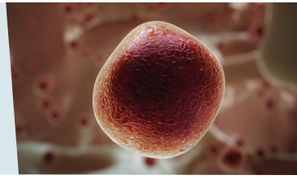
Minimal Residual Disease in Hematologic Malignancies

AMGEN®

Acute Lymphoblastic Leukemia – Pathophysiology and Epidemiology

Acute lymphoblastic leukemia (ALL) is a malignant disease which arises from the clonal uncontrolled proliferation of immature lymphoid cells. This uncontrolled proliferation causes normal cells to be displaced from the bone marrow and peripheral blood. It is a heterogeneous disease in terms of its pathology and the populations it affects.[1-3]



Immunophenotype:
ALL may be classified as B-cell precursor or T-cell lineage depending on the expression of lineage markers.[1]

B-ALL represents **85–90%** of pediatric and roughly **75–80%** of adult cases of ALL.[4, 5]

T-ALL represents **10–15%** of pediatric and approximately **20–25%** of adult cases of ALL.[1, 4, 5]

Based on American Cancer Society estimates, there will be about **6,590** new cases of ALL and about **1,430** deaths due to ALL in the US in 2016 in both children and adults.[6]

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. While current 5-year event free survival exceeds **85%**, children still suffer serious adverse effects from treatment.[4, 7]

ALL is less common in adults and the treatment outcomes are significantly lower than in children with ALL, especially those with relapsed ALL.[8]

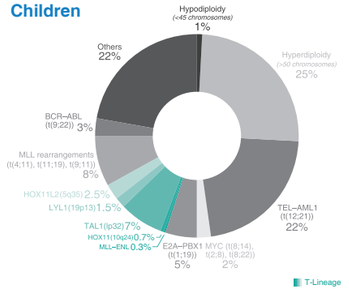
Some reasons for this difference include the higher incidence of poor prognostic cytogenetics and a lack of good cytogenetics in adults.[9]

Specific Phenotypes	Children	Adults*
Poor Prognostic		
Philadelphia-positive (Ph+)	3%[8]	20–30%[11]
**_MLL_ rearrangements	2-8%[8, 10]	5–10%[8, 10]
Good Prognostic		
TEL/AML1	50%[10]	10%[10]
Hyperdiploid	25–30%[12]	7%[8]

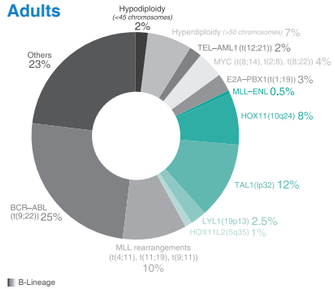
*_Adults have higher incidence of poor prognostic cytogenetics and lack good risk cytogenetics_

**_50-75% of ALL in infants [10, 12]_

Children



Adults



Adapted from: Pui CH, Relling MV, Downing JR. *N Engl J Med.* 2004;350:1535-1548

Response Categories in ALL

The following responses are based on the conventional morphologic assessment to detect the presence of lymphoblasts.

Location	Response Criteria	Features
Blood and Bone Marrow	CR[13, 14, 15]	• No circulating blasts or extramedullary disease (No lymphadenopathy, splenomegaly, skin/gum infiltration/testicular mass/CNS involvement) • < 5% BM blasts • ANC >1,000/µL • Platelets >100,000/µL
	Molecular CR[16]	Defined as the lack of MRD at a specific time point with polymerase chain reaction (PCR) assay sensitivity $\geq 10^{-4}$
	Molecular failure[16]	Defined as the persistent quantifiable presence of MRD with a PCR assay sensitivity of $\geq 10^{-4}$
	Molecular relapse[16]	Defined as reappearance of MRD within the quantitative range ($>10^{-4}$) after prior achievement of molecular CR
	M1[14]	Defined as marrow with <5% blasts
	M2[14]	Defined as marrow with 5 to 24% blasts
	M3[14]	Defined as marrow with $\geq$25% blasts
	CRh[15]	• $\leq$5% blasts in the bone marrow • No evidence of circulating blasts or extramedullary disease, partial recovery of peripheral blood counts (at least platelets >50,000/µL and ANC >500/µL)
	CRi[13]	• <5% BM blasts • Incomplete recovery of peripheral blood counts (platelets <100,000/µL and/or ANC <1,000/µL)
	CRp[13]	Subcategory of CRi where patients fulfill all criteria for CR except that platelet counts <100,000/µL
	Refractory disease[17]	Failure to achieve CR at the end of induction
	PD[18]	Increase of at least 25% in the absolute number of circulating or bone marrow blasts or development of extramedullary disease
	Relapsed disease[16]	Reappearance of blasts in the blood or bone marrow (>5%) or in any extramedullary site after CR

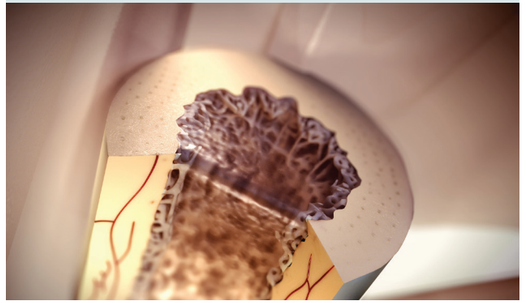
ANC, absolute neutrophil count; BM, bone marrow; CNS, central nervous system; CR, complete remission; CRi, complete remission with incomplete recovery of blood counts; CRp, complete remission without platelet recovery; MRD, minimal residual disease; PD, progressive disease

Multiple Myeloma – Pathophysiology and Epidemiology

Multiple myeloma (MM) is a B-cell malignancy in which abnormal, clonal plasma cells proliferate and accumulate in the bone marrow.[19]

These abnormal cells, referred to as myeloma cells, disrupt normal bone marrow function and invade bone. Myeloma cells produce and secrete significant quantities of monoclonal protein (M-protein) into the blood and/or urine.[19]

The clinical features of MM include hypercalcemia, renal failure, anemia, osteolytic bone lesions, and increased susceptibility to infection.[20]



MM is a genetically complex and heterogenous disease. Nearly all MM cases are preceded by an asymptomatic, pre-malignant, condition known as monoclonal gammopathy of undetermined significance (MGUS) which may progress to a smoldering MM phase. The disease course to symptomatic MM is driven by multiple genomic events within myeloma cells and changes in the bone marrow microenvironment. Several chromosomal abnormalities have been identified in patients with MM and involve translocations, deletions, or amplifications.[21]

MM is the second most common hematologic malignancy and accounts for approximately **13%** of all hematologic cancers.[22]

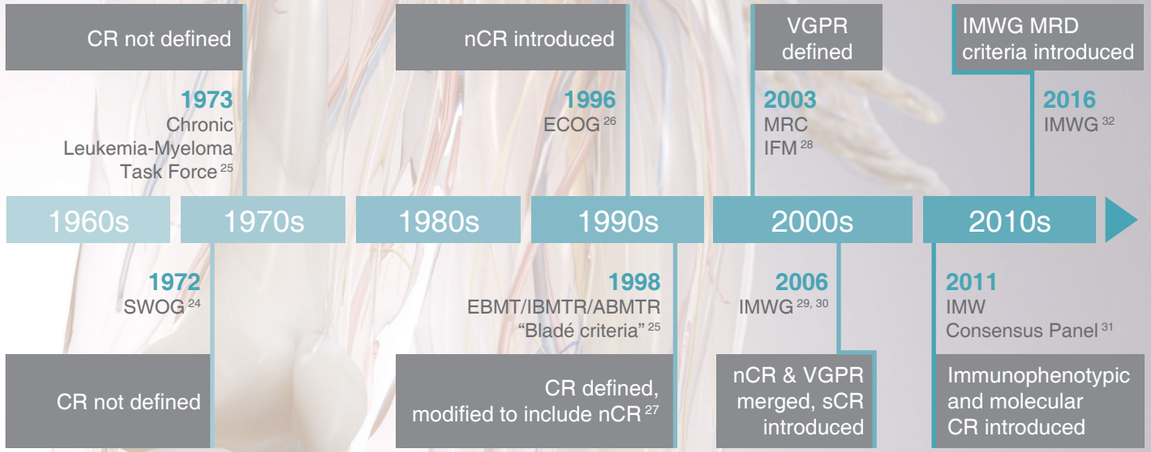
Globally, an estimated **114,251** new cases of MM are diagnosed annually, with **80,019** deaths per year attributable to the disease.[22]

MM predominantly affects elderly people, and is most frequently diagnosed between the ages of **65–74** years. MM is more common in males and African Americans compared to females and Caucasians, respectively.[23]

Overall, the **5-year survival** among adults with MM is **48.5%.**[23]

Emergence of Different Treatment Options Have Necessitated Revised Response Criteria

The evolution of MM response criteria has been driven by improvements in response with the availability of different agents and regimens.



ABMTR, Autologous Blood and Marrow Transplant Registry; CR, complete response; EBMT, European Group for Blood and Marrow Transplantation; ECOG, Eastern Cooperative Oncology Group; IBMTR, International Bone Marrow Transplant Registry; IFM, InterGroupe Francophone du Myélome; IMWG, International Myeloma Working Group; nCR, near complete response; sCR, stringent complete response; VGPR, very good partial response

Response Categories in MM [32]

Select Standard IMWG Response Criteria*	Response Criteria
sCR	CR plus normal FLC ratio and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells).
CR	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates.
VGPR	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h.
PR	≥50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≥90% or to <200 mg per 24 h; If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria; If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (SPD) of soft tissue plasmacytomas is also required.

BM, bone marrow; CR, complete response; FLC, free light chain; IMWG, International Myeloma Working Group; M-protein, monoclonal protein; PC, plasma cells; PR, partial response; sCR, stringent complete response; SPD, sum of the products of the maximal perpendicular diameters of measured lesions; VGPR, very good partial response

* Derived from international uniform response criteria for multiple myeloma. Minor response definition and clarifications derived from Rajkumar and colleagues. When the only method to measure disease is by serum FLC levels: complete response can be defined as a normal FLC ratio of 0·26 to 1·65 in addition to the complete response criteria listed previously. Very good partial response in such patients requires a ≥90% decrease in the difference between involved and uninvolved FLC levels. All response categories require two consecutive assessments made at any time before the institution of any new therapy; all categories also require no known evidence of progressive or new bone lesions or extramedullary plasmacytomas if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements. Bone marrow assessments do not need to be confirmed. Each category will be considered unconfirmed until the confirmatory test is performed. The date of the initial test is considered as the date of response for evaluation of time dependent outcomes such as duration of response.

IMWG MRD Criteria (requires a CR as defined on the left)	Response Criteria
MRD-negative	Absence of aberrant clonal plasma cells on bone marrow aspirate, ruled out by an assay with a minimum sensitivity of 1 in 10^5 nucleated cells or higher (i.e. 10^{-5} sensitivity)*
Imaging-positive MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue**
Sustained MRD-negative	MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined above, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years)

IMWG, International Myeloma Working Group; MRD, minimal residual disease; NGF next-generation flow; NGS, next-generation sequencing; PET, positron emission tomography; CT, computed tomography; SUV, standardized uptake values.

*Based on flow cytometry (EuroFlow) or next-generation sequencing (LymphoSIGHT platform or validated equivalent method).

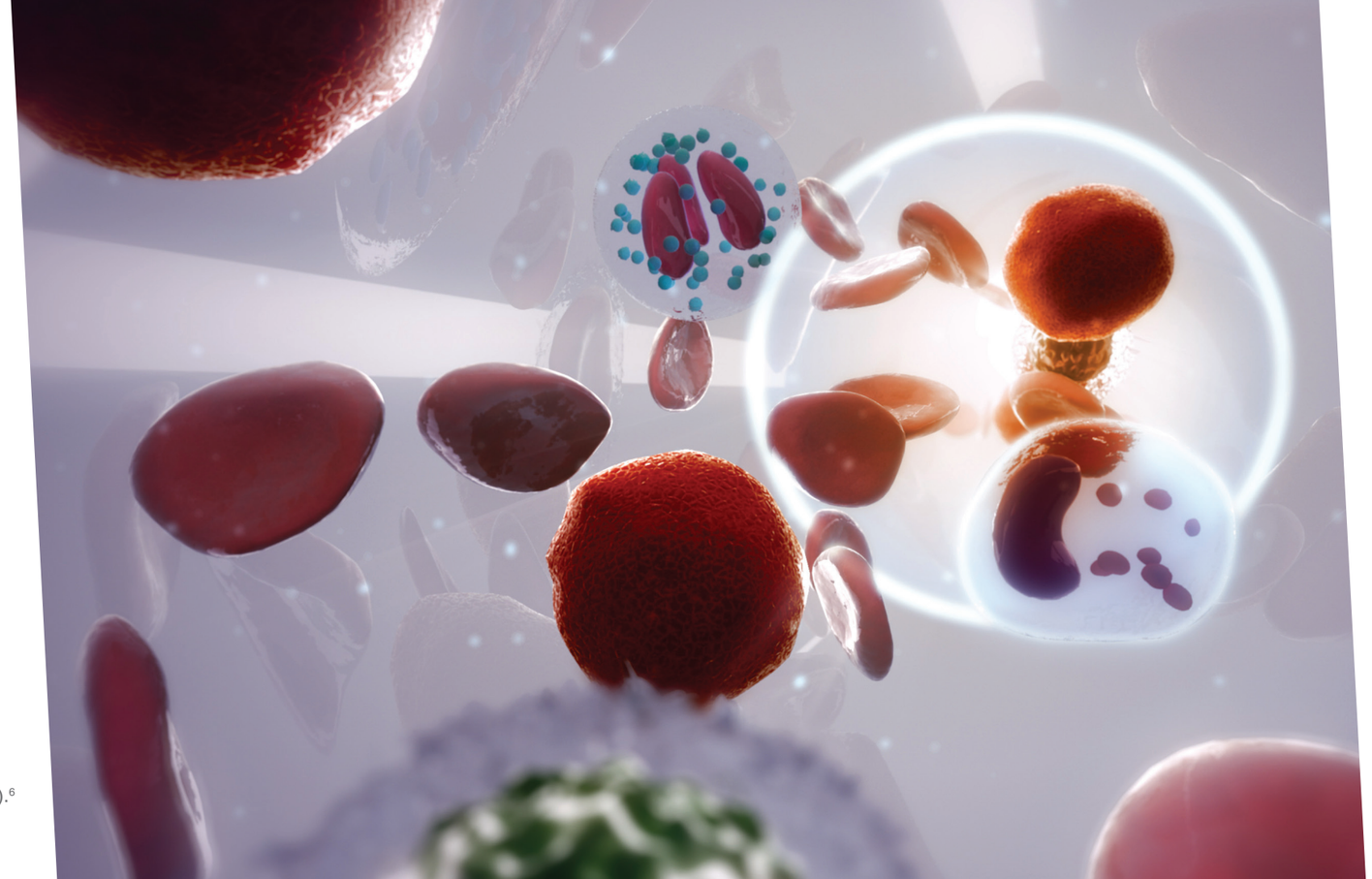
**Criteria used by Zamagni and colleagues, and expert panel (IMPetUs; Italian Myeloma criteria for PET Use). Baseline positive lesions were identified by presence of focal areas of increased uptake within bones, with or without any underlying lesion identified by CT and present on at least two consecutive slices. Alternatively, an SUVmax=2·5 within osteolytic CT areas >1cm in size, or SUVmax=1·5 within osteolytic CT areas ≤1cm in size were considered positive. Imaging should be performed once MRD negativity is determined by MFC or NGS.

Remission & Relapse

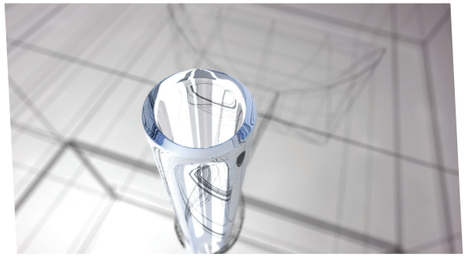
The treatment of ALL and MM has improved significantly over the last decade, resulting in many patients achieving a complete response or remission (CR; <5% of morphologically identifiable malignant cells in the bone marrow samples) to frontline therapy.[16, 33]

However, many patients may still relapse. Relapse is a result of the persistence of malignant cells in patients who achieve CR.[33]

The presence of malignant cells below the limits of detection is referred to as minimal residual disease (MRD).[6]



33% of patients with standard-risk ALL and 66% of patients with high-risk ALL relapse after achieving CR.[34]



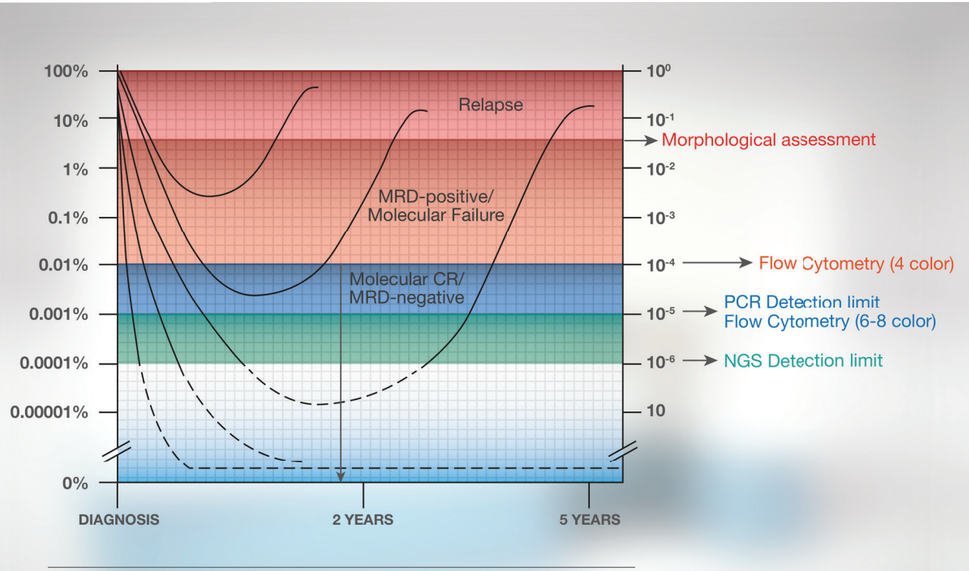
In a retrospective analysis of three clinical trials, 40% of patients with MM relapsed within 4 years after achieving CR.[35]

Introduction to MRD

Minimal residual disease (MRD) is the persistence of small numbers of residual malignant cells in patients during or following treatment, and may be a function of poor tumor cytoreduction or rapid regrowth of tumor clones with high proliferation potential. These residual malignant cells are clinically relevant, as they may lead to relapse and disease progression.[36]

Morphologic examination can miss the detection of many residual malignant cells. As the technology changes and becomes more sensitive, the limit of detection gets lower. Some of the tools used to detect these residual cells are described in the following pages.[13]

Improvements in detection techniques can enable clinicians to identify MRD throughout the disease lifecycle, which may help to assess the potential risk of relapse early on and inform treatment decisions.[13, 36, 37]



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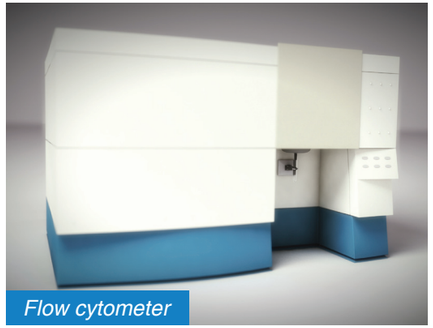
Bruggemann M, Raff T, Kneba M. *Blood.* 2012;120:4470-4481.

Methods for Detection of MRD



Flow cytometer

Multiparametric flow cytometry (MFC)

Cells from bone marrow aspirates are labeled with fluorophore-conjugated antibodies against specific cell surface antigens, and labeled cells are subjected to light of different wavelengths.[40]

Cells can then be counted and assessed according to their expression of cell surface antigens, providing quantitative information on the immunophenotype of the population of sampled cells. Flow cytometers commonly detect >4 colors, however, more sensitive >8 color flow cytometers are also available.[39]

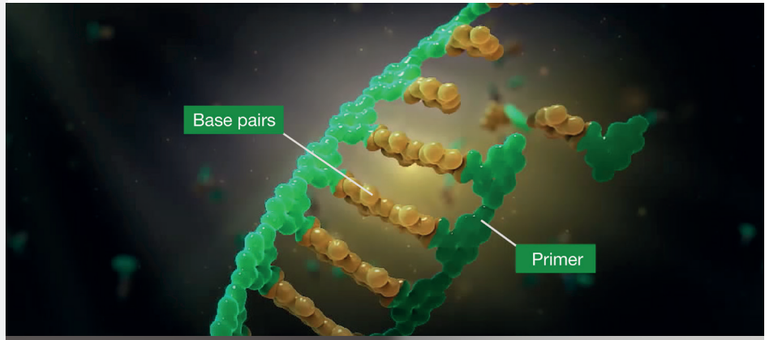
RQ-PCR machine

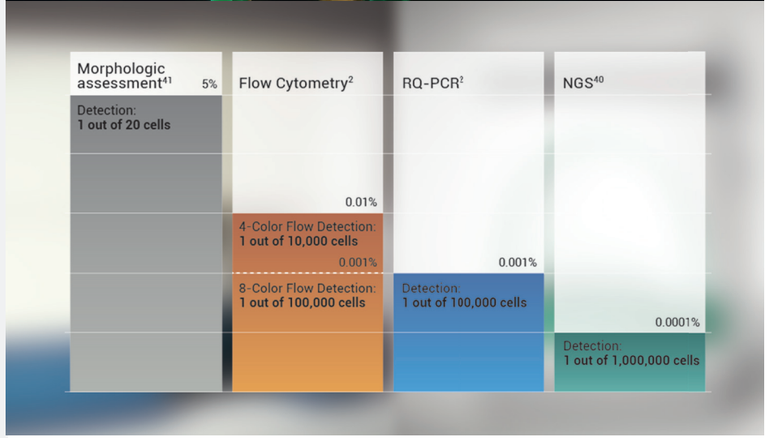
Real-time quantitative polymerase chain reaction (RQ-PCR)

Real-time quantitative PCR (RQ-PCR) is used to detect and quantify gene rearrangements in the variable region of the immunoglobulin (Ig) gene, as well as the T cell receptor (TCR) in patient blast cells.[39]

For this method, patient specific primers are required. Additionally, RQ-PCR can be used to analyze fusion transcripts such as *BCR-ABL*, or *MLL* gene fusions.[38, 39]

Gene fusions tend to occur in specific regions, not requiring primers to be generated for each patient.[2, 39]





Next-generation sequencing (NGS)

NGS uses high-throughput sequencing to detect clonal Ig *VDJ* gene rearrangements. This technique offers the potential for increased sensitivity as well as MRD assessment from peripheral blood.[40]

Clonal heterogeneity can be monitored and different clones tracked over time. NGS offers the potential for widespread applicability as data analysis is automated, however this technique still requires validation.[40, 42]

PET/CT imaging in MM

Fluorodeoxyglucose(FDG)-positron emission tomography (PET)/ computed tomography (CT) imaging permits detection of lesions demonstrating metabolic activity together with morphologic information. An advantage of PET/CT imaging is its ability to detect extramedullary disease in MM.[32, 35]

However, since not all bone lesions attributable to MM acquire FDG, both false-negatives and false-positives are possible in the setting of MM. Further research is needed to clarify the use of PET/CT imaging in monitoring MRD in MM and its role in combination with other MRD-based techniques.[32]

Features of MRD Detection Methods

Method	Applicability	Sensitivity	Important Considerations	
MFC [32, 35, 40, 43]	ALL: >90% MM: 100%	3- to 4-color: 10^{-3}–10^{-4} 6- to 9-color: 10^{-4}–10^{-6} Also depends on cell input.	• Widely applicable and available • Turnaround in hours • Relatively inexpensive • Does not require baseline sample	• Clonal heterogeneity undetectable • Standardization ongoing (EuroFlow/IMF) • Requires bone marrow aspirate • Fresh sample necessary
RQ (real-time quantitative) – PCR [32, 35, 40, 43]	ALL: 90–95% MM: 60%–70%	10^{-5}–10^{-6}	• Standardized (EuroMRD) • Fresh sample not necessary • Clonal heterogeneity undetectable • Patient-specific primers necessary	• More expensive than MFC • Requires bone marrow aspirate • Requires baseline sample • Time consuming
Fusion transcript PCR [2, 43]	BCP-ALL: 25–40% T-ALL: 10–15%	10^{-4}–10^{-5}	• Rapid • Unequivocal link with leukemic/ preleukemic clone • Stable target throughout therapy	• Possible differences in expression levels (transcripts/cells) during the course of treatment • RNA instability ➡ false negative • Risk of cross contamination ➡ false positive
NGS [32, 35, 40, 43]	>95% all lymphoid malignancies MM: ~90%	10^{-6}	• Limited clonal heterogeneity detected • Bone marrow aspirate or peripheral blood sample acceptable • Fresh sample not necessary • Not yet standardized	• Limited availability • One week or more • Expensive, but costs decreasing • Requires baseline sample or stored sample from a time point with detectable disease

ALL, acute lymphoblastic leukemia; BCP, B-cell precursor; IMF, International Myeloma Foundation; MFC, Multiparametric flow cytometry; MM, multiple myeloma; MRD, minimal residual disease; NGS, next-generation sequencing; PCR, polymerase chain reaction

Positron Emission tomography (PET)/ Computed Tomography (CT) imaging

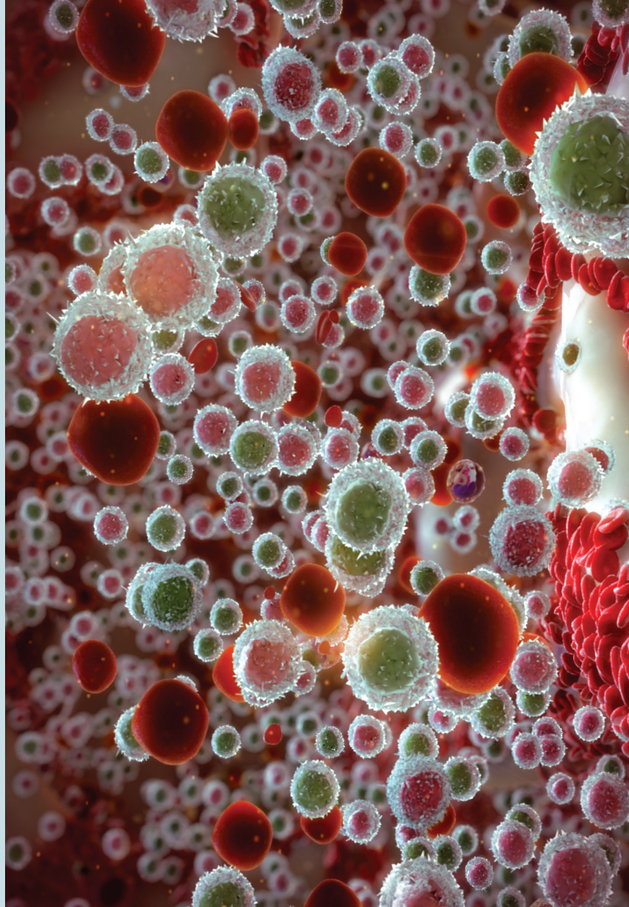
PET/CT imaging is applicable for detecting MM in 100% of patients, however sensitivity is variable. PET/CT imaging is able to detect extramedullary disease and takes a matter of hours to carry out. However, it is expensive and both false-negative and false-positive results have been seen when other coexisting infectious or inflammatory processes exist.[32, 35]

MRD in ALL Summary

Morphological assessment cannot detect very low numbers of cells representing minimal residual disease in patient samples. Consequently, techniques using flow cytometry, RQ-PCR and NGS have been developed, which allow for more sensitive detection as well as the quantification of these residual leukemic cells. These methods are highly sensitive with detection limits of 10^{-4} to 10^{-6}. [2, 41, 44, 45]

A number of studies have shown that the detection of MRD in patients with ALL, both children and adults, is an independent risk parameter of high clinical relevance.[2] This applies to patients with ALL undergoing stem cell transplant.[16]

A consensus on the timing of assessment and the definitions of common MRD terminology is becoming increasingly important when evaluating patients. Also, the standardization of MRD methodologies is important to ensure comparability within an MRD treatment protocol, as well as to provide a solid basis for the comparison of MRD data between different treatment protocols.[46]



MRD in MM Summary

Relapses in MM could potentially reflect the presence of residual disease. Recently, technologic advances in molecular testing using NGF, RQ-PCR, NGS, and imaging techniques such as PET-CT, have enabled the detection of myeloma cells with greater sensitivity.[35, 36]

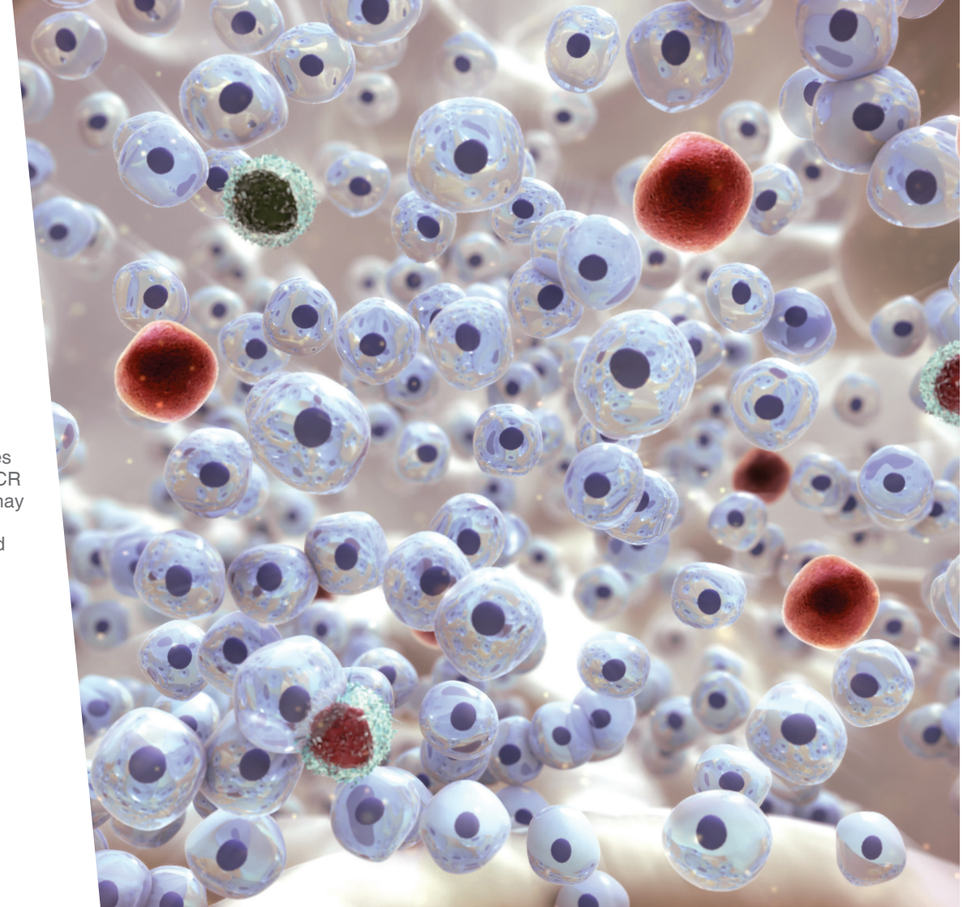
However, the heterogeneity of the disease biology may also be important in predicting the risk of relapse and sustainability of a response. Some patients who present with high-risk features at baseline may have persistent MRD despite achieving a CR, while patients with MGUS-like gene expression may experience better outcomes independent of CR status.[32]

The 2016 IMWG consensus criteria proposed definitions for MRD-negativity as the absence of clonal plasma cells using NGF or NGS with a minimum sensitivity of 10^{-5} and recommends evaluating MRD after patients achieve a CR.[32]

Ongoing studies will continue to define what level of MRD may be clinically relevant and to understand the potential value of MRD assessment throughout the treatment continuum in monitoring the course of the disease (e.g., after achieving a CR, during or after induction therapy, post-autologous stem cell transplant, or during maintenance therapy).[32, 47, 48]

The integration of imaging techniques in MRD assessment enables detection of disease outside of the bone marrow and may help mitigate biopsy bias in patients. However, identifying which patients should be assessed for extramedullary disease and whether treatment should be tailored to imaging-positive MRD remains under investigation.[32]

Further clinical trials and meta-analyses are needed to determine how current CR criteria and MRD assessment in MM may inform treatment decision-making and to validate the relationship of sustained MRD-negativity and outcomes.[32, 37]



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