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Quantifying the value of multi-gene testing in resected early-stage lung adenocarcinoma

Bharathi Muthusamy¹, Kira Raskina², Katherine T. Lofgren², Gerald Li², Khaled Tolba², Karen Schwed³, Emily Castellanos³, Richard S.P. Huang², Geoffrey R. Oxnard², Alexa B. Schrock²#, Nathan Pennell¹

1. Cleveland Clinic Foundation
2. Foundation Medicine, Inc.
3. Flatiron Health, Inc.

#Corresponding Author:
Alexa B. Schrock, PhD
Foundation Medicine, Inc.
121 Seaport Blvd
Boston, MA 02210
aschrock@foundationmedicine.com
cell phone: 734-476-3455

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Abstract

BACKGROUND: Tyrosine kinase inhibitors and immune checkpoint inhibitors (ICI), each requiring testing for precision biomarkers, have recently been approved in the adjuvant setting. We assessed the potential value of multi-gene testing in early lung adenocarcinoma (LUAD).

METHODS: Using a real-world clinico-genomic database linking deidentified electronic health record-derived clinical data to genomic data, we selected patients with LUAD who underwent tissue comprehensive genomic profiling (CGP). Using a probabilistic decision tree, we estimated the cost implications of the avoidance of adjuvant ICI in patients with PD-L1+ LUAD and an ALK/ROS1/RET driver.

RESULTS: CGP was performed on a specimen collected prior to advanced disease in 20% (1,320/6,697) of cases and ordered prior to advanced diagnosis for 12.6% (847/6,697) of patients. The prevalence of driver alterations in early and advanced stage specimens was similar, though KRAS mutations were enriched in early disease and drivers including ALK rearrangements in advanced disease. Patients who had CGP results obtained prior to vs after recurrence had less time between recurrence and the start of any first-line treatment (median 3.6 vs 6 weeks, p <0.001). Through avoidance of ICI in PD-L1+ early LUAD with an ALK/ROS1/RET driver, we estimated the universal CGP could reduce expected costs by $1,597.23 per patient relative to EGFR single-gene testing.

CONCLUSIONS: CGP can identify driver alterations and accelerate the start of first-line therapy at recurrence. It may also represent a cost-effective approach for avoiding futile adjuvant ICI in patients with drivers that have historically lacked activity with ICI in metastatic disease.
Introduction

Although around 30% of non-small cell lung cancers (NSCLC) can be completely resected with curative intent, many patients have recurrence typically requiring systemic treatment for metastatic disease. The treatment paradigm for stage IV NSCLC has changed in recent years as tyrosine kinase inhibitors (TKI) and immune checkpoint inhibitors (ICI) have become part of standard of care. Both modalities have dramatically increased overall survival in select biomarker populations of patients. The current National Comprehensive Cancer Network (NCCN) guidelines recommend evaluation of PD-L1 status for all advanced stage NSCLCs; broad genomic testing to identify oncogenic driver alterations is strongly advised for non-squamous tissue types and considered for squamous tissue types. PD-L1 and specifically EGFR mutation testing are now recommended in resectable disease as the FDA has recently approved both adjuvant atezolizumab for PD-L1+ >1% disease and adjuvant osimertinib in patients with classic EGFR driver mutations.

While smoking cessation programs are credited for the rapid drop in NSCLC incidence, recent analysis of the Surveillance, Epidemiology, and End Results (SEER) Program national database revealed that mortality from NSCLC decreased even faster than the incidence of the disease. This drop in mortality corresponded to the timing of approval of targeted therapy. As lung cancer surveillance programs gain more wide acceptance in clinical practice, more patients are expected to be diagnosed with early-stage NSCLC where emerging evidence supports the practice of applying targeted therapy in the peri-operative phase.

Though testing for PD-L1 and EGFR is necessary for choosing the optimal adjuvant treatment, multi-gene testing for other gene alterations in early-stage NSCLC may have its own benefits. For example, the NCCN recommends testing for seven other driver alterations besides EGFR mutations in metastatic disease.
comprehensive genomic profiling (CGP), these results would already be available at recurrence to guide treatment selection in a more timely manner, potentially enabling faster start of first-line therapy. Further, ICI therapy in metastatic NSCLC with EGFR, ALK, RET, or ROS1 driver alterations has not improved response rates nor survival in comparison to chemotherapy.\(^{10-14}\) Thus, most immunotherapy clinical trials exclude patients with EGFR mutations and ALK rearrangements. Although EGFR and ALK+ patients were included in the IMpower010 study which evaluated adjuvant atezolizumab and lead to its approval, it was not clear that this small subgroup had any benefit and adjuvant atezolizumab is not recommended for this population.\(^{15}\) With this information and given data from the metastatic setting, it can be reasonably inferred that ICI therapy in early-stage disease positive for these driver alterations would not improve recurrence free survival or overall survival and could lead to unnecessary side effects as well as increased risk of TKI-toxicity if disease recurs and further systemic therapy is needed. There is the appropriate concern of the costs of multi-gene testing, but this must be balanced with the costs of potentially ineffective ICI treatment. Both the potential clinical and financial benefits of CGP in patients with early-stage, resected lung adenocarcinoma (LUAD) are explored in this retrospective study.

**Methods**

*Foundation Medicine CGP*

Comprehensive genomic profiling (CGP) was performed during routine clinical care (Foundation Medicine, Inc., Cambridge, MA) on tissue specimens from early and advanced-stage LUAD. DNA was extracted from 40 microns of FFPE sections, and CGP was performed on hybridization-captured, adaptor ligation based libraries to a mean coverage depth of >550X for 315 or 324 cancer-related genes plus selected introns from genes frequently rearranged in cancer, as previously described.\(^{16}\) Tumor mutational burden (TMB) was calculated by counting the number of synonymous and non-synonymous mutations across a 0.8-1.2 megabase (Mb) region, with computational germline status filtering, and reporting the result as mutations/Mb. This method has been previously validated for accuracy against whole exome sequencing.\(^{17}\)
**PD-L1 immunohistochemistry (IHC)**

PD-L1 expression was determined by IHC performed on FFPE tissue sections. PD-L1 IHC results were available in 58.2% (3900/6697) of total cases. Missing results may be due to real world practice patterns and changing practice patterns over time. Additionally, assays that were only scored by combined positive score or immune cells were excluded. A pathologist determined the percentage of tumor cells with expression (0-100%) and the intensity of expression (0, 1+, 2+). PD-L1 expression was reported as a continuous variable with the percentage of tumor cells staining with ≥1+ intensity. PD-L1 expression was summarized as negative (<1%) or positive (≥1% of tumor cells staining with ≥1+ intensity).

**Clinico-genomic Database (CGDB)**

This study utilized real-world data from the Flatiron Health (FH)-Foundation Medicine (FM) NSCLC clinico-genomic Database (CGDB), a nationwide de-identified electronic health record (EHR)-derived database which includes patients sequenced at FM who received care within the FH network. The de-identified data originated from approximately 280 US cancer clinics (~800 sites of care). The FH-FM CGDB includes 8,378 pts with chart-confirmed NSCLC, with profiled specimen histology of LUAD who received care within the FH network between 01/2011-06/2021. Cohorts included in our analysis were limited to those who had tissue CGP (FoundationOne® or FoundationOne® CDx) at some point during cancer care. Retrospective longitudinal clinical data were derived from EHR data, comprising patient-level structured and unstructured data, curated via technology-enabled abstraction of clinical notes and radiology/pathology reports and linked to CGP data by de-identified, deterministic matching.18

Patients were excluded if their stage at diagnosis was unavailable, if stage was noted as III without further granularity, or if CGP specimen collection or report date was unavailable. 236 cases were also excluded because advanced diagnosis was noted less than 3 months from initial early diagnosis, or a specimen recorded
as collected during early disease was from a metastatic site. Early-stage disease was classified as stage I, II, or IIIA without documentation of recurrence and advanced stage disease was defined as either early-stage (I-IIIA) disease that had recurred or progressed, or initial diagnosis with stage IIIB/IIIC or IV.

IRB approval

For FM genomic analysis, approval for this study, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the WCG Institutional Review Board (IRB; Protocol No. 20152817). For FH-FM CGDB analysis, IRB approval of the study protocol was obtained prior to study conduct and included a waiver of informed consent from WCG IRB.

Statistical Analysis

We evaluated the frequency of known or likely pathogenic genomic alterations in CGP specimens for all LUAD cases and in a PD-L1+ (TPS ≥1%) subset. Oncogenes evaluated included ALK rearrangement, BRAF V600E mutation, EGFR mutation (limited to L858R, exon 19 deletion, G719X, L861Q, S768I, and exon 20 insertion), ERBB2 mutation, KRAS mutation, MET amplification and exon 14 skipping alteration, RET rearrangement, ROS1 rearrangement, and NTRK fusion. Frequencies of these driver alterations were compared using Fisher’s exact test and P values were corrected with the Benjamini-Hochberg FDR method. In patients with disease recurrence who had testing on a specimen collected in the early setting, we compared the time from recurrence to start of systemic first-line treatment for patients with the testing performed and reported before (n=203) versus after (n=439) advanced diagnosis using a Cox regression. The number of patients with an EGFR mutation, ALK, ROS1 or RET rearrangement who received matched targeted therapy as first-line treatment, and the time from recurrence to start of any first-line therapy was also compared between the two cohorts with Fisher’s exact test. Patients were excluded from these analyses if first-line treatment information was unavailable.
Timing between initial diagnosis of LUAD and CGP biopsy or report was calculated. Based on the distribution and anticipated typical clinical practice, cases classified as CGP reported in the early setting were limited to those with CGP specimen collection collected within 3 months of initial diagnosis and testing results reported within 6 months of initial diagnosis, whereas those in the late setting were those with report after recurrence or advanced initial diagnosis. For genomic analysis, early disease was classified as CGP specimen collected within 3 months of initial diagnosis and prior to recurrence.

Cost Analysis

The incremental expected cost of CGP multi-gene testing using FoundationOne®CDx compared to EGFR single-gene testing in early-stage LUAD patients was assessed with a probabilistic decision tree (Figure 5). Base-case cost parameters for diagnostic testing, drug administration, and a year of atezolizumab ICI treatment were sourced from the Centers for Medicaid and Medicare Services clinical lab fee schedules, physician fee schedules, and ASP drug pricing files (Supplementary Table 2). The prevalence of PD-L1 1%+ (TPS ≥ 1%) as well as the prevalence of ALK rearrangements among PD-L1 1%+ patients were direct estimates from the IMpower010 trial. We opted for trial-based prevalence estimates when available rather than using direct estimates from the CGDB due to the potential for a biased sample of patients receiving CGP, many of whom seek out comprehensive testing after negative biomarker results from single-gene tests or limited panels. Since RET and ROS1 prevalence estimates among PD-L1 1%+ patients were not available in the IMpower010 trial, we did use the direct estimates from the CGDB as the basis of those base-case parameter values. We also allowed for imperfect adjuvant therapy adoption (base assumption was 80% of patients consider adjuvant ICI treatment) among the eligible patient population given both the recency of the adjuvant atezolizumab approval and the role of patient preferences. A cost-neutrality threshold analysis, a one-way sensitivity analysis, and a probabilistic sensitivity analysis were conducted (Supplementary Tables 3-5 and Supplementary Figures 3-4) to
assess the influence of parameter uncertainty and base-case assumptions. The cost analysis considered only direct diagnostic and therapeutic costs with a one-year time horizon.

Results

Genomics of early disease

8,378 patients were selected with LUAD from the CGDB, of which 6,697 had CGP performed on a tissue specimen and were evaluable as detailed in the methods. Further details of cohort selection are described in Supplemental Figure 1. 20% (1,320/6,697) of patients had CGP performed on tissue collected prior to advanced diagnosis. Among this cohort 89% (1,177/1,320) had CGP performed on a specimen collected within 3 months of initial diagnosis in the absence of any advanced diagnosis, which hereafter we have defined as in the early disease setting, representing 17.6% (1,177/6,697) of the overall cohort (Supplemental Figure 2A). 80.2% (5,377/6,697) of patients had CGP performed on tissue collected in the advanced disease setting. The 143 patients with CGP performed on a specimen collected prior to any advanced diagnosis but >3 months from initial diagnosis were excluded from genomic analysis comparing the biology of early vs advanced disease. PD-L1 IHC results were available for 58.2% (3,900/6,697) cases. 3,506 (89.9%) samples were tested using the Dako 22C3 PD-L1 antibody, 200 (5.1%) were tested using other clones, and for 194 (5.0%) the PD-L1 IHC platform was unknown. Among patients with CGP testing performed on a specimen collected in the early vs advanced disease setting with PD-L1 IHC available, 54% (362/670) and 63% (1,999/3,164), respectively, of patients were PD-L1+ (TPS ≥1%).

NSCLC driver alterations were detected using CGP of specimens collected in the early disease setting (within 3 months of initial diagnosis). Compared to CGP of specimens collected in advanced disease the distribution of driver alterations was similar, although some statistically significant differences were observed.
KRAS mutations were significantly more common in the early disease setting (41.7% vs. 35.5%, adjusted p <0.01), whereas ALK rearrangements (early vs advanced: 1.8% vs 4.3%, adjusted p <0.01) and MET amplification (1.4% vs 3.1%, adjusted p <0.01) had a significantly higher prevalence in advanced disease. When evaluating only patients with PD-L1+ disease, similar results were observed, but only the differences in KRAS mutation and ALK rearrangement frequencies between the two groups were significant (Supplemental Table 1). The distribution of common EGFR and KRAS mutant isoforms was largely consistent in early and advanced disease settings (Figure 1). Alterations in a limited number of other genes including TP53, CDKN2A/B, FGF10, SMARCA4, MYC, RICTOR, and MCL1 were also enriched in advanced disease; however, these enrichments were not significant in the smaller PD-L1+ subset (Figure 2).

CGP testing patterns and Impact of early CGP on First-Line Treatment Decisions

In patients with LUAD in the CGDB, 12.6% (847/6,697) had CGP ordered prior to advanced diagnosis, for which 9.1% (608/6,697) had a specimen collected within 3 months and CGP report within 6 months of initial diagnosis, which hereafter we have defined as CGP report in the early disease setting. This included 34%, 28% and 38% of patients diagnosed with stage I, II and IIIA disease, respectively. 87.4% (5,850/6,697) of patients had CGP testing after advanced diagnosis or recurrence. Testing patterns also continue to evolve over time. The growing ubiquity of CGP in clinical care is reflected in a larger share of the analysis cohort having been tested in recent years, and more of these patients are receiving CGP testing prior to a diagnosis of advanced cancer (Figure 3). Patients with CGP testing performed in the early vs advanced disease setting tended to be older (median 69.0 vs 67.0 years old at initial diagnosis), more often female (59.4% vs 55.1%), and treated at an academic center vs in community practice (93.1% vs 88.9%) (Table 1). In patients with CGP testing prior to advanced diagnosis, CGP was done a median of 66 days (IQR 35-212 days) after initial diagnosis, and of patients who had recurred by the time of data cutoff the median time from initial diagnosis to advanced LUAD diagnosis was just under 13 months. Of those with CGP ordered prior to advanced diagnosis, 73.2% received their CGP
results within 6 months of initial diagnosis. The remaining 26.8% received CGP results >6 months after initial
diagnosis but before documentation of any recurrence, though we suspect a subset of these cases could
represent testing at suspicion of recurrence (Supplemental Figure 2B).

We evaluated the time from recurrence to the start of first-line systemic therapy in the advanced setting, hypothesizing that patients with CGP ordered any time prior to recurrence (n=174) had shorter time to
first-line treatment based on existing knowledge of actionable biomarkers compared to patients with CGP
performed on specimen collected prior to recurrence but with the testing ordered after recurrence (n=370). In
patients with CGP prior to recurrence the median time to start of first-line therapy was 25 days compared to 42
days for patients with CGP ordered on an existing specimen after recurrence (p <0.001) (Figure 4A). In the subset
of patients from each group with a targetable EGFR, ALK, RET or ROS1 driver detected, for patients with CGP
prior to recurrence the median time to start of first-line therapy was 19 days compared to 47 days for patients
with CGP ordered on an existing specimen after recurrence (p <0.001) (Figure 4B). Further, within this subset
with EGFR, ALK, RET or ROS1 drivers, 30/39 (77%) with early CGP initiated matched first-line TKI while 43/65
(66%) with CGP after recurrence received matched first-line TKI (p = 0.3), an additional 2 and 1 patients,
respectively, in each group received only an unspecified clinical study drug.

Cost Analysis

We also calculated the expected incremental cost of routine CGP testing in early LUAD as an alternative
to single gene EGFR mutation testing, with the assumption that CGP detection of ALK, ROS1 and RET driver
rearrangements could identify patients who would most likely not respond to atezolizumab, an ICI approved in
the adjuvant setting. This assumption is based on data from studies in the advanced disease setting and the
IMpower010 study showing no clear improvement in survival with ICI in patients with these oncogenic drivers.\textsuperscript{10}
A probabilistic decision tree was used to estimate the incremental cost of CGP testing (Figure 5A), where patients with \textit{ALK}, \textit{RET}, \textit{ROS1} driver alterations identified on CGP avoid adjuvant atezolizumab. Costs of CGP and \textit{EGFR} single-gene testing are estimated at $3,500 and $324.58 respectively, and treatment with atezolizumab for a year is approximately $160,199.04 (including both drug and administration costs). In the intention to treat group in IMpower010, 53.2\% of patients were PD-L1+ (\textit{TPS} \geq 1\%); 4.8\% of IMpower010 PD-L1+ patients also had an \textit{ALK} driver rearrangement. In the CGDB, among early LUAD patients with PD-L1+ disease a \textit{RET}, \textit{ROS1}, or \textit{ALK} rearrangement was detected in 1.4\%, 0.8\%, and 1.4\% of cases (Supplemental Table 1). Since they were not available from IMpower010, we used the CGDB derived estimates of \textit{ROS1} and \textit{RET} rearrangements among PD-L1+ early LUAD patients as our base case parameters for a total ICI avoidable patient population of 7\% based on \textit{ALK}, \textit{RET}, \textit{ROS1} status (Supplemental Table 2).\textsuperscript{15}

Using these estimated costs and prevalence estimates, $3,175.42 more would be spent on diagnostics per person with CGP instead of just \textit{EGFR} testing, but $4,772.65 could be saved per person on average by avoiding ICI therapy expenses in patients who are both PD-L1 1\%+ and \textit{ALK/RET/ROS1}+. Universal CGP testing for early-stage LUAD could represent an overall cost saving testing strategy per patient with a total expected incremental cost reduction of $1,597.23 (Figure 5B). These results hold under a wide range of parameter assumptions including a PD-L1+ prevalence as low as 35.4\% and CGP testing costing up to $5,097.23 (Supplemental Table 3). The most influential parameters in the one-way sensitivity analysis were \textit{ALK/RET/ROS1}+ prevalence among PD-L1+ patients when considered together as the total population identified to avoid adjuvant ICI therapy, the \textit{ALK}+ specific prevalence, the percent of eligible patients for adjuvant ICI therapy who received treatment, and the PD-L1+ prevalence (Figure 5C and Supplemental Table 4). Across ten thousand parameter draws in the probabilistic sensitivity analysis, CGP was the cost-saving testing strategy in 84\% of the simulations (Supplemental Figure 4).

\textbf{Discussion}
To the best of our knowledge, this is the first study characterizing the use of CGP in patients with early-stage lung adenocarcinoma including the evaluation of potential clinical and financial benefits. Currently, guidelines recommend broad genomic testing only for metastatic NSCLC, but with utilization of a real world database, a group of patients were selected who underwent CGP in the early disease setting with relatively even distribution of stage I, II, and IIIA diagnosis. In this population, CGP most commonly occurred within 3 months of initial diagnosis. This could suggest that there are certain practitioners/practices that are already routinely using CGP in patients with early-stage disease, and this trend appears to be increasing over time.

The prevalence of oncogenic drivers was similar between CGP specimens collected in the early vs advanced setting, though KRAS mutations were somewhat more frequent in early disease and other drivers were somewhat more frequent in advanced disease. When limited to only PD-L1+ disease, KRAS and ALK were the only oncogenes that continued to show a significant difference. While the exact reason for this difference remains elusive, it may be influenced by testing practice patterns or by screening practices by which patients with strong smoking history are more likely to qualify for lung cancer screening whereas patients with EGFR/ALK are more likely to be never smokers and thus more likely to bypass screening and present with advanced disease. These genomic findings highlight the potential utility of KRAS inhibitors in the perioperative setting and should be further explored.

This study raises several potential advantages of CGP in early-stage LUAD. First, there are potential advantages of the immediate availability of information regarding targetable genomic alterations at the time of recurrence, which leads to a decrease in time to first-line treatment of about three weeks. Although it is not clear if the difference of a few weeks would lead to improved survival, symptomatic patients may be palliated faster, and it may benefit patients from an anxiety and mental health perspective. The practitioner’s time would also be spent more efficiently. These potential benefits could be explored in a small prospective trial. Further, as new assays are developed to monitor molecular residual disease (MRD), early CGP tissue testing may provide added value as a baseline for these assays. There are also an increasing number of precision targeted
therapy trials in early lung cancer that CGP could aid in enrollment; these are summarized in Supplemental Table 5.

The difference in the percentage of patients with CGP specimen collected in early disease treated with appropriate first-line matched targeted therapy for ALK, RET or ROS1 between those with CGP testing performed in the early vs advanced setting was not significantly different from a statistical point of view, but there was a positive trend favoring those with earlier testing. About 10% more patients were treated with the guideline recommended targeted therapy when CGP was already completed at time of recurrence. It is possible this reflects the ability to initiate therapy more quickly among patients who could otherwise go untreated due to poor performance status or need to initiate therapy rapidly due to severity of disease.

Cost benefits of universal CGP compared to single-gene EGFR testing could come in the form of avoiding potentially unnecessary adjuvant atezolizumab in patients with ALK, ROS1, or RET driver alterations, an estimated average savings of $1,597.23 per patient tested with CGP. Because adjuvant atezolizumab is approved for patients with PD-L1+ disease, the prevalence of PD-L1 1%+ among LUAD patients is an important parameter to define the total addressable population. We relied on the IMpower010 study specific estimates of 53.2% PD-L1+ and 4.8% ALK+ to inform our costing analysis, estimates consistent with other trial populations. In our real-world CGDB cohort, 55% of specimens from early LUAD and 63% from advanced LUAD were PD-L1+; however, only 1.4% of early PD-L1+ cases were ALK+, perhaps reflecting a bias towards patients with positive ALK testing by IHC or FISH results not being referred for CGP. In a threshold analysis we estimated that PD-L1+ prevalence would need to be at least 35.4% and the prevalence of ALK/ROS1/RET drivers in the PD-L1+ population at least 4.7% in total for CGP to be the cost-saving or cost neutral choice.

As noted previously, the cost analysis depends on the assumption that patients with ALK, ROS1, or RET driver alterations would probably not have a survival benefit with adjuvant ICI. This is based on available data in the metastatic setting and from the IMpower010 study, where the few patients with EGFR or ALK alterations did not have a clear improvement in disease free survival (hazard ratios 0.99 and 1.04 respectively). While this
has not yet been proven definitively, and no biomarker guidance for adjuvant ICI is included yet in the NCCN guidelines, several relevant trials are ongoing.\textsuperscript{27} We believe that with the existing and anticipated new data some physicians may consider avoiding adjuvant atezolizumab in this patient population. For these practitioners in particular, our cost analysis would be relevant. Beyond costs, there is also the clinical benefit of reduced unnecessary ICI toxicity with the appropriate avoidance of adjuvant ICI. In IMpower010, 52\% of the patients receiving atezolizumab experienced an immune-related adverse event compared to just 9\% of the patients who got best supportive care, and 12\% of patients in the atezolizumab arm required treatment with systemic corticosteroids.\textsuperscript{15} Further, upon recurrence previous ICI use may lead to treatment delays and an increased risk of TKI toxicity.\textsuperscript{27-31}

There are multiple limitations that should be noted for this study. First, at this point, adjuvant therapy is recommended for patients with stage II-IIIA NSCLC. There is also a group of high-risk IB for whom adjuvant therapy can be considered, but for all IA and most IB disease adjuvant treatment is not used or recommended. These stages were not excluded in the final analysis. Assuming PD-L1 and the driver alteration rates are similar in this population though (as they are similar in both early and advanced disease), this should not change the final cost benefit results. Second, in March 2022 the FDA approved neoadjuvant nivolumab based on the results of the CHECKMATE-816 trial. Given the newness of this approval, the cost of the avoidance of neoadjuvant nivolumab was not calculated in our study, and there is no consensus in the thoracic oncology community about when to use neoadjuvant or adjuvant ICI (or maybe even both). We do feel there are patients in whom adjuvant ICI would be used, and that this analysis would still be applicable and important for this group. It would be interesting to investigate how CGP could potentially play a role in neoadjuvant selection. Third, this study is biased as all included patients were those who received CGP testing. Finally, clinical information, including receipt of therapy, was captured as documented in the EHR and events occurring outside of the Flatiron Health network and not documented in the available EHR were not captured. In particular, regarding assessing absolute rates of targeted therapy use in this study, some patients may have received targeted therapy outside the
Flatiron network, which would not have been captured, received targeted therapy in later lines, or been treated before therapies currently on-label were approved. Additionally, clinical study drugs were masked in this analysis, thus patients could have received targeted therapy on a clinical trial, and patients with stage IIIB disease may have received curative-intent chemoradiation.

**Conclusion**

Comprehensive genomic profiling in early-stage LUAD can identify *EGFR, ALK, ROS1, RET* and other driver alterations and accelerate the start of appropriate first-line TKI at recurrence. CGP could also represent a more cost-effective approach by avoiding potentially futile ICI in patients with driver alterations that have historically lacked activity with ICI in metastatic disease. Additional investigation is needed to confirm that adjuvant ICI should be avoided in these patients, and with the recent approval of neoadjuvant nivolumab, the use of adjuvant atezolizumab may be more limited. Regardless, the recent advances and ongoing trials in perioperative treatment for NSCLC highlights that early-stage disease will need a more individualized, nuanced approach. Use of CGP in early-stage disease would be helpful in making this process more efficient and effective.
References


Table 1. Characteristics of patients with CGP testing performed in the early vs advanced disease setting.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CGP report in adv disease N=5850</th>
<th>CGP report in early disease N=608</th>
<th>p value</th>
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<tr>
<td>Age at Diagnosis (median, IQR)</td>
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<td>69.0 [62.0;76.0]</td>
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<td>Community practice</td>
<td>5199 (88.9%)</td>
<td>566 (93.1%)</td>
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<td>Female</td>
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<td>361 (59.4%)</td>
<td>0.051</td>
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<tr>
<td>Race</td>
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<td></td>
<td>0.030</td>
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<td>192 (3.28%)</td>
<td>30 (4.93%)</td>
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<td>Black or African American</td>
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<td>29 (4.77%)</td>
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<tr>
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<td>391 (64.3%)</td>
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<td>Other</td>
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<td>100 (16.4%)</td>
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<tr>
<td>Not documented</td>
<td>477 (8.15%)</td>
<td>58 (9.54%)</td>
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<td></td>
<td>&lt;0.001</td>
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<td>Stage I</td>
<td>561 (9.59%)</td>
<td>207 (34.0%)</td>
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<tr>
<td>Stage II</td>
<td>308 (5.26%)</td>
<td>170 (28.0%)</td>
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</tr>
<tr>
<td>Stage IIIA</td>
<td>349 (5.97%)</td>
<td>231 (38.0%)</td>
<td></td>
</tr>
<tr>
<td>Stage IIIB/C</td>
<td>472 (8.07%)</td>
<td>0 (0.00%)</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>4160 (71.1%)</td>
<td>0 (0.00%)</td>
<td></td>
</tr>
<tr>
<td>Smoking History:</td>
<td></td>
<td></td>
<td>0.102</td>
</tr>
<tr>
<td>History of smoking</td>
<td>4595 (78.5%)</td>
<td>500 (82.2%)</td>
<td></td>
</tr>
<tr>
<td>No history of smoking</td>
<td>1241 (21.2%)</td>
<td>107 (17.6%)</td>
<td></td>
</tr>
<tr>
<td>Unknown/Not documented</td>
<td>14 (0.24%)</td>
<td>1 (0.16%)</td>
<td></td>
</tr>
</tbody>
</table>

Early disease setting refers to patients with CGP results reported within 6 months of initial diagnosis, specimen collected within 3 months of initial diagnosis, and before any advanced diagnosis. Advanced (adv) disease setting refers to CGP report after advanced diagnosis. Patients with CGP report prior to any adv diagnosis but >6 months after initial diagnosis or specimen >3 months after initial diagnosis are excluded from this analysis. Ages at diagnosis are compared using Kruskall-Wallis test, frequencies are compared using Fisher’s exact test.
Figure 1. Frequencies of driver oncogene alterations detected by CGP in tumor tissue specimens collected during early and advanced stage lung adenocarcinoma (LUAD). The prevalence of driver gene alterations listed in the National Comprehensive Cancer Network NSCLC guidelines are shown for all LUAD cases (A, B) and for the PD-L1 positive subset (C, D). B and D show the frequencies of specific EGFR and KRAS alterations. Gene alterations were compared for specimens collected in the early vs advanced (adv) disease setting. Early setting is defined as CGP specimen collected within 3 months of initial diagnosis.
Figure 2. Enrichment of co-altered genes in early and advanced lung adenocarcinoma. The prevalence of gene alterations was compared for specimens collected in the early vs advanced disease setting. Volcano plots depict all lung adenocarcinoma cases (left) and only PD-L1+ cases (right). Higher frequency alterations are shown with larger dot size. The dotted horizontal line is a significance cutoff (FDR = 0.05). P values were corrected with the Benjamini-Hochberg FDR method. Early setting is defined as CGP specimen collected within 3 months of initial diagnosis.
Figure 3. Dynamics of CGP testing patterns in the early and advanced lung adenocarcinoma disease settings over time. Incidence of CGP testing is increasing over time in both the early and advanced disease settings, and the fraction of cases tested in early disease is increasing over time. Numbers on each bar indicate cases tested. *2021 data is through 6/30/2021 only. Early vs advanced disease designation is based on CGP report date. *Early setting refers to patients with CGP report date within 6 months of initial diagnosis with specimen collected within 3 months of initial diagnosis. Other prior to advanced disease category includes patients with CGP report > 6 mos from initial diagnosis but prior to adv diagnosis or CGP report <6 mos from initial diagnosis but with specimen collection > 3 mos from initial diagnosis. If a patient had multiple CGP tests performed during their disease course the first instance is depicted here.
Figure 4. CGP prior to recurrence is associated with timely delivery of first-line therapy. (A) In LUAD patients with CGP on samples collected prior to recurrence, those with CGP results obtained any time before recurrence vs after recurrence had less time from recurrence to start of 1L therapy (median 3.6 vs 6 wks, p < 0.001). 18 patients have record of starting 1L therapy shortly (≤ 2 wks) before advanced diagnosis. (B) In LUAD patients with a targetable EGFR, ALK, RET, or ROS1 driver detected and CGP on samples collected prior to recurrence, those with CGP results obtained any time before recurrence vs after recurrence had less time from recurrence to start of 1L therapy (median 2.7 vs 6.7 wks, p < 0.001). 5 patients have record of starting 1L therapy shortly (≤ 2 wks) before advanced diagnosis.
Figure 5. Cost implications and assumptions for testing paradigms in early stage LUAD. (A) Probabilistic decision tree with alternative testing strategies of single-gene EGFR and PD-L1 IHC compared to multigene testing for at least EGFR, ALK, RET, and ROS1 using comprehensive genomic profiling (CGP). (B) Incremental cost of CGP testing strategy compared to EGFR single-gene testing in early stage LUAD shows an expected total cost reduction per patient with universal CGP testing compared to EGFR single gene testing of $1,597.23. Cost assumptions are shown in Supplemental Table 2. (C) One-way sensitivity analysis. Cost base values and minimum and maximum estimates are described in Supplemental Table 4.
Credit statement:

**Bharathi Muthusamy**: Conceptualization, Writing - Original Draft, Writing - Review & Editing, **Kira Raskina**: Formal analysis, Visualization, Data curation, Software, Writing - Review & Editing, **Katherine Lofgren**: Formal analysis, Visualization, Writing - Review & Editing, **Gerald Li**: Formal analysis, Visualization, **Khaled Tolba**: Visualization, Writing - Review & Editing, **Karen Schwed**: Writing - Review & Editing, **Emily Castellanos**: Writing - Review & Editing, **Rishard Huang**: Writing - Review & Editing, **Geoffrey R. Oxnard**: Conceptualization, Supervision, Writing - Review & Editing, **Alexa Schrock**: Supervision, Writing - Original Draft, Visualization, Writing - Review & Editing, **Karen Schwed**: Writing - Review & Editing, **Rischaard Huang**: Writing - Review & Editing, **Emily Castellanos**: Writing - Review & Editing, **Rishard Huang**: Writing - Review & Editing, **Geoffrey R. Oxnard**: Conceptualization, Supervision, Writing - Review & Editing, **Alexa Schrock**: Supervision, Writing - Original Draft, Visualization, Writing - Review & Editing, **Nathan Pennell**: Supervision, Writing - Review & Editing.