

Patient		Sample		Physician	
Name	Jane Jones	Specimen Type	Blood	Ordering Physician	John Smith
Date of Birth	11/27/1940	Collection Date	01/13/2023	Medical Facility	BillionToOne Inc
Age	82 yrs	Receipt Date	01/15/2023	Address	1035 O'Brien Drive Suite 112 Menlo Park, California 94025
Assigned Sex at Birth	Female	Accession ID	V010900AA001-1	Phone	(833) 537-1819
Diagnosis	Pancreatic Ductal Adenocarcinoma	Test Kit ID	V010900AA001-1	Fax	(833) 874-0918
Medical Record #	ID400231	Report Date	02/01/2023		
Internal Patient ID	10000090	Test Number	6		

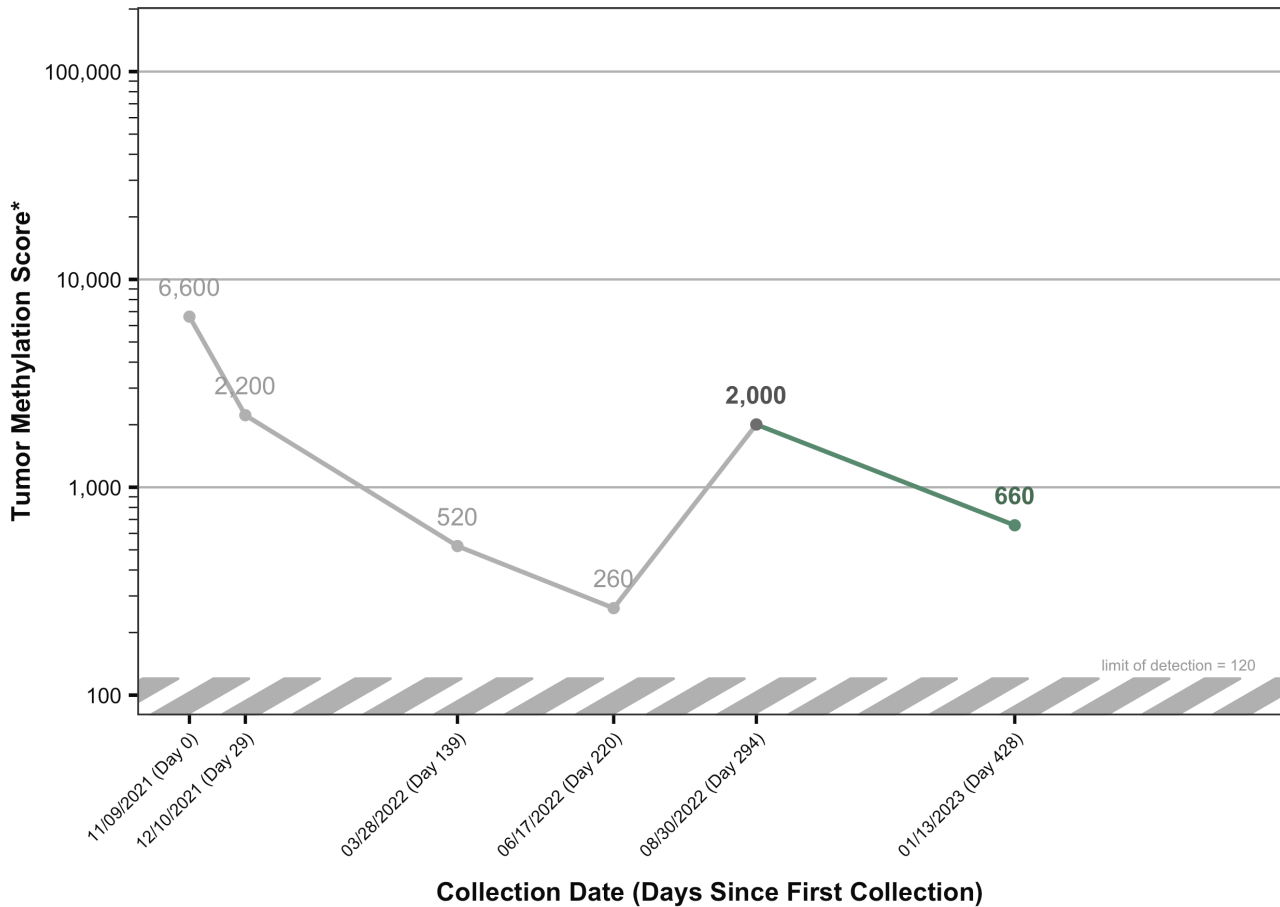
Northstar Response Results



Decrease in Tumor Methylation Score™ was detected. This corresponds to a decrease in methylated ctDNA molecules.



Fold-change in Tumor Methylation Score™ detected since the previous collection date on 11/30/2022.



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

Patient Name	Jane Jones	Date of Birth	11/27/1940	Assigned Sex at Birth	Female
Diagnosis	Pancreatic Ductal Adenocarcinoma	Internal Patient ID	1000090	Report Date	02/01/2023

Interpretation

A decrease in methylated molecules in the circulating tumor DNA (ctDNA) was detected compared to the initial measurement. Aberrantly methylated DNA is a known marker of cancer cells (PMID 15542813), and a change in methylated ctDNA corresponds to a change in tumor fraction (BillionToOne internal data). This result suggests that tumor fraction has decreased compared to the previous measurement.

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 (PMID: 31591409) 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™.

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 a 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.

Methylation may not be reported when the sample contains an insufficient amount of DNA. Results below the Tumor Methylation Score™ limit of detection (LOD), depicted on the graph by cross-hatched shading, are not interpreted and will be reported as less than the LOD. Performance specifications based on internal validation studies demonstrated that this assay can distinguish a 0.2 percentage point change in tumor fraction with 3 standard deviations of separation. 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.

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.

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