CML and Ph+ ALL Molecular Monitoring and Mutation Testing Glossary

THINK | TEST | TREAT
Because monitoring and testing matters

Molecular monitoring

Abelson murine leukaemia viral oncogene homolog 1 *(ABL1* or *ABL)*: a wild-type gene located on chromosome 9 that encodes the ABL1 protein, which functions as a tyrosine kinase involved in a variety of cellular processes, including cell division, adhesion, differentiation and response to stress.[1]

***ABL1* control gene:** a gene used as a wild-type reference in RT-qPCR to quantify the BCR-ABL1 transcript expression level.[2]

Additional chromosomal aberrations (ACAs): emergence of ACAs in addition to the hallmark of CML, which is the cytogenetic translocation of chromosomes 9 and 22 (i.e. t(9;22)(q34;q11)).[3]

***Breakpoint cluster region* (*BCR*):** a gene located on chromosome 22 that encodes the BCR protein of unknown function, which has serine/threonine kinase activity.[4]

BCR-ABL oncoprotein: the protein encoded by the *BCR-ABL1* fusion gene, which results from reciprocal translocation between chromosomes 9 and 22. The formation of constitutively active kinase activity leads to disruption of intracellular downstream signalling involved in processes such as proliferation and transcription.[5]

- **P190:** the oncoprotein formed by the translocation of *BCR* exon 1 on chromosome 22 to the *ABL* gene on chromosome 9.[6]
- **P210:** the oncoprotein formed by the translocation of *BCR* exons 1–13/14 on chromosome 22 to the *ABL* gene on chromosome 9; >95% of CML patients have the P210 BCR-ABL oncoprotein.[6]
- **P230:** the oncoprotein formed by the translocation of *BCR* exons 1–19 on chromosome 22 to the *ABL* gene on chromosome 9.[6]

***BCR-ABL1* halving time:** the time required for the level of *BCR-ABL1* transcript expression to reduce by half. *BCR-ABL1* halving time at 3 months has shown to be a relevant prognostic factor for overall survival and progression-free survival in imatinib-treated CML patients.[7]

***BCR-ABL1*/*ABL1* ratio:** the percentage ratio between *BCR-ABL1* and *ABL1* copies. This ratio is used to quantify the level of expression of the *BCR-ABL1* transcript and therefore, indicate the level of molecular response.[8]

***BCR-ABL1* transcripts:** a DNA or RNA copy of the *BCR-ABL1* gene encoding the BCR-ABL1 oncoprotein.[6]

- **e1a2:** the *BCR-ABL1* fusion transcript formed by the translocation of *BCR* exon 1 on chromosome 22 to the *ABL* gene on chromosome 9 encoding the P190 BCR-ABL1 oncoprotein.[6]
- **e13a2:** the *BCR-ABL1* fusion transcript formed by the translocation of *BCR* exons 1–13 on chromosome 22 to the *ABL* gene on chromosome 9 encoding the P210 BCR-ABL1 oncoprotein.[6]
- **e14a2:** the *BCR-ABL1* fusion transcript formed by the translocation of *BCR* exons 1–14 on chromosome 22 to the *ABL* gene on chromosome 9 encoding the P210 BCR-ABL1 oncoprotein.[6]
- **e19a2:** the *BCR-ABL1* fusion transcript formed by the translocation of *BCR* exons 1–19 on chromosome 22 to the *ABL* gene on chromosome 9 encoding the P230 BCR-ABL1 oncoprotein.[6]
- **Rare transcripts:** *BCR-ABL1* fusion transcripts resulting from rare BCR breakpoints outside of the three defined cluster regions (M-*bcr*, m-*bcr* or µ-*bcr*), or unusual *ABL1* breakpoints (e.g., e1a2, e2a2, e6a2, e19a2, e1a3, e13a3 and e14a3). Rare transcripts can go undetected with the use of primers targeting only main fusion variants of *BCR-ABL1*.[6]

Bone marrow (BM): the sponge-like connective tissue in the centre of most bones that is the primary site of haematopoiesis and consists mainly of haematopoietic cells, marrow adipose tissue and supportive stromal cells. It exists in two forms: yellow marrow which consists mainly of fat cells and red marrow which is the haematopoietic tissue involved in erythrocyte and leukocyte production.[9]

Chimerism analysis: analysis of the percentage of donor versus recipient cells in the recipient's peripheral blood or bone marrow after haematopoietic stem cell transplantation.[10]

Chromosome banding analysis (CBA; also called conventional cytogenetic testing or karyotype analysis): analysis of the number and structure (banding) of condensed chromosomes in order to identify any abnormalities. Chromosomes are stained with G-(Giemsa) banding methods which lead to characteristic staining patterns. CBA can be used to detect the Philadelphia (Ph) chromosome.[5,11]

Complementary DNA (cDNA): synthesised from a single-stranded mRNA template during reverse transcription.[12]

Cytogenetic response (CyR): a response to treatment that occurs in the bone marrow, rather than just in the blood, and refers to a reduction in Ph+ chromosome readings from the point of diagnosis.[13]

- **Complete cytogenetic response (CCyR):** when no Ph+ metaphases can be detected by conventional cytogenetic testing or <1% *BCR-ABL1* positive nuclei can be detected by fluorescence *in situ* hybridisation (FISH).[13]
- **Partial cytogenetic response (PCyR):** when 1–35% Ph+ metaphases are present within the bone marrow sample.[13]
- **Major cytogenetic response (MCyR):** when 0–35% Ph+ metaphases are present within the bone marrow sample (CCyR + PCyR).[13]
- **Minor/minimal cytogenetic response (mCyR):** when 36–95% Ph+ metaphases are present within the bone marrow.[13]

Disease progression (also referred to as progressive disease): the continued growth and spread of disease.[9]

Deoxyribose nucleic acid (DNA): contains all the information to build and maintain an organism. It is a linear molecule composed of four types of nucleotide bases: adenine, thymine, guanine and cytosine. The order of these bases determines the genetic sequence.[9]

Failure response: according to the European LeukemiaNet (ELN) recommendations for CML, treatment failure is defined as below, indicating that the patient should receive a different treatment:[3]

- At 3 months: *BCR-ABL1* transcript level >10% according to the International Scale, if confirmed within 1–3 months
- At 6 months: *BCR-ABL1* transcript level >10% according to the International Scale
- At 12 months: *BCR-ABL1* transcript level >1% according to the International Scale
- Then, and at any time: *BCR-ABL1* transcript level >1% according to the International Scale, or presence of resistance mutations or high-risk ACA.

Flow cytometry: a laser-based technique used for multiparameter analysis of cell surface and intracellular molecular expression, characterisation and definition of different cell types in a heterogeneous cell population, evaluation of sample purity, and the analysis of cell size and volume. In ALL, flow cytometry can be used to detect leukaemic immunophenotypes with a sensitivity of 0.01%.[14]

Fluorescence-activated cell sorting (FACS): the separation of a cell population by flow cytometry based on differential fluorescent labelling.[15]

Fluorescence *in situ* hybridisation (FISH): a cytogenetic technique that utilises fluorescently labelled DNA or RNA probes to identify specific DNA sequences on chromosomes. FISH can be used to detect the Philadelphia chromosome with a higher sensitivity than CBA. Furthermore, unlike CBA, peripheral blood samples (rather than bone marrow) can be used.[5,9]

***Glucuronidase beta* (also called *β-glucuronidase; GUSB*):** a control gene sometimes used as a wild-type reference in PCR to quantify the *BCR-ABL1* transcript expression level. *GUSB* may be a more accurate control gene than *ABL1*, and its expression level has been shown to mirror that of *BCR-ABL1* during tyrosine kinase inhibitor (TKI) treatment.[2]

Haematological response (HR): refers to the normalisation of blood cell counts, measured by the evaluation of complete blood counts, white blood cell (WBC) differential and assessment of spleen size.[4]

- **Complete haematological response (CHR):** in CML, CHR is a complete normalisation of the blood count, where WBC count is $<10 \times 10^9$/L, platelet count is $<450 \times 10^9$/L, there are no immature granulocytes in the differential and the spleen is non-palpable.[4]

International Scale (IS): the scale used to standardise the reporting of RT-qPCR results indicative of molecular response to treatment. This scale is based on the ratio of *BCR-ABL1* to the control gene (*ABL1* or *GUSB*), and is expressed as a log reduction.[1]

Minimal residual disease (MRD): the presence of residual leukaemic cells below the threshold of detection by conventional morphological methods.[16]

- **MRD positivity:** when a patient is in complete haematological remission, but not in complete molecular remission. MRD positivity is a strong predictor of haematological relapse following allogeneic transplantation in Ph+ ALL.[17]
- **MRD negativity:** when a patient is in complete haematological remission and molecular remission (MRD not detected by sensitive molecular probe[s]).[17]
- **Persistent MRD:** the presence of residual leukaemic cells post-consolidation therapy. This is associated with an increased chance of relapse and poor prognosis.[16]

Molecular response (MR): refers to reduced *BCR-ABL1* transcript expression, when measured by qRT-PCR, or another molecular test.[2,13]

- **Major molecular response (MMR; MR3.0):** undetectable *BCR-ABL1* mRNA transcripts by RT-qPCR in two consecutive blood samples (≥3 log reduction in *BCR-ABL1* transcript level from baseline; expression level of ≤0.1% on the International Scale).[13]
- **MR4.0:** ≥4 log reduction in *BCR-ABL1* transcript level from baseline (*BCR-ABL1* transcript expression level of ≤0.01% on the International Scale).[2]
- **MR4.5:** ≥4.5 log reduction in *BCR-ABL1* transcript level from baseline (*BCR-ABL1* transcript expression level of ≤0.0032% on the International Scale).[2]
- **MR5.0:** ≥5 log reduction in *BCR-ABL1* transcript level from baseline (*BCR-ABL1* transcription expression of ≤0.001% on the International Scale).[2]
- **Deep molecular response (DMR):** ≥4 or 4.5 log reduction in *BCR-ABL1* transcript level from baseline (MR4.0 or MR4.5).[5]
- **Early molecular response (EMR):** a *BCR-ABL1* transcript level of ≤10% on the International Scale at 3 or 6 months.[5]

Messenger RNA (mRNA): the single-stranded molecular product of transcription that carries sections of the DNA code to other parts of the cell for translation into respective proteins.[9]

Optimal response: according to the ELN recommendations for CML, optimal response is defined as below, indicating that no change of therapy is required:[3]

- At 3 months: *BCR-ABL1* transcript level ≤10% according to the International Scale
- At 6 months: *BCR-ABL1* transcript level ≤1% according to the International Scale
- At 12 months: *BCR-ABL1* transcript level ≤0.1% according to the International Scale
- Then, and at any time: *BCR-ABL1* transcript level ≤0.1% according to the International Scale.

Peripheral blood (PB): the flowing, circulating blood of the body, which is comprised of erythrocytes, leukocytes and thrombocytes.[9]

Philadelphia (Ph) chromosome: the abnormal chromosome formed from the translocation of a segment of chromosome 9 to chromosome 22. This translocation gives rise to the *BCR-ABL1* fusion gene.[9]

Real-time quantitative-polymerase chain reaction (qRT-PCR; qPCR; RQ-PCR; RT-qPCR): a variation of RT-PCR that is used not only to detect and amplify RNA transcripts, but also to characterise and quantify them within a sample. In CML, qRT-PCR is performed using a peripheral blood sample to monitor *BCR-ABL1* transcript levels during treatment.[12,18]

Reverse transcription-polymerase chain reaction (RT-PCR): a variation of PCR where RNA transcripts can be qualitatively detected and amplified via reverse transcription to cDNA. In CML, RT-PCR is performed at diagnosis using a peripheral blood sample to detect the *BCR-ABL1* transcript and assess the transcript type.[9,17]

Ribonucleic acid (RNA): a linear molecule synthesised from DNA by RNA polymerase during transcription. It is composed of ribonucleotide bases: adenine (A), cytosine (C), guanine (G) and uracil (U). RNA is often referred to as a "template" as it carries the same genetic information as DNA, but it is not utilised for long-term storage.[9]

Translocation: in genetics, a chromosome translocation is caused by the rearrangement of parts of two chromosomes. A gene fusion may be created when the translocation joins two otherwise separated genes (e.g. in CML).[9]

Treatment-free remission (TFR): ability to maintain a molecular response after stopping therapy.[19]

Warning response (also referred to as suboptimal response): according to the ELN recommendations for CML, warning is defined as below, indicating that more frequent monitoring is recommended to permit timely change in case of subsequent treatment failure:[3]

- At baseline: high-risk ACA or high-risk ELTS score
- At 3 months: *BCR-ABL1* transcript level >10% according to the International Scale
- At 6 months: *BCR-ABL1* transcript level >1–10% according to the International Scale
- At 12 months: *BCR-ABL1* transcript level >0.1–1.0% according to the International Scale
- Then, and at any time: *BCR-ABL1* transcript level >0.1–1.0% according to the International Scale, or loss of MMR.

Mutation testing

Allele-specific oligonucleotide polymerase chain reaction (ASO-PCR; ASO RQ-PCR): a variation of PCR, which utilises primers specific to a polymorphic area, usually with mutations located at its 3' end. Amplification confirms the presence of the mutation. A sensitivity of 0.001–0.01% can be achieved with ASO-PCR.[20]

Artefacts (of PCR): PCR products formed from the amplification of non-target DNA or RNA as a result of polymerase errors, generating genomic sequence changes not present in the original sample of DNA.[21]

***BCR-ABL1* kinase domain (KD):** consists of four regions: the activation loop, the TKI binding site, the catalytic site and the adenosine triphosphate (ATP)-binding loop (P-loop).[22]

Compound mutation: two or more point mutations in the same oncogene that exist in the same allele.[23]

Clonal expansion: the formation of genetically identical daughter cells that arise from the same single cell.[24]

Denaturing high performance liquid chromatography (d-HPLC): a technique that enables the comparison of two or more chromosomes from a mixture of denatured and reannealed PCR products, revealing the presence of a mutation resulting from the differential retention times of homoduplex and heteroduplex DNA. d-HPLC has a sensitivity of 0.1–10.0%, but is not as widely available as Sanger sequencing, and false positive results are possible if the mutant subclone is abundant.[20]

Digital PCR (dPCR; also called digital droplet PCR, or ddPCR): enables real-time absolute quantification of the target on a digital platform, without the need for endogenous controls. The reported sensitivity seems to be comparable with or higher than that achieved with real-time quantitative-polymerase chain reaction,[25] but expensive panels of probes are required and many reactions must run in parallel to cover all nucleotide substitutions that may lead to resistance. In CML or Ph+ ALL, digital PCR can be used in parallel with other methods to detect only the most common *BCR-ABL1* mutations.[26]

Gatekeeper mutation: mutations in the gatekeeper residue of kinases have emerged as a key way through which cancer cells develop resistance to treatment. For *BCR-ABL1*, threonine 315 (T315) is known as the gatekeeper residue, because it maps to the periphery of the nucleotide-binding site of *ABL1*. Mutations of T315 (e.g. T315I) can cause resistance to TKIs.[27]

Half-maximal inhibitory concentration (IC_{50}): the concentration of a drug needed to inhibit a biological process by half.[20]

Ligase-PCR: a DNA sequencing method that combines PCR amplification with the ligation of small oligonucleotide linkers to target DNA.[28]

Low-level mutation: a mutation that is present at a low frequency (below the detection limit of conventional detection methods) in a leukaemic cell population.[20,29]

Mass spectrometry: an instrumental method that measures the mass-to-charge ratio of fragmented molecular ions to produce a mass spectrum, which can be interpreted to determine the identity of molecules within a sample. This method can be adapted for DNA sequencing and can achieve a sensitivity of 0.05–0.5%.[20]

Mutation subclone: a subset of cells expressing a mutation that is not present in the rest of the tumour population.[24]

Next-generation sequencing (NGS): refers to high-throughput, quantitative, non-Sanger-based DNA sequencing approaches. These technologies enable millions of DNA strands to be sequenced in parallel. NGS can achieve a sensitivity of 0.5–1.0% and is able to detect compound mutations. However, it is expensive, availability is limited and results are highly dependent on RNA sample quality.[20]

P-loop mutation: a mutation within the ATP-binding loop (P-loop) of BCR-ABL1, the most commonly mutated region of BCR-ABL1. Mutations to the P-loop may lead to changes in TKI binding efficacy.[22]

Point mutation: the alteration of a single nucleotide pair within the DNA molecule.[5]

Polyclonal mutation: two or more point mutations in the same oncogene that exist separately in different alleles.[30]

Polymerase chain reaction (PCR): a DNA amplification technique used to detect the presence of a target gene within a sample. This method exploits the use of specific primers to the target DNA, combined with the synthesising properties of DNA polymerase.[31]

Primary resistance: irresponsiveness to a drug, which is caused by an inherent characteristic that is present prior to the start of treatment.[3]

Regulatory domain regions SH2, SH3: these regions are situated outside the *BCR-ABL1* kinase domain and possess the ability to regulate the kinase domain.[32]

Resistance profile: an outline of all known mutations that convey resistance to a certain drug.[33]

Sanger sequencing (SS; also referred to as direct or conventional sequencing): a technique for DNA sequencing based upon the selective incorporation of chain-terminating dideoxynucleotides (ddNTPs) by DNA polymerase during DNA replication. The technique requires the target DNA to be replicated multiple times and analyses the products via PCR in order to determine the DNA sequence. Limitations of Sanger sequencing include a sensitivity of up to 15–20% and limited value for detecting compound mutations, but it is considered an adequate assay with a reasonable compromise between sensitivity and specificity.[20,34]

Secondary resistance (also called acquired resistance): when a patient's resistance to a drug arises whilst undergoing a treatment.[4]

Sensitivity (of techniques): the lowest level of accurate detection that can be achieved by a technique.[20]

T315I: the most common point mutation found in the *BCR-ABL1* kinase domain, which is the result of an amino acid substitution (threonine to isoleucine) at position 315.[35]

Ultra-deep sequencing (UDS): an NGS technique that involves sequencing the same genomic region multiple times. UDS can achieve a sensitivity of up to 1%.[20]

EU/ICLG/NP/20/0040
UK/ICLG/P/20/0072
Date of preparation: July 2018; updated December 2020

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