

***BCR-ABL1* Translocation in Leukemia**

The UNC Hospitals Molecular Genetics Laboratory performs quantitative reverse transcription PCR (Q-rtPCR) to measure *BCR-ABL1* p210 and p190 transcripts in leukemia patients.

Chronic Myelogenous Leukemia

Chronic myelogenous leukemia (CML) is characterized by the Philadelphia chromosome (Ph¹), a shortened chromosome 22 resulting from a t(9;22) *BCR-ABL1*. The fusion gene encodes chimeric RNA that is translated into chimeric protein (called p210 because its size is approximately 210kd) functioning as an overactive ABL1 tyrosine kinase that is largely responsible for myeloid cell proliferation.

BCR-ABL1 translocation characterizes CMLs. About 5% of suspected CML cases have an occult translocation that is not evident on traditional karyotype but is detectable by molecular methods (rtPCR or FISH). The advantage of karyotype is the genome-wide survey that may reveal additional gross chromosomal abnormalities that are harbingers of accelerated phase or blast crisis. At initial diagnosis, karyotype of marrow is indicated along with baseline Q-rtPCR aimed at defining markers by which to measure residual disease during therapy.

Tyrosine kinase inhibitor therapy is considered a success if levels in blood by Q-rtPCR rapidly fall to IS % ratio <10 at 3 month & 6 month timepoints, and further fall to < 0.1 at 18 months. (0.1 is considered a “major molecular response”.) Blood levels are typically monitored every 3 months until there is a complete cytogenetic response and then every 3 to 6 months; rising levels (by ten-fold, a one-log increase) in a compliant patient trigger confirmatory testing, marrow cytogenetics, and DNA sequencing of the *ABL1* gene to identify an actionable drug resistance mutation. Following transplant, *BCR-ABL1* rtPCR is recommended every 3 months for the first 2 years, and every 6 months for the next 3 years. Levels are used to assess the efficacy of therapy and the risk of relapse, which in turn influences clinical management decisions.

Acute leukemia

Patients with *de novo* acute lymphoblastic leukemia (ALL) harboring t(9;22) *BCR-ABL1* are candidates for tyrosine kinase inhibitor therapy. Such translocation occurs in about 15% of *de novo* ALL (30% of adult and 3% of childhood ALL), of which a quarter involve the major breakpoint cluster region (p210) and the remaining three quarters involve the minor cluster region (p190). Rarely, *BCR-ABL1* is identified in acute myeloid leukemia (AML), either *de novo* AML or AML arising as blast crisis of CML. Blast crisis has a p210 breakpoint, whereas *de novo* AML usually has a p190 breakpoint and myelomonocytic differentiation. At initial diagnosis, Q-rtPCR for both p190 and p210 *BCR-ABL1* breakpoints is indicated to identify a tumor-specific marker that can be used to monitor residual disease in follow-up marrow specimens.

Laboratory Testing for *BCR-ABL1* translocation by Q-rtPCR:

Specimens must be **delivered promptly** to the laboratory to minimize RNA degradation. For CML the preferred sample is EDTA blood (3mL purple-top). In acute leukemia, EDTA marrow is preferred (0.5mL). RNA is extracted and converted to cDNA that is PCR-amplified using primers flanking the p210 or p190 translocation breakpoint, and a fluorescent *ABL1* probe permits measurement of products in real time.^{5,6} A separate control assay targeting *ABL1* cDNA normalizes for the amount of amplifiable cDNA in the sample. The *BCR-ABL1* p210 rtPCR assay is calibrated to the international standard (IS), and results are expressed in “IS % ratio” units. Assay sensitivity is estimated at .001 IS % ratio, which corresponds to about 1 translocation positive cell per 100,000 cells. *BCR-ABL1* p190 assay results are expressed as the number of “positive cells per 100,000 cells”, and assay sensitivity level is estimated at 1 in 10,000 cells. Low level translocation (< 10 in 100,000 cells) may not signify malignancy. In serial specimens, changes of 10-fold (one log) are considered to be significant.

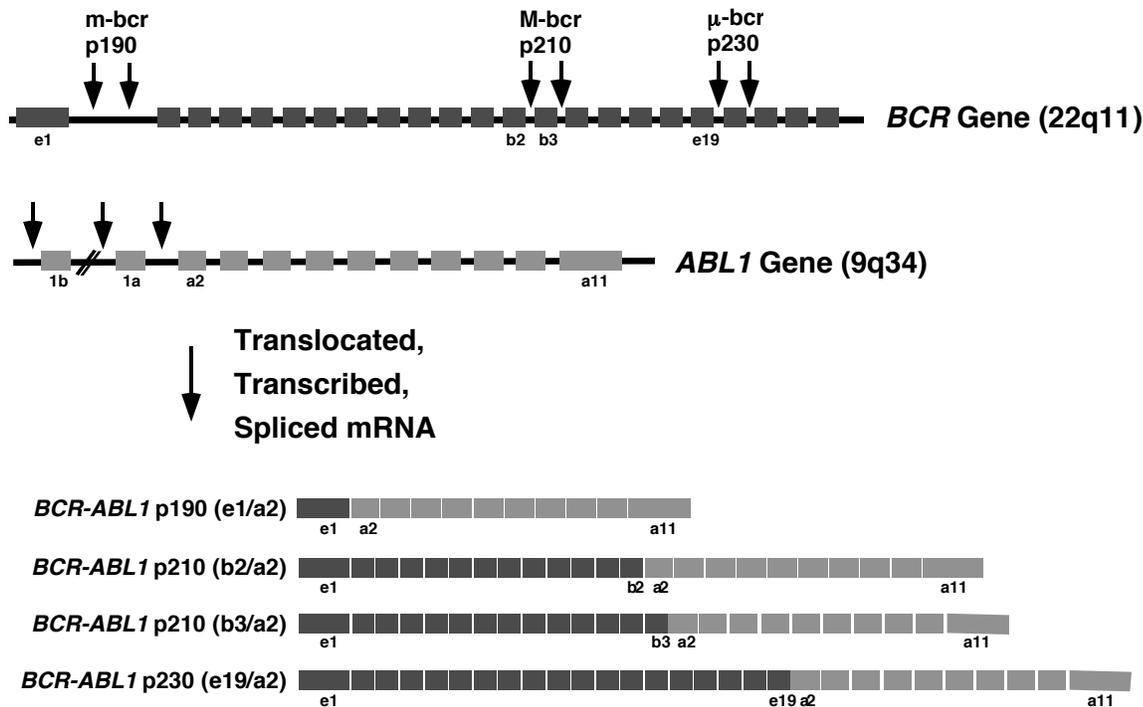


Figure 1: Maps of the *BCR* and *ABL1* genes are shown, along with four types of *BCR-ABL1* fusion transcripts that are found in various leukemias. Breakpoints in the *ABL1* gene are spread across 90 kb in intron 1, and the *ABL1* tyrosine kinase domain resides in exons 2 through 11. The *BCR* gene has three separate breakpoint cluster regions. A p210 break occurs in a 5.8kb major breakpoint cluster region (M-bcr) spanning the region around exon b3. In the resulting *BCR-ABL1* p210 chimeric transcript, *BCR* exon b2 or b3 is fused with *ABL1* exons 2 through 11. A p190 break occurs in the minor breakpoint cluster region (m-bcr) in intron 1, resulting in fusion of *BCR* exon 1 to the same *ABL1* exons. Rare leukemias express a larger p230 variant of *BCR-ABL1* protein arising from a break in the μ-bcr region that fuses *BCR* exon 19 with the same *ABL1* exons, and testing for this rare variant is available at outside laboratories. Testing for *BCR-ABL1* translocation assists in diagnosis, classification, and monitoring minimal residual disease following therapy.

References:

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To consult a pathologist about indications for testing or the significance of a result, call the Molecular Genetics Lab at (919) 966-4408 or Dr. Gulley at 843-4595. E-mail margaret_gulley@med.unc.edu

Website, http://labs.unchealthcare.org/directory/molecular_pathology/index_html