Mutational Profile from Targeted NGS Predicts Survival in LDCT Screening-Detected Lung Cancers

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Abstract

Background: The issue of overdiagnosis in low-dose computed tomography (LDCT) screening trials could be addressed by the development of complementary bio-markers able to improve detection of aggressive disease. The mutation profile of LDCT screening–detected lung tumors is currently unknown.

Methods: Targeted next-generation sequencing was performed on 94 LDCT screening–detected lung tumors. Associations with clinicopathologic features, survival, and the risk profile of a plasma microRNA signature classifier were analyzed.

Results: The mutational spectrum and frequency observed in screening series was similar to that reported in public data sets, although a larger number of tumors without mutations in driver genes was detected. The 5-year overall survival (OS) rates of patients with and without mutations in the tumors were 66% and 100%, respectively ($p = 0.015$). By combining the mutational status with the microRNA signature classifier risk profile, patients were stratified into three groups with 5-year OS rates ranging from 42% to 97% ($p < 0.0001$) and the prognostic value was significant after controlling for stage ($p = 0.02$).
Conclusion: Tumor mutational status along with a microRNA-based liquid biopsy can provide additional information in planning clinical follow-up in lung cancer LDCT screening programs.

Keywords
Low-dose computed tomography; Lung cancer; MicroRNA-based liquid biopsy; Mutational load; Next-generation sequencing

Introduction
Cigarette smoking remains the main cause of lung cancer occurrence and mortality in all countries. Lung cancer is typically asymptomatic in its early stages of development and it is often diagnosed at the metastatic phase, which is too late to enable successful treatment. Several screening trials have assessed the effectiveness of low-dose computed tomography (LDCT) for early detection of lung cancer in populations defined as high risk on the basis of age and smoking habits. The mortality reduction was around 1% per year in the LDCT arm of the National Lung Screening Trial when compared with the radiography control arm, but there was no benefit in the three smaller European studies in which an LDCT arm was compared with an observational control arm. Across studies, LDCT screening results in a high number of false positives and overdiagnosis, which has raised concern regarding the real cost-effectiveness of such methodology. Of interest, smoking habits during LDCT screening have shown a profound impact on the overall mortality of high-risk participants.

Many studies have already reported that minimally invasive molecular tests, such as microRNA (miRNA)-based liquid biopsies, can be used as complementary lung cancer diagnosis tools to improve LDCT effectiveness. In particular, we demonstrated that a plasma miRNA signature classifier (MSC) stratifying individuals undergoing lung cancer screening into three levels (high, intermediate, and low) predicted diagnosis and overall survival (OS). Moreover, we showed that the MSC test combined with LDCT reduced the false-positive rate from 19.4% to 3.7%. In the present study, to gain insight into the molecular portrait of LDCT screening–detected lung tumors, we performed targeted next-generation sequencing (NGS) of 94 LDCT screening–detected lung tumors resected from subjects participating in three screening trials conducted at our institution since 2000. We assessed whether the mutations in specific oncogenes and tumor suppressor genes present in the 94 LDCT screening–detected tumors were associated with specific clinicopathologic characteristics and the plasma MSC risk level.

Materials and Methods
Study Population
Since 2000 at our institution, three LDCT screening trials enrolling heavy-smoker (pack-years [PYs] ≥20) volunteers older than 50 years were performed: the National Cancer Institute (INT) - European Institute of Oncology (IEO) trial, the Multicentric Italian Lung
A consecutive series of 94 patients with LDCT screening–detected lung cancer with both plasma and tumor tissue samples available were selected for the present study. Both tissue and plasma specimens were collected at the time of surgery. For those patients with no available plasma samples at surgery, we considered the closest available plasma sample collected before surgery. The median follow-up considering alive patients was 3.5 years (inter-quartile range [IQR] 5.0). All samples were obtained according to the internal review and the ethics boards of the INT of Milan, and all patients provided informed consent.

**Plasma Sample Collection and RNA Extraction for MicroRNA Profiling**

Plasma samples were obtained by double centrifugation (at 1258 g at 4°C for 10 minutes) of 10 mL of whole blood collected in BD K3EDTA Vacutainer tubes (BD Biosciences, San Jose, CA). Plasma aliquots were stored at −80°C. The mirVana PARIS Kit (Thermo Fisher Scientific, Waltham, MA) was used to extract total RNA from 200 μL of plasma following the manufacturer’s protocol and eluted in 50 μL of buffer.

MiRNA expression was determined by using custom-made TaqMan MicroRNA microfluidic cards (Thermo Fisher Scientific) containing the 24 miRNAs of interest spotted in duplicates. Raw threshold cycle values of individual miRNAs were obtained by using ViiA 7 software (Thermo Fisher Scientific) with a threshold of 0.15 and an automatic baseline. The MSC algorithm was defined as previously described.18,21

**Characteristics of Tumor Tissue Samples**

NGS analysis was performed on 90 fresh frozen and four formalin-fixed, paraffin-embedded (FFPE) tumor samples available in the biobank at our institution. As reported in literature,22 no differences between the fresh frozen and FFPE samples were observed: the numbers of reads that passed the quality filters in FFPE and fresh frozen samples were similar, and the mean coverage was also comparable (>950x). Tumor histologic type, amount of tumor component, and mindbomb 3 ubiquitin protein ligase 1 (MIB-1) proliferation index were evaluated by a pathologist on available FFPE sections in 87 of 94 samples. For two samples, optimal cutting temperature compound-embedded sections were used instead of FFPE samples. The median amount of tumor component was 70%, with an IQR of 20. Neither FFPE nor optimal cutting temperature samples were available for the remaining five tumor samples.

**DNA Purification for NGS Analysis**

The DNA was extracted from whole tumor samples (not laser dissected) as described in the Supplementary Information. A total of 200 ng of DNA was shipped to Genewiz (South Plainfield, NJ) for targeted NGS analysis using the Ion AmpliSeq Cancer Hotspot Panel v.2 (Thermo Fisher Scientific). The panel consists of one primer pool of 207 amplicons covering 2855 known hotspot regions in 50 oncogenes and tumor suppressor genes.

**NGS Data Processing**

For the association of mutational status with molecular and clinicopathologic characteristics, raw sequence data were trimmed, aligned, and checked for variants using NextGENe
v2.3.4.4 and v2.4.1 software (SoftGenetics, State College, PA). FASTQ files were converted to FASTA files and reads were trimmed if the quality scores of two consecutive bases were 12 or less. In addition, reads shorter than 25 bases were removed. The resulting reads were aligned to the human genome v37p10. Additional details on the variant calling parameters and filtering strategy are provided in the Supplementary Information.

Analysis of Public Databases: TCGA

Cases from The Cancer Genome Atlas (TCGA) were selected from the Lung Adenocarcinoma and the Lung Squamous Carcinoma data sets. To reduce possible bias due to the population eligible for LDCT screening trials, only patients in the TCGA who were older than 50 years and heavy smokers (PYs ≥20) were considered. Among these, 88 were selected (Supplementary Fig. 1) in order to perfectly match with the internal (INT) series for tumor histologic type and tumor stage, match for age within a range of plus or minus 4 years, and frequency-match for smoking history and sex. To compare INT targeted sequencing data with TCGA whole exome sequencing data, the analysis was limited to the 207 amplicons that the Ion AmpliSeq Cancer Hotspot Panel v.2 (Thermo Fisher Scientific) was designed to capture (details in the Supplementary Information).

Statistical Analysis

The continuous variables were reported as the median with IQR and were compared using Mann-Whitney nonparametric tests. Associations between categorical variables were assessed by using contingency table analysis and the chi-square test or Fisher’s exact test, as appropriate. All tests were two sided and $p$ values less than 0.05 were considered statistically significant. Correlation between continuous variables was calculated with the Pearson’s correlation coefficient on log transformed variables. Survival curves were estimated by the Kaplan-Meier method, and survival distributions were compared using log-rank test. When the proportional hazard was constant over time, Cox proportional hazards models were used to evaluate the association with survival controlling for stage. Statistical analyses were carried out in GraphPad Prism version 5.02 (GraphPad Software, La Jolla, CA), except for the adjusted survival analysis, which was performed by using the survival package in R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Study Subjects and Tumor Sample Characteristics

Tumor specimens were collected at the time of surgery from 94 study subjects with lung cancer who were enrolled in three different LDCT screening trials performed in our institution (the INT cohort): the INT-IEO, MILD, and BioMILD trials. The median follow-up considering alive patients was 3.5 years (IQR 5.0) without significant differences across the three MSC risk groups, mutated versus nonmutated patients, and the other clinicopathologic subgroups. The median age and median number of PYs were 58 and 40 in the whole screening cohort of 9248 participants versus 64 and 46 in the 94 patients, respectively. Most of the tumors (74%) were adenocarcinoma (ADC) (54% with a lipidic or acinar pattern and 40% with a papillary or solid pattern); 19% were squamous cell carcinoma (SCC) and 4% were SCLC, all showing a solid pattern. The median value of the
MIB-1 proliferation index was 10% in NSCLC and 70% in the four SCLCs, with values ranging from 1% to 90% in the whole series. As expected in LDCT screening–detected series, stage I tumors were more frequent (63%) than tumors at all other (II–IV) stages (37%), and 76% of subjects were still alive at the end of the available follow-up. The MSC test results were also available from plasma samples: 39 samples (41%) had a high-risk MSC, 39 (41%) had an intermediate-risk MSC, and 13 (14%) had a low-risk MSC; the remaining three samples were hemolyzed and thus not analyzable (Table 1).

**Spectrum of Tumor Mutations**

The 94 LDCT screening–detected tumor samples were profiled by targeted NGS using the Ion AmpliSeq Cancer Hotspot Panel v.2 (Thermo Fisher Scientific), which includes the 50 genes most frequently altered in cancer. Seventy-four of the tumor samples (79%) had at least one mutation, whereas the remaining 20 tumor samples (21%) had no alteration in the amplicons considered. In the 20 tumors without mutations, tumor cell content was consistent with that observed in mutated tumors. Tumor protein p53 gene (TP53) was the most altered gene (44 cases [47%]), whereas KRAS was mutated in 27 tumors (29%), either alone (12 tumors [44%]) or along with other genes. An additional 21 genes were mutated in a smaller number of samples: serine/threonine kinase 11 gene (STK11) and cyclin-dependent kinase inhibitor 2A gene (CDKN2A) (seven tumors each); phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene (PIK3CA) (six); phosphatase and tensin homolog gene (PTEN) (four); EGFR (one L858R and three exon 19 ELREA deletions); catenin beta 1 gene (CTNNB1) (four, twice together); APC, WNT signaling pathway regulator gene (APC), BRAF, and NRAS (two each); and 12 other genes that were mutated in one sample each (Fig. 1).

**Mutations in TP53 and KRAS Are Associated with Clinicopathologic Characteristics**

Correlations between the mutated genes and clinicopathologic characteristics were examined for the subjects with LDCT screening–detected lung cancer. Nonmutated tumors were not significantly distributed according to sex, age, or smoking habits of patients; prevalent or incident nodules; or tumor histologic type (Table 2). Also, although the association with tumor stage did not reach the level of significance (Fisher’s exact test p = 0.3), an enrichment of stage I tumors in patients without mutation (75% [15 of 20]) compared with in patients with mutation (59% [44 of 74]) was observed (Table 2). Similarly, 61% of nonmutated NSCLCs (11 of 18) had an MIB-1 proliferation index below the median value of 10%, compared with the 40% of mutated NSCLCs (26 of 65) (Fisher exact test p = 0.2).

On the other hand, significant associations with clinicopathologic characteristics were found for the two genes with the highest-frequency mutations: TP53 and KRAS (Table 2). The frequency of mutations in TP53 was higher in the SCC and NE histologic types compared with in ADC (Fisher exact test p = 0.001). In contrast, KRAS mutations were found only in ADC (Fisher’s exact test p = 0.0001). Subjects with altered TP53 had a median of 51 PYs of smoking compared with a median of 43 PYs in subjects with TP53 wild type (WT) (Mann-Whitney p = 0.03 [Fig. 2A]). Subjects with KRAS mutations had a 56% 5-year OS rate after surgery compared with the 80% 5-year OS rate of subjects with KRAS WT (log-rank p = 0.04 [Fig. 2B]), even after adjusting for tumor stage (Cox’s regression p = 0.03).
Mutation Profile, MSC, and OS

The effects on OS of having mutations in the targeted genes alone or in combination with the MSC risk profile were then assessed. As described in Figure 3A, the 74 patients with at least one mutation had a 5-year OS of only 66%, whereas the 20 patients without mutations had a 5-year OS of 100% (log-rank p = 0.015). When the four SCLCs (all stages II–IV) were excluded, the analysis was still significant (log-rank p = 0.02). Of note, only one of these four tumors was nonmutated and the patient is still alive after 3 years of follow-up. When the analysis was restricted to stage I tumors, all three patients who died within 5 years had ADC with mutations in KRAS, TP53-NRAS, and TP53-KRAS–fibroblast growth factor receptor 2 gene (FGFR2)-CDKN2A, respectively.

Tumor stage and plasma MSC alone also had a significant association with 5-year OS (Supplementary Fig. 2A and B): 28% and 95% for stage II–IV and stage I, respectively (log-rank p < 0.0001), and 50%, 88%, and 91% in subjects with high, intermediate, and low MSC results, respectively (log-rank p = 0.003). When mutational load and MSC risk level were combined, very little overlap in the two groups showing the best 5-year OS was observed: only one subject had both no mutations in the targeted genes and a low-risk MSC (see Table 2). Because this overlap was small and the impact of both mutations and MSC risk level on OS may be confounded by stage, we also combined the tumor mutation profile and the MSC risk level to test for association of the three subgroups of subjects with OS controlling for stage (Fig. 3B). The 32 subjects who had either no mutations or a low-risk MSC had a 97% 5-year OS. The 32 subjects who had both mutations in the targeted genes and a high-risk MSC had the worst prognosis, namely, a 42% 5-year OS, which was significantly different from that of the group with no mutations or low-risk MSC (hazard ratio = 7.36, 95% confidence interval: 2.73–19.84) even after controlling for stage (Cox’s regression p = 0.02). In the group including 28 subjects with tumor mutations and an intermediate-risk MSC, the 5-year OS was 84%. These findings were further confirmed by a Kaplan-Meier analysis stratifying patients according to MSC, mutation status, and tumor stage into up to five groups, with the 5-year OS rate ranging from 8% to 100% (log-rank p < 0.0001 [Fig. 3C]).

Comparison of the Mutation Profile of LDCT Screening–Detected Lung Cancers and the Public TCGA Data Set

To determine whether the mutation profile in our set of screening-detected lung cancer tumors was similar to the mutation profile reported in public data sets, 88 cases from the Lung Adenocarcinoma and the Lung Squamous Carcinoma data sets of the TCGA database were selected to match for age, smoking history, tumor histologic type, and stage with the subset of 88ADCs and SCCs of the INT cohort (Supplementary Table 1).

A higher proportion of subjects died during follow-up in the TCGA cohort than in the screening series (34% versus 24%, chi-square p = 0.1), showing significant differences based on the 5-year OS when all 88 subjects were considered (log-rank p = 0.1 [Supplementary Fig. 3A]). However, when only stage I tumors were considered, the difference in 5-year OS became significant: 64% in the TCGA cohort versus 96% in the INT cohort (log-rank p = 0.001 [Supplementary Fig. 3B]). On the other hand, in stage II to IV
tumors the survival curves were almost overlapping (log-rank \( p = 0.6 \) [Supplementary Fig. 3C]).

Because sequencing was performed by using different technology in the two cohorts, both the INT and the TCGA data were analyzed to focus on genomic regions included in the 207 amplicons of the cancer panel. The most frequently mutated genes were similar between the two series, with a Pearson’s correlation coefficient of 0.96 (Supplementary Fig. 4A). Eighteen cases in the INT cohort (20%) and 12 in the TCGA cohort (14%) had no mutations (Supplementary Fig. 4B); these cases were similarly distributed according to histologic type and staging (Supplementary Table 2). However, there were 14 and six nonmutated stage I tumors in the INT and in the TCGA series, respectively. In addition, although the difference in 5-year OS between subjects with and without mutations in the screening-detected cohort was maintained when this subset of NSCLC was considered (log-rank \( p = 0.03 \) [Supplementary Fig. 4C]), the 5-year OS was not significant in the TCGA cohort (log-rank \( p = 0.8 \) [Supplementary Fig. 4D]).

**Discussion**

This article describes for the first time the mutational profile in hotspot regions of 50 commonly mutated oncogenes and tumor suppressor genes of a large series of LDCT screening–detected lung tumors. Previously, whole exome sequencing of two LDCT-detected lung tumors showed the occurrence of mutations despite a normal karyotype.\(^{24}\) In our series, by using targeted NGS analysis, we determined that the frequencies of the main genetic changes in screening-detected lung tumors were similar to those previously reported in clinically detected lung tumors.\(^{25–27}\) *TP53* was the most frequently altered gene, especially in SCC and NE tumors, and it was associated with PYs of smoking. *KRAS* mutations, which were found exclusively in ADC, were weak but significant predictors for poor prognosis, thus confirming recent data from a meta-analysis of 41 studies.\(^{28}\) Mutations of genes belonging to the *KRAS* proliferation pathway (such as *EGFR*, *BRAF*, and *NRAS*) were mutually exclusive, and *EGFR* alterations, which are very common in never-smokers,\(^{29}\) were present in four tumors in this series of heavy smokers enrolled in screening programs. Mutations in two or more genes were found in 38 samples, whereas 36 tumors had only one mutation and the remaining 20 samples showed no alterations in the 50 genes analyzed. No major clinicopathologic differences between mutated and nonmutated tumors were noted, including differences in age, sex, smoking habits, and prevalent or incident nodules. Other features commonly associated with prognosis, such as histologic patterns in ADC\(^{30}\) or proliferation index in NSCLC,\(^{31}\) did not differ between mutated and nonmutated tumors. However, despite the general low proliferation index observed in this screening series, most of the nonmutated NSCLCs (61%) had levels of MIB1-positive cells below the median value.

Whereas the mutational profile is an intrinsic characteristic of the tumor, circulating miRNA may rather reflect microenvironment-related changes eliciting lung cancer development and aggressiveness.\(^{32,33}\) In fact, inflammation and stromal activation due to smoking exposure and the tumor-associated immune response and even cachexia might modify circulating miRNA levels.\(^{34–37}\) Therefore, we assessed the combined impact of the selected mutational
profile and the results of a miRNA-based liquid biopsy, the MSC, on survival. Either plasma low-risk MSC profile or absence of mutations in tumor tissue were associated with excellent prognosis (5-year survival 91% and 100%, respectively), with only one patient overlapping between the 13 low-risk MSCs and the 20 nonmutated tumors. Conversely, either high-risk MSC or mutations in tumor were associated with worst prognosis (5-year OS rates of 50% and 66%, respectively). The difference in prognosis between groups was even larger when mutational status in the tumor and the plasma MSC score were combined; the hazard for subjects with a high-risk MSC and mutations was seven times higher than the hazard for subjects who had a low-risk MSC score or had no mutations. Given the small number of tumors detected in lung cancer screening program (less than 1% per year), we believe that splitting the series in training and validation set is not possible because the statistical power would be too weak, so we have preferred to use all samples together. The value of the plasma MSC test and tumor mutational status as complementary prognostic biomarkers will be validated in ongoing prospective studies.

In contrast to the low incidence of stage I tumors generally observed in clinical practice (16%),38 the stage I incidence observed in LDCT screening trials is typically higher, around 50% to 85%.5,39 Our series of screening-detected tumors was consistent with the expected incidence of stage I cancer (63%), and the 5-year OS of subjects with stage I tumors was much higher (96%) in this cohort than in the stage-matched TCGA series (64%). Notably, the standard of care for stage I patients is surgery without any additional medical treatment, thereby excluding bias due to different treatment regimens.40 Although these results show improvements in the detection of the disease at its earliest stage in screening trials, they suggest that at least a fraction of the indolent, possibly overdiagnosed, tumors detected by LDCT screening have a WT mutation profile in the main oncogenic drivers.

In conclusion, our findings support the use of tissue-based mutation profile along with a miRNA-based liquid biopsy to provide additional information in planning clinical follow-up in lung cancer LDCT screening programs. Noninvasive plasma MSC could serve in the preclinical setting to stratify at-risk subjects who could benefit from screening or in combination with LDCT to discriminate indolent nodules deserving less severe intervention, whereas targeted NGS could support targeted adjuvant treatments after tumor resection or tissue biopsy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References


Figure 1.
Mutation plot of 94 low-dose computed tomography screening–detected lung tumors. Co-mutation plot of genes mutated in at least one of the 94 National Cancer Institute samples (upper panel) and clinicopathologic and molecular characteristics of patients (lower panel). Genes are ranked according to the number of mutated samples. Tumor protein p53 gene (TP53) was altered in 44 cases and KRAS in 27, followed by alteration of serine/threonine kinase 11 gene (STK11) and cyclin-dependent kinase inhibitor 2A gene (CDKN2A) (seven tumors each), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene (PIK3CA) (six), phosphatase and tensin homolog gene (PTEN) (four), EGFR (one L858R and three exon 19 ELREA deletions), and catenin beta 1 gene (CTNNB1) (four, twice together); APC, WNT signaling pathway regulator gene (APC), BRAF, and NRAS (two each); and 12 other genes that were mutated in one sample each. MET, MNNG HOS Transforming gene; SMAD4, SMAD family member 4 gene; ATM serine/threonine kinase (ATM), gene; FGFR1, fibroblast growth factor receptor 1 gene; FGFR2, fibroblast growth
factor receptor 2 gene; *MLH1*, mutL homolog 1 gene; *RET*, ret protooncogene; *ERBB4*, erb-b2 receptor tyrosine kinase 4 gene; *FBXW7*, F-box and WD repeat domain containing 7 gene; *RB1*, retinoblastoma 1 gene; *SMO*, smoothened, frizzled class receptor gene; *SMARCB1*, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1 gene; *MSC*, microRNA signature classifier; *ADC*, adenocarcinoma; *SCC*, squamous cell carcinoma; neuroendocrine tumor; *NA*, not available.
Figure 2. Clinicopathologic characteristics associated with mutational status of tumor protein p53 gene (TP53) and KRAS. (A) Box plot reporting the distribution of pack-years index according to TP53 mutational status: $p$ value for two-tailed Mann-Whitney test. (B) Kaplan-Meier curve in strata of KRAS mutational status. Overall survival (OS) and $p$ value for log-rank test are reported. mut, mutation; wt, wild type.
Figure 3.
Kaplan-Meier analysis according to mutational status and microRNA signature classifier (MSC). Kaplan-Meier curves in strata of mutational status alone (A), the combination of mutational status and MSC (B), and the combination of mutational status, MSC, and tumor stage (C). MSC risk level: high (H), intermediate (I), and low (L). Overall survival (OS) and p value for log-rank test are reported.
Table 1.
Clinicopathologic Characteristics of Patients Screened for Lung Cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Patients</td>
<td>94</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>66 (70)</td>
</tr>
<tr>
<td>Median age, y (IQR)</td>
<td>64 (10)</td>
</tr>
<tr>
<td>Median pack-years (IQR)</td>
<td>46 (32)</td>
</tr>
<tr>
<td>Median years of follow-up (IQR)</td>
<td>3.5 (5)</td>
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<tr>
<td>Histologic subtype, n (%)</td>
<td></td>
</tr>
<tr>
<td>ADC</td>
<td>70 (74)</td>
</tr>
<tr>
<td>SCC</td>
<td>18 (19)</td>
</tr>
<tr>
<td>SCLC</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Other NE</td>
<td>2 (2)</td>
</tr>
<tr>
<td>ADC pattern, n (%)</td>
<td></td>
</tr>
<tr>
<td>Lepidic/acinar</td>
<td>38 (54)</td>
</tr>
<tr>
<td>Papillary/solid</td>
<td>28 (40)</td>
</tr>
<tr>
<td>NA</td>
<td>4 (6)</td>
</tr>
<tr>
<td>MIB1-positive cells</td>
<td></td>
</tr>
<tr>
<td>Median in NSCLC (IQR)</td>
<td>10% (28)</td>
</tr>
<tr>
<td>Median in SCLC (IQR)</td>
<td>70% (58)</td>
</tr>
<tr>
<td>Stage, n (%)</td>
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</tr>
<tr>
<td>I</td>
<td>59 (63)</td>
</tr>
<tr>
<td>II–IV</td>
<td>35 (37)</td>
</tr>
<tr>
<td>Status, n (%)</td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>71 (76)</td>
</tr>
<tr>
<td>Dead</td>
<td>23 (24)</td>
</tr>
<tr>
<td>MSC, n (%)</td>
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</tr>
<tr>
<td>High</td>
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<tr>
<td>Intermediate</td>
<td>39 (41)</td>
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<tr>
<td>Low</td>
<td>13 (14)</td>
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<tr>
<td>NA</td>
<td>3 (3)</td>
</tr>
</tbody>
</table>

\(^a\) Considering live patients.

\(^b\) All with a solid pattern.

\(^c\) One large cell carcinoma and one typical carcinoid.

\(^d\) Alive or dead status at the end of available follow-up for each subject.

IQR, interquartile range; ADC, adenocarcinoma; SCC, squamous cell carcinoma; NE, neuroendocrine tumor; NA, not available; MIB1, mindbomb 3 ubiquitin protein ligase 1; MSC, microRNA signature classifier.
<table>
<thead>
<tr>
<th>INT Cohort</th>
<th>Mutated</th>
<th>Nonmutated</th>
<th>p Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TP53 Mutated</th>
<th>TP53 WT</th>
<th>p Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>KRAS Mutated</th>
<th>KRAS WT</th>
<th>p Value&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>26 (37)</td>
<td>44 (63)</td>
<td>0.0026</td>
<td>27 (40)</td>
<td>43 (60)</td>
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<td>54 (77)</td>
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<td>11 (29)</td>
<td>27 (71)</td>
<td>0.2997</td>
<td>16 (42)</td>
<td>22 (58)</td>
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<td>SCC</td>
<td>16 (89)</td>
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<td></td>
<td>12 (43)</td>
<td>16 (57)</td>
<td></td>
<td>8 (29)</td>
<td>20 (71)</td>
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<tr>
<td>NE</td>
<td>4 (67)</td>
<td>2 (33)</td>
<td></td>
<td>4 (67)</td>
<td>2 (33)</td>
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<td>Lepidic/acinar</td>
<td>28 (74)</td>
<td>10 (26)</td>
<td>0.7743</td>
<td>22 (29)</td>
<td>36 (61)</td>
<td>0.0569</td>
<td>17 (29)</td>
<td>42 (71)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Papillary/solid</td>
<td>22 (79)</td>
<td>6 (21)</td>
<td></td>
<td>17 (67)</td>
<td>20 (71)</td>
<td></td>
<td>13 (40)</td>
<td>25 (71)</td>
<td></td>
</tr>
<tr>
<td>Stage, n (%)</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44 (75)</td>
<td>15 (25)</td>
<td>0.2975</td>
<td>23 (39)</td>
<td>36 (61)</td>
<td>0.0569</td>
<td>17 (29)</td>
<td>42 (71)</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>II–IV</td>
<td>30 (86)</td>
<td>5 (14)</td>
<td>21 (60)</td>
<td>14 (40)</td>
<td>0.0257</td>
<td>10 (29)</td>
<td>25 (71)</td>
<td></td>
</tr>
<tr>
<td>MIB1 in NSCLC, n (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10%</td>
<td>26 (70)</td>
<td>11 (30)</td>
<td>0.1794</td>
<td>11 (30)</td>
<td>26 (70)</td>
<td>0.0257</td>
<td>13 (35)</td>
<td>24 (65)</td>
<td>0.3320</td>
</tr>
<tr>
<td>≥10%</td>
<td>39 (85)</td>
<td>7 (15)</td>
<td></td>
<td>26 (57)</td>
<td>20 (43)</td>
<td>0.0881</td>
<td>11 (24)</td>
<td>35 (76)</td>
<td></td>
</tr>
<tr>
<td>MSC, n (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>High</td>
<td>32 (82)</td>
<td>7 (18)</td>
<td>0.2935</td>
<td>16 (41)</td>
<td>23 (59)</td>
<td>0.0881</td>
<td>11 (28)</td>
<td>28 (72)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Intermediate</td>
<td>28 (72)</td>
<td>11 (28)</td>
<td></td>
<td>17 (44)</td>
<td>22 (56)</td>
<td>0.8881</td>
<td>11 (28)</td>
<td>28 (72)</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>12 (92)</td>
<td>1 (8)</td>
<td></td>
<td>10 (77)</td>
<td>3 (23)</td>
<td></td>
<td>4 (31)</td>
<td>9 (69)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Two-tailed Fisher’s exact test p value.

<sup>b</sup>ADC pattern, MIB1 in NSCLC, and MSC data were missing for four, seven, and three patients, respectively.

MIB1, mindbomb 3 ubiquitin protein ligase 1; MSC, microRNA signature classifier; INT, National Cancer Institute; TP53, tumor protein p53 gene; WT, wild type; ADC, adenocarcinoma; SCC, squamous cell carcinoma; NE, neuroendocrine tumor.