

# MINIMAL RESIDUAL DISEASE - GENERALITIES AND PERSPECTIVES

**Florina Boldeanu<sup>1,2</sup>, Valentin L. Ordodi<sup>2</sup>, Alexandra Gruia<sup>1</sup>, Mirabela Cristea<sup>1</sup>, Elena Gai<sup>1</sup>, Margit Serban<sup>2</sup>**

## REZUMAT

Boala minimă reziduală (Minimal Residual Disease - MRD) reprezintă numărul mic de celule tumorale leucemice care rămân în pacient în timpul tratamentului sau după aceea, atunci când acesta este în remisie (fără simptome sau semne de boală). Dacă, după aproximativ patru săptămâni de chimioterapie, există mai puțin de 5% celule-blast într-un eșantion de măduvă osoasă, pacientul este în remisie clinică. Trei metode sunt disponibile pentru monitorizarea MRD: flux-citometrie imunofenotipică folosind etichetarea dublă sau triplă pentru a detecta imunofenotipurile specifice leucemiei; analiza de tip reacția în lanț a polimerazei (polymerase chain reaction (PCR)), a întreruperilor din regiunile de fuziune ale aberațiilor cromozomiale specifice leucemiei; analiza de tip PCR a regiunilor jonctionale de rearanjamente imunoglobuline (Ig) și receptorilor celulelor T (TCR). Detectia MRD asigură oportunități unice pentru intervenția terapeutică atunci când nici unul sau câțiva blaști sunt rezistenți la medicamente, în timp ce analiza MRD are câteva roluri importante: să determine dacă tratamentul cancerului îndepărtează urmele rămase; comparând eficiența diferitelor tratamente; monitorizarea statusului pacientului, remisia și recurența leucemiei; sau personalizarea tratamentului cancerului.

**Cuvinte cheie:** MRD, leucemie

## ABSTRACT

Minimal residual disease (MRD) is the small number of leukemic tumor cells that remain in the patient during treatment or afterwards, when the patient is in remission (no symptoms or signs of disease). If there are less than 5% blasts in a bone marrow sample, the patient is in clinical remission (CR) after about four weeks of chemotherapy. Three methods are available for MRD monitoring: flow-cytometric immunophenotyping using double or triple labeling to detect leukemia-specific immunophenotypes; polymerase chain reaction (PCR) analysis of breakpoint fusion regions of leukemia-specific chromosome aberrations; PCR analysis of the clone-specific junctional regions of immunoglobulin (Ig) and T-cell receptor (TCR) rearrangements. MRD detection provides unique opportunities for therapeutic intervention when none or few blasts are resistant to drugs, while MRD analysis has several important roles: to determine if treatment cancer removes remaining traces; comparing the efficacy of different treatments; monitoring patient status, remission and recurrence of leukemia; or personalization of the cancer treatment.

**Key Words:** MRD, leukemia

## MINIMAL RESIDUAL DISEASE - GENERALITIES

Minimal residual disease (MRD) is the small numbers of leukemic cells that remain in the patient during treatment, or after treatment, when the patient is in remission.<sup>1</sup> Technical approaches for MRD assessment are extensively reviewed.<sup>2</sup> Nowadays, there are very sensitive molecular biology tests, based on DNA, RNA or proteins, which can be measured in tissue samples, as low as one cancer cell in million normal cells.

MRD testing has important roles as determining whether treatment has eradicated the cancer or whether traces remain, comparing the efficacy of different treatments, monitoring patient remission status and recurrence of the leukemia or cancer and choosing the treatment that will best meet those needs.<sup>3</sup> Methods for detecting MRD (e.g. submicroscopic), can be 100 times more sensitive than morphologic examination and permit a more objective assessment of treatment response.<sup>4-6</sup>

Most research on MRD has been done on leukemia, adult chronic myeloid leukemia, and childhood acute lymphoblastic leukemia.<sup>4</sup> The success of treatment of patients with leukemia is judged by several criteria. For the patient and clinician the most desirable outcome is sustained remission from disease and long-term survival. Unfortunately it is unlikely that complete eradication of leukemic cells is ever achieved. Hematological remission is defined as fewer than 5% blast cells in the bone marrow as determined by morphology.<sup>7</sup> When interpreting residual disease one must distinguish between the postinduction situation

<sup>1</sup> Regional Center for Transplant Immunology, Emergency Clinical County Hospital Timisoara, <sup>2</sup> Victor Babes University of Medicine and Pharmacy, Timisoara

Correspondence to:  
Florina Boldeanu, Regional Center for Transplant Immunology, Emergency Clinical County Hospital, 10 Iosif Bulbuca Blvd., 300736 Timisoara  
Email: boldeanu.florina@yahoo.com

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when patients enter a morphologically defined, potentially incomplete remission and the time point at which patients have experienced maximal response to therapy and present with true MRD.<sup>8</sup>

If there are less than 5% blasts in a bone marrow sample, the patient is in “clinical remission”. Although doctors now know that clinical remission does not mean that the disease is eliminated, the term still has value as a prognostic factor in treatment.<sup>9</sup> This term came into use in the 1950’s, when this level of sensitivity was the limit of the doctors’ tests.

A majority of patients reach complete remission (CR) after about four weeks of chemotherapy.

There are techniques utilized to measure MRD in leukemia, such as DNA based tests, RNA based tests, patient specific testing, immunological tests.

DNA tests are based on detecting a leukemic specific DNA sequence. These types of tests are achieved through the use of the polymerase chain reaction. The markers used for DNA based testing are often chromosomal translocations such as t(14;18) involving BCL2 and t(11;14) involving BCL1 (CCND1). Other genes utilized for MRD detection include microsatellites, immunoglobulin and T cell receptor.<sup>10</sup>

RNA tests are based on detecting a leukemic specific RNA sequence. This is achieved through the use of reverse transcription of the RNA followed by polymerase chain reaction. RNA based tests are normally utilized when a DNA test is impractical. For example, the t(9;22) BCR-ABL translocation may occur over a large length of the chromosome which makes DNA based testing difficult and inefficient. The markers used for RNA based testing are almost exclusively chromosomal translocations such as t(9;22) BCR-ABL, t(15;17) PML-RARA and t(12;21) ETV6-RUNX1 (TEL-AML1).<sup>11</sup>

Both the DNA and RNA based tests require that a pathologist examine the bone marrow to determine which leukaemic specific sequence to target. Once the target is determined, a samples of blood or bone marrow is obtained, nucleic acid is extracted, and the sample analyzed for the leukaemic sequence. These tests are very specific, and detect leukaemic cells at levels down to 1 cell in a million, though the limit typically achieved is 1 in 10,000 to 1 in 100,000 cells. For comparison, the limit of what one can detect using traditional morphologic examinations using a microscope is about 1 cell in 100.<sup>12</sup>

Patient specific MRD detection using immunoglobulin (IG) or T cell receptors (TCR) is gaining popularity as a way of measuring MRD in leukemias that do not contain a chromosomal

translocation or other leukemic specific marker. The leukemic specific IG or TCR clone is amplified using PCR and the variable region of the IG or TCR is sequenced.<sup>13</sup>

Immunological based testing of leukaemias utilizes proteins on the surface of the cells. White blood cells (WBC) can show a variety of proteins on the surface depending upon the type of WBC. Leukaemic cells often show quite unusual and unique combinations (leukemic phenotype) of these cell surface proteins. The limit of detection of immunological tests is generally about 1 in 10,000 cells and cannot be used on leukaemias that don’t have an identifiable and stable leukaemic phenotype.<sup>14</sup>

### **IMMUNOPHENOTYPING VERSUS MOLECULAR MONITORING OF RESIDUAL DISEASE**

The most problematic aspect in the clinical relevance of a molecularly defined remission remains the small fraction of patients who can actually benefit from molecular evaluation, particularly in acute myeloid leukemia (AML). Immunophenotypic evaluation of response by multiparameter flow cytometry requires that the leukemic cells express an antigen profile, which differs from those observed on normal hematopoietic precursor cells, and are expected to be present in a remission bone marrow. The detection of aberrant markers on leukemic cells is successful in the majority of patients with acute lymphoblastic leukemia (ALL) or AML.<sup>15-17</sup> Chemotherapy may affect antigen expression patterns. However, the high incidence of leukemia-associated immunophenotypes (80–85% of cases) stands in contrast to the percentage of patients with AML, who can be followed with cytogenetic markers or their molecular equivalents (e.g. AML1/ETO, PML/RAR $\alpha$ , possibly FLT3 gene mutations). AML subtypes with traceable molecular markers account for approximately 30% of patients in clinical trials. Another fraction demonstrate chromosome abnormalities which may be monitored by fluorescence in situ hybridization (FISH) (e.g. monosomies, deletions, hyperdiploidy), but present a problem when low-level disease, below the level of sensitivity accomplished by FISH, needs to be evaluated by PCR. Although approximately 100 times more sensitive than standard cytogenetics, the sensitivity level achieved by FISH (one cell in 103) is markedly below that desired for MRD detection (one cell in 105). The possibility that genetic defects detected by interphase FISH may not be actually translated and thus reflect ‘silent’ and physiologically potentially meaningless disease adds

a significant complication to the interpretation of FISH data. The best currently known example for this concept stems from the differentiation inducing effect of all-trans retinoic acid (ATRA) therapy in APL which can lead to the finding of t(15;17) in mature granulocytes, destined to die and thus irrelevant in terms of residual disease detection.<sup>18</sup> Then there is the rather large portion of AML patients with apparently normal karyotype (30–40% of patients in most large clinical trials). In AML, the advantage offered by FISH is rather small, attributable, for instance, to the rare karyotypically normal patient with clinical and/or morphologic characteristics of APL which prompt the physician to order FISH, leading to the detection of a cryptic t(15;17) in the leukemic cells.<sup>19</sup> Of great clinical interest are patients who present with cryptic molecular markers, detected by PCR, which derive from chromosomal translocations with well-defined, prognostic significance, such as AML1/ETO (in patients lacking t[8;21]), CBF $\beta$ /MYH11 (in patients lacking inv[16]) or MLL-AF4 (in patients lacking t[4;11]), as data on their clinical implication in comparison to that of the standard cytogenetic translocations are scarce and controversial.<sup>20–24</sup>

Chances for molecular monitoring are considerably better in ALL. In pediatric ALL, approximately a quarter of patients express cytogenetically cryptic TEL/AML1 transcripts. In adult ALL, a similar percentage of patients contain BCR/ABL transcripts (resulting from the Philadelphia chromosome). Another sizable fraction of patients contain aberrations of the MLL gene which result from translocations or deletions involving chromosome 11 at q23.2. Most importantly, in ALL there is the possibility to monitor monoclonality based on immunoglobulin or T cell receptor gene rearrangements, although this approach may harbor its own challenges.<sup>25,26</sup>

## **CONCLUSIONS AND PERSPECTIVES**

MRD-detection provides unique opportunities for therapeutic intervention when none or few blasts are resistant to drugs.<sup>1</sup>

Each method of MRD has specific advantages and potential traps. Immunologic and molecular techniques are equally reliable in detecting clinically significant levels of residual leukemia, and can be applied in tandem for universal monitoring of minimal residual disease.<sup>8</sup>

Molecular markers can be used by the physician to provide an accurate prognosis and predict response, resistance, or toxicity to therapy they can give new insights into the methodology of investigation and

treatment of disease.<sup>1,8</sup>

A goal of MRD assays is to guide therapeutic decisions by recognizing patients who responded well to therapy and thus should be spared further therapy and distinguishing them from patients in whom therapy must be continued or intensified to minimize the likelihood of clinical relapse.<sup>3</sup>

MRD analysis has several important roles: to determine if treatment cancer removes remaining traces comparing the efficacy of different treatments, monitoring patient status, remission and recurrence of leukemia or cancer treatment choice that will best meet these needs (personalization of treatment).<sup>1</sup>

Important issues in molecular biology are specimen collection, modification, storage, and laboratory staff experience. Establishing a protocol and method to be followed depends on the laboratory equipment.<sup>14</sup>

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