Comprehensive Molecular and Clinicopathologic Analysis of 200 Pulmonary Invasive Mucinous Adenocarcinomas Identifies Distinct Characteristics of Molecular Subtypes

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Authors
Comprehensive Molecular and Clinicopathologic Analysis of 200 Pulmonary Invasive Mucinous Adenocarcinomas Identifies Distinct Characteristics of Molecular Subtypes

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ABSTRACT

Purpose: Invasive mucinous adenocarcinoma (IMA) is a unique subtype of lung adenocarcinoma, characterized genonomically by frequent KRAS mutations or specific gene fusions, most commonly involving NRG1. Comprehensive analysis of a large series of IMAs using broad DNA- and RNA-sequencing methods is still lacking, and it remains unclear whether molecular subtypes of IMA differ clinicopathologically.

Experimental Design: A total of 200 IMAs were analyzed by 410-gene DNA next-generation sequencing (MSK-IMPACT; n = 136) or hotspot 8-oncogene genotyping (n = 64). Driver-negative cases were further analyzed by 62-gene RNA sequencing (MSK-Fusion) and those lacking fusions were further tested by whole-exome sequencing and whole-transcriptome sequencing (WTS).

Results: Combined MSK-IMPACT and MSK-Fusion testing identified mutually exclusive driver alterations in 96% of IMAs, including KRAS mutations (76%), NRG1 fusions (7%), ERBB2 alterations (6%), and other less common events. In addition, WTS identified a novel NRG2 fusion (F11R–NRG2). Overall, targetable gene fusions were identified in 51% of KRAS wild-type IMAs, leading to durable responses to targeted therapy in some patients. Compared with KRAS-mutant IMAs, NRG1-rearranged tumors exhibited several more aggressive characteristics, including worse recurrence-free survival (P < 0.0001).

Conclusions: This is the largest molecular study of IMAs to date, where we demonstrate the presence of a major oncogenic driver in nearly all cases. This study is the first to document more aggressive characteristics of NRG1-rearranged IMAs, ERBB2 as the third most common alteration, and a novel NRG2 fusion in these tumors. Comprehensive molecular testing of KRAS wild-type IMAs that includes fusion testing is essential, given the high prevalence of alterations with established and investigational targeted therapies in this subset.

Introduction

Invasive mucinous adenocarcinoma (IMA), formerly known as mucinous bronchioalveolar carcinoma, is a unique subtype of primary lung adenocarcinoma, comprising approximately 3–5% of adenocarcinomas overall (1, 2), with distinct clinical, radiologic, histopathologic, and molecular features. Clinically and radiologically, patients with IMAs commonly present with multifocal and multilobar consolidation, frequently raising the differential diagnosis of pneumonia at the initial presentation (1, 3, 4). Histologically, IMAs are characterized by columnar or goblet-cell morphology with abundant apical intracytoplasmic mucin, basally oriented nuclei and frequent skip lesions (1, 2). Genomically, prior studies showed that KRAS mutations are reported in 50–70% of IMAs (3, 5–8). In those lacking KRAS mutations, recent studies revealed the presence of oncogenic fusions in a subset of cases, most commonly involving NRG1 (5, 6, 9). However, despite further attempts at interrogating KRAS wild-type cases using whole-transcriptome sequencing (WTS; ref. 5) or anchored multiplex PCR (6), previous studies failed to reveal a mitogenic driver alteration in the majority of such cases. Notably, the early studies frequently used molecular techniques with limited analytical sensitivity for KRAS mutation detection (3, 7, 8), and more recent studies using next-generation sequencing (NGS) techniques only examined mutations and fusions, but not copy-number alterations (5, 6, 10).

Because prior studies have shown limited survival benefits in patients with IMAs treated with conventional chemotherapy (11, 12),...
Translational Relevance
Pulmonary invasive mucinous adenocarcinoma (IMA) is a unique subtype of lung adenocarcinoma, characterized by distinct clinicopathologic and genomic features. This is the largest study to date to use comprehensive DNA- and RNA-based next-generation sequencing to systematically examine the molecular landscape of IMAs. This approach led to identification of an oncogenic driver alteration in nearly all cases. Notably, among KRAS wild-type IMAs, NRG1 and other fusions were identified in over half of the cases and led to durable responses to targeted therapies in some patients. We also describe the distribution of other mutually exclusive and potentially targetable alterations in IMAs and identify distinct histologic and clinical features of molecular subsets. Given the ineffectiveness of traditional cytotoxic approaches and high prevalence of alterations with established or investigational targeted therapies among KRAS wild-type IMAs, comprehensive DNA and RNA testing should be considered in such tumors to allow for genome-directed therapies tailored to individual patients.

Materials and Methods
Sample selection and study design
The study was performed with the approval of Institutional Review Board of Memorial Sloan Kettering Cancer Center (MSKCC). Inclusion criteria for the patient cohort were pathologic diagnosis of IMA between 2009 and 2019 for which the patients consented to molecular testing. As summarized in a consort diagram (Supplementary Fig. S1), the study included cases analyzed by two strategies. In the first cohort (diagnosed between 2009 and 2014), we identified 64 IMAs that had undergone hotspot mutation testing by Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS; ref 13). Cases that were negative for KRAS mutations by MALDI-TOF MS underwent high-sensitivity Sanger sequencing with locked nucleic acid (LNA) PCR clamping for enhanced detection of KRAS mutations, as previously described (13, 14). KRAS wild-type cases were further tested by targeted RNA sequencing (MSK-Fusion) for detection of transcript fusions. In the second cohort (diagnosed between 2014 and 2019), we identified 136 IMAs that underwent targeted DNA sequencing by Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) platform. Cases lacking mitogenic driver alterations by MSK-IMPACT were subsequently undergo MSK-Fusion testing. Cases lacking driver alterations after MSK-IMPACT and MSK-Fusion underwent further investigational testing by WES and WTS, as detailed below. Patients with insufficient tumor specimen for complete molecular analysis were excluded.

Hotspot mutation testing by MALDI-TOF MS
Samples were tested in duplicate using a series of multiplexed assays designed to detect 92 hotspot mutations in eight genes: EGFR, KRAS, BRAF, PIK3CA, NRAS, AKT1, ERBB2, and MAP2K1 (Supplementary Table S1). Genomic DNA amplification and single base pair extension steps were performed using specific primers designed with the Sequenom Assay Designer v3.1 software (Agena BioScience). The allelespecific single base extension products were quantitatively analyzed using MALDI-TOF MS on the Sequenom Mass Array Spectrometer.

Targeted DNA sequencing by MSK-IMPACT
Broad-panel targeted NGS of patient-matched tumor/normal samples was performed using the MSK-IMPACT assay, the methodology of which has been previously described (15). In brief, the MSK-IMPACT assay is a custom hybridization capture-based platform that sequences the entire coding region and select noncoding regions of 410 (v4) or 468 (v5) genes (full list in Supplementary Table S2) and identifies single-nucleotide variants, small indels, copy-number alterations, and selected structural rearrangements. Germline variants were bioinformatically filtered out based on the matched germline DNA. Manual review of KRAS codons 12, 13, and 61 for mutations below the variant allele frequency (VAF) threshold for calling was performed in cases lacking a driver alteration using Integrated Genomics Viewer (16).

Targeted RNA sequencing by MSK-Fusion
For anchored multiplex RNA sequencing (ArcherDx), the detailed procedure has been previously described (17). Unidirectional gene-specific primers were designed to target specific exons in 62 genes known to be involved in oncogenic fusions in solid tumors (Supplementary Table S3). RNA was extracted from FFPE, followed by complementary DNA synthesis and library preparation. Each RNA sample was tested using the Archer PreSeq RNA QC Assay, a qPCR-based method to assess RNA quality, before library preparation and sequencing. Three samples had QC values >28, indicating low-quality RNA, and the samples were deemed insufficient for testing and were excluded from the study. Anchored multiplex PCR amplicons were sequenced on an Illumina Miseq sequencer (Illumina), and the data were analyzed using Archer software (ArcherDx).

WES and analysis
Tumor samples lacking a mitogenic driver alteration by MSK-IMPACT and MSK-Fusion underwent WES with matched normal control. For details, please see Supplementary Method S1.

WTS and analysis
Tumor samples lacking a mitogenic driver alteration by MSK-IMPACT and MSK-Fusion were also analyzed by WTS. For details, please see Supplementary Method S2.

Clinical and histologic review
Electronic medical records were reviewed to retrieve relevant clinical data, including patient demographics, smoking and treatment history, and survival outcomes. The primary tumor size was measured pathologically in resected tumors and radiologically in unresectable tumors. Staging was performed according to the American Joint Committee on Cancer 8th edition.

The histologic slides from 200 IMAs were reviewed by two thoracic pathologists (J.C. Chang and N. Rekhtman) using the diagnostic criteria of the 2015 World Health Organization classification. Tumors were classified as pure IMA (entirely mucinous) or mixed IMAs (containing both mucinous and >10% of non-mucinous components).
The presence of tumor necrosis and stromal invasion, defined by stromal desmoplasia surrounding invasive glands or nests of tumor cells, were recorded.

Statistical analysis
Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software). P values were computed using χ² test and Student t test for categorical and continuous variables, respectively. Overall survival (OS) and recurrence-free survival (RFS) were calculated using the Kaplan–Meier approach from the time of procedure to the time of death and disease recurrence, respectively. Patients were otherwise censored at the time of last clinical follow-up. Survival curves were compared using the log-rank tests. The threshold for statistical significance was set at a P value of <0.05.

Results
Patient demographics
Baseline patient demographics are summarized in Supplementary Table S4. The median age was 68 years (range, 27–92); 61% of patients were women, 33% were never-smokers, and 28% were light smokers (≤15 pack years). The median smoking history was 8 pack years (range, 0–154). Seventy-nine percent of specimens were resections and 21% were biopsies. The baseline clinical stage distribution was as follows: Stage I 53%, Stage II 16%, Stage III 15%, and Stage IV 16%. On histologic review, 83% of IMAs were pure and 17% were mixed.

Distribution of driver alterations
In the initial cohort of tumors (n = 64) analyzed by MALDI-TOF MS for eight major oncogenes, KRAS mutations were identified in 47 cases (73%). In five cases, KRAS mutations were initially missed by MALDI-TOF MS, but detected by high-sensitivity Sanger sequencing with LNA probes. Subsequent MSK-Fusion on 17 KRAS wild-type IMAs identified fusion drivers in eight (47%) cases (Fig. 1A). Overall, driver alterations were identified in 86% of cases in this cohort.

The subsequent cohort of tumors (n = 136) analyzed by 410-gene MSK-IMPACT followed by MSK-Fusion on cases with unknown mitogenic driver revealed the following mutually exclusive driver alterations: 104 (76%) KRAS mutations, 16 (12%) fusions, and 11 (8%) other driver alterations (Fig. 1B and C; Supplementary Table S5). Overall, driver alterations were detected in 96% of cases in this cohort, with only five cases (4%) remaining with unknown mitogenic driver after combined MSK-IMPACT and MSK-Fusion testing.

Overall genomic profile of IMAs by MSK-IMPACT
MSK-IMPACT identified an average of 4 mutations (range, 0–15), 0.7 copy-number alterations (range, 0–8), and 0.1 rearrangements (range, 0–1) per case. The mean tumor mutation burden (TMB) was 3.6 mutations per megabase (mt/Mb), which is significantly lower than the mean TMB of 7.4 mt/Mb for patients with non-mucinous lung adenocarcinoma in the MSK-IMPACT database (P = 0.0001; refs. 18, 19). The mean depth of coverage of tumor DNA was 710x (range, 281–1,412).

Distribution of KRAS mutations
A total of 151 KRAS-mutant IMAs were identified (104 by MSK-IMPACT, 42 by MALDI-TOF MS, and five by Sanger sequencing with LNA probes), comprising most commonly G12D (36%), G12V (32%), and G12C (12%) variants (Fig. 1D). Overall, transition mutations (G12D, G12S, and G13D) accounted for 40% of all KRAS variants.

Distribution of gene fusions
Among all tumors tested, gene fusions were identified in a total of 24 IMAs, including 12 (50%) with NRG1, 6 (25%) with ALK, 2 (8%) with ROS1, and 1 each with ERBB2, NTRK1, FGFR2, and FGFR3 (Fig. 1A and B). Overall, fusions represented the driver alteration in 24/49 of KRAS wild-type IMAs (Fig. 1A and B). Among 16 fusions in the MSK-IMPACT cohort, six cases were only detected by MSK-Fusion; all of these cases involved NRG1, with false negatives likely due to the panel design as NRG1 introns are not captured by MSK-IMPACT due to their large size. All fusions detected by MSK-IMPACT were confirmed by an orthogonal method (MSK-Fusion, FISH, and/or IHC; Supplementary Table S6).

The most common gene partner for NRG1 fusions involved CD74 (n = 6), followed by SLC3A2 (n = 2), SDC4 (n = 2), YAMP2 (n = 1), and FI1R (n = 1). All of these genes encode a cell surface protein leading to membrane localization of the fusion protein (Fig. 2D). The partner gene for ALK (n = 6) involved EML4 in five cases and PLEKHH2 in one case.

ERBB2 and other putative driver alterations
Among cases analyzed by MSK-IMPACT, 11 tumors harbored established or putative oncogenic driver alterations that were non-KRAS and non-fusion type, which accounted for 34% (11/32) KRAS wild-type IMAs. This included ERBB2 insertion mutations (n = 4), ERBB2 amplifications (n = 3), BRAF mutations (n = 3), and ERBB3 mutations (n = 1). Overall, ERBB2 alterations (insertions and amplifications) accounted for 22% of KRAS wild-type IMAs. All four ERBB2 insertions were in-frame, including exon 20 Y772_A775dup (AYVM insertion) in-frame insertions involving the kinase domain in three cases, and an exon 17 V658_V659insR insertion involving the transmembrane domain in the fourth case. All three cases with ERBB2 amplifications represented high-level gene amplifications ranging from 6.3- to 40.2-fold changes (Fig. 3E; ref. 20). The three BRAF mutations consisted of V600E, K483E, and G469A variants, all of which are predicted to represent pathogenic variants (21). Finally, one IMA harbored concurrent ERBB3 G284R and D581N mutations, and both variants are suggested to represent oncogenic mutations (22).

Other recurrent genetic alterations
Other commonly altered non-driver genes in IMAs tested by MSK-IMPACT included NXX2-1 (n = 33, 24%), CDKN2A (n = 32, 24%), STK11 (n = 20, 15%), TP53 (n = 18, 13%), GNAS (n = 14, 10%), and SMAD4 (n = 6, 4%; Fig. 1B). All NXX2-1 mutations were truncating.

As summarized in Supplementary Table S7, comparison of the distribution of concurrent non-driver alterations in KRAS-mutant versus NRG1-rearranged IMAs was similar, although there was a trend for lower number of concurrent mutations in NRG1-rearranged tumors. Likewise, NRG1-rearranged tumors harbored lower TMB than other IMAs (1.9 vs. 4.5 mt/Mb, P = 0.016 for NRG1 vs. KRAS, respectively).

WES and WTS on tumors lacking a mitogenic driver
Five IMAs remained with unknown mitogenic driver after MSK-IMPACT and MSK-Fusion testing. These cases were further interrogated by WES and WTS. WES did not identify any additional pathogenic alterations (Supplementary Table S8). Conversely, WTS revealed an in-frame FI1R-NRG2 fusion in one case (Fig. 2E and F; Supplementary Fig. S2), and a STK11 out-of-frame fusion in another case (Supplementary Tables S9 and S10).
Comparison of clinicopathologic features between molecular subsets

As summarized in Table 1, comparison of clinicopathologic characteristics between NRG1-rearranged and KRAS-mutant IMAs revealed that NRG1 fusions were associated with lower cigarette exposure (mean 5.9 vs. 20 pack years, $P = 0.040$). Furthermore, compared with KRAS mutations, NRG1 fusions were associated with significantly larger primary tumor size (mean 7.7 vs. 3.9 cm, $P = 0.0004$) and significantly higher rate of metastasis overall ($P = 0.016$), particularly extrathoracic metastasis (50% vs. 5%, $P = 0.0006$). Furthermore, survival analysis revealed that compared with KRAS mutations, NRG1 fusions were associated with significantly worse OS in the entire cohort of patients ($P = 0.014$), and significantly worse RFS among patients with surgically resected...
tumors \( (P < 0.0001; \text{Fig. 4}) \). Median follow-up was 2.2 years (range, 0.02–16.3 years).

The heterogeneity of ERBB2 alterations precluded a comparison of survival outcomes based on this aggregated group. However, more aggressive behavior of this subset was supported by the observation that all 3 patients with ERBB2-amplified tumors had intrathoracic metastases and died within 1.7–42 months from diagnosis. Likewise, 2 of 4 patients with ERBB2 insertions had intrathoracic metastases. The clinicopathologic features and survival outcomes of ERBB2-altered cases are summarized in Supplementary Table S11.

Analysis of survival characteristics associated with concurrent genomic alterations revealed poorer outcome associated with TP53 and CDKN2A alterations (Supplementary Figs. S3 and S4); multivariable analysis was precluded by small number of patients in molecular subgroups.

Similar to most patients with NRG1-rearranged tumors, the single patient with NRG2-rearranged IMA was a never-smoker who developed contralateral intrapulmonary metastasis 5 months after initial diagnosis.

**Comparison of histologic features between molecular subsets**

Histologically, the prevalence of pure IMA histology was comparable for NRG1-rearranged versus KRAS-mutant tumors (88% vs. 83%, respectively). However, the presence of aggressive histologic features, including tumor necrosis (Fig. 2B) and/or desmoplastic stromal invasion (Fig. 2C), was more frequently seen in tumors with NRG1 fusions compared with KRAS mutations (92% vs. 54%, respectively; \( P = 0.012; \text{Table 1} \)). Similarly, all cases with ERBB2 alterations showed pure IMA morphology (Fig. 3). In contrast, all cases with ALK fusions showed mixed histology.

**Treatment response to targeted therapy**

Nine of 30 patients with potentially targetable alterations received targeted therapy; the type of targeted therapy and the best treatment
response achieved are listed in Fig. 5. Of the 5 patients with NRG1 fusions, 1 showed partial response to anti-ERBB3 mAb (GSK2849330), the details of which were previously reported (23); the remaining 4 patients were treated with afatinib, showing stable disease in 1 and progressive disease in 3. We also reviewed histologic features of 49 patients with ERBB2-altered adenocarcinomas in the previously published trial of ERBB2-directed therapies (24), and found that one of them was an IMA with ERBB2 amplification from the current study. The patient showed a lasting complete response to ado-trastuzumab emtansine.

Discussion

In this study, we have confirmed and significantly expanded on prior observations that IMAs exhibit unique genomic profiles among lung carcinomas. By a combination of sequential and complementary DNA and RNA testing techniques, we have found that mutually exclusive driver alterations can be identified in the vast majority of IMAs, including KRAS mutations (76%), NRG1 fusions (7% of cases overall and 28% of KRAS wild-type tumors), and ERBB2 alterations (6% of cases overall and 25% of KRAS wild-type tumors). In addition, we identify a novel NRG2 fusion in IMAs by performing WTS on tumors lacking a mitogenic driver by standard clinical methods. The first major novel finding in this study is that NRG1-rearranged (NRG1+) IMAs showed distinct clinicopathologic characteristics. The two largest genomic studies on IMAs to date by Nakaoku and colleagues (5) and Shim and colleagues (6) each found NRG1 fusions to account for 7% of driver alterations in predominantly Asian patient cohorts. However, the clinicopathologic characteristics of NRG1+ IMAs were not addressed in detail by these studies. To our knowledge,
this study is the largest one to date to characterize the genomic landscape of IMAs. We found that NRG1 fusions did not differ in prevalence across ethnicities, as they also accounted for 7% of driver alterations of IMAs in our predominantly Caucasian patient cohort. Although patients with NRG1þ tumors exhibited classic IMA morphology, this study reveals that NRG1 fusions were associated with several distinct characteristics. First, although IMAs in general are enriched in never/light smokers compared with non-mucinous adenocarcinomas (25), patients with NRG1þ tumors showed even lower exposure to cigarette smoking compared with those with KRASþ tumors. Second, NRG1þ tumors exhibited several more aggressive pathologic characteristics compared with KRASþ tumors, including significantly larger primary tumor size and more aggressive histology in the form of either desmoplastic stromal invasion or tumor necrosis. The presence of these histologic features has been found to correlate with worse prognosis in IMAs in a recently published study (26). Finally, NRG1þ tumors had more aggressive clinical behavior manifesting as significantly more frequent extrathoracic metastases and worse OS and RFS than KRASþ tumors. However, the survival analysis is limited by the differences in baseline stage and the relatively small number of NRG1þ tumors. Nevertheless, this is the first study to document more aggressive histologic and clinical characteristics of IMAs with NRG1 fusions.

NRG1 fusions lead to the expression of chimeric transmembrane proteins, resulting in ERBB3 activation, heterodimerization with ERBB2, and upregulation of the PIK3–AKT signaling pathway (5, 6, 23). In addition to being a major class of driver alterations in IMAs, NRG1 fusions have also been described in approximately 0.2% of solid tumors overall, including pancreatic, gallbladder, renal, bladder, ovarian, breast, and colorectal cancers (27). The interest in identifying NRG1 fusions in lung and other tumors is driven by the recent identification of novel therapeutic strategies involving ERBB3-directed therapy that have shown durable responses in patients with advanced tumors harboring this alteration (23, 28, 29). In line with these data, one of the patients with NRG1 fusions in our study demonstrated sustained clinical responses to ERBB3-directed therapies, supporting specific testing strategies to identify them.

The second major finding is the identification of a novel F11R–NRG2 fusion in an IMA lacking other mitogenic drivers. To our knowledge, this is the third reported case of lung cancer with an NRG2 fusion (30, 31), of which one had the identical F11R partner gene (31).

Table 1. Clinicopathologic comparison of IMAs with KRAS mutations, NRG1 fusions, and other alterations.

<table>
<thead>
<tr>
<th></th>
<th>KRAS N = 104</th>
<th>NRG1 N = 12</th>
<th>Other N = 28</th>
<th>P value for KRAS+ vs. NRG1+</th>
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<td>Age at diagnosis (y)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>37–84</td>
<td>34–89</td>
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<tr>
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<td>69</td>
<td>62</td>
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</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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<tr>
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<td>71 (68%)</td>
<td>6 (50%)</td>
<td>14 (50%)</td>
<td>0.21</td>
</tr>
<tr>
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<td>33 (32%)</td>
<td>6 (50%)</td>
<td>14 (50%)</td>
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</tr>
<tr>
<td>Smoking status</td>
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<tr>
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<td>2 (17%)</td>
<td>6 (21%)</td>
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<tr>
<td>Never</td>
<td>25 (24%)</td>
<td>6 (50%)</td>
<td>14 (50%)</td>
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<tr>
<td>Pack years</td>
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<tr>
<td>I</td>
<td>53 (51%)</td>
<td>2 (17%)</td>
<td>11 (39%)</td>
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<td>23 (22%)</td>
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<td>92 (88%)</td>
<td>10 (83%)</td>
<td>18 (64%)</td>
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</tr>
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<td>12 (12%)</td>
<td>2 (17%)</td>
<td>10 (36%)</td>
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<tr>
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<td>33 (32%)</td>
<td>8 (67%)</td>
<td>17 (61%)</td>
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<td>No</td>
<td>71 (68%)</td>
<td>4 (33%)</td>
<td>11 (39%)</td>
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<tr>
<td>Site of metastasis</td>
<td></td>
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<td></td>
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<tr>
<td>Intrathoracic only</td>
<td>28 (27%)</td>
<td>2 (17%)</td>
<td>12 (43%)</td>
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<td>Intrathoracic + extrathoracic</td>
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<td>6 (50%)</td>
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<td>Histologic type</td>
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<tr>
<td>Pure IMA</td>
<td>92 (88%)</td>
<td>10 (83%)</td>
<td>13 (46%)</td>
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<tr>
<td>Mixed IMA</td>
<td>12 (12%)</td>
<td>2 (17%)</td>
<td>15 (54%)</td>
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<td>Primary tumor size, cm</td>
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<td>Mean</td>
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<td>Aggressive histologic features</td>
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<tr>
<td>Present</td>
<td>56 (54%)</td>
<td>11 (92%)</td>
<td>17 (61%)</td>
<td>0.012</td>
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<td>Absent</td>
<td>48 (46%)</td>
<td>1 (8%)</td>
<td>11 (39%)</td>
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Figure 4. Comparison of overall survival (A) and recurrence-free survival (B) for IMAs with \textit{KRAS} mutations, \textit{NRG1} fusions, and other driver alterations. The \textit{P} value shown is for three-way comparison. The \textit{P} value for \textit{KRAS} versus \textit{NRG1} is 0.014 (overall survival) and <0.0001 (recurrence-free survival).

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Matched therapy</th>
<th>Best response</th>
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<td>\textit{NRG1}</td>
<td>Alectinib</td>
<td>SD</td>
</tr>
<tr>
<td>\textit{ALK}</td>
<td>Crizotinib</td>
<td>PR</td>
</tr>
<tr>
<td>\textit{CD74-NRG1}</td>
<td>Afatinib</td>
<td>SD</td>
</tr>
<tr>
<td>\textit{SDC4-NRG1}</td>
<td>Afatinib</td>
<td>PD</td>
</tr>
<tr>
<td>\textit{CD74-NRG1}</td>
<td>Afatinib</td>
<td>PD</td>
</tr>
<tr>
<td>\textit{SLC3A2-NRG1}</td>
<td>Afatinib</td>
<td>PD</td>
</tr>
<tr>
<td>\textit{CD74-NRG1}</td>
<td>GSK2849330</td>
<td>PR</td>
</tr>
<tr>
<td>\textit{CD74-ROS1}</td>
<td>Crizotinib</td>
<td>PD</td>
</tr>
<tr>
<td>\textit{ERBB2}</td>
<td>Ado-trastuzumab</td>
<td>CR</td>
</tr>
</tbody>
</table>

Figure 5. Pie chart summarizing the major classes of targetable alterations in IMAs (A) and the best clinical response achieved in patients matched to targeted therapies (B). CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.
The NRG2 gene encodes a protein that is a homologue of NRG1, which also activates ERBB2/ERBB3 (32). Thus, NRG2 fusions may be amenable to targeted therapies similar to NR1 fusions; however, this remains to be confirmed empirically or in experimental models.

We confirmed and further expanded on the spectrum and prevalence of ERBB2 alterations in IMAs, which represented a recurrent driver in 25% of KRAS wild-type IMAs, comprising exon 17 or exon 20 insertion mutations (predominantly Y772_A775dup), amplifications, and fusion. The prior study by Shim and colleagues was the first to describe ERBB2 Y772_A775dup in two IMAs, in line with our findings. However, to our knowledge, our study was the first to expand the spectrum of ERBB2 insertions and to identify other types of ERBB2 alterations (amplifications, fusion) in IMAs, thus establishing ERBB2 alterations as the third most common putative oncogenic driver in IMAs, following KRAS and NRG1. Prior studies support that ERBB2 insertions encountered in IMAs are oncogenic based on molecular modeling (33, 34). The role of ERBB2 amplification as an oncogenic driver in lung adenocarcinoma is less well established, but is supported by prior studies (35, 36). A previous study from our institution estimated that ERBB2 insertions and amplifications each accounted for approximately 2% of the driver alterations in lung adenocarcinoma overall, with ERBB2 exon 20 insertion Y772_A775dup being the most common (33). We retrospectively reviewed lung adenocarcinomas with ERBB2 insertions and amplifications, and confirmed that they occurred predominantly in conventional, non-mucinous adenocarcinomas (data not shown). Thus, unlike the strong predilection of NRG1 fusions for IMAs, ERBB2 alterations are more widely distributed among lung adenocarcinomas. ERBB2 alterations and NRG1 fusions are both thought to lead to PI3K–AKT signaling pathway upregulation by increasing homodimerization of ERBB2 and heterodimerization with ERBB3, suggesting downstream signaling convergence. Recently, anti–HER2 therapy has emerged as potential therapeutic agents in lung carcinomas harboring ERBB2 mutations or amplifications (24, 37, 38). Histologic re-review of patients in the trials revealed that one of the tumors was a classic IMA, and the patient showed a striking sustained complete response to anti–HER2 therapy (24). Similarly, the interim results from the DESTINY-Lung01 trial targeting ERBB2 alterations indicate promising clinical activity of anti–HER2 therapy (38). Thus, comprehensive molecular testing encompassing various types of ERBB2 alterations may be warranted in KRAS wild-type IMAs.

Although previous studies documented increased likelihood of finding a fusion driver in KRAS wild-type IMAs, our study confirmed and further expanded on the prevalence and spectrum of fusion alterations in this subset. In our study, fusions accounted for the driver alterations in 51% of KRAS wild-type IMAs, highlighting the utility of incorporating fusion detection in the testing algorithm of IMAs. A recent study from our institution demonstrated that MSK-Fusion identified undetected fusion in 14% of lung adenocarcinomas found to be driver-negative by MSK-IMPACT (39), prompting a recommendation that fusion testing should be considered for all driver-negative lung adenocarcinomas. This recommendation is thus particularly relevant for IMAs. Given that KRAS mutations represent the majority of driver alterations in IMAs, for laboratories that do not use upfront comprehensive NGS testing, a high-sensitivity KRAS assay may be the appropriate screening test of choice in this tumor type, followed by fusion testing in cases lacking KRAS mutations.

Similar to previous studies (5, 6), we found that beyond NRG1, the remainder of fusions in IMAs commonly involved one of the receptor tyrosine kinase genes, namely, ALK, ROS1, and NTRK1. All three fusion genes represent molecular alterations targetable by FDA-approved tyrosine kinase inhibitor therapies. We also described fusions involving FGFR2 and FGFR3 in two IMAs—a novel finding for this tumor type. These fusions have been observed in many solid tumors, and rarely in NSCLCs, most commonly squamous cell carcinomas; however, this is the first report of these fusions in IMAs. FGFR inhibitors have been recently approved for use in cholangiocarcinomas; the description of FGFR2/3 fusions further expanded the list of potentially targetable alterations in IMAs.

Prior studies from our institution have shown that despite similar comprehensive DNA and RNA interrogation, a substantial subset (12%) of conventional lung adenocarcinomas lack a major oncogenic driver (40). Conversely, by similar approaches, a major oncogenic driver is identifiable in the vast majority (97%) of IMAs, emphasizing the unique biology of this ubiquitously driver-associated tumor type. Several factors may underlie the higher prevalence of drivers in our series compared with prior NGS studies of IMAs showing up to 24% of cases without a driver (5, 6, 10). First, the high analytical sensitivity of KRAS assays used in this study minimized the chances of false-negative calls, which are frequently encountered in IMAs due to the abundance of mucin and admixed inflammatory cells in this tumor type. This issue was directly observed in this study for five of 47 tumors that initially tested negative for KRAS mutations by MALDI-TOF MS, but were subsequently found to be positive for KRAS mutations by the more sensitive method. Furthermore, the NGS assay used by the current study was able to detect hotspot mutations in KRAS down to 2% VAF. Using these high-sensitivity assays, 76% of IMAs harbored KRAS mutations in this cohort, higher than the previously reported prevalence of 50%–63% (7, 8). The second major reason for the low rate of cases with unknown drivers in this cohort is the broad NGS panel incorporating copy-number alteration and fusion detections, supplemented with dedicated fusion testing for all cases lacking a mitogenic driver. This systematic approach using our routine clinical sequencing platforms resulted in the detection of driver alterations in 96% of IMAs. Moreover, using WTS in the research setting detected an additional case with NRG2 fusion, increasing the overall driver prevalence rate to 97%.

Our findings expand on the prior observations that the molecular profile of IMAs shows close parallels with the genomic landscape of pancreaticobiliary adenocarcinomas. This includes the predominance of KRAS mutations, as well presence of recurrent alterations in ERBB2, GNAS, and SMAD4 in both tumor types. In addition, the predominance of KRAS G12D and G12V variants in IMAs mirrors the distribution of these variants in pancreatic and gastrointestinal adenocarcinomas (41). Conversely, this distribution contrasts sharply with non-mucinous lung adenocarcinomas in the Western population, where KRAS G12C represents the most common variant, accounting for approximately 40% of KRAS mutations (42), compared with only 12% in IMAs. Despite low prevalence, patients with IMAs harboring KRAS G12C mutations may be candidates for KRAS G12C inhibitor trials (43). Remarkably, recent studies have shown that KRAS wild-type pancreatic adenocarcinomas are also enriched in fusion genes, specifically those involving NRG1 (27–29).

In summary, our results uncover the high prevalence of mutually exclusive driver alterations in IMAs, comprising most commonly KRAS mutations, NRG1 fusions, and ERBB2 alterations. We show for the first time that NRG1+ tumors are associated with aggressive histologic features and worse clinical outcomes. We also identified a novel NRG2 fusion in this tumor type. As IMAs lacking a mitogenic...
driver account for a small minority of cases, comprehensive genomic profiling, including copy-number alteration and fusion detection, should be considered in KRAS wild-type IMAs to investigate for the presence of alternative driver events. The description of these alternative driver mechanisms in IMAs offers a rationale for targeted therapeutic strategies with approved and investigational agents for these tumors where traditional cytotoxic approaches are notoriously ineffective.

Authors’ Disclosures

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Authors’ Contributions

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