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ORIGINAL RESEARCH

The Impact of Prior Single-Gene Testing on Comprehensive Genomic Profiling Results for Patients with Non-Small Cell Lung Cancer

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ABSTRACT

Introduction: Tissue-based broad molecular profiling of guideline-recommended biomarkers is advised for the therapeutic management of patients with non-small cell lung cancer (NSCLC). However, practice variation can affect whether all indicated biomarkers are tested. We aimed to evaluate the impact of common single-gene testing (SGT) on subsequent comprehensive genomic profiling (CGP) test outcomes and results in NSCLC.

Methods: Oncologists who ordered SGT for guideline-recommended biomarkers in NSCLC patients were prospectively contacted (May–December 2022) and offered CGP (DNA and RNA sequencing), either following receipt

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of negative SGT findings, or instead of SGT for each patient. We describe SGT patterns and compare CGP completion rates, turnaround time, and recommended biomarker detection for NSCLC patients with and without prior negative SGT results.

Results: Oncologists in > 80 community practices ordered CGP for 561 NSCLC patients; 135 patients (27%) first had negative results from 30 different SGT combinations; 84% included *ALK*, *EGFR* and PD-L1, while only 3% of orders included all available SGTs for guideline-recommended genes. Among patients with negative SGT results, CGP was attempted using the same tissue specimen 90% of the time. There were also significantly more CGP order cancellations due to tissue insufficiency (17% vs. 7%), DNA sequencing failures (13% vs. 8%), and turnaround time > 14 days (62% vs. 29%) than among patients who only had CGP. Forty-six percent of patients with negative prior SGT had positive CGP results for recommended biomarkers, including targetable genomic variants in genes beyond *ALK* and *EGFR*, such as *ERBB2*, *KRAS* (non-G12C), *MET* (exon 14 skipping), *NTRK2/3*, and *RET* .

Conclusion: For patients with NSCLC, initial use of SGT increases subsequent CGP test cancellations, turnaround time, and the likelihood of incomplete molecular profiling for guideline-recommended biomarkers due to tissue insufficiency.

PLAIN LANGUAGE SUMMARY

Patients with non-small cell lung cancer (NSCLC) should have their tumor tissue tested for all recommended biomarkers that can help identify their best treatment options. Traditional tests look at gene biomarkers one by one (single-gene testing), and doctors can order some or all these tests individually or in a group. However, some recommended biomarkers cannot be tested by traditional single-gene tests at all. Newer technology (next-generation sequencing) covers all current recommended treatment biomarkers in one test (comprehensive genomic profiling), but this testing is more expensive and can take more time. Our study shows that NSCLC patients do not get all recommended treatment biomarkers tested when a single-gene testing approach is taken. Single-gene testing also used up some patients' tumor tissue entirely, such that further testing by comprehensive genomic profiling could not be done at all (17% vs. 7% for patients with no prior single-gene tests), resulted in more sequencing failures (13% vs. 8%), and had turnaround time for results greater than 14 days for more patients (62% vs. 29%). When comprehensive genomic profiling was completed, 46% of patients with negative results from prior single-gene testing had positive results for recommended treatment biomarkers that were not included in the initial single-gene tests. To ensure that NSCLC patients receive testing for all recommended biomarkers, comprehensive genomic profiling must be performed first.

Keywords: Comprehensive genomic profiling; Molecular diagnostics; Non-small cell lung cancer; Precision oncology

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Key Summary Points

Why carry out the study?

- Several approaches can be taken to guideline-recommended tissue-based broad molecular profiling for therapy selection in patients with advanced and metastatic non-small cell lung cancer (NSCLC).
- The efficacy of using a sequential testing approach—initial single-gene testing (SGT) followed by comprehensive genomic profiling (CGP)—has not been described.
- In our study, no NSCLC patients met guideline recommendations for biomarker testing by initial SGT alone.

What was learned from the study?

- Compared with NSCLC patients with only CGP performed, significantly more patients initially tested by SGT with negative results failed subsequent CGP DNA sequencing, or had delayed CGP results due to tissue depletion.
- Among successfully tested NSCLC patients, CGP identified a similarly high rate of guideline-recommended therapeutic targets between patients with prior negative SGT (46%), relative to patients with CGP only (53%), and should be performed first.

INTRODUCTION

Professional practice guidelines in oncology advocate for tissue-based broad molecular profiling at diagnosis to inform the therapeutic management of patients with advanced and metastatic non-small cell lung cancer (NSCLC). However, balancing limited tissue with the recommended testing for a growing list of biomarkers, including genomic variants in *ALK*, *BRAF*, *EGFR*, *KRAS*, *MET*, *NTRK1/2/3*, *RET*, and *ROS1*, and PD-L1 expression, presents a common clinical challenge. Guidelines are not prescriptive in one approach, and endorse several potential testing strategies including the use of a single comprehensive test, combining a limited number of tests, and tiered approaches that presumptively account for negative results in some genes based on anticipated oncogenic driver mutual exclusivity (between *KRAS* and other driver genes, for example) [1].

Comprehensive genomic profiling (CGP) by next-generation sequencing allows providers to simultaneously evaluate tumor tissue for all major genomic variant types (mutations, copy number alterations, rearrangements) in oncogenes recommended for testing that have Food and Drug Administration (FDA)-approved targeted therapies, as well those with emerging and potential clinical significance. Adoption of CGP by oncologists as standard-of-care testing in NSCLC has been gradual, particularly in the community setting [2, 3]. Several factors limiting CGP utilization have been cited in qualitative studies, including lack of coverage by commercial insurers, lengthy turnaround time, and reasons that can be collectively labeled as greater complexity, including interpretation of findings [4–8]. As such, oncologists continue to selectively use single-gene tests (SGT) that are individually less costly, easier to interpret, and have historically had faster turnaround time than CGP. Compared with CGP, however, these tests have inherent methodological limitations that can contribute to decreased sensitivity, and can therefore miss clinically significant variants [9, 10].

Retrospective real-world electronic health record studies confirm there is wide variation in molecular testing patterns for NSCLC in clinical practice. Over time, incremental increases in the utilization of *any* guideline-recommended biomarker testing in NSCLC have been observed, yet under-testing (not all eligible patients are tested) and incomplete testing (not all indicated biomarkers are tested) persist relative to recommendations [11–13]. These important studies provide summary estimates of biomarker positivity rates, typically at the gene level, for patients with NSCLC. However, descriptions of the specific tests performed, and

importantly, the variants those tests are analytically validated to detect, and which serve as the true denominator for biomarker prevalence, are not typically captured in electronic medical records. This is a major limitation of these data sources in accurately assessing the extent to which broad molecular profiling was attempted or achieved for eligible patients.

The fundamental prerequisite for successful broad molecular profiling in NSCLC is the availability of adequate and viable tumor tissue for testing [14, 15]. A recent data modeling study underscored the critical importance of tissue stewardship in testing. Results estimated that nearly two-thirds (64%) of newly diagnosed patients with NSCLC do not benefit from precision oncology, which could be attributed to problems with tissue procurement and quality (21%), under-testing, incomplete testing, and insurance problems (14%), assay failures and lengthy turnaround time (14%), and failure to administer biomarker-directed treatment despite test results (15%) [16]. As with other real-world electronic health record studies, however, differences attributable to specific tests and modalities like SGT and CGP were not addressed. Therefore, in the current study, we utilized prospectively collected reference laboratory data to compare two common approaches to broad molecular profiling by CGP in clinical practice for NSCLC—ordering SGT first (followed by CGP when SGT results are negative), versus only ordering CGP. As seen in Fig. 1, using SGT prior to CGP can require more than 50 slides if all tests are ordered, compared with 20 slides for CGP alone, and creates additional waste from facing the formalin-fixed, paraffin-embedded (FFPE) block prior to each use of the tissue. Given these tissue requirements, we specifically aimed to quantify the impact of an "SGT first" strategy on CGP completion rates and detected genomic findings.

METHODS

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Western Institutional Review Board (WIRB)–Copernicus Group (no. 1340120), with the need for written informed consent waived. Beginning in May 2022, Labcorp® reference laboratory information system data were used to identify oncologists who ordered one or more single-gene tests (SGT) for patients with advanced or metastatic NSCLC during standard care. Oncologists were contacted, offered, and accepted no-cost CGP testing for their patients, either in addition to SGT once SGT results were found to be negative, or instead of SGT altogether. All SGT and CGP tests were completed by December 2022, with final reports sent to ordering oncologists following standard clinical laboratory procedure.

The SGT and CGP assays included in this study (see Supplementary Material) are analytically validated, commercially available, companion diagnostic or laboratory-developed tests performed by Labcorp® in Clinical Laboratory Improvement Amendment (CLIA)-certified laboratories in the United States. The specimen requirements for each test included in the study are available online: https://oncology.labcorp.com/cancer-care-team/test-menu. In brief, SGT included FFPE tissue-based assays for genomic alterations recommended by professional practice guidelines for NSCLC patients at the time of study initiation, including *ALK*, *BRAF*, *EGFR*, *KRAS*, *MET*, *RET*, or *ROS1* for targeted therapy, and PD-L1 expression for immunotherapy [17]. Of note, some guideline-recommended predictive targeted therapy biomarkers for NSCLC are not commercially available to oncologists as individual SGTs from any reference laboratory, including *ERBB2* mutations, *MET* exon 14 skipping mutations, and *NTRK1/2/3* fusions. Testing for these alterations can only be performed by next-generation sequencing.

CGP was performed using OmniSeq INSIGHT®, a next-generation sequencing-based (Illumina® TruSight 500) in vitro diagnostic device for the detection of genomic variants in FFPE tumor tissue, as described previously [18]. Briefly, DNA was sequenced to detect small variants in the full coding region of 523 genes and for copy number alterations in 59 genes. RNA was sequenced for the detection of fusions and splice site variants in 55 genes. PD-L1 expression by IHC 22C3 was also tested and included in the CGP integrated report. CGP

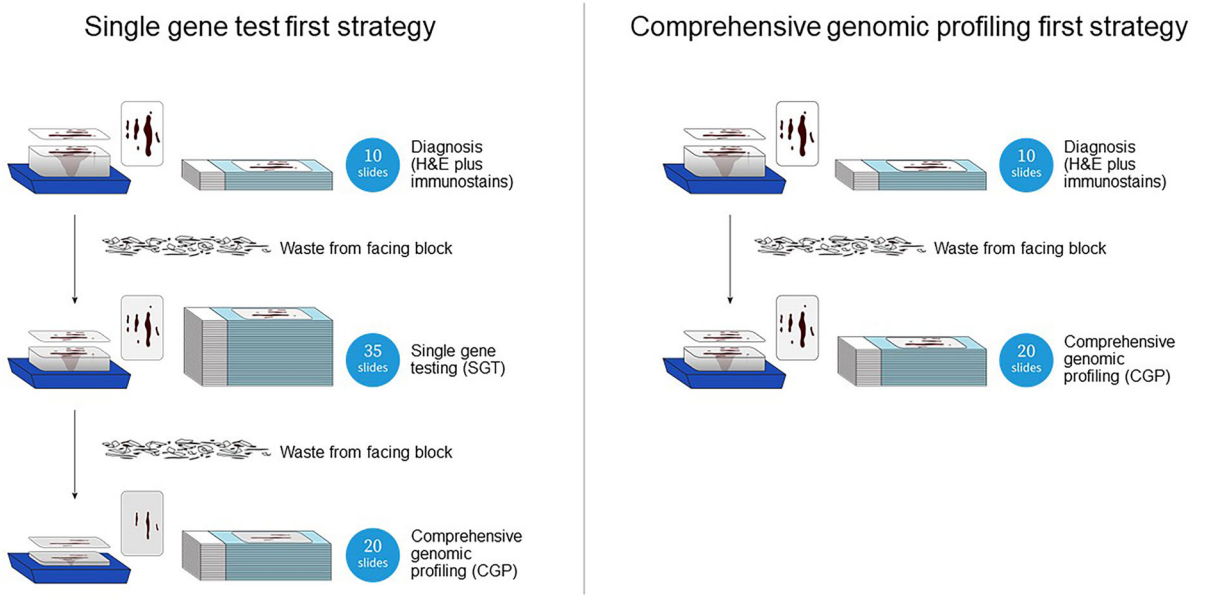


Fig. 1 Comprehensive genomic profiling (CGP) tissue utilization pathways in non-small cell lung cancer (NSCLC). All patients with advanced and metastatic NSCLC undergo a procedure for tissue procurement (biopsy, surgery, or other procedure). From the formalin-fixed paraffin-embedded (FFPE) tissue block created from the procedure, pathologists cut slides to confirm NSCLC diagnosis with hematoxylin and eosin (H&E) and additional immunostaining. When SGT is ordered (left side of figure), more than 50 slides need to be cut to cover all alterations recommended for testing. If SGT results are ultimately negative and CGP is desired as a next step, an additional 20 slides are needed. This sequential testing approach requires the block to be refaced each time slides are used, resulting in excess tissue wastage, increasing the depth into which the block must be cut and the risk of exhausting the specimen. In an alternative strategy (right side of figure), CGP is performed immediately after diagnosis, which requires facing the block only one time, with less waste, less depth, and therefore less risk of insufficient tissue

covered all guideline-recommended biomarkers for NSCLC patient therapeutic management. A complete description of the CGP test analytical performance is available online: https://oncology.labcorp.com/sites/default/files/INSIGHT_websiteABOUT.pdf

RESULTS

Initial Single-Gene Testing

Oncologists from 80 community practice sites in the United States ordered CGP for 561 patients with advanced and metastatic NSCLC; 150 (27%) of these patients had negative SGT results previously reported within 6 months of CGP orders. Oncologists ordered a wide variety of SGT prior to CGP. For ≥ 90% of patients, the most frequently ordered individual SGTs were for *ALK* rearrangements, *EGFR* mutations, and PD-L1 expression, while SGT for *MET* amplification and *RET* rearrangements were ordered the least often (Fig. 2a). All SGT for a given patient were ordered on the same day; however, SGT results for > 90% of patients were reported on multiple dates (Fig. 2b). The most common SGT combination was for *ALK*, *EGFR*, *ROS1*, and PD-L1 (28%). Only 3% of patients had all available SGTs ordered for guideline-indicated markers. Half of all SGT order combinations included *KRAS*, while 84% included *ALK*, *EGFR*, and *PD-L1* (Fig. 2c). The gamut of 30 different SGT order combinations generated wide variability in median turnaround time (TAT) calculated as days from order date to maximum report date per group. The top five most common order group combinations constituted > 70% of all SGT orders and had median TAT ranging from 8 to 26 days (Fig. 2d).

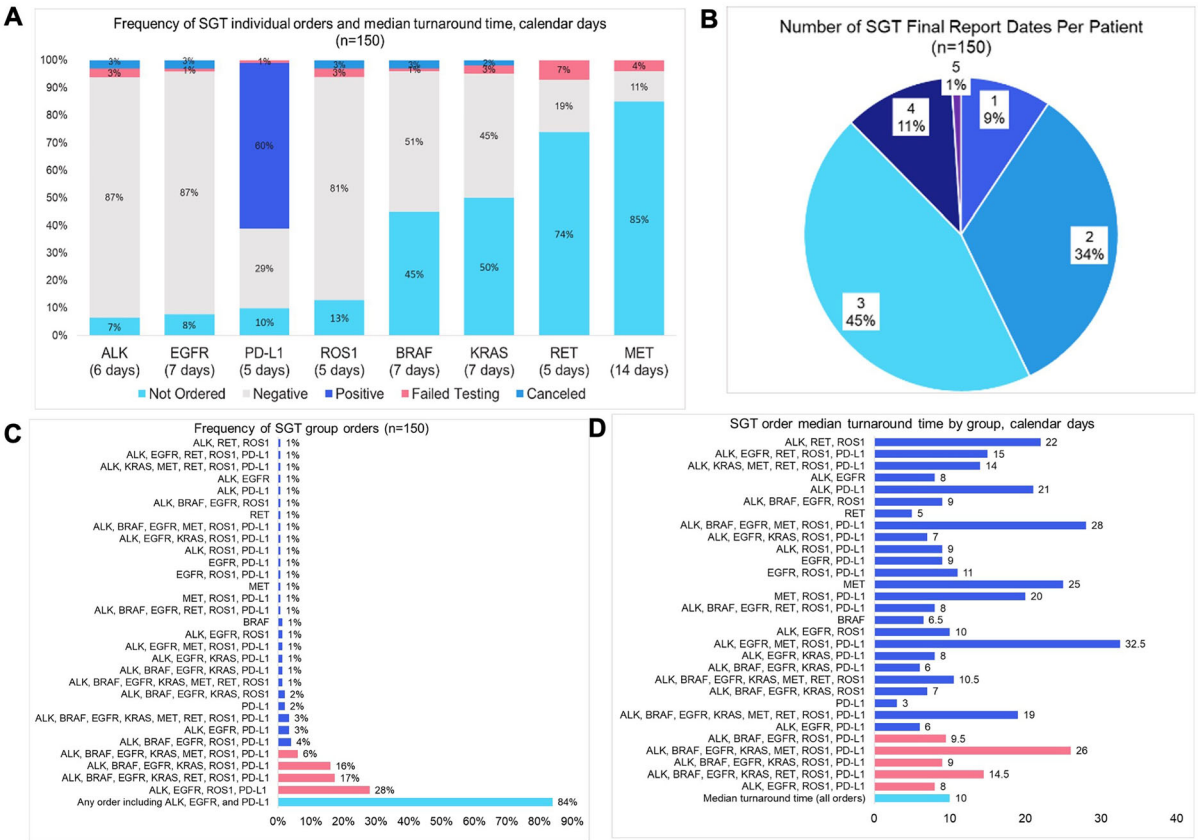


Fig. 2 Characteristics of single-gene tests (SGT) ordered prior to comprehensive genomic profiling (CGP) in patients with advanced and metastatic NSCLC (n = 150). **a** The most and least frequently ordered SGT were for *ALK* rearrangements (93%) and *MET* amplification (15%), respectively; **b** per patient, individual SGT results had between 1 and 5 different final report dates, with > 90% of patients having at least 2; **c** 30 combinations of SGT were ordered by oncologists, with 3% of patients having all available SGTs for recommended biomarkers ordered; **d** SGT median turnaround time (TAT) was 10 calendar days, and ranged from 3 days for PD-L1 alone, to 32.5 days for one patient's *ALK*, *EGFR*, *MET*, *ROS1*, and PD-L1 tests

Comprehensive Genomic Profiling Order Characteristics by Testing Strategy

CGP orders varied by testing strategy for geographic region, turnaround time, tissue type, and tissue age (Table 1). In geographic regions with higher total CGP test volume (defined as ≥ 100 orders during the study), oncologists were less likely to have ordered SGT first. The median CGP TAT was significantly longer among patients with prior SGT (16 vs. 13 days, $p < 0.001$), with significantly fewer CGP tests completed within 2 weeks of ordering relative to tests for patients with no prior SGT (38% vs. 71%). There were no significant differences in patient age, sex, or tumor histology between CGP orders with and without prior SGT. FFPE tissue specimens for patients with prior SGT were more commonly from metastatic sites, and the specimens were significantly older, with 34% of tissues more than 90 days old relative to just 4% among patients with no prior SGT, underscoring the extensive use of the same FFPE tissue block for both SGT and CGP 90% of the time.

Comprehensive Genomic Profiling Success by Testing Strategy

We defined successful completion of CGP as testing that passed pre-analytical tissue review,

Table 1 Comprehensive genomic profiling (CGP) order characteristics by single-gene testing (SGT) strategy in patients with non-small cell lung cancer (n = 561)

	Prior SGT (n = 150, 27%)	**No prior SGT (n = 411, 73%)**	**Total (n = 561)**	***P* value**
Regional test volume				
< 100 orders	68 (45.3)	73 (17.8)	141 (25.1)	< .001*
> = 100 orders	82 (54.7)	338 (82.2)	420 (74.9)	
Turnaround time (calendar days)				
≤ 14	57 (38.0)	292 (71.0)	349 (62.2)	< .001*
> 14	93 (62.0)	119 (29.0)	212 (37.8)	
Median	16	13	13	< .001**
Patient age (years)				
Average	72	71	71	0.086‡
Patient sex				
Female	69 (46.0)	214 (52.1)	283 (50.4)	0.216†
Male	81 (54.0)	197 (47.9)	278 (49.6)	
Tumor histology				
Non-squamous	114 (76.0)	338 (82.2)	452 (80.6)	0.117†
Squamous	36 (24.0)	73 (17.8)	109 (19.4)	
Tissue type				
Primary	23 (15.3)	101 (24.6)	124 (22.1)	0.021†
Metastatic	127 (84.7)	310 (75.4)	437 (77.9)	
Tissue age (calendar days)				
< 90	99 (66.0)	395 (96.1)	494 (88.0)	< .001†
≥ 90	51 (34.0)	16 (3.9)	67 (12.0)	
Median	56	12	14	0.008**
Same tissue used for SGT and CGP				
Yes	135 (90.0)	n/a	n/a	n/a

Values for age, sex, histology, specimen type, specimen age, and geographic region categories are presented as frequency (%)
*Chi-squared test
†Fisher's exact test
‡Paired t-test
**Mann–Whitney U test

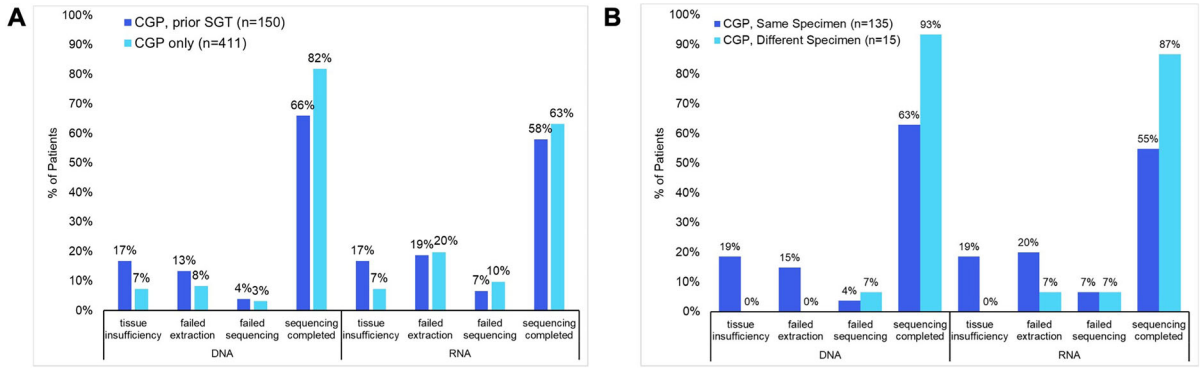


Fig. 3 Comprehensive genomic profiling (CGP) DNA/RNA sequencing success in NSCLC patients. (a) CGP with prior SGT versus CGP only (n = 561). Patients with prior SGT had less successful DNA-seq completion (66% vs. 82%), increased tissue insufficiency for DNA-seq and RNA-seq (17% vs. 7%), and increased DNA extraction failures (13% vs. 8%) than those with no prior SGT; (b) CGP with prior SGT only, same versus different specimen (n = 150). Among orders for cases with prior SGT, use of the same tissue specimen reduced successful completion of sequencing by ≥ 30% for DNA (93% vs. 63%) and RNA (87% vs. 55%). Nearly all cases of tissue insufficiency and DNA or RNA extraction failures for CGP occurred in cases where the same tissue specimens were previously used for SGT

as well as DNA or RNA extraction and sequencing, with separate presentation of findings for each. As seen in Fig. 3, across all CGP orders, DNA sequencing success by testing strategy was 66% for patients with prior negative SGT results versus 82% for patients with no prior SGT (Fig. 3a). Among patients with prior SGT (Fig. 3b), successful CGP testing was limited due to prior use of the same tissue block for SGT. Specifically, we observed a greater than 30% reduction in completion of both DNA and RNA sequencing when CGP was attempted using the same specimen, in addition to increased test cancellations due to pre-analytical tissue insufficiency and extraction failures.

Impact of Prior Single-Gene Testing on Comprehensive Genomic Profiling Variant Detection in Non-Small Cell Lung Cancer

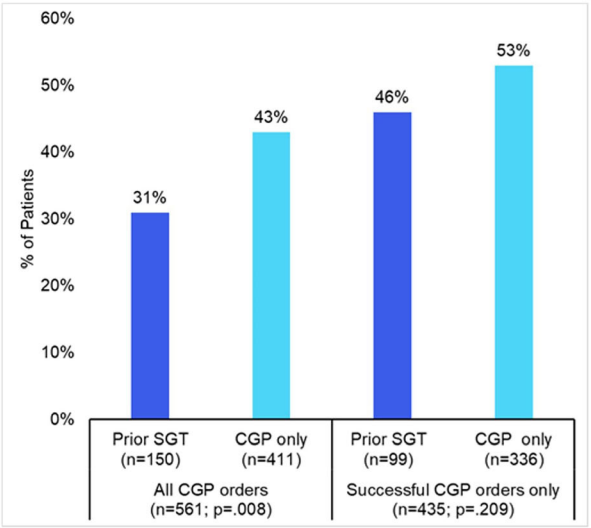
We compared the rate of guideline-recommended variant detection by CGP testing strategy at the test order level and at the variant level in NSCLC (Fig. 4). Across all CGP test orders, inclusive of test cancellations and failures, there was a significantly reduced rate of variant detection for patients with prior negative SGT (31% vs. 43%, p = 0.008). However, this difference decreased and was non-significant when only successful tests were included (46% vs. 53%, p = 0.209) (Fig. 4a).

Among patients with successful CGP orders (Fig. 4b), the frequency of guideline-recommended variant detection by CGP was lower for patients with prior negative SGT findings in genes that were most commonly assessed by SGT, particularly *EGFR* mutations, and *ALK* and *ROS1* fusions. Conversely, patients with prior negative SGT findings were enriched for variants in genes that were less frequently tested by prior SGT, including *RET* fusions and *KRAS* non-G12C mutations. However, the greatest number of alterations that were more frequently observed in these patients were not tested by prior SGT at all, including *ERBB2* copy gain and mutations, as well as *MET* exon 14 skipping mutations and fusions in *NTRK2/3*, which are only detectable by next-generation sequencing.

DISCUSSION

We assessed the impact of a conservative SGT first approach on subsequent successful completion of guideline-recommended broad molecular profiling by tissue-based CGP for

A



B

	Prior SGT	CGP Only		Total
DNA-Seq	**(n=99)**	**(n=336)**	**Ratio**	**(n=435)**
ERBB2 copy gain (amplification)	1.0%	0.3%	3.4	0.5%
ERBB2 mutations	4.0%	1.8%	2.3	2.3%
MET exon 14 skipping (splice site)	2.0%	0.9%	2.3	1.1%
KRAS non-G12C mutations	19.2%	16.7%	1.2	17.2%
KRAS G12C	11.1%	12.5%	0.9	12.2%
BRAF V600E	1.0%	1.5%	0.7	1.4%
EGFR mutations	2.0%	14.6%	0.1	11.7%
MET copy gain (amplification)	0.0%	0.6%	0.0	0.5%
RNA-Seq	**(n=87)**	**(n=260)**	**Ratio**	**(n=347)**
RET fusions	2.3%	0.0%	∞	0.6%
NTRK 2/3 fusions	1.1%	0.4%	3.0	0.6%
MET exon 14 skipping (fusions)	4.6%	3.8%	1.2	4.0%
ALK fusions	1.1%	1.5%	0.7	1.4%
ROS1 fusions	0.0%	1.5%	0.0	1.2%

Fig. 4 Non-small cell lung cancer (NSCLC) practice guideline-recommended genomic variant detection by comprehensive genomic profiling (CGP) testing strategy. **a** Detection rate for all test orders versus successful test orders. The total frequency of guideline-recommended variant detection by CGP was significantly lower for patients with prior SGT when test cancellations and failures were included in the patient denominator (31% vs. 43%), whereas there was no significant difference in the detection rate between the two test strategies when only successfully completed CGP tests were included. **b** Variant detection for successful test orders. Compared with patients with no prior SGT, detection of specific variants by CGP was lower (ratio < 1) for genes that were most often assayed by prior SGT (such as *EGFR* mutations and ALK fusions), and higher (ratio ≥ 1) for genes that were less frequently tested by prior SGT (*RET* fusions) or were not available for prior testing by SGT at all (*ERBB2* copy gain and mutations, *MET* exon 14 skipping, *NTRK2/3* fusions)

patients with NSCLC. As seen in other studies, we observed nearly universal incomplete initial biomarker testing by SGT. Some guideline-recommended biomarkers cannot be detected by SGT, such as *MET* exon 14 skipping and *NTRK* fusions. In our study, however, only 3% of patients with NSCLC had testing performed for all eight currently recommended biomarkers that can be detected by SGT. There was also extensive variability in SGT ordering practices by oncologists, with 30 different combinations of individual gene tests observed.

Tissue depletion negatively impacted successful broad molecular profiling by CGP in patients with NSCLC that had SGT performed first. Nearly all (90%) cases attempted to use the same tissue specimen for CGP that had prior SGT performed. There were more than twice as many canceled CGP tests due to tissue insufficiency (17% vs. 7%) and less successful DNA sequencing (66% vs. 82%). However, prior SGT did not appear to differentially affect successful completion of RNA sequencing. As was observed in our study, RNA is known to fail more frequently than DNA in extraction due to greater specimen instability and degradation [19–21]. Despite these pre-analytical challenges, RNA-seq presents significant advantages over single-gene protein or other DNA-based methods. In addition to the detection of known and unknown fusion variants, RNA-seq can simultaneously assess for skipping events (as was observed for MET exon 14 in our study), analyses that are not possible by single-gene tests [22].

While there were significantly more SGT cases with older specimens, specimen age did not differentially impact completion of RNA sequencing by testing strategy. Prior SGT negatively impacted CGP turnaround time (TAT), which was longer ($\geq$ 14 days) for significantly more patients (62% vs. 38%). Across all CGP orders, including those that failed due to tissue insufficiency, only 31% of patients with prior

SGT had guideline-recommended variants detected, compared with 43% of patients that only had CGP testing ordered. Further, the observed higher and lower frequency of detection of specific guideline-indicated variants by testing strategy (prior vs. no prior SGT) was mainly attributable to incomplete prior testing by SGT methods. Taken together, these findings illustrate that upfront SGT puts patients with NSCLC at risk for missed targeted therapy opportunities due to incomplete testing. First, available SGT methods do not assess all guideline-recommended alterations. Further, in cases where tissue is entirely depleted by upfront SGT, an additional biopsy would be required to adequately evaluate the tumor and is not typically feasible to procure.

Our findings have significant implications for patients with NSCLC. Under-testing and incomplete testing for oncogenic driver mutations can lead not only to underutilization of frontline targeted therapy, but also to improper use of upfront immune checkpoint inhibitors in patients with actionable genomic variants who can derive clinically meaningful benefits from biomarker-driven targeted therapy [23, 24]. Patients with NSCLC that harbor oncogenic driver mutations in *ALK*, *EGFR*, *KRAS*, *BRAF*, *ROS1*, and *MET* (exon 14 skipping) will derive minimal efficacy to checkpoint inhibitors, as these patients' tumors tend to be less immunogenic, regardless of PD-L1 expression levels [25]. Further, the efficacy of the *RET* inhibitor selpercatinib for *RET* fusion-positive NSCLC patients was very recently shown to be far superior to chemoimmunotherapy [26]. In our study, 51/150 (34%) of patients with SGT did not have successful completion of CGP (Fig. 2A), and 29/51 (57%) of these patients had positive PD-L1 expression results (data not shown). These patients were left with unknown biomarker status for targeted therapy and a positive PD-L1 result, potentially leading to inappropriate use of immunotherapy and unnecessary costs to patients and payers [27].

The availability of biomarker test results prior to initiation of frontline therapy has also demonstrated optimal NSCLC biomarker-directed treatment selection and superior outcomes relative to a host of comparison groups in several real-world data studies [28–33]. In our study, the median turnaround time (TAT) for CGP orders was higher than the TAT for SGT orders (13 vs. 10 days); however, SGT TAT had more variability. Whereas all CGP results are simultaneously reported on a single date, including PD-L1 status, results for individual SGT performed prior to CGP for these patients were reported on different dates 90% of the time, risking precipitous treatment selection without having all results in hand. Studies have also shown that while the upfront price of one CGP test is higher than that of individual SGTs, CGP is a better value because it has better efficacy for treatment selection, with minimal to no additional cost to patients and payers [34–37]. One recent analysis demonstrated that the cost of correctly identifying actionable variants was lower for next-generation sequencing than for sequential SGT for most cancer types, including NSCLC, based on the sensitivity and specificity and price estimates for the tests [38].

Community oncologists may have less information about the historical use and quality of their patients' tissue specimens when they request molecular testing. In the community practice setting, oncologists and pathologists often work in separate practices and use different electronic medical record systems. This information asymmetry may contribute to under-testing and incomplete testing and further impede CGP adoption. However, solutions can be created through cross-functional pathologist–oncologist communication such as molecular tumor boards and improved pathology report notes indicating which FFPE specimen should be used or whether tissue may be insufficient for testing. This type of optimized workflow was exemplified by a large multisite community oncology practice that implemented provider education and tools, including electronic health record consult templates and standardized order sets, and saw an increase in comprehensive biomarker testing for NSCLC patients from 68 to 93% in a single year [39]. Further, a recent study in Germany demonstrated that the delivery of CGP to NSCLC patients within a formal, large-scale precision oncology program centered on providing post-

test clinical decision support resulted in greater use of targeted therapy, less use of chemotherapy, and better overall survival than patients tested and treated outside the precision oncology program in the standard of care community setting [40].

Our study has strengths and limitations. The use of a prospectively generated data source from a large reference laboratory that offers a comprehensive oncology testing menu allowed us to characterize oncologists' ordering practices for SGT and CGP that include test details not typically captured by electronic health records. However, there may also be underrepresentation of SGT volume performed for patients with NSCLC due to the availability of no-cost CGP. The study also did not capture why oncologists who rapidly switched their test order to CGP from SGT did not order CGP to begin with. While there could be a number of reasons for this, our experience with this study suggests that oncologists value CGP and are ready to use it, but that other barriers, such as concerns that their patients' insurance will not cover the test, are at play.

A major barrier to having upfront biomarker results prior to treatment initiation is the difficulty in obtaining sufficient tissue biopsy for testing, which often leads to a failed or cancelled molecular profiling test [41]. In our study, the small number of cases with prior SGT that used a different specimen for CGP (n = 15) not only explains the lack of significance for the large differences in successful test completion in this group, but it highlights the pervasiveness of the problem of attempting additional testing with limited tissue in absolute numbers. This scenario is one where liquid biopsy can be useful, as it has been shown to provide molecular results needed to initiate treatment in a timely manner without repeat tissue biopsy. Further, studies have shown that integration of liquid biopsy and tissue testing in NSCLC leads to improved detection of biomarkers with therapeutic significance [42, 43]. Therefore, as we look into the future for NSCLC treatment, sending both tissue-based testing and liquid biopsy simultaneously at the time of diagnosis for each patient may be the most efficient approach to ensure that we have results critical for treatment decisions [44].

CONCLUSIONS

Oncologists have historically relied on SGT for patients with advanced and metastatic NSCLC. However, this approach is antiquated and results in incomplete testing of recommended biomarkers, regardless of when it is performed. CGP is needed at diagnosis for every NSCLC tumor, especially for patients with limited tissue, to avoid missed targeted therapy opportunities and potential ineffective immunotherapy.

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Declarations

Conflict of Interest. Mary K. Nesline, Rebecca A. Previs, Kyle C. Strickland, Heidi Ko, Paul DePietro, Michael D. Biorn, Maureen Cooper, Jeffrey Conroy, Sarabjot Pabla, Shengle Zhang, Zachary Wallen, Marcia Eisenberg, Brian Caveney, Eric A. Severson, and Shakti Ramkissoon are employees of Labcorp and declare the following relationships: stock ownership from Labcorp. Vivek Subbiah has had a consulting or advisory role with MedImmune, Helsinn Therapeutics, Relay Therapeutics, Pfizer, Loxo/Lilly, Research Funding from Novartis (Inst), GlaxoSmithKline (Inst), NanCarrier (Inst), Northwest Biotherapeutics (Inst), Genentech/Roche (Inst), Berg Pharma (Inst), Bayer (Inst), Incyte (Inst), Fujifilm (Inst), PharmaMar (Inst), D3 Oncology Solutions (Inst), Pfizer (Inst), Amgen (Inst), AbbVie (Inst), Multivir (Inst), Blueprint Medicines (Inst), Loxo (Inst), Vegenics (Inst), Takeda (Inst), Alfasigma (Inst), Agensys (Inst), Idera (Inst), Boston Biomedical (Inst), Inhibrx (Inst), Exelixis (Inst), Amgen (Inst), Turningpoint Therapeutics (Inst), Relay Therapeutics (Inst). Nini Wu declares that she has no competing interests. Pratheesh Sathyan is an employee of Illumina and declares the following relationships: stock ownership from Illumina. Kamal Saini is an employee of Fortrea and declares the following relationship: stock ownership from Fortrea. Kamal Saini is an Editorial Board member of *Oncology and Therapy*. Kamal Saini was not involved in the selection of peer reviewers for the manuscript or any of the subsequent editorial decisions.

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