Fast Facts: Treatment-free Remission in Chronic Myeloid Leukaemia

A Guide for Nurses

Leukaemia Care
Nurse Academy

Introduction

The tyrosine kinase inhibitor (TKI) imatinib was the first treatment to specifically target cancer cells, rather than the relatively indiscriminate effects of conventional chemotherapy on any rapidly dividing cells. This concept of targeted treatment in cancer is one of the important advances in modern medicine in the last 30 years. Indeed, treatment with TKIs has transformed chronic myeloid leukaemia (CML) from a cancer with a poor prognosis to one in which many patients can expect a normal lifespan.

Success with the TKIs has prompted the question of whether it is desirable – or feasible – for patients to remain on treatment for long periods. While the TKIs are targeted, they are associated with considerable toxicity, and long-term treatment has important economic implications for health services and patients. Thus, the concept of treatment-free remission (TFR) has emerged for patients in deep clinical remission. Clinical research over the last decade has focused on whether treatment can be stopped, how to best monitor patients while off treatment, and how to intervene before a clinical relapse. As this research progresses, the tantalising prospect of a cure for some patients seems increasingly feasible.

This new Fast Facts title explains this trail-blazing approach to the long-term management of patients living with CML in remission. It explains the concepts of molecular and haematological relapse, the highly sensitive technologies that allow disease monitoring, and how TFR is best managed in practice.

This concise educational resource is ideal for any healthcare professional involved in the treatment of patients with CML who wants to understand TFR, particularly clinical nurse specialists and pharmacists who increasingly help clinicians to run CML clinics.

If you would like any information on the sources used for this booklet, please email **communications@leukaemiacare.org.uk** for a list of references.

In this booklet

About Leukaemia Care

Leukaemia Care is a national charity dedicated to ensuring that people affected by blood cancer have access to the right information, advice and support.

Our services

Helpline

Our helpline is available 8:30am – 5:00pm Monday - Friday and 7:00pm – 10:00pm on Thursdays and Fridays. If you need someone to talk to, call **08088 010 444**.

Alternatively, you can send a message via WhatsApp on **07500068065** on weekdays 9:00am – 5:00pm.

Nurse service

We have two trained nurses on hand to answer your questions and offer advice and support, whether it be through emailing **nurse@leukaemiacare.org.uk** or over the phone on **08088 010 444**.

Patient Information Booklets

We have a number of patient information booklets like this available to anyone who has been affected by a blood cancer. A full list of titles – both disease specific and general information titles – can be found on our website at **www.leukaemiacare.org.uk/support-and-information/help-and-resources/information-booklets/**

Support Groups

Our nationwide support groups are a chance to meet and talk to other people who are going through a similar experience. For more information about a support group local to your area, go to **www.leukaemiacare.org.uk/support-and-information/support-for-you/find-a-support-group/**

Buddy Support

We offer one-to-one phone support with volunteers who have had blood cancer themselves or been affected by it in some

way. You can speak to someone who knows what you are going through. For more information on how to get a buddy call **08088 010 444** or email **support@leukaemiacare.org.uk**

Online Forum

Our online forum, **www.healthunlocked.com/leukaemia-care**, is a place for people to ask questions anonymously or to join in the discussion with other people in a similar situation.

Patient and carer conferences

Our nationwide conferences provide an opportunity to ask questions and listen to patient speakers and medical professionals who can provide valuable information and support.

Website

You can access up-to-date information on our website, **www.leukaemiacare.org.uk**.

Campaigning and Advocacy

Leukaemia Care is involved in campaigning for patient well-being, NHS funding and drug and treatment availability. If you would like an update on any of the work we are currently doing or want to know how to get involved, email **advocacy@leukaemiacare.org.uk**

Patient magazine

Our magazine includes inspirational patient and carer stories as well as informative articles by medical professionals: **www.leukaemiacare.org.uk/communication-preferences/**

Abbreviations

- **ABL1:** ABL proto-oncogene 1
- **ALL:** acute lymphoblastic leukaemia
- **ASCT:** allogeneic stem cell transplantation
- **ATP:** adenosine triphosphate
- **BCR:** breakpoint cluster region (gene)
- **cDNA:** complementary DNA
- **CLL:** chronic lymphocytic leukaemia
- **CML:** chronic myeloid leukaemia
- **CMR:** complete molecular response
- **DNA:** deoxyribonucleic acid
- **ELN:** European LeukemiaNet
- **FISH:** fluorescence in situ hybridisation
- **gDNA:** genomic DNA
- **IS:** International Scale
- **LSC:** leukaemic stem cell
- **MR:** molecular response
- **mRNA:** messenger RNA
- **NGS:** next-generation sequencing
- **NK:** natural killer (cell)
- **PCR:** polymerase chain reaction
- **RNA:** ribonucleic acid
- **RT-qPCR:** quantitative reverse transcriptase polymerase chain reaction
- **TFR:** treatment-free remission
- **TKI:** tyrosine kinase inhibitor

The concept of treatment-free remission

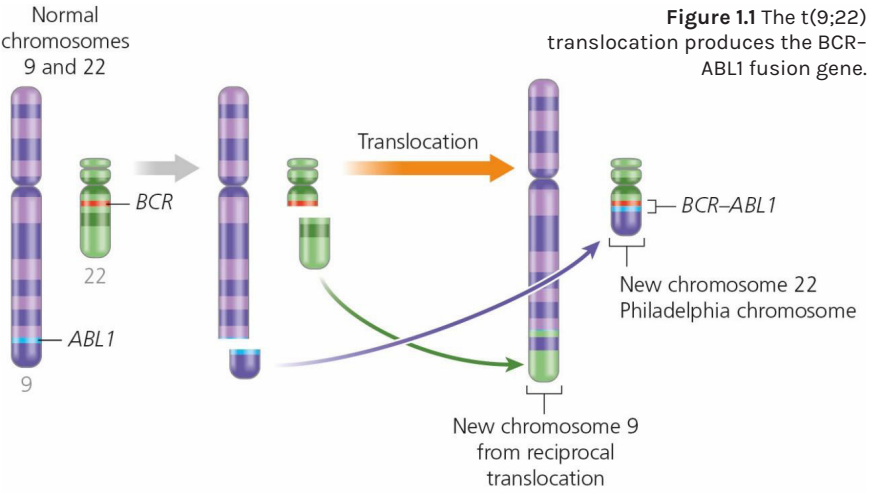

Figure 1.1 The t(9;22) translocation produces the BCR-ABL1 fusion gene.

Chronic myeloid leukaemia

Chronic myeloid leukaemia (CML) affects white blood cells in the myeloid lineage. About 750 new cases of CML are diagnosed in the UK each year. CML used to have a poor prognosis, with a 5-year relative survival of 19–74% in Europe. However, it is now considered to be a model disease in which targeted therapies are giving many patients the possibility of long-term remission and a normal life span.

The Philadelphia chromosome

In 1960, an abnormal chromosome was identified in bone marrow-derived white blood cells from patients with CML – the Philadelphia chromosome. Further research revealed that parts of the long arms of chromosomes 9 and 22 are exchanged – termed the t(9;22) translocation (Figure 1.1).

BCR–ABL1 translocation

The t(9:22) translocation results in fusion of the genes for the breakpoint cluster region (BCR) and ABL proto-oncogene 1 (ABL1), creating the BCR–ABL1 fusion gene.

ABL1 codes for a tyrosine kinase which, when phosphorylated, recruits other proteins involved in cell signalling. The fusion

The concept of treatment-free remission (cont.)

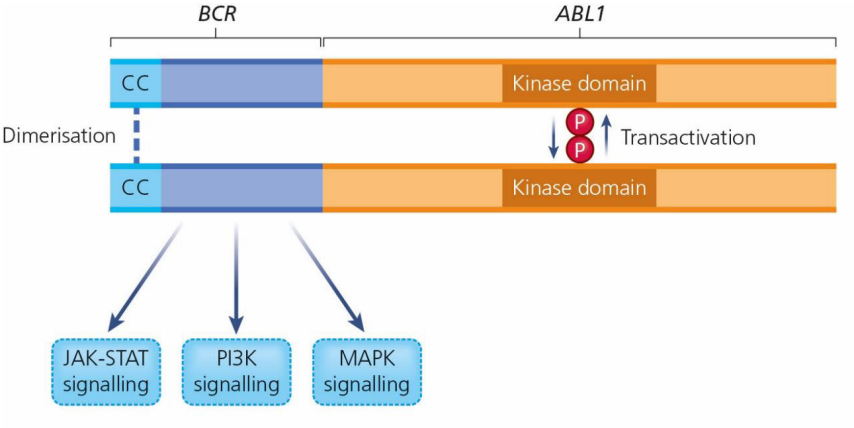

Figure 1.2 Dimerisation of the coiled–coiled (CC) domain of BCR–ABL1 allows autophosphorylation (P) of the kinase domain and activation of downstream signalling pathways. JAK-STAT, Janus kinase-signal transducer and activator of transcription proteins; PI3K, phosphoinositide 3-kinase; MAPK, mitogen-activated protein kinase.

with BCR prevents the shuttling of ABL1 between the nucleus and cytoplasm so it stays in the cytoplasm where it can continuously recruit signalling proteins.

BCR contains a coiled–coiled domain that allows two BCR–ABL1 proteins to join (dimerisation) (Figure 1.2). This allows the ABL1 kinase domain (the active domain) to phosphorylate itself, promoting the further recruitment of signalling proteins and the activation of signalling pathways such as mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K) and Janus kinase-signal transducer and activator of transcription proteins (JAK-STAT).

The overall effect of the BCR–ABL1 fusion protein is that cells proliferate before they differentiate (i.e. become mature), leading to the accumulation of immature leukaemic blasts and myeloid progenitor cells in the blood. The three phases of CML are shown in Table 1.1. The accelerated phase and/or blast crisis are often associated with the accumulation of further mutations in genes such as RUNX1, BCOR, IKZF1 and NOTCH1 which further blocks differentiation, accelerating the accumulation of leukaemic

blasts.

TABLE 1.1 The three phases of chronic myeloid leukaemia according to the blast count*

Phase	Blast count
Chronic phase	< 10%
Accelerated phase	10–29%
Blast crisis	≥ 30%

Values are the proportion of nucleated (immature) cells in the bone marrow or peripheral blood.

*European LeukemiaNet criteria.

The t(9;22) translocation also gives rise to a reciprocal ABL1–BCR translocation on chromosome 9, although a functional role for the ABL1–BCR protein has not been found.

Atypical CML refers to about 1% of patients who do not have the t(9:22) translocation – exclusively adults and with a male preponderance. Atypical CML is associated with a number of cytogenetic and molecular changes:

- trisomy 8 (three copies of chromosome 8)
- del(20q) (deletion of the q arm of chromosome 20)
- mutations in genes coding for epigenetic modifiers (which affect phenotype through mechanisms that do not involve DNA, such as SETBP1 [involved in histone methylation] and ASXL1 [involved in chromatin remodelling]), metabolic enzymes (ETNK1) and signalling proteins (CSF3R and RAS).

Tyrosine kinase inhibitors

Tyrosine kinases are a subclass of protein kinase – enzymes that transfer a phosphate group from ATP to a protein in a cell, which operates as an on/off switch for many cellular functions. This is an important mechanism in communicating signals within a cell (signal transduction) and regulating cellular activity, such as cell division. However, protein kinases can become mutated such that they become stuck in the 'on' position, leading to unregulated cell growth. The tyrosine kinase inhibitors (TKIs) bind to the active site of the tyrosine kinase, inhibiting ATP binding, so that the tyrosine kinase protein cannot phosphorylate itself (autophosphorylation). This prevents the recruitment of

The concept of treatment-free remission (cont.)

Generation	TKI	Dose	Frequency
First	Imatinib	400 mg	Once daily
Second	Nilotinib	300 mg	Twice daily
	Bosutinib	500mg	Once daily
	Dasatinib	100mg	Once daily
Third	Ponatinib	45 mg	Once daily

TABLE 1.2 Tyrosine kinase inhibitors approved for the treatment of chronic myeloid leukaemia

signalling proteins. Several TKIs are approved for the treatment of CML (Table 1.2); each bind slightly differently to the active site in the tyrosine kinase molecule.

A key challenge with TKIs is that mutations can develop in the active site of the tyrosine kinase enzyme, which prevents TKI binding; specific mutations confer resistance to particular TKIs. The T315I mutation, which accounts for up to about 20% of tyrosine kinase mutations, confers resistance to all first- and second-generation TKIs.

Imatinib was the first TKI, and indeed the first targeted treatment, to be used in cancer. It was evaluated against interferon alfa and cytarabine in the Phase III IRIS trial: after 18 months' follow-up, the rate of major cytogenetic remission (when the Philadelphia chromosome could not be seen in any bone marrow white cells) was 87% in the imatinib arm, compared with 35% in the interferon/cytarabine arm. Given this profound improvement in response with imatinib, most patients receiving interferon/cytarabine crossed over to the imatinib arm.

Nilotinib is a second-generation

Trial	TKI	Major molecular response rate at 12 months, newer TKI vs imatinib
ENESTnd11	Nilotinib	44% vs 22%
DASISION12	Dasatinib	46% vs 28%
BFORE13	Bosutinib	47% vs 37%
EPIC14	Ponatinib	Some benefit over imatinib in terms of response but trial terminated early because of excess vascular events in the ponatinib arm

TABLE 1.3 Comparison of second- and third-generation tyrosine kinase inhibitors with imatinib

TKI which binds more potently and specifically to the ABL tyrosine kinase domain than other TKIs.

Dasatinib and bosutinib are also more potent than imatinib and inhibit the Src family of tyrosine kinases in addition to the ABL tyrosine kinases. Src tyrosine kinases are upregulated in patients who develop resistance to imatinib and nilotinib, without gaining new mutations, allowing continued signalling activation. Thus, dual ABL–Src TKIs overcome this.

Ponatinib is a third-generation TKI that can overcome resistance mediated by the T315I mutation. Its efficacy was confirmed in the PACE trial, with 40% of patients achieving a major molecular response.

Several trials have compared the second- and third-generation TKIs versus imatinib (Table 1.3).

However, the choice of TKI is not based purely on efficacy, depth of response or ability to overcome tyrosine kinase domain mutations; patients may not be able to tolerate the side effects of particular TKIs. Table 1.4 lists common side effects for individual TKIs.

The concept of treatment-free remission (cont.)

TKI	Common side effects
Imatinib	Abdominal pain, nausea, diarrhoea, vomiting, hypophosphataemia Muscle spasm Peripheral oedema, pleural effusion Nilotinib Rash, alopecia, pruritus Headache Deranged liver function tests Pancreatitis Cardiovascular events, worse glycaemic control, hyperlipidaemia, prolonged QT interval, heart failure Peripheral arterial disease
Dasatinib	Neutropenia, thrombocytopaenia with impaired platelet function Pleural effusion Haemorrhagic gastrointestinal colitis
Bosutinib	Diarrhoea Deranged liver function tests
Ponatinib	Rash, dry skin Pancreatitis Thrombocytopaenia with impaired platelet function Arterial thrombosis – stroke, myocardial infarction, peripheral vascular disease

TABLE 1.4 Side effects commonly reported for indivdual tyrosine kinase inhibitors

Treatment-free remission

Treatment with TKIs achieves remission in many patients. However, up to one-third experience moderate to severe side effects (see Table 1.4) and many wish to stop treatment, when it is safe to do so.

None of the TKIs are indicated for use in pregnancy or while breastfeeding. Preclinical studies in rats treated with any TKI showed considerable teratogenicity, including skeletal malformations. A retrospective study of 180 pregnant women who took imatinib for any condition during pregnancy reported 18 spontaneous abortions and eight live births with congenital abnormalities.

A treatment-free remission (TFR) can also save patients a lot of money in countries where they have to pay for treatment. In the EURO-SKI trial (n = 758), which evaluated the efficacy of TFR, 50% of patients remained treatment free for 24 months, which resulted in an estimated potential saving of €22 million for healthcare systems. The sense of

normality for patients when they are able to stop treatment and their perceived improvements in their quality of life cannot be underestimated.

Knowing which patients are eligible to stop treatment and continue on close monitoring to make sure they stay in remission is of paramount importance and is discussed in Chapter 3.

Key points – the concept of treatment-free remission

- Chronic myeloid leukaemia (CML) mostly results from a t(9;22) translocation leading to the fusion of the BCR and ABL1 genes; this leads to constitutive activation of signalling and abnormal cell proliferation.
- The tyrosine kinase inhibitors (TKIs) have revolutionised the treatment of CML by inducing prolonged deep molecular responses.
- This has led to the concept of offering treatment-free remission to some patients, providing respite from TKI side effects and financial benefits.

Measurement of disease burden

Method	Type of remission	Definition
Full blood count – levels of each cell type on blood film (Figure 2.1a)	Complete haematological remission	Platelet count < $450x10^9$/L White cell count < $10x10^9$/L No immature granulocytes Basophils < 5% of all white blood cells
Giemsa banding karyotyping to identify the Philadelphia chromosome; cells are treated with colchicine to arrest as many as possible in metaphase of mitosis (Figure 2.1b)	Complete cytogenetic remission	No visible Philadelphia chromosomes
FISH: different colour fluorescent probes are hybridised to BCR and ABL1 (Figure 2.1c)	Complete cytogenetic remission	In normal cells, the two probes appear slightly separated on confocal microscopy as BCR and ABL1 are on different chromosomes In CML cells with the BCR–ABL1 transcript, the two probes appear in the same place
PCR: used to detect BCR–ABL1 transcript	Complete molecular remission	No detectable BCR–ABL1 transcripts
NGS: sensitive to 1 abnormal cell in 100 million normal cells	Complete NGS remission	No detectable BCR–ABL1 transcripts

TABLE 2.1 Methods for identifying remission, in order of increasing sensitivity

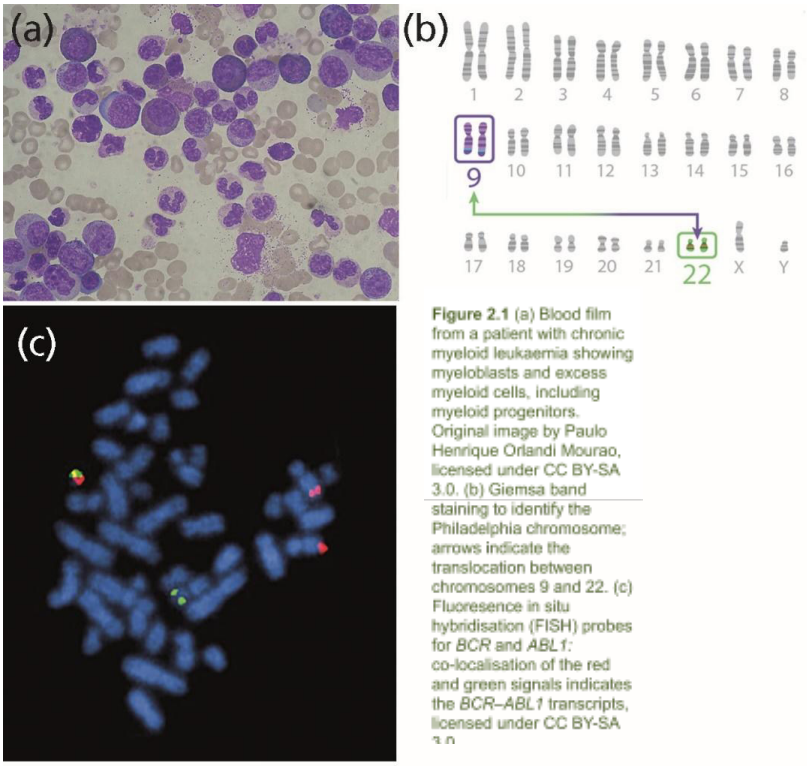

Figure 2.1 (a) Blood film from a patient with chronic myeloid leukaemia showing myeloblasts and excess myeloid cells, including myeloid progenitors. Original image by Paulo Henrique Orlandi Mourao, licensed under CC BY-SA 3.0. (b) Giemsa band staining to identify the Philadelphia chromosome; arrows indicate the translocation between chromosomes 9 and 22. (c) Fluoresence in situ hybridisation (FISH) probes for *BCR* and *ABL1*; co-localisation of the red and green signals indicates the *BCR–ABL1* transcripts, licensed under CC BY-SA 3.0

To be eligible to attempt a treatment-free remission (TFR) a patient must have reached remission, defined according to the number of residual leukaemic cells relative to healthy cells. The methods for defining different classes of remission, in increasing order of sensitivity, are presented in Table 2.1. Most trial data are derived from molecular testing using the polymerase chain reaction (PCR). Although next-generation sequencing (NGS) is more sensitive – BCR–ABL1 transcripts can be detected to a level of 1 chronic myeloid leukaemia (CML) cell in 100 million normal cells – it is currently too expensive for routine clinical use.

Several methods are used to test the disease burden in clinical practice, including the level of BCR–ABL1 transcripts. The European LeukemiaNet (ELN) guidelines recommend quantification of BCR–ABL1 transcripts by quantitative reverse transcriptase PCR (RT-qPCR), karyotyping for the Philadelphia chromosome and

Measurement of disease burden (cont.)

Time	Optimal response	Warning	Failure
Baseline		High-risk clonal chromosomal abnormalities	
3 months	BCR–ABL1IS ≤ 10% and/or Ph+ ≤ 35%	BCR–ABL1IS > 10% and/or Ph+ 36–95%	No CHR and/or Ph+ > 95%
6 months	BCR–ABL1IS < 1% and/or Ph+ 0%	BCR–ABL1IS 1–10% and/or Ph+ 1–35%	BCR–ABL1IS > 10% and/or Ph+ > 35%
12 months	BCR–ABL1IS ≤ 0.1%	BCR–ABL1IS > 0.1–1%	BCR–ABL1IS > 1% and/or Ph+ > 0%
Any subsequent time	BCR–ABL1IS ≤ 0.1%	New –7 or –7q karyotype	Any of: Loss of CHR, or cytogenetic remission on one measurement Loss of BCR–ABL1IS ≤ 0.1% on two consecutive measurements Tyrosine kinase domain mutations New clonal chromosomal abnormalities

TABLE 2.2 European LeukemiaNet guidelines' stratification of responses to tyrosine kinase inhibitors

assessment of haematological responses by full (complete) blood count. The response to treatment is stratified as 'optimal', 'warning' or 'failure' (Table 2.2).

Polymerase chain reaction

Given that most human cells contain only about 6 picograms of DNA, amplification is required to identify particular genes or transcripts. PCR can double the amount of DNA in each cycle of amplification, exponentially increasing the amount of DNA to detectable levels (Figure 2.2).

PCR requires template DNA from the patient's cells, a heat-stable DNA polymerase purified from bacteria, nucleotides from which

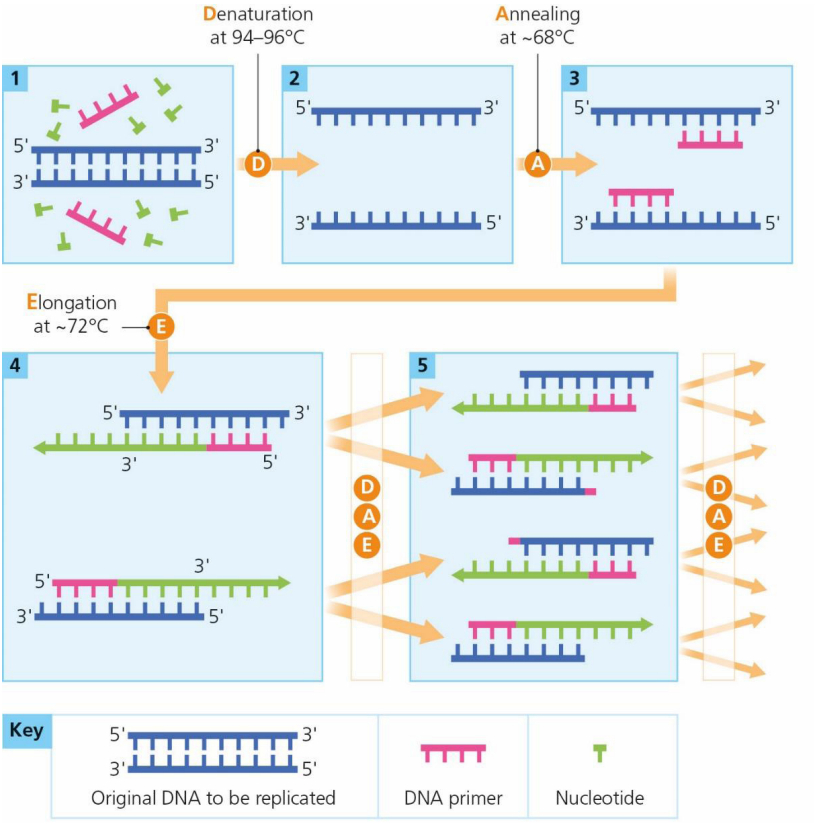

Figure 2.2 Amplification of template DNA during the polymerase chain reaction (PCR). Multiple DNA copies are produced using DNA polymerases under controlled conditions. (D) Denaturation: the reaction is heated to 94–96oC for 20–30 seconds, which disrupts the hydrogen bonds of the template DNA to produce single-stranded DNA. (A) Annealing: the reaction temperature is lowered to about 68oC for 20–40 seconds, allowing DNA primers to anneal to the single-stranded DNA molecules. A good bond is formed when the primer sequence closely matches the original DNA sequence. (E) Elongation: the DNA polymerase synthesises a new DNA strand. This process is repeated 20–30 times until the DNA is amplified to detectable levels.

the new DNA is assembled, and primers that target the regions to be amplified. The reaction is run through cycles at different temperatures, which are optimised to enable separation of the two strands of DNA (denaturation), primer annealing to the template DNA, and the joining of nucleotides to the newly replicating strand of DNA (elongation) (see Figure 2.2). In total, 20–30 cycles of PCR are performed to amplify DNA to

Measurement of disease burden (cont.)

Figure 2.3 Standard polymerase chain reaction (PCR) requires different primers for different patients whereas the PCR of complementary DNA (cDNA) can use the same primer sets for most patients. Arrows indicate regions where primers anneal to the DNA.

detectable levels.

Primers determine the sections of DNA to be amplified and are designed to amplify short segments of only a few hundred DNA bases; attempts to amplify longer segments of DNA can lead to errors and non-specific amplification.

Direct amplification of BCR–ABL1 DNA in CML cells is challenging because the breakpoint where BCR joins on to ABL1 on the Philadelphia chromosome differs between patients, so a standard set of primers cannot be used. However, this is overcome using reverse transcriptase (RT)-PCR (described below).

RT-PCR

A key cellular process is the transcription of DNA to RNA by RNA polymerase. RNA introns (non-coding regions) are spliced out and the exons (coding regions) are joined to give

messenger RNA (mRNA). mRNA can then be reverse transcribed using a reverse transcriptase to form complementary DNA (cDNA), providing a template for PCR that lacks introns (Figure 2.3).

Exon 1 of ABL1 is joined to exon 13 or 14 of BCR in about 95% of CML cases, allowing use of a standard primer set for 95% of patients with CML.

Quantitative RT-PCR (RT-qPCR)

To quantify the BCR–ABL1 cDNA as it is amplified, a fluorescent dye such as Sybr Green is added to the reaction and is incorporated into the DNA. The level of fluorescence (captured by a light detector) is proportional to the amount of BCR–ABL1 cDNA.

Reference genes

A reference gene is a gene that is transcribed to a constant level in all cells at the same time, providing a reference point for the measurement of fluorescence intensity and allowing comparison between tests done at different times during monitoring of a patient. An ideal reference gene is expressed consistently between different cell types and patients, and over the course of treatment. It should also be expressed at a level similar to that of BCR–ABL1 and be broken down at a similar rate, since delays between blood sampling and testing can differentially alter levels of different mRNAs. ABL1 is an ideal reference gene, so BCR–ABL1 transcript burden is normally reported as the BCR–ABL1:ABL1 ratio; some laboratories use -glucuronidase as a reference gene instead.

Practical considerations

Sample quality

The quality of RT-qPCR detection depends on the quality of the blood or bone marrow sample provided. The red blood cells are lysed and RNA extracted from the remaining cells for RT-qPCR. However, if delivery or processing of the sample is delayed, or the sample has clotted because of prolonged marrow extraction or difficult venepuncture, many of the cells will be dead

Measurement of disease burden (cont.)

or dying and will therefore have low levels of poor-quality RNA, reducing transcription.8 However, transcription of the BCR–ABL1 transcript is reduced proportionally to the ABL1 reference gene, so BCR–ABL1:ABL1 ratios are maintained and RT-qPCR results may still be valid.8 Nevertheless, the sensitivity threshold for detection may be poorer and it may not be possible to detect the very low levels of BCR–ABL1 needed to identify deep molecular responses.

Number of cells

If very little sample is provided (e.g. < 5mL blood or bone marrow) or the white blood cell count is very low, there is unlikely to be sufficient RNA to extract for testing. This may be a particular issue when testing samples from the few patients with refractory CML who undergo allogeneic stem cell transplantation, as there may be insufficient sample during induction therapy and in the early stages of engraftment and immune reconstitution. Experts recommend that at least 10 million white blood cells are needed for testing with high sensitivity.

Type of sample

A further consideration is whether to obtain blood or bone marrow for testing. Patients generally prefer peripheral blood testing because it is less invasive and less painful than bone marrow aspiration. However, blood cell production (haematopoiesis) begins in stem cells in the bone marrow, and it takes a while for cells to mobilise to the peripheral blood. A bone marrow sample may therefore give an earlier snapshot into disease status, allowing earlier detection of a molecular relapse.

Variation in laboratory techniques and standardisation

Samples may be tested at sites remote from where patients are seen, and the results generated by different laboratories may vary for a number of reasons, including:

- method of isolating white blood cells (e.g. red blood cell lysis,

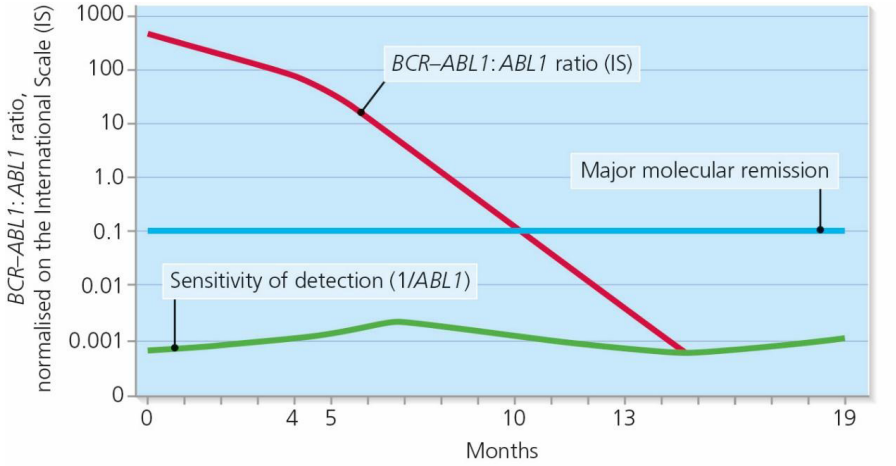

Figure 2.4 Example report of a patient with chronic myeloid leukaemia being monitored for disease burden based on the BCR–ABL1:ABL1 transcript ratio (measured using quantitative reverse transcriptase polymerase chain reaction [RT-qPCR]), shown by the red line. The horizontal blue line represents the threshold for major molecular remission (3-log molecular response). The green line represents the sensitivity of detection, which varies at each RT-qPCR measurement depending on the quality of the sample and practical reasons. Adapted from Cross et al., 2018.

density gradient centrifugation)

- method of extracting RNA (e.g. phenol–chloroform, column purification)
- choice of reverse transcriptase and DNA polymerase enzymes
- choice of primers
- source of fluorescence (Sybr Green, Taqman probes)
- RT-qPCR machine used
- quantification method (e.g. standard curve, delta-delta cycle threshold)

Standardisation is achieved by normalising results to the International Scale (IS), which is based on a set of standards from patients in the IRIS trial of imatinib in CML. Laboratories either calibrate their equipment based on RNA standards from an IS laboratory or use a conversion factor. In the UK, the National External Quality Assessment Services is responsible for standardisation between laboratories, and results are normally calibrated to the IS Laboratory at Sheffield Teaching

Measurement of disease burden (cont.)

Hospitals NHS Foundation Trust.

Interpretation of results

The BCR–ABL1 transcript burden ranges across several orders of magnitude, so results are best visualised on a log scale (as in Figure 2.4). Each log-decrease in the BCR–ABL1:ABL1 ratio represents a tenfold decrease in level. For example, a 2-log decrease means a 100-fold decrease; a 3-log decrease means a 1000-fold decrease and so on. This is designated as a superscript number, so MR3 means a 3-log reduction in BCR–ABL1:ABL1 ratio. Table 2.3 presents the categories for depth of response. The deeper the response, the better the prognosis.

TABLE 2.3 Molecular response categories

BCR–ABL:ABL (IS)	Log	Category
≤ 0.1%	3	MR3 (major
≤ 0.01%	4	MR4
≤ 0.0032%	4.5	MR4.5
≤ 0.001%	5	MR5

Key points – measurement of disease burden

- The burden of disease (i.e. the level of BCR–ABL1 transcript) can be assessed by several methods, including full (complete) blood count, Giemsa banding karyotyping to identify presence of the Philadelphia chromosome, fluorescence in situ hybridisation, polymerase chain reaction (PCR) and next-generation sequencing.
- The most commonly used method is quantitative reverse transcriptase PCR, which

detects the ratio of abnormal BCR–ABL1 transcripts to normal ABL1 transcripts.

- An International Scale has been developed for standardisation of results, so that disease burden can be compared at different times even if there is variation in laboratory equipment or the quality or type of sample.

Clinical practice

Patients started on first-line imatinib

The concept of treatment-free remission (TFR) emerged from small observational studies in which some patients who stopped treatment with a tyrosine kinase inhibitor (TKI) because of intolerance or by preference remained in remission for at least several months. This led to larger studies, such as the Stopping Imatinib (STIM) study,1 in which 100 patients who had been in complete molecular response (CMR) for at least 2 years (> 5-log reduction in BCR–ABL and ABL levels and undetectable transcripts on quantitative reverse transcriptase polymerase chain reaction [RT-qPCR]) stopped treatment. After cessation of treatment, 41% were free from molecular relapse (defined as 1-log rise in BCR–ABL1 transcripts) at 12 months. Relapses generally occurred in the first 6 months after stopping treatment, with only three late relapses (after 18 months). Importantly, those who experienced relapse responded to subsequent imatinib, with 16 of 42 (38%) going on to achieve a second CMR.

Similar results were reported in the Australasian TWISTER study,2 which investigated patients in molecular remission to a sensitivity of 4.5 logs (i.e. MR4.5): 47% remained in molecular remission at 24 months.

However, these studies were conducted in 'ideal' patients who were clearly in molecular remission, whereas in real-world practice patients often hover just below MR4.5 with persistent low levels of BCR–ABL1 transcripts following cessation of treatment. This cohort of patients were investigated in the French A-STIM trial;3 molecular relapse was defined as BCR–ABL1 transcripts above MR3. Despite this, the TFR rate was 37% at 36 months, which is highly comparable to the results from the STIM and TWISTER studies.

The EURO-SKI trial had even less stringent inclusion criteria, allowing patients to discontinue TKI treatment after 3 years with MR4. The molecular relapse-free

rate was 50% at 24 months.4 While this allowed more patients to stop TKI treatment, subgroup analysis demonstrated that the longer the duration of treatment, the deeper the remission and the greater the probability of maintaining remission to MR3.

Thus, an attempt at TFR may be considered for patients in remission below MR4 and who have taken a TKI for at least 3 years, although some international experts, such as Fran ois-Xavier Mahon at the Institut Bergonié, Bordeaux, France, tend to be more conservative, given that longer treatment is associated with deeper and more persistent remission (Table 3.1).5 However, practice is informed by local guidelines, which vary widely. For example, the US National Comprehensive Cancer Network guidelines require only MR4 for 2 years.

TABLE 3.1 Criteria for attempting treatment-free remission in patients with chronic myeloid leukemia

Centrer requirements	Declaration to a national or international registry Ability to monitor BCR–ABL1 transcripts to IS sensitivity of ≥ 4 log
Patient requirements	Chronic phase of CML ≥ 5 years' treatment with TKI No prior history of resistance to TKI treatment MR4 for ≥ 3 years or MR4.5 for ≥ 2 years
Molecular monitoring during TFR	Monthly for the first 12 months Every 2 months in year 2 Every 3 months thereafter
Molecular relapse	Loss of molecular remission to MR3 Re-challenge with the same TKI within a month

Clinical practice (cont.)

Study	TKI treatment	Discontinuation criterion	MR threshold	Molecular relapse-free survival at 12 months
DADI	2L dasatinib > 1 year	MR4.5	MR4.5	48%
STOP 2G-TK	1L/2L dasatinib/ nilotinib > 3 years	MR4.5 > 2 years	MR3	45%
DASFREE	1L/2L dasatinib	MR4.5 > 1 year	MR3	63%
ENESTop	2L nilotinib ≥ 3 years	MR4.5 ≥ 1 year	MR3	58%
D-STOP	1L/2L dasatinib ≥ 2 years	MR4 ≥ 2 years	MR4.5	63%
ENESTFreedom	1L nilotinib ≥ 3 years	MR4.5 ≥ 1 year	MR3	52%
ENESTPath	2L nilotinib ≥ 2 or 3 years	MR4.5 ≥ 1 year	MR3	43%
STAT2	2L nilotinib ≥ 2 years	MR4.5 ≥ 2 years	MR4.5	68%
Nilst	2L nilotinib ≥ 2 years	MR4.5 ≥ 2 years	MR4.5	59%

TABLE 3.2 Treatment-free remission with second-generation tyrosine kinase inhibitors

Second-generation TKIs

The advent of the second-generation TKIs prompted the question of whether TFR could be attempted in patients who received these agents as first-line treatment or as second-line treatment after failure of imatinib. Table 3.2 summarises outcomes for patients in TFR following treatment with a second-generation TKI. In all trials, a second molecular response could

be achieved after re-initiation of the same TKI following a first molecular relapse.

Predicting successful TFR

Clinical trials have identified factors that predict a successful (i.e. long-lasting) TFR, such as response to first-line TKI, duration of TKI treatment and duration of deep molecular remission. Further analyses have suggested several other factors.

Early molecular response (i.e. low BCR–ABL1IS)

This is associated with a better prognosis. Ratios over 10% at 3 months were associated with shorter progression-free and overall survival in trials of dasatinib.

Type of TKI

Most studies suggest that the molecular response rate is higher with the second-generation TKIs, so more patients become eligible for attempting TFR. However, results from the trials of imatinib and the second-generation TKIs discussed above indicate that a longer TFR achieved by the use of second-generation TKIs does not always translate into longer overall survival. It is therefore important to consider whether any adverse effects of the TKIs may compromise survival compared with that expected based on their molecular response.

Risk stratification

Several scoring systems can be used to stratify the risk of chronic myeloid leukaemia (CML), including Sokal, EUTOS and EURO. Sokal is perhaps the best known and takes into account the patient's age, spleen size, blast count and platelet count. While the STIM trial seemed to show some correlation between duration of TFR and Sokal score, most trials since, including the large EURO-SKI trial,4 have not shown an association between TFR success and Sokal score.

Patient education

Before attempting to achieve TFR, patients need to be fully informed about and understand the risks and benefits. They should understand that a molecular relapse (a significant increase in BCR–ABL1 transcripts, usually above MR3) is often a precursor to haematological relapse, when cells proliferate abnormally. They must therefore be prepared to attend regular monitoring and appointments so that molecular

Clinical practice (cont.)

relapse can be identified and treatment restarted to avoid full haematological relapse.

TFR may cause anxiety about the risk of haematological relapse by withdrawing a treatment that a patient knows works well. Indeed, an Italian survey of over 1100 patients reported that nearly 50% had such concerns.17 Not all patients will want to stop treatment, and many will prefer to manage on treatment with minimal side effects.

Patients should be aware that about half of those who stop treatment are likely to have a molecular relapse within the first year and will have to restart treatment. However, molecular relapse does not mean that the TKI has failed, only that it needs to be used for longer. Many TFR trials have shown that patients usually respond to the TKI that they initially achieved a deep molecular remission with. Furthermore, patients undergoing TFR may go into a blast crisis, whereupon achieving a second remission with just TKI therapy is unlikely. Indeed, one patient who underwent TFR in the A-STIM trial experienced a lymphoid blast crisis.

It is important for patients to understand that staying in deep molecular remission does not necessarily mean that they are cured, as CML stem cells are particularly hard to eradicate and may survive quiescently for many months. Indeed, trials have reported the occasional late relapse at 18–24 months of TFR.

Risks of attempting TFR

In clinical trials, including EURO-SKI and ENESTFreedom, 25–30% of patients who stopped taking any TKI experienced a 'TKI withdrawal syndrome' – pain localised to various parts of the body, including the hips, shoulders and extremities, resembling polymyalgia rheumatica. Most patients can manage these symptoms with paracetamol and non-steroidal anti-inflammatory drugs, although some require steroids. It is thought that off-target effects of TKIs in

inhibiting the receptor tyrosine kinase cKIT relieves some joint inflammation, which returns upon TKI withdrawal. Supporting this link, some patients with spondyloarthritis achieve pain relief with nilotinib.

Eligibility for attempting TFR

CML without the classic BCR–ABL1 fusion site

Monitoring to a very high sensitivity using techniques such as RT-qPCR is crucial during TFR in order to assess the depth of molecular remission and to identify early re-emergence of BCR–ABL1 transcripts. Most BCR–ABL1 translocations occur between exon 1 of ABL1 and exon 13 or 14 of BCR, and primers for RT-qPCR are designed for such transcripts. Occasionally, however, the BCR–ABL1 fusion may occur at a different point, meaning that transcripts may not be detectable using conventional primers. While patients with these transcripts may respond to TKIs and achieve remission, they are less suitable for TFR because early molecular relapse cannot be detected using standard techniques, so the patient is at risk of full haematological relapse.

Atypical CML

This is not driven by the BCR–ABL1 translocation and is associated with a variety of chromosomal aberrations and mutations. Issues with monitoring preclude these patients from TFR, as described above.

History of TKI resistance

While clinical trials have shown that patients who have a molecular relapse during TFR are sensitive to the TKI that they achieved remission with, this has not been confirmed in patients with TKI resistance mutations. These patients may develop further mutations (compound heterozygotes) that would render them resistant to any TKI. Thus, there is reluctance to attempt TFR in patients with known TKI resistance.

Children

CML is quite rare in childhood. While the same theoretical

Clinical practice (cont.)

principles should apply, whereby a deep remission correlates with a successful TFR, there have been no clinical trials to demonstrate this. In addition, factors such as growth and fertility need to be considered, which may not be adequately explored in adult trials. TKIs are known to affect other growth receptor molecules; whether alternating between treatment and no treatment would have any deleterious effect on growth or fertility is unknown.

Alternatives to TFR

Patients who have not been taking a TKI for long enough (3–5 years) and those who do not have a deep enough molecular remission are not eligible for TFR. It is important that these patients understand why they cannot attempt TFR, and that CML is a potentially fatal blood cancer; therefore continuing with daily TKI treatment is vital to keep their cancer in remission. We should be clear that TFR is only attempted in patients for whom it is believed to be safe, based on extensive clinical trials. Many patients will have to wait until they have taken TKI for long enough and have a deep enough remission.

For patients who want or need to alter their treatment, dose reduction or a treatment holiday can be considered.

Dose reduction

This can be considered for patients who do not fit the criteria for TFR but experience side effects with TKIs. Generally, reducing the dose of TKI is known to ameliorate side effects, but this should not be at the risk of disease relapse. In the DESTINY trial, patients who had been on the same TKI (imatinib, nilotinib or dasatinib) for 3 years and were in MR3 were eligible to halve their dose for a year, followed by TFR. During the half-dose phase, the molecular relapse-free survival rate was 93% and all of these patients went on to TFR. Patients reported that most side effects improved within the first 3 months of the half-dose phase.

Treatment holiday

Some patients can be given a

deliberate treatment holiday of a few days, or possibly weeks, in order to be free from side effects for a special occasion, or if they are unwell for other reasons. Treatment is restarted after a short break. However, longer treatment holidays are not recommended. Patients who want to become pregnant should be counselled about the need for a planned approach, and the need to meet the criteria for attempting TFR.

Patients may also be considered for regular treatment holidays of 2 days a week (e.g. weekends). The Phase III DasaHIT trial, which is currently recruiting patients on dasatinib, is investigating whether molecular remission rates and toxicity profiles are affected by regular treatment holidays.

Key points – clinical practice

- Treatment-free remission (TFR) can be considered for patients who achieve deep molecular remission (at least MR4) with first- or second-generation tyrosine kinase inhibitors (TKIs).
- The risks and benefits must be discussed thoroughly with patients before starting TFR, and patients must agree to regular monitoring to identify early molecular relapse and thereby avoid a full haematological relapse.
- Some patients experience joint pains after stopping TKI treatment.
- Patients with atypical CML or a history of TKI resistance, and children, are not eligible for TFR.
- Dose reduction or a deliberate treatment holiday can be considered for patients who are not eligible for TFR.

Future directions

Second attempt at treatment-free remission

Currently, patients who experience disease relapse during treatment-free remission (TFR) are advised to take a tyrosine kinase inhibitor (TKI) for life. However, the French Re-STIM multicentre trial evaluated a second TFR in 70 patients who had a molecular relapse in their first TFR. Patients were required to have achieved molecular response (MR)4.5 upon resumption of a TKI before a second attempt at TFR. Forty-six percent of patients were molecular relapse-free at 12 months but relapses continued steadily after this time, with some occurring over 3 years after cessation of TKI treatment. Nevertheless, 36% of patients remained in long-term molecular remission, suggesting that there is value in attempting a second TFR.

Biomarkers

In many tumour types, biomarkers have been identified that help predict response to treatment, and this is an intense area of research in chronic myeloid leukaemia (CML). Biomarkers are needed that will enable us to identify the patients likely to respond best to treatment and hence achieve a deep remission, and those who may benefit from TFR. Several approaches are being explored but have not yet reached clinical trials.

In vitro analysis

Patients' CML cells can be cultured in vitro and treated with different concentrations of imatinib; the lower the concentration of imatinib needed to kill 50% of all CML cells (the IC50), the better the patient is likely to respond to imatinib.

OCT-1 (organic cation transporter 1)

This is the major transporter protein that imports TKIs into cells. Patients with high OCT-1 activity have high molecular

remission rates whereas patients with low OCT-1 activity show resistance to most TKIs.

CIP2A (cancerous inhibitor of protein phosphatase 2A) is an oncoprotein that promotes proliferation of cancer cells, anchorage-independent cell growth and resistance to apoptosis. CML with high CIP2A expression is more likely to transform to accelerated or blast crisis.

GFI-1 (growth factor independent 1 transcriptional repressor)

This inhibits cell proliferation; CML with low GFI-1 tends to be more aggressive and more likely to transform.

BIM polymorphism

This refers to a common intronic deletion polymorphism in the gene that encodes BCL2-like 11 (BIM), a pro-apoptotic member of the B-cell chronic lymphocytic leukaemia (CLL)/lymphoma 2 (BCL2) protein family; its upregulation is required for TKIs to induce apoptosis in kinase-driven cancers. In Asian populations, a polymorphism in BIM leads to relative resistance of CML cells to TKIs.

T cell cytotoxicity and natural killer cell numbers

Once patients achieve remission, the immune system can kill off many of the residual CML cells. In particular, natural killer (NK) and cytotoxic T cells can kill CML cells through the secretion of perforin and granzyme. In the STIM trial, the numbers of NK cells and level of cytotoxicity was lower in patients who relapsed during TFR than in those who did not.

Quantification of BCR–ABL1 transcripts

Deeper molecular remission is more likely to result in a longer lasting TFR. BCR–ABL1 transcripts down to MR5 can be measured using quantitative reverse transcriptase polymerase chain reaction (RT-qPCR), which

Future directions (cont.)

corresponds to just one cancer cell in every 100 000 normal cells. However, most patients with deep molecular remissions are not 'cured' and remain at risk of relapse. The development of even more sensitive techniques may make it possible to identify patients who are likely to have a long TFR. It may also be possible to identify patients who are fully cured. While cure is unlikely with TKIs only, it may be possible with upcoming novel therapies.

As discussed in Chapter 2, RT-qPCR is a useful technique because a common set of primers can be used to test most patients in a standardised way. A more sensitive method that directly tests for the fused BCR–ABL1 genomic DNA (gDNA) would be ideal but would require custom primers for each patient's specific BCR–ABL1 translocation, which would be time- and labour-intensive.

A superior way to test gDNA would be to perform high-coverage next-generation sequencing (NGS), which enables detection to MR6. NGS is often performed on IlluminaTM platforms which can provide reads up to 600 bp in length; however, this would still require custom primers for each patient. The use of NGS platforms that can offer very long reads could potentially overcome this limitation. For example, nanopore sequencing can provide reads of up to 2 million bases, meaning that just one set of primers targeting the start of exon 1 of BCR and the end of exon 11 of ABL1 would allow detection of any BCR–ABL1 fusion in any patient. The feasibility of this approach in CML has been established in a study involving ten patients, one of whom had a complex variant BCR–ABL1 translocation, in which all patients could be monitored to a high sensitivity.

Such improvements may allow us to offer TFR to patients with atypical BCR–ABL1 fusions (these patients are not currently eligible). Intriguingly, we may also be able to identify patients who have no

remaining cells with the BCR–ABL1 translocation and who are therefore cured.

Combination therapy

The duration of TFR depends on the depth of molecular remission, as discussed in Chapter 3 and as seen in the EURO-SKI and other trials. While the second-generation TKIs can induce deeper molecular remission than imatinib, adding a second agent to work synergistically with the TKI may enable an even deeper response.

Studies by the Nordic CML study group and the French SPIRIT study showed that the addition of pegylated interferon alfa to imatinib led to higher rates of molecular response and deeper molecular remissions. It is hypothesised that molecular relapse largely derives from quiescent leukaemic stem cells that are insensitive to TKIs. Interferon alfa drives these stem cells out of quiescence and into the cell cycle, when they are likely to become sensitive to TKIs. Unfortunately, however, the German CML IV study did not corroborate the findings of the other two studies, as it did not show any advantage of adding interferon alfa to a TKI; thus, the value of this therapeutic strategy remains unclear.

Treatment-free remission with novel therapies

New therapies emerging for CML may alter the selection of patients to whom we offer TFR. As discussed in Chapter 3, patients with known TKI resistance mutations may not be eligible for TFR. However, the development of non-ATP site allosteric inhibitors such as asciminib have shown good efficacy even in patients with highly resistant tyrosine kinase mutations, particularly in combination with current TKIs such as ponatinib. Thus, we may be able to offer TFR to patients with compound tyrosine kinase mutations in the future, although

Future directions (cont.)

the safety of this approach needs to be evaluated in clinical trials.

Eradication of quiescent leukaemic stem cells

Quiescent leukaemic stem cells (LSCs) are known to survive the deep remission induced by TKIs. Thus, ways to eradicate these cells are being explored. The greatest experience has been in allogeneic stem cell transplantation (ASCT). In ASCT, following high-dose chemotherapy (with or without radiotherapy), haematopoietic stem cells from a donor are transplanted into the patient with CML. The donor cells can engraft and reconstitute haematopoiesis in the patient, and donor-derived T cells and natural killer cells attack the patient's cancer cells, including LSCs. Interestingly, there is some evidence that continuing TKI therapy after transplant can improve outcomes. It will therefore be interesting to determine options for TFR in these patients and whether any experience a relapse.

Other ways to eradicate LSCs in patients with CML are also being explored:

- targeting signalling pathways such as JAK-STAT with the JAK inhibitor ruxolitinib
- inhibiting the interaction of LSCs with their microenvironment
- targeting epigenetic proteins such as EZH2
- promoting apoptosis of LSCs using venetoclax
- targeting metabolic pathways in LSCs.

Re-definition of eligibility criteria

The ever-improving technologies for disease detection and the development of novel therapies mean that the eligibility criteria for TFR are also evolving. Further trials using these new technologies and treatments will help us establish the optimal

eligibility criteria for routine clinical practice.

Treatment-free remission in other haematological malignancies

CML can be considered a trail-blazing disease in which the TFR paradigm has been established, prompting exploration of TFR in other haematological malignancies.

Chronic lymphocytic leukaemia

Recent trials in CLL have shown good outcomes with targeted therapies. For example, in a recent Alliance trial, median progression-free survival was longer with ibrutinib than with rituximab plus chemotherapy in patients over 65 years; the 4-year progression-free survival rate was over 70% in the ibrutinib arm, compared with about 45% in the chemotherapy arm.

Patients continue ibrutinib until disease progression and most patients continue to have detectable minimal residual disease, including those who do not relapse. This good outcome without being cured raises parallels with patients with CML who continue to have detectable BCR–ABL1 transcripts without relapsing. It would therefore be interesting to explore whether patients with CLL receiving ibrutinib can be offered TFR, whether they relapse off treatment and whether the disease remains sensitive to ibrutinib if re-challenged.

Acute lymphoblastic leukaemia (ALL)

Adults with ALL in remission who do not undergo ASCT usually receive about 2 years' maintenance treatment with, for example, daily mercaptopurine, weekly methotrexate and steroid courses. However, some patients experience relapse when maintenance therapy is stopped. The GMALL trials showed that measuring immunoglobulin and T cell receptor clonogenicity by quantitative PCR enabled

Future directions

impending relapse to be predicted. A better strategy for these patients may be to continue maintenance treatment for longer until a deep remission is achieved and only then offer TFR.

Other lymphomas

Patients with follicular lymphoma receive maintenance with rituximab for 2 years. Two years' maintenance with lenalidomide has been shown to improve progression-free survival in patients with diffuse large B-cell lymphoma.23 Rather than these arbitrary durations, a superior strategy may be to continue therapy until circulating tumour DNA is cleared from the bloodstream and then attempt TFR.

Key points – future directions

- The feasibility of a second treatment-free remission (TFR) is being explored.
- Biomarkers are being identified that may enable us to identify patients who are more likely to have a long-lasting TFR.
- Advances in detection technology may enable monitoring of patients with atypical BCR–ABL1 translocations, allowing the option of TFR, and earlier detection of likely relapse.
- The duration of TFR may be improved by novel therapies or by combinations of treatments that lead to deeper molecular remission.
- The success of the TFR approach in chronic myeloid leukaemia has paved the way to explore this approach in other haematological malignancies.

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