Unipath
SPECIALTY LABORATORY Ltd.

CML
Query????????

BCR-ABL TESTING

Department of Molecular Biology

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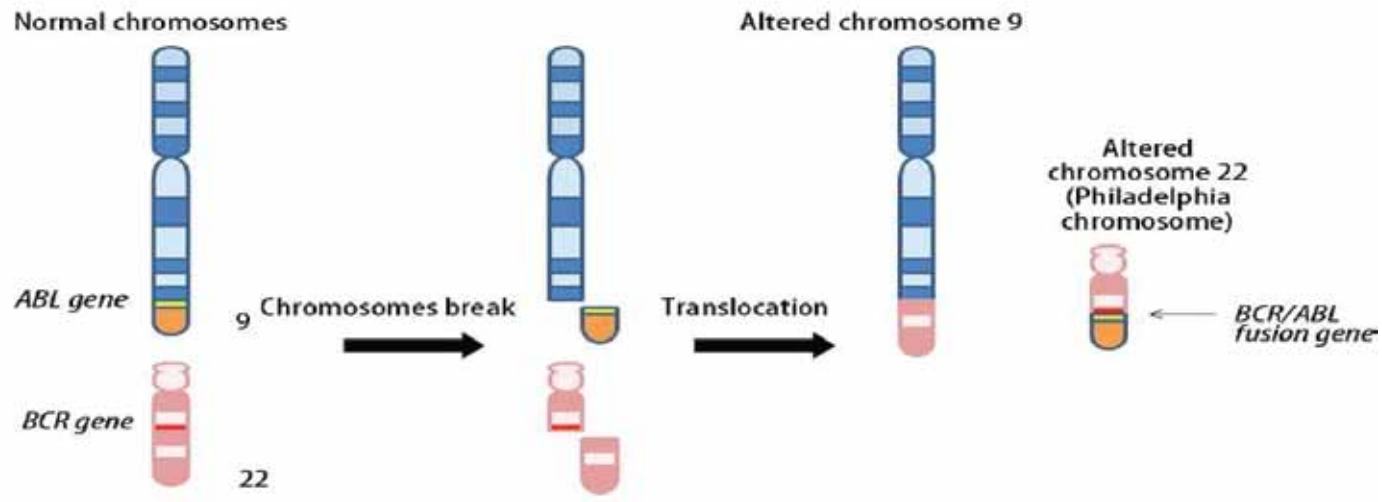
Background:

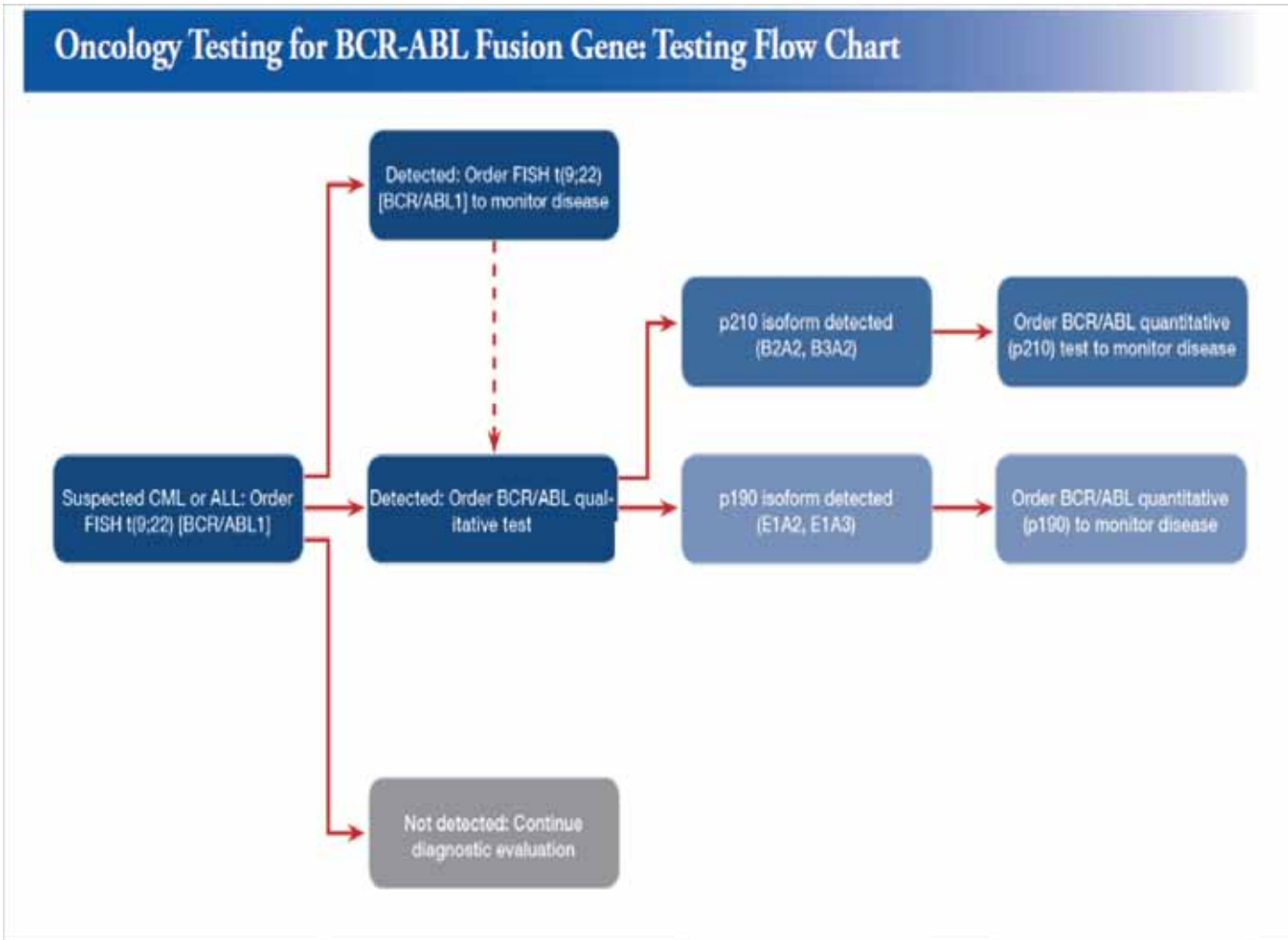
The Philadelphia chromosome (Ph), a derivative chromosome 22 resulting from a translocation between chromosomes 9 and 22 causing the BCR-ABL fusion, is present in approximately 95% of chronic myeloid leukemia (CML) and 25-30% of adult acute lymphoblastic leukemia (ALL) cases. This fusion is also seen in 2-4% of children with ALL. Chronic neutrophilic leukemia (CNL) is also associated with the Philadelphia chromosome and a specific BCR-ABL transcript. In general, the ratio of the fusion to a reference control can be used for diagnosis, prognosis, and



The BCR-ABL rearrangement results in the generation of fusion protein with constitutive tyrosine kinase activity. Based on the specific breakpoints of the rearrangement, several different isoforms of the BCR-ABL fusion protein can be generated, which correlate with different leukemic phenotypes. According to recommendations from the National Comprehensive Cancer Network, "identification of specific recurrent genetic abnormalities is critical for disease evaluation, optimal risk stratification and treatment planning" (NCCN Guidelines Version 2.2012, "Acute Lymphoblastic Leukemia"). In approximately 95% of CML cases, the major rearrangement is present and the p210 fusion protein is produced. In most children with Ph+ ALL, the minor rearrangement generates the p190 fusion protein. Adults with Ph+ ALL may have either the p190 or the p210 transcript.

For CML patients, associations have been established between BCR-ABL/ABL international scale (IS) values and assessment of treatment milestones and therapeutic efficacy. For the p210 fusion transcript, BCR-ABL/ ABL IS values indicating a major molecular response (MMR) correlate with excellent progression-free survival. For patients who are suspected to have emerging drug resistance or who fail to achieve a MMR initially, evaluation of ABL kinase domain mutations may identify the cause of resistance and potentially guide treatment changes.

For Ph+ ALL patients, associations for p190 IS values have not been established. For the p190 fusion transcript, an increasing BCR-ABL/ABL ratio may indicate a poor initial response in Ph+ ALL patients. Evaluation of ABL kinase domain mutations in recurrent Ph+ ALL can help guide changes in therapy.



AVAILABLE TESTS FROM UNIPATH SPECIALTY LABORATORIES:

BCR-ABL FISH cytogenetic testing:

This test detects the presence of the t (9; 22) translocation and the BCR-ABL fusion gene within somatic cells. This test may also identify an additional copy of Ph in cells or an atypical 9/22 translocation. FISH cytogenetic testing is indicated as an initial evaluation for patients suspected to have CML or ALL and then for monitoring disease status, however, it does not differentiate between the different isoforms (p190 and p210).

Qualitative BCR-ABL molecular testing:

This is indicated as an initial evaluation for patients known to have a positive FISH cytogenetic test for BCR-ABL. The qualitative BCR-ABL test can determine the specific type (isoform) of the Philadelphia chromosome present which is important for appropriate diagnosis and treatment. Our qualitative BCR-ABL test detects the presence of the p190 and p210 isoforms; however, the qualitative test does not measure the levels of the transcripts.

Quantitative BCR-ABL (p210) molecular testing:

This test is indicated for monitoring of disease for any patient positive for the p210 BCR-ABL fusion gene by qualitative assay. Our laboratory uses a methodology that measures the number of copies of the p210 BCR-ABL fusion gene present relative to the number of ABL gene transcripts in the same sample. Comparing the ratio of disease fusion gene copies to normal gene copies allows physicians to have a numerical measure of response to therapy and allows for a more sensitive monitoring for possible disease relapse.

p210 isoform International Scale (IS) :

A major molecular response is considered to be a 3-log reduction in the ratio from baseline of diagnosis (for example, 100% BCR-ABL/ABL ratio must reduce to 0.1% BCR-ABL/ABL ratio or 10 copies of fusion gene compared to 10,000 copies of the ABL gene). To facilitate comparison of quantitative RT-PCR results for the p210 BCR-ABL fusion gene between laboratories and platforms, International Scale (IS) reference materials were established by the World Health Organization and results of this assay are reported on the IS.

IS reference materials have not been established for the p190 BCR-ABL fusion gene.

TESTING STRATEGY:

Initial evaluation:

BCR-ABL FISH cytogenetic testing is performed to detect the BCR-ABL fusion gene as well as to provide an estimate of the percentage of cells carrying the fusion gene. Qualitative BCR-ABL testing is then performed to determine which isoform is present. A baseline sample should then be sent for quantitative BCR-ABL testing for the isoform identified.

Monitoring of disease status:

Disease can be monitored by BCR-ABL FISH cytogenetic testing until the assay can no longer detect the fusion gene, and then a sample for quantitative BCR-ABL testing should be sent for continued monitoring of disease, if the patient has the p190 or p210 isoform of the BCR-ABL fusion gene

TESTING METHODOLOGY:

BCR-ABL FISH cytogenetic testing:

Fluorescence in situ hybridization with probes for the t(9;22) translocation.

Qualitative BCR-ABL (p210/p190) testing:

RNA isolation with reverse transcription nested PCR and gel detection of PCR products.

Quantitative BCR-ABL testing:

RNA isolation with RQ-PCR reaction to precisely quantitate the amount of control and fusion gene transcripts.

Sensitivity and Accuracy:

BCR-ABL FISH cytogenetic testing:

For initial analysis, 250 nuclei are counted. For disease monitoring, with a previously abnormal result, up to 500 nuclei may be counted.

Qualitative BCR-ABL (p210/p190) molecular testing:

One cell with fusion transcripts per 10,000 normal cells.

Quantitative BCR-ABL (p210) testing: Sensitivity studies within the laboratory have shown that a ratio as low as 0.1% can be detected. Because of variability of the RNA quantity within a specimen and assay variability, only changes of 0.5 log or greater should be considered significant. Therefore results of 1% BCR-ABL/ABL would be considered equivalent to any results between 3% and 0.3%.

SPECIMEN REQUIREMENT:

BCR-ABL FISH cytogenetic testing:
1-3 ml bone marrow OR peripheral blood in green (Na Hep) tube.

Qualitative BCR-ABL (p210/p190) testing:
3 ml peripheral blood OR bone marrow in purple (EDTA) tube (Please note that samples must be received within 48 hours after collection).

Quantitative BCR-ABL testing:
5-10 ml peripheral blood OR 3-5 ml bone marrow in purple (EDTA) tube (Please note that samples must be received within 24 hours after collection).

TAT AND ORDERING INFORMATION:

TEST NAME	METHOD	SPECIMEN	REPORTING TIME
Qualitative BCR-ABL minor (p190)	REAL TIME PCR	EDTA WB/BM	48 Hours
Qualitative BCR-ABL Major (p210)	REAL TIME PCR	EDTA WB/BM	48 Hours
IS BCR-ABL Major Quantitative	REAL TIME PCR	EDTA WB/BM	12 DAYS
ABL Kinase Domain Mutations (IRMA)	PCR SEQUENCING	EDTA WB/BM	15 DAYS

RESULTS:
Each test report includes a detailed interpretation of the genetic findings, the clinical significance of the result, and specific recommendations for clinical management and additional testing, if warranted. Results will be reported to the referring physician or health care provider as specified on the test requisition form.

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