Liquid biopsy mutation panel for non-small cell lung cancer: analytical validation and clinical concordance

Lee S. Schwartzberg1,2, Hidehito Horinouchi3, David Chan3, Sara Chernilo4, Michaela L. Tsai5, Dolores Isla6, Carles Escriu7, John P. Bennett8, Kim Clark-Langone8, Christer Svedman8, Pascale Tomasini9 and Oncotype SEQ® Study Investigators and Program Team*

Molecular testing for genomic variants is recommended in advanced non-small cell lung cancer (NSCLC). Standard tissue biopsy is sometimes infeasible, procedurally risky, or insufficient in tumor tissue quantity. We present the analytical validation and concordance study of EGFR variants using a new 17-gene liquid biopsy assay (NCT02762877). Of 144 patients enrolled with newly diagnosed or progressive stage IV nonsquamous NSCLC, 140 (97%) had liquid assay results, and 117 (81%) had both EGFR blood and tissue results. Alterations were detected in 58% of liquid samples. Overall tissue-liquid concordance for EGFR alterations was 94.0% (95% CI 88.1%, 97.6%) with positive percent agreement of 76.7% (57.7%, 90.1%) and negative percent agreement of 100% (95.8%, 100%). Concordance for ALK structural variants was 95.7% (90.1%, 98.6%). This assay detected alterations in other therapeutically relevant genes at a rate similar to tissue analysis. These results demonstrate the analytical and clinical validity of this 17-gene assay.

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INTRODUCTION

Improved understanding of the molecular basis of cancer has enabled the personalized treatment of patients with agents targeting cancer-specific gene alterations. For example, in non-small cell lung cancer (NSCLC), targeted therapies are currently approved and preferred over cytotoxic chemotherapy for patients with sensitizing EGFR mutations1–4, ALK and ROS1 gene rearrangements5–8, and BRAF variants9. There are additional alterations in other genes (ERBB2, RET, MET) with available targeted therapies and emerging evidence that are included in treatment guidelines10, including NTRK fusions11. The approval of osimertinib for patients with emergence of EGFR T790M resistance mutations highlights the growing importance of assessing and targeting emerging mutations associated with sensitivity or resistance12,13. Assessment of patient tumor genomic alteration status, at diagnosis and throughout the course of metastatic disease, is now necessary for optimal therapy.

Even though mutation assessment for EGFR, ALK, and ROS1 has been standard of care for several years, testing of patients with advanced disease is suboptimal; reported proportions of patients without EGFR testing surpass 25% in some countries13,14. In addition, only a low proportion of patients have mutation test results available at their first oncology consultation, resulting in delayed initiation of therapy or initiation of chemotherapy before diagnostic test results are available15. The causes of suboptimal mutation assessment are multifactorial, including patient comorbidities, complications of lung biopsy such as bleeding, pneumothorax, and infection, and frequently inadequate sampling leading to costly rebiopsy in about a third of patients15,16. Furthermore, increasing understanding of tumor heterogeneity calls into question the representativeness of a biopsy of a single metastatic site17,18. Thus, alternative, less invasive methods to assess the genomic alterations of tumors are warranted.

Circulating cell-free DNA (cfDNA) from blood can be used to detect tumor-specific genomic alterations in the metastatic setting in various tumor types, and current sequencing technologies allow for rapid identification of a large number of genomic alterations from a modest volume of blood19,20. In addition to reducing discomfort for the patient, assessing genomic alterations in blood versus tumor tissue may more accurately reflect the mutational landscape of metastatic lesions at different sites and the molecular heterogeneity among various tumor sites and regions. Recent data also indicate that liquid biopsy may be useful for monitoring the development of alterations associated with acquired resistance, a potential that carries implications for treatment decisions21,22.

The reported concordance of assessing genomic alterations in blood versus tumor tissue varies widely due to multiple factors, such as the methods used—including analytical platform, varying tumor burden (lower sensitivity in patients with lower tumor burden and earlier stages of disease), and genomic coverage of the regions of interest. It is therefore of clinical importance to characterize the analytical assay performance in the intended use population by assessing the concordance of key actionable genomic alterations detected in plasma with those found in tissue (biopsy/cytology/excision), the current standard of care. The ideal plasma ctDNA mutation panel assay would be prospectively validated in the intended use population and provide clinically actionable results for all genomic mutations that are associated with benefit from targeted therapies (that are FDA-approved, in...
ASC0, CAP, NCCN guidelines, or in late-stage clinical development with evidence of efficacy and safety).

Here we report the analytical validation of a 17-gene next-generation sequencing (NGS) liquid biopsy panel that assesses genomic alterations in plasma, including validated alterations that, when present in the tumor, may portend benefit from targeted agent treatment approved by regulatory authorities. The panel also assesses select genes that are targets of therapies in late-stage clinical trials and genomic alterations that may be relevant for treatment selection but are supported by preliminary or preclinical evidence only.

We conducted a prospective study assessing EGFR variant concordance between liquid and tissue biopsy in patients with nonsquamous NSCLC for whom the clinical utility of a liquid biopsy-based mutation assessment is high. Many such patients may have tumors that are difficult to biopsy or that yield poor-quality biopsies but for whom knowing the status of genomic alterations is necessary to select optimal therapy. We present here the interim analysis of the concordance study.

RESULTS

Analytical validation
Detection thresholds were set to ensure >99% per-sample specificity. The lowest target amount for 95% detection rate (i.e., limit of detection or LOD95) was determined for each variant type and were as follows: insertions/deletions (indels), 0.1% allelic fraction (AF); single-nucleotide variants (SNVs), 0.37% AF; structural variants (SVs), 0.44% AF; and copy number variants (CNVs), ≥3 copies. Using a combination of reference standards and samples shown to harbor a mutation by digital droplet polymerase chain reaction, the positive percent agreement (PPA) and technical positive predictive value were both 98.9%. In the repeatability and reproducibility study, all expected variants were observed, indicative of 100% PPA and reproducibility. The detailed results on specificity, sensitivity, accuracy, repeatability and reproducibility, interfering substances, stability, contamination, and cross talk from the analytical validation are provided in the Supplementary Tables 1–12 and Supplementary Notes.

Clinical concordance study
The clinical concordance study enrolled 157 patients from 16 sites between April 2016 and October 2017. Thirteen patients were excluded due to failure to satisfy eligibility criteria, including absence of tissue samples, presence of treatment between tissue and liquid biopsies, withdrawal of consent, or death prior to blood draw. The vast majority (140/144) of protocol-eligible patients had evaluable liquid assay results including 121 cohort A patients (those with newly diagnosed metastatic disease or progressive disease on any-line non-EGFR-targeted therapy) and 19 cohort B patients (those with progressive disease on EGFR-targeted therapy). Four samples were excluded due to blood preprocessing issues; there were no laboratory failures. In cohort A, 117 patients had concurrent EGFR tissue results, with a median of 27 days (interquartile range 14–35) between tissue and blood collection.

Patient demographics and clinical characteristics are presented in Table 1. In cohort A, which was used for the primary analysis of tissue/liquid concordance for EGFR mutational status, 61% of patients were white and 50% female. The average age was 66 years (range 42–94 years). A large majority of these patients (93%) were newly diagnosed with stage IV NSCLC. Nearly half (48%) of patients had two or more organs with metastases and about three-fourths (74%) had two or more metastatic lesions in all organs.

Alterations reported by the liquid biopsy assay are presented in Figs. 1 and 2. Overall, the liquid biopsy assay identified 120 alterations across 13 genes in 81/140 (58%) patients, including EGFR (29%), KRAS (16%), MET (7%), ALK (4%), ERBB2 (4%), RET (2%), BRAF (1%), and ROS1 (<1%). More than half (54%) of alterations were SNVs, 23% indels, 14% CNVs, and 8% SVs. Most (51/81) patients had only one alteration reported.

In cohort A, the liquid biopsy assay identified 90 genomic alterations in 66 (55%) patients. Among the 90 alterations were 34 EGFR variants, which included 13 exon 19 deletions and 14 SNVs. Ten of the EGFR SNVs are included in the primary analysis (L858R [n = 6], G719A [n = 2], L861Q [n = 2]). Four other EGFR SNVs were found, including one T790M. In 19 cohort B patients (progressing on erlotinib, gefitinib, or afatinib), the assay detected 30 genomic alterations in 66 (55%) patients. Among the 90 alterations were 34 EGFR variants, which included 13 exon 19 deletions and 14 SNVs. Ten of the EGFR SNVs are included in the primary analysis (L858R [n = 6], G719A [n = 3], L861Q [n = 1]). The prevalence of EGFR in ERBB2, KRAS, and PIK3CA. The prevalence of EGFR in
patients progressing on EGFR-targeted therapy was 32% (95% CI: 13–57%).

In the primary concordance analysis of 117 cohort A patients with evaluable tissue and liquid results, there was substantial variation in the tissue tests used (Supplementary Table 13) and the methodology: polymerase chain reaction (52.1%), NGS (32.5%), Sanger (14.5%), and restriction fragment length polymorphism (0.9%). Of 157 enrolled patients, the central Clinical Laboratory Improvement Amendments (CLIA) laboratory test (FoundationOne®, Foundation Medicine, Cambridge, MA) was ordered in 33 patients. Eleven (33%) of these tests failed due to insufficient tumor, DNA yield, or tumor purity metrics. In some patients, a small amount of residual material was available after local tissue assessment, increasing the risk of failure due to biopsy volume.

Concordance results for alterations in EGFR and ALK are shown in Table 2. Thirty patients had the prescribed EGFR mutations (exon 19 deletions, L858R, L861Q, G719X, and S768I) in tissue and 23 had the same alterations detected in plasma. All patients with EGFR alterations detected in plasma had the same alterations detected in tissue, resulting in a PPA of 76.7% (95% CI: 57.7–90.1%) and a negative percent agreement (NPA) of 100% (95.8–100%). The overall concordance was 94.0% (88.1–97.6%). Patients with EGFR alterations reported in tissue but not in plasma tended to have lower tumor burden as indicated by lower DNA yields from blood and a lower proportion of patients with evidence of disease in two or more organs (Supplementary Fig. 1).

Ten patients had ALK translocations detected in tissue, five of whom had ALK alterations detected in liquid, with a resulting PPA of 50.0% (18.7–81.3%), NPA of 100.0% (96.5–100.0%), and overall percent agreement (OPA) of 95.7% (90.1–98.6%). In one patient with an alteration detected in tissue only, the liquid biopsy signal for ALK translocation was just below the threshold for calling the variant. The details of concordance for each gene in the panel are presented in Supplementary Table 14. The number of assessments in tumor tissue for some of the genes was limited.

Among patients with key EGFR alterations (listed in Table 2) in both tissue and liquid (n = 23) the best response (by imaging and/or clinical assessment) in patients treated with targeted therapy was available in 21 patients. According to both imaging and clinical assessment, there was partial response in 11 patients (52%) and stable disease in 10 (48%). In patients with EGFR alterations detected in tissue only, the response rate was captured in six of seven patients. Best response (by imaging and/or clinical assessment) was partial response in three patients (50%) and stable disease in three patients (50%).

An ad hoc analysis was performed to examine the association of response rate with AF. In patients with AF below 1%, 2/8 (25%) had partial responses and 6/8 (75%) had stable disease. In patients with an AF of 1% or above, 9/13 (69%) had partial responses and 4/13 (31%) had stable disease.

Best response to first therapy after liquid biopsy was available in five of five patients with ALK alterations detected in both tissue and liquid. All patients received ALK-targeted therapy. Two patients (40%) had complete responses and two patients had partial responses (40%) by imaging. One patient (20%) had progressive disease by clinical assessment. Among the five patients with ALK alterations detected in tissue only, four had best response data available: one had a complete response, one had stable disease, and two had progressive disease.

DISCUSSION
There are many potential uses of liquid biopsy testing in oncology, from screening of early-stage disease to monitoring of treatment effect. Presently, there is relatively little evidence supporting the
utility of such testing for most of these purposes, except for patients with advanced or metastatic disease who are candidates for systemic targeted therapy but in whom tissue testing cannot be performed successfully. Such patients may have insufficient diagnostic tissue for testing or may be difficult to biopsy because of tumor location, comorbidities, bleeding disorders, or a strong personal preference. Even in these patients, only limited data from prospective studies exist to support use of tests assessing actionable genomic alterations across multiple genes, despite a high unmet need. Data also support liquid biopsy assessment in patients progressing on targeted therapy where there are identified acquired mutations associated with resistance and sensitivity to other drugs and where the patient is challenging to biopsy.

In previously reported prospective clinical concordance studies, the sensitivity and specificity for detecting EGFR mutations in liquid versus tissue was similar to that reported for the approved Cobas EGFR test. The analytical validation of the liquid biopsy assay described herein demonstrated high sensitivity, specificity, and reproducibility for detecting SNVs, indels, SVs, and CNVs. Importantly, there were no EGFR alterations found in liquid that were not present in tumor tissue in newly diagnosed patients, leading to an NPA of 100%. The PPA below 80% supports reflex tumor tissue testing when a liquid test is negative, similar to the Cobas EGFR test. The OPA for all SNVs, indels, and CNVs in the panel was comparable with that for EGFR (SNV 89.7% [82.8–94.6%], indels 95.7% [90.3–98.6%], CNVs 90.3% [74.2–98.0%]). The OPA for translocations was also similar to EGFR, while the PPA based on 14 positive patients was somewhat lower (50% [23.0–77.0%]). Gene rearrangements can be more challenging to identify than SNVs or small indels when using hybrid capture versus amplicon-based enrichment methodologies. It is thus possible that the true concordance between cfDNA and tissue indeed may be lower for gene rearrangements than for SNVs when using hybrid capture technologies.

Overall, the data across many studies using a variety of modern methods demonstrate sensitivities for EGFR alterations assessed in liquid biopsy versus tumor tissue to be in the 70–85% range in patients with metastatic disease. The consistency of our results with published data suggests that this sensitivity limit is driven in part by biological constraints, perhaps disparity in overall tumor burden or differences in DNA shedding from tumor cells in various tissue locations. Our planned analysis by tumor burden revealed that failure to detect alterations in liquid was associated with lower tumor burden, as indicated by lower DNA yields from blood and evidence of disease in fewer than two organs. These findings are consistent with the literature. It is important to recognize that the theoretical biological limit of sensitivity to detect tumor mutations is a function of both tumor mutant AF and the total amount of DNA. While some assays, in principle, may have an LOD95 of 0.001% using contrived samples containing more genome equivalents than present in a typical patient sample, it is unlikely that this can be consistently achieved in clinical samples. Thus, samples containing a representative number of genome equivalents are recommended when establishing LOD95. The ability to make correct variant calls at very low AF is important, as recent evidence shows that patients with very low variant AFs may have excellent responses to targeted therapy. In our study, although the number of patients with an AF below 1% was small, a high proportion of these had partial response or stable disease consistent with benefit from EGFR-directed therapy.

The results from the cohort of patients progressing on erlotinib, gefitinib, or afatinib demonstrate that the assay can be used to detect alterations associated with resistance/sensitivity in patients progressing on therapy. The reported rate of EGFR T790M of 32% is on the lower end of that reported in the literature based on tumor tissue testing, possibly reflecting the small sample size or the high proportion of Japanese patients in this cohort (the T790M mutation rate in Japanese patients may be lower than in Caucasians). In addition, the cohort is somewhat enriched in EGFR-mutant patients due to the participation of a Japanese site, as well as sites that are referral centers for EGFR-mutant patients. The patient population is thus not completely representative of an all-comer patient population in either Caucasians or East Asians, but the enrichment of EGFR-mutant patients was important in order to make study accrual feasible.

The FDA recently approved a broad tumor tissue mutation panel, which will likely increase the use of broad mutational profiling. However, the sample requirements for broad panel testing can lead to high test failure rates in small samples: over 28% for endoscopic biopsies and 51% for fine-needle aspirates. This is an especially important issue in lung cancer, where there are many actionable alterations yet the NGS test failure rate is over 26%. This could create an increased need for rebiopsy, a possible shift toward larger biopsies with higher risks of adverse events, or an alternative assessment by liquid biopsy. In our study, the clinical outcome captured in patients mutation-positive by liquid biopsy is consistent with what has been reported for patients mutation-positive by tumor tissue biopsy. Given 100% NPA and a PPA of less than 80% for the liquid biopsy assay, a negative liquid biopsy should trigger a reflex tumor tissue biopsy if feasible. It should be emphasized that tissue biopsy still has the advantage of providing diagnostic/histological information and suitable material for the assessment of PD1/PDL1 status and other histologic assessments, such as tumor-infiltrating lymphocytes. Given current guidelines that strongly recommend first-line treatment with targeted agents rather than immunotherapy for patients with sensitizing alterations in EGFR, ALK, ROS1, and BRAF, an initial liquid biopsy assessment may be medically appropriate in many patients and could potentially reduce delays in initiation of treatment that cause anxiety in patients. Patient preference regarding liquid biopsy versus tissue biopsy is not well documented but is a field where research is warranted, as it seems likely that many patients would strongly prefer a less invasive and risky procedure.

There are limitations to the conclusions that can be drawn from this concordance study. While a comparison of liquid biopsy to tumor tissue biopsy results is needed for assay validation, the clinical advantages of liquid biopsy may be most obvious for...

### Table 2. Positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) with 95% confidence intervals for key EGFR and ALK alterations in cohort A.

| Alteration | n   | PPA         | NPA         | OPA         |
|------------|-----|-------------|-------------|-------------|
| EGFR       |     |             |             |             |
| Exon 19 deletions, L858R, L861Q, G719X, or S768I | 117 | 76.7% (57.7%, 90.1%) | 100.0% (95.8%, 100%) | 94.0% (88.1%, 97.6%) |
| ALK        |     |             |             |             |
| Structural variants | 115 | 50.0% (18.7%, 81.3%) | 100.0% (96.5%, 100%) | 95.7% (90.1%, 98.6%) |

(continued...
patients who do not have tissue results, either because of tissue assay failure or because the tumors are challenging to biopsy. Thus, the study population may not be fully representative of a primary target population for clinical use. The large majority of patients in this study had de novo metastatic disease, and it is possible that relapsing patients have a differential expression of liquid versus tissue genomic alterations. Location of the primary tumor and metastatic lesions is likely the most common reason making biopsy difficult and risky. As data in the literature and our results indicate, a lower tumor burden may be associated with a lower sensitivity. In addition, the study permitted local assessment of mutation status in tumor tissue and many different methods were used. Central assessment using an NGS panel was offered for all patients, but only ~30% of patients underwent testing with the central NGS panel. The reasons for this fairly low rate of central testing may include a preference for faster local results or the lack of sufficient residual material after local testing. The heterogeneity of tests and methods used for tumor tissue assessment can be seen as a strength of this study, however, as it reflects current clinical practice. Importantly, that the NPA was 100% and that the concordance was similar when compared with either central NGS tests or local tests indicate that the results were not negatively impacted.

In conclusion, the 17-gene liquid biopsy panel is analytically validated with consistent performance across SNVs, indels, translocations, and CNVs. The concordance with tumor tissue biopsy for clinically relevant alterations in EGFR is comparable with that of the approved liquid Cobas EGFR test. In addition, the 17-gene panel can detect alterations in other genes relevant for treatment decisions in NSCLC. Liquid biopsy assessment may be clinically helpful in the substantial proportion of patients for whom obtaining a tissue biopsy is challenging, there is a strong patient preference, or tissue biopsy or analysis has failed.

**METHODS**

The 17-gene liquid biopsy assay was performed in a single CLIA-certified laboratory at Genomic Health, Inc. (Redwood City, CA). Plasma was obtained from whole blood (2 × 10 ml tubes, Cell-Free DNA BCT, Streck) using the standard double spin method; blood was spun for 10 min at 1500 g at 4°C, plasma isolated and re-spun at 3000 g for 10 min, and transferred to a clean tube. Extraction was performed using a proprietary methodology based on the MagMAX Cell-Free DNA Isolation Kit (Thermo Fisher Scientific). DNA quantitation was performed using the Quant-iT dsDNA kit (Thermo Fisher Scientific). To mimic cfdNA, reference control genomic DNA was sheared using the E220 Focused-Ultrasonicator (Covaris) followed by size selection using AMPure XP Beads (Agencourt/Beckman Coulter). The resulting DNA was assessed using the 2100 Bioanalyzer (Agilent) and samples required to be >100 bp and >200 bp. Whole genome libraries were prepared by a proprietary methodology using the KAPA Hyper Prep Kit (Kapa Biosystems/Roche) and dual-indexed adapters (Integrated DNA Technologies). Resulting pool libraries were quantified and quality controlled using the 4200 TapeStation Instrument (Agilent Genomics). Hybrid capture was performed using a modified SeqCap EZ HyperCap (NimbleGen/Roche) workflow and baits (Integrated DNA Technologies) designed to cover the 17-gene target regions. The genes and alterations covered in the panel are detailed in Table 3. Enriched libraries were quantified using the 4200 TapeStation Instrument (Agilent Genomics).

**Clinical concordance study**

We conducted a global multicenter prospective clinical study (NCT02762877) to characterize the concordance of key clinically relevant genomic alterations in DNA extracted from formalin-fixed, paraffin-embedded tumor tissue (biopsy/excision/cytology) and in cfdNA from liquid biopsy (blood), and the frequencies of genomic alterations identified in liquid biopsy (listed in Table 3) in patients with stage IV nonsquamous NSCLC. Patients seeking treatment at 16 oncology centers in the United States of America, Europe, and Japan who were identified to meet eligibility criteria were enrolled. The study enrolled two cohorts: cohort A, patients who were either newly diagnosed with metastatic disease or progressive disease on non-EGFR-targeted therapy (any line); and cohort B, patients with progressive disease on EGFR-targeted therapy (erlotinib, gefitinib, afatinib). Full inclusion and exclusion criteria are in Supplementary Table 15. All samples were collected with institutional review board approval (Asentral IRB) and written informed patient consent. Concordance analysis focused on cohort A. Tissue biopsy and blood collection were less than 8 weeks apart with no new systemic antitumoral treatment given in the interval between the tissue biopsy and blood collection (local therapy, such as radiation, was permitted). Tissue analysis by a central CLIA laboratory (Foundation Medicine, Inc.) was offered but not required. The results of the central laboratory assessment were used in the concordance

### Table 3. Genes/alterations included in the 17-gene liquid biopsy panel.

| Gene       | Variant type(s) reported |
|------------|--------------------------|
| ALK        | Targeted and de novo SNVs, targeted and de novo SVs |
| AR         | Targeted and de novo SNVs |
| BRAF       | Targeted and de novo SNVs, targeted and de novo indels |
| BRCA1      | Targeted and de novo SNVs, targeted and de novo indels |
| BRCA2      | Targeted and de novo SNVs, targeted and de novo indels |
| EGFR       | Targeted and de novo SNVs, CNV gains, targeted and de novo indels |
| ERBB2      | Targeted and de novo SNVs, CNV gains, targeted and de novo indels |
| ESR1       | Targeted and de novo SNVs, CNV gains |
| KIT        | Targeted and de novo SNVs, CNV gains, targeted and de novo indels |
| KRAS       | Targeted and de novo SNVs, targeted and de novo indels |
| MET        | Targeted and de novo SNVs, CNV gains, targeted and de novo indels |
| NRAS       | Targeted and de novo SNVs |
| PDGFR A    | Targeted and de novo SNVs, CNV gains, targeted and de novo indels |
| PIK3CA     | Targeted and de novo SNVs, CNV gains, targeted and de novo indels |
| PTEN       | Targeted and de novo indels |
| RET        | Targeted and de novo SNVs, targeted and de novo indels, targeted and de novo SVs |
| ROS1       | Targeted and de novo SNVs, targeted and de novo SVs |

The panel includes detection of single-nucleotide variants (SNVs), copy number variant (CNV) gain or loss, targeted insertions/deletions (indels), and targeted structural variants (SVs).
analysis. If no central laboratory result was available, the results from the local assessment of genomic alteration status in tissue were used. In addition, detection of EGF T790M alterations in plasma was characterized in cohort B patients where tissue sample collection was not required. Patients were followed to collect treatment given after liquid biopsy, best response to this treatment (complete response, partial response, stable disease, or progressive disease), and date of clinical or radiological progression for up to 12 months after liquid biopsy. Presence of any of the prespecified clinically actionable EGFR alterations (exon 19 deletions, L858R, L861Q, G719X, and S768I) was considered as EGF-positive for the primary analysis. PPA, NPA, and OPA were calculated. Two-sided 95% Clopper-Pearson confidence intervals were reported. The interim analysis was prespecified to occur after enrollment of at least 30 patients with EGF alterations. Individuals involved in laboratory analysis of liquid samples were blinded to clinical data and tissue biopsy results. Data were analyzed using SAS software, version 9.4, of the SAS System for Windows (Copyright 2018 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA).

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA AVAILABILITY
The data that support the findings of the clinical concordance study are available from the corresponding author upon reasonable request. Technical details of the analytical validation study are available in the Supplementary File. Proprietary bioinformatics methods will remain confidential and will not be shared.

CODE AVAILABILITY
The analyses presented in this paper used standard SAS procedures and data steps. No custom code or algorithms were created.

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Additional information

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Correspondence and requests for materials should be addressed to L.S.S.

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Author contributions

Each author confirms that he or she has made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; drafted the work or substantially revised it; approved the submitted version (and any substantially modified version that involves the author’s contribution to the study); and agreed both to be personally accountable for the author’s own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

Competing interests

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