A novel liquid biopsy assay with 88% detection rate of genomic alterations across CNS tumors

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BACKGROUND

Non-invasive detection of challenging tumors like those in the CNS is needed

Genomic assessment of central nervous system (CNS) tumors, including glioblastomas (GBM), is necessary to inform clinical decision making, especially for genes which have prognostic and treatment implications such ad *IDH1/2*.

Tissue biopsy remains the current standard for histological and genomic analyses, however there are limitations:

- **Tumor location** renders sample extraction infeasible, or when available, limited in size
- **High heterogeneity** in tumors such as GBMs makes it difficult to fully capture the diversity of the genomic profile

Plasma-based liquid biopsies have emerged as a complement or alternative to tissue. However, ctDNA from CNS tumors can be difficult to detect due to:

- **Low ctDNA shed rates** inherent to CNS tumor biology
- **Blood brain barrier** impedes ctDNA permeability into peripheral blood

These factors make CNS tumor-derived ctDNA difficult to detect, with current literature suggesting 27 to 55% detection for any alteration, including variants of uncertain significance (VUS):

Study	Patients (n)	ctDNA Detection Rate*
Schwaederle et al. PMID: 26848768	33	27%
Piccioni et al. PMID: 30855176	222	55%
Zill et al. PMID: 29776953	107	51%
Bagley et al. PMID: 31666247	20	55%

Table 1. Studies reporting ctDNA sequenced by next-generation sequencing (NGS) from plasma obtained from GBM patients. *Includes all alterations, including actionable, pathogenic and VUS.

Currently, there are no assays on the market approved for use in CNS tumors because of this poor detection rate. Therefore, there is unmet clinical need for a highly sensitive liquid biopsy assay to genomically profile CNS tumors to overcome the above limitations.

REFERENCES

1. Tsao, D.S., Silas, S., Landry, B.P. et al. A novel high-throughput molecular counting method with single base-pair resolution enables accurate single-gene NIPT. Sci Rep 9, 14382 (2019). https://doi.org/10.1038/s41598-019-50378-8
2. Deveson IW, Gong B, Lai K, et al. Evaluating the analytical validity of circulating tumor DNA sequencing assays for precision oncology. Nat Biotechnol. 2021;39(9):1115-1128. doi:10.1038/s41587-021-00857-z
3. LOD defined as lowest concentration of analyte that can be detected >95% probability.

METHODS

Highly sensitive, plasma-based genomic profiling assay

Northstar Select is a plasma-based, comprehensive genomic profiling test that leverages Quantitative Counting Template™ (QCT) technology[1], optimized chemistry and panel design, as well as bioinformatics innovations to increase sensitivity. From January to December 2023, 62 patients with diagnosed CNS tumors were submitted commercially to BillionToOne for Northstar Select analysis.

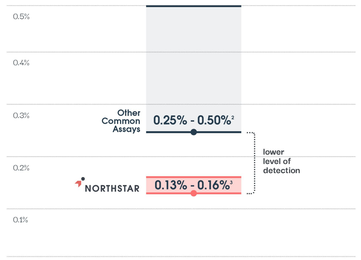


Figure 1: Cited single-nucleotide variant (SNV) limits of detection (LODs) of common assays in the market range from 0.25% to 0.50% VAF[2] ●. Northstar Select LOD ranges from 0.13-0.16% VAF[3] ●.

SNVs / Indels 82 genes

AKT1	*CCNE1*	*EZH2*	*JAK2*	*MYC*
AKT2	*CD274* (PD-L1)	*FANCA*	*JAK3*	*NF1*
ALK	*CDH1*	*FBXW7*	*KIT*	*NOTCH1*
APC	*CDK12*	*FGFR1*	*KRAS*	*NPM1*
AR	*CDK4*	*FGFR2*	*MAP2K1* (MEK1)	*NRAS*
ARAF	*CDK6*	*FGFR3*	*MAP2K2* (MEK2)	*NTRK1*
ARID1A	*CDKN2A*	*FGFR4*	*MET*	*PALB2*
ATM	*CDKN2B*	*GATA3*	*MLH1*	*PDGFRA*
BRAF	*CHEK2*	*GNA11*	*MPL*	*PIK3CA*
BRCA1	*CTNNB1*	*GNAQ*	*MSH2*	*PMS2*
BRCA2	*DDR2*	*GNAS*	*MSH6*	*PTEN*
BRIP1	*EGFR*	*HRAS*	*MTOR*	*PTPN1*
CCND1	*ERBB2* (HER2)	*IDH1*	*PIK3CA*	*RAD51C*
CCND2	*ESR1*	*IDH2*	*RET*	*RAD51D*

CNAs: Amplifications 19 genes

AR	*ERBB2*	*MET*
BRAF	*ESR1*	*MYC*
CCNE1	*FGFR1*	*PDGFRA*
CD274 (PD-L1)	*FGFR2*	*PIK3CA*
CDK4	*KIT*	*RAF1*
CDK6	*KRAS*	*RET*
EGFR		

Fusions 9 genes

ALK	*NTRK1*
BRAF	*NTRK2*
FGFR2	*NTRK3*
FGFR3	*RET*
	ROS1

CNAs: Losses 5 genes

ATM	*CDKN2A*
BRCA1	*PTEN*
BRCA2	

Biomarker

MSI

Table 2. Northstar Select gene panel

DATA

Increased sensitivity results in high detection rate of clinically actionable alterations in CNS tumors

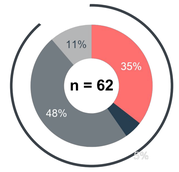
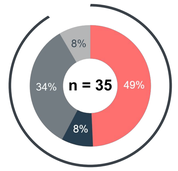
	All CNS Cases	GBM Cases Only
Total Alteration Detection Rate	**88% of Patients**	**92% of Patients**
Clinically actionable pathogenic alteration Pathogenic, not clinically actionable alteration VUS only No alterations		
Number of Actionable Alterations Identified (therapy, trial, or resistance marker)	**43**	**33**
Median VAF (25th - 75th percentile)	**0.17%** (0.10 - 2.02%)	**0.17%** (0.11 - 1.50%)

Figure 2: Summary of alterations detected by Northstar Select in CNS tumors

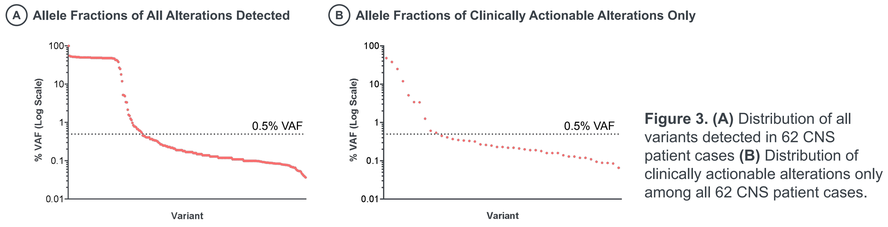
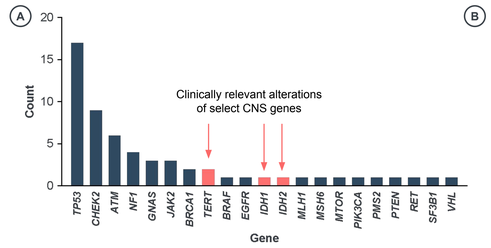


Figure 3. (A) Distribution of all variants detected in 62 CNS patient cases **(B)** Distribution of clinically actionable alterations only among all 62 CNS patient cases.

CLINICAL CASE STUDIES

Clinically informative alterations in select genes



B

Patient Age, Sex, Diagnosis	Gene cdot,pdot	Allele Fraction
66, male, GBM	***TERT*** c.-54C>A, p.?	**0.21%**
53, female, oligodendroglioma	***IDH1*** c.395G>A, p.R132H	**0.088%**
47, male, GBM	***IDH2*** c.419G>A, p.R140Q	**1.26%**

Figure 4. (A) Pathogenic / likely pathogenic alterations detected by Northstar Select **(B)** Patient-level details on clinically relevant alterations of 3 select genes: *TERT* and *IDH1/2*.

RESULTS

88% detection rate in CNS tumors, 91% in GBMs only

In this study, Northstar Select's detection rate of any alteration was 88% in patients with CNS tumors and 92% among the GBM patients.

When limiting analysis to clinically actionable alterations (i.e. there is a drug, treatment implication, or clinical trial available), 36% of cases had at least one alteration detected, and in GBMs, this number increased to 49% of cases.

The majority of the alterations found were below 0.5% VAF

The assay's uniquely low LOD of 0.13% to 0.16% VAF, which is below the 0.17% median VAF demonstrated across CNS tumors in this study, enabled a high detection rate among patients with CNS tumors.

CONCLUSIONS

Higher detection rates uncover more low-abundance alterations

Current literature indicates an unmet clinical need for a highly sensitive liquid biopsy assay to determine the genomic landscape of CNS tumors and to aid clinical decision making.

Northstar Select has a detection rate of 88.7% in all cases and 91.4% in GBMs, nearly doubling current rates.

Nearly 50% of detected alterations in GBMs were actionable, supporting the clinical utility of Northstar Select to inform clinical decisions either as a complement to imaging and tissue analyses, or independently when tissue biopsies are infeasible.

These data further suggest that the clinical utility of Northstar Select may be expanded into metastatic brain disease and other low ctDNA shedding tumors.