Analytical and clinical validity of Northstar Select, a quantitatively enhanced liquid biopsy assay for comprehensive genomic profiling of solid tumors

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INTRODUCTION

Comprehensive Genomic Profiling (CGP) liquid biopsy has emerged as a vital tool in precision oncology, improving treatment selection for late-stage solid tumor cancer patients by allowing for the detection of clinically actionable somatic variants via circulating tumor DNA (ctDNA). However, current liquid biopsies are generally unreliable for the detection of variants below 0.5% variant allele fraction (VAF),[1] which limits their utility in cancers that shed low levels of ctDNA. Nearly ½ of SNVs are present at <0.5% VAF, and ¼ are present at <0.25% VAF.[2] Furthermore, patients have been shown to respond to targeted therapy regardless of driver mutation VAF level in blood,[2-5] underscoring the need for a highly sensitive liquid biopsy that can detect actionable variants at <0.5% VAF.

Northstar Select is a CGP test that leverages Quantitative Counting Template™ (QCT) technology, optimized chemistry and panel design, and bioinformatics innovations to increase sensitivity and thereby address this unmet need. Here, we present the design and validation of the assay, including a 'head-to-head' comparison against existing liquid biopsies.

METHODS

- Analytical Validation
  - Analytical and reference samples were used to evaluate the sensitivity and specificity of Northstar Select.
  - A Limit of Detection (LoD) study was performed for each class of variants (e.g. SNVs, CNVs, fusions) to determine the signal level at which each can be reliably detected.
- Clinical Validation
  - Blood specimens were concurrently assayed by both Northstar Select and the physician's choice of a commercially available comparator CGP liquid biopsy ordered as part of standard of care (n=182 patients).
  - All procedures were carried out in accordance with the Declaration of Helsinki and protocols were approved by WCG IRB #20230250.

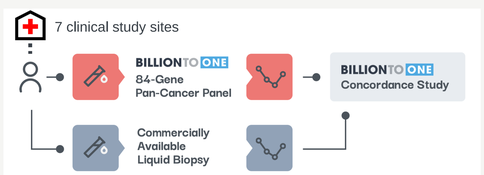

Fig 1. Head-to-head clinical validation study design.

Acknowledgements

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References

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INNOVATIONS

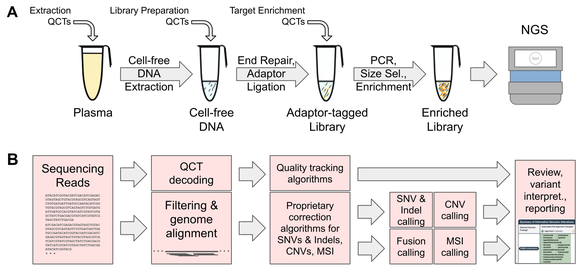

Fig 2. Northstar Select assay, bioinformatics, and reporting.
A. Isolated plasma is processed through DNA extraction, library preparation, and sequencing. Quantitative Counting Templates (QCTs) are added at multiple steps to encode sample integrity at each assay step. **B**. Sequencing reads corresponding to QCTs are decoded and passed into a custom quality tracking workflow, enabling re-queuing of deficient samples. Sequencing reads are processed through proprietary calling algorithms for each variant type which enhance sensitivity and specificity.

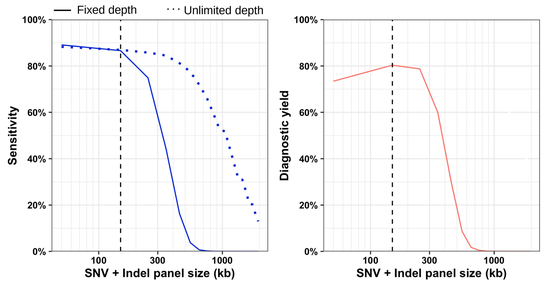

Fig 3. In silico analysis of sensitivity and specificity shows diagnostic yield is maximized at ~150 kb panel size. The effect of panel size on sensitivity was evaluated in a model that incorporates sequencing read depth, false positive rate, and the frequency of somatic variants across the genome. Larger panels are expected to exhibit a higher rate of false positives per patient due to the corresponding increased number of base calls. Therefore, more stringent filters that account for multiple hypothesis testing are required to maintain a 95% specificity, resulting in poorer per-variant sensitivity at larger panel sizes. Notably, the sensitivity trend was preserved both when the depth of NGS sequencing was fixed at 100M reads (fixed depth) and when sequencing depth scaled with panel size (unlimited depth). Larger panel sizes can detect greater numbers of somatic variants per patient across the genome. However, due to the loss of per-variant sensitivity at larger panel sizes, the probability of detecting at least one variant for a given patient (diagnostic yield) peaks at a ~150kb panel.

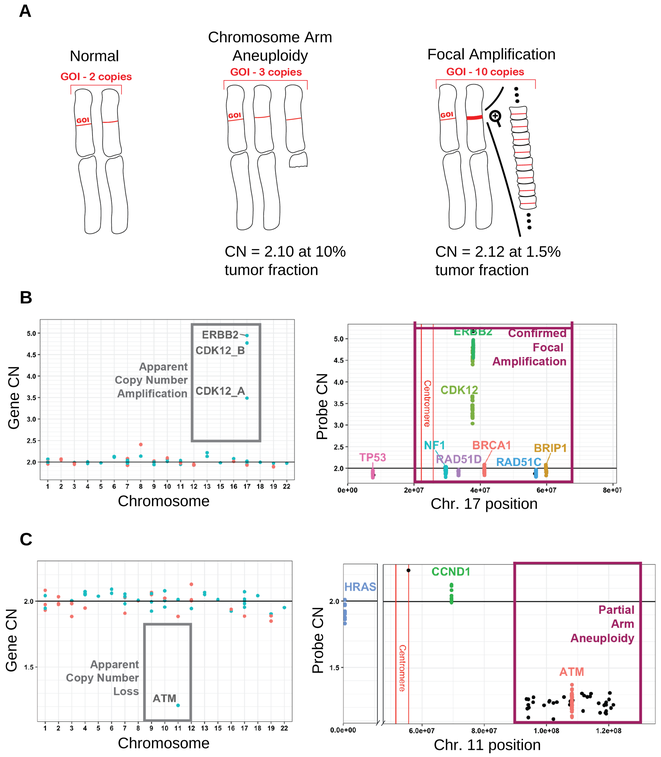

Fig 4. Distinguishing between focal copy number variations and aneuploidies. **A**. Schematic of gene copy number gain due to aneuploidy or a focal amplification and required tumor fraction to achieve ~2.1 copy number (CN) in plasma, corresponding to Northstar Select's LoD of copy number amplification. **B**. Example of *ERBB2* and *CDK12* focal amplification with plasma CN of 5, in which neighboring genes on Chr. 17 (e.g. *BRCA1*) have normal copy number. Interestingly, a consistent step change in copy number was found in the interior of *CDK12 (*compare CDK12_A and CDK12_B*)*, suggestive of a breakpoint in that region. **C.** Example of a large (30Mb+) deletion on Chr. 11 that encompasses *ATM*, potentially indicative of copy number instability.

ANALYTICAL RESULTS

Table 1. Analytical sensitivity, specificity, and LoD.

Variant Type	# Variants for sens. & spec. Total (Unique)	Sensitivity	Specificity	LoD_{95}
SNV	1128 (181)	100%	>99.999% (3,168,175 / 3,168,181 bp)	0.13% - 0.16% VAF
Indel	1173 (155)	100%	100%	
CNV	17 (10)	100%	100%	2.11 copies (Amp.) 1.8 copies (Loss)
Fusion	80 (10)	100%	100%	0.30% TF
MSI-H	27 (17) samples	100%	100%	0.07% TF

CONCLUSIONS

- The development of Northstar Select incorporated many innovations in hybrid capture liquid biopsy technology and design.
  - QCT tracking and panel design both serve to increase sensitivity.
  - CNV noise reduction and calling strategies result in a novel ability to determine the biological nature of copy number signals.
- These innovations translated to an analytical and clinical performance that surpasses that of current CGP liquid biopsies.
  - High sensitivity resulted in remarkably low LoD.
    - **0.13-0.16% VAF for SNV & Indels, 2.11 copies for CN amp.**
  - Northstar Select demonstrated a >40% increase in sensitivity for SNVs & Indels. Over 2x as many CNVs were detected via both increased sensitivity and expanded coverage.
- Northstar Select is therefore uniquely useful for cancers with low ctDNA level, addressing an unmet clinical need.

CLINICAL RESULTS

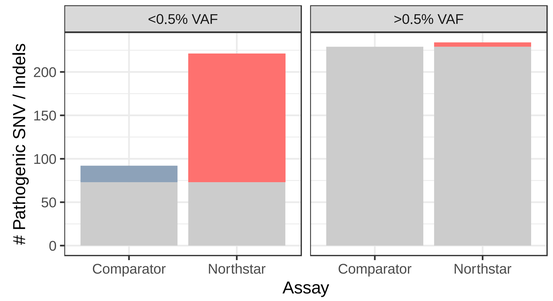

Fig 5. Northstar Select detected more pathogenic SNVs and indels, especially at low VAF. Northstar Select and comparator assay results were reported for 182 patients. Six patients were found to be MSI-H and harbored numerous indels; concordance analysis excluded the 6 MSI-H patients to avoid biasing results. At VAF >0.5%, the results were 95%+ concordant. Northstar Select detected over 2x as many SNVs and indels below 0.5% VAF. Overall, Northstar Select detected >40% more SNV and indel variants relative to comparators. Only genes covered by both Northstar Select and comparator panels were included in this analysis.

Comparator alone	Both	Northstar alone
BCL2L1 amp (1)*	*ERBB2* amp (4)	*CDKN2A* loss (8)
EGFR amp (1)	*MYC* amp (4)	*PTEN* loss (5)
ERCC2 amp (1)*	*FGFR1* amp (3)	*AR* amp (4)
MDM2 amp (1)* ...	*KRAS* amp (3) ...	*CCNE1* amp (2) ...

* Not included in Northstar Select 84 gene panel

Table 2. Representative examples of CNVs detected by Northstar Select and comparators.
Examples are shown of the genes for which copy number amplifications and losses are most frequently detected, along with the number of patients in which they were detected. Northstar Select found CDKN2A or PTEN losses in 13/182 (7%) of patients, none of which were detected by comparators.

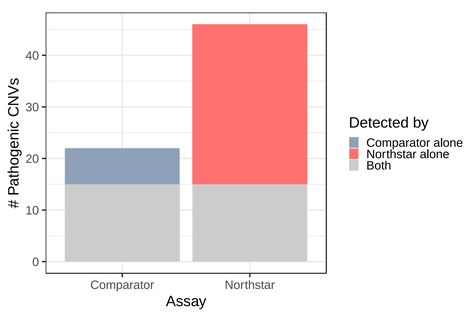

Fig 6. Northstar Select detected ~2x as many pathogenic CNVs relative to comparators. Northstar Select detected 6 copy number amplifications and losses that were missed by comparators, including 2 patients with *CCNE1* amplifications. Additionally, Northstar Select detected CNVs in genes that are not covered by comparators, including 4 patients with *AR* amplifications. Overall, Northstar Select's improved sensitivity and expanded panel coverage for CNVs resulted in detection of 109% more pathogenic CNVs.