PRECIPIO EXPECT MORE
203-787-1717
203-901-1289

MOLECULAR

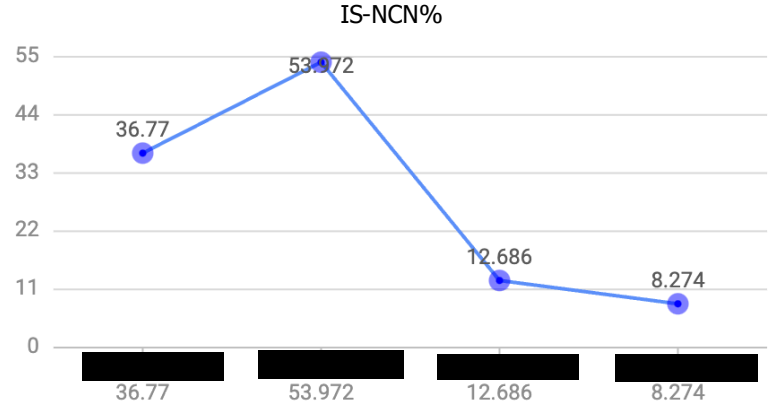
Patient: John A. Doe
DOB/Gender: 10/10/44 (74 yrs) - Male
Patient ID/MRN: 123456
Date Collected: 01/02/2024

Case# XX-00000
Status: Final
Report Category:
Detected

Provider: Jane Smith, M.D.
Hospital Medical Center
Tel: 800-123-4567
Fax: 800-765-4321

DIAGNOSIS:

Peripheral blood:
- BCR::ABL1 p210 fusion was detected at 8.274% IS-NCN.
- No BCR::ABL1 p190 fusion was detected.
- No BCR::ABL1 p203 fusion was detected.
- No BCR::ABL1 p230 fusion was detected.
-ABL1-Resistance Status: ABL1 exon 8 variant detected (p.E459K).



INTERPRETATION

(Bloodhound BCR::ABL1): Mutations within the BCR/ABL1 kinase domain of patients with chronic myeloid leukemia or acute lymphoblastic leukemia with Philadelphia chromosome are the most commonly identified mechanism associated with resistance to kinase inhibitors. It has been reported that most patients with detectable BCR/ABL1 kinase domain mutations are imatinib resistant or resistant to other kinase inhibitors.

High Resolution Melt analysis was performed to identify BCR/ABL1 fusion isoforms (p190, p210, p230, p203) for diagnostic, therapeutic, monitoring and drug-response of Philadelphia chromosome positive leukemic cells. BCR/ABL1 translocations in the major breakpoint cluster region resulting in fusion protein are seen in nearly all cases of chronic myelogenous leukemia (CML), acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML) and myeloproliferative neoplasms (MPN/MDS).

p190 BCR/ABL1 fusion encodes micro transcripts e19a2 common in Philadelphia-positive B-ALL and has been reported in 1% of CML cases. p203 BCR/ABL1 fusion encodes transcripts e13a3 (b2a3), a precursor to p210 fusion. p210 BCR/ABL1 fusion encodes major transcripts e14a2 (b2a2) or e13a2 (b3a2) proteins common in CML. p230 BCR/ABL1 fusion encodes transcripts e19a2 is known as µ-BCR/ABL1 transcript and common in neutrophilic-chronic myeloid leukemia.

(ABL1 reflex): Resistance to the targeted treatment of TKI and subsequent therapeutic failure is driven by the presence of mutations in the translocated ABL1 region. Chronic myeloid leukemia (CML) is a myeloproliferative neoplasia associated with a molecular alteration, the fusion gene BCR-ABL1, that encodes the tyrosine kinase oncoprotein BCR-ABL1. This led to the development of tyrosine kinase inhibitors (TKI), with Imatinib being the first TKI approved. Although the vast majority of CML patients respond to Imatinib, resistance to this targeted therapy contributes to therapeutic failure and relapse.

The mutations covered:
ABL1 Exon 4: c.730A>G; p.M244V; c.742C>G; p.L248V; c.749G>A; p.G250E; c.757T>C; p.Y253H; c.763G>A; p.E255K; c.764A>T; p.E255V
ABL1 Exon 5: c.895G>A; p.V299L; c.895G>T; p.V299L
ABL1 Exon 6: c.943A>G; p.T315A; c.944C>T; p.T315I; c.949T>C; p.F317L; c.949T>G; p.F317V; c.949T>A; p.F317I; c.950T>G; p.F317C; c.951C>G; p.F317L; c.951C>A; p.F317L; c.1052T>C; p.M351T; c.1075T>G; p.F359V; c.1075T>A; p.F359I; c.1076T>G; p.F359C
ABL1 Exon 7: c.1187A>G; p.H396R
ABL1 Exon 8: c.1375G>A; p.E459K

REFERENCES:
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PMID: 33677711; DOI: 10.1007/s00432-021-03569-8
2. Rossari, Federico, Filippo Minutolo, and Enrico Orciuolo. "Past, present, and future of Bcr-Abl inhibitors: from chemical development to clinical efficacy."
PMID: 29925402; DOI: 10.1186/s13045-018-0624-2
3. Kohla, Samah, et al. "P190 BCR::ABL1 in a Patient with Philadelphia Chromosome Positive T-Cell Acute Lymphoblastic Leukemia: A Rare Case Report and Review of Literature."
PMID: 34326740; DOI: 10.1159/000516270
4. Reckel, Sina, et al. "Differential signaling networks of Bcr–Abl p210 and p190 kinases in leukemia cells defined by functional proteomics."
PMID: 28111465; DOI: 10.1038/leu.2017.36
5. Chootawiriyasakul, Kanokon, Chitima Sirijerachai, and Kanchana Chansung. "Frequency of BCR-ABL Fusion Transcript Types with Chronic Myeloid Leukemiaby Multiplex PCR in Srinagarind Hospital, Khon Kaen Thailand."
DOI: 10.33425/2639-944X.1095
6. Hamid, Mohammad, and Hanieh Bokharaei. "The frequency of BCR::ABL1 fusion transcripts in iranian patients with three different types of leukemia."
DOI: 10.5812/zjrms.10197
7. Adnan-Awad, Shady, et al. "Characterization of p190-Bcr-Abl chronic myeloid leukemia reveals specific signaling pathways and therapeutic targets."
PMID: 33168949; DOI: 10.1038/s41375-020-01082-4
8. Gong, Z., et al. "Clinical and prognostic significance of e1a2 BCR::ABL1 transcript subtype in chronic myeloid leukemia."
PMID: 28708130; DOI: 10.1038/bcj.2017.62
9. Verstovsek, Srdan, et al. "Neutrophilic-chronic myeloid leukemia: Low levels of p230 BCR/ABL mRNA and undetectable p230 BCR/ABL protein may predict an indolent course."
PMID: 12015767; DOI: 10.1002/cncr.10490
10. Pienkowska-Grela, Barbara, et al. "Complete cytogenetic and molecular response after imatinib treatment for chronic myeloid leukemia in a patient with atypical karyotype and BCR-ABL b2a3 transcript."
PMID: 17452251; DOI: 10.1016/j.cancergencyto.2006.11.021
11. Liu, Li-Gen, et al. "Chronic myelogenous leukemia with e13a3 (b2a3) type of BCR-ABL transcript having a DNA breakpoint between ABL exons a2 and a3."
PMID: 14635208; DOI: 10.1002/ajh.10429

METHOD:

(Bloodhound BCR::ABL1): Total RNA is isolated and converted to cDNA. The HRM primers are designed to detect the major (p210) BCR/ABL1 breakpoint as well as minor breakpoints p190, p203, p230. Quantification of the p210 BCR/ABL1 breakpoint is performed utilizing qPCR.

High resolution melting (HRM), produces curves using dyes that fluoresce in the presence of DNA. As the temperature increases, the fluorescence decreases as a result of the denaturation of the DNA, producing a characteristic melt profile.

(p210 quantification): BCR/ABL1 p210 fusions are quantitated by real-time PCR amplification using the BCR/ABL1 Mbcr IS-MMR Kit from Ipsogen®. The primers are designed to detect the major (p210) BCR/ABL1 breakpoint including fusions between BCR exon 13 and ABL1 exon 2 (b2a2) and BCR exon 14 and ABL1 exon 2 (b3a2). The qPCR assay includes standards for BCR/ABL1 and the ABL1 control. Normalized copy number (NCN) (BCR/ABL1 copies/ABL1 copies) are reported for each sample. The NCN is further converted to a value on the international scale (IS) using validated reference material that has been calibrated against the NIBSC WHO certified primary reference material (International Genetic Reference Panel for the quantitation of BCR/ABL1 translocation by RQ-PCR (1st I.S.)).

(ABL1 reflex): Melting curve analysis in combination with real-time PCR is a natural extension of continuously monitored PCR within each cycle. During high-resolution DNA melting analysis (HRM or HRMA), melting curves are produced using dyes that fluoresce in the presence of double-stranded DNA (dsDNA). Using specialized instruments designed to monitor fluorescence during heating; as the temperature increases, the fluorescence decreases, producing a characteristic melting profile.
This assay can detect mutations with a minimum sensitivity of 5% depending on the wild-type background in the specimen. Although molecular testing is highly accurate, rarely false-positive and false-negative diagnostic errors may occur.

HRM analysis was performed using HRM v3.1 Thermo Fisher software to discriminate DNA sequences based on their composition, length, GC content, or strand complementarities.

LIMITATIONS:
(Bloodhound BCR::ABL1): The limit of detection of the HRM assay is one BCR/ABL1 positive cell in 105 normal cells. These results must always be interpreted in the context of morphologic and other relevant data and should not be used alone for a diagnosis of malignancy.

Electronically Signed By: Frank Bauer, MD, Precipio, Inc. (01/10/2024 09:19)

The technical component was performed by Precipio, Inc., New Haven, CT (CLIA #07D2030009). Analysis and professional components of these tests were completed by Seble Chekol, located at Remote Site #4 (CLIA# 11D2288796). The adequacy of staining is verified by the appropriate positive and negative controls. The reagents used for these assays are analyte specific reagents (ASR). Their performance characteristics have been validated by Precipio, Inc., New Haven, CT. They have not been reviewed by the FDA. The FDA has deemed that such approval is unwarranted. These assays are for clinical use and should not be viewed as experimental or "research use only".

CLINICAL DATA

ICD-10: C92.10, D50.0, D75.9. Chronic myeloid leukemia, BCR/ABL-positive, not having achieved remission. Iron deficiency anemia secondary to blood loss (chronic).

Received CBC, reported on 12/21/2023: WBC 2.60; RBC 4.18; HGB 11.0; HCT 35; MCV 82.5; MCH 26.2; MCHC 31.8; RDW 28.5%; PLT 231; MPV 7.9; LYM NP; GRAN NP; MID NP; MON NP; NEU NP; EOS NP; BAS NP; (NP = not provided)

Disclaimer: The adequacy of staining is verified by the appropriate positive and negative controls. The reagents used for this assay are analyte specific reagents (ASR) or research use only (RUO). Their performance characteristics have been validated by Precipio, Inc.,in its locations (New Haven, CT & Omaha, NE). They have not been reviewed by the FDA. The FDA has deemed that such approval is unwarranted. These assays are for clinical use and should not be viewed as experimental or "research use only". This laboratory is CLIA & CAP certified to perform high complexity clinical testing.

Patient: John A. Doe

Case #: XX-00000

Received Information: 1 lavender-top tube(s)

Received: 01/03/2024 11:45

Reported: 01/10/2024 10:30