Clinical and Molecular Characteristics of NF1-Mutant Lung Cancer

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Abstract

Purpose: NF1 is a tumor suppressor that negatively regulates Ras signaling. NF1 mutations occur in lung cancer, but their clinical significance is unknown. We evaluated clinical and molecular characteristics of NF1 mutant lung cancers with comparison to tumors with KRAS mutations.

Experimental Design: Between July 2013 and October 2014, 591 non–small cell lung cancer (NSCLC) tumors underwent targeted next-generation sequencing in a 275 gene panel that evaluates gene mutations and genomic rearrangements. NF1 and KRAS cohorts were identified, with subsequent clinical and genomic analysis.

Results: Among 591 patients, 60 had NF1 mutations (10%) and 141 (24%) had KRAS mutations. 15 NF1 mutations (25%) occurred with other oncogenic mutations [BRAF (2); ERBB2 (2); KRAS (9); HRAS (1); NRAS (1)]. There were 72 unique NF1 variants. NF1 tumor pathology was diverse, including both adenocarcinoma (36, 60%) and squamous cell carcinoma (10, 17%). In contrast, KRAS mutations occurred predominantly in adenocarcinoma (136, 96%). Both mutations were common in former/current smokers. Among NF1 tumors without concurrent oncogenic alterations, TP53 mutations/2-copy deletions occurred more often (33, 65%) than with KRAS mutation (46, 35%; P < 0.001). No difference between cohorts was seen with other tumor suppressors.

Conclusion: NF1 mutations define a unique population of NSCLC. NF1 and KRAS mutations present in similar patient populations, but NF1 mutations occur more often with other oncogenic alterations and TP53 mutations. Therapeutic strategies targeting KRAS activation, including inhibitors of MAP kinase signaling, may warrant investigation in NF1 mutant tumors. Tumor-suppressor inactivation patterns may help further define novel treatment strategies.

Introduction

NF1 is a tumor-suppressor gene located on chromosome 17 encoding a protein known as neurofibromin. Neurofibromin negatively regulates Ras activity and has been well characterized in the clinical context of neurofibromatosis type 1, an autosomal dominant genetic disorder with an estimated incidence of 1 in 3,000 individuals (1–3). Classically, neurofibromatosis patients may develop neurofibromas, café-au-lait macules, optic gliomas, and iris hamartomas, although the disorder can also be associated with other cardiovascular, musculoskeletal, and nervous system abnormalities (4). NF1 was first cloned in 1990 and is one of the largest genes in the human genome, with 60 exons representing a total of 350 kb of genomic DNA (5, 6).

Functional studies have demonstrated that neurofibromin functions as a Ras-GTPase activating protein (GAP) (7). Furthermore, several NF1 mouse models have demonstrated that loss of normal neurofibromin function causes a predisposition to tumor development in mice that is very similar to what is seen in human patients with neurofibromatosis (7, 8).

A critical region in the neurofibromin protein is encoded by exons 20–27 that represents a ras-guanosine-triphosphate (GTP)-ase activation protein (GAP)–related domain (GRD). This GRD region of neurofibromin can bind to Ras proteins and downregulate their activity through the conversion of the active Ras–GTP complex to inactive Ras–GDP. Negative regulation of Ras subsequently prevents the downstream activation of Ras effector pathways, specifically the MAPK and PI3K–Akt–mTOR pathways that drive the pro-proliferation, survival, and differentiation effects associated with Ras activation (3, 8, 9).

Indeed, the tumor-suppressor function of neurofibromin has historically been attributed primarily to its ability to negatively regulate Ras function, although recent reports have also begun to expand our understanding of the cellular role of neurofibromin. Neurofibromin has been shown to have proapoptotic effects that are both Ras dependent and Ras independent (10). Neurofibromin helps regulate cell adhesion, migration, and survival (11), whereas analysis of neurofibromas from patients with neurofibromatosis suggests that loss of neurofibromin may contribute to the epithelial–mesenchymal transition

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(EMT) and activation of the heat shock response (12). Together, these data suggest a complex cellular role for neurofibromin as a tumor suppressor that can function through modulation of Ras activity and influence many other intracellular signaling events.

Notably, patients with neurofibromatosis are known to have up to a 4-fold increased risk of malignancy throughout their lifetime when compared with the general population (4). These malignancies do not typically include lung cancer, but a number of relatively rare tumors are prevalent in this cohort, including malignant peripheral nerve sheath tumor (MPNST), optic pathway glioma (OPG), and juvenile myelomonocytic leukemia (JMML). However, it was not until the publication of the Cancer Genome Atlas (TCGA) data that the frequency of NF1 mutations in human tumors, including lung cancers, became more apparent. The clinical and molecular features of non–small cell lung cancer (NSCLC) tumors containing an NF1 mutation remain unknown, but TCGA data from lung cancers demonstrate that NF1 mutations are frequently seen in both adenocarcinoma (8.3%) and squamous cell carcinoma (12%; refs. 13, 14).

The link between neurofibromin and Ras signaling suggests that NF1 mutations may be of great clinical significance in lung cancer. KRAS is the single most frequently mutated oncogene found in NSCLC and can be seen in tumors from both smokers and never-smokers. Although identification of activating mutations or rearrangements in targetable oncogenes such as the EGFR (15, 16) or the anaplastic lymphoma kinase (ALK; ref. 17) have led to targeted therapies with dramatic clinical implications, similar efforts to target KRAS mutations remain a challenge. As a pleiotropic GTPase, KRAS is upstream of several critical intracellular signaling pathways (18), thus one strategy to target Ras signaling in lung cancer has been selective inhibition of downstream pathways. Recent efforts have demonstrated that inhibition of MAP kinase signaling may be effective in KRAS-mutant NSCLC (19–21). A recent randomized phase II trial demonstrated a clinically meaningful improvement in progression-free survival in patients with KRAS mutations who received docetaxel plus the MEK inhibitor selumetinib, compared with docetaxel alone (22). A randomized phase III clinical trial further evaluating this approach is ongoing (ClinicalTrials.gov: NCT01933932). Importantly, tumors harboring a mutation in NF1 may also be sensitive to this strategy of downstream inhibition because of the negative regulation of Ras activity associated with functional neurofibromin.

In this study, we evaluate the clinical and molecular features of a cohort of NSCLC characterized by NF1 mutation and compare the results with a cohort of tumors characterized by KRAS mutation. Our findings begin to identify a novel cohort of NSCLC defined by NF1 mutation and suggest that ongoing therapeutic targeting strategies for KRAS tumors may also have efficacy in this population.

**Translational Relevance**

Mutations in the tumor-suppressor NF1 occur in lung cancer, but their clinical significance and molecular characterization remain unknown. NF1 functions as a negative regulator of Ras signaling, suggesting that lung cancers with NF1 mutations may also be characterized by downstream activation of Ras signaling. Further evaluation of lung cancers defined by NF1 mutation may reveal a unique cohort of tumors, distinct from KRAS mutant lung cancers, for which therapeutic targeting of pathways downstream of activated Ras may have clinical efficacy.

**Materials and Methods**

Next-generation sequencing and identification of patient cohorts

Between July 2013 and October 2014, 4,267 patients underwent targeted next-generation sequencing (NGS) at the Dana-Farber Cancer Institute/Brigham and Women’s Hospital under an IRB-approved research protocol. All patients provided written informed consent before sequencing of their tumors. Among this population, 591 patients with NSCLC were identified using the Oncology Data Retrieval System (OncDRS), an internal system developed at the Dana-Farber Cancer Institute to integrate clinical and genomic data. No germline sequencing was performed in this cohort.

Sequencing was performed on tumor DNA extracted from fresh, frozen, or formalin-fixed paraffin-embedded samples and evaluated for single nucleotide variants (SNV), copy-number variations, and structural variants (including rearrangements; ref. 23). The initial gene panel surveyed all exons of the 275 genes in the panel, along with 91 introns across 30 genes for rearrangement detection. The full list of genes is available as Supplementary Data (Supplementary Fig. S1). DNA was isolated from tissue containing at least 20% tumor nuclei and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq 2500 sequencer. Data were analyzed by an internally developed bioinformatics pipeline composed of reconfigured publically available tools and internally developed algorithms. SNVs were called using MuTect (24) and indels using Indelocator (http://www.broadinstitute.org/cancer/nga/indelocator). Annotation was performed using Onco-locator (25). Tumor tissues were tested without a paired normal from individual patients, thus additional informatics steps were taken to identify common SNPs: any SNP present at >0.1% in Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP; Seattle, WA; URL: http://evs.gs.washington.edu/EVS/ accessed May 30, 2013) or present in dbSNP was filtered. Variants also present in COSMIC were rescued for manual review. VisCap Cancer calls copy-number changes based on log, ratios that are calculated using a normalized depth of coverage against a median from a panel of normal (non-cancer) samples. Samples with a mean target coverage of <50× were failed and excluded from further analysis. Individual variants present at <10% allele fraction or in regions with <50× coverage were flagged for manual review and interpreted by the reviewing laboratory scientists and molecular pathologists based on overall tumor percentage, read depth, complexity of alteration, and evidence for associated copy-number alterations.

**Genetic mutation analysis**

Identified mutations in NF1 were analyzed using the Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and Human Splicing Finder (http://www.umd.be/HSF/) programs to evaluate whether a given mutation was predicted to have a benign
or damaging effect on the NF1 protein. The introduction of a stop codon or predicted loss of function was also determined. The NF1 mutations identified in our cohort were cross-referenced with reported NF1 variants in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), COSMIC (http://cancer.sanger.ac.uk/cosmic/), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), ExAC (http://exac.broadinstitute.org), Exome Variant Server (http://evs.gs.washington.edu), and PubMed (http://www.ncbi.nlm.nih.gov/pubmed). An institutional instance of cBioPortal was used to visualize the data across specimens (26).

Clinical characteristics

Following identification of patients with NF1 and KRAS mutations, clinical and molecular characteristics were determined. Patient age, gender, date of diagnosis, stage at diagnosis, location of metastatic lesions at diagnosis, smoking status at diagnosis, and date of death were determined from a review of the medical record and the social security death index. NGS data from each patient cohort were then reviewed to identify concurrent alterations in other known oncogenic drivers with relevance in lung cancer (EGFR, ALK, ROS1, NRAS, HRA5, BRAF, and ERBB2). Sequencing data were also reviewed to identify mutation or two-copy deletion in commonly altered tumor suppressors, including TP53, LKB1, PTEN, RB1, CDKN2A, and CDKN2B.

Statistical analysis

Overall survival (OS) is defined as the time in months from the date of diagnosis of metastatic disease to the date of death from any cause; patients alive at the time of analysis have been censored at their last known follow-up date. The Kaplan–Meier method was used to estimate event-time distributions. The Fisher's exact test was used to compare frequency of gene mutations in tumor suppressors and selected clinical variables between the NF1 and the KRAS cohorts. The Mann–Whitney test was used to compare the age distribution between the NF1 and the KRAS cohorts.

Results

NF1 mutations are heterogeneous and widely distributed throughout the NF1 gene

Seventy-two unique NF1 nucleotide variants were identified in our cohort of 60 distinct tumors. The majority of these variants have not been previously reported or functionally characterized (Supplementary Table S1). Variants in NF1 included missense mutations (n = 32), nonsense mutations (n = 15), splice site mutations (n = 13), and frameshift mutations (n = 12; Fig. 1A). Polyphen-2 or Human Splicing Finder was used to predict the functional effect of identified NF1 variants. All splice site mutations result in a broken acceptor site with predicted loss of function of the NF1 protein. Of the 59 missense, nonsense, and frameshift variants identified by NGS in our cohort, 11 variants were predicted to be benign and 48 were predicted to have a damaging effect. Notably, 27 of the 59 variants result in the generation of a stop codon with predicted loss of function of the NF1 protein (Fig. 1B). Nine tumors had more than one NF1 variant, with 7 tumors having 2 variants and 2 tumors having 3 variants (Supplementary Table S2). The NF1 mutations found concurrently with other driver mutations (n = 15 tumors, 17 NF1 variants) were noted to be representative of the spectrum of NF1 variants found in the larger cohort, with a similar proportion of missense mutations, nonsense mutations, splice site mutations, and frameshift mutations (Fig. 1C). The distribution of all identified NF1 mutations in our cohort is represented in Fig. 1D. Mutations are spread throughout all exons of the NF1 gene. Similarly, 13 KRAS mutations were identified in our cohort of 141 distinct tumors (Supplementary Table S3). No tumors had more than one KRAS mutation, and the majority of mutations identified represent known activating mutations in exons 1 or 2 (Fig. 1E).

NF1 mutations do exist with concurrent oncogenic alterations, but the majority of NF1 mutations in NSCLC occur independently

Forty-five of 60 tumors (75%) with an NF1 mutation did not have any concurrent oncogenic alterations. Among the 15 NF1 tumors with a concurrent oncogenic alteration, 9 tumors had a coexisting KRAS mutation; 1 tumor had an NRAS or HRA5 mutation; and 2 tumors had a BRAF or an ERBB2 mutation (Table 1). Concurrent KRAS mutations were all found in the G12 or G13 position, with three G12D and G12V mutations; two G13C mutations; and one G13V mutation. The HRA5 mutation was noted to be a Q61H mutation, and the NRAS mutation was identified as a G13R. BRAF mutations included the previously identified G469V mutation and a C685S variant. C685S is predicted to be a benign variant with a Polyphen-2 prediction score of 0.271. ERBB2 mutations included the previously identified S310Y mutation and a V308L variant, both found in the furin-like, cysteine-rich region of exon 8. V308L is predicted to be a possibly damaging mutation with a Polyphen-2 score of 0.691.

NF1 and KRAS cohorts share similar clinical characteristics with some differences in tumor histology

Median age, gender, smoking status, stage at diagnosis, locations of metastases at diagnosis, and tumor histology were determined for both the NF1 and the KRAS cohort (Table 2). In total, tumors with NF1 mutations represented 10% of our NSCLC cohort and tumors with KRAS mutations represented 24% of our NSCLC cohort. Overall, the NF1 and KRAS cohorts shared very similar clinical profiles. Median age was similar, at 62 years (range, 44–83) for the NF1 cohort and 65 years (range, 42–86) for the KRAS cohort (P = 0.14, Mann–Whitney test). Gender distribution included 52% women in the NF1 cohort (31/60) compared with 59% women in the KRAS cohort (83/141; P = 0.36, Fisher’s exact test). Current or former smokers represented the largest subgroup within both cohorts (53/60, 88% for NF1 and 135/141, 96% for KRAS), but there were more never-smokers in the NF1 cohort (7/60, 12%) compared to the KRAS cohort (6/141, 4%), with a trend toward significance (P = 0.06, Fisher’s exact test).

A majority of patients in both groups presented with advanced Stage III/IV disease (NF1 = 38/60, 63%; KRAS = 72/141, 51%). A greater percentage of patients with an NF1 mutation presented with stage IV disease compared with patients whose tumors harbored a KRAS mutation (NF1 = 27/60, 47%; KRAS = 53/141, 38%), but this difference was not found to be statistically significant (P = 0.34, Fisher’s exact test). Patients with metastatic disease at diagnosis presented with a similar distribution of metastatic sites, including bone (NF1 = 13/60, 22%; KRAS = 28/141, 20%);
Figure 1. Characterization of identified NF1 mutations reveals a heterogeneous population with distribution throughout the gene. A, a pie chart demonstrating mutation classification of all 72 identified NF1 variants in our cohort. B, a pie chart demonstrating functional classification of all 72 identified NF1 variants with functional prediction by Polyphen-2 analysis. C, a pie chart demonstrating mutation classification of 17 NF1 variants identified in 15 tumors with concurrent NF1 variants and additional oncogenic alterations (n = 17 variants). D, a lollipop plot demonstrating distribution of all identified NF1 variants in our cohort. Green circles, missense mutations; red circles, known truncating mutations (nonsense, frameshift, and splice site); purple circles, residues affected by multiple mutation types. Indicated in green is the RasGAP domain and indicated in red is the CRAL-TRIO domain, which functions as a lipid binding pocket. E, a lollipop plot demonstrating distribution of all identified KRAS mutations in our cohort. Green circles, missense mutations; red circles, known truncating mutations (nonsense, frameshift, and splice site); purple circles, residues affected by multiple mutation types.
(NF1 = 5/60, 8%; KRAS = 14/141, 10%); and visceral disease (NF1 = 25/60, 42%; KRAS = 48/141, 34%). No statistically significant differences between the NF1 and KRAS cohorts were observed when comparing the location of metastatic sites for patients with stage IV disease.

Tumor histology was also evaluated for all tumors in both NF1 and KRAS cohorts. The majority of tumors in both cohorts represented were adenocarcinomas (NF1 = 36/60; 60%; KRAS = 136/141, 96%), but there was substantially more diversity in histopathology in the NF1 cohort. Most notably, compared with KRAS tumors (2/141, 1%), NF1 mutations were also found in squamous cell carcinoma (10/60, 17%, P < 0.001, Fisher’s exact test). Both NF1 tumors and KRAS tumors included large cell neuroendocrine tumors and giant cell carcinoma at low frequency. The NF1 cohort also included several tumors with poorly differentiated/undifferentiated histology (11/60, 18%) that was not seen in any of the KRAS tumors.

Concurrent tumor suppressor mutations show varying frequencies in the NF1 and KRAS NSCLC cohorts

The frequency of mutations or 2-copy deletions in key tumor suppressors was identified in nonoverlapping NF1 and KRAS cohorts. Nine tumors were found to have both an NF1 and a KRAS mutation, thus the NF1 cohort for this analysis included 51 tumors whereas the KRAS cohort included 132 tumors. TP53 and LKB1 were the most frequently mutated tumor suppressors found in tumors harboring either NF1 or KRAS mutations. Furthermore, among the nonoverlapping NF1 and KRAS cohort, TP53 mutations or 2-copy deletions were found to occur more frequently in the NF1 cohort (33/51, 65%) than in the KRAS cohort (46/132, 35%), with P < 0.001 (Fig. 2A). In contrast, no significant difference was noted between the frequency of LKB1 mutations or 2-copy deletions in the NF1 cohort (8/51, 16%) compared to the KRAS cohort (33/132, 25%), P = 0.24 (Fig. 2A). All mutations in TP53 and STK11 are shown collectively for the NF1 cohort (Fig. 2B) and the KRAS cohort (Fig. 2C).

When compared with TP53 or LKB1, mutation/deletions in RB1, PTPN, CDKN2A, or CDKN2B were rare in both cohorts (Fig. 2D). RB1 alterations were found in 3 of 51 NF1 tumors and 4 of 132 KRAS tumors; PTPN alterations were found in 1 of 51 NF1 tumors and 2 of 132 KRAS tumors; CDKN2A alterations were found in 6 of 51 NF1 tumors and 9 of 132 KRAS tumors; and CDKN2B alterations were found in 4 of 51 NF1 tumors and 5 of 132 KRAS tumors (all nonsignificant).

Overall survival for patients with NF1 mutant tumors is similar to OS for patients with KRAS-mutant tumors

Kaplan–Meier analysis was used to determine the OS of patients with stage IV NSCLC at diagnosis and with either an NF1 or KRAS mutation (Fig. 3). OS in both stage IV patient cohorts was similar, with patients whose tumors harbored an NF1 mutation having an OS of 12.4 months, whereas stage IV patients whose tumors harbored a KRAS mutation had an OS of 11.6 months.

Discussion

Advances in our understanding of the genomic drivers of NSCLC have profoundly altered the diagnostic workup, treatment options, and prognosis for this disease. Genetic testing at time of diagnosis is now standard-of-care, and targeted therapy options for activating mutations in EGFR, ALK, and ROS1 have significantly improved outcomes for these patients. Ongoing trials continue to develop strategies for targeting other oncogenic drivers.
alterations (27–30). In the last decade, NSCLC has gone from a disease in which genetic characterization was limited to identification of KRAS as a frequently occurring oncogene without targeted treatment options, to a disease in which an ever-growing number of low frequency yet targetable mutations have been identified (31).

However, despite the success of this approach, there are still many patients for whom treatment options for NSCLC remain limited. First, the advances of the targeted therapy era have yet to make a substantial difference for patients with KRAS mutations. As a GTPase, activated KRAS is not amenable to the direct targeting approach that works well for many kinases. Second,
Despite the growing number of small cohorts of NSCLC defined by targetable activating mutations, some 30% to 40% of NSCLC is still characterized by the lack of a clearly identifiable oncogenic alteration. For these patients, cytotoxic chemotherapy remains the mainstay of oncology care. Finally, the histology of NSCLC helps determine the likelihood that a patient will have targeted therapy options, as targetable oncogenic alterations are found almost exclusively in adenocarcinoma. TCGA data demonstrate that the squamous subset of NSCLC is enriched for mutations in several tumor suppressors (TP53, CDKN2A, PTEN, and RB1), along with PIK3CA and NOTCH1, yet none of these are easily targeted (13). Against this landscape, the identification of NF1 as an oft-mutated tumor suppressor in both the adenocarcinoma and squamous subsets of NSCLC is particularly intriguing (13, 14). NF1 is a widely studied tumor suppressor, but research in the field to date has been primarily in the context of Type I neurofibromatosis. However, if downstream signaling caused by NF1 mutations can be targeted in NSCLC, this treatment approach could have relevance for patients with both adenocarcinoma and squamous tumors.

In our cohort, the frequency of KRAS mutations is generally consistent with published reports (31), whereas our observed frequency of NF1 mutations is congruent with TCGA data (13, 14). Importantly, we demonstrated that, although there is some overlap between tumors harboring both mutations, the NF1 cohort is at least partially independent, with 75% of mutations (45/60) occurring independently from other oncogenic alterations. Notably, the institutional database used to identify the NF1 and KRAS cohorts described here is biased against including EGFR mutant tumors because many of these patients would have undergone rapid PCR-based testing for clinical decision-making, thus it may be important to reconsider NF1 variants specifically in the setting of concurrent EGFR mutations (32). However, in light of the well-characterized role of NF1 in regulating Ras signaling through the MAPK–ERK pathway, it is not surprising that all of the concurrent driver mutations identified with NF1 mutations in this cohort also represent genes involved with MAPK/ERK signaling (KRAS, HRAS, NRAS, BRAF, and ERBB2).

The specific mutations affecting NF1 were found to be highly variable and distributed throughout the genome, as expected for mutations in a tumor suppressor. Genomic prediction programs were used to evaluate each individual mutation, with the majority of identified variants predicted to have damaging effects. Some of the variants identified in our cohort do correspond to previously reported SNPs at the same NF1 codon, but this represents a minority of the 73 variants in our cohort. A similar pattern of NF1 mutations was also seen in the tumors containing a concurrent oncogenic alteration. However, in the absence of paired germline assessment, it is impossible to completely exclude variants that represent germline inheritance instead of somatic changes in the tumor. Furthermore, the analysis reported here does not include the functional validation of NF1 mutations that will be an important next step in preclinical evaluation. However, it is notable that the majority of the variants identified in this NSCLC cohort have not been previously reported as germline variants or functionally characterized.

In comparison with patients with KRAS-mutant tumors, several clinical similarities were noted in the NF1 cohort, a finding that is not surprising, given the functional activation of downstream Ras signaling that would be anticipated in both populations. However, there was a clear difference seen in the histopathology observed in each cohort, with NF1-mutant tumors displaying greater histologic diversity. Further distinctions between the two cohorts were identified in an evaluation of concurrent tumor-suppressor mutations. Mutations in TP53 and LKB1 have previously been identified in KRAS-mutant tumors (33), with some suggestion that prognostic outcome in these tumors, particularly the success of MEK inhibitors, can be influenced by whether or not a tumor has intact LKB1 signaling (33, 34). Notably, in the NF1 cohort, both TP53 and LKB1 mutations were identified. However, when compared with the KRAS tumors, a statistically significant increase in TP53 mutations was noted without a significant difference in the frequency of LKB1 mutations. In light of prior studies evaluating LKB1 in KRAS-mutant tumors, this finding provides some suggestion that NF1 tumors may be enriched in a population that is more likely to respond to targeted therapies acting downstream of Ras activation. Interestingly, the MPNST that develop in neurofibromatosis patients are also frequently found to have concurrent mutations in TP53, whereas mutations in LKB1 have not been widely reported (35). Overall, these findings continue to emphasize that NSCLC tumors defined by NF1 mutation share many overlapping features and characteristics with KRAS-mutant tumors, but are also a tumor cohort with some unique distinctions that may be exploitable for therapeutic benefit.

Intriguingly, recent clinical trials in neurofibromatosis patients support the potential efficacy of a targeted therapy approach in tumors driven by NF1 mutations. Preclinical studies of neurofibromas in a genetically engineered murine model of neurofibromatosis have shown that tumor size can be controlled with the MEK inhibitor PD-0325901 (36). Similar findings have also been observed in xenograft models of...
human MPNSTs (37). Interestingly, in animal models of NF1 deficiency with a myeloproliferative disorder similar to the juvenile myelomonocytic leukemia seen in pediatric neurofibromatosis patients, treatment with PD-0325901 also demonstrated improvement in clinical measures (38). A phase I trial evaluating the MEK inhibitor selumetinib in pediatric patients with inoperable plexiform neurofibromas resulted in all 11 patients having some response on restaging exam (39), and a phase II trial evaluating PD-0325901 in these patients is ongoing (Clinical Trials identifier: NCT02096471). Notably, NF1 mutations have also been noted in melanoma, with suggestion of dependence upon MEK signaling (40, 41), although the potential applications in this clinical setting remain under investigation (42). The safe use of MEK inhibitors in NSCLC patients has been previously established in patients with BRAF or KRAS mutations (27, 43), and it remains to be determined whether MEK inhibitors may also be effective in NSCLC harboring NF1 mutations. Preclinical studies in animal models of neurofibromatosis have also identified a role for the mTOR pathway in facilitating the growth of NF1-deficient tumors. TORC1 is an essential component of tumorogenesis in neurofibromatosis-associated malignancies (44), and mouse models of NF1-deficient tumors are sensitive to treatment with an Hsp90 inhibitor combined with the mTOR inhibitor rapamycin (45). Preclinical studies have revealed that combination MEK and mTORC1 inhibition is required for optimal growth inhibition in human MPNST cell lines and a genetically engineered mouse model of MPNST (46). Moving forward, it is possible that in the right genetically defined subset of tumors with an NF1 mutation, dual inhibition of mTOR signaling along with inhibition of a parallel pathway may lead to greater clinical efficacy.

The complex and overlapping milieu of Ras signaling pathways suggests that effective downstream targeting in lung cancer may depend upon identifying subpopulations of patients who are sensitive to inhibition of one pathway over another. Multiple lines of evidence indicate that variables as divergent as specific exon/base-pair KRAS mutation (47) or co-existing mutations in key tumor-suppressor genes (21, 48, 49) may help shape the biologic heterogeneity of tumors with a KRAS mutation. Although KRAS has historically been considered an "undruggable" target (50), novel approaches to silencing Ras-driven pathways are currently ongoing (43). The biologic function of NF1 suggests that the same targeting strategies under ongoing evaluation in KRAS tumors may also have efficacy in NF1-mutant tumors. Our findings identify the clinical and molecular characteristics of a new cohort of NSCLC defined by NF1 mutation and reveal both similarities and differences with tumors characterized by KRAS mutation. These findings establish a role for further preclinical studies to validate the functional activity of NF1 mutations in NSCLC and to investigate the efficacy of targeted inhibition of downstream Ras signaling pathways in such tumors.

Disclosure of Potential Conflicts of Interest

P.A. Janne has ownership interest (including patents) in Gatekeeper Pharmaceuticals, is a consultant/advisory board member for AstraZeneca, Chuang, Pfizer and Roche, reports receiving commercial research grants from Astellas and AstraZeneca, and receives post-marketing royalties from DFCI owned intellectual property on EGFR mutations licensed to Lab Corp. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A.J. Redig, M. Capelletti, P.A. Janne
Development of methodology: A.J. Redig
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.J. Redig, M. Capelletti, S. Mach, P.A. Janne
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