Patient

Name	**Jane Jones**
Date of Birth (Age)	**11/27/1940 (84 yrs)**
Assigned Sex at Birth	**Female**
Diagnosis	**Non-Small Cell Lung Carcinoma**
Medical Record #	**ID400231**
Internal Patient ID	**10000090**

Sample

Specimen Type	**Blood**
Collection Date	**10/16/2025**
Receipt Date	**10/18/2025**
Accession ID	**V010900AA001-1**
Report Date	**10/23/2025**
Test Number	**8**

Physician

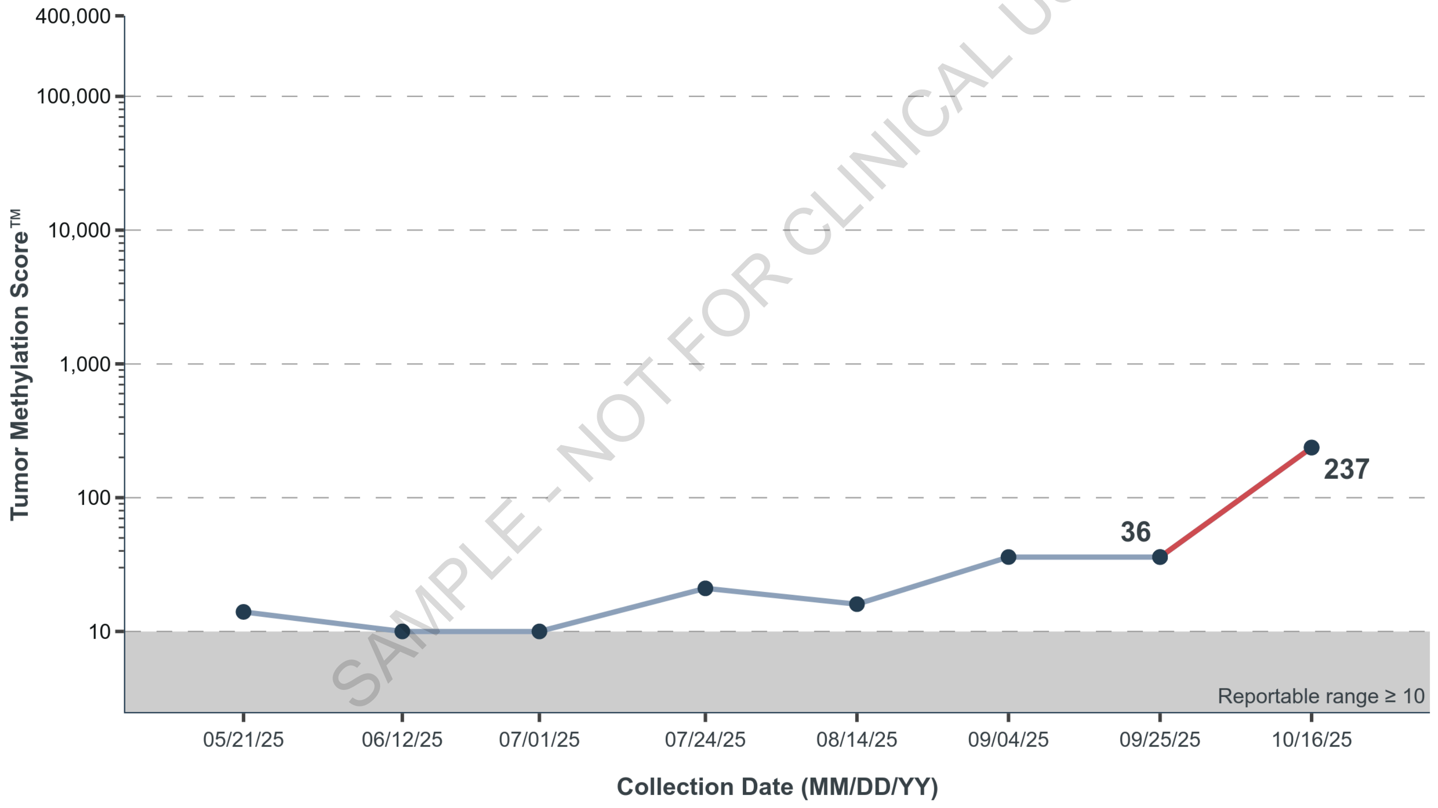
Ordering Physician	**John Smith**
Medical Facility	**BillionToOne Inc**
Address	**1035 O'Brien Drive Menlo Park, California 94025**
Phone	**(833) 537-1819**
Fax	**(833) 874-0918**

Northstar Response Results

↗ **INCREASE** in Tumor Methylation Score™ was detected based on an increase in methylated ctDNA molecules.

6.6-fold ↗ Fold-change in Tumor Methylation Score™ detected since the previous collection date on 09/25/2025.

Tumor Methylation Score™ Represents the normalized sum of methylated molecules at >2000 loci that are hypermethylated in circulating tumor DNA (ctDNA).



Interpretation

An increase in methylated molecules in the circulating tumor DNA (ctDNA) was detected compared to the previous measurement. This result suggests that tumor fraction has increased compared to the previous measurement. Differential methylation from normal epigenetic DNA patterns are known markers of cancer cells [1], and changes in methylated ctDNA corresponds to a change in tumor fraction [2].

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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. Methylated molecules were quantified in both cfDNA and gDNA using BillionToOne's QCT™ molecular counting technology [3] at >2000 locations in the genome known to be hypermethylated in cancer compared to non-cancerous tissue and blood. Methylation detected in the gDNA was used to adjust the cfDNA methylation signal, accounting for background from non-tumor DNA. The remaining cfDNA methylated molecules are aggregated and normalized across hypermethylation locations to calculate the Tumor Methylation Score™ [2].

The Tumor Methylation Score (TMS) from the current collection was compared to the most recently reported TMS to determine an increase, decrease, or no change call. The change in TMS must exceed an analytical, statistical significance threshold in order to be reported as an increase or a decrease. No interpretive calls for change in TMS are made for baseline tests without any prior collections. TMS may not be reported when the sample contains an insufficient amount of cfDNA. 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.

Results below the TMS reportable range, depicted on the graph by shading, are not interpreted, and will be reported as less than the reportable range. Results below the reportable range do not provide quantitative information regarding the amount of ctDNA; the assay is not intended for minimal residual disease or early cancer detection use. Northstar Response was designed for quantifying TMS in patients with solid tumors; results for liquid tumors such as leukemias are not valid. Results may vary or be invalid if the patient is pregnant, 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. Ye PP, Viens RA, Shelburne KE, Langpap SS, Bower XS, Shi JJ, 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. *Sci Rep.* 2025;15:5869. PMID:39966612.
3. 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.

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Appendix: Northstar Response Historical Results

Collection Date	Days since baseline	TMS	Fold-change
10/16/2025	148	**237**	**6.6 fold increase**
09/25/2025	127	**36**	No significant change
09/04/2025	106	**36**	No significant change
08/14/2025	85	**16**	No significant change
07/24/2025	64	**21**	No significant change
07/01/2025	41	**<10**	Below reportable range
06/12/2025	22	**<10**	No significant change
05/21/2025	0	**14**	Baseline measure

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).

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