JLB 9 (2025) 100322

Contents lists available at ScienceDirect

The Journal of Liquid Biopsy

journal homepage: www.sciencedirect.com/journal/the-journal-of-liquid-biopsy

Validation of a liquid biopsy assay with increased sensitivity for clinical comprehensive genomic profiling

Xavier S. Bower [a], Jan Wignall [a], Matthew G. Varga [a], Joyce Zhu [a], Michael O'Sullivan [a], Naomi E. Searle [a], Lenny K. Hong [a], Turgut Dogruluk [a], Zeqian Li [a], Tiffany E. Farmer [a], Emilio Rosas-Linhard [a], Jason Luong [a], Esther Lin [a], Marie Erica Simon [a], David S. Tsao [a], John R. ten Bosch [a], Gary Palmer [a], Ajeet Gajra [b], Chanh Huynh [c], Wen Zhou [a,*]

[a] BillionToOne, Inc., Menlo Park, CA, USA
[b] Hematology Oncology Associates of Central NY, East Syracuse, NY, USA
[c] Cancer Care Associates of York, York, PA, USA

ARTICLE INFO

Keywords:
Liquid biopsy
Circulating tumor DNA
Comprehensive genomic profiling
Next generation sequencing
Precision oncology

ABSTRACT

Recent advancements in precision oncology have affirmed the need for comprehensive genomic profiling (CGP) liquid biopsy assays with increased sensitivity, especially for detecting alterations in tumors which shed circulating tumor DNA (ctDNA) at low abundance. Such tests could address the limitations of tissue biopsies while simultaneously enhancing the detection of clinically actionable variants. This study included analytical and clinical validation studies of Northstar Select, a plasma-based, tumor-naive CGP assay covering 84 genes. The assay detects SNV/Indels, CNVs (gain and loss), fusions, and microsatellite instability (MSI-H). A retrospective analysis of 674 analytical patient samples collected during routine care in the United States, covering various solid tumor types, was conducted to investigate performance across tumor types. In addition, clinical validation was conducted in a prospective head-to-head comparison study of 182 patients, assessing the performance of Northstar Select and on-market CGP liquid biopsy assays. Analytical validation demonstrated a 95 % Limit of Detection of 0.15 % variant allele frequency (VAF) for SNV/Indels, which was confirmed by digital droplet PCR. Northstar Select demonstrated sensitive detection of CNVs in liquid down to 2.11 copies for amplifications and 1.80 copies for losses, and 0.30 % for gene fusions, addressing a key challenge in liquid biopsy testing. It outperformed on-market CGP assays, identifying 51 % more pathogenic SNV/indels and 109 % more CNVs. Additionally, this resulted in 45 % fewer null reports with no pathogenic or actionable results. The majority (91 %) of additional clinically actionable SNV/indels found were detected below 0.5 % VAF. Northstar Select demonstrated analytical and clinical validity, with high sensitivity across all variant classes. The low LOD allows for reliable detection of variants at lower VAFs compared to existing commercial assays. Northstar Select can therefore enhance clinical decision-making by providing the opportunity to identify more actionable genomic alterations, which may be especially beneficial for patients with low-shedding tumors.

1Introduction

The landscape of oncology has evolved significantly with the integration of genomic profiling into treatment decision-making [1]. While tissue-based comprehensive genomic profiling (CGP) remains the historical standard, it faces notable limitations. These include insufficient tissue quantity or quality—with up to 26 % of solid tumor samples failing to meet testing requirements—as well as procedural complexities and extended turnaround times that can delay treatment initiation [2,3]. In addition, some patients may not tolerate biopsy, such as patients with advanced NSCLC or COPD, brain tumors, or those at risk of bleeding complications. Furthermore, tumor heterogeneity, both within individual tumors and across metastatic sites, can result in missed actionable variants and subsequent treatment failures [4].

These constraints have catalyzed the emergence of liquid biopsy as both a complementary and independent diagnostic tool [5,6]. The non-invasive nature of liquid biopsies, combined with their rapid turnaround times and ability to capture tumor heterogeneity, has led to their

* Corresponding author. BillionToOne, Inc, 1035 O'Brien Drive, Menlo Park, CA, 94025, USA.
E-mail address: wzhou@billiontoone.com (W. Zhou).

https://doi.org/10.1016/j.jlb.2025.100322
Received 30 June 2025; Received in revised form 30 July 2025; Accepted 6 August 2025
Available online 7 August 2025

incorporation into clinical guidelines [7]. The added utility of liquid biopsy is exemplified in a study of over 8,000 non-small cell lung cancer (NSCLC) cases where there was an observed 65 % increase in the detection rate of driver mutations using ctDNA testing, with therapy response rates comparable to tissue-based assays [8]. This clinical value has been recognized through FDA approval of five liquid biopsy companion diagnostic tests spanning multiple cancer types [9]. In addition, professional guidelines increasingly support the routine use of liquid biopsy as part of the standard of care in 4 cancer types to date (NSCLC, breast, mCRC and prostate) [10] For example, in advanced NSCLC, guidelines recommend liquid biopsy when either the patient is unfit for invasive tissue sampling, there is insufficient tissue available, or when timing of tissue acquisition is uncertain. Additionally, the FDA's January 2023 approval of elacestrant for ER+/HER2− metastatic breast cancer with an *ESR1* mutation prompted an immediate ASCO guideline update recommending next-generation DNA sequencing for *ESR1* mutations at all stages of ER+/HER2− metastatic breast cancer progression, with a preference for liquid biopsies due to their greater sensitivity [11]. These changes to national guidelines, recommending the routine use of liquid biopsies as part of standard care, can be attributed to their increasing clinical utility. These guidelines also stress the importance of improving sensitivity at low variant allele frequency (VAF) levels, as low VAF somatic alterations can be just as clinically actionable as high VAF alterations and be similarly used to predict response to biomarker directed therapy [12,13].

Despite these advances, current liquid biopsy assays exhibit variable performance, particularly in sensitivity and positive predictive value at variant allele frequencies (VAF) below 0.5–1 % [8,14–16]. This limitation is significant given that many cancer types have variants at low VAF. For example, over 25 % of somatic mutations in NSCLC are detected below 0.2 % VAF, while most current commercial assays maintain a limit of detection (LOD) above 0.2 % [8,15]. The need for enhanced sensitivity is further emphasized by evidence that variants detected at low VAF respond similarly to targeted therapies as those with higher signals, making accurate VAF detection for personalized care delivery [12,15,17,18]. Copy number variant (CNV) detection presents additional challenges in liquid biopsy analysis. While high tumor fraction samples (20–35 %) show strong concordance with tissue assays, sensitivity decreases dramatically (28–35 %) in samples with lower tumor fraction [19–22]. Improving CNV detection requires not only enhanced sensitivity at lower concentrations but also better discrimination between focal events and chromosomal aneuploidies [23]. This distinction is crucial as focal alterations often drive oncogenesis, while broad aneuploidies may indicate therapy resistance [24].

To address these challenges, we developed BillionToOne's Northstar Select®, a tissue-naive CGP assay that employs proprietary Quantitative Counting Template™ (QCT) technology [25], allowing for detailed performance analysis across multiple stages of sample processing. This leads to optimized cfDNA extraction and target enrichment, minimizing errors and enhancing variant detection. The assay also employs novel bioinformatic pipelines developed to further improve sensitivity and reduce noise, particularly in CNV analysis. These innovations and refinements represent an advancement in liquid biopsy capabilities, addressing the critical need for improved detection of clinically relevant variants in precision oncology [11]. We present the clinical validation study of Northstar Select, evaluating its performance and diagnostic sensitivity against five tumor-naive liquid biopsy assays in 182 patients with diverse variants and cancer types. The analytical validation demonstrates that Northstar Select has an LOD for SNV/Indel variants of 0.15 %, which was orthogonally confirmed via independent ddPCR assays. Furthermore, we demonstrate that improved variant detection stems from enhanced sensitivity rather than artifact detection due to clonal hematopoiesis (CH).

2Results

2.1. Northstar Select assay and analytical validation

Northstar Select's 84 gene panel coverage was designed to optimize the sensitivity of the assay (Table S1, Fig. S1). In addition, Northstar Select implements a custom sequencing protocol that is enhanced using proprietary QCT technology and bioinformatic innovations (Fig. S2). To validate the analytical performance of the assay, including sensitivity, specificity, and Limit of Blank (LOB), analytical samples, reference samples and confirmed negative clinical samples were assayed (Tables S2–S5, Fig. S2), as described in the Methods. To establish the limit of detection, we first conducted a range-finding experiment in which Northstar Select was evaluated on contrived materials covering a wide range of VAFs, (0.06 %–0.35 %). 95 % or more variants were detected for SNVs in a bin of 0.13–0.16 % VAF (Table S2, Methods). Similarly for CNVs and MSI, expected sensitivity limits were established through physically and computationally contrived samples (Methods). Next, we confirmed the limit of detection by focusing on the VAF ranges identified with additional samples. The 95 % Limit of Detection (LOD) was determined for each class of alteration using analytical samples. The results demonstrated high sensitivity and specificity for SNV/Indels, CNVs (both amplification and loss), as well as fusions and MSI (Table 1).

2.2. Head-to-head comparison to on-market assays

To further evaluate the performance of Northstar Select in a clinical setting, a prospective, head-to-head comparative study was conducted in which advanced stage patients who were planning to receive an on-market CGP liquid biopsy assay as part of standard of care were enrolled. Over 200 patients were recruited from 6 community oncology clinics and one large hospital from across the USA. At the time of sampling for the clinician's choice of comparator assay, blood was drawn simultaneously from the same venipuncture for comparison to Northstar Select (Fig. 1A). Comparators were NGS-based ctDNA assays offered by four CLIA/CAP laboratories with extensive analytical and clinical validation and/or FDA approvals. This head-to-head comparison aimed to directly evaluate the impact of the assay's technological advancements on improving identification of therapeutic options for patients with solid tumors.

In total, 182 patients (Table S6) across >17 tumor types (Fig. 1B) were analyzed. Due to Northstar Select's low LOD for multiple classes of alterations (SNV/Indel, CNV, etc), the proportion of patients with no pathogenic or clinically actionable alterations detected (negative report) was nearly half that of comparators (11 % with no pathogenic alterations by Northstar Select vs 20 % in comparators), despite comparators generally having larger panels than Northstar Select (Fig. 1C–Table S10). Twenty-one patients had pathogenic results detected only with Northstar Select and among those patients, 19 had clinically actionable pathogenic variants detected (Table S7).

Since it has been previously shown that ctDNA-based assays are largely concordant above 0.5 % VAF but can have significant discordance below 0.5 % VAF [15,16], the concordance of Northstar Select with comparator assays at different VAF levels was evaluated. Above 0.5 % VAF, the positive predictive agreement (PPA) and negative

Table 1
LOD/LOB of Northstar Select.

Variant Type	Specificity (LOB)	LOD_{95}
SNV	>99.9999 % (8,750,343/8,750,344 bp)	0.15 % VAF
Indel	>99.9999 % (8,750,343/8,750,344 bp)	
CNV	>99.9 %	2.11 copies (amplification) 1.8 copies (loss)
Fusion	>99.9 %	0.30 % Tumor Fraction
MSI-H	>99.9 %	0.07 % Tumor Fraction

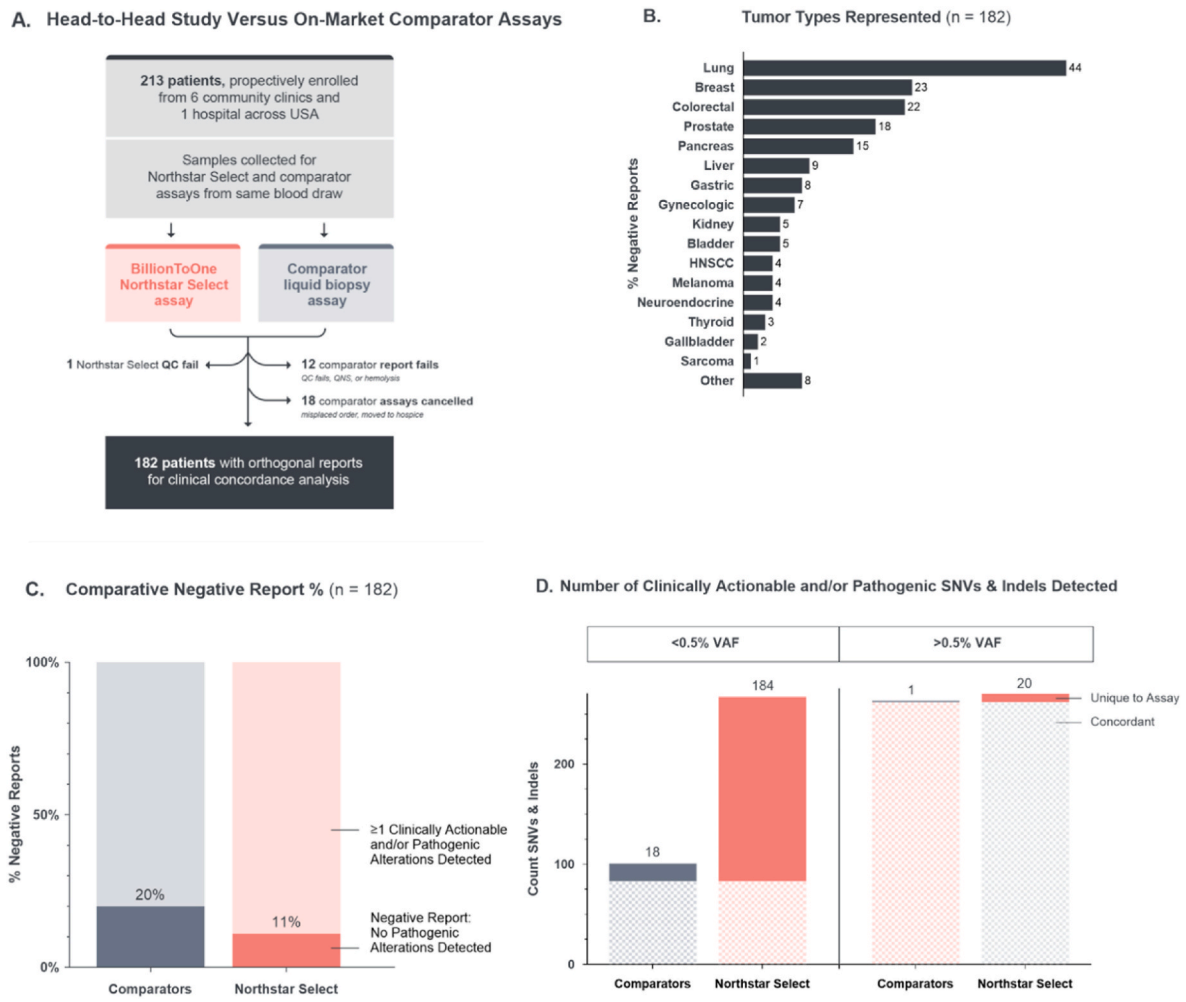


Fig. 1. Head-Head Comparison Study Design and summary data **(A)** CONSORT diagram of the prospective head-to-head observational study conducted to compare Northstar Select with on-market comparator assays. **(B)** Histogram of cancer types used in the head-to-head comparison analysis versus on-market comparator assays. **(C)** For all patients who had both Northstar Select and comparator assays available, diagnostic yield was measured as the proportion of patients for whom at least one clinically actionable and/or pathogenic variant was called by both Northstar Select and the comparators. Differing variant classification was considered such that concordant variants that were called as VUS for one of the assays were not counted towards that assay's diagnostic yield. **(D)** Of the reports with pathogenic alterations detected, the number of clinically actionable and pathogenic SNV/Indels by each assay are shown and categorized using a 0.5 % VAF threshold.

predictive agreement (NPA) were 93 % and >99 %, respectively. Below 0.5 % VAF, Northstar Select detected more than twice as many variants (267 vs 101, Fig. 1D).

Increased sensitivity for SNV/Indels is not due to coverage differences:

Driven in large part by the additional detections below 0.5 % VAF, Northstar Select detected more variants for SNV/Indels than the comparators, with 51 % more pathogenic and clinically actionable alterations combined over genes covered by both the comparator and Northstar Select (Fig. 2A and B, Table S9). Therefore, the coverage matched head-to-head analysis is focused on SNV/indel sensitivity of detection and is not biased against assays with differing coverage. Further analysis of the alterations uniquely identified by Northstar Select revealed that a significant proportion (68.6 %) were clinically actionable (Fig. 2B). When evaluating uniquely identified alterations on the patient level, 103 patients (103/182, 56.6 %) had alterations detected only by Northstar Select, of which 84 patients (84/103, 81.6 %) had clinically actionable variants.

The enhanced sensitivity observed with Northstar Select is independent of coverage differences and is particularly pronounced for the most clinically relevant variants. Northstar Select detected 17 % (62 vs. 53) more SNV/Indels with FDA approved/guideline recommended treatments for the patient's indication (Tier 1A, on-label). When expanding to FDA approved/guideline recommended treatments indicated in another tumor type (Tiers 1A and 2C.1, Table 2), the number of variants detected by Northstar Select was 56 % higher (179 vs. 115), accounting for matched coverage with comparators (Tables S8 and S10). Of note, some key variants where Northstar Select detected more than comparators included *KRAS* G12 (NS: 37, Comp: 32), *BRAF* V600 (NS: 5, Comp: 4), *KRAS* G13 (NS: 3, Comp: 2), *PIK3CA* E542 (NS: 4, Comp: 3), and *PIK3CA* H1047 (NS: 10, Comp: 9). In summary, Northstar Select delivers enhanced detection performance even when accounting for coverage differences, affirming its capability to reliably identify well-characterized variants across patient samples.

2.2.1. *Comparative detection of copy number variants*

Finally, when assessing the whole panel of comparators and Northstar Select, Northstar Select detected 2.1x (109 %) more CNVs versus the comparator assays (Fig. 2C). Across matched coverage, Northstar Select detected 37.5 % more CNVs versus the comparator assays. The utility of Northstar Select's ability to more sensitively detect copy number loss is exemplified in the 5 patients in whom Northstar Select detected a *PTEN* loss, which is potentially treatable with the *PI3K/AKT1/PTEN* pathway inhibitor capivasertib, which was granted FDA approval in late 2023.

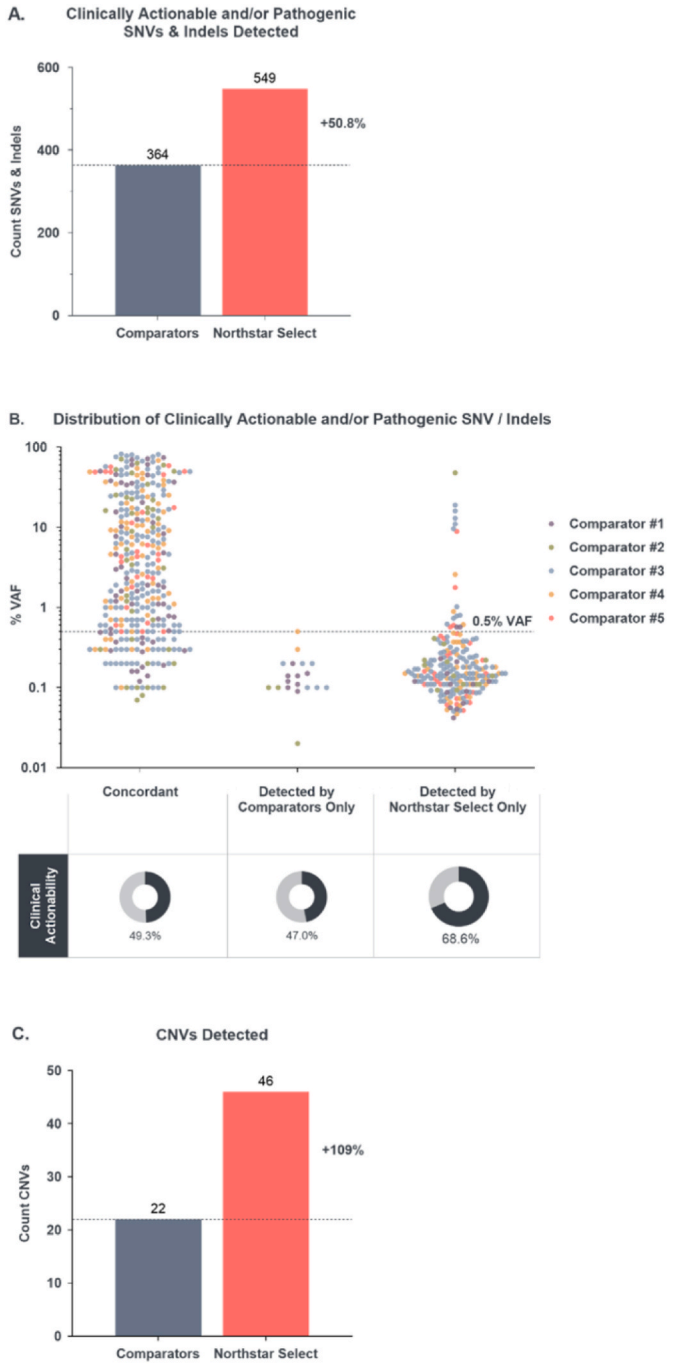


Fig. 2. Northstar Select demonstrates higher sensitivity than comparator liquid biopsies in head-to-head comparison. **(A)** To compare sensitivity, the number of SNVs and Indels was taken across all samples. Only variants on coverage regions matching between the comparator and Northstar Select were included in the comparison. Northstar Select detected 50.8 % more pathogenic variants relative to the comparators. **(B)** The VAF distribution of pathogenic SNV/Indels detected by both assays, the comparator assay alone, or by Northstar Select alone. Dotted line shows 0.5 % VAF threshold. Among the pathogenic SNV/Indels shown, the percent of clinically actionable alterations as defined by Northstar or the comparator clinical reports are shown below the x-axis. (**C**) The total number of CNVs detected is shown for each assay. Variants with differing variant classification between the assays are excluded. Northstar Select detected 109 % more pathogenic CNVs, not accounting for coverage differences between assays.

Table 2
Difference in variants with FDA-approved or guideline recommended on-label therapies for the patient's indication, or in another indication, detected by Northstar Select vs. comparator, grouped by gene. Matched coverage.

Gene	# detected Northstar Select	# detected Comparator	% Detected by Northstar Select Over Comparators
SNV/Indels			
ATM	24	8	200.0
BRAF	5	4	25.0
BRCA1	4	5	−20.0
BRCA2	11	6	83.3
BRIP1	2	1	100.0
CHEK2	8	3	166.7
EGFR	2	2	0.0
ERBB2	3	2	50.0
ESR1	6	4	50.0
EZH2	1	1	0.0
FGFR3	3	2	50.0
IDH1	3	1	200.0
IDH2	3	1	200.0
KIT	2	0	+
KRAS	44	38	15.8
MET	1	1	0.0
MLH1	3	2	50.0
NF1	5	1	400.0
NRAS	4	3	33.3
PALB2	4	2	100.0
PIK3CA	37	27	37.0
TSC1	2	0	+
VHL	2	1	100.0
SNV/Indel Sum	179	115	54.3
CNV			
ERBB2 Amp	4	4	0.0 %
PTEN Loss	1	0	+
CNV Sum	5	4	25.0 %

However, 4 of the 5 patients with a *PTEN* loss detected by Northstar Select were run on comparator assays that did not cover *PTEN* loss (Table 2).

2.2.2. *Comparative detection of fusions*

A total of 20 samples with clinically significant gene fusions were detected by either comparator assays or Northstar Select in *ALK*, *BRAF*, *FGFR2*, *FGFR3*, *NTRK1*, *NTRK2*, *RET*, and *ROS1*. Three of these fusions were from samples in the prospective head-to-head study. All three fusions in the head to head were detected by both Northstar Select and the comparator assays. Due to the small sample size and low incidence of fusions in an open observational trial design, we supplemented the cohort with an additional 17 commercially submitted clinical samples where Northstar Select detected fusions or comparator results showed a fusion. These additional samples were compared to an orthogonally validated NGS assay performed by an independent CLIA/CAP laboratory, or pathology reports procured from the medical record. In total Northstar Select detected 19 out of the 20 (95 %) fusions. Northstar Select detected one additional fusion (*FGFR3-TACC3*) that was not detected by the comparator assays.

2.2.3. *Comparative detection of MSI-H*

Additionally, 6 samples were detected as MSI-H by comparator assays, and Northstar Select detected MSI-H in all 6 samples and 1 additional sample, leading to 100 % sample-level PPA and 99.4 % sample-level NPA. The discrepant sample, which Northstar Select called MSI-H, also had a mutation detected within the Mismatch Repair (MMR) gene *MSH2* at low VAF (0.13 %), adding confidence to the validity of Northstar Select over the comparator's report which did not include this gene in its panel coverage.

2.3. Clinical utility of enhanced sensitivity: ctDNA shedding and detection of clinically actionable alterations in a retrospective study

To elucidate the clinical utility of the Northstar Select assay's enhanced sensitivity in detecting clinically significant variants and facilitating the identification of biomarker-directed treatments and clinical trials, 674 unique samples were obtained from BillionToOne's internal biobank, spanning a multitude of tumor types, and retrospectively analyzed (Fig. 3A). The distribution of pathogenic variants across the genome revealed by Northstar Select aligns with similar liquid biopsy assay studies [26–28]; as expected, canonically highly altered genes made up the majority of the top alterations by detection rate (Fig. S3). On average, there was a high variant detection rate for pathogenic and/or clinically actionable alterations across all classes of variants (Fig. S4).

Next, the median VAF of the pathogenic and clinically actionable SNV/Indels, a surrogate for ctDNA shedding, was calculated for each patient and the distribution of this measure was examined in each cohort that had at least 30 patients (Fig. 1B). For each of these tumor types, there was a wide distribution of the median VAF, whereby even canonically high shedding tumor types, such as CRC [29], had patients below 0.5 % median VAF. In addition, for patients with canonically low shedding tumor types, such as CNS tumors [30], the majority of patients had a median VAF of the detected clinically actionable and/or pathogenic alterations below 0.5 %. Overall, the distribution of median VAF ranged from under 0.1 % to over 70 %, per cancer type (Fig. 3B). In total, 162 out of the 674 patients (24 %) had a median VAF below 0.5 % for pathogenic and/or clinically actionable SNV/Indels. The rate of low shedders for cancer types with more than 30 patients is, in descending order: 40 % in Prostate, 26 % in Pancreas, 23 % in Brain & CNS, 22 % in Gastric and Esophageal, 21 % in Breast, 21 % in Lung, 19 % in Ovarian and Gynecologic, and 18 % in Colorectal. The low shedding cancer types are in general agreement with existing studies [19,29,31,32].

Subsetting the shedding analysis to only activating variants within oncogenes showed similar VAF distributions (Fig S5 A). The gene-level mutational landscape by diagnosis, for both SNV/Indel and CNV variants, is concordant with published large-scale clinical genomic landscape studies (Fig S5B, Fig S6) [27].

To further establish the clinical utility of Northstar Select, the clinical reports from all 674 patients were curated and the alterations were categorized into clinically actionable, pathogenic but not clinically actionable, or VUS. There was a high detection rate across the whole cohort where only 13 patients (1.9 %) had no alterations of any kind (actionable, pathogenic, or VUS) detected, of which 8 (62 %) were from patients with CNS tumors (Fig. 3C). Clinically actionable alterations were detected in all tumor types tested, with 68 % of patients having at least one clinically actionable alteration (Fig. 3C). Of note, this includes patients with CNS tumors, which, due to sensitivity limitations, are not included in analyses by current on market assays [33]. The current literature reports a 27–55 % detection rate of any alteration (including VUS) in CNS tumors [34–37], whereas Northstar Select detection rate was 87 % for all alterations, and a 30 % detection rate of clinically actionable alterations. When patients with CNS tumors are excluded from analysis to parallel current literature, 71.8 % of patients had at least one clinically actionable alteration. In summary, Northstar Select is able to detect clinically actionable alterations, including those in low shedding tumors, across cancer types.

2.4. Validation of enhanced sensitivity

2.4.1. Orthogonal ddPCR

To confirm the increased sensitivity of Northstar Select, ddPCR orthogonal testing was performed. ddPCR targets were selected according to clinical actionability, prevalence, and PCR probe design feasibility. Samples for ddPCR confirmation were selected according to existing orthogonal ctDNA assay results and availability of sufficient banked plasma from the retrospective study. Seventeen individual ddPCR assays were validated to maximize the number of clinical samples that could be feasibly tested. Samples tested included 65 clinical samples with ddPCR determined VAFs ranging from 0.03 % to 10.58 % VAF. 64/65 were concordant between Northstar Select and ddPCR (98 %). VAF was consistent between methods (Fig. 3D, **pink dots**). Orthogonal ddPCR testing was also performed on a subset of 12 patient samples obtained from the head-to-head study (Fig. 3D, black dots, Table S9) where the variant was not detected by the comparator assay and the detected allele fraction was below the LOD of the comparator assay. ddPCR assays on these variants resolved 11/12 variants in favor of Northstar Select. Taken together, the number and diversity of variants confirmed by ddPCR on all 77 samples demonstrate Northstar Select's enhanced sensitivity and strongly suggest that extrapolation into remaining additional variants detected by Northstar Select would also be true positives.

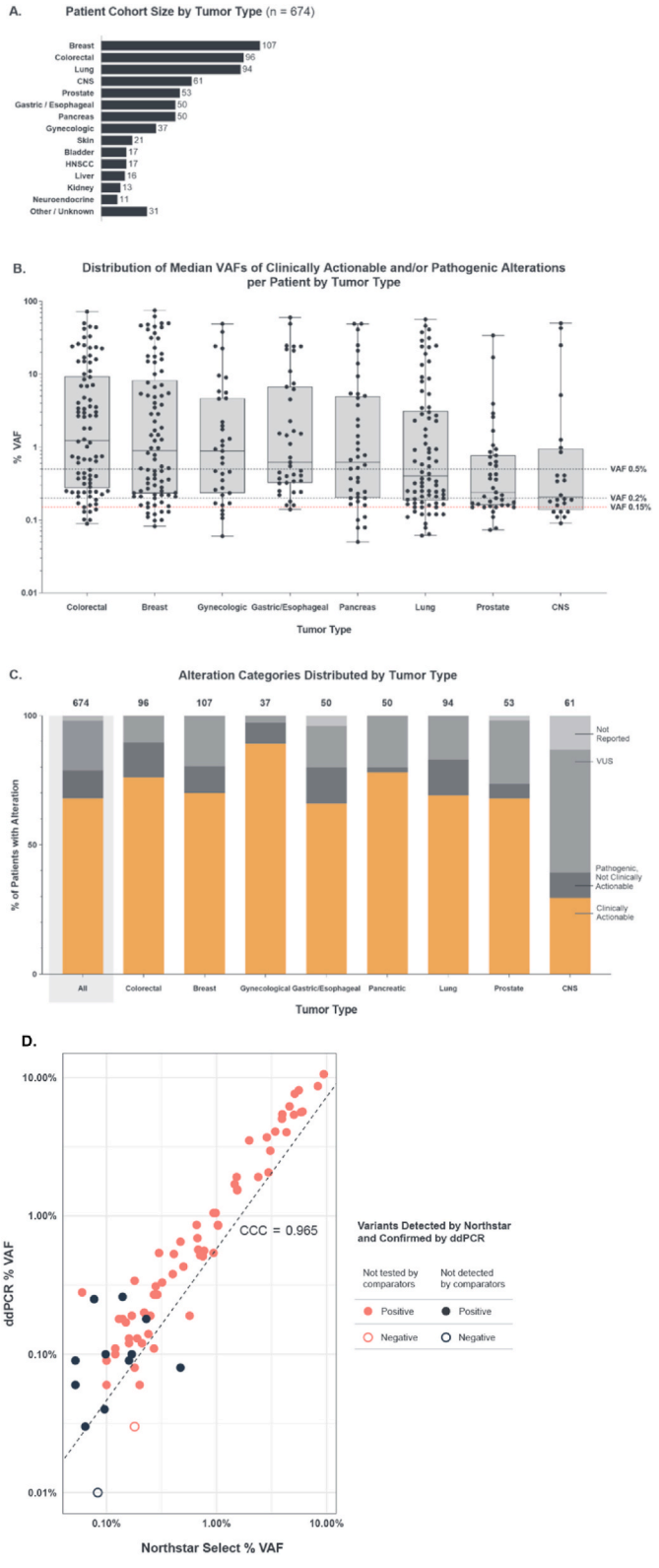
2.4.2. Increased sensitivity is not due increased CH detection

Furthermore, to ensure that additional variant detections were not a result of clonal hematopoiesis (CH), Northstar Select assay was run on buffy coat gDNA with slight modification (sonic shearing of gDNA for input into the assay) using a subset of samples from the head to head study for which there was buffy coat remaining (n = 28). Variants detected in the buffy coat were compared against the plasma-based clinical reports of comparators and Northstar Select for matched coverage genes. When compared against the buffy coat, the detection rate of CH variants was similar for both the comparator assays and for Northstar Select. Northstar Select reported CH mutations in 19.0 % ± 7.6 % (19/100) of SNVs and Indels, versus 17.2 % ± 9.2 % (11/64) by comparator assays (Fisher's Exact test odds ratio of 1.13, P-value = 0.838). To further establish that the high sensitivity of the assay was not due to the detection of more CH variants, we analyzed an additional 17 paired cfDNA and buffy coat gDNA samples (total n = 45) from the larger 674-cohort of commercial samples, examining prevalence at different VAFs. In total, pathogenic variants with VAF below 10 % revealed a CH prevalence of 34 % (59/171). Among low VAF (<0.5 %) variants, 32 % (40/124) were attributed to CH. Samples were also tested that were expected to be CH negative. In order to parallel current literature which demonstrated that cfDNA *KRAS* G12D variant detection had low impact from CH, a similar analysis was conducted [38]. *KRAS* G12D is a common mutation commonly found in pancreatic, colorectal, and lung cancers with several targeted therapies currently in development. Across both cohorts, 32 cfDNA *KRAS* G12D positive patients were identified with a broad VAF range (0.05 %–56 %). The gDNA obtained from the buffy coats of these patients were assayed and all 32 variants were found to be absent, confirming that the variants detected in the patients' plasma were tumor derived and not of CH origin.

3. Discussion

The transition toward liquid biopsies as the standard of care represents a significant advancement in oncology, addressing limitations of tissue biopsies including invasiveness, availability, and potential sampling bias due to tumor heterogeneity. This study demonstrates that Northstar Select provides superior sensitivity compared to existing liquid biopsy assays, detecting approximately 50 % more clinically actionable and/or pathogenic SNV/Indels with similar fusion detection and MSI calling across matched coverage variants. The assay also has the added benefit of more sensitive CNV detection, with 37.5 % more CNVs detected on matched variants and 109 % more in unmatched variant coverage, in part due to a higher number of CN losses in the panel.

The overall enhanced sensitivity of the assay stems from two key technical achievements. First, Northstar Select demonstrates superior limits of detection for SNVs/Indels, particularly at low variant allele frequencies (<0.5 % VAF). The validity of these additional detected variants was independently confirmed through ddPCR validation in a



(caption on next column)

Fig. 3. Northstar Select detects informative variants in the majority of patients and returns over 70 % clinically actionable results in certain tumor types. **(A)** Histogram of patients in each cancer type cohort. **(B)** For the cohorts with over 30 patients, the median variant allele fraction (VAF) of the pathogenic SNVs and Indels for each patient is plotted as a single point. The distribution of this measure, excluding patients with no pathogenic SNVs and Indels, across each cohort is shown. Dashed lines for 0.15 %, 0.2 %, and 0.5 % VAF are shown for reference. **(C)** Proportion of patients in each cancer type cohort for whom their clinical reports contained at least one clinically actionable alteration, a pathogenic variant not clinically actionable, VUS only, or null report. Note that only tumor types with n > 30 are broken out in this figure, but 'All' contains all groups regardless of size. **(D)** A scatterplot of the ddPCR data of 65 retrospective clinical patients (pink dots), and the 12 patients in the 182 patient head-to-head study (black dots) where Northstar Select detected a variant missed by the comparator. The dashed line is a Passing Bablok Regression, and Lin's Concordance Coefficient is 0.965, indicating a high degree of correlation.

subset of samples, while analysis of matched buffy coat samples demonstrated that the increased sensitivity is not an artifact of increased CH detection compared to less sensitive assays. It should be noted that these developments require significant sequencing depth (average of ~40,000x), which is higher than many other assays (typically 8,000–20,000x), potentially impacting assay cost and throughput. Second, the assay employs an advanced CNV calling algorithm that can differentiate between focal events and chromosomal aneuploidies (Fig. S7). This attribute, coupled with a low LOD, enables detection of copy number changes in liquid as subtle as 2.11 copies for amplification or 1.8 copies for losses.

The assay's enhanced CNV detection capabilities address a key challenge in liquid biopsy testing. For any given gene, driver copy number differences are typically focal and contribute to tumor biology through altered transcription levels, while "passenger" copy number differences may arise from large-scale aneuploidies present in approximately 90 % of solid tumors [39]. While both can result in similar copy number measurements in liquid biopsy, distinguishing between focal versus distributed changes is crucial for clinical interpretation. Northstar Select's sensitive algorithm considers multiple genes when determining if a copy number change represents aneuploidy or a true focal event, enabling more informed clinical decisions particularly in low shedding samples.

The clinical utility of this enhanced sensitivity is particularly evident in patients with low shedding tumors, defined as those with maximum pathogenic VAF below 0.5 %. In our 674-patient cohort, 24 % of patients (162/674) met this criterion, with prevalence ranging from 18 % to 40 % depending on cancer type. For these patients, the improved detection capability of Northstar Select could mean the difference between identifying or missing actionable variants. Of note, Northstar Select detected clinically actionable variants in 30 % of patients with CNS tumors, a notoriously low shedding tumor type where liquid biopsy is not routinely used due to sensitivity constraints. Furthermore, in the head-to-head cohort, approximately 10 % more pathogenic genomic anomalies were detectable compared to commercial comparators. Nearly two-thirds of the additionally detected alterations were clinically actionable, with a 17 % increase in detected variants, even when restricted to Tier 1A variants with FDA-approved or guideline-recommended treatments. This increased to 53 % when expanding to all variants with FDA-approved drugs for on-label and off-label use (Tiers1A-2C.1), described in the Variant Interpretation section of the Methods.

This validation study has intrinsic constraints that should be acknowledged. First, the study was not designed to track clinical outcomes such as response or survival. Because our primary intent was to establish assay validity and performance against on-market assays, longitudinal follow-up would be outside the scope of the current study and will be more appropriately investigated in future clinical utility studies. Second, we did not design a matched tissue concordance analysis. Tissue-liquid concordance studies have been previously established

and the cross-comparability, advantages and disadvantages thoroughly discussed [7,10,16,21]. As such, this assay platform is deliberately tissue-agnostic and is meant to be deployed when tumor material is scarce or unobtainable; accordingly, all comparator assays were themselves plasma-only assays. Third, raw read-level data from the comparator laboratories were not accessible. While granular data can illuminate the mechanistic basis of concordance or discordance, clinical laboratories ultimately act on the final, curated variant calls reported to treating physicians. Assessing agreement at the report level therefore represents the most pragmatic and clinically relevant metric for clinical validation.

4. Conclusion

Northstar Select offers an improved liquid biopsy tool in the clinical oncology landscape. By enhancing sensitivity, particularly at low VAFs and for copy number variants, this assay addresses critical gaps in current CGP approaches. The ability to detect a broader range of clinically actionable SNV/Indels, and precisely detect and differentiate CNVs across an expanded panel of genes, offers oncologists powerful insights that allow them to tailor treatments more effectively. Taken together, these data suggest that the improvements put forward by Northstar Select will enable more patients to benefit from precision medicine therapies based on a deeper, more sensitive, and more holistic view of the tumor genomic profile.

5. Materials and methods

5.1. Assay design

5.1.1. Hybrid capture panel design

The Northstar Select panel is an 84-gene NGS panel which detects SNVs and indels across 150.7 kilobases on 82 genes (40 have whole coding sequence and splice sites coverage while the others have hotspot coverage). The panel is also designed to cover copy number amplification for 19 genes and copy number loss for 5, gene fusions of 9 genes, and MSI-H detection, bringing the total targeted coverage to ~255 kilobases.

5.1.2. cfDNA extraction

The blood volume for each sample was verified to be $>$ 3 mL, and plasma was isolated via double centrifugation. Plasma was stored at -20 °C until proceeding. Isolated plasma specimens were thawed, vortexed, and briefly centrifuged. Plasma volume was verified to be $>$ 1.5 mL. cfDNA extraction was performed centrally. Extraction QCTs were added to each plasma sample before beginning extraction. The minimum sample input after extraction was 5 ng and the sample inputs were capped at 50 ng maximum going into each subsequent library preparation reaction.

5.1.3. gDNA extraction

After centrifugation, buffy coat was separated from the rest of the whole blood, and stored at -20 °C until processing. Sample gDNA was extracted using the QIAamp DNA Blood Kit – Genomic DNA Extraction™ according to manufacturer specifications. Samples were verified to be greater than 12 ng/μL before proceeding. For CH samples, the extracted buffy coat gDNA was sonically sheared and up to 60 ng is input into library preparation.

5.1.4. Library preparation

Library preparation was a modified version of the xGen™ FFPE cfDNA Library Preparation Kit (IDT) manufacturer-provided protocol. Briefly, ligation times for adapters were increased from 15 to 45 min. Library preparation QCTs were added before the end repair step to assess library preparation quality, and target enrichment QCTs were added before indexing PCR. Potential gDNA contamination was removed using a double-sided selection.

5.1.5. Hybridization capture and target enrichment

Libraries were pooled together for a single target enrichment reaction at 500 ng input each. The hybridization and target enrichment were performed with xGen™ Hybridization and Wash Kit (IDT) and KAPA HiFi HotStart ReadyMix (Roche) according to manufacturer-provided protocol, using a custom probe panel. The final enriched pool was quantified using a Qubit 1X dsDNA HS Assay Kit on an Invitrogen Qubit 4 Fluorometer, and the average fragment length determined using an Agilent 4150 TapeStation System.

5.1.6. Sequencing

Sequencing was performed on Illumina NextSeq 2000 with P3 flow cells and paired-end 150 sequencing (300 cycle) kits and/or the Illumina NovaseqX+ with 10B flowcell and paired end 150 sequencing (300 cycle) kits. Up to 0.1–5 % PhiX was spiked into the libraries, targeting 100 million paired end reads per sample. Sequencing read depth averages 40,000x. Base calling was performed with Real-Time Analysis (RTA) 3 software. Raw base call files were demultiplexed and converted to fastqs using DRAGEN BCL Convert (v3.8.4 on NS2000 and v4.2.7 on NovaseqX+).

5.1.7. ddPCR for CH

60 ng of DNA was added to a reaction containing a PCR master mix and primers and Taqman probes targeting the potential CH locus. Following droplet generation, an endpoint thermal cycling was performed. A droplet reader measures the fluorescence of each analyte droplet-by-droplet, classifies each droplet as positive or negative for each template, and uses a statistical formula to arrive at the variant allele frequency (VAF) of the variant in the mixture of DNA. If a mutation identified in Northstar Select is also detected in the buffy coat via ddPCR, it can be confirmed as CH.

5.2. Bioinformatics

5.2.1. Bam file creation

First, demultiplexed fastq files for each sample undergo QCT sequence extraction with SeqKit (v2.1.0). The remaining non-QCT reads undergo adapter trimming with Trimmomatic (v0.38). After adapter trimming, BWA-MEM (v0.7.17) is used for sequence alignment against the reference genome Hs37d5. The resulting aligned BAM files of raw reads are utilized for CNV and Fusion variant analysis, as described further below. Furthermore, the raw read alignment files were analyzed with Picard (v2.26.11) to collect target enrichment QC metrics. For SNVs Indel variants, and MSI calling, Fgbio (v2.0.2) is used to generate a processed bam file.

5.2.2. QCT analysis

The QCT data is processed from raw FASTQ files using a custom pipeline. Key steps include merging reads to identify full QCT sequences, filtering sequences based on criteria such as merging metrics, and calculating unique QCT counts using barcodes. These counts are corrected for sequencing saturation and compared to QCT molecule counts and their distribution in control runs. Finally, base error rates for PCR and sequencing are determined from expected sequences in QCT components.

5.2.3. SNV and Indel calling

VarDict (v.1.8.3) was used for SNV and Indel calling against reference genome Hs37d5, specifying custom exonic and splice regions. Custom adaptive filtering reduces false positives by considering polymerase false positive modes such as different false positive rates of base substitutions, context specific false positive mutation frequencies, and filtering based on the number of mutant molecules and total molecules detected, using a binomial statistical model. Training of adaptive

thresholds for false positive expectations of different variant locations and base substitution was done with repeated healthy plasma samples.

5.2.4. CNV calling

CNVs are identified and quantified using a custom comparative genomic hybridization-like method. Three forms of normalization are applied to control for technical noise and batch effects: batch normalization across probes in each batch, focal sample normalization, and genome-wide single sample normalization across probes in individual samples. Normalization algorithms were established on individual negative samples. These normalizations correct for read depth differences and GC bias. Per-sample normalization applies a peak-fitting algorithm and determines genome-wide aneuploidy calibration levels. Next, a within-chromosome focal normalization is used to separate CNVs that are focal as opposed to resulting from chromosome arm aneuploidy. Aneuploidy calls are determined for genes that do not meet the threshold for an amplification or loss call, but are above the thresholds for a negative call.

5.2.5. Fusion calling

For structural variants, primarily gene fusions, SViCT (v1.0.1) [40] is used to call potential fusion breakpoints based on the non-deduplicated mapped BAM file. To improve specificity, downstream filtering and fusion orientation correction custom scripts are implemented to computationally remove non-specific breakpoint calls. The orientation of the fusion calls represented in VCF 4.2 format is also corrected by examining the bam file reads on both sides of the breakpoints.

5.2.6. MSI

MSI-High detected/not detected calls are made with a custom algorithm that examines 96 informative microsatellite locations to calculate an MSI score. These 96 sites were curated from an internal database of approximately 300 sites compiled from publicly available lists of microsatellites [41–43]. The identified microsatellites overlap with several established microsatellite instability biomarkers such as BAT25 and BAT26 from the NCI 1997 Bethesda Guidelines [44]. For calling, an MSI specific indel mutation list is generated using Vardict on the BAM file with the reference genome Hs37d5, with non MSI specific variants removed. An MSI score is calculated using the weighted sum of the count of mutations in each sample. Samples are reported as MSI-H if the MSI score is above an empirically derived static threshold based on hundreds of MSS patients.

5.2.7. CH calling

For CH studies, if a variant was detected in the buffy coat of the sample and in the cell free DNA, it was classified as CH. For the head to head comparison study, assessments were made compared against the comparator clinical report and the Northstar Select clinical report.

5.2.8. Variant interpretation

SNVs and Indels were reported across approximately 150 kb of genomic coverage. Clinical actionability of variants was defined by Qiagen's QCI Interpret (Software Build: 9.2.1.20231012), based on FDA, professional guidelines, and primary literature. VCF files were uploaded and variants were annotated and filtered. QCI Interpret Tiers 1A, 1B, 2C and 2D were classified as 'clinically actionable', according to previously published guidelines for interpretation and reporting [45]. 2C.1 was used to describe 2C variants that had drugs approved with both on-label and off-label use.

5.3. Analytical validation

5.3.1. Analytical sensitivity and specificity

All thresholds for setting sensitivity and specificity were conducted on a separate set of samples prior to locking the assay and conducting the validation.

First, a sensitivity limit estimation was performed for small variants such as SNVs and indels using diluted Horizon Discovery reference standards. To assess where the 95 % sensitivity range would be, high VAF reference standards with many replicates were run and diluted to obtain a wide VAF spread below 1 %. The LOD study was performed for SNVs and indels using two reference materials. Horizon Discovery Multiplex cfDNA reference standards (HD780) containing 8 variants (6 SNV and 2 Indel) confirmed by ddPCR were run at 0.15 % and 0.25 % VAF (1 and ~1.5x LOD) at three different inputs: 10 ng, 30 ng, and 50 ng. Twist cfDNA pan-cancer reference v2 contains 111 variants (71 SNV and 40 Indels) over 42 genes, confirmed by NS550 sequencing and ddPCR. These were diluted in cfDNA to 0.15 % and 0.25 % VAF, and run at 10, 30, and 50 ng inputs.

Seventeen individual ddPCR assays were designed, validated, and performed by an independent CLIA/CAP accredited laboratory (PacificDX). Variants tested were: *KRAS* G12D, *KRAS* G12S, *KRAS* G12C, *KRAS* G12A, *KRAS* G12V, *PIK3CA* E545K, *PIK3CA* E542K, *PIK3CA* Q546K, *PIK3CA* H1047L, *BRAF* V600E, *JAK2* V617F, *EGFR* L858R, *EGFR* E746_L747delinsIP, *TP53* R175H, *TP53* Y220C, *TP53* R273C, and *TP53* R273H. 65 samples were assessed, containing variants between 0.06 % and 9.43 % VAF.

A LOD study was performed for CN amplification and CN losses using well-characterized reference materials. Two cell-line reference materials from ATCC, SNU-16 and NCI-H2170, both containing CDKN2A losses and two further reference materials, Horizon HD836 Prostate Cancer Panel cfDNA Reference Standard and HD837, both containing *PTEN* losses confirmed by Horizon Discovery via ddPCR, were chosen for the CN loss study. For copy number amplifications, two ddPCR confirmed standards from Seracare, Seraseq CNV Breast Mix (containing *EGFR*) and Seraseq Lung and Brain CNV Mix (containing *ERBB2* and *MET*), were assayed. Horizon Discovery HD836 also contains a ddPCR confirmed AR amplification and thus was run as part of the CN Amplification LOD study. This totaled 6 unique reference materials tested. The reference materials were diluted in NA12878, to target LOD of 2.11 CN and 2.15 CN for amplification, and 1.8 and 1.5 CN loss. Each standard was tested at three different input masses of 10, 30, and 50 ng, with five replicates at each condition. This totaled eight expected CN variants in six genes (2x *CDKN2A*, 2x *PTEN*, 1x *MET*, 1x *AR*, 1x *EGFR*, 1x *ERBB2*).

To assess the LOD of fusion in Northstar Select, six fusion variants were designed and contrived to mimic ctDNA fusion breakpoints. An SNV was inserted 10bp from the fusion breakpoint to assess an accurate allele fraction upon sequencing. Each fusion was spiked into a fusion-free sonically sheared healthy gDNA sample at various allele frequencies, targeting 0.1–2.0 % VAF. 20 replicates were run through the assay at a 30 ng input. The SNV-INDEL LOD study reference material (Twist cfDNA Pan-cancer) also contains 10 fusion variants in 2 genes. Fusions tested were: *TRP-ALK*, *EML4-ALK* x 3 breakpoint variants, *CCDC6-RET* x 3 breakpoint variants, *KIF5B-RET* x 2 breakpoint variants, and *NCOA4-RET*. These were analyzed at the three inputs and two VAFs already described in the SNV LOD study.

Eight MSI-H solid tumor tissue samples were ordered from Accio Biobank and confirmed MSI-H via IHC or PCR, along with their paired buffy coats. The LOD was computationally estimated by bioinformatically combining reads from the buffy coat and the tumor in allele fractions of 0.03, 0.05, 0.07, 0.1, 0.15, 0.2, 0.25, 0.50, and 1.00 %. Twenty replicates of an MSI-H sample were run at 0.07 % tumor fraction. Tumor fraction was estimated as the maximum VAF of driver variants in the tumor.

To assess the Limit of Blank, 58 pre-screened plasma samples from healthy volunteers were run on the Northstar Select assay. These were analyzed on a base-wise, panel-wide basis for small variants. CNVs, MSI-H detection, and fusion presence were also analyzed.

5.3.2. Repeatability and reproducibility

For repeatability and reproducibility, 20 samples were tested. 10 samples were assessed at 50 ng input and 0.15–0.25 % range, and 10

samples were assessed at 10 ng input and 0.4–1.5 % range, the ranges set for testing detection limits at both input levels. All 20 samples were repeated 3 times within a batch, and an additional 2 times on different days with different operators, for a total of 100 samples.

5.3.3. Robustness

To test the robustness of the assay to the carry through of blood components, the Twist reference material was subjected to Hematin contaminations of 0, 0.001, 0.01, 0.1, 1, and 10 μM, at two replicates each.

To demonstrate that QCT addition had no effect on Northstar Select, Twist reference material was run in duplicate with and without QCTs by two different operators on different days, sequenced on different machines for a total of 8 samples. All 279 variants were called identically in all eight samples.

5.4. Clinical validation

5.4.1. Clinical study cohorts

A retrospective analysis of archived liquid biopsies (Northstar Select) ordered within the USA between May 23, 2023 and January 9, 2024 collected during routine clinical care was conducted. If multiple tests were ordered for the same patient, only the first result was analyzed. In total, results for 674 patients were included. The purpose of this study was to analyze the differences of detection and shedding patterns between diagnoses. Authors did not have access to information that could identify individual participants during or after data collection.

For the prospective, observational, head-to-head comparison study, 182 patients with advanced stage solid tumors who were due to receive a currently on-market CGP assay (the comparator) as part of standard of care were enrolled across 6 community oncology clinics and one large hospital system across the USA from May 15, 2023 through Mar 31, 2024. In total, 5 different comparator tests from 4 testing laboratories were used, with assay panel sizes ranging between 74 and 523 genes. The comparator assay selection was the choice of the ordering physician as a part of the patient's standard of care, and included the three most commonly used liquid biopsy treatment selection assays in the United States [46–48]. The blood samples for Northstar Select and the respective comparator assay were collected concurrently (from the same blood draw) for each patient, such that the results from the two assays reflect a similar input quantity and quality of ctDNA. Approval for this observational study was obtained from the WCG Institutional Review Board (protocol 20230250), and each patient provided written informed consent. Owing to potential differences in variant classification of SNVs and CNVs, a variant was considered pathogenic and included in comparative analyses if one or both assays classified the variant as pathogenic. MSI and fusions were evaluated as a binary detected/not-detected. Variants on genes not covered by one assay were not counted against that assay ("matched coverage"). All numbers, unless otherwise stated, are "coverage matched", meaning that assays were compared only considering variants covered by both assays. An independent team, with no access to lab or bioinformatic operations and blinded to the Northstar Select results, manually entered the orthogonal data into a database. The operators who conducted the lab, bioinformatics and variant interpretation processes of Northstar Select were fully blinded from the orthogonal data and reports. Authors did not have access to information that could identify individual participants during or after data collection.

5.4.2. ddPCR confirmations

Twelve patients harboring variants detected only by Northstar Select from the Clinical Validation were assayed using the 17 validated ddPCR assays from the analytical validation. cfDNA extracted from the plasma were sent to PacificDx to run the ddPCR assays.

5.4.3. Comprehensive genomic profiling

The Northstar Select CGP assay was performed in a Clinical Laboratory Improvement Amendments CLIA)–certified, College of American Pathologists (CAP)–accredited laboratory (BillionToOne, Inc. Menlo Park, CA).

All studies were conducted in accordance with the Declaration of Helsinki.

Author contributions

Conceptualization: Wen Zhou, Xavier Bower, Jan Wignall, Joyce Zhu.

Data Curation: Wen Zhou, Xavier Bower, Lenny Hong, Naomi Searle, Turgut Dogruluk

Formal Analysis: Xavier Bower, Wen Zhou, Jan Wignall, Joyce Zhu, Michael O'Sullivan, Naomi Searle, Lenny Hong, Turgut Dogruluk, **Investigation:** Wen Zhou, Xavier Bower, Jan Wignall, Joyce Zhu, Michael O'Sullivan, Zeqian Li, Matthew Varga, Gary Palmer, Ajeet Gajra, Chanh Huynh.

Methodology: Wen Zhou, Xavier Bower, Matthew Varga.

Project Administration: Wen Zhou, David Tsao, Gary Palmer.

Resources: Ajeet Gajra, Chanh Huynh.

Software: Wen Zhou, Xavier Bower, Zeqian Li, Jan Wignall, Joyce Zhu.

Supervision: Wen Zhou.

Validation: Xavier Bower, Jan Wignall, Michael O'Sullivan, Joyce Zhu, Zeqian li, Jason Luong, Esther Lin, Marie Erica Simon, Emilio Rosas-Linhard, Turgut Dogruluk, John Ten Bosch.

Visualization: Xavier Bower, Jan Wignall

Writing – Original Draft Preparation: Xavier Bower, Jan Wignall, Wen Zhou, Matthew Varga, Tiffany Farmer.

Writing – Review and Editing: Xavier Bower, Jan Wignall, Wen Zhou, David Tsao, Matthew Varga, Naomi Searle, Lenny Hong, Turgut Dogruluk, Zeqian Li, John Ten Bosch, Chanh Huynh, Ajeet Gajra, Gary Palmer, Michael O'Sullivan.

Data availability statement

Raw data was obtained across real-world health care settings and are subject to controlled access for privacy and proprietary reasons. The data that support the findings of this study may be available on reasonable request from the corresponding author, [WZ].

Funding

No external funding was provided for this study. The research was funded entirely by BillionToOne, Inc.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Xavier Bower reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Jan Wignall reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Matthew G. Varga reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Joyce Zhu reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Michael O Sullivan reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Naomi E. Searle reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Lenny K. Hong reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Turgut Dogruluk reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Zeqian Li reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Tiffany E. Farmer

reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Emilio Rosas-Linhard reports a relationship with BillionToOne Inc that includes: employment. Jason Luong reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Esther Lin reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Marie Erica Simon reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. David S. Tsao reports a relationship with BillionToOne Inc that includes: board membership, employment, and equity or stocks. John R. ten Bosch reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Gary Palmer reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. Ajeet Gajra reports a relationship with BillionToOne Inc that includes: consulting or advisory. Chanh Huynh reports a relationship with BillionToOne Inc that includes: consulting or advisory. Wen Zhou reports a relationship with BillionToOne Inc that includes: employment and equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jlb.2025.100322.

References

[1] Schram AM, Reales D, Galle J, Cambria R, Durany R, Feldman D, et al. Oncologist use and perception of large panel next-generation tumor sequencing. Ann Oncol 2017;28:2298–304. https://doi.org/10.1093/annonc/mdx294.

[2] Tomlins SA, Hovelson DH, Suga JM, Anderson DM, Koh HA, Dees EC, et al. Real-world performance of a comprehensive genomic profiling test optimized for small tumor samples. JCO Precis Oncol 2021:1312–24. https://doi.org/10.1200/PO.20.00472.

[3] Chan HT, Chin YM, Low S-K. Circulating tumor DNA-based genomic profiling assays in adult solid tumors for precision oncology: recent advancements and future challenges. Cancers 2022;14:3275. https://doi.org/10.3390/cancers14133275.

[4] Gerlinger M, Catto JW, Orntoft TF, Real FX, Zwarthoff EC, Swanton C. Intratumour heterogeneity in urologic cancers: from molecular evidence to clinical implications. Eur Urol 2015;67:729–37. https://doi.org/10.1016/j.eururo.2014.04.014.

[5] Gibbs SN, Peneva D, Cuyun Carter G, Palomares MR, Thakkar S, Hall DW, et al. Comprehensive review on the clinical impact of next-generation sequencing tests for the management of advanced cancer. JCO Precis Oncol 2023:e2200715. https://doi.org/10.1200/PO.22.00715.

[6] Mosele F, Remon J, Mateo J, Westphalen CB, Barlesi F, Lolkema MP, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO precision medicine working group. Ann Oncol 2020;31:1491–505. https://doi.org/10.1016/j.annonc.2020.07.014.

[7] Rolfo C, Mack P, Scagliotti GV, Aggarwal C, Arcila ME, Barlesi F, et al. Liquid biopsy for advanced NSCLC: a consensus statement from the international association for the study of lung cancer. J Thorac Oncol 2021;16:1647–62. https://doi.org/10.1016/j.jtho.2021.06.017.

[8] Mack PC, Banks KC, Espenschied CR, Burich RA, Zill OA, Lee CE, et al. Spectrum of driver mutations and clinical impact of circulating tumor DNA analysis in non–small cell lung cancer: analysis of over 8000 cases. Cancer 2020;126:3219–28. https://doi.org/10.1002/cncr.32876.

[9] Febbo PG, Allo M, Alme EB, Cuyun Carter G, Dumanois R, Essig A, et al. Recommendations for the equitable and widespread implementation of liquid biopsy for cancer care. JCO Precis Oncol 2024:e2300382. https://doi.org/10.1200/PO.23.00382.

[10] Iams WT, Mackay M, Ben-Shachar R, Drews J, Manghnani K, Hockenberry AJ, et al. Concurrent tissue and circulating tumor DNA molecular profiling to detect guideline-based targeted mutations in a multicancer cohort. JAMA Netw Open 2024;7:e2351700. https://doi.org/10.1001/jamanetworkopen.2023.51700.

[11] Medford AJ, Ellisen LW. Optimizing access to liquid biopsy in the present and future cancer landscape. JCO Precis Oncol 2024:e2300609. https://doi.org/10.1200/PO.23.00609.

[12] Jacobs MT, Mohindra NA, Shantzer L, Chen IL, Phull H, Mitchell W, et al. Use of low-frequency driver mutations detected by cell-free circulating tumor DNA to guide targeted therapy in non–small-cell lung cancer: a multicenter case series. JCO Precis Oncol 2018:1–10. https://doi.org/10.1200/PO.17.00318.

[13] Kim ST, Banks KC, Lee S-H, Kim K, Park JO, Park SH, et al. Prospective feasibility study for using cell-free circulating tumor DNA–guided therapy in refractory metastatic solid cancers: an interim analysis. JCO Precis Oncol 2017:1–15. https://doi.org/10.1200/PO.16.00059.

[14] Volckmar A, Sültmann H, Riediger A, Fioretos T, Schirmacher P, Endris V, et al. A field guide for cancer diagnostics using cell-free DNA: from principles to practice and clinical applications. Genes Chromosomes Cancer 2018;57:123–39. https://doi.org/10.1002/gcc.22517.

[15] Deveson IW, Gong B, Lai K, LoCoco JS, Richmond TA, Schageman J, et al. Evaluating the analytical validity of circulating tumor DNA sequencing assays for precision oncology. Nat Biotechnol 2021;39:1115–28. https://doi.org/10.1038/s41587-021-00857-z.

[16] Stetson D, Ahmed A, Xu X, Nuttall BRB, Lubinski TJ, Johnson JH, et al. Orthogonal comparison of four plasma NGS tests with tumor suggests technical factors are a major source of assay discordance. JCO Precis Oncol 2019:1–9. https://doi.org/10.1200/PO.18.00191.

[17] Tran HT, Lam VK, Elamin YY, Hong L, Colen R, Elshafeey NA, et al. Clinical outcomes in Non–small-cell lung cancer patients treated with EGFR-tyrosine kinase inhibitors and other targeted therapies based on tumor versus plasma genomic profiling. JCO Precis Oncol 2021:1241–9. https://doi.org/10.1200/PO.20.00532.

[18] Abida W, Armenia J, Gopalan A, Brennan R, Walsh M, Barron D, et al. Prospective genomic profiling of prostate cancer across disease states reveals germline and somatic alterations that may affect clinical decision making. JCO Precis Oncol 2017:1–16. https://doi.org/10.1200/PO.17.00029.

[19] Razavi P, Li BT, Brown DN, Jung B, Hubbell E, Shen R, et al. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. Nat Med 2019;25:1928–37. https://doi.org/10.1038/s41591-019-0652-7.

[20] Tukachinsky H, Madison RW, Chung JH, Gjoerup OV, Severson EA, Dennis L, et al. Genomic analysis of circulating tumor DNA in 3,334 patients with advanced prostate cancer identifies targetable BRCA alterations and AR resistance mechanisms. Clin Cancer Res 2021;27:3094–105. https://doi.org/10.1158/1078-0432.CCR-20-4805.

[21] Wyatt AW, Annala M, Aggarwal R, Beja K, Feng F, Youngren J, et al. Concordance of circulating tumor DNA and matched metastatic tissue biopsy in prostate cancer. JNCI J Natl Cancer Inst 2017;109. https://doi.org/10.1093/jnci/djx118.

[22] Tsui DWY, Cheng ML, Shady M, Yang JL, Stephens D, Won H, et al. Tumor fraction-guided cell-free DNA profiling in metastatic solid tumor patients. Genome Med 2021;13:96. https://doi.org/10.1186/s13073-021-00898-8.

[23] Kumaki Y, Oda G, Ikeda S. Targeting MET amplification: opportunities and obstacles in therapeutic approaches. Cancers 2023;15:4552. https://doi.org/10.3390/cancers15184552.

[24] Replogle JM, Zhou W, Amaro AE, McFarland JM, Villalobos-Ortiz M, Ryan J, et al. Aneuploidy increases resistance to chemotherapeutics by antagonizing cell division. Proc Natl Acad Sci 2020;117:30566–76. https://doi.org/10.1073/pnas.2009506117.

[25] Tsao DS, Silas S, Landry BP, Itzep NP, Nguyen AB, Greenberg S, et al. A novel high-throughput molecular counting method with single base-pair resolution enables accurate single-gene NIPT. Sci Rep 2019;9:14382. https://doi.org/10.1038/s41598-019-50378-8.

[26] Jovelet C, Ileana E, Le Deley M-C, Motté N, Rosellini S, Romero A, et al. Circulating cell-free tumor DNA analysis of 50 genes by next-generation sequencing in the prospective MOSCATO trial. Clin Cancer Res 2016;22:2960–8. https://doi.org/10.1158/1078-0432.CCR-15-2470.

[27] Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 2017;23:703–13. https://doi.org/10.1038/nm.4333.

[28] Vasseur D, Arbab A, Giudici F, Marzac C, Michiels S, Tagliamento M, et al. Genomic landscape of liquid biopsy mutations in TP53 and DNA damage genes in cancer patients. npj Precis Oncol 2024;8:51. https://doi.org/10.1038/s41698-024-00544-7.

[29] Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6. https://doi.org/10.1126/scitranslmed.3007094.

[30] Ronvaux L, Riva M, Coosemans A, Herzog M, Rommelaere G, Donis N, et al. Liquid biopsy in glioblastoma. Cancers 2022;14:3394. https://doi.org/10.3390/cancers14143394.

[31] Zhang Y, Yao Y, Xu Y, Li L, Gong Y, Zhang K, et al. Pan-cancer circulating tumor DNA detection in over 10,000 Chinese patients. Nat Commun 2021;12:11. https://doi.org/10.1038/s41467-020-20162-8.

[32] Weber ZT, Collier KA, Tallman D, Forman J, Shukla S, Asad S, et al. Modeling clonal structure over narrow time frames via circulating tumor DNA in metastatic breast cancer. Genome Med 2021;13:89. https://doi.org/10.1186/s13073-021-00895-x.

[33] LCD. MolDX: plasma-based genomic profiling in solid tumors (L38168). Available: https://www.cms.gov/medicare-coverage-database/view/lcd.aspx?LCDId=38168. [Accessed 16 December 2024].

[34] Schwaederle M, Husain H, Fanta PT, Piccioni DE, Kesari S, Schwab RB, et al. Detection rate of actionable mutations in diverse cancers using a biopsy-free (blood) circulating tumor cell DNA assay. Oncotarget 2016;7:9707–17. https://doi.org/10.18632/oncotarget.7110.

[35] Piccioni DE, Achrol AS, Kiedrowski LA, Banks KC, Boucher N, Barkhoudarian G, et al. Analysis of cell-free circulating tumor DNA in 419 patients with glioblastoma and other primary brain tumors. CNS Oncol 2019;8:CNS34. https://doi.org/10.2217/cns-2018-0015.

[36] Bagley SJ, Nabavizadeh SA, Mays JJ, Till JE, Ware JB, Levy S, et al. Clinical utility of plasma cell-free DNA in adult patients with newly diagnosed glioblastoma: a pilot prospective study. Clin Cancer Res 2020;26:397–407. https://doi.org/10.1158/1078-0432.CCR-19-2533.

[37] Zill OA, Banks KC, Fairclough SR, Mortimer SA, Vowles JV, Mokhtari R, et al. The landscape of actionable genomic alterations in cell-free circulating tumor DNA from 21,807 advanced cancer patients. Clin Cancer Res 2018;24:3528–38. https://doi.org/10.1158/1078-0432.CCR-17-3837.

[38] Roma C, Sacco A, Forgione L, Esposito Abate R, Lambiase M, Dotolo S, et al. Low impact of clonal hematopoiesis on the determination of RAS mutations by cell-free DNA testing in routine clinical diagnostics. Diagnostics 2022;12:1956. https://doi.org/10.3390/diagnostics12081956.

[39] Weaver BA, Cleveland DW. Does aneuploidy cause cancer? Curr Opin Cell Biol 2006;18:658–67. https://doi.org/10.1016/j.ceb.2006.10.002.

[40] Gawroński AR, Lin Y-Y, McConeghy B, LeBihan S, Asghari H, Koçkan C, et al. Structural variation and fusion detection using targeted sequencing data from circulating cell free DNA. Nucleic Acids Res 2019;47. https://doi.org/10.1093/nar/gkz067. e38–e38.

[41] Takehara Y, Nagasaka T, Nyuya A, Haruma T, Haraga J, Mori Y, et al. Accuracy of four mononucleotide-repeat markers for the identification of DNA mismatch-repair deficiency in solid tumors. J Transl Med 2018;16:5. https://doi.org/10.1186/s12967-017-1376-4.

[42] Crary-Dooley FK, Xi L, Van Der Merwe R, Furtado NM, Li J, Lin J, et al. Abstract 6530: preferential low frequency allele enrichment with lower sequencing depth requirement using KAPA HyperPETE specialized primer designs. Cancer Res 2023;83. https://doi.org/10.1158/1538-7445.AM2023-6530. 6530–6530.

[43] Han X, Zhang S, Zhou DC, Wang D, He X, Yuan D, et al. MSIsensor-ct: microsatellite instability detection using cfDNA sequencing data. Briefings Bioinf 2021;22:bbaa402. https://doi.org/10.1093/bib/bbaa402.

[44] Umar A, Boland CR, Terdiman JP, Syngal S, Chapelle ADL, Ruschoff J, et al. Revised bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. JNCI J Natl Cancer Inst 2004;96:261–8. https://doi.org/10.1093/jnci/djh034.

[45] Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the association for molecular pathology, American society of clinical oncology, and college of American pathologists. J Mol Diagn JMD 2017;19:4–23. https://doi.org/10.1016/j.jmoldx.2016.10.002.

[46] Chennamadhavuni A, Kasi PM. Circulating tumor DNA in identifying resistant subclones post EGFR blockade: implications for EGFR rechallenge. Front Oncol 2022;12:847299. https://doi.org/10.3389/fonc.2022.847299.

[47] Kuderer NM, Burton KA, Blau S, Rose AL, Parker S, Lyman GH, et al. Comparison of 2 commercially available next-generation sequencing platforms in oncology. JAMA Oncol 2017;3:996–8. https://doi.org/10.1001/jamaoncol.2016.4983.

[48] Woodhouse R, Li M, Hughes J, Delfosse D, Skoletsky J, Ma P, et al. Clinical and analytical validation of FoundationOne liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. PLoS One 2020;15:e0237802. https://doi.org/10.1371/journal.pone.0237802.