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Targeted Tissue and Cell-Free Tumor DNA Sequencing of Advanced Lung Squamous-Cell Carcinoma Reveals Clinically Significant Prevalence of Actionable Alterations

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Abstract

Actionable alterations were identified in 10.5% of patients (primarily by cell-free circulating DNA) from a real-world cohort of primarily advanced-stage lung squamous-cell carcinoma (LUSC). Objective response was observed in all 3 evaluable patients who received targeted therapy. Accurate histopathologic assessment in advanced LUSC can be challenging. Evaluating the genomic landscape in this setting is warranted to potentially identify underappreciated treatment options.

Background: Major guidelines do not recommend routine molecular profiling of lung squamous-cell carcinoma (LUSC) because the prevalence of actionable alterations is thought to be low. Increased utilization of next-generation sequencing (NGS), particularly with cell-free circulating tumor DNA, facilitates reevaluation of this premise. **Patients and Methods:** We retrospectively evaluated the prevalence of actionable alterations in 2 distinct LUSC cohorts totaling 492 patients. A total of 410 consecutive patients with stage 3B or 4 LUSC were tested with a targeted cell-free circulating DNA NGS assay, and 82 patients with LUSC of any stage were tested with a tissue NGS cancer panel. **Results:** In the overall cohort, 467 patients (94.9%) had a diagnosis of LUSC, and 25 patients (5.1%) had mixed histology with a squamous component. A total of 10.5% of the LUSC subgroup had somatic alterations with therapeutic relevance, including in *EGFR* (2.8%), *ALK/ROS1* (1.3%), *BRAF* (1.5%), and *MET* amplification or exon 14 skipping (5.1%). Sixteen percent of patients with mixed histology had an actionable alteration. In the LUSC subgroup, 3 evaluable patients were treated with targeted therapy for an actionable alteration; all of them experienced partial response. **Conclusion:** In this large, real-world LUSC cohort, we observed a clinically significant prevalence of actionable alterations. Accurate local histopathologic assessment in advanced-stage LUSC can be challenging. Further evaluation of the genomic landscape in this setting is warranted to potentially identify underappreciated treatment options.

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Introduction

Lung squamous-cell carcinoma (LUSC) is the second most common histology (20%-25%) of non–small-cell lung cancer in the United States and worldwide.[1,2] While there have been substantial advances over the past decade in targeted treatments for lung adenocarcinoma, vascular endothelial growth factor and epidermal growth factor receptor (EGFR) monoclonal antibodies represent the only US Food and Drug Administration (FDA)-approved targeted therapies in LUSC and have provided modest survival benefit.[3,4]

Actionable genomic alterations, generally defined here as somatic alterations that have a demonstrated clinical response to FDA-approved or investigational therapies, occur in 20% to 60% of lung adenocarcinoma patients (depending on demographic factors).[5] In contrast, these alterations were historically thought to be rare in LUSC.

Currently, genomic alterations in *EGFR*, *ALK*, *ROS1*, and *BRAF* have approved targeted treatment options for non–small-cell lung cancer patients.[6] Clinical activity has also been observed with agents targeting other alterations such as *MET* amplifications and exon 14 skipping,[7,8] *BRAF* non-V600E mutations,[9] and *RET* and *NTRK* translocations.[10,11] These actionable alterations have been observed at a lower rate in LUSC, although the reported prevalence has varied across studies. In the pan–lung cancer whole-exome sequencing LUSC cohort (comprised primarily of resected specimens, including samples previously published by The Cancer Genome Atlas Research Network), actionable alterations in *EGFR* (0.8%), *MET* (exon 14 skipping [0.2%] or amplification [1%]), and *BRAF* (1.2%) appear to be uncommon.[12] In other LUSC cohorts tested by conventional molecular profiling methods such as immunohistochemistry (IHC), PCR, fluorescence in-situ hybridization (FISH), and RNA expression, *EGFR* (0%) mutations and *ALK* (0%-1.4%), *ROS1* (0%), and *RET* (0%) rearrangements were rare.[13-17] However, a much higher rate of *EGFR* sensitizing mutations have been recently reported in advanced LUSC tumors, including in 6% of stage IIIB and IV LUSC patients from LUX-8, a phase 3 clinical trial.[18] However, only half of these were *EGFR* mutations of known clinical significance. Furthermore, a separate analysis showed improved progression-free and overall survival in the subset of patients with *ERBB* gene alterations treated with a pan-*ERBB* inhibitor.[19] Additionally, 2.1% of an LUSC cohort exhibited *MET* exon 14 skipping as detected by tissue next-generation sequencing (NGS).[20]

Difficulty in the accurate histopathologic diagnosis of LUSC may be a reason for disparate findings across case series. For example, poorly differentiated adenocarcinomas with a solid growth pattern are not common but can resemble LUSC. During central pathology review, 17% of (primarily resected) tumors submitted as LUSC to The Cancer Genome Atlas project were reclassified as nonsquamous.[21] Challenges in accurate diagnosis are likely exacerbated in the metastatic setting, as reliance on small biopsy specimens may incompletely sample mixed adenosquamous histology.[13] These issues lead to high interobserver variability and have resulted in updated recommendations for IHC staining in all cases of solid adenocarcinoma and nonkeratinizing squamous-cell carcinoma.[22,23]

While current guidelines do allow for consideration of *EGFR*, *ALK*, and *ROS1* testing in cases of never smokers, small biopsy specimens, and mixed histology or in patients with other clinical features that may suggest a higher probability of an oncogenic driver (eg, younger age), routine comprehensive molecular profiling is currently not recommended for LUSC.[6,24] Notably, *BRAF* and *MET* exon 14 mutations may actually be more common in smokers.[25,26] Given increased accessibility and utilization of comprehensive NGS, particularly with cell-free circulating DNA (cfDNA), we sought to reevaluate the prevalence of actionable alterations in a primarily metastatic cohort, where histopathologic challenges are often accentuated and targeted therapies for these alterations are typically employed. Additionally, we aimed to characterize the pattern of single nucleotide variants (SNVs) and copy number amplifications (CNAs) obtained by targeted cfDNA NGS and compare it to the previously described genomic landscape of LUSC.

Patients and Methods

Study Design

We retrospectively evaluated the prevalence of actionable alterations in 2 distinct LUSC cohorts totaling 492 patients. The first cohort consisted of 410 consecutive cfDNA-tested patients with stage 3B or 4 (American Joint Committee on Cancer, 7th edition) LUSC (squamous, squamous-like, or mixed-squamous histology per submitted pathology report) from 172 community and academic practices within the United States, Asia, Europe, and the Middle East. From October 2014 to October 2016, these patients had blood samples sent for proprietary cfDNA NGS testing described in further detail below. This real-world cfDNA cohort had limited clinical annotation and no central pathology review. We retrospectively analyzed a second cohort consisting of 82 consecutive LUSC patients of any stage (no mixed histology), with detailed clinicopathologic annotation, that were seen at MD Anderson Cancer Center between February 2010 and February 2017 and had tissue tested by a Clinical Laboratory Improvement Amendments (CLIA)-certified institutional 50- to 134-gene NGS panel and selected PCR and/or FISH testing.

Actionable alterations were specifically defined in this study as those that have at least Level 3B (compelling clinical evidence for predictive response to the biomarker in another indication) or Level R1 (biomarker predictive of resistance to standard of care drug in this indication) evidence by proprietary annotation (N-of-One Inc, Concord, MA), using Levels of Evidence terminology as defined in the open access OncoKB database.[27] Supplemental Table 1 in the online version details the specific actionable alterations that were detected in this study.

Tissue Tumor DNA Assay

Molecular profiling of tissue samples in the MD Anderson Cancer Center (MDACC) cohort was obtained by CLIA-certified institutional NGS tests. The majority of cases in this tissue cohort (85%) were tested with a previously described 46 gene hotspot panel.[28] In general, 10 ng of formalin-fixed, paraffin-embedded DNA was used as template to amplify 740 mutational hotspots using the Ion Torrent AmpliSeq cancer panel and Ion Torrent Personal Genome Machine (Thermo Fisher Scientific; Waltham, MA). Cases after January 4, 2016 (with formalin-fixed, paraffin-embedded DNA of at least 20 ng) were analyzed with the

Oncomine Cancer Panel (Thermo Fisher Scientific; Waltham, MA) at an average amplicon depth of at least 100×, providing critical exon coverage of 143 genes for the detection of SNVs and indels and CNAs of 49 genes.[29] *ALK* and *ROS1* rearrangements were separately detected by FISH break-apart probes.

Cell-Free Circulating Tumor DNA Assay

Guardant 360 is a proprietary cfDNA NGS assay that detects SNVs of 54 to 70 genes as well as selected actionable or informative CNAs, indels, and fusions (Supplemental Table 2 in the online version).[30-32] Samples tested during October 2014 to January 2015 were analyzed on a 54-gene panel; from February to October 2015 by a 68-gene panel; and after October 2015 by a 70-gene panel. At least 5 ng of cell free DNA was required for hybrid capture-based next generation paired-end sequencing of 160 to 170 bp DNA strands with average coverage of 8000× to 15,000×, depending on the test version. Germline variants were quantitatively excluded, as previously described.[30]

Circulating Tumor DNA Landscape

In order to compare the landscape of all nonsynonymous alterations detected by the cfDNA assay with the known tissue landscape of LUSC, we accessed SNV, indel, and copy-number variation data from the pan–lung cancer cohort of 484 LUSC patients via cBioPortal.[12,33,34]

Statistical Analysis

We tested categorical associations (eg, sex) between patients with and without actionable alterations by the Fisher exact test. For SNVs and CNAs of greatest significance in LUSC, we evaluated the frequency correlation between our cfDNA cohort and the pan–lung cancer cohort by the Spearman rank test. All statistical tests were 2 sided. Two-tailed $P < .05$ was considered to be statistically significant. Statistical analyses were conducted using GraphPad Prism 7 software. This retrospective study was conducted in accordance with the institutional review board at the University of Texas MD Anderson Cancer Center.

Results

Baseline Characteristics

Table 1 shows the baseline clinical characteristics of the patients in this study. Generally consistent with known epidemiology of LUSC, most of the patients in our study were male (60.8%) and with median age of 69 at time of testing. Smoking history was not reported in the cfDNA cohort, but patients in the MDACC cohort had a median 42 pack-year smoking history. The overwhelming majority of the patients in our study had metastatic disease (94.9%) and no mixed histology per their formal pathology report (94.9%). Central pathology review was not performed in this study. Patients in the MDACC cohort were generally diagnosed by morphology only.

Most cfDNA samples (75.5%) were analyzed with the 70-gene panel. The vast majority of cfDNA patients (96.3%) were tested at only a single time point. The cfDNA sample was drawn a median of 284 days (and average of 499 days) from the time of diagnosis (data available for 46% of cases). A total of 379 cfDNA patients (92.4%) had at least one somatic alteration detected.

Table 1 Baseline Characteristics of Overall Study Cohort

Characteristic	cfDNA (N = 410)	MDACC (N = 82)	Total (N = 492)
Age (y), median (range)	69 (34-96)	68 (32-84)	69 (32-96)
Sex			
Male	253 (61.7)	46 (56.1)	299 (60.8)
Female	157 (38.3)	36 (43.9)	193 (39.2)
Pack-years, median (range)	NR	42 (0-125)	NR
Stage			
I or II	0 (0)	25 (30.5)	25 (5.1)
III or IV	410 (100)	57 (69.5)	467 (94.9)
Histology			
Squamous	385 (93.9)	82 (100)	467 (94.9)
Adenosquamous	18 (4.4)	0 (0)	18 (3.7)
Other mixed squamous	7 (1.7)	0 (0)	7 (1.4)

Data are presented as n (%) unless otherwise indicated.
Abbreviations: cfDNA = cell-free circulating DNA; MDACC = MD Anderson Cancer Center; NR = not reported.

Actionable Alterations in LUSC Subgroup

The prevalence of patients with actionable alterations in our overall study cohort was 10.0%. This includes a small number of cases with mixed-squamous histology, as described below. In order to best address the clinically relevant question of actionable alterations in LUSC, we restricted our major analyses to cases that had no mention of mixed histology (ie, the LUSC subgroup).

When considering only the 467 patients in the LUSC subgroup, 10.5% (cfDNA 12.2%, MDACC 8.5%) had somatic alterations with therapeutic relevance (Table 2). The median age (68) of these patients with actionable alterations is consistent with that of the overall study cohort. There was a trend towards an association between female sex and the presence of actionable alterations in squamous patients (hazard ratio = 1.70; 95% confidence interval, 0.92-3.05; $P = .09$). MDACC squamous patients with actionable alterations collectively had a median smoking history of 25 pack-years. *MET* amplifications and *EGFR* SNVs/indels were the most common actionable alterations identified (4.3% and 2.8%, respectively). Activating *BRAF* mutations were next most common (1.5% of patients) though none with V600E, followed by *MET* exon 14 skipping (1.3%), *ALK* fusions (0.9%), and *ROS1* fusions (0.4%). *RET* fusions were not detected in the cfDNA cohort and were not tested in the MDACC cohort. Notably, *ALK* rearrangements and *EGFR* alterations were most common (combined 4.9% of patients) in the MDACC cohort. Of the 7 patients with actionable alterations in the MDACC cohort, molecular profiling was performed at time of diagnosis and upon metastatic recurrence for 5 and 2 patients, respectively. All of these patients had molecular testing on tissue obtained from their initial diagnosis or resection. None had prior targeted therapy.

Concomitant actionable alterations were rare (Figure 1). In the cfDNA cohort, there were 5 patients with actionable comutations (1.1% of LUSC subgroup), all involving *EGFR* alterations. The *EGFR* coalterations were primarily *EGFR* exon 19 deletions comutated with T790M and/or C797S resistance mutations. Another patient had *EGFR* L858R/T790M and *MET*

Table 2 Clinical and Molecular Characteristics of LUSC Patients With Actionable Alterations

Characteristic	cfDNA (N = 42)	MDACC (N = 7)	Total (N = 49)
Age (y), median (range)	67 (44-90)	68 (53-83)	68 (44-90)
Sex			
Male	21 (50.0)	3 (42.9)	24 (49.0)
Without actionable alterations	215 (62.7)	44 (58.7)	259 (62.0)
Female	21 (50.0)	4 (57.1)	25 (51.0)
Without actionable alterations	128 (37.3)	31 (41.3)	159 (38.0)
Smoking status			
Never-smoker		2 (28.6)	
≥ 30 pack-years		3 (42.9)	
<30 pack-years		2 (28.6)	
Pack-years, median (range)	NR	25 (0-38)	NR
Stage			
I or II	0	2 (28.6)	2 (3.7)
III or IV	42 (100.0)	5 (71.4)	52 (96.3)
Alterations[a]			
EGFR activating SNV or indel	11 (2.9)	2 (2.4)	13 (2.8)
ALK fusion	2 (0.5)	2 (2.4)	4 (0.9)
ROS1 fusion	1 (0.3)	1 (1.2)	2 (0.4)
MET exon 14 skipping[b]	4 (1.0)	NR	4 (1.3)
MET amplification	19 (4.9)	1 (1.2)	20 (4.3)
BRAF SNV (non-V600E)	6 (1.6)	1 (1.2)	7 (1.5)
RET fusion	0	NR	0

Data are presented as n (%) unless otherwise indicated.
Abbreviations: cfDNA = cell-free circulating DNA; MDACC = MD Anderson Cancer Center; NR = not reported; SNV = single nucleotide variant.
[a]Percentage of patients with only squamous histology in each cohort; sum may be greater than number of patients with actionable alterations in each cohort because of comutations.
[b]Adjusted percentage to reflect patients for which testing was actually available. Testing for *MET* exon 14 skipping available only with 70-gene panel of G360 and not available in MDACC NGS panel.

amplification. We also observed one instance of *EGFR* G719A/S768I comutation. No concomitant *MET* exon 14 skipping and amplification was observed. Comutations were not observed in the MDACC cohort.

Minor Impact of Mixed-Squamous Histology

The MDACC cohort had no cases of mixed histology. There was a small percentage of patients with mixed-squamous histology in the cfDNA cohort (25 patients, 6.1%). Seventy-two percent of these cases were adenosquamous (3.7% of overall study cohort). The rest were classified as mixed glandular and squamous (3 patients), neuroendocrine and squamous (1 patient), large cell carcinoma and squamous (2 patients), and poorly differentiated squamous with possible urothelial features (overall clinical picture consistent with lung primary; 1 patient). In this mixed-squamous subset of 25 patients, we observed 6 actionable alterations in 4 patients (16% of mixed histology patients). All of those alterations had adenosquamous histology and involved *EGFR* SNVs or indels, including 2 patients with T790M concurrent with their driver mutation.

Clinical Response to Targeted Therapy

Clinical outcome data were generally not available for patients in the cfDNA cohort. However, we were able to obtain clinical details for a select patient in that cohort (Supplemental Table 3 in the online version). This patient had IHC-confirmed LUSC (TTF-1 negative, p40 and p63 positive) and cfDNA-detected *EZR-ROS1* fusion that was subsequently verified by tissue NGS analysis. She had a major partial response, per Response Evaluation Criteria in Solid Tumors v1.1, to crizotinib that has persisted for over 10 months.[35] For the 7 patients in the MDACC cohort with actionable mutations, 4 have not yet been treated with the associated targeted therapy due to adequate disease control from other treatment. Of the patients who have been treated with targeted therapy, one patient had very limited *ALK* inhibitor exposure because of intractable drug related gastrointestinal toxicity and was considered not to be evaluable for the purposes of this analysis. Two patients with separate sensitizing *EGFR* exon 19 deletions (detected in both cfDNA and tissue) achieved partial response. The first of these patients had a total of 23 months of disease control on erlotinib and then afatinib. The second patient recently started erlotinib, with partial response after 2 months.

Tissue and cfDNA NGS Concordance

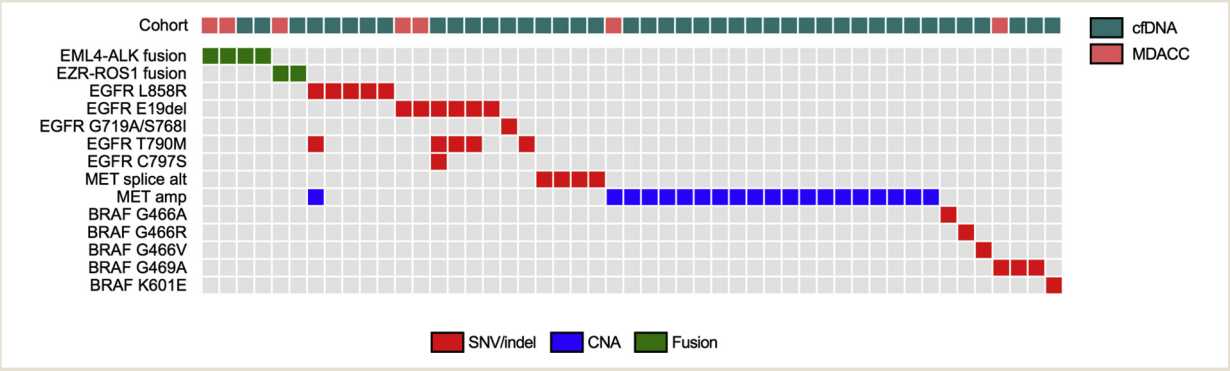
Previous retrospective studies (including one with over 15,000 patients) have demonstrated concordance between tissue and cfDNA NGS-detected alterations (tested on the Guardant platform) in the range of 70% to 87%.[31,36-38] Due to the limited number of patients in our overall cohort that had both tissue and cfDNA NGS testing, concordance analysis was not possible in this study. For all 3 cases in our cohort that did have tissue and cfDNA NGS results, the respective driver alteration was detected by both modalities (Supplemental Table 3 in the online version).

Somatic cfDNA Landscape of LUSC

Overall, the frequency of SNVs and CNAs in cfDNA positively correlates with the pan−lung cancer LUSC cohort (Spearman $r = 0.62$, $P < .001$). All nonsynonymous SNVs were included in this comparison, regardless of clinical relevance. In cfDNA, the most commonly observed SNVs were in *TP53* (62.6%), *NF1* (13.5%), *PIK3CA* (12.2%), *ARID1A* (11.9%), and *EGFR* (11.7%). Except for *EGFR*, all of these frequently mutated SNVs in the cfDNA cohort (frequency > 10%) are also considered to be particularly relevant in LUSC (q value < 0.1 per MutSig2CV analysis in pan−lung cancer LUSC data set) (Figure 2A). The frequency of SNVs in cfDNA correlates with the pan−lung cancer LUSC cohort with near statistical significance (Spearman $r = 0.59$, $P = .06$, Figure 2B).

The CNAs tested in our cfDNA panel that are considered significant in LUSC (q value < 0.25 per GISTIC 2.0 analysis in the pan−lung cancer LUSC data set) are *EGFR*, *FGFR1*, *MYC*, *CCNE1*, *CDK6*, *ERBB2*, *PDGFRA*, *CCND1*, *KIT* (Figure 3A). Amplifications in *PDGFRA*, *CCND1*, *KIT* were not frequently seen. The frequency of CNAs correlates between cfDNA and the

Figure 1 Lung Squamous Patients With at Least One Actionable Alteration Detected by Comprehensive Next-Generation Sequencing



pan–lung cancer LUSC tissue landscape (Spearman $r = 0.53$, $P = .02$, Figure 3B).

Discussion

Actionable driver alterations in genes such as *EGFR*, *ALK*, and *KRAS* are known to be present at lower rates in LUSC than LUAD, although the existing data are derived primarily from surgically resected, early stage LUSC, typically with central pathology review. There are limited data regarding the frequency of targetable alterations in advanced LUSC patients diagnosed in a real-world, or predominantly community-based, setting. Molecular profiling is not routinely obtained for LUSC and the use of targeted therapies directed at genomic alterations has been limited in this histology. However, the increased utilization of comprehensive NGS by cfDNA in clinical practice presents an opportunity to profile advanced-stage disease in a large-scale manner and facilitates reexamination of the prevalence of actionable alterations in LUSC.

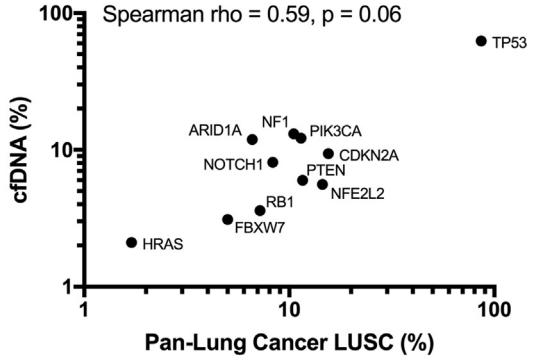
The real-world population assessed here differs in important ways from the predominantly early stage, surgically resected tumors reported in the pan–lung cancer project. In this study, the lack of central pathology review and the dependence on generally more limited tissue specimens increases the likelihood of greater variability in the diagnosis of LUSC. This is more representative of the range of advanced LUSC tumors that are treated with systemic therapies in clinical practice. Well-known challenges in the histopathologic diagnosis of LUSC suggest that the true prevalence of actionable alterations may be lower than what was observed in our real-world cohort. However, the prevalence of actionable alterations in the tertiary-care, single-institution subgroup (MDACC cohort) is comparable to that of the cfDNA cohort, suggesting that any reclassification of

Figure 2 Frequency of Significant SNVs in cfDNA ctDNA Cohort Compared to Pan–Lung Cancer LUSC. Significance Was Defined as MutSig2CV $q < 0.1$ in Pan–lung Cancer LUSC. (A) SNV Frequency (Ordered by Decreasing Significance). Bold SNVs Are Frequent (> 10%) in cfDNA. (B) Spearman Correlation for Significant SNVs Detectable by cfDNA

A

SNV	cfDNA (%)	Pan-Lung LUSC (%)
TP53	**62.6**	**86.4**
CDKN2A	9.4	15.5
NFE2L2	5.6	14.5
PTEN	6.0	11.6
MLL2	NR	23.6
RB1	3.6	7.2
FAT1	NR	14.3
NOTCH1	8.1	8.3
RASA1	NR	6.0
NF1	**13.1**	**10.5**
ARID1A	**11.9**	**6.6**
KDM6A	NR	5.0
PIK3CA	**12.2**	**11.4**
CUL3	NR	5.0
HRAS	2.1	1.7
IRF6	NR	2.9
FBXW7	3.1	5.0
ARHGAP35	NR	5.8
PASK	NR	3.9
NSD1	NR	5.8

B



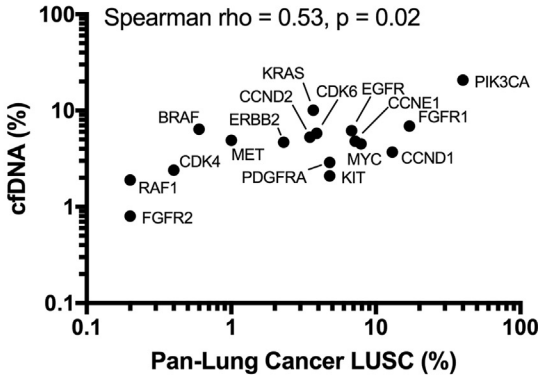
Abbreviations: cfDNA = cell-free circulating DNA; ctDNA = circulating tumor DNA; LUSC = lung squamous-cell carcinoma; SNV = single nucleotide variant.

Figure 3 Frequency of CNAs Detectable in cfDNA Compared to Pan-Lung Cancer LUSC. (A) cfDNA CNA Frequency. Bold CNAs Are Considered Significant in LUSC ($q < 0.25$ per GISTIC Analysis In Pan—Lung Cancer LUSC). (B) Spearman's Correlation For CNAs Detectable By cfDNA. AR Not Shown (Not Amplified In Pan—Lung Cancer LUSC) But Is Included In Correlation Analysis

A

CNA	cfDNA (%)	Pan-Lung LUSC (%)
PIK3CA	20.7	40.1
KRAS	10.1	3.7
FGFR1	**6.9**	**17.1**
BRAF	6.4	0.6
EGFR	**6.2**	**6.8**
CDK6	**5.8**	**3.9**
CCND2	5.3	3.5
MET	4.9	1.0
CCNE1	**4.8**	**7.2**
ERBB2	**4.7**	**2.3**
MYC	**4.5**	**7.9**
CCND1	**3.7**	**13.0**
PDGFRA	**2.9**	**4.8**
CDK4	2.4	0.4
KIT	**2.1**	**4.8**
RAF1	1.9	0.2
AR	1.1	0
FGFR2	0.8	0.2

B



Abbreviations: AR = androgen receptor; cfDNA = cell-free circulating DNA; CNA = copy number amplification; LUSC = LUSC = lung squamous-cell carcinoma.

histology upon central testing likely would not significantly impact the overall magnitude and clinical implications of our findings. If there was significant reclassification of histology in the study cohort, this would underscore the on-going difficulties in LUSC diagnosis in advanced-stage disease and the potential peril of limiting genomic profiling in patients diagnosed with this histology.

We only have limited patient-level data for the cfDNA cohort that comprises the majority of our study. Thus, we are not able to make major conclusions about other clinical characteristics that may be associated with actionable alterations in LUSC (such as smoking history) or comprehensively assess clinical outcomes for patients who may have been treated with appropriate targeted therapy. Three patients in the cohort that have been treated with targeted therapy and were evaluable for response; all 3 had measurable disease response.

Compared to prior LUSC studies that were limited by conventional molecular profiling or focused on resected/early-stage disease, our cohort of primarily advanced-stage LUSC patients tested mostly with cfDNA NGS may better represent the impact of metastatic disease, intratumor heterogeneity, and practical challenges in the histopathologic diagnosis of LUSC. The clinically significant prevalence of actionable alterations that we observed suggest that there are underappreciated treatment options in patients diagnosed with LUSC in the real-world setting. This hypothesis will be directly evaluated in the on-going Lung Cancer Master Protocol (Lung-MAP), which includes genomic profiling from advanced LUSC patients (using both cfDNA and tissue NGS) in a clinical trial framework testing different targeted and immune therapies.[39]

Conclusion

Targeted therapy in LUSC is an unmet need. In our cohort of LUSC patients, comprehensive NGS revealed a clinically significant prevalence of actionable alterations in 10.5% of patients. Additionally, we observed that SNVs and CNAs from cfDNA profiling were largely consistent with the known tissue LUSC landscape. Our findings suggest that routine genomic profiling in advanced LUSC merits further evaluation to potentially uncover underappreciated treatment options in a setting where accurate histopathologic assessment has well-known challenges. Moreover, advanced-stage LUSC patients should continue to be included in appropriate matched therapy studies in order to build upon these findings and expand the overall understanding of outcomes from targeting molecular driver alterations in LUSC.

Clinical Practice Points

- Major guidelines currently do not recommend routine molecular profiling for LUSC.
- Actionable alterations in LUSC are thought to be rare. However, those data are primarily derived from resected specimens tested at large academic centers.
- There are limited data regarding the frequency of targetable alterations in advanced LUSC patients diagnosed in a real-world setting. This is relevant because there are well-known challenges in the histopathologic diagnosis of LUSC.
- We report a clinically significant prevalence of actionable alterations in a large, real-world LUSC cohort.
- These alterations represent underappreciated treatment options and suggest that the prevalence actionable alterations in advanced LUSC should be further evaluated.

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Supplemental Data

Supplemental tables accompanying this article can be found in the online version https://doi.org/10.1016/j.cllc.2018.08.020.

Disclosure

K.C.B., R.B.L., and A.T. are employees with stock ownership in Guardant Health Inc. N.P. consults for and receives honoraria from Guardant Health Inc. J.V.H. is an advisory board member for Guardant Health Inc. The other authors have stated that they have no conflict of interest.

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Supplemental Data

Supplemental Table 1 Actionable Alterations Detected

Alteration	Level of Evidence
EGFR	
Exon 19 del	1[a]
L858R	1
G719A	1
T790M	R1[b]
C797S	R1
S768I	1
EML4-ALK fusion	1
EZR-ROS1 fusion	1
MET	
Exon 14 skipping	2A[c]
Amplification	2A
BRAF	
K601E	3B[d]
G466A/R/V	3B
G469A	3B

[a]US Food and Drug Administration (FDA)-recognized biomarkers that are predictive of response to FDA-approved drug in this indication.
[b]Standard of care biomarkers that are predictive of resistance to FDA-approved drug in this indication.
[c]Standard of care biomarkers that are predictive of response to FDA-approved drug in this indication.
[d]Biomarkers that are predictive of response to novel targeted agents that have shown promising results in clinical trials in another indication.

Supplemental Table 2 Guardant cfDNA Gene Panels

Alteration Type	Jun 2014 to Feb 2015 Panel Composition (54 Genes Total)						Mar 2015 to Oct 2015 Additions (68 Genes Total)				Nov 2015 to Apr 2016 Additions (70 Genes Total)	
SNVs	*ABL1*	*AKT1*	*ALK*	*APC*	*AR*	*ATM*	*ARAF*	*CCNE1*	*MAP2K1*	*RHEB*	*RB1*	*TSC1*
	BRAF	*CDH1*	*CDKN2A*	*CSF1R*	*CTNNB1*	*EGFR*	*ARID1A*	*CDK4*	*MAP2K2*	*RHOA*		
	ERBB2	*ERBB4*	*EZH2*	*FBXW7*	*FGFR1*	*FGFR2*	*BRCA1*	*CDK6*	*NF1*	*RIT1*		
	FGFR3	*FLT3*	*GNA11*	*GNAQ*	*GNAS*	*HNF1A*	*BRCA2*	*CDKN2B*	*NFE2L2*	*ROS1*		
	HRAS	*IDH1*	*IDH2*	*JAK2*	*JAK3*	*KDR*	*CCDN1*	*ESR1*	*NTRK1*			
	KIT	*KRAS*	*MET*	*MLH1*	*MPL*	*MYC*	*CCND2*	*GATA3*	*RAF1*			
	NOTCH1	*NPM1*	*NRAS*	*PDGFRA*	*PIK3CA*	*PROC*						
	PTEN	*PTPN11*	*RB1*	*RET*	*SMAD4*	*SMARCB1*						
	SMO	*SRC*	*STK11*	*TERT*	*TP53*	*VHL*						
CNAs	*EGFR*	*ERBB2*	*MET*				*AR*	*BRAF*	*CCNE1*	*CDK4*	*CCND1*	*CCND1*
							CDK8	*FGFR1*	*FGFR2*	*KIT*		
							KRAS	*MYC*	*PDGFRA*	*PIK3CA*		
							RAF1					
Fusions	None						*ALK*	*RET*	*ROS1*	*NTRK1*	*FGFR2*	*FGFR3*
Indels	None						*EGFR*				*ERBB2*	*MET*

Abbreviations: cfDNA = cell-free circulating DNA; CNA = copy number amplification; SNV = single nucleotide variant.

Supplemental Table 3 Clinical Details of Patients in MDACC Cohort With Actionable Alterations and a Selected cfDNA Cohort Patient

Age (y)	Sex	Smoking (Pack-Years)	Stage	Alteration	Results of Tissue IHC/Molecular Analysis	Best Response to Targeted Therapy	Clinical Course
MDACC Cohort							
68	F	25	IIa	*ALK* fusion	*ALK* fusion confirmed by FISH	NA	NED after resection then adjuvant chemotherapy
55	M	38	IV	*ALK* fusion	*ALK* fusion confirmed by FISH	PD	1L chemotherapy then 2L crizotinib and ceritinib, very limited duration of treatment due to intolerance
67	F	25	IIIa	*BRAF* G469A	*BRAF* alteration confirmed in tissue NGS	NA	NED after definitive chemoradiation
74	M	30	IV	*ROS1* fusion	*ROS1* fusion confirmed by FISH	NA	Metastatic recurrence after definitive trimodal treatment; SD on nivolumab
69	M	50	IV	*MET* amp	*MET* copy number 5.7, *MET*:*CC7* 1.3 by FISH	NA	1L chemotherapy then 2L pembrolizumab with PR
53	F	0	IV	*EGFR* ex19del[a]	TTF-1 negative; p40, p63, CK5/6 positive; *EGFR* alteration confirmed in tissue NGS	PR	1L erlotinib (13 mo) with PR, then 2L afatinib (10 mo) with metabolic response before PD
83	F	0	IV	*EGFR* ex19del	TTF-1, Napsin A negative; p40, CK5/6 positive; *EGFR* alteration confirmed in tissue NGS	PR	1L reduced dose erlotinib with PR × 7 mo then PD. Repeat biopsy affirmed LUSC but now with T790M. Recently started on osimertinib
cfDNA Cohort							
63	F	0	IV	*ROS1* fusion	TTF-1 negative; p40, p63 positive. *EZR-ROS1* fusion confirmed in tissue NGS	PR	Dramatic response to 1L crizotinib within 3 wk and continues to have PR and metabolic CR (10 mo)

Abbreviations: cfDNA = cell-free circulating DNA; CR = complete response; FISH = fluorescence in-situ hybridization; IHC = immunohistochemistry; MDACC = MD Anderson Cancer Center; NA = not applicable; NED = no evidence of disease; NGS = next-generation sequencing; PD = progressive disease; PR = partial response; SD = stable disease.