Clinical Cancer Research

The Impact of Smoking and TP53 Mutations in Lung Adenocarcinoma Patients with Targetable Mutations—The Lung Cancer Mutation Consortium (LCMC2)

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Abstract

Purpose: Multiplex genomic profiling is standard of care for patients with advanced lung adenocarcinomas. The Lung Cancer Mutation Consortium (LCMC) is a multi-institutional effort to identify and treat oncogenic driver events in patients with lung adenocarcinomas.

Experimental Design: Sixteen U.S. institutions enrolled 1,367 patients with lung cancer in LCMC2; 904 were deemed eligible and had at least one of 14 cancer-related genes profiled using validated methods including genotyping, massively parallel sequencing, and IHC.

Results: The use of targeted therapies in patients with *EGFR, ERBB2,* or *BRAF* p.V600E mutations, *ALK, ROS1*, or *RET* rearrangements, or *MET* amplification was associated with a survival increment of 1.5 years compared with those with such mutations not receiving targeted therapy, and 1.0 year compared with those lacking a targetable driver. Importantly, 60 patients with a history of smoking derived similar survival benefit from targeted therapy for alterations in *EGFR*/*ALK*/*ROS1*, when compared with 75 never smokers with the same alterations. In addition, coexisting *TP53* mutations were associated with shorter survival among patients with *EGFR, ALK*, or *ROS1* alterations.

Conclusion: Patients with adenocarcinoma of the lung and an oncogenic driver mutation treated with effective targeted therapy have a longer survival, regardless of prior smoking history. Molecular testing should be performed on all individuals with lung adenocarcinomas irrespective of clinical characteristics. Routine use of massively parallel sequencing enables detection of both targetable driver alterations and tumor suppressor gene and other alterations that have potential significance for therapy selection and as predictive markers for the efficacy of treatment. *Clin Cancer Res; 24(5); 1038–47. ©2017 AACR.*

Introduction

Lung adenocarcinoma is the most common histologic type of lung cancer and is diagnosed in 130,000 patients in the United States and 1 million persons worldwide each year (1). Lung adenocarcinomas are frequently characterized by different oncogenic driver mutations that affect a variety of kinases and their downstream signaling pathways (2–15), many of which are targetable using both standard-of-care FDA-approved and promising investigational therapies (16). For these reasons, systematic testing for oncogenic driver mutations is standard of care at

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Translational Relevance

Characterization of lung adenocarcinomas by multiplex genomic profiling for multiple mutations is now standard of care. Here we show that the survival benefit of targetable mutation detection and directed therapy is similar for both never smokers and current and former smokers with lung adenocarcinomas. We also demonstrate that concurrent TP53 mutation is associated with poorer survival among lung adenocarcinoma patients with EGFR, ALK, or ROS1 alterations. Hence, routine use of massively parallel sequencing enables rapid detection of all types of clinically significant sequence variants for the care of lung adenocarcinoma patients, accelerating both targeted therapy selection and prognostic assessment.

diagnosis of metastatic lung adenocarcinomas and has been formally recommended by multiple molecular pathology guideline panels (16, 17).

The Lung Cancer Mutation Consortium (LCMC) was established in 2010 as a multi-institutional effort to investigate the frequency of different oncogenic drivers in lung adenocarcinoma, facilitate clinical protocol enrollment especially for rare molecular subsets, enable exchange of information and protocols for reproducibility of molecular testing among institutions, and thereby to accelerate further development of personalized treatment for lung adenocarcinoma across the United States (18–20).

Here, we report on the tumor genomic patterns and patient outcomes from a second cohort of LCMC subjects (LCMC2). This second cohort of subjects was enrolled because additional oncogenic drivers were identified that could be targeted with novel genotype-specific agents. Patients were prospectively enrolled to perform tumor genotyping of the 10 oncogenic drivers studied in LCMC1, as well as assays for *ROS1* (*ROS1r*) and *RET* (*RETr*) rearrangements (21–23), and IHC analysis for PTEN and MET expression. PTEN and MET IHC analyses were included on the basis of the promise of therapies for these alterations that were in clinical trials, including PI3K inhibitors and antibodies against MET. During the course of LCMC2 enrollment, most institutions switched from focused or serial testing to highly multiplexed genetic testing using massively parallel sequencing (MPS; also known as next-generation sequencing; refs. 24–27). This development enabled simultaneous analysis of mutations in several other genes in lung cancer that are biologically important, but not currently targetable (specifically *TP53*), that may be prognostically relevant when present concomitantly with oncogenic driver mutations.

Materials and Methods

Patient recruitment, enrollment, and IRB approval

These studies were conducted in accordance with the ethical principles present in the Belmont Report. Sixteen clinical sites participated in LCMC2 (Supplementary Table S1). All sites obtained Institutional Review Board approval for this study. Eligible patients met the following criteria: stage IV or recurrent lung adenocarcinoma; Southwest Oncology Group performance status of 0, 1, or 2; expected survival of more than 6 months; no prior treatment with targeted therapy; diagnosis of metastatic disease after May 1, 2012; and adequate tissue for molecular analyses. All subjects enrolled provided written informed consent. Of 1,367 patients enrolled, 1,009 were deemed eligible (Supplementary Fig. S1). Epidemiologic and clinicopathologic data were prospectively collected, including age, sex, race, cigarette smoking history, stage at diagnosis, metastatic sites, and survival from the time of documented metastatic disease.

Pathology evaluation

Anatomic pathologists at each institution confirmed a diagnosis of lung adenocarcinoma, assessed tumor content, and determined specimen adequacy for molecular diagnostic testing. Central confirmation of lung adenocarcinoma diagnosis was based on review of an hematoxylin- and eosin-stained histology slide or a scanned whole-slide image (Leica Biosystems Inc.) and the pathology report, when available (I.I. Wistuba or J. Fujimoto).

Mutational analyses

All mutational analyses were performed in Clinical Laboratory Improvement Amendments (CLIA)–certified diagnostic laboratories, using a variety of methods (Supplementary Table S2). The mutations studied consisted of four small indels and 93 point mutations occurring in eight genes: *AKT1*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MAP2K1*, *NRAS*, and *PIK3CA* (Supplementary Table S3), hereafter denoted the eight core genes and 97 core alleles. During the course of this study, many diagnostic laboratories converted from single gene testing to MPS methods (Supplementary Table S4). MPS technologies at each site were independently validated to CLIA standards for both wet-bench and bioinformatics components. A total of 460 subjects' samples were analyzed by MPS, from which 431 MPS reports or variant call files were centrally reviewed to confirm the extent of assay coverage, including coverage data for *TP53*, *STK11*, and *PTEN* (Supplementary Fig. S2) and to exclude technical artifacts or germline variants, which may have been reported on the basis of automated mutation calling algorithms. Systematic evaluation for *MET* exon 14 skipping variants was not performed. All results are shown in Supplementary Table S5.

Rearrangement detection

FISH was performed using assays for fusions/rearrangements in *ALK*, *ROS1*, and *RET*, as described previously (12, 19, 28). Rearrangement results were also accepted from laboratories using hybrid capture–based MPS as the principal detection method. FISH or silver *in situ* hybridization (Roche/Ventana) for assessment of *MET* amplification was also performed (29), and amplification was considered to be present when the *MET*/centromere 7 ratio was at least 2.0 (30).

IHC for PTEN and MET

IHC for PTEN (clone 138G6; Cell Signaling Technology) and MET (clone SP44, Roche/Ventana) was performed at 12 study sites. Individual sites were responsible for assay validation on their local staining platforms within a CLIA-certified laboratory. PTEN results were scored as intact ($\geq$90% tumor cells staining), lost (<10% tumor cells staining), or heterogeneous (between 10%–90% tumor cell staining). MET IHC was defined as positive if the sample had an H score of $\geq$200, following previously published scoring methods (31). Both PTEN and MET IHC scoring involved pathologist

training and interlaboratory proficiency testing (Supplementary Methods).

Classification of *EGFR* mutations

We considered *EGFR* p.L858R, exon 19 in-frame deletions and insertions, p.G719S/C/A, and p.L861Q mutations as sensitizing to therapy with EGFR tyrosine kinase inhibitors (TKI; sensitizing *EGFR*, s*EGFR*; ref. 18). We considered p.E709A, exon 20 in-frame insertion or deletion, and p.T790M mutations as nonsensitizing to TKIs, a category we labeled "other" (o*EGFR*; refs. 32, 33, 34). With the exception of combinations including *de novo* p.T790M mutations, all examples of compound sensitizing and nonsensitizing mutations were categorized as s*EGFR*.

Analysis of *TP53* mutations

TP53 mutations were categorized as "disruptive" as described previously: (i) all inactivating mutations (i.e., nonsense, frameshift, splice-site); or (ii) nonconservative missense mutations occurring within the DNA-binding domain L2 (codons 163–195) or L3 (codons 236–251; Supplementary Table S6; ref. 35). All other variants were considered nondisruptive. All combinations of disruptive and nondisruptive mutations were categorized as disruptive.

Targeted therapy

We considered targeted therapy to be any treatment provided as standard of care or within a clinical trial that was a kinase inhibitor or antibody directed at a specific genomic alteration. This included therapies directed at the following alterations: s*EGFR*, *ERBB2* exon 20 insertions or missense mutations, *BRAF* p.V600E *(*ve*BRAF)*, *ALK*r, *ROS1*r, *RET*r, and *MET* amplification (*MET*amp), hereafter denoted as "the targeted therapy cohort."

Survival analysis and statistical methods

Descriptive statistics, including median for continuous variables, and percentages and frequencies for categorical variables, are presented. Group comparisons were analyzed using the Wilcoxon rank sum or Kruskal–Wallis tests for continuous variables and χ^2 test for categorical variables. Survival curves were calculated from the Kaplan–Meier method, and differences in survival were tested by the log-rank test. To evaluate whether driver gene mutation effects were similar between smoker and nonsmoker groups, Cox proportional hazards model analysis was performed including driver gene mutation, smoking status, and their interaction. Statistical analyses were performed using R version 3.3.1.

Results

Subjects and molecular analyses

From January 1, 2013, to December 1, 2015, 1,367 subjects were enrolled, of which 1,009 (74%) met all eligibility criteria. Reasons for exclusion are indicated in Supplementary Fig. S1.

Of the 907 confirmed adenocarcinomas cases, 904 had at least one mutation analysis, 866 had at least one FISH assay, and 830 had at least one IHC assay completed (Supplementary Fig. S2). Of 904 patients for whom at least one biomarker was assessed ("any genotyping"), 54% were female, 92% had an ECOG performance status of 0 or 1, 63% were former smokers, and 25% of patients were never smokers (Table 1). A total of 423 cases had "full" genotypes reported for all 14 drivers assessed, including MET and PTEN IHC (Table 2).

Mutation findings

Rates of genomic alterations among patients with "any" genotyping ($n = 904$) and "full" genotyping ($n = 423$) and numbers of patients enrolled on targeted therapies are shown in Table 2. A driver oncogenic alteration, when including *KRAS* mutations, was observed in 544 (60%) patients overall and in 273 (65%) patients with full genotyping (Fig. 1A). *RET*r and *ROS1r* each were seen in 11 cases [2.8%; 95% confidence interval (CI), 1.3–3] of the full genotyping cohort. Tumors containing two putative oncogenic drivers were detected in 22/904 (2.4%) in the overall cohort, and in 10/423 (2.4%) in the full genotyping cohort (Supplementary Table S7). *MET*amp was observed as a concurrent oncogenic driver event in 8% of ve*BRAF*, 3.0% of *KRAS*, and 2.5% of *EGFR*-mutated cases and

Table 1. LCMC2 patient characteristics

	Genotyping		Driver[a] and treatment status		
Patient characteristics	**Any**	**Full**	**No driver**	**Driver + Tx**	**Driver No Tx**
Sex	$n = 904$	$n = 423$	$n = 337$	$n = 162$	$n = 74$
Male	416 (46)	213 (50.4)	176 (52.2)	67 (41.4)	34 (45.9)
Female	488 (54)	210 (49.6)	161 (47.8)	95 (58.6)	40 (54.1)
Age at enrollment, mean (range)	64 (22–90)	64 (22–90)	64 (34–90)	61 (35–86)	63 (22–90)
Performance status					
0	247 (27.6)	109 (25.8)	77 (22.9)	47 (29.2)	21 (28.4)
1	574 (64.1)	273 (64.7)	226 (67.3)	105 (65.2)	44 (59.5)
2	74 (8.3)	40 (9.5)	33 (9.8)	9 (5.6)	9 (12.2)
Missing	9 (1.0)	1 (0.2)	1 (0.3)	1 (0.7)	0
Cigarette smoking history					
Never	219 (24.6)	101 (24.1)	45 (13.4)	86 (53.4)	22 (29.7)
Former	556 (62.5)	274 (65.4)	242 (72.2)	69 (42.9)	41 (55.4)
Current	115 (12.9)	44 (10.5)	48 (14.3)	6 (3.7)	11 (14.9)
Missing	14 (1.5)	4 (1)	2 (0.6)	1 (0.7)	0
Prior therapy					
Surgery	389 (43.9)	175 (41.6)	149 (44.3)	49 (30.4)	33 (45.2)
Chest radiotherapy	137 (15.5)	55 (13.1)	54 (16.2)	14 (8.7)	14 (18.9)
Chemotherapy	487 (61.6)	223 (57.5)	205 (65.7)	81 (53.6)	40 (59.7)
Time from metastatic disease dx to enrollment, mean (years)	0.31	0.3	0.34	0.28	0.31

[a]Driver in this table refers to sensitizing *EGFR, BRAF* V600E, and *ERBB2* mutation, *ALK, ROS1*, and *RET* rearrangement, and *MET* amplification.

Table 2. Summary of mutation and expression findings in LCMC II

	Any genotyping (n[a])	% (based on n for each assay)	CI	Full genotyping ($n = 423$; %)	CI	Targeted therapy[c]
Major targetable alterations						
EGFR						
sEGFR	116 (862)	13.5%	11–16	65 (16.7%)	12–19	100
L858R	50			31		
Exon 19 in/del	56			30		
G719X or L861Q	10			4		
MET amplification	33 (689)	4.8%	3–7	19 (4.9%)	3—7	6
ALK rearrangement	36 (843)	4.3%	3–6	17 (4.4%)	2—6	30
BRAF V600E	26 (860)	3.0%	2–4	17 (4.4%)	2—6	10
ROS1 rearrangement	18 (832)	2.2%	1.3–3	11 (2.8%)	1.3–5	8
RET rearrangement	18 (817)	2.2%	1.3–4	11 (2.8%)	1.3–5	9
ERBB2	16 (647)	2.5%	1.4–4	12 (3.1%)	1.5–5	6
Other alterations						
KRAS	269 (862)	31.2%	28–34	113 (29.0%)	23–31	2
oEGFR	20 (861)	2.3%	1.4–4	11 (2.8%)	1.3–5	0
NRAS	6 (860)	0.7%	0.3–2	5 (1.3%)	0.4–3	0
BRAF (non-V600E)	8 (860)	0.9%	0.4–2	2 (0.5%)	0.1–2	0
AKT1	0 (708)	0.0%		0		
Known cooccurring alterations						
MET expression (IHC)	482 (827)	58.3%	55–62	235 (60.3%)	51–60	
TP53 mutation	218 (431)[b]	50.5%	46–56	136 (274)[b] (49.6%)	44–56	
PTEN loss (IHC)	54 (646)	8.3%	6–11	40 (10.3%)	10–18	
PIK3CA	23 (860)	2.7%	2–4	15 (3.8%)	2–6	
MAP2K1	2 (765)	0.3%	0–1	0		

[a]n denotes the number of subjects whose cancers were tested for each alteration.
[b]For TP53 mutation detection rate, only cases in which NGS testing was performed are considered.
[c]Number of patients receiving a targeted therapy for the indicated molecular alteration in the any genotyping subset.

was present at a low level (*MET* to CEP7 ratio of 2-3.3) in all *KRAS* and *veBRAF* cases. Three tumor specimens with s*EGFR* mutation also had *de novo MET*amp, of which two were high level (ratios of 15 and 4.7). Combined s*EGFR* and *KRAS* activating mutations were observed in three cases. Dual *EGFR* and fusion alterations were observed in three cases (s*EGFR*/*ALKr* = 2, s*EGFR*/*RETr* = 1), and *KRAS* mutations/*ROS1* fusions were observed in two cases; corroborating evidence for a rearrangement was limited in all cases.

Comutation plot and analysis

Use of MPS allowed us to perform analyses of mutations in other genes in 460 samples, including tumor suppressor genes *TP53*, *STK11*, and *PTEN*. We examined concurrence of mutations in detail in 154 subjects with complete genotyping of core alleles, as well as *TP53*, *STK11*, and *PTEN*. In this set, mutations in the core alleles were mutually exclusive, except for one sample with an s*EGFR* and *KRAS* p.Q61R mutation, and five samples that had both *MET*amp and another driver mutation (Fig. 1B; Supplementary Table S7). *TP53* mutations were identified in 14 of 35 (40%) *EGFR* and 22 of 44 (50%) *KRAS*-mutant tumors, and were rare in *ALKr*, *ROS1r*, or *RETr* tumors (1/11, 9%, *ROS1r*). *STK11* mutations were observed in 11% of cases, exclusively in *KRAS*-mutated and driver oncogene-negative cases. Only one of 17 cases with PTEN loss of expression by IHC (see below) had an identifiable *PTEN* mutation. In 41 (27%) cases, *PIK3CA*, *TP53*, or *STK11* mutation and/or PTEN loss was identified in the absence of a coexisting oncogenic driver alteration. No variants were detected in the examined genes in 14 (9%) of cases.

PTEN and MET IHC

Central pathology review was performed for 646 PTEN-stained tumors: PTEN was lost in 54 (8%; 95% CI, 6–11), intact in 526 (81%) and heterogeneous in 66 (11%; Supplementary Fig. S3). Heterogeneous cases rarely demonstrated abrupt loss of expression, as has been reported in prostate adenocarcinoma (36). Instead, these cases typically showed a gradient of staining, which was interpreted as intact expression. MET IHC results were reported in 827 cases and were considered positive (H score $\geq$ 200) in 482 (58%; 95% CI, 55–62; to be reported in detail elsewhere).

Clinicopathologic associations with specific mutations

Supplementary Figure S5 displays associations between oncogenic driver mutations and clinical characteristics. Multiple nominally significant associations were identified that should be considered exploratory in this analysis but are consistent with previously published observations.

Survival in the presence of targetable alteration

Survival was longer in 162 subjects with mutations in any targetable driver gene [s*EGFR* ($n = 95$), *ERBB2* ($n = 6$), ve*BRAF* ($n = 9$), *ALKr* ($n = 28$), *ROS1r* ($n = 8$), *RETr* ($n = 8$), *MET*amp ($n = 2$)], or multiple drivers ($n = 6$) who received targeted therapy in comparison with patients with such mutations who did not received targeted therapy, and in comparison with those without a driver identified ($P < 0.001$, Fig. 2). As expected, patients with *sEGFR* alterations received benefit from EGFR-targeted therapy, compared with those who did not receive therapy ($P < 0.001$), with 1.7-year improvement in median survival from 1.3 to 3 years (Supplementary Fig. S6).

Molecular modulators of survival in targeted therapies

Neither PTEN loss nor MET positivity by IHC were associated with a difference in overall survival for the targeted therapy cohort ($P = 0.944$, Supplementary Fig. S7A; $P = 0.729$, Supplementary

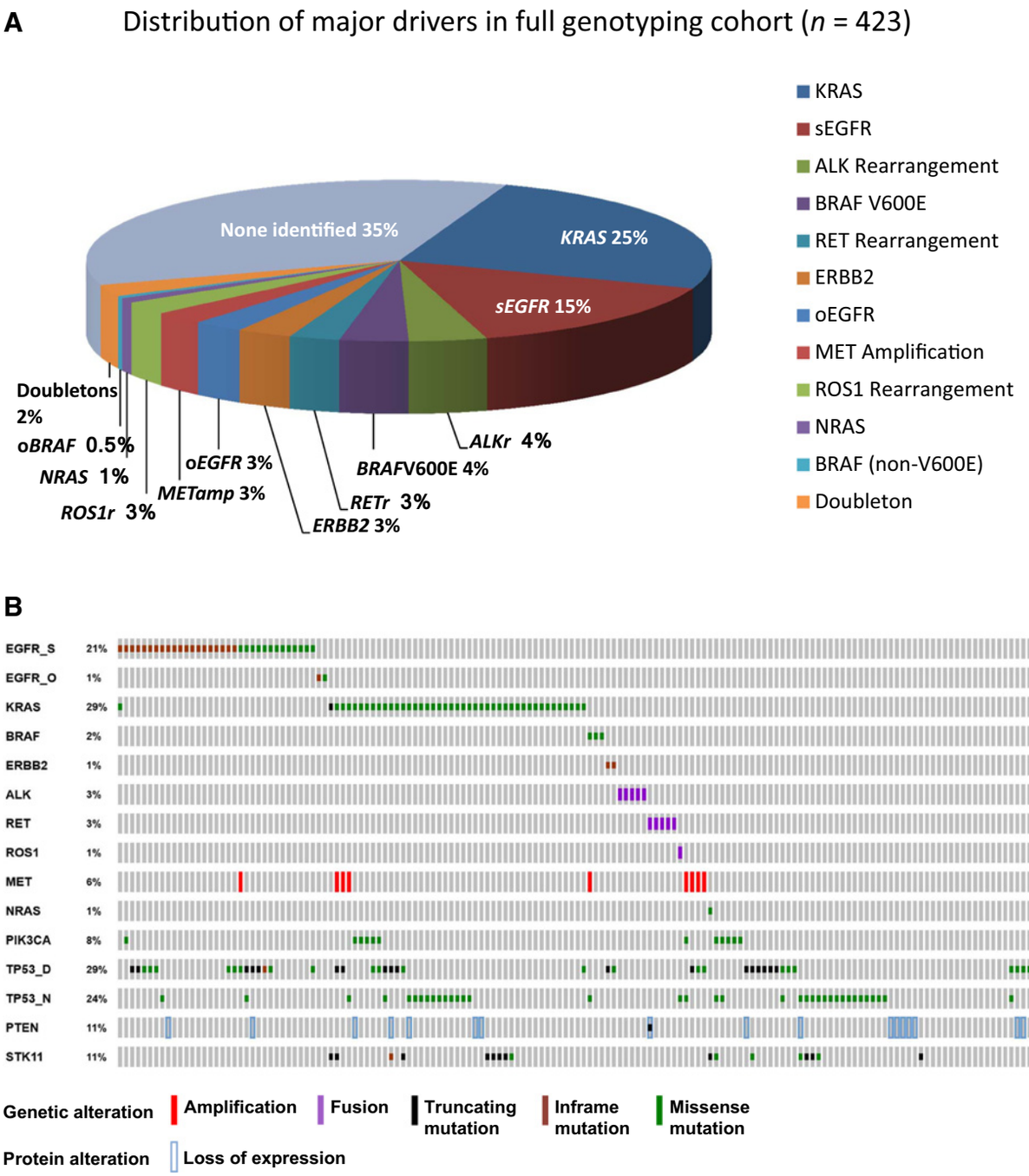

Figure 1.

Mutations and comutation plot in LCMC II. **A,** Distribution of oncogenic drivers in full genotyping cohort. The relative proportion of the various driver mutations is shown for the 423 subjects with complete testing for 12 genes. No *AKT1* or *MAP2K1* mutations were detected in this set. s*EGFR*, sensitizing EGFR mutation; o*EGFR*, other EGFR mutations; *ALK*r, *RET*r, and *ROS1*r denote rearrangements in the respective genes; *MET*amp denotes amplification of *MET;* o*BRAF* denotes a mutation other than V600E; and doubletons denotes samples with two or more of the oncogenic drivers shown here. **B,** Comutation plot. Genetic and expression alterations in the 14 core genes plus key tumor suppressor genes in 154 lung adenocarcinomas with complete analysis. No *AKT1* or *MAP2K1* mutations were detected in this set. EGFR_S, sensitizing *EGFR* mutations; EGFR_O, other *EGFR* mutations; TP53_D, disruptive alterations; TP53_*N*, nondisruptive alterations. Prepared using http://www.cbioportal.org/oncoprinter.jsp#.

Fig. S7B); however, analysis of PTEN is limited by the small number of cases with loss of expression. In addition, when considering the following events as a class, PTEN loss by IHC, *PTEN* mutation, *PIK3CA* mutation or *TP53* mutation, no significant effect on survival was noted in the overall targeted therapy cohort (Supplementary Fig. S7C).

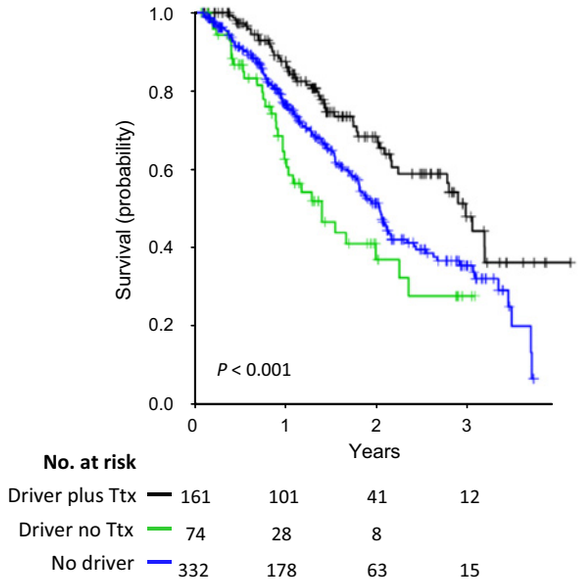

Figure 2.

Survival comparisons according to targeted therapy. Survival curves for subjects with any of s*EGFR*, *ERBB2*, *BRAF p.V600E (veBRAF)*, *ALK*r, *ROS1*r, *RET*r, or *MET*amp alterations who received targeted therapy (Ttx), versus those with similar alterations who did not receive targeted therapy (no Ttx), versus those with no mutations in any of these genes.

However, as previous reports have suggested that *TP53* mutation might adversely affect the survival of patients treated with targeted therapy for oncogenic driver mutations in lung cancers (37, 38), we explored this possibility, specifically focusing on patients in whom MPS testing had been performed, *TP53* status manually curated, and survival data were available. Patients with s*EGFR* treated with targeted therapy harboring a *TP53* mutation displayed a trend toward shorter survival compared with those without a *TP53* mutation [2.9 years vs. not reached ($P = 0.06$); Fig. 3A]. To examine this further, we divided *TP53* mutations into disruptive and nondisruptive types (see Materials and Methods). Disruptive *TP53* mutations were associated with a reduction in survival (median survival = 2.6 years) in comparison with no *TP53* mutation (median survival not reached) in those with s*EGFR* mutations ($P = 0.055$; Fig. 3B; Supplementary Fig. S8A). Given these results, we extended this analysis to the set of patients with any of s*EGFR*, *ALK*r, and *ROS1*r alterations. Any *TP53* mutation was associated with reduced survival (median 2.6 years) in the *EGFR-ALK*r-*ROS1*r subset, compared with no *TP53* mutation (median not reached, $P = 0.014$, Fig. 3C), and this difference was enhanced by consideration of *TP53*-disruptive mutations only (median survival 2.6 years versus not reached, $P = 0.009$, Fig. 3D). In addition, for the *EGFR-ALK*r-*ROS1*r subset, survival differed according to the presence of a *TP53*-disruptive mutation versus a *TP53*-nondisruptive mutation versus no *TP53* mutations ($P = 0.033$; Supplementary Fig. S8B).

Effect of smoking history on mutation frequency and targeted therapy benefit

Despite a correlation between targetable driver mutations and nonsmoking status, all mutation types were also seen in current and/or former smokers (Supplementary Fig. S4). We examined the benefit of targeted therapy for s*EGFR-ALK*r-*ROS1*r alterations in patients with and without a cigarette smoking history. As expected, targeted therapy conferred a major survival benefit to never smoker patients with an s*EGFR-ALK*r-*ROS1*r alteration (Fig. 4A, $P = 0.011$). Notably, a similar improvement in survival was seen in former and current smokers with an s*EGFR-ALK*r-*ROS1*r alteration who received targeted therapy in comparison with those who did not (Fig. 4B, $P = 0.003$). Furthermore, the survival benefit from targeted therapy was similar in the never smoking and current/former smoking subgroups ($P = 0.975$, Cox proportional hazards model).

Discussion

The LCMC was formed to expand and formalize molecular genetic testing of lung adenocarcinoma specimens for targetable driver mutations to enable broader dissemination of personalized therapy for this malignancy. In the current study, we have expanded the panel of genetic alterations examined to include *ROS1*r and *RET*r and added additional assays to explore PTEN expression and MET expression in this new cohort of 904 patients. The PTEN and MET analyses were added as at the time the study was planned, both PI3 kinase inhibitors and onartuzumab were promising therapies for alterations in these two genes, respectively. Subsequent studies failed to show benefit from these agents in patients with lung carcinoma. However, newer compounds and treatment strategies targeting these alterations are under continuing investigation (39, 40). Currently, MET-directed TKIs are thought to have potential benefit for patients with MET amplification, with a suggestion that high-level MET amplification may be the most predictive marker (41). MPS-based panel testing was incorporated into routine diagnostic practice at most sites during LCMC2, enabling us to examine the effect of comutations on outcomes following targeted therapy in that subset.

Similar to our analyses of the LCMC1 population, we found that persons with oncogenic drivers in their tumors who were treated with targeted therapy experienced a longer survival than those who did not receive such therapy (18). Although patients with an identified driver mutation typically receive targeted therapy, a variety of factors may prevent the therapeutic intervention including rapid clinical decline after enrollment and loss to follow-up at the institution where the testing was performed. However, the reduced survival of untreated patients was not clearly attributable to early death after enrollment (Fig. 2; ref. 40). We acknowledge that as this population did not derive from a randomized trial, there is potential for bias, and all observations made here should be considered in that context (25, 26, 42, 43).

Although prior studies have suggested a correlation between *TP53* mutation and worse outcomes among *EGFR*-mutated lung adenocarcinomas (38, 44, 45), this is the first study to demonstrate the adverse prognostic impact of *TP53* mutations on patients treated with targeted therapy directed against s*EGFR*, *ALK*r, or *ROS1*r alterations. Similar findings have been recently observed in a cohort of *EGFR* mutation–positive patients (45). In our study, this association was enhanced when disruptive *TP53* mutations only were considered in comparison with subjects with no *TP53* mutation ($P = 0.009$). However, the total number of evaluable subjects for this analysis was small and, therefore, this correlation should be considered preliminary. Additional studies are needed to confirm the prognostic impact of *TP53* mutation in this setting. *TP53* mutation testing is included in many MPS assays

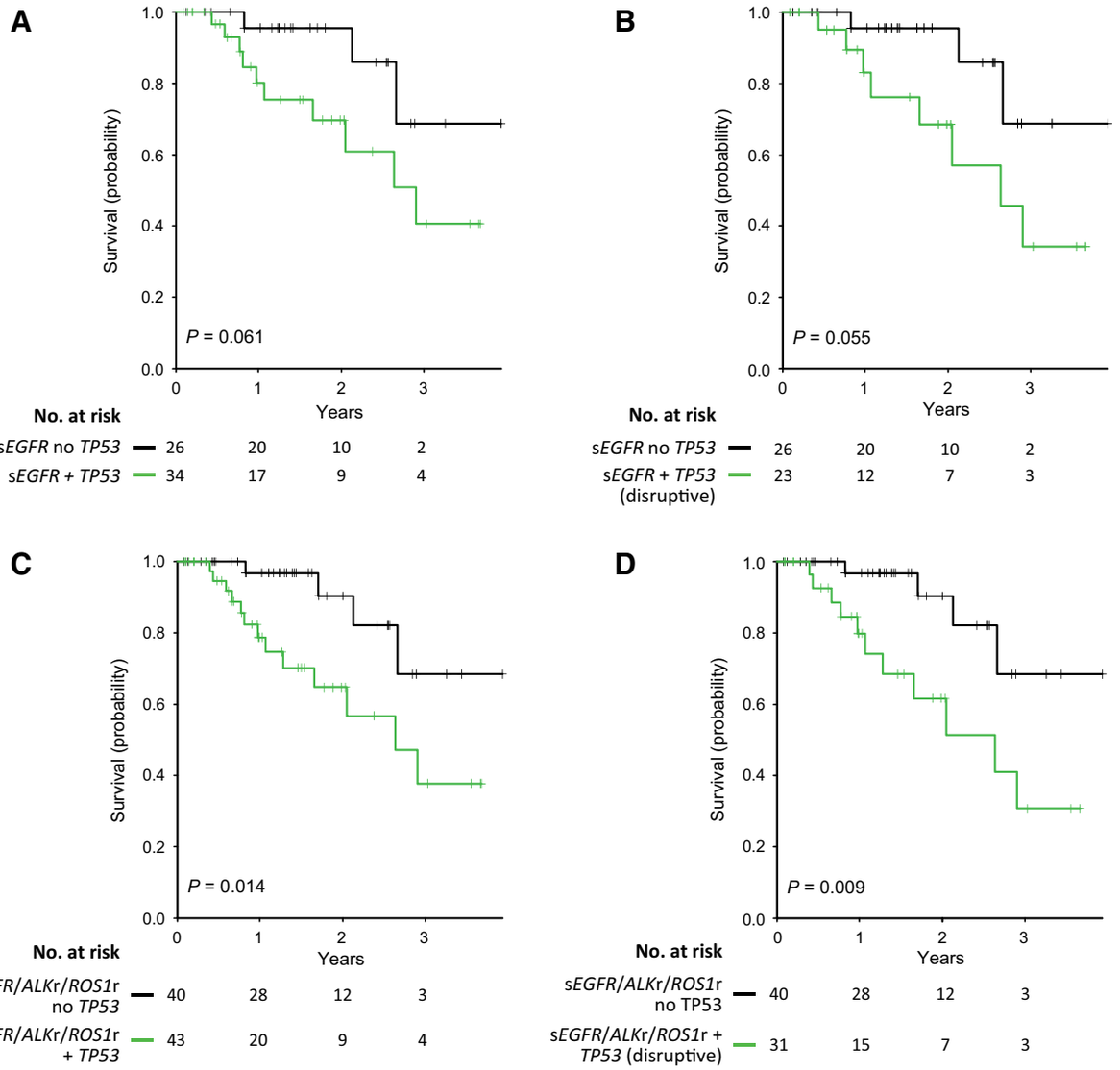

Figure 3.

Survival comparisons according to presence or absence of *TP53* mutation. **A,** Comparison of survival among 60 subjects with s*EGFR* mutations with and without *TP53* mutation. **B,** Comparison of survival among 49 subjects with s*EGFR* mutations with a disruptive *TP53* versus those without any *TP53* mutation. **C,** Comparison of survival among 83 subjects with s*EGFR*, *ALK*r, or *ROS1*r mutations with and without *TP53* mutation. **D,** Comparison of survival among 71 subjects with s*EGFR*, *ALK*r, or *ROS1*r mutations with a disruptive *TP53* versus those without any *TP53* mutation.

used currently in the United States, so this information is often available to clinicians. The molecular basis that underlies the potential prognostic value of TP53 mutations in this setting is not certain. Mechanistically, we suggest that *TP53* mutation leads to genome instability in lung adenocarcinoma and, thus, may accelerate the development of multiple mechanisms of resistance to targeted therapy in these patients, leading to shorter survival (46).

Although MPS is a powerful and informative technology now in wide use for lung cancer care, caution is appropriate in the interpretation of reported findings. In this dataset, we performed manual curation to review findings in *TP53*, *STK11*, and *PTEN*, due to the inclusion of both germline variants and artifacts in the initial molecular reports (and/or variant call files). Although automated approaches to this review process may be helpful, careful review by a knowledgeable human expert is still required at this time. Nonetheless, we strongly advocate MPS analysis of lung adenocarcinoma specimens for all patients with advanced disease, as it is the most efficient means to rapidly identify diverse driver mutations, enabling access to a broader portfolio of targeted therapies. We note that the panel of genes with proven targetability continues to expand, with the most recent additions being *veBRAF* and *MET* exon 14 skipping mutations (7, 8, 47–51).

We observed that the presence of a s*EGFR*, *ALK*r, or *ROS1* alteration that was treated with targeted therapy led to benefit in both smoking and never smoking populations of equivalent magnitude. Although these targetable alterations are much more

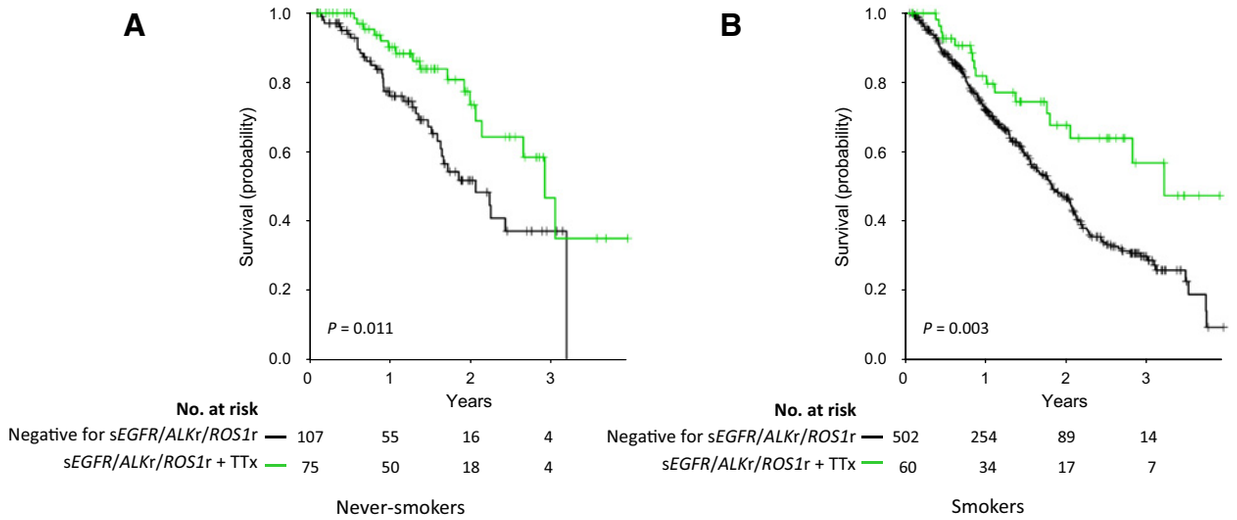

Figure 4.

Survival comparisons among subjects with s*EGFR*, *ALK*r, or *ROS1*r mutations according to smoking status. **A,** Survival of never smokers without s*EGFR-ALKr-ROS1*r mutation, or with s*EGFR-ALKr-ROS1*r mutation who received targeted therapy. **B,** Survival of former and current smokers without s*EGFR-ALKr-ROS1*r mutation, or with s*EGFR-ALKr-ROS1*r mutation who received targeted therapy.

prevalent in never smokers, to our knowledge, this is the first study to directly compare outcomes between smokers and never smokers. These findings underscore the importance of testing patients regardless of smoking history, as all patients with a targetable alteration, such as s*EGFR*, *ALK*r, or *ROS1*r, stand to benefit from targeted therapy.

Disclosure of Potential Conflicts of Interest

D.L. Aisner is a consultant/advisory board member for AbbVie, Bristol-Myers Squibb, and Inivata and reports receiving commercial research support from Genentech. L.C. Villaruz is a consultant/advisory board member for Pfizer. K. Politi is an inventor on a patent that was licensed by MSKCC to Molecular MD, is a consultant/advisory board member for AstaZeneca, Merck, Novartis, and Tocagen, and reports receiving commercial research grants from AstraZeneca and Roche. E. Garon reports receiving commercial research support from AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Genentech, Merck, Mirati, Novartis, and Pfizer. B.E. Johnson reports receiving commercial research grants from Novartis and Toshiba. M.G. Kris is a consultant/advisory board member for AstraZeneca. D.J. Kwiatkowski is a consultant/advisory board member for AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

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