BCR::*ABL1* Testing for Initial Diagnosis of Chronic Myeloid Leukemia

Version: 1.0
Date: August 31, 2025

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SYNOPSIS AND RELEVANCE

Suspected cases of myeloproliferative neoplasia require *BCR*::*ABL1* testing to diagnose or exclude chronic myeloid leukemia (CML). Several molecular techniques can be used to detect the fusion gene with varying sensitivity and utility depending on transcript heterogeneity and clinical scenario. This module outlines appropriate utilization practices and provides recommendations to reduce complexity and avoid unnecessary testing for ordering clinicians. Adherence to the strategies outlined in this module will:

- Educate healthcare providers on appropriate initial *BCR::ABL1* testing in the setting of suspected chronic myeloid leukemia (CML).
- Optimize initial utilization of qualitative *BCR*::*ABL1* testing for efficient CML diagnosis.
- Impact patient care with appropriate quantitative *BCR::ABL1* testing needed for future disease monitoring.

OBJECTIVES

- Know the indications for different types of *BCR*::*ABL1* tests.
- Understand the utility and limitations of peripheral blood *BCR::ABL1* testing for suspected CML.
- Enact strategies to maximize efficient and appropriate test ordering for CML diagnosis.

BACKGROUND

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm defined by the presence of a reciprocal translocation of the long arms of chromosomes 9 (*ABL1* gene located at 9q34.1) and 22 (*BCR* gene at 22q11.2), resulting in t(9;22)(q34.1;q11.2) and formation of the *BCR::ABL1* fusion gene and deregulated ABL1 tyrosine kinase activity.[1] The fusion product may also be referred to as the Philadelphia (Ph) chromosome or Ph translocation , discovered by Nowell and Hungerford at Fox Chase Cancer Center in Philadelphia. The disease results in myeloid hyperplasia of the bone marrow with left-shifted granulocytosis with/without thrombocytosis in the peripheral blood and splenomegaly due to extramedullary hematopoiesis. Patients typically present in the chronic phase, but left untreated, may progress to the accelerated phase and/or blast crisis within 3 to 5 years.[1] Fortunately, CML is a highly treatable disease due to the widespread use of targeted tyrosine kinase inhibitor (TKI) therapy. Therefore, diagnosis is essential to allow timely initiation of treatment and prevent disease progression.[2] Quantitation of *BCR*::*ABL1* transcripts is used to monitor response to therapy. While not the focus of this module, appropriate testing at diagnosis is required to determine which test should be used for monitoring.

In the appropriate clinical context, demonstration of the *BCR::ABL1* fusion gene establishes the diagnosis of CML, however it is also important to identify the transcript so that appropriate quantitative assays can be used to monitor for residual or recurrent disease. The National Comprehensive Cancer Network (NCCN) recently published updated guidelines on the diagnosis, treatment and monitoring of CML.[2] Comparable practice guidelines have been established by European LeukemiaNet (ELN) and European Society for Medical Oncology. [3,4] Along with other appropriate laboratory studies (ie, CBC/differential, chemistry profile, etc.), the initial evaluation begins with verifying the presence of the *BCR::ABL1*. ELN recommends qualitative multiplexed reverse transcription polymerase chain reaction (RT-PCR) as initial testing, though FISH may also be used and has the benefit of detecting rare transcripts. The qualitative RT-PCR assay determines if a *BCR::ABL1* fusion is present and specifies which messenger RNA

(mRNA) transcript is present, the e13a2 and e14a2 transcripts (which encode for the 210-kDa protein, p210) or the e1/a2 and e1/a3 transcripts (which encode for p190).[5] These transcripts account for 95% and 1-2% of CML, respectively. The qualitative results then direct which quantitative RT-PCR (qPCR) assay, which are specific for each protein length, is appropriate to determine the baseline transcript level at the time of diagnosis and thereafter during monitoring. Some other strategies may include performing both p190 and p210 quantitative PCR, since they will detect 96-97% of case, but reflexing to another test which will detect other transcripts than those that produce these proteins if there is a high clinical suspicion for CML. Importantly, the e1a2 transcript (encoding for p190), if dominant, has been shown to be a predictor of inferior survival.[6] Only the dominant, clinically relevant, transcript need be followed for clinical management (**see Appendix A: Example of a *BCR::ABL1* Testing Algorithm at Diagnosis**). Of note, in 2-5% of cases, rare variant translocations involving multiple chromosomes or variant transcripts, such as the e19/a2 *BCR::ABL1* fusion form encoding for p230, [5,7] may lead to discordant results between fluorescence in situ hybridization (FISH) and qPCR. Monitoring these patients may be challenging as currently no standardized qPCR assays are available for these variant cases,[8,9] although newer technologies, such as genomic DNA qPCR and multiplex droplet digital qPCR, have been explored and may offer alternative monitoring approaches.[10,11]

FISH may also be used to detect t(9;22), and is agnostic to the breakpoints.[2, 12] Depending on the laboratory test menu and workflow, FISH results may be available more quickly than PCR-based testing. This may be helpful in cases where therapy needs to be initiated more quickly as in blast phase CML, acute myeloid leukemia with *BCR*::*ABL1* or B lymphoblastic leukemia (B ALL) with *BCR*::*ABL1*. Metaphase karyotyping will also detect most *BCR*::*ABL1*, though rare cases may be cryptic and turnaround time is slower, so other tests are usually preferred for diagnosis. Bone marrow karyotype is an important prognostic indicator in CML and may indicate disease progression. Thus, bone marrow examination and karyotype are recommended at baseline.[2,13]

Anchored multiplex reverse transcriptase PCR based NGS is a newer technology which can identify fusions and breakpoints. This has the benefit of not only identifying the usual p190, p210, and p230 forms but also other lymphoid/myeloid neoplasms with kinase fusions such as *ETV6*::*ABL1* which may clinically mimic CML.[14] A summary of the various uses and limitations of the different assays in presented in **Appendix B**.

The information in this module will allow pathologists to assist their clinical colleagues with the appropriate use of peripheral blood *BCR::ABL1* testing, emphasizing the need for qualitative testing to detect and identify transcript present at diagnosis to diagnose CML and facilitate future monitoring.

INSIGHTS

1. RT-PCR to detect *BCR*::*ABL1* e13a2 and e14a2 (p210) transcripts is an appropriate test for diagnosis in suspected CML. A qualitative test multiplexed with reactions for other transcripts including those encoding for p190 can be performed first, then reflexed to the appropriate quantitative assay. This strategy is typically offered by large reference laboratories.
2. Locally available testing may vary, regardless of how the presence of *BCR*::*ABL1* is confirmed, it is essential to determine which transcript is present for future monitoring. Quantitative RT-PCR at baseline is also recommended.
3. Use of multiple quantitative tests (eg. both p190 and p210) for diagnosis may miss 2-5% of cases but could be used for initial diagnosis if that is the most readily available assay for your laboratory. If these tests are negative and there is a high clinical concern for CML, additional testing to detect other transcripts is required.
4. FISH is widely available and has a fast turnaround time. It is also positive in unusual fusion variants, but this testing must also be followed by identification of the transcripts present.

INTERVENTIONS

1. Emphasize the use of the diagnostic algorithm best suited for your laboratory.
    a. Arrange search results so that the qualitative test appears first when searching for "*BCR*::*ABL1*"
    b. Rename test to "*BCR*::*ABL1* for CML diagnosis"
2. Ensure appropriate follow-up testing to identify the transcript present.
    a. Build a reflex to the appropriate test(s) after a positive assay **See Appendix A** for an example algorithm.

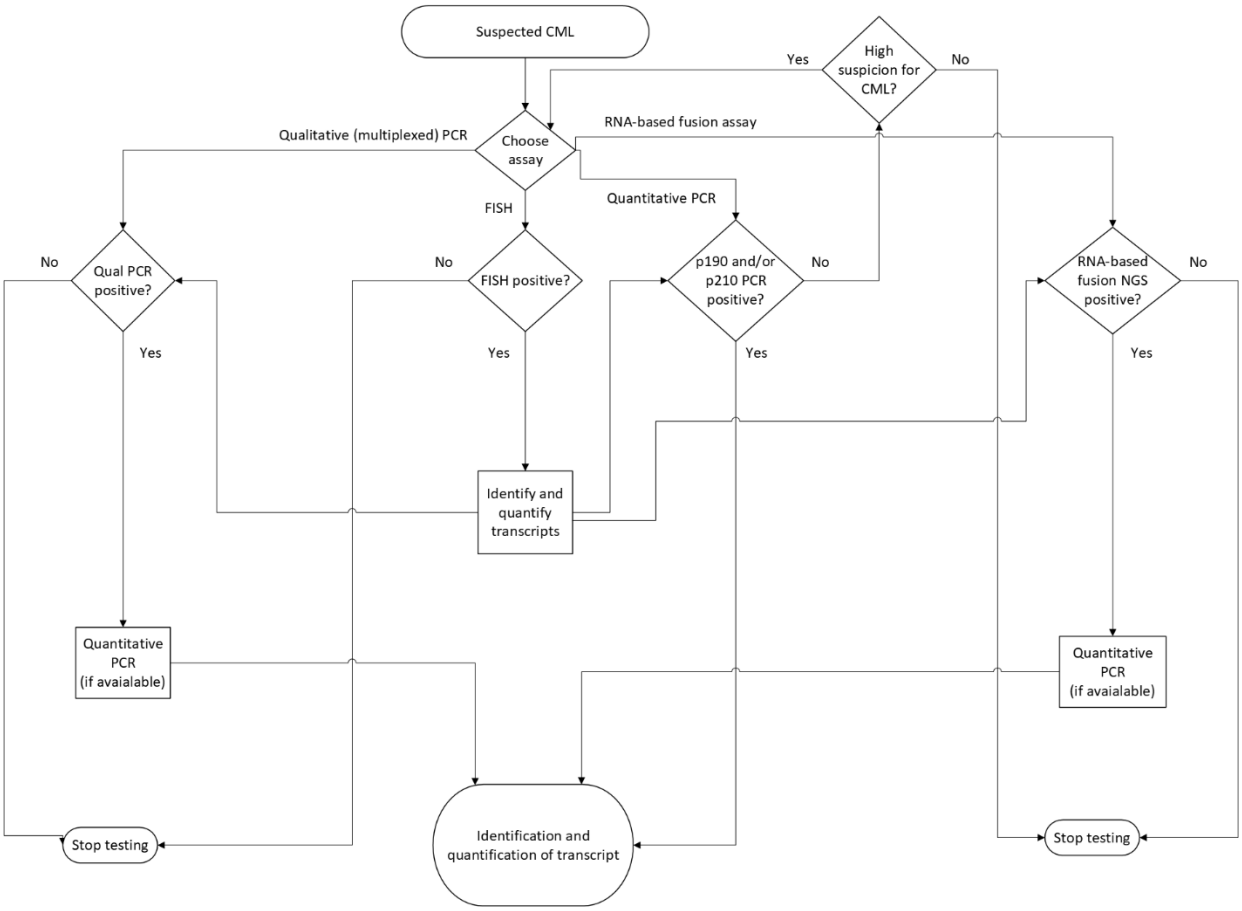
b. Ensure that the interpretive report for qualitative testing includes appropriate follow-up testing. For example, in the 5-7% of CML which show a dominant p210 transcript and low-level minor p190 transcript, recommend using the quantitative p210 assay for monitoring.

3. Educate that a negative assay for *BCR*::*ABL1* p210 does not exclude CML, such as a comment applied to negative results.

INTERVENTION ANALYSIS

Monitor how often quantitative *BCR*::*ABL1* testing is ordered prior to the qualitative test being resulted before and after the intervention. If the quantitative test is frequently being ordered prior to qualitative, this may indicate the need for targeted education or a stronger intervention such as a “best practice alert” or other EMR intervention.

APPENDIX A
EXAMPLE OF A *BCR*::*ABL1* TESTING AT DIAGNOSIS ALGORITHM



Karyotyping may be used in place of FISH but generally has slower turnaround time and some fusions may be cryptic. CML, chronic myelogenous leukemia; FISH, Fluorescence in situ hybridization; NGS, next-generation sequencing; PCR, polymerase chain reaction; Qual PCR: multiplexed, qualitative PCR RNA, ribonucleic acid.

APPENDIX B
Capabilities and limitations of different *BCR*::*ABL1* assays.

	Detects *BCR*::*ABL1*?	**Identifies specific transcript?**	**Quantifies transcript?**	**Subsequent/ reflex testing needed**	**Comments**
FISH	Yes	No	No	1. Identify transcript 2. Quantify transcript	Fast TAT, specimen more stable, widely available. Can detect, but not identify rare breakpoints
Multiplexed PCR	Yes	Yes	No	1. Quantify transcript	Available from some reference laboratories
Quantitative PCR	Yes if p190 or p210. No for others	Yes if p190 or p210. No for others	Yes	If negative and high clinical suspicion, NGS or multiplexed PCR to detect rare transcript	Detects, identifies, and quantifies transcript present for majority of cases
RNA-based fusion NGS	Yes	Yes	No	1. Quantify transcript	Mostly in academic centers and reference laboratories

QUESTIONS AND ANSWERS

QUESTION 1 OBJECTIVE
Understand the indications for different *BCR*::*ABL1* tests.

QUESTION 1
A 58-year-old man presents with neutrophilic leukocytosis, left shift without blasts and thrombocytosis. Which of the following statements regarding testing for *BCR*::*ABL1* fusion are correct?
A. Quantitative testing should be performed initially because one test will detect any transcript type present.
B. Identification of the transcript by multiplexed qualitative RT-PCR at baseline is sufficient.
C. The transcript should be identified and quantified at baseline.
D. Conventional karyotyping has no role in this scenario.
E. PCR testing is not indicated, FISH is required due to decreased turnaround time

The correct answer is C. The transcript should be identified and quantitated for subsequent monitoring of response to therapy.
A is incorrect. Quantitative RT-PCR tests are designed to detect specific transcripts. If a CML has a p210 and only p190 is tested, this will be negative and not diagnostically useful.
B is incorrect. The baseline transcript number is needed prior to therapy to monitor disease response.
D. is incorrect. Karyotype does provide prognostic information in CML, however due to slow turnaround time is not typically used for initial diagnosis.
E. is incorrect. FISH results may be available more quickly but will neither identify nor quantitate the BCR::ABL1 transcript.

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QUESTION 2 OBJECTIVE

Understand the limitations of molecular *BCR*::*ABL1* assays for CML diagnosis.

QUESTION 2

A 53-year-old man presents with an enlarged spleen and persistent neutrophilia. Testing is performed to evaluate for chronic myeloid leukemia (CML). *BCR::ABL1* rearrangement is identified on a peripheral blood sample by fluorescence in situ hybridization (FISH), however, qualitative *BCR::ABL1* PCR for p210 and p190 transcripts are negative. Possible causes for these discordant cytogenetic and molecular results are:

A. Peripheral blood is not a suitable specimen for *BCR::ABL1* testing and may cause unreliable results.
B. The patient has a variant p210 or a p230 *BCR::ABL1* transcript.
C. The FISH result is a false positive.
D. Quantitative PCR (qPCR) should have been ordered instead of qualitative PCR.
E. The *BCR::ABL1* transcript level is below of the analytic sensitivity of the PCR assay.

The correct answer is B. Discordant cytogenetic and molecular results may indicate the presence of a variant *BCR::ABL1* transcript (i.e. p230 or rare p210 variants).
A is incorrect. Peripheral blood is a suitable specimen, closely mirroring *BCR::ABL1* testing on bone marrow.
C is incorrect. FISH reliably detects standard and variant *BCR::ABL1* rearrangements.
D is incorrect. At diagnosis, qualitative PCR should be ordered first with reflex to a quantitative assay.
E is incorrect. Prior to TKI therapy (i.e., at diagnosis), the *BCR::ABL1* transcript level will be high and sufficiently above the limit of detection of the PCR assay.

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QUESTION 3 OBJECTIVE

Understand the utility and limitations of different *BCR*::*ABL1* tests.

QUESTION 3

Which of the following is true regarding tests for *BCR*::*ABL1*?

A. Multiplexed qualitative RT-PCR can detect all known *BCR*::*ABL1* transcripts.
B. *BCR::ABL1* will only be detected in and is specific for CML
C. Turnaround time less than one week is not needed for *BCR*::*ABL1* testing.
D. Conventional karyotyping can detect all *BCR*::*ABL1* fusions.
E. Rare transcripts may only be detectable by specialized testing (e.g. NGS fusion panel).
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The correct answer is E. There are some rare fusions which may be identifiable only via specialized testing and may not have specific RT-PCR tests available.
A is incorrect. Multiplexed qualitative PCR is designed for the most common *BCR*::*ABL1* transcripts but does not detect all.
B and C are incorrect. *BCR*::*ABL1* can also be present in acute myeloid leukemia (AML) and B ALL. In this disease, and CML presenting in blast phase, it may be necessary to have results more quickly to initiate TKI therapy.
D is incorrect. Some *BCR*::*ABL1* fusions may be cryptic on karyotyping.

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