Fast Facts: Measurable Residual Disease

A Guide for Nurses

Leukaemia Care
Nurse Academy

Introduction

Conventional methods of detecting disease remission in cancers such as leukaemia rely on microscopic analysis of tissue samples. Measurable (or minimal) residual disease describes the presence of disease beyond the levels of sensitivity afforded by microscopy. Fast Facts: Measurable Residual Disease describes what is meant by measurable residual disease, the opportunities and challenges afforded by its identification and the advantages and disadvantages of the methods used for detection.

We also draw on specific examples to illustrate the implications of detecting measurable residual disease in different disease scenarios, with emphasis on acute myeloid and acute lymphoblastic leukaemias. The recognition and monitoring of measurable residual disease are likely to play an increasingly important role in disease prognosis and subsequent treatment direction – this accessible resource is ideal for any healthcare professional wanting to know more about this exciting and fast-moving area.

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

- **ALL:** acute lymphoblastic leukaemia
- **AML:** acute myeloid leukaemia
- **APML:** acute promyelocytic leukaemia
- **CBF:** core binding factor
- **CHIP:** clonal haematopoiesis of indeterminate potential
- **CLL:** chronic lymphocytic leukaemia
- **CML:** chronic myeloid leukaemia
- **CR:** complete remission
- **DNA:** deoxyribonucleic acid
- **ELN:** European LeukemiaNet
- **FISH:** fluorescent in situ hybridisation
- **MRD:** measurable residual disease (also referred to as minimal residual disease)
- **NGS:** next-generation DNA sequencing
- **NICE:** (England and Wales) National Institute for Health and Care Excellence
- **OS:** overall survival
- **PFS:** progression-free survival
- **RT-qPCR:** quantitative reverse transcriptase polymerase chain reaction

What is measurable residual disease?

As every patient has a different response to treatment, a fundamental question is how to assess this treatment response. The assessment of response to treatment has enormous implications for what the patient can expect for the future and whether they might require further monitoring or treatment. For some malignancies such as lymphoma, imaging to assess tumour size will be critical. However, for leukaemias, which are fundamentally diseases of the blood and bone marrow, there can be many levels of assessment.

The simplest level is that of the full blood count – has the count been corrected so that the patient can become independent of blood and platelet transfusion support?

The next level is that of the bone marrow. Assessment can take place using light microscopy so that the haematologist/pathologist can physically count cells to determine whether there is complete remission (CR; sometimes referred to as morphological remission). In acute myeloid leukaemia (AML), for example, CR is said to have occurred if blasts account for fewer than 5% of cells overall.1 Below the level of sensitivity afforded by this direct physical assessment, it is difficult to ascertain by microscopy alone whether any leukaemia cells remain. Despite cells having the characteristic appearance of a 'blast', it is not always clear whether they are normal regenerating stem/progenitor cells or residual leukaemic disease. The implications are clear in acute leukaemia: patients often achieve CR, as assessed by light microscopy, after initial induction chemotherapy, but they inevitably relapse in the absence of further treatment.

Critically, the assessment of measurable (historically called minimal) residual disease (MRD) allows a dynamic evaluation of a patient's risk of relapse and

What is measurable residual disease? (cont.)

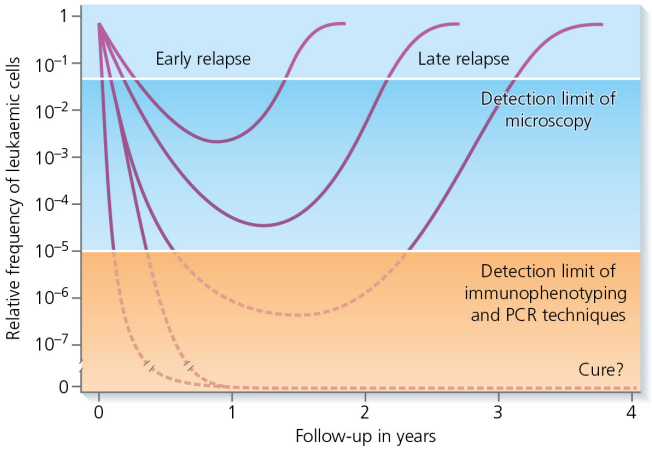


Figure 1.1 The clinical trajectories and associated levels of measurable residual disease that are possible for a patient with acute lymphoblastic leukaemia. PCR, polymerase chain reaction. Adapted from van Dongen et al. 2015.

treatment failure over time, beyond the initial assessment of risk based on genetic and clinical factors. It is to be hoped that this will inform patient/clinician discussions about disease management options.

Assessing measurable residual disease

Assessments developed to quantify MRD are based on a range of sensitive techniques that, essentially, detect small numbers of disease cells. Improved sensitivity allows better prognostication and treatment stratification for patients. The move from describing this residual disease as 'minimal' to 'measurable' is predicated on the findings of studies that have shown 'minimal' to be misleading, as its presence tends to be associated with poor prognosis.

MRD assessment, like all tests, is subject to limitations: a negative MRD result does not necessarily mean a patient is cured, nor does a positive MRD result imply relapse will definitely occur.

The different levels of disease quantification and the limits of different forms of assessment are shown in Figure 1.1.

MRD technology is based on the identification of a disease marker (for example, a surface protein or DNA mutation) at diagnosis that can be quantified during treatment. Ideally, this marker should remain stably present on or in the cell throughout the disease course. MRD techniques are developing rapidly, but a brief overview of the different methods is given in the next chapter.

CR with MRD negativity

This has been suggested as a new form of CR in many clinical trial response criteria.1 It may also be increasingly useful in conditions such as chronic lymphocytic leukaemia (CLL), where the treatments are now extremely effective and it is difficult to show significant differences in overall survival in clinical trials. This is also referred to as a 'surrogate endpoint'.

Challenges

Each test needs to be calibrated to a certain standard and performed in a reproducible manner to ensure the level of sensitivity.

Interpreting the results depends on expertise. As a consequence, MRD samples may need to go to regional or national laboratories for analysis. There is also the expense of collecting, processing and interpreting the information.

National trial protocols, particularly those developed for acute lymphoblastic leukaemia (ALL) and AML trials, have helped clinicians act on results of MRD analysis. International collaborations such as European LeukemiaNet (ELN) have produced helpful guidelines. Chronic myeloid leukaemia (CML) is characterised by the presence of a t(9;22) chromosomal translocation which produces a fusion protein BCR–ABL. The fusion transcripts from the fusion gene BCR–ABL can be used to monitor the presence of disease. ELN guidelines have been produced to support the interpretation of BCR–ABL results. Similarly, international collaborations have attempted to set best-practice standards for MRD assessment in AML.

Limitations

Many MRD technologies are specific for disease subtypes. MRD assessment is not suitable

What is measurable residual disease? (cont.)

for all disease subtypes, and alternative forms of disease assessment are required (for example, imaging and histology). Also, MRD may be prognostic, but it is not always clear how this should influence therapy. For example, it is not known whether intensifying treatment for patients with MRD before an allogeneic stem cell transplant necessarily improves outcomes. Finally, MRD quantification (the degree of MRD) needs to be interpreted based on the type of specimen, disease, mutation, treatment and patient context.

Patient considerations

Awaiting the results of MRD assessment can be an anxious time for the patient, who also has the stress and inconvenience of repeat blood or bone marrow sampling. MRD positivity and negativity need to be communicated clearly. A negative MRD result does not necessarily mean the patient is cured, though it may increase the chance of achieving a cure over time. Similarly, a positive MRD result does not necessarily mean that they have relapsed, but the risk of relapse may be increased.

Key points – what is measurable residual disease?

- Measurable (or minimal) residual disease (MRD) refers to the low number of disease cells that may remain despite the patient being in remission.
- Different methods of MRD assessment have distinct advantages and disadvantages.
- Results need to be interpreted based on many factors, including the testing method as well as the patient and disease context. Guidelines are available to help interpret MRD levels.

How is MRD measured?

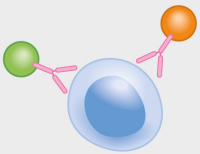
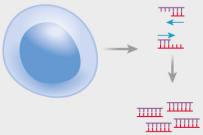
MRD modality	Advantages	Disadvantages
Multiparameter flow cytometry 	• Fast turnaround • Can be used in majority of patients	• Relies on expertise and skills of reporting laboratory • Phenotype of leukaemic cell may change during monitoring period
Quantitative PCR 	• Highly sensitive • Allows comparisons with previous results • Routinely established in many laboratories, with standardisation	• Restricted molecular targets • Requires expertise
Next-generation sequencing 	• Widely applicable	• Development is ongoing • Interpretation depends on many factors, including the gene in question • Limited sensitivity (but improving) • Requires expertise

Figure 2.1 The advantages and disadvantages of the main methods used to analyse measurable residual disease (MRD). AML, acute myeloid leukaemia; PCR, polymerase chain reaction.

There is no single optimal measurement of measurable residual disease (MRD). In this chapter, we describe the principles behind some of the common and emerging techniques and discuss the advantages and disadvantages of each one. One common point is that dilution of the bone marrow with blood (haemodilution) can affect the results (just as a diluted sample might affect the morphological assessment). Therefore, a common recommendation is that the 'first pull' bone marrow is used for MRD analysis.

The advantages and disadvantages of different methods of MRD measurement are summarised in Figure 2.1.

How is MRD measured? (cont.)

Multiparameter flow cytometry

Flow cytometry is a technique whereby antibodies are used to bind to different cell markers on the outside (and sometimes on the inside) of cells. Individual antibodies bind specifically to target proteins. These antibodies are tagged (conjugated) with a fluorescent molecule called a fluorochrome. The treated cells then pass, cell by cell, through a laser beam. If the cell is coated with the antibody, the fluorochrome emits a specific signal that is detected by the flow cytometer. This allows the identification of specific proteins present on the cell. To identify different populations of cells (such as leukaemic cells and T cells), multiple fluorochrome-conjugated antibodies that can bind different targets are used. These groups of antibodies may be described as a panel.

Panels of fluorochrome-conjugated antibodies are designed for different diseases (for example, the antibody panel designed for acute myeloid leukaemia [AML] is different from that designed for chronic lymphocytic leukaemia [CLL]). These panels typically include eight different targets, though some laboratories use multiple panels and can detect more targets.

Different analytical techniques are possible: for example, one way of analysing AML cells is to look for specific abnormal expression patterns of surface markers at diagnosis and then track these markers, and hence the cells, through treatment. In some patients, though, some cell markers may be lost and so this may miss changes in the appearance or profile (phenotype) of the cells during treatment. A combination of techniques to enable capture of both diagnostic and relapse-associated phenotypes is recommended.

Advantages

The benefit of this technique is that it can capture other information, such as the viability of the cell sample – whether cells are alive, rapidly proliferating or dead. Also, because a range of markers are analysed, the results can provide a perspective on the identity of the cell. For example, one area of interest in AML is the identification of leukaemic stem cell populations. Leukaemic stem cells are thought to be a reservoir of cells that may be resistant to chemotherapy; persistence of these cells can lead to relapse, with blasts forming the bulk of the leukaemia. Many ways have been used to describe leukaemic stem cells, including the presence on their cell surface of different protein markers. It is unsurprising that researchers have tried to correlate the presence of different leukaemic cells with outcomes. Using flow cytometry to monitor the leukaemic stem cell population and to assess MRD has been shown to have additional prognostic value for some patients before allogeneic stem cell transplant.

The fast turnaround of the sample is also an advantage of this technique. Most laboratories can run the test within 24 hours of receiving the sample, though interpretation of the results may take longer.

Limitations

Although flow cytometry is a readily available technique in many laboratories, selecting the correct panel of antibodies and the subsequent analysis and interpretation of the results depend on expertise. Consequently, not all laboratories can perform multiparameter flow cytometry for MRD assessment.

Polymerase chain reaction

Quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) is a standard molecular biological technique. DNA is amplified and the copies of the target sequence are detected

How is MRD measured? (cont.)

using a fluorescent marker that binds newly synthesised DNA strands in the reaction, allowing quantification (Figure 2.2).

As a starting point, a target needs to be identified. This is usually a known mutated gene. The polymerase enzyme copies this target using a set of primers, synthesised to match the start and end of the target sequence, and free nucleotides. Repeated cycles are performed to produce multiple copies of the target. To quantify the DNA, the number of copies produced is compared with the levels of a standard, often a housekeeper gene (one that is essential for cell function and is therefore expressed at a reproducibly constant level). The starting quantity of mutant DNA is calculated in comparison with the standard, and a level of disease can be inferred.

Applications

RT-qPCR is firmly established in clinical practice for certain diseases: for example, in the monitoring of the PML–RARA fusion transcripts in acute promyelocytic leukaemia (APML) and BCR–ABL fusion transcripts in chronic myeloid leukaemia (CML) and acute lymphoblastic leukaemia (ALL) (fusion transcripts arise when a mutation brings together two unrelated genes to produce a new fusion gene). These applications are well standardised with good quality control methods implemented, so results are consistent across international centres. With many monitoring targets, the sensitivity is greater than with flow cytometry and the target can be quantified to very low levels such as 1 in 100 000 cells.

RT-qPCR is also used to track the disease course in ALL. Indeed, this type of RT-qPCR is the primary form of MRD assessment in ALL and is more widely used than multiparameter flow cytometry. Normally, each lymphocyte expresses a particular T-cell receptor/immunoglobulin that allows it to bind specifically to a

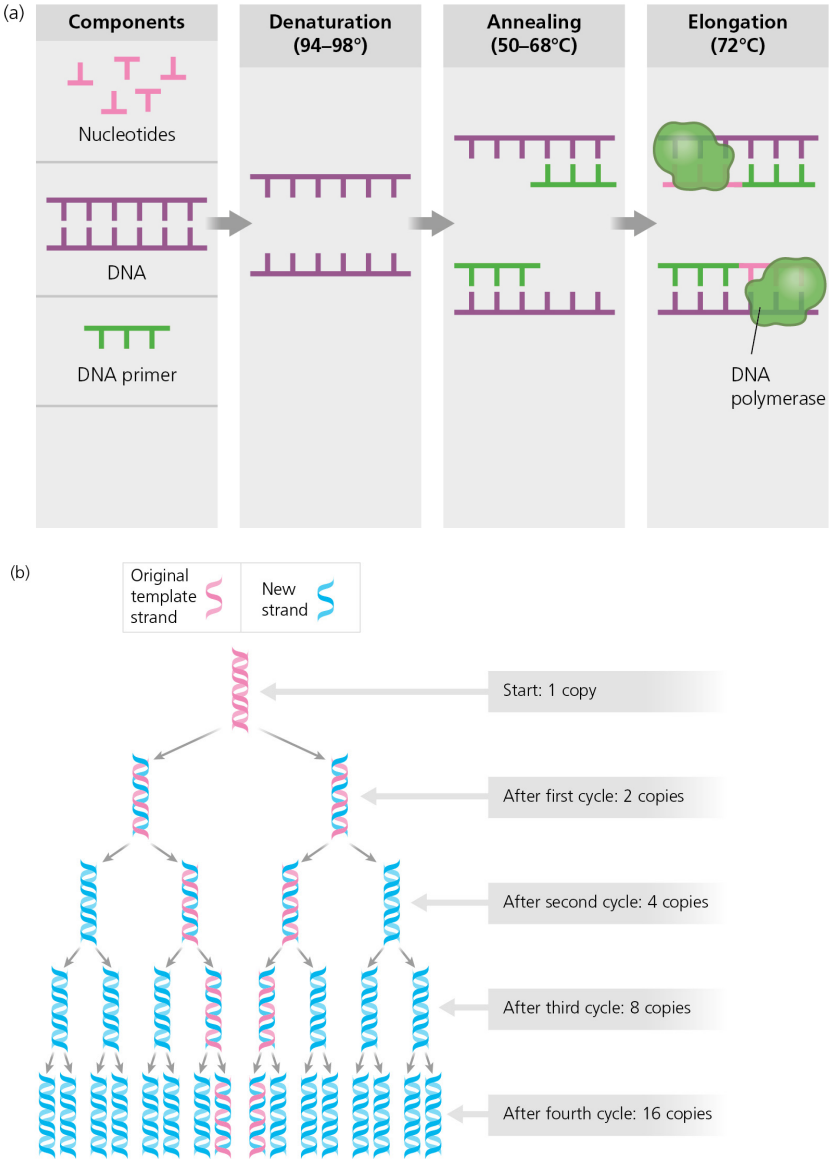


Figure 2.2 (a) Using primers that are complementary to the target sequence allows the polymerase enzyme to produce a new strand of complementary DNA. (b) The polymerase chain reaction results in an exponential increase in the number of DNA strands, which can be quantified against a standard.

How is MRD measured? (cont.)

target. This specificity arises as a consequence of the immune system evolving to recognise a panoply of foreign pathogens. During lymphocyte development, the genetic material used as a general template for the receptor/immunoglobulin is cut and edited differently to produce a specific receptor/immunoglobulin. This unique identifier can be used to identify a cell as being clonal (a clonal population comprises identical cells arising from a single cell precursor), as happens in leukaemic populations. RT-qPCR can be used to monitor the specific genetic rearrangements associated with particular receptors/immunoglobulins, and the technique gives sensitive detection of disease levels during the course of treatment.

When leukaemia progresses, further secondary mutations can lead to the development of different subpopulations – subclones – of cancerous cells. As subclones may be lost during treatment because of selection pressures, it is good practice, where possible, to monitor more than one receptor/immunoglobulin genetic rearrangement.

Limitations

Determining the specific genetic rearrangements requires expertise. It is important that the correct diagnostic samples reach the MRD laboratory so that the rearrangements can be identified (many diseases specifically require bone marrow). This is important as, without the correct sample from before treatment, an MRD laboratory may subsequently be unable to identify a marker for MRD monitoring.

The results of RT-qPCR often take days to weeks to come through, as the experiments are often performed in batches. The results then have to be validated and authorised by the clinical scientist. Interpretation also often requires knowledge of the preceding result, among many other factors, and sequential

testing may be needed to ensure detection of disease that progresses on a molecular level.

In AML, there is not a clear target for RT-qPCR in all disease subtypes. Targets that are well characterised include:

- PML–RARA
- mutations affecting NPM1, encoding nucleophosmin (NPM1), that give rise to the mutated protein, NPM1-cytoplasmic (NPM1c)
- AML1–ETO (also known as RUNX1–RUNX1T1)
- mutations giving rise to the fusion oncoprotein core binding factor β (CBFβ)–smooth muscle myosin heavy chain 11 (SMMHC)
- MLL fusion genes

However, these mutations are present in only a minority of people with AML. That an RT-qPCR target is not present in the majority of people with AML limits the application of this technology.

Next-generation DNA sequencing

At diagnosis

Next-generation DNA sequencing (NGS) is a new but well-established technology used at diagnosis. The technique is carried out in many clinical laboratories as a standard diagnostic procedure to identify mutations in either a few genes (targeted sequencing) or the entire genome. The ability to identify the genes that have prognostic value is particularly useful where the genetic heterogeneity of the disease is striking, such as in AML, and NGS is now firmly established in diagnosis and in prognostic algorithms. An AML with an NPM1 mutation is, for example, likely to have a very different response to treatment from that of an AML with a mutation in the RUNX1 gene.

Although NGS requires a number of different experimental steps, it is now performed routinely. The DNA is initially broken down

How is MRD measured? (cont.)

into smaller fragments; known sequences of DNA, known as adaptors and supplied by the manufacturer, are adjoined to the DNA fragments. This allows the small amount of DNA to be amplified using a polymerase enzyme similar to that described in the qPCR section above. The fragments of DNA with the adaptors can then attach to the surface of the sequencing machine. Millions of these fragments are read in parallel (at the same time) and the sequence of the DNA is reconstructed by bio-informaticians.

The cost of testing is falling rapidly. Often, samples from a number of patients are pooled into a single experimental run to minimise costs.

To monitor MRD

NGS may become a useful tool as it increases the number of patients with an MRD target that can be monitored, as nearly all patients will have an identifiable cancer-causing mutation. However, the utility of NGS as a technique to monitor MRD after a treatment course is not well established. Standardisation of the technique for monitoring purposes, which is important for quality management, is still being developed.

Improvements to sensitivity are in development; currently, NGS is far behind RT qPCR in its ability to detect very low levels of disease. The major limitation to sensitivity arises from an in-built sequencing error – the polymerase enzyme that is used has an error rate. Potentially, correcting for this error can increase sensitivity and hence the value of this assay in MRD monitoring.

Furthermore, clinical interpretation of the results is still not clearly defined. For example, the detection of mutations in certain genes, such as DNMT3A, may not significantly affect prognosis because these mutations are also common in healthy elderly individuals. Clonal haematopoiesis of indeterminate potential (CHIP) is common with ageing – genetically distinct

subpopulations of cells with somatic mutations occur in the absence of haematologic disease. The exclusion of these CHIP mutations is thought to improve the sensitivity of NGS MRD mutation analysis in AML MRD monitoring,6 but such improvements have not been replicated consistently across studies.

Other techniques

Fluorescent in situ hybridisation (FISH)

This is another surrogate measurement of disease presence. This technique uses fluorescence-labelled 'probes', which are large stretches of DNA complementary to the target of interest. FISH is less sensitive than flow cytometry or RT-qPCR, but it can be useful for large chromosomal rearrangements and is more sensitive than routine chromosomal karyotyping. There is, however, a cost associated with the use of the probes and FISH also requires the time and skill of a clinical scientist to set up and interpret the tests.

Chimerism

Following transplantation, patients will have chimerism of their bone marrow or peripheral blood monitored. This is usually done by looking at polymorphisms in the donor cells and comparing them with those in the recipient cells. An example is PCR detection of recognised microsatellite regions, which are usually identified before the transplant and then used to track the donor:recipient cell mix in the transplanted haematopoietic system. This is not an MRD measurement, as it is not a disease marker in itself, but rapidly falling donor chimerism is associated with an increased risk of disease relapse.

Sample requirements

Samples of bone marrow are often stipulated for MRD analysis, rather than blood (for example, in ALL monitoring). Many protocols for MRD assessment (for example, in AML and ALL) suggest using the first pull of bone marrow, with

How is MRD measured? (cont.)

a set volume, to prevent sample haemodilution with peripheral blood, as this may affect the sensitivity of the measurement. For some tests, such as BCR–ABL monitoring of CML in the chronic phase, monitoring of MRD using peripheral blood is acceptable.

Key points – how is MRD measured?

- The optimal technique to assess measurable residual disease (MRD) depends on the patient and disease context, as each method has advantages and disadvantages.
- MRD measurement requires specialist laboratories but it is now widely standardised internationally.
- The most commonly used technologies are based on flow cytometry and quantitative reverse transcriptase polymerase chain reaction (RT-qPCR).
- Emerging technologies will likely provide new methods of disease assessment.

What is the significance of MRD?

A result from testing for measurable residual disease (MRD) can be informative in different ways. At the simplest level, MRD testing can be used to determine the prognosis of a patient. Most initial studies of MRD relied on showing that the presence of MRD correlates with increased relapse rates and reduced overall survival (OS). MRD can also be used to monitor therapy, but this depends on the availability of alternative therapeutic options. Finally, in some patients, MRD can be used to define an undetectable level of disease at which therapy may safely be de-escalated or stopped. This chapter focuses on the acute leukaemias, for which the principles for MRD are best established. However, MRD monitoring is rapidly developing in chronic lymphocytic leukaemia (CLL) and myeloma, and clinical trials will use MRD analysis with increasing frequency.

Acute lymphoblastic leukaemia

MRD monitoring in acute lymphoblastic leukaemia (ALL), particularly in paediatric management, has been a paradigm for the use of this technology during treatment (Figure 3.1).

In children

In children, outcomes have improved over recent decades, such that patients designated as low risk can have OS above 90%. MRD results have been used to determine the need for treatment de-escalation in patients with low-risk disease and treatment escalation for those with high-risk disease. This is founded on the establishment of MRD as a strong independent prognostic factor during treatment – for example, after induction chemotherapy – that can accurately inform the risk of disease relapse.

MRD assessment enables better personalisation of treatment, thereby reducing treatment-related toxicities in patients for whom a less intensive management pathway is appropriate. The UKALL 2003 pediatric ALL treatment trial found

What is the significance of MRD? (cont.)

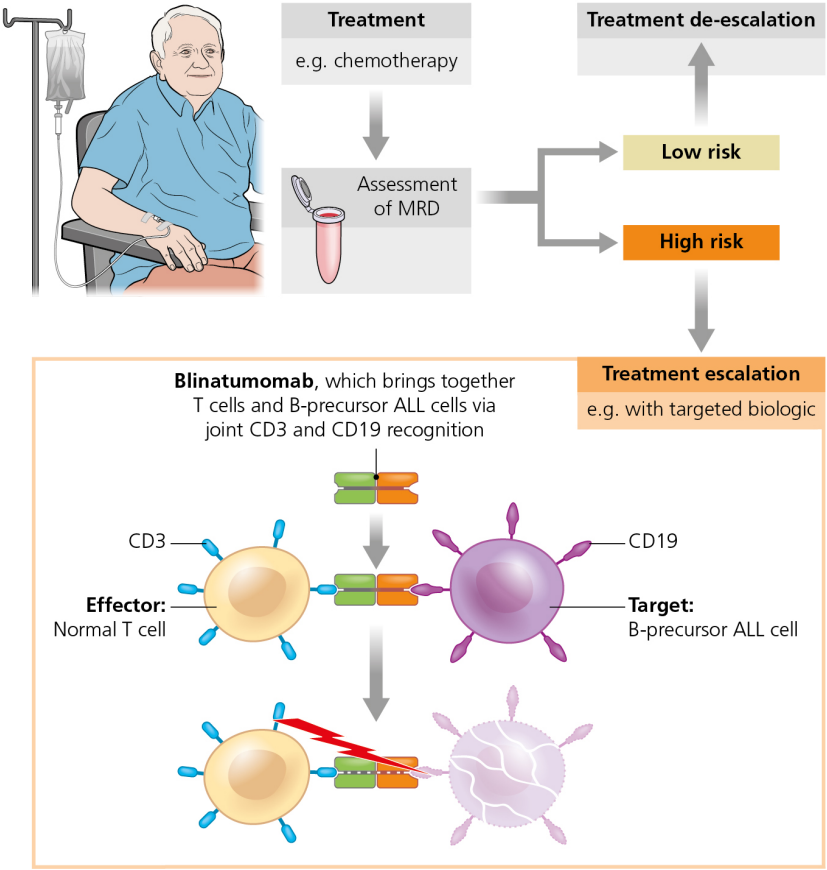


Figure 3.1 Assessment of measurable residual disease after treatment is used to support decision-making for further treatment. ALL, acute lymphoblastic leukaemia; MRD, measurable residual disease.

that patients with low-risk ALL, as defined by a low/undetectable MRD at the end of induction therapy, had equivalent outcomes whether they received one or two cycles of delayed intensification chemotherapy. In contrast, patients with persistently high levels of MRD had better disease control with an augmented chemotherapy regimen.

In adults

In adults, MRD is also a strong and independent prognostic factor in ALL. Some data suggest that patients with MRD do better with an allogeneic stem cell transplant than those unable to have a transplant (for example, because of the lack of a donor). However, data also suggest a poor outcome after transplant for patients who enter the treatment with MRD-positive disease.

The first MRD-dependent treatment for ALL has been accepted by the National Institute for Health and Care Excellence (NICE [England and Wales]) and the Food and Drug Administration (FDA [USA]). Blinatumomab is a bispecific antibody that binds CD19, expressed on B-ALL cells, and CD3, expressed on T cells. Consequently, the antibody causes T cells to directly kill leukaemic cells (see Figure 3.1). NICE recommends blinatumomab for patients with Philadelphia chromosome-negative (Ph–) B-ALL expressing CD19 in their first complete response (CR1) with an MRD level of at least 0.1%. This recommendation is based on a study showing that in patients in complete remission (CR) but with MRD, one cycle of blinatumomab treatment could produce high rates of response in terms of MRD (that is, MRD became undetectable). Patients who had undetectable MRD after treatment with blinatumomab had improved OS.

Furthermore, MRD studies in ALL have shown the importance of interpreting the level of MRD based on the timepoint of measurement as well as the result itself. However, the difficulty of using MRD as a marker of disease is that its utility depends on an individual's preceding results and previous treatments and therefore results cannot be easily compared across different treatment protocols.

What is the significance of MRD? (cont.)

Acute myeloid leukaemia

The best-established use for MRD assessment in acute myeloid leukaemia (AML) is in the management of acute promyelocytic leukaemia (APML). This leukaemia is characterised by the presence of the t(15;17) translocation that generates the PML–RARA fusion gene. The mutation can be detected by quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) and its level used to monitor patients at the end of treatment; the presence of persistent or progressing MRD is a strong risk factor for relapse and an indication for salvage treatment. As a result, the standard of care for these patients is to have 3-monthly bone marrow aspirates to check PML–RARA levels for 3 years. Salvage regimens include chemotherapy and arsenic trioxide.

RT-qPCR monitoring

RT-qPCR monitoring of NPM1 mutations and chromosomal rearrangements inv(16) and t(8;21) (in core binding factor [CBF] AML) has also improved patient management, especially as these patients are often spared allogeneic stem cell transplantation as a result. Consequently, adequate monitoring is important for these individuals as they may have only limited cycles of chemotherapy. There is a suggestion that performing 3-monthly bone marrow aspirates for 2 years is sufficient to detect impending relapse. This monitoring approach may, for example, allow the delivery of salvage therapy and stem cell transplant before overt relapse. If carried out after stem cell transplantation, monitoring can support the modification of immunosuppression and use of donor lymphocyte infusions.

Although mutant transcripts may still be present at remission in CBF AML, it is the degree of molecular response that is important: a 3-log reduction in MRD following the second course of chemotherapy is needed – without this there is

a high risk of relapse. For some patients, MRD assessment may indicate that allogeneic stem cell transplantation is needed to consolidate the remission. However – illustrating the difficulties with applying these data – in a study testing the value of MRD, although relapse rates were far higher with increasing levels of MRD, the impact of the MRD result on survival was limited by the high rates of remission from salvage chemotherapy. Running trials to test the use of MRD-directed treatment is challenging for many reasons, including participant numbers and suitable choices of intervention.

Multiparameter flow cytometry

This has also been shown to be a powerful prognostic marker in both younger and older patients with AML. In time, this may help improve the selection of those patients with intermediate-risk cytogenetic AML who should proceed to an allogeneic stem cell transplant. This is particularly important for patients who do not have a definitive prognostic cytogenetic or genetic mutation marker. At present, MRD detected at or above 0.1% at timepoints after induction therapy, including pre-transplantation, indicates a high risk of relapse. How this should direct treatment is a question tested in a number of clinical trials. The ongoing UK National Cancer Research Institute (NCRI) AML18 trial for elderly patients uses MRD assessment to direct treatment intensification.

MRD has also been shown to be a strong prognostic marker before and after transplantation. The presence of MRD before the transplant, detected using flow cytometry, has been shown to strongly affect the outcomes of an allogeneic stem cell transplant. Indeed, the data suggest that patients with MRD-positive disease who had an allogeneic stem cell transplant had poor outcomes similar to those of patients with morphologically detectable disease who underwent transplantation. Clearance of the mutation at an early timepoint after the

What is the significance of MRD? (cont.)

transplant, detected by new-generation DNA sequencing (NGS), had implications for relapse risk. However, at present, MRD assessment strategies need to be prospectively embedded in clinical trials to determine the optimal use of this technology. It is not yet clear, for example, whether improving patients' MRD response before the transplant – by intensifying conditioning regimens or giving pre-transplant consolidation chemotherapy – will improve outcomes.

Finally, it may be that a combination of these MRD-monitoring technologies will be used in the future to optimally manage patients with AML. A large study of patients with AML has shown that flow cytometry and NGS monitoring of MRD have additive prognostic power.

Use of MRD in other diseases

Analysis of MRD is increasingly used in myeloma and CLL treatment. Novel methods are being developed. One example is the assessment of circulating tumour DNA from patients' plasma, a technique that has been developed largely in the solid tumour and lymphoma field. This is useful in CLL, where nodal disease is a burden. The effectiveness of new treatment combinations for CLL has necessitated a longer assessment period to ascertain progression-free survival (PFS) in comparisons of new treatment strategies. There have been developments by regulatory authorities to allow the use of CR with MRD negativity to 1 in 10 000 cells as a criterion they will consider while the full PFS is ascertained. It is hoped that this will allow effective drugs to be evaluated more rapidly.

In myeloma treatment, flow cytometry has been used to assess MRD for a considerable period of time. However, many patients have relapsed following a result of 'MRD-undetectable disease'. Advances in flow cytometry have led to improvements in MRD measurements and alternatives now exist, including NGS to identify and quantify

immunoglobulin rearrangements. As in other areas of haematology, the need for MRD assessment has been driven by the need for a discriminating response criterion for the increasing number of therapies with high conventionally measured response rates. This will, in turn, lead to the assessment of MRD-directed therapy in upcoming clinical trials.

Key points – what is the significance of MRD?

- Risk stratification using measurable residual disease (MRD) is important for patients undergoing intensive treatment.
- MRD is a key decision driver in acute lymphoblastic leukaemia (ALL), especially in paediatrics, to ensure the right intensity of treatment and for optimal timing of treatments.
- MRD monitoring is important for detecting and preventing relapse of overt disease in acute myeloid leukaemia (AML), particularly in acute promyelocytic leukaemia (APML).
- MRD monitoring is now a key part of risk stratification in most clinical trials involving patients with leukaemia or myeloma.

Leukaemia Care is a national charity dedicated to providing information, advice and support to anyone affected by a blood cancer.

Around 34,000 new cases of blood cancer are diagnosed in the UK each year. We are here to support you, whether you're a patient, carer or family member.

Want to talk?

Helpline: **08088 010 444**
(free from landlines and all major mobile networks)

Office Line: **01905 755977**

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