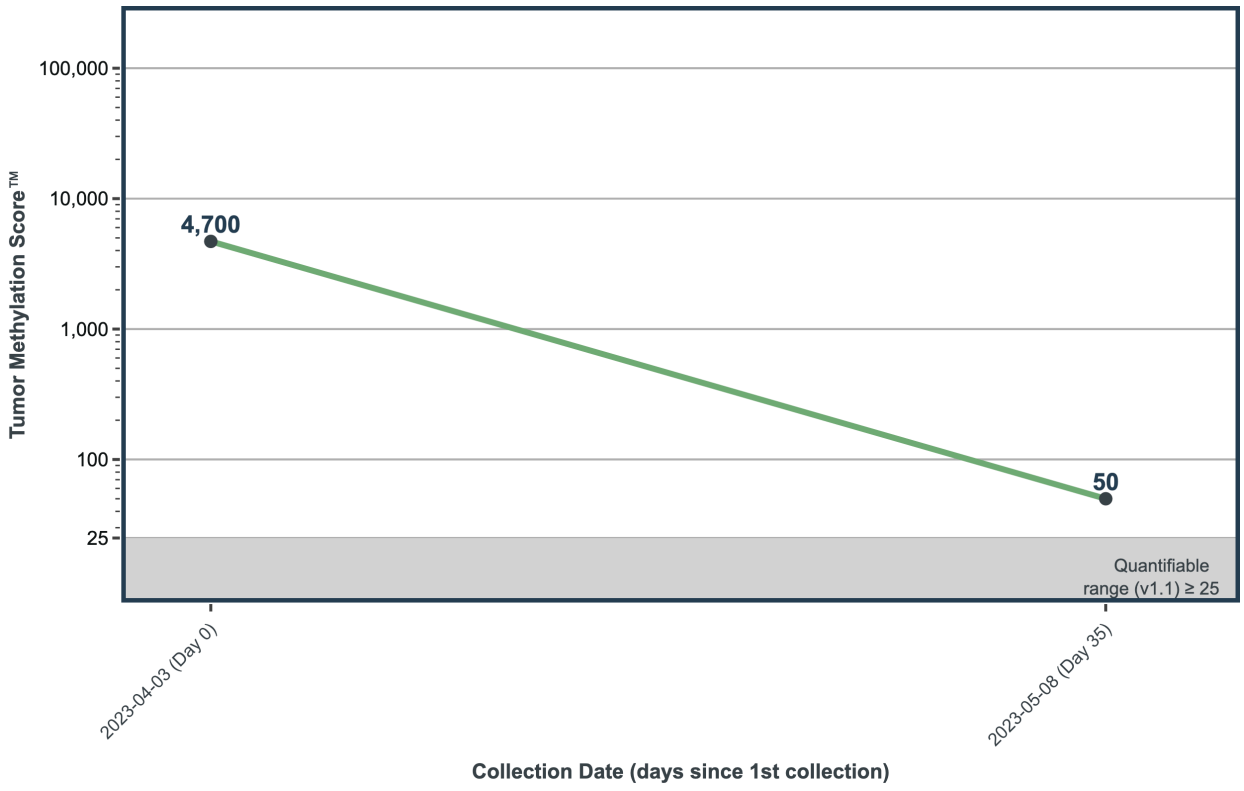
Patient		Sample		Physician	
Name	**Patient Doe**	Specimen Type	**Blood**	Ordering Physician	**Dr. Jane Smith**
Date of Birth (Age)	**09/02/1947 (75 yrs)**	Collection Date	**01/01/2023**	Medical Facility	**UC San Diego Health**
Assigned Sex at Birth	**Female**	Receipt Date	**01/02/2023**	Address	**3855 Health Sciences Drive La Jolla, CA 92037**
Diagnosis	**Non-Small Cell Lung Carcinoma**	Accession ID	**VEE0121700003**	Phone	**(858) 822-6100**
Medical Record #	**MRN12345**	Report Date	**02/12/2024**	Fax	**(858) 822-6100**
Internal Patient ID	**ID12345**	Test Number	**2**		

Northstar Response Results

DECREASE in Tumor Methylation Score™ was detected based on a decrease in methylated ctDNA molecules.

94-fold Fold-change in Tumor Methylation Score™ detected since the previous collection date on 10/03/2022.

Tumor Methylation Score™ Represents the normalized sum of methylated molecules at >500 loci that are hypermethylated in circulating tumor DNA (ctDNA).



Interpretation

A decrease in methylated, circulating tumor DNA (ctDNA) molecules was detected compared to the previous measurement. This result suggests that tumor fraction has decreased compared to the previous measurement. Compared to normal methylation patterns in the DNA of healthy cells, there are distinct and differential methylation patterns associated with cancer cells [1], and changes in quantity of methylated ctDNA corresponds to a change in the patient's tumor fraction [3].

Patient Name	**Patient Doe**	Date of Birth (Age)	**09/02/1947 (75 yrs)**	Assigned Sex at Birth	**Female**
Diagnosis	**Non-Small Cell Lung Carcinoma**	Internal Patient ID	**ID12345**	Report Date	**02/12/2024**

Methods and Limitations

Northstar Response™ is a next generation sequencing (NGS)-based test designed to measure the change in methylated tumor molecules in a cancer patient from a blood draw. In particular, Northstar Response™ quantifies the methylated ctDNA (circulating-tumor DNA) molecules isolated from cell-free DNA (cfDNA) at loci known to be hypermethylated in tumors compared to healthy tissue.

Plasma and buffy coat were isolated from whole blood collected in a Streck cell-free DNA tube. Cell-free DNA (cfDNA) was extracted from the plasma, and genomic DNA (gDNA) was extracted from the buffy coat. The number of methylated molecules was quantified in both cfDNA and gDNA using BillionToOne's QCT molecular counting technology [2] at >500 locations in the genome known to be hypermethylated in cancer compared to non-cancerous tissue and blood. Methylation measured in gDNA is subtracted from cfDNA methylation in order to remove background from the ctDNA signal. The remaining cfDNA methylated molecules are summed across all hypermethylation locations to calculate the Tumor Methylation Score™ [3].

The Tumor Methylation Score™ from the current collection was compared to the most recently reported Tumor Methylation Score™ to determine an increase, decrease, or no change call. The change in Tumor Methylation Score™ must exceed an analytical, statistical significance threshold in order to be reported as an increase or a decrease. No interpretive calls for change in Tumor Methylation Score™ are made for baseline tests without any prior collections. Results should be discussed with a medical professional and interpreted in conjunction with the patient's complete clinical history within the context of multiple timepoints. The Tumor Methylation Score™ is highly dependent on tumor shedding patterns, which are influenced by cell-free DNA (cfDNA) composition in the blood, ctDNA shedding variability (due to many factors, such as individual biology and/or tumor location) or low circulating tumor molecules in the bloodstream.

Methylation may not be reported when the sample contains an insufficient amount of cfDNA. Results detected below the Tumor Methylation Score™ quantifiable range, depicted on the graph by gray shading, are not interpreted and will be reported as detected below the quantifiable range. Performance specifications demonstrated that this assay can distinguish a 0.25% absolute change in tumor fraction with 3 standard deviations of separation [3]. Northstar Response™ was designed for quantifying Tumor Methylation Score™ in patients with solid tumors; results for liquid tumors such as leukemias are not valid. Results may vary or be invalid if the patient has undergone recent blood transfusion, stem cell transplant, or other procedures that may significantly affect the composition of cfDNA or buffy coat gDNA.

References

1. Das PM, Singal R. DNA methylation and cancer *J Clin Oncol.* 2004;22(22):4632-42. PMID:15542813.
2. Tsao DS, Silas S, Landry BP, Itzep NP, Nguyen AB, Greenberg S, Kanne CK, Sheehan VA, Sharma R, Shukla R, Arora PN, Atay O. A novel high-throughput molecular counting method with single base-pair resolution enables accurate single-gene NIPT *Sci Rep.* 2019;9(1):14382. PMID:31591409.
3. Ye PP, Viens RA, Shelburne KE, Langpap SS, Bower XS, Zhou W, Wignall JC, Zhu JJ, Woodward BD, Husain H, Tsao DS, Atay O. Molecular counting enables accurate and precise quantification of methylated ctDNA for tumor-naive cancer therapy response monitoring. *medRxiv.* doi:10.1101/2023.05.31.23290555.

This NGS-based assay was developed and its performance characteristics determined by BillionToOne, Inc. It has not been cleared or approved by the U.S. Food and Drug Administration. BillionToOne, Inc. is regulated under CLIA. This test is used for clinical purposes. It should not be regarded as investigational or for research. This test was performed using BillionToOne's patented technology (www.billiontoone.com/patents).

BillionToOne, Inc.
1035 O'Brien Drive
Menlo Park, CA 94025

Laboratory Director	**Joseph Michael Anderson, MD**	Phone	**(833) 537-1819**
Lab ID	**CLF-90008579**	Fax	**(833) 874-0918**
CLIA ID	**05D2275351**	Email	**support@northstaronc.com**

Joseph Michael Anderson, MD
Laboratory Director
California License: A 98379