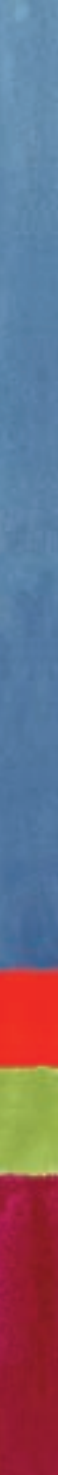


# Diagnosing, Testing, and Monitoring Chronic Myeloid Leukaemia

A guide for patients





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This booklet is dedicated to the memory of **Professor John M Goldman** (1938-2013), DM, FRCP, FRCPath, FMedSci, Emeritus Professor and Senior Research Investigator at the Division of Investigative Science Imperial College, London, UK, co-founder and Chair of the International CML Foundation (iCMLf). John Goldman's major disease interest was CML. An erudite and profoundly empathetic man, many have benefited from his observant, elegant and ever curious mind. For that gift we remain eternally grateful.

# 1. Background

## Introduction

This booklet provides an overview of the quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR) test. It includes a description of the qRT-PCR **International Standardisation (IS) project** within the context of the recently updated (2013) **ELNet** Recommendations and **NCCN** Treatment Guidelines for the treatment and management of Ph+CML based on molecular testing by qRT-PCR<sup>18</sup>.

This primer provides a useful resource for patients and others with an interest in this important topic and will contribute to the better understanding and interpretation of qRT-PCR results and response to treatment. For simplicity the test is referred to as q-PCR throughout.

We hope this primer also serves as an exemplar in a healthcare environment where patients face a lifetime on therapy, enabling an in depth understanding, not just of the disease and its current treatments, including those in development, but also test methodologies used to monitor responses to therapy.

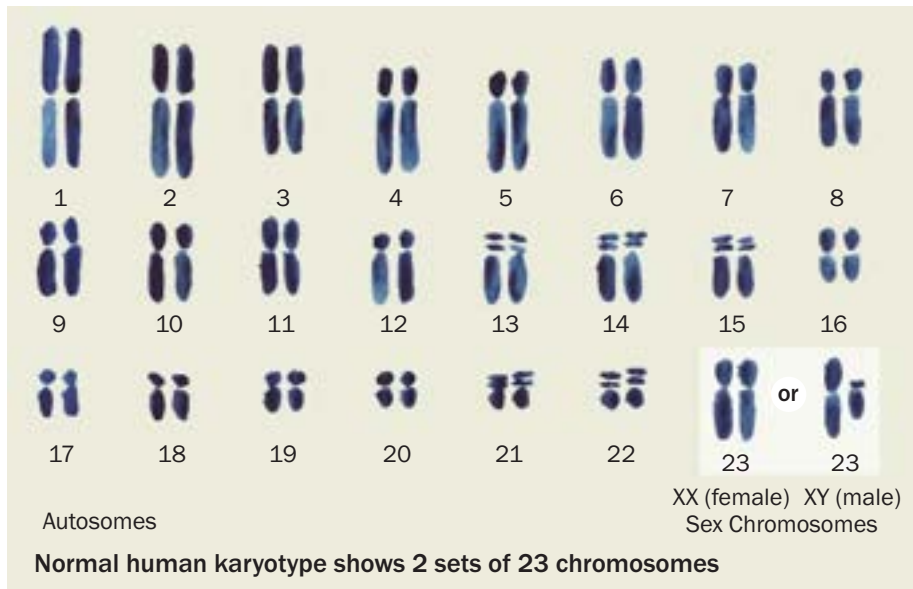
This primer has been developed following a suggestion from patient self-educators as a way to encourage a better understanding of q-PCR testing. The draft was further developed by Sandy Craine who is the author of the text on behalf of the CML Support Group.

## Inside the cell

Normally each of us inherits 23 pairs of 'homologous' chromosomes (twin pairs with the same basic structure) one set from our father and one set from our mother, making 46 chromosomes in total. Each pair is numbered from 1 to 22 according to size. Number 23 is the pair that defines gender, containing either an X and a Y chromosome in men, or two X chromosomes in women.

All 23 pairs are found in the nucleus or 'engine' of all blood cells apart from red cells.

Each chromosome has a constriction point (centromere) dividing it into two sections or 'arms'. The short arm is labelled the 'p' arm (for petite); q (chosen as the next letter after p in the alphabet) indicates the long arm. The location of the centromere gives each chromosome its characteristic shape and helps describe where specific genes are located. Changes to the basic structure of chromosomes can sometimes occur, possibly through exposure to ionizing radiation, environmental toxins or for other unknown reasons. A 'Genome' describes a complete set of DNA code; the human genome has been estimated to contain approximately 30,000 genes.



Chromosomes are not visible in the nucleus unless the cell is in its dividing phase. They are actually long strands of DNA which, during the process of cell division become tightly packed; only then are they visible under a microscope. Most of what we know about chromosomes was learned by researchers observing cells during the dividing cycle.

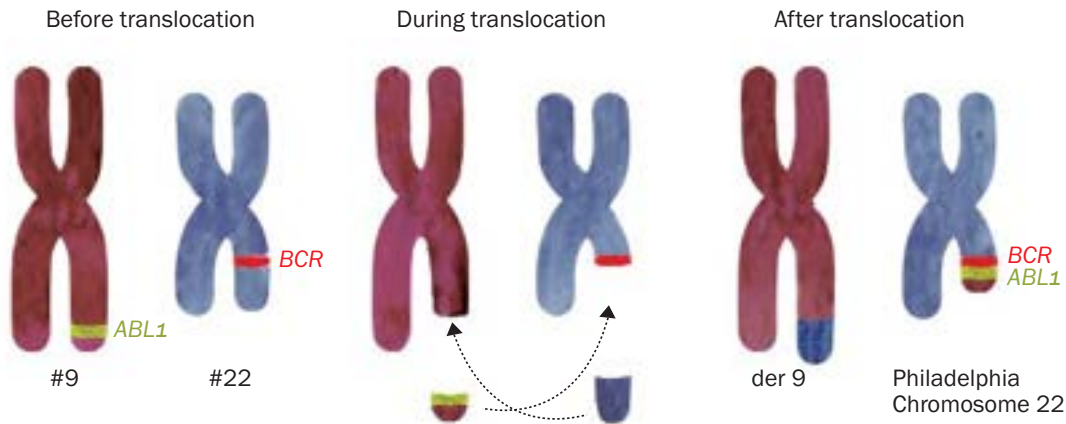
## Chronic Myeloid Leukaemia and the Philadelphia Chromosome

The Philadelphia chromosome is an acquired abnormality and is the definitive marker for CML. It is formed when chromosomes 9 and 22 swap one part each with the other. It is not yet understood why this happens. CML is not an inherited condition and as such cannot be passed on to children.

Known as a reciprocal translocation, a piece containing the *ABL1* gene from the bottom part of chromosome 9 breaks off and attaches to a region on chromosome 22 where the *BCR* gene is located, thus forming a new chromosome 22 containing the abnormal fusion gene *BCR-ABL1*

The new shortened chromosome 22 is called the Philadelphia chromosome. You might also see it written as t(9; 22). The *BCR-ABL1* fusion gene makes a protein called a tyrosine kinase. These kinds of proteins are located on or near the surface of cells and send signals that speed up cell division.

Ph+CML is a rare disease with an incidence of 1 to 1.5 new cases per 100,000 people each year. It is very rare in young people under 19 and ultra-rare in young children.



**A 'reciprocal translocation' of *ABL1* and *BCR* results in an abnormal chromosome 22—The Philadelphia Chromosome**

In approximately 95% of cases, the Ph chromosome is detected by cytogenetic analysis. In around 5% of the remaining suspected cases, the Ph chromosome is not visible. However, the *BCR-ABL1* fusion gene can be identified by molecular testing with q-PCR in approximately half of those cases. For simplicity, any disease that contains the *BCR-ABL1* fusion gene is referred to as Ph+ CML. If you do not have the Ph chromosome or evidence of the *BCR-ABL1* gene then you do not have CML.

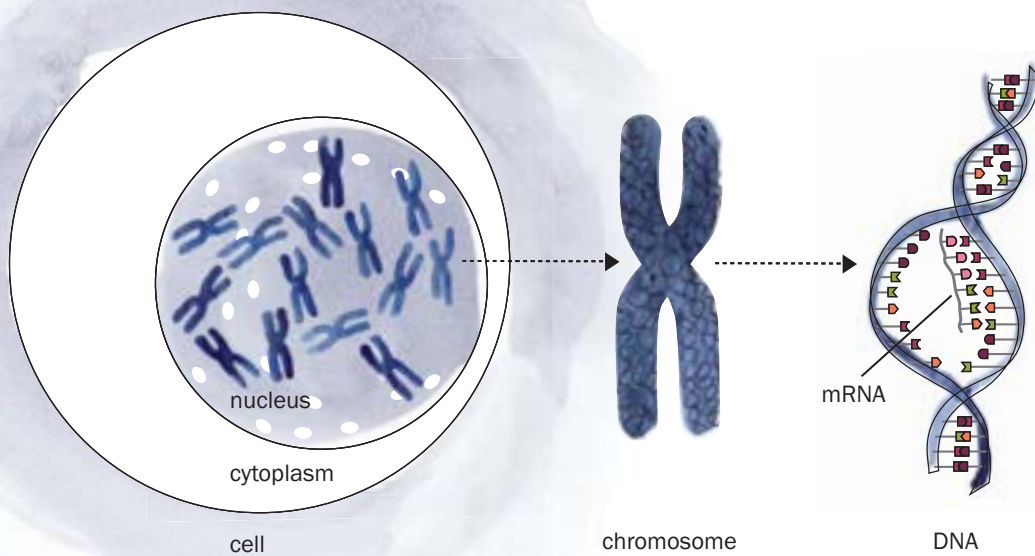
It has been shown that the *BCR-ABL1* fusion gene is the single definitive marker for Ph+ CML and remains the key abnormality throughout the chronic phase of the disease.

The *BCR-ABL1* fusion gene translates as a protein tyrosine kinase, referred to as Bcr-Abl1. The activity of tyrosine kinases is typically controlled by other molecules, but the mutant tyrosine kinase produced by the *BCR-ABL1* gene means the signal for cell division is always 'switched on'. Its continuous activity sets up a cascade of events that allows for unregulated cell division (cancer). Over time the Ph+ cells overpopulate the marrow, crowding out normally functioning cells.

Since the introduction of targeted therapy with tyrosine kinase inhibitors (TKIs), it is increasingly evident that, at least in chronic phase, when cells containing the *BCR-ABL1* gene are reduced to very low levels, the risk of disease progression is significantly reduced.

Genes are denoted with italicized capitals as in *ABL1*; *BCR* etc., whereas proteins are denoted with non-italicized capitals, sometimes followed by lower case, as in Bcr-Abl1.





A cell nucleus showing chromosomes, DNA and its duplicate mRNA (visible inside the two strands)

## Genetic Tests used at diagnosis and in the first months of TKI therapy

Some sort of genetic testing will be done to look for the Philadelphia chromosome and/or the *BCR-ABL1* gene. The following types of tests can confirm or deny a diagnosis of CML.

### **Cytogenetics: also called Karyotyping**

Chromosomes can only be seen when cells are in the dividing phase. Blood or marrow samples are cultured in the lab so that the cells begin to grow and divide, although this is not always successful. The dividing cells are looked at under a microscope to assess the number of immature vs mature cells as well as changes to chromosomes (pieces of DNA) and, in the case of CML, to detect the Philadelphia chromosome. Sensitivity is limited, typically detecting 1 out of 20 cells tested. Even when the Philadelphia chromosome is not seen, other tests can confirm the presence of the *BCR-ABL1* gene.

## Qualitative PCR

The polymerase chain reaction based qualitative test is used to diagnose Ph+CML by confirming whether or not *BCR-ABL1* gene transcripts (copies) are present in a blood and/or bone marrow sample. It can detect very small amounts of *BCR-ABL1*, even when the Philadelphia chromosome is not detected in bone marrow cells with cytogenetic testing.

## FISH: Fluorescence in situ hybridization

A more sensitive method than cytogenetics, testing upwards of 50 to 200 cells. FISH uses probes labelled with fluorescent dyes which 'light up' the fused *BCR-ABL1* gene sequence. Fluorescent probes are sections of single strands of DNA complementary to the specific portions of the DNA of interest, in this case the *ABL1* and *BCR-ABL1* genes. When slides are examined using a special microscope, the genes that match the DNA probe appear as bright spots on a dark background. The test determines the percentage of cells in a sample containing the *BCR-ABL1* gene. It can be used on either blood or bone marrow samples without the need to culture the cells, so results are available more quickly than with conventional cytogenetics.

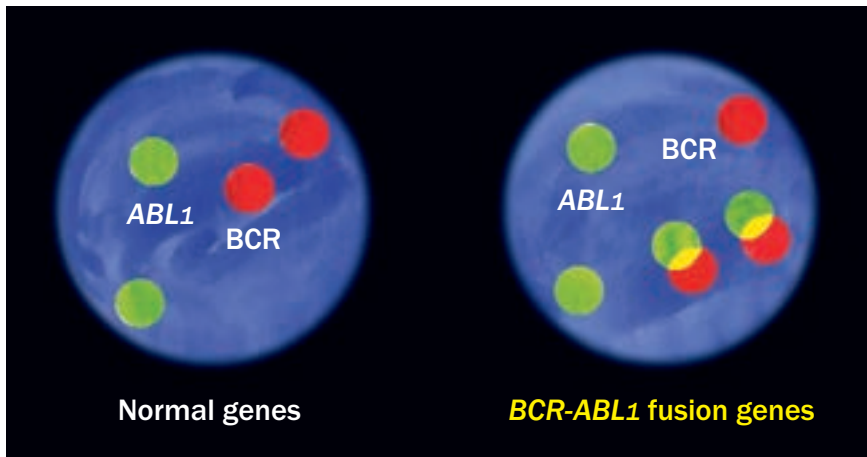


Illustration of a FISH test: the fusion gene *BCR-ABL1* shows up as yellow – as in example on the right

## 2. Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR)

### What the test measures and its relationship to other tests.

At diagnosis, virtually every white cell in a blood or marrow sample will be leukaemic (Ph+) so the result should, in theory, be 100% Ph+. However, because there are higher levels of Ph+ cells present at diagnosis, q-PCR testing is not accurate, which is why Ph positivity varies between 50% and 100%. This test may be used to establish a baseline value of Ph+ cells at diagnosis.

After the start of therapy q-PCR is used at specific time points after cytogenetic/FISH tests. Once tests show that the Ph+ cell population has reduced to less than 10%, q-PCR testing can more accurately quantify the amount of residual disease left in the marrow.

The goal of TKI therapy is to reduce the abnormal *BCR-ABL1* gene to a deep molecular level, preferably to at least 0.1% (MMR/MR3).

During the first 3, 6, 9 and 12 months of therapy Ph+ cells should reduce significantly. When the level of Ph+ cells falls below 1% q-PCR testing is extremely accurate and will be used to monitor the stability of a molecular response. Under ideal conditions, this test can detect 1 Ph+ cell in every 100,000 cells, although more commonly it detects 1 Ph+ cell in every 10,000.

### The *BCR-ABL1* gene and its mRNA, the Protein Tyrosine Kinase Bcr-Abl1

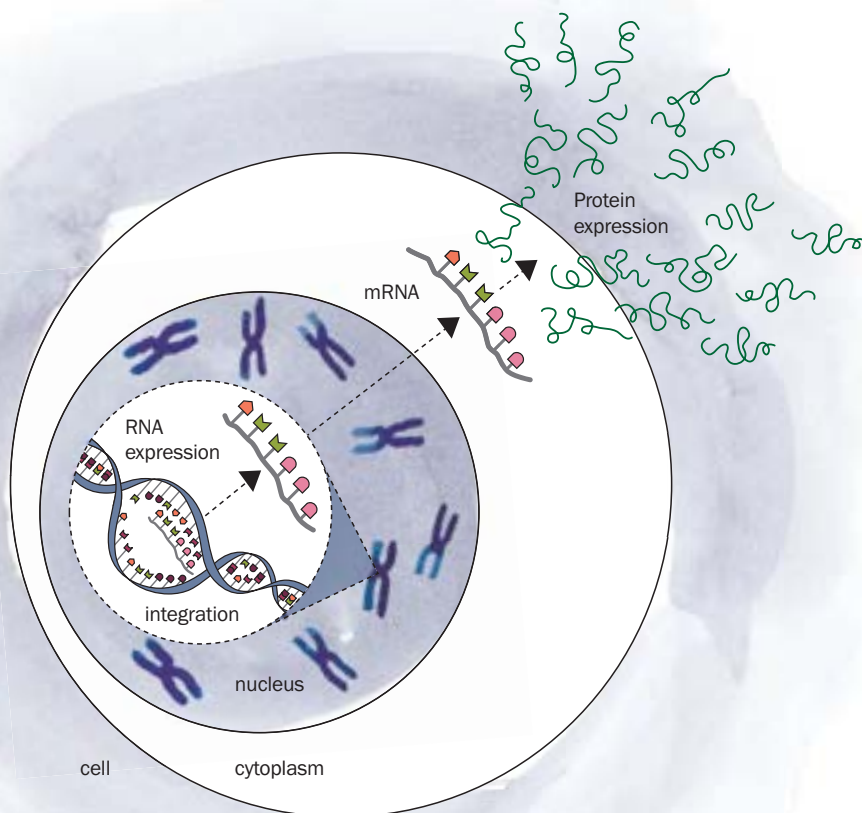
Chromosomes are found in a cell's nucleus and are made up of tightly wound stretches of DNA, the genetic code essential for the life of the cell and therefore the life of the individual.

The nucleus is a protected environment, nothing can get inside it and neither can DNA move outside it. In order to deliver instructions (as code) for a myriad of cell processes, short stretches of DNA are

duplicated as molecules known as RNAs, of which there are several forms. The RNA we are interested in here is messenger RNA or mRNA.

RNA travels outside the nucleus into the cell cytoplasm where protein tyrosine kinases are formed. Proteins express signals, setting in motion a variety of cell processes including division, proliferation and cell death (apoptosis).

In CML the *BCR-ABL1* gene duplicates its coded instruction as a messenger RNA (mRNA). In this form the DNA code moves outside of the nucleus into the cytoplasm where the protein Bcr-Abl1 signals the Ph+ cells to divide in a deregulated (leukaemic) manner.



Tyrosine kinase inhibitors (TKIs) target the abnormal protein Bcr-Abl1, effectively blocking the signal for continuous Ph+ cell division. This reduces the abnormal Ph+ cell population to very low levels along with the clinical manifestations of CML.

Continued TKI therapy is highly effective over the longer term allowing the majority of patients to live out their normal life-span.

## How Q-PCR testing works in practice

Q-PCR testing extracts the available mRNA in a blood or marrow sample. A test result is expressed as a percentage showing the ratio between of mRNA from normal control gene transcripts, for example *ABL1* or *BCR*, compared to mRNA from the abnormal *BCR-ABL1* gene transcripts present in a sample.

To perform the test, samples of blood or bone marrow are sent to the molecular pathology laboratory where mRNA is extracted from the white cells. There are a variety of ways of doing this and methods vary to a greater or lesser degree between laboratories.

To ensure results that more accurately reflect the number of Ph positive cells present in an individual, samples taken from patients must contain adequate numbers of copies of a control (normal) gene.

At least 10,000 copies of a control gene, such as *ABL1* or *BCR*, are needed in every sample sent for testing if the percentage ratio of *BCR-ABL1* is to be correctly assessed.

The control genes most commonly used are *ABL1*, *BCR* or *GUSB*. There is no consensus as to which of these normal genes is the best control to use. The choice lies entirely with the laboratory performing the test.

## Common factors affecting the suitability of a sample sent for q-PCR testing

- If a blood sample has been in transit for several days, or has been stored for too long after collection, the cells it contains will already be in the process of dying and the mRNA will have started to degrade. This means there is a greater chance of an inaccurate result and many labs will not report results from such samples if the control gene is at too low a level.
- The lowest acceptable level for a control gene in any one sample is 10,000 copies (transcripts).
- Test results will show a relative proportion, (expressed as a percentage) of how many *BCR-ABL1* transcripts are present over the total number of cells analysed in the blood sample.
- A sample taken from a patient who is responding well to therapy is more likely to contain a good amount of normal *ABL1* gene transcripts and a much lower amount of abnormal *BCR-ABL1* transcripts. This is because cells containing the abnormal fusion gene will have been killed during therapy and would be very few in number.

### 3. Response to treatment: the role of q-PCR testing

Currently q-PCR is the most accurate test used to monitor response to a particular therapy and to detect any significant rise in *BCR-ABL1* transcripts. Test results are used to make evidence based decisions in the context of the 2013 ELNet recommendations and NCCN Guidelines for the treatment of Ph+ CML.

Both ELNet and NCCN identify a major molecular response (MMR / MR<sub>3</sub>) within 12 months to be an optimal response and a realistic goal of TKI therapy.

Under the best lab conditions q-PCR can detect as little as 0.001%<sup>IS</sup> (MR<sub>5</sub>) *BCR-ABL1* transcripts in a sample, allowing for better detection of residual disease as well as the identification of patients who may be at risk of treatment failure or suboptimal response. Consistently rising levels of *BCR-ABL1* transcripts identifies a need to address a probable cause, such as primary or acquired resistance or the possible lack of adherence to therapy.

Regularly missing more than three daily doses in one month is likely to affect optimal responses to therapy. In patients whose adherence to therapy was monitored, those whose adherence rate was greater than 90%, meaning that they took more than 90% of their prescribed doses in a month, were more likely to achieve the lower molecular levels of remission required for optimal response, such as MR<sub>3</sub>; MR<sub>4.5</sub> or lower. <sup>3</sup>

#### Levels of Molecular Response

Until recently the ultimate goal for CML patients treated with TKI therapy was to achieve a 'PCR negative' result, also known as a Complete Molecular Response (CMR). However, the use of the word 'complete' is now considered to be misleading because it is often interpreted to mean that there has been a total eradication of disease. Recently, international CML experts and clinical groups have agreed to stop using the term CMR replacing it with MR followed by a log reduction number, as in the definition on the next page.

## Q-PCR test results reported according to the International Standard

% of <i>BCR-ABL1</i> detected by qRT-PCR testing	Equivalent Log reduction from 100% <sup>IS</sup>
0.1%	MR <sup>3</sup>
0.01%	MR <sup>4</sup>
0.0032%	MR <sup>4.5</sup>
0.001%	MR <sup>5</sup>

### Why results may differ between testing laboratories

In its present form, q-PCR testing is technically challenging to perform requiring a high level of skill, a consistent method of sample collection and timely delivery to the laboratory. Several factors can adversely influence a result, which often makes it difficult to be confident that it is an accurate reflection of the actual level of residual disease.

Factors include:

- The quality of the sample, also related to time taken for the sample to reach the lab
- Adequate amounts of a control gene – there should be at least 10,000 transcripts in a sample
- The control gene used—*ABL1*, *GUSB*, *BCR* or other
- The method a lab uses to extract mRNA, related to the chemicals and type of equipment used

Even if the method used is consistent, the quality of a sample and the efficient extraction of mRNA are variable. Results, even from the same laboratory, may fluctuate up as well as down. Doctors are only likely to recommend a change of treatment if there is a rise in the % of *BCR-ABL1* transcripts from 2 or 3 consecutive q-PCR results generated from samples containing adequate numbers of control gene transcripts such as *ABL1* or *BCR*.



## 4. The International Standardisation Project (IS)

The q-PCR Standardisation Project is designed to address the inconsistencies of q-PCR results reported between labs in different regions and countries. Professor Tim Hughes and his colleagues, co-founders of the International CML Foundation (iCMLf), initiated the global standardisation project seeking a wide adoption of the International Scale (IS). The aim of the project is to recruit labs to an internationally standardised scale for reporting q-PCR in as many cooperating countries as possible.

The IS enhances the ability to accurately gauge whether a patient's response meets the internationally agreed TKI therapy goals and milestones CCyR and MR3, as well as deeper molecular responses, MR4, MR4.5 and MR5.

It is important to remember that the above levels of molecular responses can only be reported when there are adequate numbers of control gene transcripts present in a sample.<sup>4</sup>

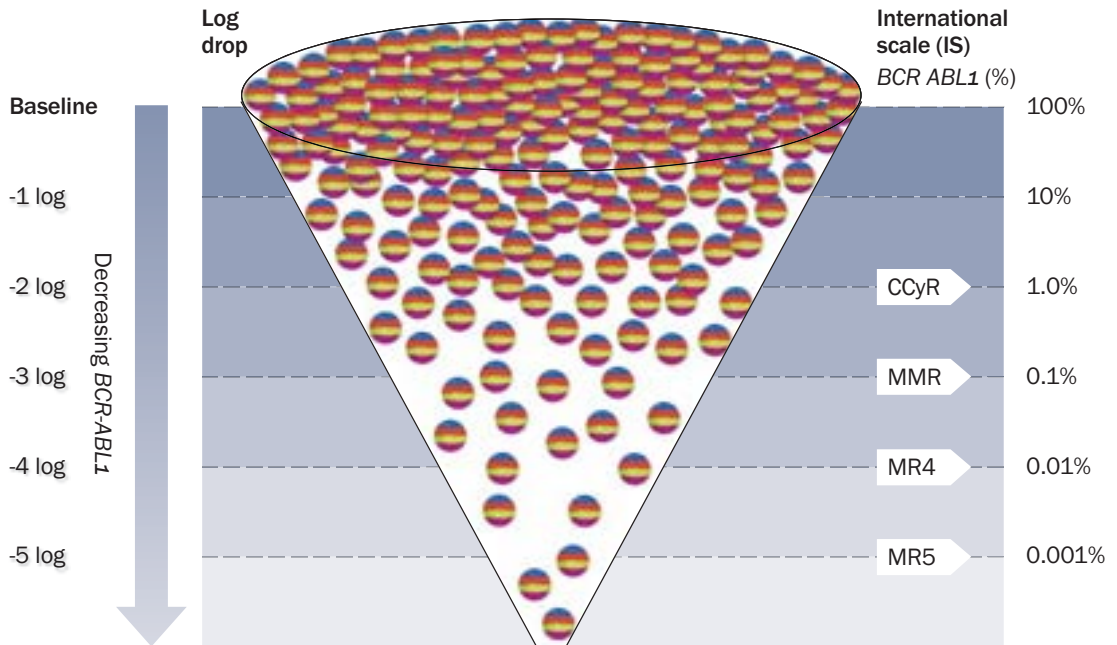
Early detection of a significant rise in *BCR-ABL1* gene transcripts is agreed by both European LeukaemiaNet and NCCN, as an important indication of a potential loss of response, suboptimal response or treatment failure.

### Establishing an International Standard

The strength of an International Scale (IS) is its potential to be used as a common reference baseline against which an individual's response to therapy can be accurately measured. Currently there are upwards of 200 labs that are validated on the IS. However there are still over 1000 labs that are not currently using the International Scale, and even where they do, not all doctors report q-PCR results according to the IS to their patients. This causes confusion for patients who try to assess their response to a particular therapy according to the ELNet recommendations or NCCN guidelines.

## The foundation of the IS method

Samples taken at diagnosis from a group of 30 patients enrolled in the IRIS trial (2000) were measured by three different laboratories: Adelaide in Australia, Manheim in Germany and Hammersmith in London. An average of the 3 reported values were agreed as the baseline reference value and defined as 100% IS. Test results from the IRIS study were then reported in 1 log (tenfold) reductions from this baseline



Both log drop and IS percentage (%) tell you how much your *BCR-ABL1* level has decreased – the lower, the better.

## Conversion Factor: How a specific local lab value is calculated to allow for conversion to IS

“In order to convert a given local result to the international scale, it is necessary to use a conversion factor (CF). This is calculated as follows:  $CF = 0.1\%$  divided by  $MMR^{Eq}$  (since  $0.1\%$  is the agreed value for MMR on the international scale). Once a laboratory-specific conversion factor has been derived, it can be used to convert all local values to the international scale. (This calculation will be invalid if the reproducibility or linearity of the assay is poor, in which case the methodology will need to be optimized.)”

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Laboratory	$MMR^{Eq}$ , %	$0.1\%$ divided by $MMR^{Eq}$ , %  = CF	Conversion Formula  ( $BCR-ABL1^L \times CF$ = $BCR-ABL1^{IS}$ )
<b>Adelaide</b>	0.08	$0.1/0.08 = 1.25$	$BCR-ABL1^L \times 1.25$
<b>Mannheim</b>	0.12	$0.1/0.12 = 0.83$	$BCR-ABL1^L \times 0.83$
<b>London</b>	0.045	$0.1/0.045 = 2.22$	$BCR-ABL1^L \times 2.22$

## The International Standard: its usefulness as a prognostic tool

Currently most patients need to continue to take TKI therapy on a daily basis, even after achieving a stable molecular response shown by q-PCR testing.

Whilst it is agreed that deeper molecular responses within specific timelines reduce the risk of progression or development of resistance to therapy, issues around the measurement of residual disease still remain.

## Four key variables necessary for optimal q-PCR testing:

- i. The sensitivity of the method used and requirement of a high level of technical expertise
- ii. The correct method of sample collection – in transit cells start to die and mRNA degrades
- iii. The quality of the blood or marrow sample: an adequate number (at least 10,000) of control gene transcripts should be present in any sample
- iv. Reliability- can the result be repeated?

*BCR-ABL1* transcript levels are undoubtedly an important indicator of clinical response to TKI therapy. When assessing results from q-PCR testing it is important to look at the trend of several results from a consistent testing source rather than any one single test result.

## Switching between testing laboratories

The quality of samples as well as differences in ‘control’ genes, sensitivity of machines, baseline references and reagents, all conspire to make it difficult to compare results reported by different laboratories. A case in point is that of a patient who switched testing from a non-validated lab in the USA to an IS validated lab in the UK. The difference in results went from 0.0032% (MR<sup>4.5</sup>) reported by the non-validated lab to 0.1% (MR<sup>3</sup>) reported by the IS validated lab. This represented an increase of 2 logs and was understandably very unsettling for the patient involved.

For CML patients whose disease is monitored by q-PCR, the message from this and other examples is that ‘not all laboratories are equal’. As an informed patient, it is important to know the pedigree of your q-PCR results. Switching labs may well be unavoidable and is a complex problem, particularly in the USA where health insurance companies often determine the choice of lab. Should you need to change your doctor, in order to ensure consistency in your test results you should request that your samples are sent to the same laboratory used prior to your move.

## 5. Summary

Your oncologist/hematologist is a trained professional and should be comfortable with discussing your lab results with you, in boring detail if necessary.

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Being aware of sample collection and the method used to generate your results is part of being an informed patient.

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Because q-PCR testing is so sensitive, it is normal for percentages of *BCR-ABL1* to fluctuate a little over time.

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A “log drop” means *BCR-ABL1* transcripts have reduced by 10 fold from a standardized baseline of 100%<sup>IS</sup> at diagnosis

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MMR is a 3-log (1000-fold) reduction in *BCR-ABL1* transcripts

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Achievement of MMR (0.1%<sup>IS</sup>) within 12 months is, according to ELNet recommendations and NCCN guidelines (2013), an optimal response with very low risk of progression.

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There is significant variability among laboratories using different assays and test platforms.

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Q-PCR testing for *BCR-ABL1* transcripts should be performed by the same laboratory or referred to a specialist laboratory that follows universal reporting criteria.

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Results from several tests that show a trend of rising or falling levels of *BCR-ABL1* transcripts is more important than one single test result.

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Samples of both blood and bone marrow are often evaluated at diagnosis, but the majority of follow-up monitoring is performed on peripheral blood samples.

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**To monitor your progress against the recently updated NCCN Guidelines and ELNet Recommendations<sup>5</sup>, consider asking your doctor the following questions:**

- Are my blood samples sent to a specialist lab for testing by q-PCR?
- Does this lab report the results according to the IS?
- If not, how sensitive is their q-PCR method?
- Has the lab calculated an IS conversion factor?
- Can my results be converted to the IS?
- If not, how can I be sure my response is meeting the updated ELNet Recommendations or NCCN Guidelines?
- Can you provide a printed copy of my lab report?

## 6. Ensuring an optimal response to TKI therapy

### Adherence: the importance of taking daily therapy

TKI therapies keep the levels of *BCR-ABL1* transcripts very low, so it is vitally important to adhere to therapy by taking the tablets at a regular time every day. Less than 90% adherence per month (approx. 3 doses) increases the risks of losing stability of response, developing resistance or disease progression.

“In practice, no CMRs were observed when adherence was <90% and no MMRs were observed when adherence was <80%”...“In patients with CML treated with imatinib for some years, poor adherence may be the predominant reason for inability to obtain adequate molecular responses.” Marin D, et al. *J Clin Oncol.* 2010;28:2381-2388.<sup>3</sup>

### Drug Resistant Mutations.

Mutations can sometimes develop in part of the *BCR-ABL1* gene, which can affect how well a particular tyrosine kinase inhibitor can block the protein that signals for Ph+cell division.

“The prevalence of *ABL1* mutations increases with ‘disease time’: that is, rare in newly diagnosed chronic phase and increasing with late chronic-phase and advanced-phase disease (i.e. with increasing Sokal score). Thus, *Abl1* mutations occur as part of the natural history of CML, rather than as merely a manifestation of selective pressure from TKI therapy.” J.P Radich<sup>2</sup>

Mutation testing is an important tool to assess why a patient fails to meet a particular treatment milestone, or loses an established response in spite of continuous treatment without adherence issues. Identification of specific mutations can help indicate a particular TKI that would be a more effective treatment option.<sup>2</sup>

## 7. The Future

### New and Advanced Monitoring: q-PCR techniques; can we do better?

At the moment probably not, but in the future we should expect some changes. The recently published updated ELNet Recommendations and the 2013 edition of the NCCN Guidelines both suggest that strict molecular monitoring by standardised q-PCR testing should be performed at 3, 6, 9 and 12 month time points. A number of international studies that have provided robust evidence that *BCR-ABL1* transcripts below 10% by q-PCR, (or <35% Ph+ by cytogenetics) at 3 months and below 1% at 6 months, is predictive of event free survival (EFS) over the longer term. <sup>4 (table 3,2c)</sup>

According to ELNet 2013 recommendations, MMR (0.1%IS), as a stable molecular response over the longer term is, in general, a reasonable goal to be achieved within 12 months of starting therapy. <sup>5</sup>

- A reduction in time taken to report results is important because *BCR-ABL1* transcript levels at 3 and 6 months are increasingly used as a basis to drive changes in therapy.
- A great deal of attention has recently been focused on reaching agreement of a more precise definition of minimal, or measurable, residual disease (MRD) such as MR3, MR4, MR4.5 and MR5
- More precise definitions of MRD would present a basis to enrol patients in clinical studies leading to dose de-escalation and/or discontinuation with the possibility of remaining in treatment free remission (TFR). Ideally this would be achieved with newer testing methods such as digital q-PCR, a method that does not require a control gene or standardisation, and thus detects *BCR-ABL1* transcripts with even greater precision. However, it may not be widely adopted for some years yet.



### **GeneXpert® System by Cepheid:**

An automated pre-prepared cartridge based system, requiring only 250 micro-litres of blood per sample.

- Automatically extracts mRNA from a sample
- Converts results to the International Standard if required
- The Adelaide lab has found equivalent sensitivity to the present method.
- A newer version has increased sensitivity even further
- It can be used for testing for other diseases making its use more cost effective.

### **Digital PCR:**

- A newer approach to nucleic acid detection using a different method of absolute quantification and rare allele detection compared to conventional q-PCR.
- Allows researchers to explore beyond the limits of q-PCR
- It counts individual molecules for absolute quantification by partitioning a sample into many individual real-time PCR reactions. Some portions of the reactions will test positive for the target molecules, others will test negative. Without having to use reference standards or endogenous controls, the fraction of negative answers generates an absolute answer reflecting the exact number of target molecules in a sample.
- Nevertheless, both technologies are complementary

### **DNA PCR:**

The Adelaide lab in Australia is studying DNA PCR, a method that tests at a unique (personalised) DNA level. An assay can be designed to fit an individual patient. DNA PCR is 1 to 1.5 logs more sensitive than conventional q-PCR method using mRNA. It represents a way of identifying patients who can successfully stop treatment without losing response; however it is presently too impractical to use on the wider CML population and remains in the realm of research for now.

## Then and Now: the post imatinib era

“Imatinib produced a big breakthrough in the course of CML. Today, after less than 15 years, we know more, we have more, and we want more. If on one hand, we must realistically stay at standard treatment recommendations, on the other hand we should continue to design new treatment protocols and to enrol new patients in prospective studies. This is not easy, because of the high efficacy of standard treatment. In any case, the treatment of CML must be guided by healthcare professionals with specific training and specific interest in CML that are necessary for the optimisation of the treatment and a proper utilisation of the resources. The home physicians must be involved more and more in the care of the CML patient, because an optimal treatment ensures an average life expectancy, and the patient should no longer be considered as a patient at risk of dying of cancer, but as any other individual.”

Michele Baccarani et al, *Mediterr J Hematol Infect Dis* v.6(1); 2014  
PMC3894838.<sup>1</sup>

# Resources

## 8. Glossary

**ABL1** – A human proto-oncogene located on chromosome 9. The designation is taken from name of the scientist, Herbert Abelson MD, who discovered the gene. *ABL1* activates a number of cell cycle-controlling proteins and enzymes. Mutations in the *ABL1* gene are associated with chronic myelogenous leukaemia (CML).

**Adherence** – TKI adherence is the extent to which therapy is taken as prescribed. Good adherence to therapy has a direct impact on the effectiveness of treatment.

**Allele** – one of two or more versions of a gene. Individuals inherit two alleles for each gene, one from each parent.

**Apoptosis** – the process of programmed cell death, in adults it rids the body of cells that have been damaged beyond repair.

**Autosome** – any one of the numbered pairs of chromosomes, as opposed to the sex chromosomes. Humans have 22 pairs of autosomes numbered roughly in relation to their size.

**BCR** – normal gene located at the 'breakpoint cluster region' on chromosome 22.

**BCR-ABL1** – fusion gene found on the Philadelphia chromosome, the definitive marker for CML.

**Bcr-Abl1** – the protein transcribed by the *BCR-ABL1* gene that signals for a continuous division of Ph<sup>+</sup> cells. It displays high protein tyrosine kinase activity (this activity is due to the *Abl1* half of the protein). The unregulated expression of Bcr-Abl1 activates other proteins that are involved in cell cycling and cell division.

**Blast Cells** – immature cells such as myeloblasts and lymphoblasts develop into mature white blood cells. Represent about 1 to 5 % of normally developing marrow cells.

**Bone marrow** – spongy tissue in the hollow central cavity of the bones where blood cells are formed from stem cells.

**Cancer** – a group of diseases characterized by uncontrolled cell growth.

**Centromere** – central region of a chromosome separating it into a short arm (p) and a long arm (q).

**CCyR** – complete cytogenetic response.

**Chromosome** – within the nucleus of cells DNA is organized into long strands forming structures called chromosomes. During cell division chromosomes are duplicated in the process of DNA replication, providing each cell its own complete set of chromosomes.

**CML** – chronic myeloid leukaemia or chronic myelogenous leukaemia is a cancer that starts in the blood forming cells in the marrow causing too many abnormal white blood cells to form.

**Conversion Factor** – a local labs molecular testing value calculated to convert to the IS.

**Cytogenetics** – process of analysing the number and size of chromosomes under a microscope to detect structural and/or numerical abnormalities.

**Cytoplasm** – liquid composed of water, salts and organic molecules that fills the inside of a cell.

**DNA** – deoxyribonucleic acid is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses. Most DNA molecules consist of two biopolymer strands coiled around each other to form a double helix. The two DNA strands are known as polynucleotides since they are composed of simpler units called nucleotides. Each nucleotide is composed of a nitrogen containing nucleobase, either guanine (G), adenine (A), thymine (T), or cytosine (C).

**ELNet** – European Leukaemia Network of expert Haematologists.

**EFS** – Event free survival. A measure of the proportion of people who remain free of a particular complication of disease (called an event) after treatment that is designed to prevent or delay that particular complication.

**FISH** – Fluorescence in Situ Hybridization, uses a special microscope to visualise cells in a sample with the *BCR-ABL1* fusion gene.

**Genes** – carried in the DNA of every cell, genes hold all the information to build and maintain an organism and pass genetic traits to offspring. All organisms have genes corresponding to various biological traits, some of which are instantly visible (eye colour) some of which are not, (blood type). Derived from the Greek word *genesis* meaning “birth”, or *genos* meaning “origin”.

**Genetic code** – the genetic code specifies the correspondence during protein translation between codons and amino acids. The genetic code is nearly the same for all known organisms.

**GUSB** – A control or ‘housekeeping gene’ used by some labs in qRT-PCR testing.

**Haematopoiesis** – a continuous process of blood cell development from stem cells in the marrow. Stem cells differentiate into immature blood cells of various types. The immature blood cells develop into mature fully functional blood cells which enter the blood and circulate throughout the body. Most blood cells live for short periods and must be steadily replaced. Red cells die within four months, platelets within 10 days and most neutrophils in one to three days. About 100 billion blood cells are made each day.

**IRIS** – the International Randomized Study of Interferon and STI571 (imatinib/Glivec).

**IS** – International Scale.

**Karyotype** – the systematic arrangement of the 46 chromosomes in the human cell in 22 matched pairs (maternal and paternal member of each pair) by length from longest to shortest and other features, with the sex chromosomes (#23) shown as a separate pair (either XX or XY). The 22 pairs are referred to as autosomes.

**Log Reduction** – 1 log reduction is a 10-fold (one decimal) or 90% reduction in numbers. A 3-log reduction is equivalent to a 1000-fold decrease.

**Minimal Residual Disease (MRD)** - Small amounts of abnormal cells remaining after treatment, even when blood and marrow may appear to be normal. Residual cells can only be identified by molecular testing techniques like q-PCR.

**Molecular Targeted Therapy** - see Tyrosine Kinase Inhibitor

**MR** – molecular response e.g. MR3 (0.1%); MR4 (0.01%); MR4.5 (0.032%); MR5 (0.001%).

**MMR**– major molecular response also written as log reduction: MR<sup>3</sup> or a percentage: 0.1% *BCR-ABL1*.

**Nucleus** – all cells, apart from red cells, have a nucleus which is where chromosomes reside.

**NCCN** – National Comprehensive Cancer Network (USA).

**Oncogene** – mutated gene that is a cause of a cancer. Several subtypes of acute myeloid leukaemia, acute lymphoblastic leukaemia and lymphoma and nearly all cases of chronic myeloid leukaemia are associated with an oncogene.

**OS** – Overall survival. Indicates the proportion of people within a group who are expected to be alive after a specified time. Accounts for death due to any cause – both related and unrelated to the disease in question.

**Proto-oncogene** – a normal gene (e.g. *ABL1*) that can become an oncogene by a relatively small modification of its original function such as a chromosomal translocation event, as in CML, with increased cancerous/oncogenic activity.

**Peripheral blood** – blood that circulates throughout the body via the venous system.

**PFS** – progression free survival, a survival rate that measures the length of time, during or after treatment, during which the disease does not get worse (progress)

**Philadelphia chromosome (Ph)** – an abnormal shortened chromosome 22 found in the marrow and blood cells of patients with chronic myeloid leukaemia and a small proportion of patients with acute lymphoblastic leukaemia. First described in 1960 by Peter Nowell from University of Pennsylvania School of Medicine and David Hungerford from the Fox Chase Institute for Cancer Research and named after Philadelphia, the city in which both facilities are located.

**Platelets** – type of blood cell that controls bleeding through its clotting mechanism.

**Proteins** – products of genes produced in the cytoplasm of the cell, they signal for a variety of processes including cell division, proliferation and cell death (apoptosis).

**Qualitative PCR** – polymerase chain reaction test used to confirm the presence of *BCR-ABL1* gene transcripts.

**QRT-PCR** – qRT-PCR, quantitative reverse transcriptase (real time) polymerase chain reaction, a sensitive molecular test used to measure the ratio of *BCR-ABL1* versus normal gene transcripts such as *ABL1* or *BCR*.

**Red Blood Cells** – non nucleated cells that carry oxygen throughout the body.

**RNA** – a second type of nucleic acid that is similar to DNA. The expression of genes encoded in DNA begins by their duplication into RNA molecules. RNA is less stable than DNA and is typically single-stranded.

**mRNA** – messenger RNA: in CML the oncogene *BCR-ABL1* duplicates as mRNA (*Bcr-Abl1*) which moves out of the nucleus into the cytoplasm where it translates as a signalling protein, a receptor tyrosine kinase which is constitutively active, leading to uncontrolled cell proliferation.

**Stem Cell** – called blood stem cells or haematopoietic stem cells, are primitive cells found in the bone marrow from which all types of blood cell develop.

**Tyrosine Kinase** – proteins that play a key role in cell signalling processes.

**TKI** – Tyrosine Kinase Inhibitor (TKI), a molecular targeted therapy designed to block specific proteins that cause leukaemic cells to divide. Imatinib was the first generation in this class of oral therapy; dasatinib, nilotinib, bosutinib and ponatinib are second generation TKIs now used as 1st, 2nd, 3rd and 4th line therapy.

**Translocation** – an event that occurs when part of one chromosome breaks off and attaches to a different chromosome. In 1973, Janet D. Rowley at the University of Chicago described the mechanism by which the Philadelphia chromosome arises as a reciprocal translocation between chromosomes 9 and 22.

**White Blood Cells** – are part of the immune system and help fight infections. There are three major types of white cell in the marrow: monocytes, lymphocytes and granulocytes. Neutrophils, eosinophils, and basophils are granulocytes (a type of white cell that has small particles). White cells are also called leukocytes.

< – less than.

> – more than.

## 9. Citations

- 1. Treatment Recommendations for Chronic Myeloid Leukaemia**  
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<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3894838/>
- 2. How I monitor residual disease in chronic myeloid leukemia:**  
J.P Radich <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3654782>
- 3. Long-term Adherence to Imatinib Critical for Achieving Molecular Response-** Marin D, et al. J Clin Oncol. 2010;28:2381-<http://jco.ascopubs.org/content/28/14/2381.full.pdf>
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<http://bloodjournal.hematologylibrary.org/content/116/19/3758.full.pdf>
- 5. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013**  
<http://bloodjournal.hematologylibrary.org/content/122/6/872.full>



## 10. References and useful links

- **Pocket Card LeukemiaNet recommendations for the Management of CML 2013** [http://www.leukemia-net.org/content/leukemias/cml/recommendations/e8078/infoboxContent10260/PocketCard\\_UPDATE2013\\_105x165\\_final.pdf](http://www.leukemia-net.org/content/leukemias/cml/recommendations/e8078/infoboxContent10260/PocketCard_UPDATE2013_105x165_final.pdf)
- **NCCN Guidelines for CML patients: Version 1:2014:** <http://www.nccn.org/patients/guidelines/cml/index.html#1>
- **Oncogene/proto-oncogene:** <http://en.wikipedia.org/wiki/Oncogene>
- **Bcr-Abl1 Tyrosine Kinase Inhibitors:** [http://en.wikipedia.org/wiki/Bcr-Abl\\_tyrosine-kinase\\_inhibitor](http://en.wikipedia.org/wiki/Bcr-Abl_tyrosine-kinase_inhibitor)
- **National Human Genome Research Institute:** <http://www.genome.gov/glossary/index.cfm?id=25>

### Other educational resources on qRT-PCR:

- **qRT-PCR on the International Scale** by Dr. Michael Mauro [http://www.whatismypcr.org/FAQ.aspx#.U6Asw\\_mwLYh](http://www.whatismypcr.org/FAQ.aspx#.U6Asw_mwLYh)
- **“What is MY PCR?” International Campaign** coordinated by The MAX Foundation [http://www.whatismypcr.org/FAQ.aspx#.U6Asw\\_mwLYh](http://www.whatismypcr.org/FAQ.aspx#.U6Asw_mwLYh)
- **CML Society of Canada—’PCR’** <http://cmlsociety.org/monitoring-the-disease/pcr-polymerase-chain-reaction/>
- **Educational Video ‘The CML Society of Canada Presents ‘PCR and monitoring CML’** <https://www.youtube.com/watch?v=ZwgyKh6J5U>

**[www.cmlsupport.org.uk](http://www.cmlsupport.org.uk)**

## 11. CML Support Group

Charity Registration Number 1114037

The CML Support Group was formed in 1999 by CML patients. Publication of a web based forum followed in April 2000. Support, advice and information to all those affected by CML is provided through this online resource, as an essential supplement to good professional health care. Informed patients make better choices that have a tangible impact on survival. We encourage everyone involved to become self-educators by learning about all aspects of their disease, including its current best treatment and management options. All clinical information contained on our site is published in the public domain and is regularly updated.

Registration is not required, however if you wish to interact with others on the discussion forum you must register by providing an email address and choosing a username.

**[www.cmlsupport.org.uk](http://www.cmlsupport.org.uk)**

**[cmlsupportgroup@gmail.com](mailto:cmlsupportgroup@gmail.com)**

**Facebook: /CMLSupportGroup**

**Twitter: @CMLSupportGroup**

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**cmlsupport**  
chronic myeloid leukaemia support group