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: 01/12/2021

Case#/Status: P21-00123 - Final

Report Category:
Detected

Provider: John Doe, M.D.
Hematology Oncology Associates
Tel: 800-123-4567
Fax: 800-765-4321

DIAGNOSIS:

Peripheral blood:
- BCR-ABL1 Fusion Transcripts (p210 forms) were detected by RT-qPCR
  BCR-ABL/ABL1: 0.059% (IS-NCN)
  Molecular Response: Major
- ABL1 resistance mutation screening: Detected

COMMENT

ABL1 mutation was detected on a single nucleotide variant at base change c.944C>T affecting protein change T315I. ABL1-T315I mutation is a predictive biomarker for use of ponatinib, bosutinib, dasatinib, imatinib, and nilotinib in patients.

Our results showed that the ratio of BCR-ABL/ABL1 is 0.059 on IS (%, International Scale). 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.

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.

INTERPRETATION

This result confirms the existence of persistent transcriptionally active Philadelphia chromosome positive leukemia cells in patients undergoing therapy targeted to BCR-ABL1. CML patients with BCR-ABL1 RNA levels between 2 and 3 logs below the standardized baseline (0.1-1.0% on the IS) have a better prognosis than patients not achieving a 2 log-drop (1.0% on the IS). Results however should be interpreted with standard precautions that are inherent to quantitative PCR assays.

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-ABL 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.

TEST INFORMATION:
(BCR-ABL): This assay quantifies BCR-ABL1 transcripts (b2a2 and b3a2) for therapeutic monitoring of Philadelphia chromosome positive leukemic cells. BCR-ABL1 translocations in the major breakpoint cluster region resulting in the p210 fusion protein are seen in nearly all cases of chronic myelogenous leukemia (CML) and in a few cases of acute lymphoblastic leukemia (ALL). To facilitate interlaboratory comparison of findings and the assessment of molecular milestones (major molecular response; MMR), results are reported using 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)

(ABL): This assay includes analysis of 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 6 months of therapy in CML patients often reflects the presence of mutations or a high probability that mutations will subsequently be detected.

METHODS:
(BCR-ABL): Total RNA is isolated and converted to cDNA; BCR-ABL1 fusions are quantitated by real-time PCR amplification using the BCR-ABL 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 PCR assay includes a standard curve for BCR-ABL and the ABL1 control. Normalized copy number (NCN) (#BCR-ABL1 cDNA molecules/#ABL1 cDNA molecules) 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 to a standard set of diagnostic specimens.

(ABL): ABL1 mutation analysis uses nested RT-PCR/sequencing methodology. The BCR-ABL1 allele was amplified from patient's RNA using one step RT-PCR. The entire ABL1 kinase domain is sequenced using Sanger sequencing. Changes of nucleotides and amino acids in the coding sequence are reported. The sensitivity of detecting BCR-ABL1 allele in the background of normal alleles is about 1:100,000. Intron-derived insertions within the BCR-ABL1 kinase domain that cause a frame shift (such as the common 35-bp insertion) have not been shown to be associated with TKI-resistance; therefore, they will not be reported.

If the nested does not work since we are looking at exons 4-8.

ABL1 mutation analysis is performed on the original cDNA synthesis product. Two amplicons covering the entire ABL1 kinase domain were designed. Detection is done by Sanger Sequencing. Changes of nucleotides and amino acids in the coding sequence are reported. The sensitivity of detecting BCR-ABL1 allele in the background of normal alleles is about 1:100,000. Intron-derived insertions within the BCR-ABL1 kinase domain that cause a frame shift (such as the common 35-bp insertion) have not been shown to be associated with TKI-resistance, therefore they will not be reported.

LIMITATIONS:
(BCR-ABL): The limit of detection of this assay is 1 BCR-ABL1 positive cell in 106 normal cells. The limit of quantification is 1 x 10-5 or 0.69 % IS. This assay does not detect transcripts resulting from BCR-ABL1 rearrangements with a BCR breakpoint resulting in the p190 or p230 fusion proteins. The results of this test must always be interpreted in the context of morphologic and other relevant data and should not be used alone for a diagnosis of malignancy.

DISCLAIMER:
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. CML.

Received CBC, reported on 01/12/2021: WBC 13.77; RBC 3.6; HGB 10.7; HCT 34.2; MCV 94.0; MCH 29.4; MCHC 31.3; RDW 13.8%; PLT 260; LYM 13.3%; IG 0.50%; MON 6.2%; NEU 78.6%; EOS 1.20%; BAS 0.20%

Electronically Signed By: Frank Bauer, MD, Precipio, Inc. (01/31/21 10:50)

Patient: John A. Doe
Received: 01/12/21
Case #: P21-00123
Reported: 01/31/21
Received Information: 2 Green-top tubes, 1 lavender-top tube