#3072 - Clinical validation of Northstar Select, a novel liquid biopsy assay for comprehensive genomic profiling of solid tumors

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BACKGROUND

The field of oncology has increasingly embraced comprehensive genomic profiling (CGP) to enhance treatment decision-making for late-stage solid tumor cancer patients. In particular, *liquid biopsy CGP assays:*

- Detect clinically actionable somatic mutations via circulating tumor DNA.
- Alleviate issues with sample availability, tumor heterogeneity, and turnaround time in tissue-based CGP testing.

However, **current liquid biopsies are generally unreliable** for the detection of variants **below 0.5% variant allele fraction** (VAF)[1], leading to an ***unmet clinical need for a highly sensitive liquid biopsy***:

- Many cancers shed little ctDNA - ½ of SNVs are <0.5%, ¼ <0.25% VAF.[2]
- Patients are similarly likely to respond to targeted therapy regardless of driver mutation VAF level in blood.[2-5]

Northstar Select is a CGP assay that leverages Quantitative Counting Template™ (QCT) technology and innovations in chemistry, panel design, and bioinformatics **for greatly increased sensitivity**. Here, we present:

- The clinical relevance of variants called by Northstar Select and their indication of ctDNA shedding rates, across cancer types.
- Head-to-head comparison of results against on-market liquid biopsies.

METHODS

- ctDNA Shedding & Clinical Actionability
  - 674 unique samples spanned a multitude of cancer types.
  - Blood specimens were assayed by at BillionToOne in Menlo Park, CA.
  - ctDNA shedding level was estimated as the average VAF of pathogenic SNVs and Indels for each patient.
  - Clinically actionable variants include those associated with targeted therapy, clinical trial and/or diagnostic/prognostic relevance.
- Comparison to On-Market Liquid Biopsies
  - Blood specimens were concurrently assayed by Northstar Select and the physician's choice of commercially available comparator CGP liquid biopsy as part of standard of care (n=182 patients).
  - Buffy coat genomic DNA was used to identify variants due to clonal hematopoiesis of indeterminate potential (CHIP) (n=28 patients).
  - All procedures were carried out in accordance with the Declaration of Helsinki and protocols approved by WCG IRB #20230250.

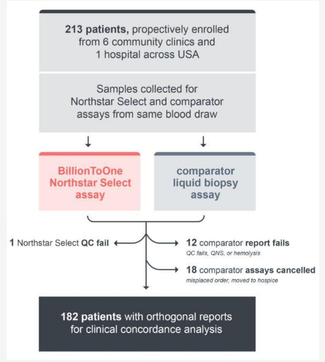

Fig 1. Head-to-head comparison study against on-market assays.

CTDNA SHEDDING & CLINICAL ACTIONABILITY

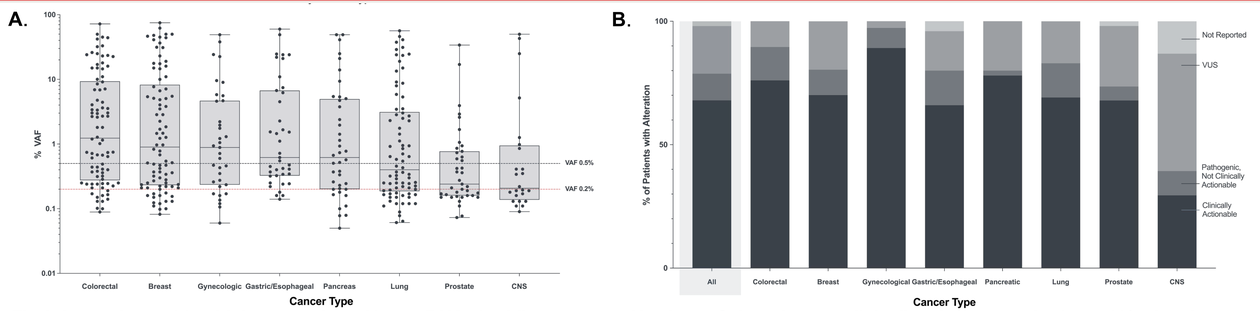

Fig 2. In a cohort with a high proportion of low ctDNA shedding cancers, Northstar Select detects clinically relevant results for ~70% of patients.
A. Median VAF of pathogenic SNVs and Indels for each patient estimates shedding rate. Reference values of 0.2% and 0.5% VAF reveal the number of 'low shedder' cancers.
B. Proportion of patients with at least one reported variant that is clinically actionable, pathogenic but not clinically actionable, or VUS, per cancer type.

HEAD-TO-HEAD COMPARISON TO ON-MARKET LIQUID BIOPSIES

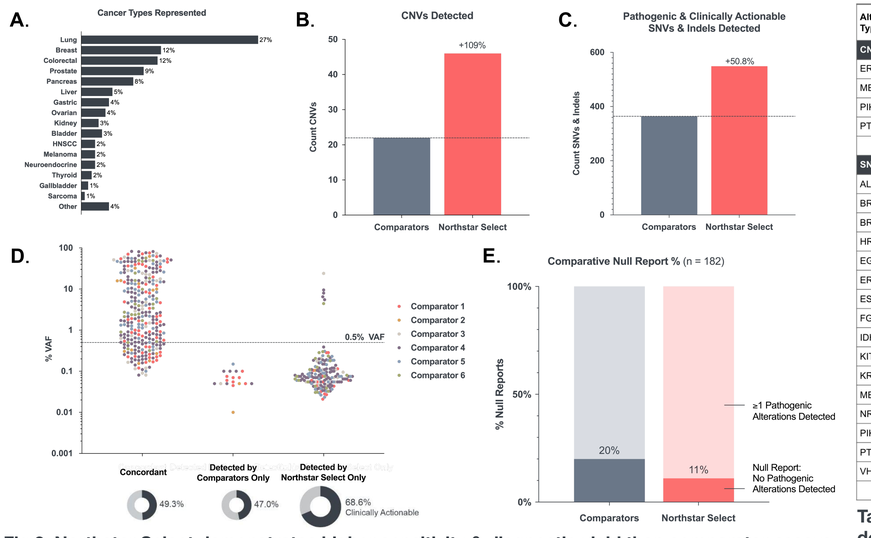

Fig 3. Northstar Select demonstrates higher sensitivity & diagnostic yield than comparator assays.
A. Histogram of cancer types used in the head-to-head analysis versus on-market assays.
B. Total number of CNVs detected for all patients, excluding those with differing variant classification between assays.
C. Total number of pathogenic and clinically actionable SNVs and Indels for all patients, on matched coverage regions.
D. VAF distribution of pathogenic and clinically actionable SNVs and Indels detected by both assays or uniquely by either.
E. Diagnostic yield is the inverse of the fraction of 'null reports', i.e. containing 0 pathogenic or clinically actionable variants. Concordant variants that were called VUS by one of the assays were not counted towards that assay's diagnostic yield.

Alteration Type	# Detected by Northstar Select	# Detected by Comparators	% Detected by Northstar Select Over Comparators
CNV			
ERBB2 (amplification)	4	4	0%
MET (amplification)	1	1	0%
PIK3CA (amplification)	3	1	200%
PTEN (loss)	5	0	+
CNV Sum	13	6	116%
SNV / Indels			
ALK	1	0	+
BRAF	8	6	33%
BRCA1/2	15	11	36%
HRR Genes*	42	18	133%
EGFR	6	4	50%
ERBB2	6	5	20%
ESR1	6	4	50%
FGFR3	3	2	50%
IDH1	3	1	200%
KIT	2	0	+
KRAS	44	38	16%
MET	2	2	0%
NRAS	4	3	33%
PIK3CA	37	27	37%
PTEN	30	18	67%
VHL	2	1	100%
SNV / Indels Sum	211	140	51%

Table 1. Variants with FDA-approved therapies detected by Northstar Select vs. comparators.

Assay	# CHIP	# Somatic	Percent CHIP (CI)
Northstar Select	22	66	25.0% (± 9.0%) †
Comparators	13	40	24.5% (± 13.3%) †

† - NS (p >.05) by Fisher's Exact (odds ratio = 1.02, p > 0.9999)

Table 2. CHIP variant rate comparison.

SUMMARY

Across all analyzed solid tumor types, **a significant proportion of cancers are 'low shedders' of cell-free circulating tumor DNA**, as evidenced by variant allele fractions (VAFs) of <0.5% on liquid biopsy. Even in the lowest shedding cancer types, **Northstar Select is highly sensitive for clinically actionable variants**.

In a head-to-head clinical validation, **Northstar Select demonstrates higher sensitivity than on-market liquid biopsies**, due in large part to the **detection of more variants at <0.5% VAF**, resulting in a **~2x reduced null report rate**.

The majority of additional SNVs, Indels, and CNVs detected by Northstar Select are clinically actionable, with an equivalent CHIP variant detection rate and **improved detection of variants with FDA-approved therapies** across >15 genes.

Therefore, **Northstar Select has unique utility for CGP testing of low shedder cancers**, addressing an unmet clinical need in treatment selection.

Acknowledgements

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