


Real-World Clinical Performance of a DNA-Based Comprehensive Genomic Profiling Assay for Detecting Targetable Fusions in Nonsquamous NSCLC

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Abstract

Background: Genomic fusions are potent oncogenic drivers across cancer types and many are targetable. We demonstrate the clinical performance of DNA-based comprehensive genomic profiling (CGP) for detecting targetable fusions.

Materials and Methods: We analyzed targetable fusion genes in >450 000 tissue specimens profiled using DNA CGP (FoundationOne CDx, FoundationOne). Using a de-identified nationwide (US-based) non-small cell lung cancer (NSCLC) clinico-genomic database, we assessed outcomes in patients with nonsquamous NSCLC (NonSqNSCLC) who received matched therapy based on a fusion identified using DNA CGP. Lastly, we modeled the added value of RNA CGP for fusion detection in NonSqNSCLC.

Results: We observed a broad diversity of fusion partners detected with DNA CGP in conjunction with targetable fusion genes (*ALK*, *BRAF*, *FGFR2*, *FGFR3*, *NTRK1/2/3*, *RET*, and *ROS1*). In NonSqNSCLC with oncogenic *ALK*, *NTRK*, *RET*, and *ROS1* fusions detected by DNA CGP, patients treated with a matched tyrosine kinase inhibitor had better real-world progression-free survival than those receiving alternative treatment regimens and benefit was observed regardless of the results of orthogonal fusion testing. An estimated 1.3% of patients with NonSqNSCLC were predicted to have an oncogenic driver fusion identified by RNA, but not DNA CGP, according to a model that accounts for multiple real-world factors.

Conclusion: A well-designed DNA CGP assay is capable of robust fusion detection and these fusion calls are reliable for informing clinical decision-making. While DNA CGP detects most driver fusions, the clinical impact of fusion detection is substantial for individual patients and exhaustive efforts, inclusive of additional RNA-based testing, should be considered when an oncogenic driver is not clearly identified.

Key words: DNA sequencing; RNA sequencing; gene fusion; next-generation sequencing; lung cancer.

Implications for Practice

When specific design elements and analytics are incorporated, DNA-based next-generation sequencing (NGS) assays are capable of detecting clinically important fusions, including novel structures and fusion partners. In certain scenarios, RNA-based NGS may extend or complement DNA-based NGS for fusion detection, and may be of substantial benefit when (1) a DNA NGS assay is not optimally designed or (2) in tumors where an oncogenic driver alteration has not been detected at the DNA level. Given the increased technical challenges inherent to RNA as an analyte, it is important that DNA assays are designed for maximal fusion detection.

Introduction

Two key developments in the clinical management of patients with solid tumors include the mainstream availability of comprehensive genomic profiling (CGP) and the identification of oncogenic drivers amenable to small molecule inhibition using targeted therapy.^{1–4} In no indication has this transformation been more impactful than in non-small cell lung cancer (NSCLC), where the identification of numerous oncogenic drivers and a rapidly growing landscape of matched targeted therapies has extended overall survival for a high percentage

of patients.^{5,6} Even still, it is estimated that approximately 30% of NSCLC adenocarcinoma lack a well-defined oncogenic driver alteration.^{7–10}

Historically, CGP has been performed on DNA derived from tumor specimens. However, as the importance of oncogenic fusions as cancer drivers has been realized,^{11,12} particularly in NSCLC,¹³ attention has turned to RNA as an important analyte for the detection of driver fusions that might be missed using DNA-based assays.^{14,15} Given such reports, both the National Comprehensive Cancer Network (NCCN) and the

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American Society of Clinical Oncology (ASCO) recommend pursuing RNA-based fusion profiling for driver-negative NSCLC as determined by initial DNA-based next-generation sequencing (NGS).^{16,17} Potential advantages of RNA-based fusion detection include the ability to determine whether a fusion product is expressed at the RNA level and avoidance of often complex methodology required to sequence introns and over large chromosomal regions. However, major limitations of RNA profiling are the lower stability, quality, and quantity of the RNA analyte compared to DNA which can result in high sequencing failure rates,¹⁸⁻²⁰ especially for formalin-fixed archival material.²¹ Thus, there are advantages and disadvantages to either testing methodology.

The current realities of CGP testing necessitate that many patients rely on DNA-based CGP for fusion detection, and it is thus critical that DNA-based assays are optimally designed to identify these important driver events which can significantly influence treatment decisions and patient outcomes. Moreover, it is of value to identify the clinical scenarios in which patients are most likely to benefit from additional RNA-based testing. In this study, we demonstrate that robust detection of fusions is possible using carefully designed DNA-based sequencing methodologies and that fusions detected using high-quality DNA CGP assays are associated with clinical benefit from matched targeted agents. We also attempt to quantify the potential added value of RNA CGP for fusion detection following DNA CGP for patients with nonsquamous NSCLC (NonSqNSCLC).

Materials and Methods

Comprehensive Genomic Profiling

Comprehensive genomic profiling using FoundationOne CDx (F1CDx) or FoundationOne (F1) was performed on hybridization-captured, adaptor ligation-based libraries using DNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor in a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited, New York State-approved laboratory (Foundation

Medicine, Inc., Cambridge, MA). Approval for this study, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817). Samples were interrogated for alterations in 324 (F1CDx) or 406 (F1) cancer-associated genes.²² Alteration types detected by the assay include base substitutions, short insertions and deletions, copy number amplifications and homozygous deletions, and large genomic rearrangements, as well as microsatellite instability and tumor mutational burden (TMB). TMB was determined on up to 1.24 Mb of sequenced DNA.²³ A number of deliberate assay design features (Figure 1; Supplementary Table S1 with detailed description in Results) support rearrangement detection.

Study Cohorts

Genomic-Only Cohort: Pan-Solid Tumor Fusion Partner Diversity Analysis Utilizing Foundation Medicine Genomic Database

In the Foundation Medicine (FMI) genomic database, we interrogated pan-solid tumor tissue biopsy specimens submitted for CGP (Foundation Medicine, Inc.) using F1CDx or F1 during routine clinical care between August 2014 and December 2022. For patients with multiple tissue CGP results, a single specimen was chosen on the basis of quality metrics. Patients with predicted gene fusions (in-strand and in-frame rearrangements involving distinct target and partner genes) were queried and compared to fusions reported in the AACR Project Genomics Evidence Neoplasia Information Exchange (GENIE)^{24,25} database (v13.1). AACR Project GENIE is an international pan-cancer registry collecting clinical-grade cancer genomic data together with clinical outcomes data for >140 000 patients (v13.1 release). Only assays/samples with structural variant profiling in GENIE were included and data from GENIE institutions that utilize FMI testing was excluded. Profiling of hematological malignancies and profiling with heme-specific panels were also excluded. GENIE data used in this study included

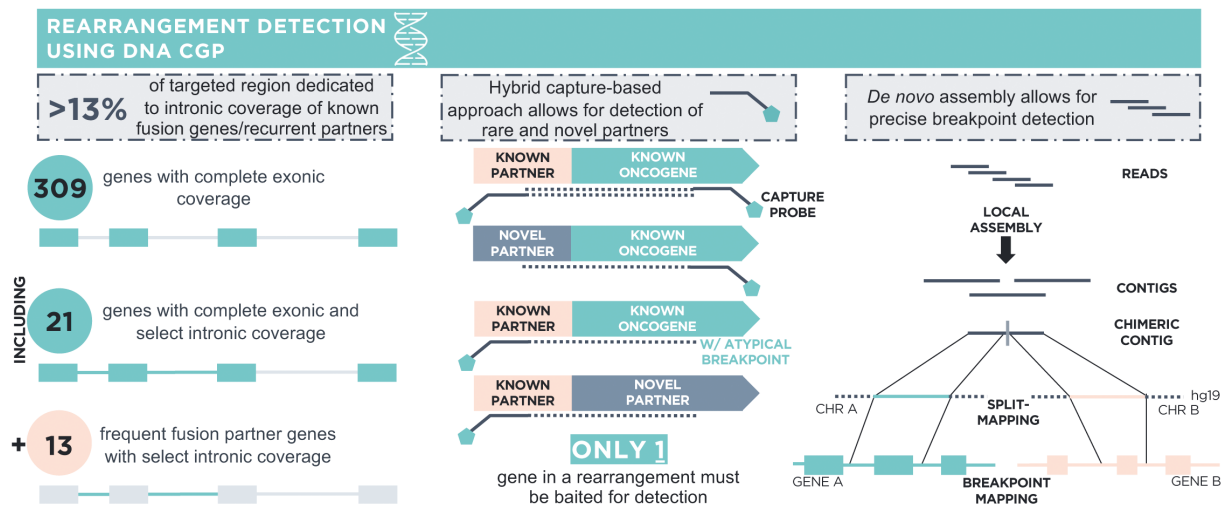


Figure 1. F1CDx assay design enables robust DNA-based rearrangement detection. Design features of F1CDx that support rearrangement detection include (1) select intronic coverage (Supplementary Table S1) for $N = 34$ genes including both common fusion genes ($N = 21$) and frequent fusion partners ($N = 13$), (2) the use of a hybrid capture-based sequencing approach which only requires that one gene involved in a fusion be baited allowing for detection of known as well as rare and novel fusion partners, and (3) the use of a de novo assembly approach in the bioinformatics pipeline which facilitates precise breakpoint detection compared to reference-based methods.

structural variant profiling for 97 592 patients with solid tumors using 18 different assays/assay versions submitted by 7 academic institutions. This included 96 552 patients (98.9%) with DNA-only profiling, 929 (0.9%) with DNA/RNA profiling, and 111 (0.1%) with RNA-only profiling. Note that *NTRK* genes were profiled in most, but not all, patients (97 106/97 592, 99.5%), whereas all other analyzed genes were covered on all included assays.

Clinico-Genomic Cohort: NSCLC Genomic Driver Landscape and Outcomes Analysis on Targeted Therapy Utilizing Flatiron Health-Foundation Medicine Clinico-Genomic Database

To assess the adequacy of DNA-only fusion detection to support clinical decision-making, we studied a cohort of patients with confirmed diagnosis of NSCLC included in the nationwide US-based Flatiron Health (FH)-FMI de-identified NSCLC clinico-genomic database (CGDB). All patients underwent genomic testing using FMI CGP assays. De-identified clinical data originated from approximately 280 US cancer clinics (approximately 800 sites of care). Retrospective longitudinal clinical data were derived from electronic health records, comprising patient-level structured and unstructured data, curated via technology-enabled abstraction of clinical notes and radiology/pathology reports, which were linked to genomic data derived from FMI testing by de-identified, deterministic matching.²⁶ The data were de-identified and subject to obligations to prevent re-identification and protect patient confidentiality. Clinical data included demographics, self-reported smoking status, clinical and laboratory features, timing of treatment exposure, and mortality. Institutional Review Board approval of the study protocol was obtained prior to the study conduct and included a waiver of informed consent.

Patient records were included in this study if they received an F1CDx or F1 CGP report between August 2014 and December 2022 and the histological and genomic characteristics of their disease (as assessed by chart abstraction and the tissue CGP report, respectively) were consistent with NonSqNSCLC. Clinical characteristics for the NonSqNSCLC cohort ($N = 10\,761$) are presented in [Supplementary Table S2](#). A subset of patients received additional non-FMI biomarker testing (“orthogonal testing”) as part of routine care using a variety of testing methodologies (eg, fluorescence in situ hybridization [FISH], immunohistochemistry [IHC], polymerase chain reaction [PCR], and NGS). Where orthogonal testing results were available, these have been included in the dataset for analysis limited to *KRAS*, *EGFR*, *BRAF*, *MET*, *ALK*, *RET*, *ROS1*, and *NTRK1/2/3*.

Analysis

Real-World Progression-Free Survival

Real-world progression-free survival (rwPFS) was calculated from the start of treatment to the first progression date >14 days after treatment start or to death. Real-world progression events were abstracted from patient charts using a clinician-anchored approach supported by radiology report data, as previously described.²⁷ Mortality information in the dataset has been externally validated in comparison to the National Death Index.²⁸ Patients were censored at their last clinic note date if no progression or death was observed. Median rwPFS values were estimated in months with 95% CI.

In these analyses, oncologist-defined, rule-based lines of therapy were classified into those including a fusion-specific FDA-approved TKI for NSCLC (ie, alectinib, brigatinib, ceritinib, crizotinib, entrectinib, larotrectinib, lorlatinib, seliperparin, and pralsetinib) or not. These rules allow early switches from non-TKI-containing into TKI-containing lines of therapy without being considered the start of a new line (eg, after receipt of biomarker test results not available at the start of therapy). Analysis was limited to the first line of therapy in the advanced setting such that a patient who received a non-TKI in their first line and received a TKI in a later line would be considered a non-TKI patient for this analysis.

Statistical Analysis and Interpretation

Differences in time-to-event outcomes were assessed with the log-rank test and univariable and multivariable Cox proportional hazard models. Multivariable Cox models included adjustment for clinical characteristics that could impact prognosis including line number, socioeconomic status, practice type, gender, race, stage at diagnosis, self-reported smoking history, age, ECOG performance status, opioid prescription immediately prior to therapy (a proxy for burden of disease), and presence of metastases pre-therapy (in bone, central nervous system, liver, adrenal, or other). Missing values for adjustment covariables were not imputed, causing some patients to be excluded from multivariable analyses. R version 4.2.2 software was used for all statistical analyses.

RNA CGP Fusion Detection Modeling

We calculated the number of patients with driver fusion-positive NonSqNSCLC expected to be uniquely identified using RNA CGP following DNA CGP ($\text{fusions}_{\text{RNA,only}}$) as a function of several real-world factors (equation 1):

$$\text{fusions}_{\text{RNA,only}} = (\text{prev}_{\text{fusions}}) \times (1 - \theta_{\text{DNA}}) \times (p_{\text{DNA}}) \times (p_{\text{RNA}} | \text{DNA}_{s=1}) \times (\text{selection.bias}_{\text{RNA}})$$

where $\text{prev}_{\text{fusions}}$ is the estimated prevalence of driver fusions in the unselected patient population with NonSqNSCLC, θ_{DNA} is the probability of detecting an oncogenic driver on DNA CGP, p_{DNA} is the probability of DNA assay technical success, and $p_{\text{RNA}} | \text{DNA}_{s=1}$ is the probability of RNA assay technical success conditional on DNA assay technical success. To account for possible selection bias toward a higher underlying fusion prevalence in the DNA CGP driver-negative patient population, we included a prevalence multiplier ($\text{selection.bias}_{\text{RNA}}$) in sensitivity analyses. In addition to our base case calculation, we conducted both one-way deterministic and probabilistic sensitivity analyses to assess the influence of our base case assumptions and to characterize uncertainty in the estimate of RNA-only identified fusions. Estimates and justifications for the base case parameter assumptions and the deterministic and probabilistic sensitivity analyses are summarized in [Supplementary Table S3](#). We assumed perfect test characteristics for RNA fusion detection as a conservative assumption. Our base estimates and sensitivity values for the prevalence of *ALK*,^{11,29-34} *RET*,^{11,33-36} *ROS1*,^{11,30,33,34,37} and *NTRK*^{11,34,38,39} fusions in patients with NSCLC were based on the published literature. While *BRAF*, *EGFR*, *FGFR*, *NRG1*, and other fusions do occur in NSCLC, these are rare events such that the combined prevalence of NCCN fusion drivers (*ALK* + *NTRK* + *RET* + *ROS1*) was expected to produce a reasonable estimate of the overall prevalence of oncogenic

driver fusions in NSCLC. The probability of detecting a driver alteration in NonSqNSCLC using DNA CGP was determined using FMI DNA CGP results for patients in CGDB based on a select list of NSCLC receptor tyrosine kinase (RTK)/mitogen-activated protein kinase (MAPK) driver alterations (Supplementary Table S4) as described in Results. The probability of DNA/RNA sequencing success was based on FoundationOne Heme historical data with the upper bound based on real-world experience with DNA NGS reflexed to RNA NGS testing in a study by Benayed et al.¹⁴ The base case calculation assumes no selection bias for fusions in the DNA CGP-negative patient population (ie, a prevalence multiplier of 1×). The upper bound (1.2× the base case fusion prevalence) was derived from the percentage of fusion-positive patients identified using DNA CGP and RNA CGP in the study by Benayed et al.¹⁴ The minimum and maximum values for the parameters in equation 1 (Supplementary Table S3) were used to generate deterministic one-way sensitivity values where a single parameter was modified from its base case value to either the minimum or maximum value. The parameter distributions specified in Supplementary Table S3 were used to simultaneously draw

parameter values for all 5 parameters across 10 000 simulations to characterize a joint uncertainty estimate.

Results

FMI DNA CGP Detects Fusions With a Rich Diversity of Fusion Partners

We analyzed genomic profiling data from 459 751 patients with diverse cancer types to characterize the landscape of targetable oncogenic fusions detected in tissue biopsies using a DNA CGP assay purposefully designed for rearrangement detection (Figure 1). We identified predicted fusions in *ALK* (*n* = 2089), *BRAF* (*n* = 1092), *FGFR2* (*n* = 1283), *FGFR3* (*n* = 1100), *NTRK1/2/3* (*n* = 244), *RET* (*n* = 910), and *ROS1* (*n* = 378) across all solid tumors (Fig. 2A). We observed a rich diversity of fusion partners, both previously reported and novel, across target genes (Fig. 2B, 2C; Supplementary Fig. S1; Supplementary Table S5). We used the AACR Project GENIE database (v13.1)^{24,25}—inclusive of 97 592 patients with structural variant profiling using primarily DNA-based assays—as a point of reference for our partner diversity analysis. The most recurrent partners for each target gene were

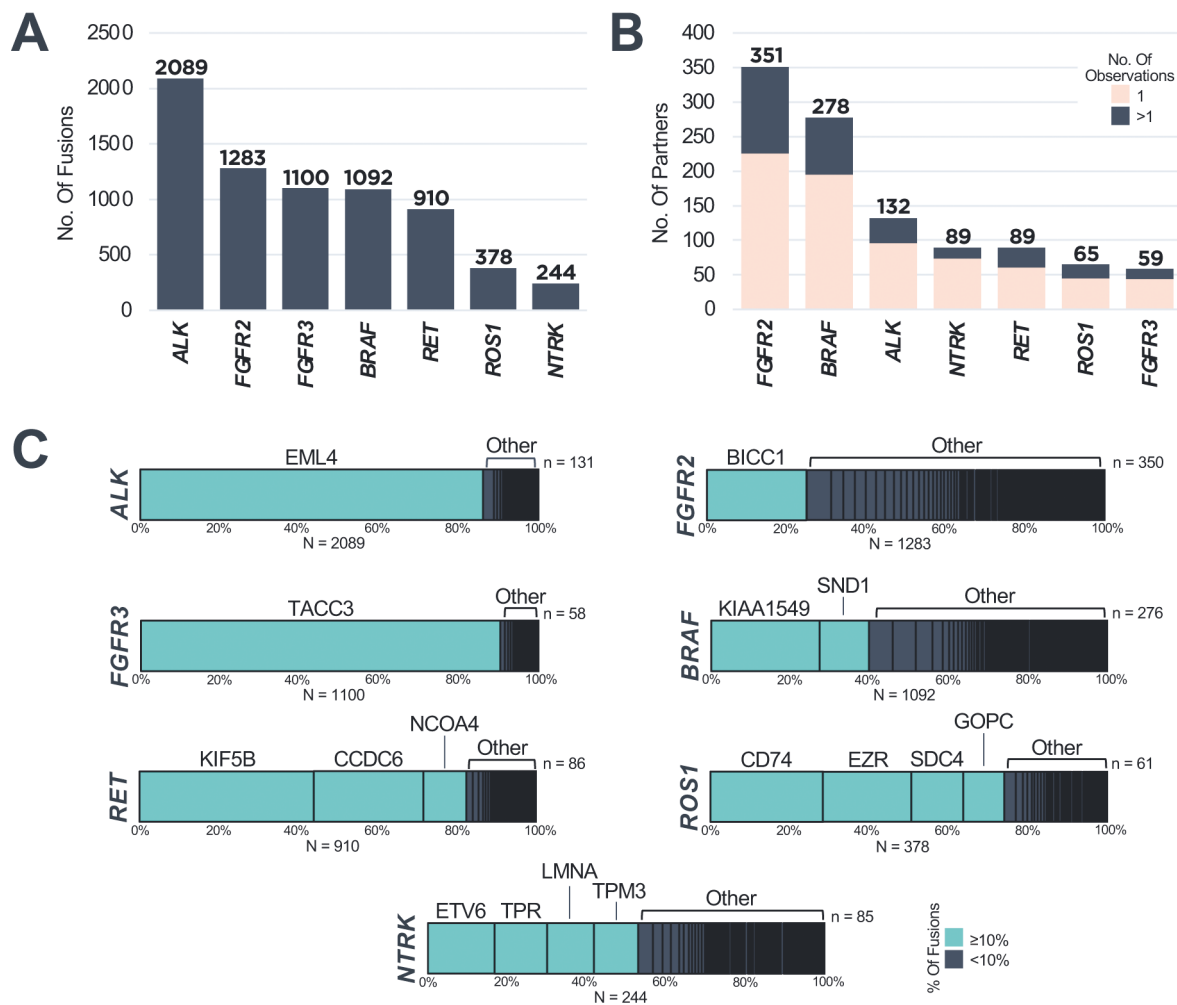


Figure 2. FMI DNA CGP detects fusions with a rich diversity of fusion partners. (A) Number of fusions detected involving *ALK*, *BRAF*, *FGFR2*, *FGFR3*, *NTRK1/2/3*, *RET*, and *ROS1* across solid tumors. (B) Number of partner genes observed with *ALK*, *BRAF*, *FGFR2*, *FGFR3*, *NTRK1/2/3*, *RET*, and *ROS1* across solid tumors. (C) Distribution of partner genes detected in association with each indicated fusion gene across solid tumors. Recurrent partners accounting for ≥10% of detected fusions are indicated in teal.

common among the data sets: *EML4* with *ALK*; *KIAA1549* with *BRAF*; *BICC1* and *TACC2* with *FGFR2*; *TACC3* with *FGFR3*; *ETV6*, *TPR*, *LMNA*, and *TPM3* with *NTRK1/2/3*; *KIF5B*, *CCDC6*, and *NCOA4* with *RET*; and *CD74*, *EZR*, *SDC4*, and *GOPC* with *ROS1* (Supplementary Fig. S1; Supplementary Table S5). Due to the relative size of the FMI DNA CGP cohort, a plethora of unique partner genes (observed as partner genes in the FMI but not the GENIE cohort) was detected (*ALK* $n = 104$, *BRAF* $n = 232$, *FGFR2* $n = 295$, *FGFR3* $n = 44$, *NTRK* $n = 76$, *RET* $n = 71$, *ROS1* $n = 50$; Supplementary Fig. S1; Supplementary Table S5). With FMI DNA CGP, 64%-83% of fusion partners detected for each gene were observed in only a single sample, highlighting the capability of DNA CGP for the detection of fusions involving rare and novel partners (Supplementary Fig. S2A). In addition, detection of a subset of *NRG1* fusions ($n = 67$, 0.02% of the cohort, $N = 316\ 152$) is enabled through baiting of common partner genes *CD74* and *SDC4* on F1CDx (Supplementary Fig. S2B).

Targetable Fusions in Patients With NonsqNSCLC Detected Using FMI DNA CGP Gain More Benefit From Matched Therapy Than Patients With Fusions Detected Using Other Testing Methods

We compared the clinical benefit from targeted therapy using matched tyrosine kinase inhibitors (TKI) for oncogenic fusions detected using tissue-based FMI DNA CGP and/or an orthogonal assay (eg, FISH, IHC, PCR, and non-FMI NGS). In the total cohort of patients analyzed who received orthogonal fusion testing and were assessed for progression after starting first-line therapy for advanced disease ($N = 3959$; Table 1; Supplementary Fig. S3), the most common orthogonal testing method used was FISH followed by non-FMI liquid biopsy DNA NGS, with a lesser degree of IHC, PCR, and RNA-based NGS testing performed (Fig. 3A). Similar trends were observed when limiting to the cohorts in which FMI DNA CGP and orthogonal diagnostic assay results were discordant (Supplementary Fig. S4).

Among 193 patients for whom oncogenic fusions in *ALK*, *NTRK1/2/3*, *RET*, or *ROS1* were detected on both FMI and orthogonal assays and for whom rwPFS data were available, those who received a matched TKI in the advanced first line of therapy had nominally (nonsignificantly) better rwPFS than those who received an alternative treatment regimen (univariable HR 0.81, 95% CI 0.59-1.11, $P = .19$; Supplementary Fig. S5A). After adjustment for known prognostic factors in a multivariable Cox model, this difference was statistically significant (multivariable HR 0.53, 95% CI 0.35-0.79, $P = .002$; Fig. 3B; Supplementary Fig. S6A).

Among the 148 patients whose *ALK*, *NTRK*, *RET*, and *ROS1* fusions were detected by FMI DNA CGP but were not observed by orthogonal testing, patients treated with a matched TKI had better rwPFS than those receiving an alternative treatment regimen (univariable HR 0.59, 95% CI 0.39-0.90, $P = .012$; Multivariable HR 0.60, 95% CI 0.37-0.99, $P = .05$; Fig. 3B; Supplementary Fig. S5B; Supplementary Fig. S6B). However, among the 54 patients for whom FMI DNA CGP did not detect an actionable fusion in *ALK*, *NTRK*, *RET*, or *ROS1* when an orthogonal assay did detect a fusion, patients who received a matched TKI had worse rwPFS than those who did not (univariable HR 3.48, 95% CI 1.75-6.91, $P < .001$; multivariable HR 4.59, 95% CI 1.29-16.32, $P = .019$; Fig. 3B; Supplementary Fig. S5C; Supplementary Fig. S6C).

Across all patients who received a matched TKI and had at least one test which detected an *ALK*, *NTRK*, *RET*, or *ROS1* fusion (FMI DNA CGP and/or orthogonal, $n = 185$), those with fusions detected by FMI DNA CGP had significantly longer rwPFS than those with fusions not detected by FMI DNA CGP, regardless of the result of orthogonal testing (relative to FMI DNA CGP-/orthogonal+: FMI DNA CGP+/orthogonal+ univariable HR 0.25, 95% CI 0.14-0.45, $P < .001$; FMI DNA CGP+/orthogonal- univariable HR 0.18, 95% CI 0.09-0.35, $P < .001$; Fig. 3C). These results were more pronounced when applying a multivariable Cox model adjusting for various known prognostic factors (Supplementary Fig. S6D).

Expected Detection of Clinically Actionable Fusions Using Complementary RNA CGP in NonSqNSCLC

To quantify the added value of RNA CGP following DNA CGP for detecting clinically important driver fusions in NonSqNSCLC, we used a mathematical model which takes into account several real-world factors. We started with the assumption that patients with an oncogenic driver detected on DNA CGP are unlikely to have an additional driver identified on RNA CGP since drivers in NSCLC tend to be mutually exclusive. To estimate the probability of identifying an oncogenic driver on DNA CGP, we analyzed genomic profiling data from 10 761 patients with NonSqNSCLC who received tissue-based FMI DNA CGP. We stratified the cohort by self-reported smoking status given established genomic differences between these 2 NSCLC subpopulations^{10,40,41} to inform our sensitivity analyses (Table 2; Supplementary Fig. S3). We explored the prevalence of fusion and nonfusion oncogenic driver alterations in RTK genes and the MAPK pathway. Notably, this included all patients with actionable driver alterations as defined in the NCCN guidelines for NSCLC¹⁶ as well as additional known biological drivers (Supplementary Table S4). As defined, an oncogenic driver alteration was not detected in 12% of never-smokers and 33% of ever-smokers with NonSqNSCLC (Supplementary Fig. S7). These prevalence estimates were used to inform one parameter of the model (θ_{DNA}), the probability of detecting an oncogenic driver on DNA CGP.

Our modeling estimates that approximately 1.3% of patients with NSCLC are expected to have an actionable driver fusion uniquely identifiable on RNA CGP (Fig. 4A). This estimate assumes (1) an underlying driver fusion prevalence of 6.0% in NSCLC based on the reported prevalence of *ALK*, *RET*, *ROS1*, and *NTRK* fusions in NSCLC and (2) a 70.8% probability of detecting an oncogenic driver using DNA CGP in the unselected patient population with NSCLC. (The full set of assumptions, parameter sources, and calculation approach are available in Methods, Supplementary Table S3, and Supplementary Fig. S8.) To determine the most influential parameters underlying the base case estimate of 1.3%, we conducted one-way sensitivity analyses (Fig. 4A). The most influential parameters were (1) underlying NSCLC fusion prevalence and (2) the probability of a driver alteration being detected on DNA CGP. However, even with an increase in the population-level fusion prevalence estimate from the base case of 6% to the maximum of 10%, we estimate a newly identified fusion will be detected on RNA CGP in only 2.2% of patients. Across all minimum and maximum potential parameter values for the one-way sensitivity analysis, this estimate ranged from 0.34% to 2.22% (Supplementary Table S6). We also conducted a probabilistic

Table 1. Clinical characteristics of patients with NonSqNSCLC stratified by fusion testing results.

Clinical characteristics	Orthogonal testing performed (N = 3959)	FMI DNA CGP-/ Orthog- (N = 3564)	FMI DNA CGP-/ Orthog+ (N = 54)	FMI DNA CGP+/ Orthog- (N = 148)	FMI DNA CGP+/ Orthog+ (N = 193)
Age at 1L start (years), Median (IQR)	65.9 (10.6)	66.7 (10.2)	64.0 (9.99)	61.2 (11.5)	56.1 (12.6)
Sex, n (%)					
Female	2237 (56.5%)	2017 (56.6%)	29 (53.7%)	88 (59.5%)	103 (53.4%)
Male	1722 (43.5%)	1547 (43.4%)	25 (46.3%)	60 (40.5%)	90 (46.6%)
Self-reported race, n (%)					
Asian	163 (4.1%)	143 (4.0%)	1 (1.9%)	3 (2.0%)	16 (8.3%)
Black or African American	257 (6.5%)	233 (6.5%)	2 (3.7%)	10 (6.8%)	12 (6.2%)
Hispanic or Latino	5 (0.1%)	5 (0.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Other race	583 (14.7%)	525 (14.7%)	4 (7.4%)	24 (16.2%)	30 (15.5%)
White	2687 (67.9%)	2414 (67.7%)	43 (79.6%)	100 (67.6%)	130 (67.4%)
Unknown/not documented	264 (6.7%)	244 (6.9%)	4 (7.4%)	11 (7.4%)	5 (2.6%)
AJCC stage at diagnosis, n (%)					
I	355 (9.0%)	339 (9.5%)	2 (3.7%)	7 (4.7%)	7 (3.6%)
II	238 (6.0%)	225 (6.3%)	4 (7.4%)	3 (2.0%)	6 (3.1%)
III	694 (17.5%)	635 (17.8%)	11 (20.4%)	24 (16.2%)	24 (12.4%)
IV	2598 (65.6%)	2296 (64.4%)	36 (66.7%)	112 (75.7%)	154 (79.8%)
Other/unknown/not documented	74 (1.9%)	69 (1.9%)	1 (1.9%)	2 (1.4%)	2 (1.0%)
Smoking history, n (%)					
History of smoking	2958 (74.7%)	2789 (78.3%)	40 (74.1%)	59 (39.9%)	70 (36.3%)
No history of smoking	998 (25.2%)	772 (21.7%)	14 (25.9%)	89 (60.1%)	123 (63.7%)
Unknown/not documented	3 (0.1%)	3 (0.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Practice type, n (%)					
Academic	534 (13.5%)	451 (12.7%)	9 (16.7%)	30 (20.3%)	44 (22.8%)
Academic/community	372 (9.4%)	326 (9.2%)	10 (18.5%)	13 (8.8%)	23 (11.9%)
Community	3053 (77.1%)	2787 (78.2%)	35 (64.8%)	105 (70.9%)	126 (65.3%)
Socioeconomic status (SES), n (%)					
1—Lowest SES	472 (11.9%)	433 (12.1%)	10 (18.5%)	13 (8.8%)	16 (8.3%)
2	647 (16.3%)	598 (16.8%)	7 (13.0%)	17 (11.5%)	25 (13.0%)
3	818 (20.7%)	724 (20.3%)	6 (11.1%)	42 (28.4%)	46 (23.8%)
4	886 (22.4%)	805 (22.6%)	16 (29.6%)	24 (16.2%)	41 (21.2%)
5—Highest SES	850 (21.5%)	750 (21.0%)	12 (22.2%)	36 (24.3%)	52 (26.9%)
Unknown	286 (7.2%)	254 (7.1%)	3 (5.6%)	16 (10.8%)	13 (6.7%)
ECOG PS at 1L start, n (%)					
0	1265 (32.0%)	1121 (31.5%)	19 (35.2%)	53 (35.8%)	72 (37.3%)
1	1654 (41.8%)	1506 (42.3%)	27 (50.0%)	57 (38.5%)	64 (33.2%)
2	369 (9.3%)	345 (9.7%)	3 (5.6%)	10 (6.8%)	11 (5.7%)
3	84 (2.1%)	78 (2.2%)	0 (0.0%)	2 (1.4%)	4 (2.0%)
Unknown	587 (14.8%)	514 (14.4%)	5 (9.3%)	26 (17.6%)	42 (21.8%)
1L therapy class, n (%)					
No TKI Received	3761 (95.0%)	3551 (99.6%)	40 (74.1%)	101 (68.2%)	69 (35.8%)
Received TKI	198 (5.0%)	13 (0.4%)	14 (25.9%)	47 (31.8%)	124 (64.2%)

sensitivity analysis which accounts for uncertainty in all input parameters simultaneously (Fig. 4B; see distribution details in Supplementary Table S3 and individual parameter

draws in Supplementary Fig. S9). Across 10 000 simulations, 95% of estimates were at or below 2.2% (minimum 0.1%, maximum 5.9%).

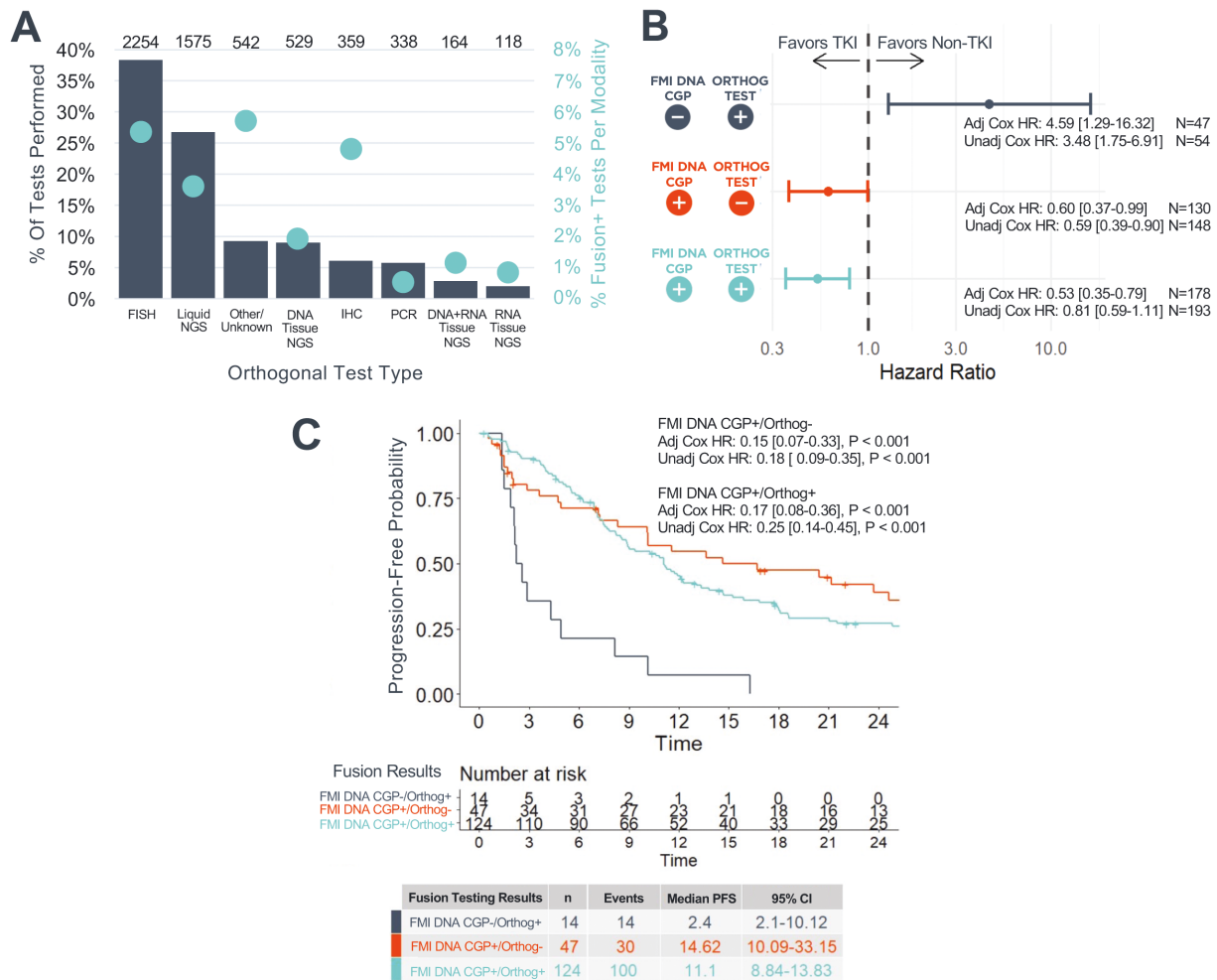


Figure 3. Targetable fusions detected using FMI DNA CGP are associated with improved clinical benefit from matched therapy compared to fusions detected using other testing methods. (A) Distribution of orthogonal testing modalities (N = 5879 tests; left axis, gray bars) and results (right axis, teal dots) from 3959 patients with NonSqNSCLC who were assessed for progression and underwent both FMI DNA CGP and additional fusion testing. Each testing modality is only counted once per patient. However, a single patient could be counted toward multiple modalities if a patient underwent multiple types of orthogonal testing such that the sum of all bars may exceed 100%. Ns above bars indicate the number of patients who underwent each type of testing. The fusion-positive percentage was calculated based on the total number of tests performed in each category (N = 7299 tests across all modalities), even if multiples of the same testing modality were performed for a single patient. (B) Forest plot depicting the adjusted rwPFS HR of receiving a matched TKI in the 1L for NonsqNSCLC subcohorts defined by concordance/discordance between FMI DNA CGP and orthogonal fusion testing results. (C) Among patients who received 1L-matched TKI, patients who were negative for *ALK*, *NTRK*, *RET*, and *ROS1* fusions on FMI DNA CGP had shorter rwPFS than patients who were positive by FMI DNA CGP. Unadjusted Kaplan-Meier plot is shown. All analyses are indexed to the start of 1L therapy. In addition to univariable Cox model HRs, adjusted HRs are presented for a multivariable Cox model that includes established prognostic variables (Supplementary Fig. S6). A small number of cases were excluded from multivariable analyses due to missingness of covariables. Abbreviations: FMI DNA CGP, Foundation Medicine Tissue DNA comprehensive genomic profiling; HR, hazard ratio; TKI, tyrosine kinase inhibitor; rwPFS, real-world progression-free survival; 1L, first advanced line of Tx.

Discussion

FMI DNA CGP assays employ a multifaceted approach to enable robust DNA-based rearrangement detection (Figure 1). In addition to baiting breakpoint hotspot introns in important fusion genes, the platform also baits breakpoint hotspot introns in previously reported fusion partner genes. Intentional baiting of specific partners allows for the detection of fusions involving clinically important oncogenes that are difficult to bait directly,^{14,42} eg, detection of a subset of *NRG1* fusions is achieved through baiting of *CD74* and *SDC4* and detection of a subset of *NTRK3* fusions is achieved through baiting of *ETV6*. The platform includes select intronic targeting for 34 genes (Supplementary Table S1). In total, over 13% of the targeted region is dedicated to intronic coverage

for the express purpose of rearrangement detection. Another feature of the assay that supports rearrangement detection is the use of a hybrid capture-based sequencing approach which requires that only one gene involved in a fusion be baited for detection, whereas its partner can be any gene in the genome even if not explicitly targeted by the assay. This allows for the detection of fusions involving recurrent or rare known partners as well as novel partners. Together with select partner gene baiting, this approach also supports the detection of known oncogenes with rare or novel breakpoints outside of baited hotspot intronic regions and oncogenes with hotspot intronic regions that cannot be efficiently baited (eg, *NRG1* and *NTRK3*). The bioinformatics pipeline used for fusion calling also contributes to the capabilities of the

Table 2. Clinico-genomic characteristics of patients with NonSqNSCLC stratified by smoking history.

Clinico-genomic characteristics	Smoking status known (N = 8747)	Former or current smoker (N = 7081)	Never-smoker (N = 1666)	P
Age at diagnosis (years), median (IQR)	67.0 (60.0;74.0)	68.0 (60.0;74.0)	66.0 (57.0;74.0)	<.001
Age at diagnosis (categorical), n (%)				
<45	192 (2.2%)	97 (1.4%)	95 (5.7%)	<.001
≥45	8555 (97.8%)	6984 (98.6%)	1571 (94.3%)	
Sex, n (%)				
Female	4801 (54.9%)	3682 (52.0%)	1119 (67.2%)	<.001
Male	3946 (45.1%)	3399 (48.0%)	547 (32.8%)	
Self-reported race, n (%)				
Asian	301 (3.4%)	126 (1.8%)	175 (10.5%)	<.001
Black or African American	595 (6.8%)	480 (6.8%)	115 (6.9%)	
Hispanic or Latino	8 (0.1%)	6 (0.1%)	2 (0.1%)	
Other race	1207 (13.8%)	978 (13.8%)	229 (13.7%)	
White	5807 (66.4%)	4819 (68.1%)	988 (59.3%)	
Unknown/not documented	829 (9.5%)	672 (9.5%)	157 (9.4%)	
AJCC stage at diagnosis, n (%)				
I	1055 (12.1%)	893 (12.6%)	162 (9.7%)	<.001
II	700 (8.0%)	602 (8.5%)	98 (5.9%)	
III	1639 (18.7%)	1394 (19.7%)	245 (14.7%)	
IV	5093 (58.2%)	3984 (56.3%)	1109 (66.6%)	
Other/unknown/not documented	260 (3.0%)	208 (2.9%)	52 (3.1%)	
Practice type, n (%)				
Academic	1051 (12.0%)	784 (11.1%)	267 (16.0%)	<.001
Academic/community	498 (5.7%)	383 (5.4%)	115 (6.9%)	
Community	7198 (82.3%)	5914 (83.5%)	1284 (77.1%)	
Socioeconomic status, n (%)				
1—Lowest SES	1199 (13.7%)	1049 (14.8%)	150 (9.0%)	<.001
2	1519 (17.4%)	1298 (18.3%)	221 (13.3%)	
3	1755 (20.1%)	1446 (20.4%)	309 (18.5%)	
4	1811 (20.7%)	1430 (20.2%)	381 (22.9%)	
5—Highest SES	1694 (19.4%)	1238 (17.5%)	456 (27.4%)	
Unknown	769 (8.8%)	620 (8.8%)	149 (8.9%)	
ECOG PS at diagnosis, n (%)				
0	1768 (20.2%)	1416 (20.0%)	352 (21.1%)	<.001
1	1839 (21.0%)	1545 (21.8%)	294 (17.6%)	
2	479 (5.5%)	423 (6.0%)	56 (3.4%)	
3+	121 (1.4%)	106 (1.5%)	15 (0.9%)	
Unknown	4540 (51.9%)	3591 (50.7%)	949 (57.0%)	
Tumor mutational burden (Mut/Mb), n (%)				
TMB < 10	5690 (65.2%)	4121 (58.3%)	1569 (94.5%)	<.001
TMB (10-20)	2029 (23.2%)	1964 (27.8%)	65 (3.9%)	
TMB ≥ 20	1011 (11.6%)	985 (13.9%)	26 (1.6%)	

assay for rearrangement detection. Initially, clusters of discordant read pairs are used to identify potential genomic rearrangement candidates. These candidates are further refined using a de novo local genomic assembly approach.^{22,43} This method facilitates precise breakpoint detection compared

to reference-based methods because rearrangement breakpoints often feature novel sequences introduced by DNA repair mechanisms that cannot be accurately mapped. More accurate breakpoint detection allows for better predictions regarding the functional effect of rearrangement events.

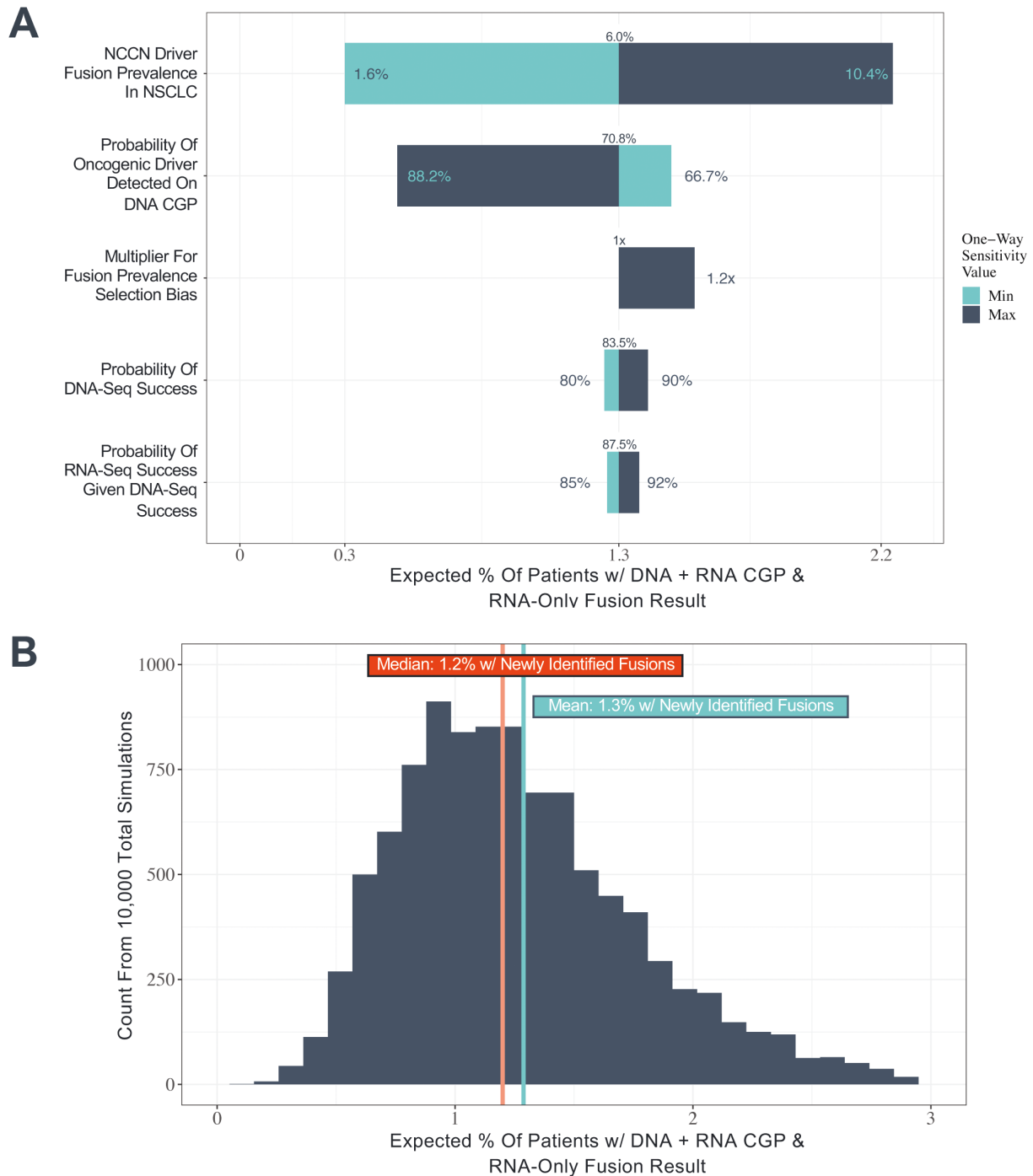


Figure 4. 1.3% of patients with NonSqNSCLC are expected to have an actionable fusion detected by RNA CGP that is not detected by DNA CGP. We conducted (A) one-way sensitivity analyses on key model parameters including the prevalence of oncogenic driver fusions in *ALK*, *NTRK*, *RET*, and *ROS1* in NSCLC, the probability an oncogenic driver is detected in NSCLC using DNA CGP, the probability of successful DNA and RNA sequencing, and the potential selection bias for fusion-positive patients among those that do not have oncogenic driver alterations detected on DNA CGP and (B) a probabilistic sensitivity analysis incorporating parameter uncertainty jointly with mean and median estimates across 10 000 simulations indicated.

Together, these design features enable robust detection of rearrangements and fusions with a DNA-based assay. We observed a broad diversity of both common and novel fusion partners associated with clinically actionable fusion genes including *ALK*, *BRAF*, *FGFR2/3*, *NTRK1/2/3*, *RET*, and *ROS1* using FMI DNA CGP. Moreover, we established that *ALK*, *NTRK*, *RET*, and *ROS1* fusions detected using FMI DNA CGP were highly correlated with favorable outcomes on matched TKI, whereas fusions solely detected using

orthogonal methods were not, illustrating that FMI DNA CGP is a robust modality for fusion detection compared to other methods, consistent with prior reports.^{44,45}

While most highly recurrent fusion partners were common to both the FMI and AACR GENIE datasets, one glaring discrepancy was the high frequency of *FGFR2-NPM1* fusions reported in GENIE. While *FGFR2-NPM1* rearrangements were detected using FMI DNA CGP, they were considered variants of uncertain significance. The *FGFR2-NPM1*

rearrangements detected on FMI DNA CGP had breakpoints in *FGFR2* intron 9 without retention of the kinase domain and showed a cancer type distribution distinct from known pathogenic *FGFR2* fusions, ie, found in NSCLC, colorectal (CRC), and other cancers but only infrequently in cholangiocarcinoma or other pancreaticobiliary cancers (data not shown). Furthermore, there is no published functional characterization of *FGFR2-NPM1* fusions to our knowledge. While these fusion events have been described in a prior study, the breakpoints and domain organization of these fusions were not detailed and they likewise were found in cancer types not typically associated with *FGFR2* fusions (CRC $N = 2$, large cell lung carcinoma $N = 1$).⁴⁶ Notably, all *FGFR2-NPM1* fusions reported in GENIE were from a single institution, likely reflecting differences in reporting rules between institutions/assays. Information about the intronic breakpoints for these fusions was not provided. The bioinformatic pipeline used for rearrangement calling/curation and manual review/interpretation are also key factors affecting assay reliability as not all structural variants are potentially pathogenic. Thus, the dependability of DNA-based rearrangement detection is reliant on both the technical capabilities of the assay as well as the reporting rules.

Both DNA and RNA CGP have advantages and shortcomings for fusion detection. While well-designed DNA CGP assays can detect a wide diversity of rearrangements and fusions, RNA CGP is better poised to detect certain rearrangement events. Whereas DNA CGP is capable of robust detection of canonical fusions with recurrent breakpoints since established breakpoint hotspots can be intentionally baited, RNA CGP can detect atypical fusions with breakpoints outside of hotspot regions. RNA CGP can also detect emergent fusions in genes not included on established DNA panels (eg, *NRG1*) due to technical limitations associated with baiting of certain fusion genes, eg, challenges related to long intronic regions which are prohibitive of efficient tiling as well as repetitive intronic sequence elements.^{14,42} Notably, detection of fusions involving these genes using a DNA CGP assay is still possible through baiting of fusion partners, eg, a subset of *NRG1* fusions are detected using FMI DNA CGP due to baiting of partner genes *CD74* and *SDC4*. RNA CGP is also ideal for identifying diagnostic fusions not commonly covered on DNA panels (eg, in sarcomas⁴⁷ and salivary gland tumors⁴⁸⁻⁵⁰) as the multitude and cancer type specificity of such fusions makes it infeasible to bait intronic regions of all involved genes due to panel size restrictions. In addition, RNA profiling can clarify the functional significance of detected rearrangements since expression at the mRNA level is confirmed, whereas expression of fusions detected through DNA profiling is merely predicted, complicating interpretation of noncanonical rearrangements.⁵¹ However, the technical difficulty of working with RNA is a significant limitation to RNA CGP.¹⁸⁻²¹ In addition to general issues of stability and quality, a low level of mRNA expression in cells can limit the detection of alterations resulting in false negatives.²⁰ Notably, DNA CGP can also be performed on circulating tumor DNA from liquid biopsies when tissue material is not readily available,⁵²⁻⁵⁴ whereas RNA profiling using liquid biopsies is not widely offered.⁵⁵

It is estimated that approximately 30% of NSCLC adenocarcinoma lack a well-defined oncogenic driver alteration.⁷⁻¹⁰ Using tissue-based FMI DNA CGP, we observed that approximately 12%-33% of NonSqNSCLC tumors lack a clear

oncogenic driver with driver alterations enriched in never-smokers (88%) compared to ever-smokers (67%), consistent with prior studies.¹⁰ However, it is important to clarify that the absence of a known oncogenic driver on DNA CGP does not necessarily equate to a *missed* driver, eg, a known oncogenic driver fusion that was not detectable on DNA CGP but may be detectable on RNA CGP. We expect such cases to represent a much smaller percentage of the overall NSCLC population with the remainder of driver-negative cases explained by as yet undiscovered biology.⁹ Accounting for several real-world factors associated with DNA/RNA CGP and the reported landscape of driver fusions in NSCLC, we estimate that approximately 1.3% of patients with NonSqNSCLC would be expected to have a driver fusion identified on RNA CGP but not on DNA CGP. This estimate is consistent with prior studies in which (1) reflex to RNA CGP after driver-negative DNA CGP identified an additional 1.1% of patients with NSCLC with targetable fusions¹⁴ and (2) concurrent DNA- and RNA-seq identified 0.3% of additional patients with actionable fusions across 20 cancer types.⁵⁶ While this yield may appear relatively low in all-comers, RNA CGP may be of greater value in select subpopulations, eg, non-smokers with NSCLC¹⁰ and driver-negative NSCLC with low TMB.¹⁴ In our study, the estimated increase in fusion detection afforded by RNA CGP represents approximately 4.4% of cases that would otherwise have been classified as driver-negative NonSqNSCLC.

Limitations of this study include the use of CGP results from tissue biopsies submitted during routine clinical care which introduces certain biases, compounded by comparison to an external database consisting of multiple patient cohorts each with its own inherent biases. While AACR Project GENIE data represent multiple assays from multiple institutions which complicates comparison to results from a single assay, the GENIE database is one of the largest repositories of publicly available clinically annotated genomic data in the world, including data from leading academic medical centers with established high-quality in-house NGS platforms, as well as other commercial assays, and thus represents a reasonable point of reference for our analysis. Additionally, our mathematical modeling approach was based on a number of assumptions and our parameter estimates were based on data/studies with their own respective sets of limitations. A more accurate estimate would require formal analysis of samples analyzed with both DNA and RNA CGP which is beyond the scope of the current study but of high interest for future investigations.

Conclusion

This study shows the robust capability of DNA CGP to detect clinically important fusions. There should be high confidence in the reliability of fusion calls from a well-designed DNA CGP assay to inform treatment decisions. However, variation in assay performance is affected by differences in design strategies, validation standards, and reporting rules among manufacturers and academic laboratories and not all DNA CGP assays are equally reliable. While we predicted an additional 1%-2% of patients with actionable driver fusions could potentially be detected with the addition of RNA CGP to a DNA CGP assay in NonSqNSCLC, the actual population prevalence in different tumor types and clinical scenarios must be studied in

large cohorts in which DNA and RNA are profiled concurrently and would necessarily be dependent on the capabilities of the included assays. While the expected yield of additional fusion detection using RNA CGP may be low in the unselected cancer population, the potential clinical benefit of detecting an actionable fusion driver for an individual patient is significant and every effort should be made to identify these drivers. Therefore, reflex to RNA CGP should be considered when DNA CGP does not reveal a clear oncogenic driver.

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Conflict of Interest

P.C.M. is on the advisory board for Guardant Health, has received honoraria from Amgen, and consults for Vivace Therapeutics. R.B.K.-E., G.L., K.T.L., A.B.S., S.E.T., J.M.A., K.T., and R.S.P.H. are employees of Foundation Medicine, a wholly owned subsidiary of Roche, and have an equity interest in Roche. G.R.O. is an employee of Lilly and has an equity interest in Roche.

Author Contributions

Conception/design: R.B.K.-E., G.L., K.T., G.R.O., R.S.P.H. Provision of study material or patients: R.S.P.H. Collection and/or assembly of data: R.B.K., G.L., K.T.L. Data analysis and interpretation: P.C.M., R.B.K., G.L., K.T.L., A.B.S., J.M.A., K.T., G.R.O., R.S.H.P. Manuscript writing and final approval of manuscript: All authors.

Data Availability

The authors declare that all relevant aggregate data supporting the findings of this study are available within the article and its supplementary information files. The data that support the findings of this study originated from Flatiron Health, Inc. and Foundation Medicine, Inc. Requests for data sharing by license or by permission for the specific purpose of replicating results in this manuscript can be submitted to PublicationsDataaccess@flatiron.com and cgdb-fmi@flatiron.com. In accordance with the Health Insurance Portability and Accountability Act, we do not have IRB approval or patient consent to share individualized patient genomic data, which contains potentially identifying or sensitive patient information and cannot be reported in a public data repository. Foundation Medicine is committed to collaborative data analysis and has well established and widely used mechanisms by which qualified researchers can query our core genomic database of >700 000 de-identified sequenced cancers. Academic researchers can submit a proposal to the Foundation Medicine Data Collaborations Committee and, if approved, the researcher/institution will be required to complete a Data Usage Agreement. More information and mechanisms for data access can be obtained by contacting

the corresponding author or the Foundation Medicine Data Governance Council at data.governance.council@foundationmedicine.com. This study made use of publicly available packages with R version 4.2.2. Code used to perform statistical analyses and generate figures is available upon request.

Supplementary Material

Supplementary material is available at *The Oncologist* online.

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