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: 01/02/2023

Case# P23-00323
Status: Final
Report Category:
Detected

Provider: Jane Smith, M.D.
Hematology Oncology Associates
Tel: 800-123-4567
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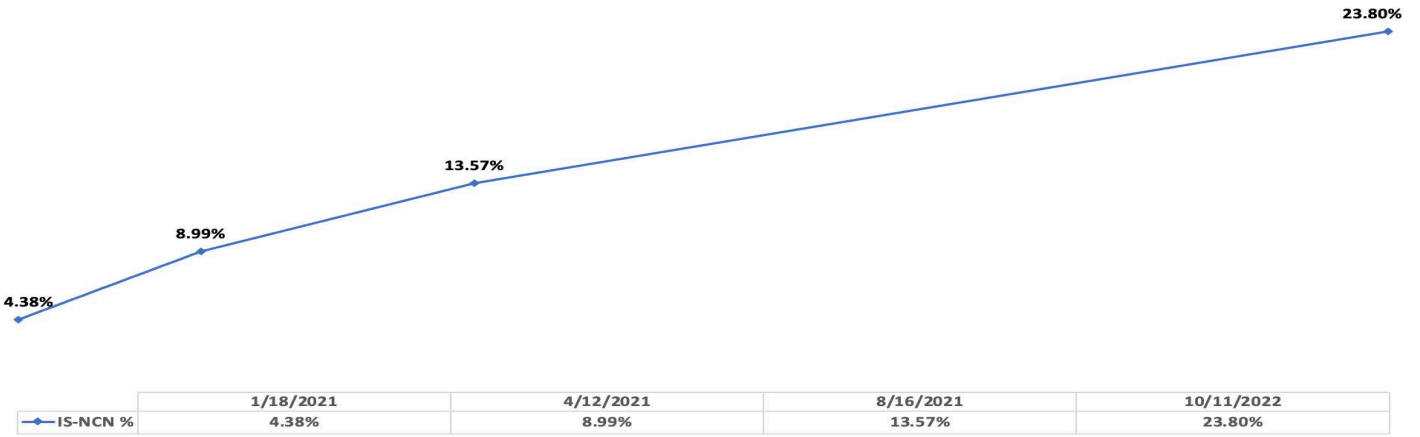
DIAGNOSIS:

Peripheral blood:
- BCR/ABL1 p210 fusion was detected at 23.801% IS-NCN
  No BCR/ABL1 p190 fusion was detected.
  No BCR/ABL1 p203 fusion was detected.
  No BCR/ABL1 p230 fusion was detected.
- ABL1 mutation analysis: No variant detected



	1/18/2021	4/12/2021	8/16/2021	10/11/2022
IS-NCN %	4.38%	8.99%	13.57%	23.80%

INTERPRETATION

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.

(p210 quantification): Molecular analysis was performed to detect and quantify the BCR/ABL1 p210 fusion gene transcripts.

Our results show that the normalized copy number of BCR/ABL1 is 23.801 on the International Scale (%IS-NCN). To monitor patient response to treatment or MRD (Minimal Residual Disease), we recommend evaluating changes from the baseline with time rather than the absolute ratio from a single time point.

To facilitate interlaboratory comparison of findings and the assessment of molecular milestones (major molecular response; MMR), results are reported using the standardized "international scale" (IS) of BCR/ABL1 transcript measurement that has been recommended by an international consensus of CML experts.

The international scale (IS) defines a BCR/ABL1 RNA level as follows:
< 0.05% IS-NCN level : Major Molecular Response (MMR)
> 0.05% IS-NCN <0.15 : Gray zone around MMR cutoff, resulting in inconclusive results.
> 0.15% IS-NCN level : No Major Molecular Response (MMR)

(ABL1): This assay includes screening for targeted ABL1 mutations, which is the most commonly reported imatinib resistance mechanism and also confers resistance to two second generation tyrosine kinase inhibitors, nilotinib and dasatinib. Failure to achieve cytogenetic response in the first six months of therapy in CML patients often reflects the presence of mutations or a high probability that mutations will subsequently be detected.

METHOD:
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): ABL1 mutation analysis uses standard PCR and Sanger sequencing methodology to target hot spots within exons 4, 5, 6, 7, and 8. Two amplicons designed to cover this region were used to amplify cDNA synthesized from the patient's RNA, then analyzed via Sanger sequencing. The sensitivity of detecting mutant ABL1 allele in the background of normal alleles is about 1:100,000.

LIMITATIONS:
The limit of detection of the HRM assay is one BCR/ABL1 positive cell in 105 normal cells. The limit of quantification via the qPCR assay is 1 x 10-5 with a %IS-NCN reportable limit of 0.0069%. 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.

REFERENCES:
1. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. Am J Hematol. 2018;93(3):442-459.
2. Jones D, Kamel-Reid S, Bahler D, et al. Laboratory practice guidelines for detecting and reporting BCR/ABL1 drug resistance mutations in chronic myelogenous leukemia and acute lymphoblastic leukemia: a report of the Association for Molecular Pathology. J Mol Diagn. 2009;11(1):4-11.
3. Simona Soverini, et al. Contribution of ABL Kinase Domain Mutations to Imatinib Resistance in Different Subsets of Philadelphia-Positive Patients: By the GIMEMA Working Party on Chronic Myeloid Leukemia. Clin Cancer Res 2006;12(24): 7374-7379.

Electronically Signed By: Frank Bauer, MD, Precipio, Inc. (01/06/2023 11:54)

CLINICAL DATA

ICD-10: C92.10. CML.

Received CBC, reported on 12/30/2022: WBC 9.2; RBC 5.06; HGB 16.4; HCT 48.2; MCV 95; MCH 32.4; MCHC 34.1; RDW 13.2%; PLT 289; MPV 7.4; LYM 28.5%; GRAN NP; MID NP; MON 9.1%; NEU 58.9%; EOS 2.4%; BAS 1.1%; (NP = not provided)

Disclaimer: The reagents used for these assays are for research use only (RUO). 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 for clinical use. These assays should be viewed as experimental and/or research use only.

Patient: John A. Doe
Case #: P23-00323
Received Information: 1 lavender-top tube(s)
Received: 01/03/2023 10:30
Reported: 01/06/2023 12:30