

Therapy Response Monitoring FOR SOLID TUMORS

A Tumor-Naive Therapy Response Monitoring Assay

Northstar Response™ is a next generation sequencing (NGS)-based test designed to detect hundreds of genomic loci uniquely methylated in cancer cells. This new test is a tumor-naive assay. Using only blood draws from a patient with advanced-stage cancer, Northstar Response measures the change in methylated tumor molecules, reflecting tumor fraction changes, over time. Across different cancer types and therapy modalities, Northstar Response has demonstrated promising potential as a therapy monitoring tool^{1,11}.

>10x Stronger Signal than SNV-Based ctDNA Monitoring Assays

Northstar Response looks at tumor-derived cell-free DNA (cfDNA) with epigenetic alterations. Current literature suggests that changes in methylated circulating tumor DNA (ctDNA) are a predictive and prognostic biomarker for cancer treatment response and progression, as a surrogate for tumor burden²⁻⁹. Distinct from individual, more sparse mutations that are the basis for single nucleotide variant (SNV)-based ctDNA monitoring, methylated ctDNA is a global and additive marker that allows for a more robust and cumulative measurement of ctDNA. Typical SNV-based ctDNA monitoring assays such as tumor-informed MRD assays look at on average 9 SNVs, while Northstar Response quantifies on average 90 methylated loci, which is 10-fold increase in signal¹⁰. Serial quantification of this methylated signal may provide an early indication of response or progression of the cancer to systemic therapy, complementing information provided from scans or tissue biopsy.

Powered by Single-Molecule Level Quantification

BillonToOne pioneered single-molecule precision counting technology using Quantitative Counting Templates™ (QCT™). Using QCT, the Northstar Response assay can count the number of methylated molecules, reflecting the tumor fraction in the sample. The Tumor Methylation Score™ is provided on each report and represents the normalized sum of methylation at >500 loci that are hypermethylated in ctDNA. Through serial collections, the Tumor Methylation Score can accurately distinguish changes in tumor fraction over time.

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- Bratman SV, Yang SVC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nat Cancer*. 2020 Sep;1(9):873-881. doi: 10.1038/s43018-020-0096-5. Epub 2020 Aug 3. PMID: 35121950.
- Nassar FJ, Mshelik ZS, Nasr RR, Temraz SN. Methylated circulating tumor DNA as a biomarker for colorectal cancer diagnosis, prognosis, and prediction. *Clin Epigenetics*. 2021 May 17;13(1):111. doi: 10.1186/s13148-021-01095-5. PMID: 34001239.
- de Vos L, Jung M, Koerber RM, et al. Treatment Response Monitoring in Patients with Advanced Malignancies Using Cell-Free SHOX2 and SEPT9 DNA Methylation in Blood: An Observational Prospective Study. *J Mol Diagn*. 2020 Jul;22(7):920-933. doi: 10.1016/j.jmoldx.2020.04.205. Epub 2020 Apr 30. PMID: 32361006.
- Jin S, Zhu D, Shao F, et al. Efficient detection and post-surgical monitoring of colon cancer with a multi-marker DNA methylation liquid biopsy. *Proc Natl Acad Sci U S A*. 2021 Feb 2;118(5):e2017421118. doi: 10.1073/pnas.2017421118. PMID: 33495330.
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- Lianidou E. Detection and relevance of epigenetic markers on ctDNA: recent advances and future outlook. *Mol Oncol*. 2021 Jun;15(6):1683-1700. doi: 10.1002/1878-0261.12978. Epub 2021 May 14. PMID: 33942482.
- Reinert T, Henriksen TV, Christensen E, et al. Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer. *JAMA Oncol*. 2019;5(8):1124-1131. doi:10.1001/jamaoncol.2019.0528
- Internal data on file, Dec 2022.

- Distinguishes changes as small as 0.2% tumor fraction

In analytical validation, the assay precisely distinguished a 0.2 percentage point changes of tumor fraction^{1,11}.

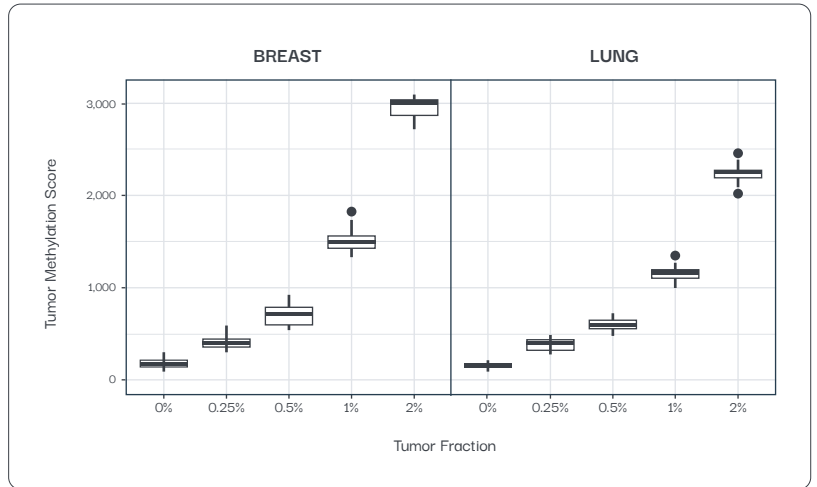


Figure 1. Median and interquartile range of Tumor Methylation Scores at each tumor fraction. Sheared gDNA from tumor cells were added to sheared gDNA from healthy cells from the same subject at different tumor fraction.

- Achieves consistent accuracy across 10 cancer types

Northstar Response was able to report accurate changes in tumor fraction in >98% of contrived tumor samples spanning 10 cancer types¹¹.

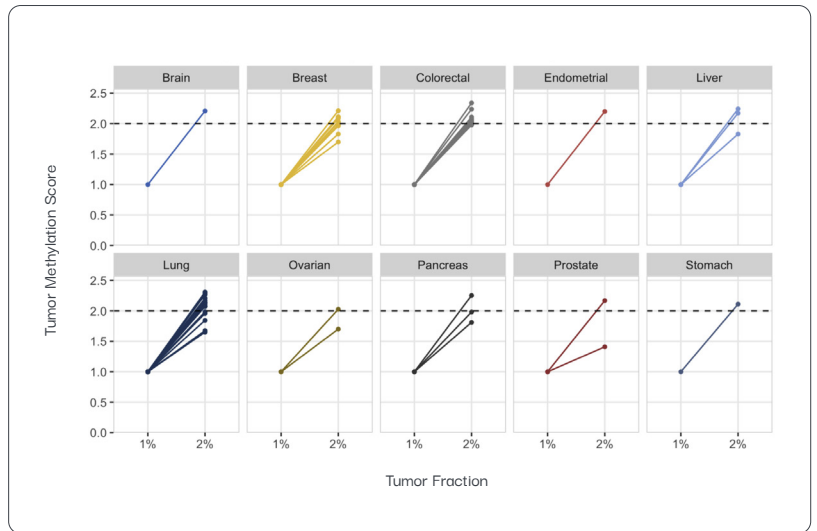


Figure 2. Tumor Methylation Scores for 1% and 2% tumor DNA samples. Sheared tumor DNA samples were created by mixing sheared gDNA extracted from tumor with sheared gDNA from matched buffy coat.

BILLIONTO ONE is a precision diagnostics company with the mission to make molecular diagnostics more accurate, efficient, and accessible for all. Powered by the QCT™ platform, BillionToOne has a series of unique cfDNA based assays including the first and only commercial single-gene non-invasive prenatal test for recessive conditions.

BillionToOne continues to build clinical evidence for Northstar Response.

If you are interested in collaboration opportunities, please email medical@northstaronc.com.

CASE EXAMPLES

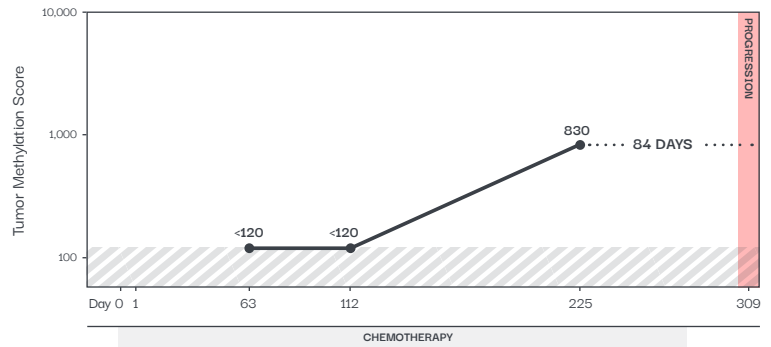
Northstar Response has demonstrated utility across different cancer types in detecting changes in ctDNA that are concordant with patient outcomes. Below is a selection of individual clinical case examples, not representative of all patient outcomes¹¹.

CASE 01 Detected ctDNA increase 84 days before the scan image detected progression

Stage IV Colorectal Adenocarcinoma

Chemotherapy + Immunotherapy

- fluorouracil / leucovorin / bevacizumab

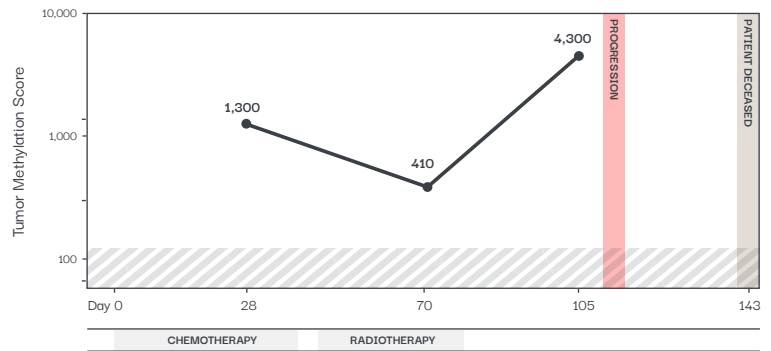


CASE 02 Detected ctDNA increase with patients undergoing radiotherapy

Late Stage Lung Small Cell Neuroendocrine Carcinoma

Chemotherapy + Radiotherapy

- cisplatin / etoposide / neulasta

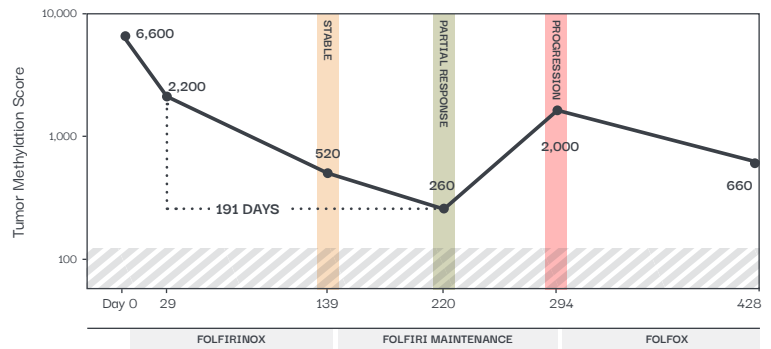


CASE 03 Detected partial response 191 days earlier and also detected progression afterward

Stage IV Pancreatic Ductal Adenocarcinoma

Chemotherapy

- folfirinox
- followed by folfiri maintenance
- followed by folfox



CASE 04 Detected partial response 58 days before the scan

Stage IV Non-Small Cell Lung Cancer

Chemotherapy + TKI

