of the 9) had NF1 alterations, including 4 heterozygous point mutations (1 non-sense, 2 missense, and 1 splice site mutation), and one large deletion. Patients with
NF1 were significantly more often treated with chemotherapy: 6.6% (5/35) of patients without NF1 alterations received chemotherapy while 14.3% (9/137) of patients with NF1 mutations received chemotherapy before surgery (P = 0.01). The median age of these five NF1 patients was 55.1 years (46.1–69.5). Of the five patients, three were men and three were current or former smokers (Tables S1 and S2). In two of the five NF1-mutated tumors, paired pre- and post-chemotherapy samples were analyzed. The same NF1 mutation was identified in both pre- and post-chemotherapy paired samples.

4 | DISCUSSION

In our series, NF1 mutations and deletions were found in 17.5% and 8% of lung adenocarcinoma surgical specimens, respectively. Among the 24 NF1 mutations, 4 (16.7%) were homozygous. Most NF1 mutations were exclusive of KRAS and EGFR mutation (19 of the 24 samples), and one third co-occurred with TP53. These results are consistent with previous published studies.14,23,24 However, the occurrence of NF1 mutations was higher in our study (17.5%) compared to the study by Redig et al (10%).14 The population of these two studies was different: Redig et al study described patients with metastatic adenocarcinomas and squamous cell cancers and our cohort only included patients with metastatic adenocarcinoma. This difference may explain the higher occurrence of NF1 mutations in our cohort. Moreover, the method used for variant selection was different between the two studies. Redig et al24 used a MAF ≥ 10% and a minimum sequencing depth ≥50X for variant detection. In the present study, calling parameters were a MAF ≥ 5% and a minimum sequencing depth ≥200X. In our study, four NF1-mutated samples have a MAF comprised between 5% and 10%. If we exclude these samples, NF1 mutations are found in 20 of the 137 samples (14.6%) in our cohort (Table S1).

Co-occurrence of NF1 alterations with EGFR and KRAS mutations were rare in our series, suggesting a driver role. Even in cases of co-mutations in RAS-MAPK pathway genes, NF1 alterations were predicted to be deleterious (Table S1).

The clinical profile of NF1-mutated patients was similar to the one of KRAS-mutated patients who were mainly males, and current or former smokers. Previous studies demonstrated that KRAS mutation had a negative prognostic impact, especially in early stage lung cancer.26,27 Here, we observed that NF1-mutated patients showed significantly increased DFS and OS vs KRAS-mutated patients in univariate analysis (Figure 3). In multivariate analyses for OS, KRAS mutations were found to be associated with poorer survival both in NF1 WT and NF1-altered status. NF1-altered patients with no KRAS mutation had the same prognosis than NF1 WT/KRAS WT patients. Few data are available concerning NF1-mutated patients’ prognosis. Redig et al and Pan et al did not find differences in survival

### TABLE 3 Multivariate Cox Model on overall survival

| Characteristics                        | Hazard ratio | 95% CI      | P     |
|----------------------------------------|--------------|-------------|-------|
| Age (per year)                         | 1.01         | 0.98-1.03   | 0.58  |
| Tumor stage (I-II vs III-IV)           |              |             |       |
| I-II (ref)                             | 1            | –           | –     |
| III-IV                                 | 2.91         | 1.79-4.71   | <0.0001|

**Mutations**

|                            | Hazard ratio | 95% CI      | P     |
|---------------------------|--------------|-------------|-------|
| KRAS WT/NF1 WT (ref)      | 1            | –           | –     |
| KRAS mutations/NF1 WT     | 2.47         | 1.33-4.59   | 0.004 |
| NF1 alterations/KRAS WT   | 1.44         | 0.78-2.67   | 0.24  |
| NF1 and KRAS co-mutations | 3.35         | 1.03-5.36   | 0.04  |

ref, reference; WT, Wild type.

Bold values mean that the result is statistically significant.
between NF1- and KRAS-mutated patients. However, the studied populations were different than in the present study. Our results have to be confirmed in a larger cohort.

The clinical and molecular profiles of NF1-deleted (11/137) and NF1-mutated (24/137) patients were different. Patients with NF1 deletions included less smokers, younger patients, more females, and showed a trend for a better survival compared to patients with NF1 point mutation (Table S5). To our knowledge, no previous study had reported this observation. In our cohort, three NF1 deletions (27.3%) were associated with EGFR mutations. Only few previous data described NF1 CNAs in lung cancers and no co-occurrence of NF1 deletions and EGFR mutations was found. The co-occurrence of NF1 deletions with EGFR mutations could explain the observed associated phenotype in our cohort.

Only surgery specimen samples were analyzed in our cohort explaining the small proportion of stage IV disease (9.5%) and the small proportion of patients receiving chemotherapy (6.6%) at the time of the surgery. However, we report no significant difference in disease stages according to NF1 molecular status maybe because of the small cohort size. Interestingly, we found a significant higher proportion of NF1 mutations in tumors from chemotherapy-treated patients vs treatment-naive patients. Even if emergence of NF1 mutations following targeted therapies was previously described in melanoma, and the downregulation of NF1 tumors from chemotherapy-treated patients vs treatment-naive patients, more females, and showed a trend for a better survival compared to patients with NF1 point mutation (Table S5). To our knowledge, no previous study had reported this observation. In our cohort, three NF1 deletions (27.3%) were associated with EGFR mutations. Only few previous data described NF1 CNAs in lung cancers and no co-occurrence of NF1 deletions and EGFR mutations was found. The co-occurrence of NF1 deletions with EGFR mutations could explain the observed associated phenotype in our cohort.

Our results have to be confirmed in a larger cohort explaining the small proportion of stage IV disease (9.5%) and the small proportion of patients receiving chemotherapy (6.6%) at the time of the surgery. However, we report no significant difference in disease stages according to NF1 molecular status maybe because of the small cohort size. Interestingly, we found a significant higher proportion of NF1 mutations in tumors from chemotherapy-treated patients vs treatment-naive patients. Even if emergence of NF1 mutations following targeted therapies was previously described in melanoma, and the downregulation of NF1 expression was observed in EGFR-mutated lung adenocarcinoma, we do not confirm this observation in our cohort. The same NF1 mutations were identified in the two available pre- and post-chemotherapy paired samples. Our observation of a significant higher proportion of NF1 mutations in tumors from chemotherapy-treated patients needs to be confirmed in a larger cohort.

We identified 4 of the 24 (16.7%) NF1 compound heterozygous mutations. The four patients with biallelic NF1 mutations had the same clinical characteristics than patients with monoallelic NF1 alterations. Only one patient had a co-mutation with KRAS (Tables S1-S3). In a previous study, Redig et al also reported 15% (9/60) of lung adenocarcinoma samples with biallelic NF1 mutations. Little is known on functional consequences of NF1 mutation allelic balance in cancer cells. It has become apparent that not all consistent loss-of-function hits in tumor suppressor genes are accompanied by obvious aberrations on the WT allele, in discordance with the two-hit hypothesis affecting tumor suppressor genes. Single-copy loss may have a role in tumorigenesis, and haploinsufficiency effect may be highly tissue specific and context dependent. An appreciation of the functional role of NF1 gene dosage in lung adenocarcinoma development will be important for prediction of response to therapeutics. Notably, preclinical and clinical data have suggested efficacy of targeted therapies including MEK or mTOR inhibitors alone or in combination in NF1-mutated tumors. It will be important not only to assess the presence of NF1 mutation in a tumor, but also to accurately assess the potential therapeutic impact of NF1 point mutations, copy number, and the ratio of mutant to normal as predictive biomarkers.

5 | CONCLUSION

Our results are consistent with previous published data and confirm the implication of NF1 somatic alterations in lung adenocarcinoma with distinct molecular and clinical characteristics. These findings need to be confirmed in a larger cohort and functional consequences to be studied for a better management of available treatments including chemotherapy, targeted therapies, or immunotherapies.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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**SUPPORTING INFORMATION**

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

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