T 833.537.1819 **F** 833.874.0918 **E** support@northstaronc.com **northstaronc.com**

Patient		Sample		Physician	
Name	**Jane Jones**	Specimen Type	**Blood**	Ordering Physician	**John Smith**
Date of Birth (Age)	**11/27/1940 (83 yrs)**	Collection Date	**09/18/2024**	Medical Facility	**BillionToOne Inc**
Assigned Sex at Birth	**Female**	Receipt Date	**09/20/2024**	Address	**1035 O'Brien Drive Menlo Park, California 94025**
Diagnosis	**Non-Small Cell Lung Carcinoma**	Accession ID	**V010900AA001-1**	Phone	**(833) 537-1819**
Medical Record #	**ID400231**	Report Date	**02/28/2025**	Fax	**(833) 874-0918**
Internal Patient ID	**10000090**	Test Number	**2**		

Northstar Select Results

2 **informative genomic alterations identified**

Summary of Informative Genomic Alterations

Detected Genomic Findings§	Associated FDA-Approved and/or Guideline Recommended Therapies			Clinical Trials§	VAF / Copy number
	✓ **Approved** in indication	✓ **Approved** in other indication	✗ Associated with **resistance**		
EGFR L747_P753delinsS	• **afatinib** • **afatinib/cetuximab**G • **amivantamab**G • **bevacizumab/erlotinib**G • **dacomitinib** • **erlotinib** • **erlotinib/ramucirumab** • **gefitinib** • **osimertinib**			**10**	0.15%
TP53 F338Sfs*4				**0**	0.17%

G Treatment listed is based upon recommendation from professional guidelines only. Please consult professional guidelines and FDA indications for complete details.
§ For additional information, please see the **Variant Details** and **Clinical Trials** section of the report.

Microsatellite Instability-High

Microsatellite Instability-High (MSI-H) is reported here as detected/not detected based upon mutation analysis at curated genomewide MSI sites and subsequent bioinformatic algorithm calculation.

Not Detected Detected

Summary of Guideline-Recommended Genes Evaluated

The following guideline-recommended genes for Non-Small Cell Lung Carcinoma were evaluated. Variants not listed in the tables above are considered 'Not Detected'. For a complete list of genes tested, refer to the Methods section of the report.

ALK *BRAF* *EGFR* *ERBB2 (HER2)* *KRAS* *MET* *NTRK* *RET* *ROS1*

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Genes with copy number signal indicating potential for aneuploidy

Changes in copy number due to biological factors including aneuploidy can confound the accurate detection of clinically informative copy number alterations (CNAs). The following genes have deviations in copy number signal but are not called for copy number amplification/loss due to observed patterns of copy number signal (i.e. aneuploidy) within the chromosomal arm and/or other chromosomal arms of the sample.

Copy number signal indicating potential aneuploidy was not detected in any gene.

Variants of Unknown Significance (VUS)

The clinical relevance of these variants is currently unknown. Therefore, the functional impact of targeting these variants cannot be determined at this time.

No Variants of Unknown Significance detected.

Clinical Trial Availability

Clinical trial matches are displayed based upon somatic variant detection and patient demographic and diagnostic information provided on the test requisition form. Trials are matched within 500 miles of the ordering provider's location. This list is neither comprehensive nor a guarantee of eligibility, as many other requirements must be met prior to enrollment.

For further details on eligibility, please visit **www.clinicaltrials.gov** and enter the NCT#.

Phase 3 NCT06396065	A Randomized, Double-blind, Multi-center, Phase III Study of AK112 or Placebo Combined With Pemetrexed and Carboplatin in Patients With EGFR-mutant Locally Advanced or Metastatic Non-squamous NSCLC Who Have Failed to EGFR-TKI Treatment **EGFR L747_P753delinsS**	**Bethesda, Maryland (20817)** American Oncology Partners
Phase 3 NCT04181060	Randomized Phase III Study of Combination Osimertinib (AZD9291) and Bevacizumab Versus Osimertinib (AZD9291) Alone as First-Line Treatment for Patients With Metastatic EGFR-Mutant Non-Small Cell Lung Cancer (NSCLC) **EGFR L747_P753delinsS**	**Hanover, Pennsylvania (17331)** WellSpan Medical Oncology and Hematology
Phase 2 NCT05498428	A Phase 2, Open-Label, Parallel Cohort Study of Subcutaneous Amivantamab in Multiple Regimens in Patients With Advanced or Metastatic Solid Tumors Including EGFR-mutated Non-Small Cell Lung Cancer **EGFR L747_P753delinsS**	**District of Columbia, District of Columbia (20016)** Johns Hopkins Office of Capital Region Research - Sibley Memorial Hospital
Phase 2 NCT04410796	A Phase 2 Randomized Study of Osimertinib Versus Osimertinib Plus Chemotherapy for Patients With Metastatic EGFR-Mutant Lung Cancers That Have Detectable EGFR-Mutant cfDNA in Plasma After Initiation of Osimertinib **EGFR L747_P753delinsS**	**Basking Ridge, New Jersey (07920)** Memorial Sloan Kettering Basking Ridge (Limited Protocol Activities)
Phase 2 NCT03786692	TH-138: Phase II Randomized Trial of Carboplatin + Pemetrexed + Bevacizumab, With or Without Atezolizumab in Stage IV Non-squamous NSCLC Patients Who Harbor a Sensitizing EGFR Mutation or Have Never Smoked **EGFR L747_P753delinsS**	**Philadelphia, Pennsylvania (19111)** Fox Chase Cancer Center
Phase 2 NCT03586453	A Phase II Study of Osimertinib With On-study and Post-progression Biopsy in the First Line Treatment of EGFR Inhibitor naïve Advanced EGFR Mutant Lung Cancer **EGFR L747_P753delinsS**	**Boston, Massachusetts (02215)** Beth Israel Deaconess Medical Center
Phase 1/Phase 2 NCT05908734	A Phase 1/2 Study Evaluating the Safety and Efficacy of Amivantamab and Cetrelimab Combination Therapy in Metastatic Non-small Cell Lung Cancer **EGFR L747_P753delinsS**	**Fairfax, Virginia (22031)** Virginia Cancer Specialists

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Phase 1/Phase 2 NCT05519293	A Phase I/IIa, Open-label, Dose-escalation and Expansion Study to Evaluate the Safety, Tolerability, Pharmacokinetic and Preliminary Anti-tumor Activity of H002 in Patients With Active EGFR Mutation Locally Advanced or Metastatic NSCLC **EGFR L747_P753delinsS**	**Fairfax, Virginia (22031)** NEXT Virginia
Phase 1/Phase 2 NCT05109442	A Phase 1/2a Open Label, Multicenter Study to Assess the Safety, Tolerability, Pharmacokinetics, and Efficacy of AFM24 in Combination With Atezolizumab in Patients With Selected Advanced/ Metastatic EGFR-expressing Cancers **EGFR L747_P753delinsS**	**Baltimore, Maryland (21287)** Johns Hopkins University
Phase 1 NCT04077463	An Open-label Phase 1/1b Study to Evaluate the Safety and Pharmacokinetics of JNJ-73841937 (Lazertinib), a Third Generation EGFR-TKI, as Monotherapy or in Combinations With JNJ-61186372, a Human Bispecific EGFR and cMet Antibody in Participants With Advanced Non-Small Cell Lung Cancer **EGFR L747_P753delinsS**	**Fairfax, Virginia (22031)** Virginia Cancer Specialists

Variant Details

EGFR

EGFR activating mutations or amplification may predict sensitivity to Egfr-targeted therapies, including inhibitors of multiple ErbB family members, and several have received agency approval in some tumor types [1, 2, 3]. The Egfr TKIs erlotinib, afatinib, gefitinib, osimertinib, and dacomitinib, as well as the combination of amivantamab plus lazertinib, have been approved by the FDA for the treatment of non-small cell lung cancer (NSCLC) with exon 19 deletion or L858R EGFR mutations; osimertinib has additionally been approved for the treatment of NSCLC with EGFR T790M [4, 5, 6, 7, 8, 9, 10, 1, 11]. Afatinib has additionally been FDA-approved for the treatment of NSCLC with S768I, L861Q, and/or G719X mutations [12]. The combination of erlotinib and ramucirumab as well as osimertinib plus platinum-based chemotherapy have been FDA-approved for the treatment of metastatic NSCLC patients with tumors harboring an EGFR exon 19 deletion or the exon 21 L858R mutation [13, 14]. Amivantamab in combination with carboplatin and pemetrexed has been FDA-approved for the treatment of adult patients with locally advanced or metastatic NSCLC harboring EGFR Exon 19 deletions or Exon 21 L858R substitution mutations whose disease has progressed on or after treatment with an EGFR TKI [11]. Amivantamab has also been approved by the FDA for NSCLC patients with EGFR exon 20 insertions, whose disease has progressed on or after platinum-based chemotherapy and as frontline therapy in combination with carboplatin and pemetrexed. The accelerated FDA approval of mobocertinib for NSCLC patients with EGFR exon 20 insertions has been withdrawn due to lack of progression-free survival benefit in the confirmatory Phase 3 trial [15]. Although one study has reported the emergence of EGFR L747_P753delinsS in a non-small cell lung cancer patient with disease progression after osimertinib treatment, a separate study has reported response to osimertinib in 4/7 patients with EGFR L747_P753delinsS who acquired EGFR T790M mutation [16, 17].

EGFR L747_P753delinsS

Gene	**EGFR**
Nucleotide	**NM_005228.5: c.2240_2257del**
Amino Acid	**p.L747_P753delinsS**
Exon	**19**
Biomarker Type	**activating**

EGFR L747_P753delinsS is an in-frame deletion in exon 19 that occurs in the tyrosine kinase domain of the Egfr protein (UniProt, IGV). This alteration and other exon 19 deletions have been shown to activate Egfr tyrosine kinase activity, result in increased cell growth as compared with wild-type Egfr, and confer sensitivity to Egfr tyrosine kinase inhibitors such as erlotinib, afatinib, dacomitinib, and gefitinib [18, 19, 20, 21, 22, 23]. EGFR L747_P753delinsS has been reported as an uncommon exon 19 indel alteration in NSCLC patients and has been observed to exhibit median progression-free survival of 15-20 months following first-line Egfr TKI therapy, longer than patients with common EGFR exon 19 deletions [17, 24]. Although one study has reported the emergence of EGFR L747_P753delinsS in a non-small cell lung cancer patient with disease progression after osimertinib treatment, a separate study has reported response to osimertinib in 4/7 patients with EGFR L747_P753delinsS who acquired EGFR T790M mutation [16, 17]. A study has reported reduced sensitivity to osimertinib as compared with erlotinib and gefitinib in NSCLC preclinical models with EGFR L747_P753delinsS, however, tumor growth was still reduced significantly with osimertinib. In a cohort of NSCLC patients with uncommon EGFR exon 19 delins mutations, including EGFR L747_P753delinsS, reduced progression-free and overall survival was observed with first-line Egfr therapies as compared with third-line therapies [25].

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Interpretation

Genomic alterations (SNVs/indels/fusions) were detected in the cell-free DNA (cfDNA) isolated from the patient's blood specimen. The variant frequency of the mutations, reported as variant allele fraction (VAF), is calculated and reported for detected SNVs and indels. These alterations may be informative to cancer treatment response and/or clinical trial eligibility. While somatic actionability is of primary concern when determining report inclusion, some variants may be included without matched actionable outcomes. These variants may be critical to carcinogenesis in the patient's tumor.

Methods and Limitations

Northstar Select® is a next generation sequencing (NGS)-based in vitro diagnostic test for detection of substitutions (SNVs), small insertion and deletion alterations (indels), selected genes' copy number alterations (CNAs, including amplifications and deletions) and selected gene-rearrangements in a total of 84 genes (Table 1), as well as microsatellite instability-high (MSI-H) status. Cell-free DNA (cfDNA) is extracted from plasma, and the targeted regions, encompassing >250kb, are amplified and sequenced. The sensitivity of the test is 100% (95% CI: [99.84%,100.00%]) for SNVs and indels for variant allele fraction (VAF) ≥0.5% and fusions examined in the assay analytical validation, and 100% (95% CI: [76.84%,100.00%]) for CNAs. The limit of detection for the assay is 0.15% VAF for SNVs and indels. However, key actionable variants may be reported at lower VAFs where technically feasible. The overall base-wise specificity of the Northstar Select® test is >99.99%, leading to high confidence in true positive reporting for variants with VAF >0.2%, despite the expansive genomic loci targeted and assayed. For CNAs, the LOD for gene amplifications is 2.11 copies and for loss is 1.8 copies. CNA LOD is subject to aneuploidy noise, with less aneuploid samples having a superior LOD. The copy number displayed on the report represents gene copies in liquid. Thresholds have been set to report homozygous loss for deletions, focal amplifications, or high-level amplifications (e.g. >6 copies estimated in the solid tumor). Observed increased or decreased copy number may not be called as CNAs despite potentially reflecting a characteristic (ie aneuploidy) of the tumor biology. These genes with copy number signal indicating potential for aneuploidy are listed in the corresponding section of the report (except for the AR gene located on the X-chromosome, which will not be subject to this additional analysis). MSI score is calculated using a count of somatic indel mutations in targeted microsatellite sites; based upon meeting threshold, MSI-H status is reported when detected. The sensitivity of the MSI component is 100% (95% CI: [97.72%,100.00%]) for MSI-H at a tumor fraction of ≥0.5%, and the limit of detection of the assay for MSI-H is 0.07-0.4% tumor fraction. An MSI-Indeterminate result is an inconclusive result as it does not suggest the presence or absence of MSI-H in the patient. For certain cfDNA sample or variant characteristics, such as low cfDNA input or high level of cancer-associated chromosomal copy abnormality, the analytical sensitivity may be reduced.

Variants detected in the cfDNA are aligned to the hg19 reference genome. Informative genomic alterations are potentially actionable or biologically relevant variants based on evidence from medical and scientific literature. Variants of unknown significance (VUS) are genomic alterations that do not have sufficient evidence to determine biological/clinical significance. Variants classified as benign, likely benign, and variants likely originating from a pseudogene are not reported.

Genomic profiling of tumors can detect alterations not associated with the tumor itself but due to clonal hematopoiesis (CH). This assay cannot differentiate whether variants detected in the cfDNA are derived from a patient's solid tumor or clonal blood cells. Variants indicating CH, which are found most commonly in specific genes, may be detected, especially in older individuals or previously systemically treated patients (e.g., chemotherapy), and may impact targeted treatment efficacy. Clinical guidelines state that caution is warranted in interpreting ctDNA-only somatic alterations in genes indicated for PARP inhibitors and actionable tumor suppressor genes [49]. In alignment with guideline recommendations, detected alterations in these genes and other genes that are most CH-prone are flagged: *ATM, BRCA1, BRCA2, BRIP1, CDK12, CHEK2, FANCA, JAK2, MLH1, NF1, PALB2, PTEN, RAD51C, RAD51D,* and *SF3B1* [49, 50, 51, 52]. Buffy coat enriched for white blood cells is currently stored by the laboratory in case further testing is necessary. This report should not be used in place of a dedicated hematological evaluation. Any interpretations should take into consideration the patient's clinical context.

Reported treatments and trials should be comprehensively evaluated by the medical provider, as inclusion in the report is neither a guarantee of treatment/trial match, nor intended to be fully comprehensive. Treatments and trials are reported based upon the information provided at the time of test requisition, including diagnosis, sex, age, and location, and may not account for all elements of the patient's medical history. Certain treatments may have specifications surrounding other clinical factors, such as HR/HER2-status or germline mutation origin. The ordering provider should consult official FDA-approval information for full approval specifications, as well as professional guidelines for complete recommendations. Inclusion in Northstar Select® does not guarantee that reported treatments or trials will have an impact on clinical outcome.

This assay is validated for the detection of somatic variants, but was not developed to distinguish germline variants, which may predispose the patient to certain types of cancer, from detected somatic variants. Incidental findings will be interpreted and reported in the context of somatic disease. For variants with a VAF reported at >40%, separate germline testing may be recommended to identify and characterize such variants that may have hereditary implications.

Table 1: Genes on the Northstar Select® panel

Northstar Select® reports single nucleotide variants, insertion and deletion variants (indels), and splice site mutations for clinically relevant exons in 82 genes, copy number amplifications in 19 genes, and copy number loss in 5 genes, and fusion events in 9 genes, as detailed in the table below. This assay was designed for blood-based molecular profiling of solid tumors; while indicated for use, gene coverage may not meet guideline recommendations for certain cancer types such as sarcomas and lymphomas.

AKT1, *AKT2*, *ALK*^, *APC*, *AR*+, *ARAF*, *ARID1A*, *ATM*−, *BRAF*^+, *BRCA1*−, *BRCA2*−, *BRIP1*, *CCND1*, *CCND2*, *CCNE1*+, *CD274*+, *CDH1*, *CDK12*, *CDK4*+, *CDK6*+, *CDKN2A*−, *CDKN2B*, *CHEK2*, *CTNNB1*, *DDR2*, *EGFR*+, *ERBB2*+, *ESR1*+, *EZH2*, *FANCA*, *FBXW7*, *FGFR1*+, *FGFR2*^+, *FGFR3*^, *FGFR4*, *GATA3*, *GNA11*, *GNAQ*, *GNAS*, *HRAS*, *IDH1*, *IDH2*, *JAK2*, *JAK3*, *KIT*+, *KRAS*+, *MAP2K1*, *MAP2K2*, *MET*+, *MLH1*, *MPL*, *MSH2*, *MSH6*, *MTOR*, *MYC*+, *NF1*, *NOTCH1*, *NPM1*, *NRAS*, *NTRK1*^, *NTRK2*^^, *NTRK3*^^, *PALB2*, *PDGFRA*+, *PIK3CA*+, *PMS2*, *PTEN*−, *PTPN11*, *RAD51C*, *RAD51D*, *RAF1*+, *RB1*, *RET*^+, *RHOA*, *RIT1*, *ROS1*^, *SF3B1*, *SMAD4*, *SMO*, *STK11*, *TERT*, *TP53*, *TSC1*, *VHL*

^ Northstar Select® also reports fusion events for this gene
^^ Northstar Select® reports only fusion events for these genes, no SNVs and indels will be reported; *NTRK3* is limited to *NTRK3-ETV6* fusions only
+ Northstar Select® also reports copy number amplifications of this gene
− Northstar Select® also reports copy number deletions of this gene