

Patient ID SA00167523	Patient Name ATLASAB, NGCLN	Birth Date 1966-04-03	Sex F	Age 58
Order Number SA00167523	Client Order Number SA00167523	Ordering Physician CLIENT,CLIENT	Report Notes	
Account Information C7234570 SQA Manual Account		Collected 03 Apr 2024 11:53		

Chronic Lymphoid Neoplasms, NGS, V

Specimen Type

Peripheral blood

MCR

Indication for Test

NA

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Pathogenic Mutations Detected

1. BTK: ChrX(GRCh37):g.100611165A>T;
NM_000061.2(BTK):c.1441T>A; p.Cys481Ser (22%)

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No other pathogenic mutations were detected in the other genes tested on the panel. See below for Variants of Unknown Significance and Additional Information. Please see the section of "Panel Gene List" below for the complete list of genes tested.

Interpretation

1. BTK: ChrX(GRCh37):g.100611165A>T;
NM_000061.2(BTK):c.1441T>A; p.Cys481Ser (22%)

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Normal Gene/Protein Function: The BTK gene, located on chromosome Xq22.1, encodes the protein Bruton's tyrosine kinase which is a cytoplasmic tyrosine kinase playing a pivotal role in B-cell development, differentiation, signaling, and activation. Upon antigen stimulation of the cell surface B-cell receptor (BCR), signal transduction through BCR-associated tyrosine kinases (e.g., LYN and SYK) leads to phosphorylation of BTK. One major downstream target of BTK is phospholipase C Gamma2 (PLCG2) which catalyzes the cleavage of membrane phosphatidyl-inositol 4,5 bisphosphate (PIP2) into inositol triphosphate (IP3) and diacylglycerol (DAG), leading to activation of the NFAT, NFkB, and MAPK pathways. BTK also participates in the chemokine receptor signaling of lymphocyte trafficking and Toll-like receptor signaling of immune response (Petro et al., 2000, 10811867; Merolle et al., 2018, 29861875; Zhang et al., 2015, 25858358; Kenny et al., 2013, 23967355). Loss-of-function BTK mutations result in X-linked agammaglobulinemia, an immunodeficiency characterized by significantly reduced B lymphocytes and failure to generate plasma cells, with markedly decreased immunoglobulin production and antibody responses (Ochs et al., 1996, 8982147).

Mutation Effect: The c.1441T>A (C481S) variant occurs in amino acid residue C481 in the active site of the BTK tyrosine kinase domain. The covalent BTK inhibitor ibrutinib irreversibly binds to this residue and abrogates BTK activation and signaling (Honigberg et al., 2010, 20615965). C481S is the most frequent mutation seen in B-cell neoplasm patients developing ibrutinib resistance. In vitro studies have shown that the mutant exhibits enhanced kinase activity, disrupts the irreversible binding of ibrutinib to BTK, and results in loss of BTK inhibition (Furman et al., 2014, 24869597; Woyach et al., 2014, 24869598).

Disease Associations: BTK signaling is essential in the survival and proliferation of B-cell neoplasms (BCN). Although somatic mutation of BTK is not a common mechanism of lymphomagenesis, BTK is frequently upregulated and/or constitutively active in BCNs such as chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), Waldenstrom's Macroglobulinemia (WM) and multiple myeloma (MM) (Hendricks et al., 2014, 24658273; Cinar et al., 2013, 23962569). BTK inhibition has shown efficacy in inhibiting BCN growth and BTK inhibitors (BTKi) have been approved for treatment in MCL, CLL, WM

Performing Site Legend

Code	Laboratory	Address	Lab Director	CLIA Certificate
MCR	Mayo Clinic Laboratories - Rochester Main Campus	200 First Street SW, Rochester, MN 55905	William G. Morice M.D. Ph.D	24D0404292

Patient ID SA00167523	Patient Name ATLASAB, NGCLN	Birth Date 1966-04-03	Sex F	Age 58
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and marginal zone lymphoma. However, acquired resistance to BTKi with disease progression has been observed in some patients, most harboring BTK and/or PLC??2 mutations (Furman et al., 2014, 24869597; Woyach et al., 2014, 24869598; Chiron et al., 2015, 25082755). BTK mutations are seen in approximately 70% of CLL patients progressed on ibrutinib therapy. Similar resistance has also been reported in patients on 2nd generation covalent BTK inhibitor acalabrutinib therapy (Lee et al., 2020, 32615167; Kanagal-Shamanna et al., 2019, 30508305; Byrd et al., 2016, 26641137; <https://doi.org/10.1182/blood-2019-127674>).

Variants of Unknown Significance

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None.

The VUS variants listed here (with approximate variant allele %) are not sufficiently characterized in the current literature and therefore are of uncertain or unknown clinical significance at this time. They are reported here for future reference in the event they become clinically significant in light of new scientific data.

Additional Information

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None.

Clinical Trials

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Information regarding possible clinical trials for this patient can be found at the following sites:

- 1). ClinicalTrials.gov:
<http://clinicaltrials.gov/ct2/search/advanced>
- 2). Mayo Clinic:
<http://www.mayo.edu/research/clinical-trials>
- 3). National Cancer Institute:
<http://www.cancer.gov/clinicaltrials/search>
- 4). The Leukemia and Lymphoma Society's Clinical Trial Support Center: <https://www.hematology.org/education/clinicians/clinical-trial-support-center>

Method Summary

1 MCR

A portion of the testing process was performed at Mayo Clinic Laboratories site.

DNA is extracted from validated specimen sources. Library preparation for Next Generation Sequencing (NGS) is performed followed by probe hybridization and capture. Sequencing of the final sample library is performed on a NGS instrument. Following bioinformatic processing of the sequencing data, the sequencing results are interpreted to provide a final clinical report. Genomic alterations are called according to human genome reference build GRCh37 (hg19).

Performance characteristics of NGS panel:

Single base substitutions: accuracy >99%; reproducibility >99% (intra- and interassay); sensitivity 5% variant allele fraction with a minimum depth of coverage of 500X.

Insertion/deletion events (less than or equal to 1000 bps in size): accuracy 100%; reproducibility >99% (intra- and interassay); sensitivity 5%

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variant allele fraction with a minimum depth of coverage of 500X. Some genetic or genomic alterations such as very large insertion/deletion events, copy number alterations (CNA) and gene translocation events are not detected by this assay.

Disclaimer

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CLINICAL DISCLAIMER

Mutation calls detected between 5–10% variant allele fractions (VAF) may indicate low-level (i.e. subclonal) tumor populations, although the clinical significance of these findings may not be clear. Some apparent mutations classified as VUS may represent rare or low frequency polymorphisms. Prior treatment for hematologic malignancy could affect the results obtained in this assay. In particular, prior allogeneic hematopoietic stem cell transplant (HSCT) may cause difficulties in resolving somatic or polymorphic alterations, or in assigning variant calls correctly to donor and recipient fractions, if pertinent clinical or laboratory information (e.g. chimerism engraftment status) is not provided. Multiple additional factors may also impact the observed VAF including copy number alterations of the genetic locus, chromosome ploidy changes or subclonal composition. Correlation with clinical, histopathologic and additional laboratory findings is required for final interpretation of these results. This assay does not distinguish between somatic and germline alterations in analyzed gene regions, particularly with VAF near 50% or 100%. If nucleotide alterations in genes associated with germline mutation syndromes are present and there is also a strong clinical suspicion or family history of malignant disease predisposition, additional genetic testing and appropriate counseling may be indicated. This report interpretation is based on current medical and scientific literature, but clinical significance may not be completely established for all reported target gene abnormalities identified. The final interpretation of results for clinical management of the patient is the responsibility of the managing physician. At this time, it is not standard practice for the laboratory to systematically review likely pathogenic variants or variants of uncertain significance that have been previously detected and reported. The laboratory encourages health care providers to contact the laboratory at any time to learn how the status of a particular variant may have changed over time.

TECHNICAL DISCLAIMER

The depth of sequencing coverage may be variable for some target regions, but assay performance below the minimum acceptable criteria, or for failed regions is noted. Analysis of rare (low allele frequency) polymorphisms may be problematic in some cases. A low tumor cell percentage in the sample may affect the true mutation VAF and/or sensitivity. Suboptimal-performing regions (i.e. less than the expected minimum depth of coverage) may affect analytic sensitivity for detecting lower level mutations. In the rare event of suboptimal measured DNA input quantity for library preparation (e.g. due to limited tissue amount), detection sensitivity for subclonal variants near the assay limit of detection may be decreased, despite the presence of otherwise acceptable sequencing quality metrics. This is a qualitative test. The variant read fractions are provided for information only and represent a relative proportion of mutated alleles, but do not indicate a measure of analytical sensitivity for the given genes; assay sensitivity is as stated in the method summary. Some genetic or genomic alterations, such as very large insertion/deletion events, copy number alterations (CNA) and gene translocation events are not detected by this assay.

Panel Gene List

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ATM (NM_000051.3) Exons 2–63, BCL2 (NM_000633.2) Exons 2–3, BIRC3 (NM_001165.4) Exons 2–9, BRAF (NM_004333.4) Exons 11–18, BTG1 (NM_001731.2) Exons 1–2, BTK (NM_000061.2) Exons 2–19, CCND1 (NM_053056.2) Exons 1–5, CDKN2A (NM_000077.4) Exons 1–3, CXCR4 (NM_003467.2) Exons 1–2, DDX3X (NM_001356.4) Exons 1–17, EZH2 (NM_004456.4) Exons 2–20, FBXW7 (NM_001349798.2) Exons 4–14, KLF2 (NM_016270.3) Exons 1–3, KRAS (NM_033360.3) Exons 2–4, MAP2K1 (NM_002755.3) Exons 2–3, 6, MYD88 (NM_002468.4) Exons 1–5, NOTCH1 (NM_017617.3) Exons 26–27, 34, NOTCH2 (NM_024408.4) Exons 26–27, 34, NRAS (NM_002524.4) Exons 2–4, PIK3CA (NM_006218.2) Exons 2–11, 14–21, PLCG2 (NM_002661.3) Exons 2–33, SF3B1 (NM_012433.2) Exons 13–16, TNFAIP3 (NM_006290.3) Exons 2–9, TP53 (NM_000546.4) Exons 4–11, XPO1 (NM_003400.3) Exons 14–18.

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For some genes, the transcript IDs used in the analysis may not be the same as in other cancer mutation databases, such as COSMIC
<http://cancer.sanger.ac.uk/cosmic>

Reviewed By
MCR

Signing Pathologist: SHANDA VOELTZ

NGCLN Result
MCR

See Interpretation

Received: 03 Apr 2024 13:31

Reported: 03 Apr 2024 13:53

Laboratory Notes

- 1 This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

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