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REPORT

NCI First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation: Report from the Committee on Disease-Specific Methods and Strategies for Monitoring Relapse Following Allogeneic Stem Cell Transplantation. Part II: Chronic Leukemias, Myeloproliferative Neoplasms, and Lymphoid Malignancies

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Relapse has become the major cause of treatment failure after allogeneic hematopoietic stem cell transplantation. Outcome of patients with clinical relapse after transplantation generally remains poor, but intervention prior to florid relapse improves outcome for certain hematologic malignancies. To detect early relapse or minimal residual disease, sensitive methods such as molecular genetics, tumor-specific molecular primers, fluorescence in situ hybridization (FISH), and multiparameter flow cytometry (MFC) are commonly used after allogeneic stem cell transplantation to monitor patients, but not all of them are included in the commonly employed disease-specific response criteria. The highest sensitivity and specificity can be achieved by molecular monitoring of tumor- or patient-specific markers measured by polymerase chain reaction-based techniques, but not all diseases have such targets for monitoring. Similar high sensitivity can be achieved by determination of recipient-donor chimerism, but its specificity regarding detection of relapse is low and differs substantially among diseases. Here, we summarize the current knowledge about the utilization of such sensitive monitoring techniques in chronic leukemias, myeloproliferative neoplasms, and lymphoid malignancies based on tumor-specific markers and cell chimerism and how these methods might augment the standard definitions of posttransplant remission, persistence, progression, relapse, and the prediction of relapse. Critically important is the need for standardization of the different residual disease techniques and to assess the clinical relevance of minimal residual disease and chimerism

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surveillance in individual diseases, which in turn must be followed by studies to assess the potential impact of specific interventional strategies.

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INTRODUCTION

This is the second part of disease-specific methods and strategies for monitoring relapse following allogeneic hematopoietic stem cell transplantation (alloHSCT). In the first part, we focused on disease-specific monitoring of acute leukemias and myelodysplastic syndrome (MDS) [1]. Here, in this second part we will review disease-specific monitoring for chronic leukemias, chronic myeloproliferative neoplasms, and lymphoid malignancies.

Methodologic and technologic advances allow sensitive detection of minimal residual disease (MRD) and early recognition of recurrence after alloHSCT. Importantly, intervention prior to florid relapse improves outcome for certain hematologic malignancies [2,3]. This manuscript by the *Workshop Committee on Disease-Specific Methods and Strategies for Monitoring Relapse following Allogeneic Stem Cell Transplantation* is derived into 2 parts and reviews disease-specific detection methods and available data with the use of such after alloHSCT. Given the critical importance to the goals of this *Workshop*, standard disease-specific response and relapse criteria are summarized. Outside of the alloHSCT setting, international working groups have developed standard diagnostic criteria that are widely employed in the definition of relapse for the different hematologic malignancies [4]. These are based primarily on morphologic investigations of peripheral blood (PB) and/or bone marrow (BM) imaging, and/or specific laboratory findings. After alloHSCT, more sensitive methods such as molecular genetics, tumor-specific molecular primers, fluorescence in situ hybridization (FISH), multiparameter flow cytometry (MFC), and/or chimerism (see part I) are commonly used to monitor patients with respect to relapse. Some of these have clearly been shown to be predictive of outcome in specific diseases (eg, chronic myelogenous leukemia [CML]). However, the utility of the array of available tools in the monitoring of disease status after alloHSCT has not yet been fully elucidated across all hematologic malignancies. It is anticipated that sensitive MRD detection will allow for earlier therapeutic intervention, and it is hoped that treatment prior to overt relapse may improve outcome of alloHSCT for hematologic malignancies. Critically important is the need to assess the clinical relevance of MRD surveillance in individual diseases, which in turn must be followed by studies to assess the potential impact of specific interventional strategies. Recommendations for the utilization of sensitive monitoring techniques to augment the standard definitions of posttransplant remission, persistence, progression, and relapse, and to predict of relapse are proposed, based on current, available evidence whenever possible. From the point of view of this *Committee*, the use of these proposed definitions and methods should facilitate future studies of the natural history of relapse (*Committee on Epidemiology and Natural History of Relapse*), therapeutic interventions to prevent clinical relapse (*Committee on Strategies/Therapies Used to Prevent Relapse*), and the treatment of relapse (*Ccommittee on Disease-Specific Treatment of Relapse*). Finally, major deficits and important questions for further clinical research will be addressed.

METHODS TO DETECT AND MONITOR DISEASE RESPONSE, PERSISTENCE, PROGRESSION, AND RELAPSE

A wide variety of techniques are available to monitor residual disease after therapy, including in the posttransplant setting (Table 1), although the applicability varies by the specific disease subtype and the predictive value of each method is currently not well defined for most diseases. Some of these techniques are difficult to standardize, which is essential to the conduct of multicenter studies to assess the utility in the prediction and possible prevention of overt relapse.

Broadly, posttransplant monitoring of disease status is assured by 2 different methodologies: specific MRD detection and characterization of chimerism. The last characterizes the origin of posttransplant hematopoiesis, whereas MRD detection measures the malignant clone directly. For each approach, a variety of techniques are available, although in general there have been more studies looking directly at markers of residual malignancy than at chimerism. Issues of applicability, standardization, sensitivity, and specificity are discussed separately for each technique in detail in part I [1].

DISEASE-SPECIFIC DEFINITIONS AND MONITORING OF RELAPSE AFTER ALLOHSCT

Standard diagnostic criteria have been established to define response and relapse for the hematologic

Table 1. Diagnostic Methods to Monitor Residual Disease and Relapse of Hematologic Malignancies after alloHSCT

	Tumor Marker Detection					Chimerism	
Method	*Chromosomal Banding*	*FISH*	*Flow Cytometry*	*Antigen Receptor PCR*	*Translocation or Other RT-PCR*	*XY FISH*	*qPCR/STR-PCR*
Applicability	Subset of all types	Subset of all types	ALL; most AML; CLL; myeloma	ALL; lymphoma; CLL	CML; Subset of ALL; subset of AML; subset of lymphoma	Sex mismatched alloHSCT	All types with differences in donor/recipient polymorphisms
Sensitivity	10^{-1}	10^{-2}	10^{-3}-10^{-4}	10^{-4}-10^{-5}	10^{-3}-10^{-6}	10^{-2}	10^{-3}-10^{-6}

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; qPCR, quantitative real-time PCR; RT-PCR, reverse transcription PCR; STR, short tandem repeats.

malignancies. These criteria have historically been based on morphologic BM investigations (eg, blast count in acute leukemias), imaging methods (eg, occurrence of new lymph nodes on fluorodeoxyglucose (FDG)-positron emission tomography (PET) scans for non-Hodgkin lymphoma [NHL]), and/or specific laboratory findings (eg, increased paraprotein by immunofixation and electrophoresis in multiple myeloma [MM]). Recently, more sensitive methods have been utilized to assess patients for disease response. Some, but not all, of these approaches have been integrated into response criteria definitions for various hematologic malignancies. Herein, we propose criteria for incorporation of currently available methodologies in the definitions for disease response, persistence, progression, relapse, and the prediction of relapse after alloHSCT.

CML

Remission definitions for CML after alloHSCT are well defined (Table 2). Relapse constitutes the main cause of failure after alloHSCT [5], occurring in 10%-25% of patients transplanted in chronic phase and up to 70% in patients transplanted in blast phase [6]. The incidence of relapse has remained relatively stable over the years, although the negative impact of relapse on survival has been declining, suggesting an improved management of relapse over time [6].

Although the Philadelphia chromosome is usually not seen in cytogenetic analysis patients following alloHSCT, this does not exclude the presence of residual leukemic cells. Usually, relapse of CML after alloHSCT is a slow gradual process, although sudden growth of CML cells is not uncommon, especially in patients reaching alloHSCT in advanced phases [7,8]. Relapse characteristically is first detectable only by using molecular methods indicating a low level of residual disease [9,10]. Subsequently, it can be detected using cytogenetic analysis, FISH, or conventional blood analyzers. Relapse of CML can occur in chronic, accelerated, or blast phase following a pattern of progression similar to newly diagnosed CML (Table 3), although the interval between phases may be shorter [7,8,11].

Qualitative reverse-transcription polymerase chain reaction (RT-PCR)

It is now well established that the detection of the chimeric *BCR-ABL1* mRNA transcript by RT-PCR is a powerful predictor of subsequent relapse [9,10,12,13]. Several studies had attempted to assess the clinical significance and predictive value of detecting *BCR-ABL1* transcripts by RT-PCR assay after alloHSCT. However, the majority of the early studies were based on qualitative RT-PCR, and the results had been conflicting [14-18].

Using a nested primer technique Roth et al. [16] analyzed 64 CML patients after alloHSCT and detected *BCR-ABL1* transcripts at 1 time point in 37 patients. They concluded that nested RT-PCR could

Table 2. Remission Definitions for Chronic Myeloid Leukemia

Complete Molecular Remission

Using a quantitative real-time PCR (qPCR) method, the *BCR-ABL1* fusion mRNA is not detectable in the peripheral blood and/or the bone marrow, by an assay with a sensitivity to allow detection of 1 Ph^+ cell in 10^5 to 10^6 normal cells. The results should be confirmed by 2 consecutive tests done at least 4 weeks apart. The duration of molecular remission is defined as the time from the first negative RT-PCR assay.

A "nested" PCR assay should be used for confirmation of a negative RT-PCR if the sensitivity of the qPCR is $<10^{-5}$.

Complete Cytogenetic Remission (must be confirmed by a second assay).

It should be measured using conventional cytogenetic analysis or hypermetaphase FISH. The definition of complete cytogenetic remission requires 0% Ph^+ metaphases. A minimum of 20 analyzable metaphases must be assessed for appropriate evaluation of a cytogenetic remission. Remission should be confirmed with a repeated cytogenetic analysis within 4 to 12 weeks. The duration of cytogenetic remission is defined as the time from first negative assay.

Complete Hematologic Remission (must be confirmed by a second assay).

All of the following:
- WBC $<10 \times 10^9$/L;
- Hemoglobin >11 g/dL;
- Platelets $<450 \times 10^9$/L;
- Normal WBC differential (<1% precursor cells);
- No disease-related symptoms;
- No palpable splenomegaly;
- No extramedullary disease;
- Normalization of the bone marrow appearance.

PCR indicates polymerase chain reaction; FISH, fluorescence in situ hybridization; Ph^+, Philadelphia chromosome positive; qPCR, quantitative real-time PCR; RT-PCR, reverse transcription PCR.

Table 3. Definition of Relapse in CML

Molecular Relapse (The date of molecular relapse is the date of the first positive RT-PCR assay).†
Is said to be present in a CML patient lacking any other evidence of the disease (ie, patient in hematologic remission and cytogenetic remission) at least 4 months after alloHSCT when any of the following apply:
- Three samples over a minimum of 4 weeks show a *BCR-ABL1/ABL1* ratio higher than 0.02% as measured by qPCR tests.*
- Three samples over a minimum of 4 weeks show clearly rising levels of *BCR-ABL1/ABL1* ratio with the last 2 higher than 0.02% as measured by qPCR tests.*
- Two samples over a minimum of 4 weeks show a *BCR-ABL1/ABL1* ratio higher than 0.05% as measured by qPCR tests.*

Cytogenetic Relapse
Any of the following in a patient lacking any clinical or hematologic evidence of the disease (ie, patient in hematologic remission):
- Presence of 1 or more Ph^+ metaphases with standard cytogenetic analysis or hypermetaphase FISH.
- >2% cells with the *BCR-ABL1* fusion gene by interphase FISH.

Hematologic Relapse
All of the following:
- Abnormal blood or marrow counts or morphology consistent with CML.
- Cytogenetic and/or molecular confirmation of the presence of the disease.
  Hematologic relapse is subclassified into chronic phase, accelerated phase, or blast phase according to following WHO criteria:
- Chronic Phase: None of the features of accelerated phase or blast crisis.
- Accelerated Phase: Any of the following:
  - Blasts 10%-19% of WBCs in peripheral blood and/or nucleated bone marrow cells.
  - Peripheral blood basophils ≥20%.
  - Persistent thrombocytopenia (<100 × 10^9/L) unrelated to therapy.
  - Persistent thrombocytosis (>1000 × 10^9/L) unresponsive to therapy.
  - Increasing spleen size and increasing WBC count unresponsive to therapy.
  - Cytogenetic evidence of clonal evolution.‡
- Blast Phase: Any of the following:
  - Blasts ≥20% of peripheral blood white cells or of nucleated bone marrow cells.
  - Extramedullary blast proliferation.
  - Large foci or clusters of blasts in the bone marrow biopsy.

Progression
The definition of progression of CML is based upon the above definitions of relapse. Once the CML has fulfilled the criteria for relapse at any level (molecular, cytogenetic, or hematologic), the patient remains at risk of developing disease progression.
Disease progression can thus be defined as any of the following:
- Molecular relapse progressing into cytogenetic or hematologic relapse.
- Cytogenetic relapse progressing into hematologic relapse.
- Hematologic relapse progressing from chronic phase to accelerated phase.
- Hematologic relapse progressing from chronic phase to blast phase.
- Hematologic relapse progressing from accelerated phase to blast phase.

CML indicates chronic myelogenous leukemia; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; qPCR, quantitative real-time PCR; RT-PCR, reverse transcription PCR; alloHSCT, allogeneic hematopoietic stem cell transplantation.
*Other control genes such as *BCR*, *GUS*, and *G6PDH* have been used in several laboratories. No published data exists regarding the use of these control genes although the same cutoff for the *BCR-ABL1*/control gene ratio could be applied.
†RT-PCR assays referred to in this table were not done according to the International Scale.
‡Clonal evolution refers to the appearence of new chromosomal abnormailities not previously detected.

define subgroups of patients in apparent clinical remission (CR) but with an increased risk of disease recurrence. The Hammersmith group showed that RT-PCR positivity within 6 months after transplantation did not predict a worse outcome, whereas RT-PCR positivity later than 6 months after transplantation did [17]. Radich et al. [9] presented a comprehensive multivariate analysis of 346 patients after alloHSCT; they identified RT-PCR-positivity at 6 to 12 months post-alloHSCT as 1 independent variable influencing subsequent relapse. The significance of the presence of *BCR-ABL1* transcripts in predicting disease recurrence was, however, lost in patients who tested positive more than 36 months post-alloHSCT [9].

Quantitative RT-PCR

The clinical value of monitoring MRD has been greatly improved by the use of quantitative PCR (qPCR) and the establishment of consensus thresholds of residual disease above which a patient is likely to relapse [19-23]. Serial qPCR techniques can distinguish those PCR positive patients who have low or falling *BCR-ABL1* levels from those whose levels are increasing [17]. Patients destined not to relapse after alloHSCT have persistently undetectable, low, or falling *BCR-ABL1* levels on sequential analysis. After 6 to 12 months, *BCR-ABL1* transcripts are usually undetectable and remain so indefinitely. In contrast, increasing or persistently high levels of *BCR-ABL1* mRNA precede relapse, often several months before the cytogenetic detection of the Philadelphia chromosome positive BM metaphases. Provided assays are performed with sufficient frequency; rising or persistently high numbers of *BCR-ABL1* transcripts can be detected prior to frank relapse, and this information may be used for early therapeutic intervention. Several studies have demonstrated that the molecular burden of *BCR-ABL1* transcripts, and the kinetics of increasing *BCR-ABL1*, predict relapse. Lin et al. [17] demonstrated that the kinetics of *BCR-ABL1* level over time described both impending relapse and response to donor lymphocyte infusion (DLI). Low (or no) residual *BCR-ABL1* was associated with a very low risk of relapse (1%), compared to 75% relapse rate in patients

with increasing or persistently high *BCR-ABL1* levels. Olavarria et al. [10] studied 138 CML patients "early" (3-5 months) posttransplant and showed that the *BCR-ABL1* level was highly correlated with relapse. Patients with no evidence of *BCR-ABL1* had a 9% risk of subsequent relapse, whereas patients defined as having a "low" burden of disease or "high" level of transcripts had a cumulative relapse rate of 30% and 74%, respectively. These results are consistent with a study of 379 CML patients "late" (>18 months) posttransplant performed by Radich et al. [24]. Ninety patients (24%) had at least 1 assay positive for *BCR-ABL1*, and 13 of 90 (14%) patients relapsed. Only 3 of 289 patients who were persistently *BCR-ABL1* negative relapsed [24].

The highest risk of relapse associated with *BCR-ABL1* MRD appears to be associated with "early" (≤12 months) detection after transplant; however, there may be a need for life-long monitoring. The Hammersmith group analyzed 243 patients who had *BCR-ABL1* transcripts monitored by qPCR after alloHSCT for a median of 84.3 months [11]. Patients were allocated to 1 of 4 categories: (1) 36 patients were "persistently negative" or had a single low-level positive result; (2) 51 patients, "fluctuating positive, low level," had more than 1 positive result but never more than 2 consecutive positive results; (3) 27 patients, "persistently positive, low level," had persisting low levels of *BCR-ABL1* transcripts but never more than 3 consecutive positive results (therefore, never fulfilled the definition for molecular relapse); and (4) 129 patients relapsed. In 107 of these, relapse was based initially only on molecular criteria; in 72 (67.3%) patients, the leukemia progressed to cytogenetic or hematologic relapse either prior to or during treatment with DLI. Their conclusions were that the pattern of *BCR-ABL1* transcript levels after allograft is variable; that only a minority of patients who had fluctuating or persistent low levels of *BCR-ABL1* transcripts long term eventually relapsed and that the majority of patients who had a molecular relapse were likely to progress further [11].

Moreover, occasional CML patients who were treated by alloHSCT in chronic phase have relapsed more than 10 years after an otherwise "successful" transplantation [25], and data collated by the CIBMTR show that the cumulative incidence of relapse at 15 years for patients in remission at 5 years after alloHSCT was 17% [11,26].

Cytogenetic analysis and FISH

The role of conventional cytogenetic analysis (ie, G-banding) and FISH in the monitoring of patients with CML undergoing alloHSCT is relatively limited [7,27]. These techniques are necessary for the characterization of the stage of the relapse and confirmation that the morphologic changes observed in the PB and/or BM correspond to CML. Patients who fulfill the criteria for molecular relapse (Table 3), must have an assay to confirm or exclude the presence of the Philadelphia chromosome. This could be FISH in PB/BM or conventional cytogenetic analysis of BM aspirates. In addition, conventional cytogenetic analysis is necessary to assess the progression to accelerated phase (ie, presence of clonal evolution), and FISH analyses may be useful in assessing chimerism status in sex mismatched alloHSCT.

Chimerism studies

There is scant information regarding the use of chimerism studies in the monitoring of CML patients after alloHSCT. It is widely accepted that in CML, relapse occurs in the context of mixed or decreasing T cell chimerism, and given the hematopoietic potential of CML cells, this is also true for myeloid chimerism [28,29]. However, relapses have been described in the presence of 100% donor chimerism [30,31]. Chimerism studies could be of some value in predicting the response to the treatment of relapse (especially after DLI) and in monitoring the response to DLI or tyrosine kinase inhibitors (TKIs). Less than 10% donor chimerism predicts for lack of response to DLI [32]. Achievement of 100% donor chimerism may be associated with long-term remission [27,33]. However, there is a need to investigate this field further in future studies.

Application of MRD studies in prospective CML trials

It is now well established that PCR-based monitoring methods play a significant role in the management of CML patients undergoing alloHSCT. qPCR allows for the detection of molecular relapse and accurately predicts for disease progression. Future clinical trials should evaluate the role of MRD monitoring in patients reaching the transplant after failure to respond to the new TKIs. In this context, clinical trials are needed to determine the value of qPCR in conjunction with the use of first- or second- generation TKIs either in a prophylactic or preemptive fashion. Furthermore, prospective clinical trials should address the potential synergistic combination of the graft-versus-leukemia (GVL)/tumor effect of DLI and TKIs in relapsed CML. Finally, there is a need to validate all the RT-PCR results mentioned in the previous paragraphs in the era of standardization of the RT-PCR assays and the development of an International Scale [34].

Myeloproliferative Neoplasms

This section on myeloproliferative neoplasms (MPNs; a.k.a myeloproliferative disorders) will focus only on myelofibrosis (primary myelofibrosis [PMF] or myelofibrosis after either polycythemia vera [PV] or essential thrombocythemia [ET]), because alloHSCT is rarely indicated in uncomplicated PV or

Table 4. IWG-MRT Complete Remission and Progression Criteria for Myelofibrosis [35]

Complete Remission (CR): requires all of the following:
- Complete resolution of disease-related symptoms and signs including palpable hepatosplenomegaly.
- Peripheral blood count remission defined as hemoglobin level of at least 11 g/dL, platelet count of at least 100×10^9/L, and absolute neutrophil count of at least 1.0×10^9/L. In addition, all 3 blood counts should be no higher than the upper normal limit.
- Normal leukocyte differential including disappearance of nucleated red blood cells, and immature myeloid cells in peripheral smear in the absence of splenectomy.
- Bone marrow histologic remission defined as the presence of age-adjusted normocellularity, no more than 5% myeloblasts, and an osteomyelofibrosis grade no higher than 1.

Complete Cytogenetic Response: CR with failure to detect a preexisting cytogenetic abnormality.

Major Molecular Response: CR with absence of a previously detected specific disease-associated mutation in peripheral blood granulocytes.

Partial Remission (PR): requires all of the above criteria for CR except the requirement for bone marrow histologic remission. However, a repeat bone marrow biopsy that does not fulfill the criteria for CR is required.

Progressive Disease: requires 1 of the following:
- Progressive splenomegaly that is defined by the appearance of a previous absent splenomegaly that is palpable at greater than 5 cm below the left costal margin or a minimum of 100% increase in palpable distance for baseline splenomegaly of 5-10 cm or a minimum of 50% increase in palpable distance for baseline splenomegaly of >10 cm.
- Leukemic transformation confirmed by bone marrow blast count of at least 20%.
- Increase in peripheral blood blast percentage of at least 20% that lasts for 8 weeks.

Relapse: Changes from CR to PR or CR/PR to clinical improvement.*

*Clinical improvement is defined as: absence of PD or CR/PR with improvement in peripheral blood (hemoglobin, absolute nutrophil count (ANC), and platelets, as well as 50% reduction of splenomegaly (for details see [35]).

ET. Definitions for remission and relapse/progression have recently been published by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT; Table 4) [35].

Definitions of remission and relapse for myelofibrosis after alloHSCT

There is no approved definition of relapse after alloHSCT for myelofibrosis patients. Based on the recent IWG-MRT consensus definitions in PMF, it may be possible to define remission after alloHSCT, and therefore persistent disease, relapse, and disease progression could be subsequently inferred. It is beyond the scope of this review to discuss in detail the different MPNs and the new molecular classifications [36,37]. Until recently, the majority of MPN patients undergoing alloHSCT had been diagnosed with PMF or atypical MPN, with very few patients having PV or ET, and almost none diagnosed on the basis of the molecular defect [38]. In PMF, the situation is confounded by the fact that only 50%-60% of patients show regression of the BM fibrosis in the early posttransplant period, making it difficult to define relapse [39]. Given the slow kinetics of progression of PMF, it is acceptable to assume that relapse after alloHSCT would follow a similar pattern to CML, although little is known about this [40]. Furthermore, there is increasing evidence to suggest that in PMF, relapse occurs initially at the molecular level, followed by progression to cytogenetic and hematologic relapse, imitating what happens in CML [41]. Clearance of the JAK2 mutation level in PB after alloHSCT as a time-dependent variable significally predicts clinical relapse [41,42].

Hematological remission and relapse. Hematologic remission requires normalization of the BM cellularity, blast counts, and degree of fibrosis on a BM biopsy. According to this last criterion, only 60% of patients will enter remission within the first 3 months after reduced-intensity conditioning (RIC) alloHSCT; the proportion increasing to nearly 90% after 12 months. However, hematologic CR also requires normalization of the PB counts, which in alloHSCT patients may be influenced by graft-versus-host disease (GVHD), poor engraftment, infections, drug toxicity, and other posttransplant complications, thus rendering these criteria invalid in many patients. In this way, the diagnosis of hematologic relapse in a patient who had previously achieved a CR is relatively straightforward. However, in a patient with persistent (but otherwise decreasing) fibrosis, the detection of a hematologic relapse could prove extremely difficult in the absence of other cytogenetic or molecular markers of disease relapse.

Cytogenetic remission and relapse. In the minority of patients with PMF who have karyotypic abnormalities, cytogenetic CR is defined as the absence of the preexisting abnormalities at any given time after treatment, whereas minor cytogenetic response is defined by a reduction of at least 50% in the proportion of positive cells. Whether these criteria could be applied in practice to PMF patients after alloHSCT remains unclear. Cytogenetic relapse is thus defined as the reappearance of the previously known chromosomal abnormality (with or without new abnormalities) in a patient who was previously in cytogenetic remission.

Molecular remission and relapse. Although a substantial number of myelofibrosis patients today are known to have a molecular defect, there are no clear definitions of molecular remission making the definition of molecular relapse after alloHSCT an important area of future research. However, new data suggest that it should be possible to define molecular remission in *JAK2*V617F positive myelofibrois patients using qPCR in a similar way to CML. *JAK2*V617F mutation is found in about 50% of patients with myelofibrosis. Kröger et al. [41] described 17 such patients who became PCR negative after alloHSCT using a highly sensitive RT-PCR method. Only 2 patients subsequently relapsed at the molecular level, 1 of whom progressed to overt hematologic relapse within 6 weeks. Alchalby et al. [42] showed that rapid clearance of JAK2 level in PB significantly reduced the risk of relapse after alloHSCT. Steckel et al. [43] reported 15 *JAK2*V617F positive PMF patients, 12

of whom became PCR negative after alloHSCT. Other molecular markers, such as the *MPL*W515L/K mutation, are seen in only 5% of myelofibrosis patients and a smaller series reported rapid clearance after alloHSCT [44].

It is proposed that molecular remission could be defined as a negative PCR for the presence of the *JAK2*V617F mutation, with an assay of at least 10^{-3} sensitivity, in the PB and/or the BM of a patient who was positive for the mutation prior to transplant. The results should be confirmed by 2 consecutive tests done at least 4 weeks apart. The duration of molecular remission should be defined as the time from the first negative RT-PCR assay. Molecular relapse could then be defined as 2 consecutive positive PCR tests, at least 4 weeks apart in a patient who had previously achieved molecular remission. It is not possible at present to establish a quantitative cutoff below which a patient with a positive PCR assay should remain in molecular remission.

Relapse after alloHSCT for myelofibrosis: Methods of MRD detection qPCR

As noted before, the activating mutation V617F of the *JAK2* gene is an obvious target for monitoring MRD in patients with PMF after alloHSCT [45]. There are emerging data suggesting that, similar to *BCR-ABL1* in CML, PCR negativity for *JAK2*V617F correlates with prolonged remission and that reappearance of a detectable *JAK2*V617F clone is associated with relapse [41-43,46-49]. Furthermore, quantification of the tumor burden by qPCR may be a useful technique to monitor MRD after alloHSCT and guide possible therapeutic interventions. Kröger et al. [50] showed that it is possible to use qPCR successfully to guide DLI in PMF patients. Complete disappearance of the *JAK2*V617F mutation was achieved in a significant proportion of patients receiving 1 or more DLIs. Similar to what occurs in CML, it would seem that DLIs are more effective if performed at a level of molecular residual disease. In addition, there is evidence that persistence of the *JAK2*V617F mutation measured by qPCR correlates with mixed chimerism after alloHSCT [46,50].

PCR-based assays for the detection of *JAK2*V617F lack standardization. In a recent study by Lippert et al. [51], 16 laboratories performed 11 different assays for the quantification of *JAK2*V617F allelic burden. The results showed great variability among laboratories, low sensitivity in sequencing techniques, and strong discrepancies with 4 techniques, which could be attributed to inadequate standards or to different modes of expression of results. After calibration of assays with common *JAK2*V617F standards (dilutions of UKE-1 cells in normal leukocytes) there was good correlation among 4 quantitative Taq-Man allele specific PCR assays, 2 of which were able to detect levels of 0.2% *JAK2*V617F. It is highly desirable that a standardized international scale for measuring *JAK2*V617F transcripts be established. Lippert et al. [51] recommended combining plasmid DNA dilutions, which allow precise quantification of the number of copies of JAK2, with at least 1 well-calibrated genomic DNA sample as an internal control. Finally, there is no consensus as to whether PB samples or BM samples are the best samples to analyze. Some investigators suggest that purified blood granulocytes are preferable [52].

Quantification of the level of the *JAK2*V617F mutation can also be done by methods other than qPCR. Koren-Michowitz et al. [53] measured levels of *JAK2*V617F by mass-spectrometry in 60 patients with the *JAK2*V617F mutation undergoing alloHSCT and found that patients in CR had significantly better survival.

There are a number of other molecular targets that may prove useful in monitoring MRD in patients with MPN. As noted earlier, around 5% of PMF cases have a mutation in the *MPL* gene, although the proportion of patients with the *MPL*W515 mutation undergoing alloHSCT may be different. This mutation can be also used for monitoring molecular disease after alloHSCT [44]. Given the paucity of data with this and other mutations, the use of PCR or other methods to monitor MRD needs to be evaluated further.

Cytogenetic analysis and FISH. The role of conventional cytogenetic analysis and FISH in the monitoring of patients with MPN undergoing alloHSCT is dependent on the presence of an abnormal karyotype or other chromosomal abnormalities detectable by FISH at diagnosis. The frequency ranges from 80% in cases of progression to acute leukemia, 40% in PMF, and >5% in molecularly defined MPN [46]. However, the incidence of chromosomal abnormalities in patients undergoing alloHSCT might be higher.

In PMF and other MPN, the need to assess BM histology makes performance of BM cytogenetic studies routine in many centers. There is, however, no consensus on the frequency of these studies after alloHSCT. In contrast to CML, cytogenetic analysis is not necessary to assess the progression to an accelerated phase of the disease.

Chimerism studies. There is very limited information regarding the use of chimerism studies in the monitoring of MPN patients after alloHSCT. It widely accepted that relapse occurs in the context of mixed or decreasing T cell chimerism, although relapses have been described in the presence of 100% donor chimerism [54,55]. Chimerism studies could be of some value in predicting or monitoring the response to the treatment of relapse (eg, after DLI).

Overlapping of MRD Detection Methods

In any given patient, it is likely that some combination (rather than a single test) of the methods described

before will be used for the monitoring of the disease response after alloHSCT. The integration of sometimes discrepant results provides an enormous challenge. Typically, a PMF patient at 3 to 6 months posttransplant could have persistence of BM fibrosis, signs such as splenomegaly, and, at the same time, a negative PCR test for the presence of the *JAK2*V617F mutation previously detectable before alloHSCT. Another possible scenario includes 1 in which PCR-based chimerism studies show 100% donor chimerism in both the myeloid and lymphoid lineages, whereas cytogenetic analysis studies confirm the persistence of a previously noted chromosomal abnormality. Kröger et al. [41] described 5 patients who had a persistently positive RT-PCR for *JAK2*V617F after alloHSCT, of which 4 patients fulfilled the criteria for hematologic CR of the IWG-MRT. In the same study, Kröger et al. [41] found a highly significant inverse correlation between donor chimerism and *JAK2*V617F PCR negativity. At the other extreme, in *JAK2*V617F negative MPN without chromosomal abnormalities, chimerism studies may be the only test on which to base therapeutic decisions apart from histopathologic review of the BM. These issues constitute an important research field in the monitoring of MPN patients undergoing alloHSCT and should be evaluated prospectively.

Applications of MRD Monitoring Methods in Prospective Clinical Trials for MPN

One of the most challenging hurdles in monitoring MRD in MPN patients after alloHSCT is the definition of the different levels of relapse: molecular, cytogenetic, and hematologic. Another pitfall is the lack of standardization of the qPCR methods. Currently available methods have only a sensitivity of 1%-5%, which is clearly unsatisfactory for posttransplant monitoring. Highly specific qPCR methods have been developed and should be made widely available. Future transplant studies should be ready to address the value of MRD monitoring using quantitative qPCR in predicting relapse and disease progression. In addition, an important goal for those studies should be the evaluation of the role of PCR-based MRD monitoring in guiding the use and evaluation of response to DLI. Such approaches should be applied to the evaluation of safety and efficacy of *JAK2* tyrosine kinase inhibitors before and after alloHSCT.

Chronic Lymphocytic Leukemia (CLL)

The definitions for relapse or progression of CLL after alloHSCT have traditionally used clinical and hematologic parameters and have been recently updated by the International Working Group CLL (IWCLL) (Table 5). The guidelines now also incorporate a definition of MRD negativity as assessed by MRD flow or allele specific oligonucleotides (ASO) primer immunoglobulin heavy chain (IgH) qPCR. The IWCLL/NCI-Working Group defined MRD negativity as <1 CLL cell in 10,000 benign leukocytes in PB or BM [56]. Tumor cell increases at the MRD level do not constitute clinical CLL progression or relapse unless they exceed 5 B cells/nL in peripheral blood.

Table 5. Relapse Definition for Chronic Lymphocytic Leukemia

Relapse: Progression occurring 6 months or later after having achieved CR or PR

Progression: iwCLL/NCI-WG criteria for CLL progression (at least 1 must apply)

- Appearance of any new lesion such as enlarged lymph nodes (>1.5 cm), splenomegaly, hepatomegaly, or other organ infiltrates;
- Increase of lymphadenopathy by 50% or more in greatest determined diameter of any previous site, or an increase of 50% or more in the sum of the product of diameters of multiple nodes;
- Increase in the liver or spleen size by 50% or more or the de novo appearance of hepatomegaly or splenomegaly;
- Increase in the number of blood lymphocytes by 50% or more with at least 5/nL B cells;
- Transformation to a more aggressive histology (eg, Richter's syndrome);
- Occurrence of cytopenia (neutropenia, anemia, or thrombocytopenia) attributable to CLL.

Complete MRD Response: Clinical remission in the absence of 1 CLL cell per 10,000 leukocytes in the peripheral blood or bone marrow.

MRD Relapse: Tumor cell recurrence or increases at the MRD level that does not exceed 5 B cells/nL in the peripheral blood.

CLL indicates chronic lymphocytic leukemia; MRD, minimal residual disease; CR, complete remission; PR, partial remission.

Methods for MRD Detection in CLL

Ideally, an assay for measurement of MRD should be CLL-specific, highly sensitive even in the presence of a majority of physiological B cells, broadly applicable, easily standardized between laboratories, and capable of quantification. During recent years, 2 main approaches of MRD assessment in CLL have been followed: flow cytometry, taking advantage of the unique immunophenotype of CLL, and PCR-based strategies using the clonal rearrangement of the hypervariable complementary determining region 3 (CDR3) of the variable (V_H) part of the IgHgene [57-61].

Consensus PCR

CLL is a clonal disorder of mature B cells characterized by a clone-specific rearrangement of the IgH CDR3. With appropriate primers annealing to consensus V_H framework regions (FR) and joining regions (JH), respectively, the CLL clone-specific CDR3 rearrangement can be amplified and detected by appropriate methods, such as gene scanning or heteroduplex analysis [60]. This technique does not rely on sequencing of the individual CDR3 region and thus has the advantage of being relatively simple and rapid. Because it has to detect the clonal tumor product against a polyclonal background of normal B cells, however, it is less sensitive than PCR assays based on allele-specific primers. Consensus IgH PCR is reported to detect 1 monoclonal B cell in 100-1000 benign leukocytes.

Furthermore, the sensitivity of consensus IgH PCR for a particular sample cannot be predicted precisely, as it depends on both the number of benign B cells in the sample and the length of the PCR product [58-60]. Another disadvantage of consensus IgH PCR is that it does not allow quantification of the CLL clone and, thus, of the MRD level. The specificity and sensitivity of the approach was somewhat improved using a combination of Southern blotting and labeled patient specific probes for detection [62].

Clone-specific PCR—nested

Unlike the bcl2/IgH rearrangement in follicular lymphomas (FLs) and the bcl1/IgH rearrangement in mantle cell lymphomas (MCLs), CLL has no hallmark genetic abnormality that can be used as a universal PCR marker. Therefore, only primers addressing the CLL-specific CDR3 rearrangement can be used for specific amplification of the CLL clone. If such an allele-specific approach is combined with a first-step consensus IgH PCR ("nested IgH PCR"), CLL cells can be detected with a very high sensitivity of up to 10^{-6} provided a sufficient amount of DNA is analyzed [63]. Limitations of nested IgH PCR include the need for individual sequencing of the V_H gene and the fact that it is not a quantitative method.

Clone-specific PCR

Quantitative measurement of clone-specific IgH rearrangement, copy numbers, and thus MRD levels, can be achieved by qPCR using allele-specific primers similar to the nested PCR together with consensus JH or FR consensus backward primers and JH or FR consensus probes [58,60]. The technique can be used on stored DNA and data interpretation is now standardized [64], but qPCR is labor-intensive because of the need for CDR3 sequencing and individual clone specific primer design and it is not as sensitive as nested PCR (10^{-4} to 10^{-5}) [58,61,65].

Flow cytometry

Recently, 4-color flow cytometry using the characteristic immunophenotype of CLL cells (MRD flow) has been introduced as a sensitive and quantitative tool for MRD detection in CLL. Appropriate CLL-specific antibody combinations allow for sensitivities of 10^{-4} to 10^{-5} [57,58,66]. Close quantitative correlation ($r = 0.95$) and high qualitative concordance with qPCR for detection of CLL above 10^{-4} could be demonstrated by experienced operators, even in the presence of anti-CD20 antibodies [67]. Although it is less reliable than qPCR below the 10^{-4} threshold, MRD flow is simple, fast, and applicable to all sample types and therapeutic regimens without need for a priori-probe construction from a sample known to be MRD-positive. Based on an international standard [66], MRD flow is currently the most widely used method for routine MRD assessment in CLL. However, the method requires 20-fold more total leukocytes to achieve the same sensitivity as qPCR and requires the availability of fresh samples.

Chimerism analysis

Taken together, both MRD flow and allele-specific qPCR are excellent assays for MRD quantification with high sensitivity and specificity in CLL. Consequently, any method of nonspecific chimerism determination offers no advantage, and thus chimerism assays do not play a relevant role for MRD measurement after alloHSCT for CLL. Nevertheless, chimerism analyses can provide very valuable additional information on GVL activity and resistance. Although individual cases of sustained MRD negativity in the absence of complete chimerism can occur [65], data from the 37 patients of the German CLL Study Group CLL3X study evaluable for this endpoint suggest that MRD clearance by month +12 is almost always associated with complete donor chimerism, implying that GVL-mediated MRD clearance is based on graft-versus-hematopoiesis effects. On the other hand, in the patients with MRD persistence at 12 months, complete chimerism was frequently achieved, indicating lack of GVL despite effective graft-versus-hematopoiesis activity (Dreger et al., unpublished data). In conclusion, incomplete or decreasing chimerism after alloHSCT for CLL seems to be a predictor of an insufficient GVL effect, and vice versa, of a high relapse risk. Thus, MRD and chimerism assessment are complementary tools essential for guiding post-transplant immune modulation in CLL.

Applications of MRD Monitoring after alloHSCT in CLL

Based on the high sensitivity and specificity of the MRD assays available for CLL, as well as their easy applicability to PB samples, they have been investigated for a variety of clinical purposes. MRD is important as potential marker for early prediction of long-term treatment outcome, and for guidance of posttransplant preemptive therapy.

Clinical trials in CLL are hampered by the fact that because of the general indolent course of the disease it may take very long until clinical endpoints are reached. In particular, this limits the ability to rapidly assess the effectiveness of treatments such as immunochemotherapy and transplantation. Theoretically, endpoints considering MRD responses or kinetics may be used to replace clinical endpoints, such as progression-free survival (PFS) or overall survival (OS), allowing for much faster identification of patients with high or low likelihood of long-term disease control. Moreover, in potentially curative treatment approaches, such as alloHSCT, MRD might emerge as a surrogate marker

for permanent disease eradication, thereby providing an early predictor of cure. Because CLL is susceptible to GVL effects and antibody-based immune modulation [65,67-73], MRD monitoring could be used for guiding preemptive immunomodulating interventions, such as immunosuppression tapering, DLI, and rituximab administration. Given the unique possibility of "real-time" monitoring of GVL efficacy provided by continuous MRD assessment, MRD measurement could help to delineate mechanisms of GVL activity and resistance by correlating MRD responses with the occurrence of potential effectors of GVL, such as CLL-specific T cells and allo-reactive T cells [74].

Prognostic Value of MRD Kinetics after AlloHSCT for CLL

The prognosis of CLL is essentially determined by the clinically relevant endpoints survival and disease progression/relapse. According to the recently updated NCI-Working Group guidelines [56], progressive disease is defined by at least 1 of the criteria listed in Table 5. Relapse is defined as CLL progression occurring 6 months or later after having achieved CR or partial remission (PR). It is important to stress that these are purely clinical criteria, that is, tumor cell increases at the MRD level will not count as CLL progression or relapse unless they exceed 5 B cells/nL in the PB.

HSCT was the first treatment modality that far exceeded the efficacy of conventional therapy, thereby establishing the need for more sensitive tools for response assessment. Transplantation is a good model to illustrate that the predictive value of MRD assessment is strongly dependent on the treatment modality actually used, that is, MRD negativity after autologous HSCT (autoHSCT) has a prognostic meaning different from that after alloHSCT. Moreover, lessons learned from alloHSCT provide evidence that MRD kinetics is more important than absolute MRD levels.

MRD measurement in CLL was first introduced by Gribben and coworkers [62] in the context of the Dana-Farber Cancer Institute CLL transplant program. This group used a PCR methodology based on a consensus primer CDR3 PCR plus a patient-specific oligonucleotide applied to blood and BM samples obtained after autoHSCT and alloHSCT. They found a strong correlation between achievement of MRD negativity and relapse risk in patients who had undergone autoHSCT with B cell-depleted BM grafts or alloHSCT with CD6-depleted BM grafts after myeloablative (MA) treatment [75].

A number of groups have reported quantitative MRD assessment following alloHSCT. Ritgen et al. [76] performed an analysis of MRD kinetics using qPCR and/or MRD flow in 32 patients who had undergone RIC alloHSCT and demonstrated that, in the majority of cases, achievement of MRD negativity was clearly linked to immune intervention, such as tapering of immunosuppression (n = 12) or DLIs (n = 6). Four additional patients became MRD-negative immediately post-alloHSCT, and 3 other patients who had MRD samples available only from months 5, 33, and 46 onward were also found to be MRD-negative. With a median follow-up of 72 (41-101) months, only 1 clinical relapse was observed in these 25 patients, whereas 6 of the 7 patients remaining MRD-positive relapsed. Using a highly sensitive nested PCR method, Farina et al. [60] found permanent MRD negativity or a "mixed pattern" (not consistently negative, but without significant increase over time) in 16 of 29 patients (55%) in clinical CR after RIC alloHSCT for CLL. All 3 patients with mixed pattern who were tested with qPCR in parallel were MRD-negative. Only 1 of these 16 patients relapsed during a follow-up time of 40 (12-85) months, whereas 8 of 13 MRD-positive patients developed clinical relapse. Four additional patients were always MRD-positive by nested PCR, but showed decreasing or stable levels by qPCR. None of these 4 relapsed. More recently, Khouri et al. and Sorror et al. reported absent or apparently reduced recurrence in 21 and 14 patients, respectively, who achieved MRD negative clinical CR after RIC alloHSCT as documented by polyacrylamide gel electrophoresis-based allele-specific CDR3 PCR [77,78]. Moreno et al. [79] reported complete MRD clearance or "mixed pattern" (and C. Moreno, personal communication, May 2009) by MRD flow or qPCR in 9 of 15 patients with CLL (60%) after MA alloHSCT. At a follow-up of 6-120 months, only 1 of these 9 patients had relapsed clinically. Caballero et al. [80] observed a complete clearance of CLL by MRD flow in the context of acute or chronic GVHD (aGVHD, cGVHD) in 6 patients by day 360 subsequent to being MRD-positive at day 100.

In summary, MRD assessment after alloHSCT is predictive for durable freedom from CLL progression if: (1) MRD levels are below 10^{-4} 1 year posttransplant; or (2) show decreasing or stable kinetics within the quantitative range. Taken together, in all studies employing quantitative MRD assessment, MRD negativity 1-year posttransplant was not only predictive for virtual absence of clinical relapse (except for 2 patients with extranodal disease recurrence), but also durable over the whole follow-up in >90% of patients. In conclusion, MRD monitoring after alloHSCT for CLL is capable of identifying those patients with a very low risk of disease recurrence. Moreover, the fact that conversions or increases of MRD are rarely observed in those patients who became negative upon immune modulation strongly suggests that these individuals have a high probability of permanent CLL control. Therefore, MRD negativity might represent a surrogate marker for cure in this subset.

Research Perspectives

MRD-guided preemptive immune modulation and treatment

As successfully applied in CML [31,81], posttransplant quantitative MRD monitoring might be used for preemptive CLL-specific immune intervention or targeted therapy. Although the study by Ritgen et al. [76] suggests that minimal residual CLL persisting after alloHSCT can be successfully eliminated by induction of GVL following cyclosporine tapering or DLI, conclusive results from a prospective study on posttransplant MRD-guided preemptive treatment are still lacking. Although the results of DLI observed in the Ritgen study and those of DLI given for mixed chimerism in the absence of clinical disease after T-depleted alloHSCT [82] are promising, it remains to be shown if preemptive strategies can indeed improve the overall poor results of therapeutic DLI in CLL [82-86].

Mechanisms of GVL onset and resistance

The studies that have employed quantitative MRD monitoring have demonstrated either correlations between posttransplant MRD kinetics and activity of cGVHD [60,76,80], or at least a delayed clearance of MRD suggestive of GVL [79]. Thus, in the absence of other variables influencing the tumor load, MRD kinetics could serve as a "real-time" marker of GVL efficacy and resistance. Accordingly, a promising research perspective is to perform longitudinal studies of the donor-derived effector cell compartment to identify and characterize those cell populations that emerge upon onset of MRD decrease and/or cGVHD. This could lead to delineation of the mechanisms responsible for GVL effects in CLL, thereby opening the avenue for more specific and less toxic cellular therapies. In turn, analyses of CLL cells and the composition of the donor effector cells during increasing MRD levels despite ongoing GVHD activity might lead to better understanding of GVL resistance and ways to overcome it [76]. MRD flow appears particularly suited to assess those markers expressed on MRD cells that might be associated with GVL resistance.

MRD-Guided Preemptive Intervention

Because more than one-third of patients undergoing RIC alloHSCT for poor-risk CLL will experience relapse, strategies for improvement of long-term leukemia control are a very important research area. As noted earlier, a promising but poorly investigated approach to this end is MRD-guided preemptive CLL-specific therapy posttransplant. Prospective studies should be devloped to address the benefit of predefined MRD-triggered immune modulation. These trials should not only focus on nonspecific maneuvers such as immunosuppression tapering and DLI, but also on other forms of targeted posttransplant interventions. For example, rituximab given concomitantly with RIC or DLI might facilitate control of the CLL clone [73]. This could result not only from direct cytotoxicity of rituximab to CLL cells but also to modulation of the GVL activity. Because rituximab is rather poorly effective in CLL, this approach should be extended to other antibodies or CLL-specific molecules, such as ofatumumab [87]. An even more powerful way of redirecting donor T cells to residual CLL cells could be posttransplant administration of bispecific antibody constructs targeting both B and T cell antigens, such as blinatumomab [88].

NHL and Hodgkin Lymphoma (HL)

For NHL and HL, physical examination, imaging, and BM morphology are utilized in the assessment of response and relapse in accordance with the revised response criteria by International Harmonization Project on Lymphoma (IHPL), which are also applied in the relapse setting (Table 6) [89].

Detection of Lymphoma Relapse

Physical examination

Frequent physical examinations are performed in the posttransplant setting with evaluation of lymphadenopathy or the presence of masses, or asking about unusual symptoms. Any finding suggestive of lymphoma recurrence would lead to further testing and imaging.

BM biopsies

BM biopsies are usually performed in patients with lymphoma at the time of initial evaluation and at relapse. However, if the patient's BM has always been uninvolved, it is unlikely to be positive at the restaging points posttransplant. When performing BMs it is important to get an adequate specimen, either a 2″ core biopsy or 2 1″ core biopsies as recommended by the IHPL response criteria [89].

Timing of the Evaluations

Most transplant centers repeat the previously positive tests around day +100, 6 months, and 1 year posttransplant. Many centers continue to repeat these tests on at least a yearly basis typically for 5 years posttransplant. The clinical benefit of frequent repeated tests is unknown.

Imaging Studies

Computed tomography (CT), magnetic resonance imaging (MRI), and PET are standard methods to evaluate disease extent and response in lymphoma and have

Table 6. Response Criteria for Lymphoma Patients according to Cheson et al. [89]

Response	Definition	Nodal Masses	Spleen, Liver	Bone Marrow
CR	Disappearance of all evidence of disease.	(a) FDG-avid or PET positive prior to therapy; mass of any size permitted if PET negative. (b) Variably FDG-avid or PET negative; regression to normal size on CT.	Not palpable, nodules disappeared.	Infiltrate cleared on repeat biopsy. If indeterminate by morphology, immunohistochemistry should be negative.
PR	Regression of measuable disease and no new sites.	≥50% decrease in SPD of up to 6 largest dominant masses; no increase in size of other nodes. (a) FDG-avid or PET positive prior to therapy; 1 or more PET positive at previously involved site. (b) Variably FDG-avid or PET negative; regression on CT.	≥50% decrease in SPD of nodules (for single nodule in greatest transverse diameter); no increase in size of liver and spleen.	Irrelevant if positive prior to therapy. Cell type should be specified.
SD	Failure to attain CR/PR or PD.	(a) FDG-avid or PET positive prior to therapy; PET positive at prior sites of disease and no new sites on CT or PET. (b) Variably FDG-avid or PET negative; no change in size of previous lesions on CT.		
Relapsed disease or PD	Any new lesion or increase by ≥50% of previously involved sites from nadir.	Appearance of a new lesion(s) >1.5 cm in any axis, ≥50% increase in SPD of more than 1 node, or ≥50% increase in longest diameter of a previously identified node >1 cm in short axis. Lesions PET positive if FDG-avid lymphoma or PET positive prior to therapy.	> 50% increase from nadir in the SPD of any previous lesions.	New or recurrent involvement.

CR indicates complete remission; FDG, fluorodeoxyglucose; PET, positron emission tomography; CT, computed tomography; PR, partial remission; SPD, sum of the product of the diameters; SD, stable disease; PD, progressive disease.

also been used to monitor MRD following alloHSCT. However, CT, MRI, and PET scans are all known to potentially have false positive and false negative results in some patients. Examples of false positive CT and MRI scans include residual lymphadenopathy or masses associated with fibrotic tissue. Such would normally be negative on a PET scan; therefore, these modalities are complementary. PET scans may also have false positives, for example, secondary to infection, inflammation, thymic recovery, or scans done too soon after therapy. False negatives can occur, although this is less likely with PET scans in comparison to CT and MRI.

Confirmation of Results

Confirmation of results with a pathologic biopsy is necessary to diagnose recurrence of lymphoma based upon a suspicious scan.

Chimerism

The relationship between disease response and donor chimerism by day 90 after transplantation was evaluated in FL [90]. Seventeen of 33 patients had mixed chimerism by day +90, yet all experienced CR, and there was no additional risk of relapse compared with those patients who had full donor chimerism at this time point. This observation suggests that achievement of early full donor chimerism is not a requirement for disease control in indolent lymphoma after T cell-replete transplantation. Also, the use of DLI for treatment of mixed chimerism should be avoided, unless a rapid decrease in donor chimerism of more than 20% is observed. Whether this same strategy can be applied to other histological types remains to be determined.

Clinical Importance of MRD in Lymphoma

The vast majority of B cell malignancies are characterized by clonal IgH rearrangements, which could serve as potential targets for MRD detection using methods similar to those used in acute lymphoblastic leukemia (ALL) and CLL. In addition, specific chromosomal translocations detectable by PCR amplification, particularly t(11;14) and t(14;18) translocations, are present in specific NHL entities [91]. The t(14;18) translocation is a major pathogenetic mechanism of FL causing deregulation of the bcl-2 protooncogene, which induces prolonged cell survival and inhibition of apoptosis. The t (11;14) translocation fuses the bcl-1 locus with the IgH locus on chromosome 14 and is the characteristic translocation for MCL. Remarkably, at (14;18) translocation is also detectable by PCR at low levels in 10%−25% of healthy individuals [92]. This fact underlines the necessity of serial qPCR approaches in the setting of clinical MRD studies.

The recent development of assays for quantitative molecular MRD assessment has allowed comparison of the relative impact of different treatment modalities on tumor load and has provided insights into the kinetics of tumor regrowth in FL and MCL. Indeed, this impact has been observed within the setting of conventional chemotherapy, monoclonal antibodies, and autoHSCT.

The significance of MRD in patients with relapsed FL or MCL has not been fully explored, as the incidence of relapse after non-T cell-depleted transplant has been relatively low (<15%), at least in patients who received their transplant during chemosensitive disease. Survival in patients with diffuse large B cell lymphoma who relapse after a nonmyeloablative or RIC alloHSCT is dismal (Khouri, unpublished data). For this reason, PCR monitoring of MRD might in theory be helpful to evaluate molecular relapse, which could allow interventions such as programmed immunomodulation before patients experience clinical or radiographic evidence of disease recurrence, although to date there are no data to support this. For HL, neither cytogenetic analysis, nor flow cytometry, nor molecular testing are helpful for assessing residual disease.

Overall, the clinical impact of MRD detection in different lymphomas remains to be determined. Although MRD has proven to be an independent prognostic factor in other hematologic malignancies, the clinical relevance of MRD assessment in lymphoma is still unclear. Further studies are required to obtain additional MRD information for these patients in the setting of alloHSCT.

Multiple Myeloma (MM)

Disease-specific laboratory parameters and imaging studies are employed in standard response criteria definitions for MM (Table 7) [93]. Beside these conventional criteria, cytogenetic analysis (including FISH), lineage-specific chimerism, flow cytometry, and molecular methods are more sensitive markers to monitor residual disease and relapse after hematopoietic alloHSCT. Furthermore, imaging methods play an important role in the detection of extramedulllary disease.

Achievement of CR is a major goal of all therapeutic interventions in treatment of MM. Several studies suggest that those patients who achieve CR, especially after high-dose chemotherapy, have longer survival [94,95]. Compared with other treatment modalities in MM, alloHSCT induces the highest rate of clinical CR. The CR rate of alloHSCT after standard MA and after RIC ranged between 27% and 81% [96-102]. These differences result from different definitions of CR. The most commonly used are the definition proposed by the European Group for Blood and Marrow Transplantation (EBMT) and of the International Working Group (IWG) on MM, which introduced a so called stringent definition of CR (sCR) that has not yet been validated (Table 7) [93,103].

Table 7. Complete Response and Relapse Criteria for Multiple Myeloma According to the European Group for Blood and Marrow Transplantation (EBMT) and the International Working Group (IWG) Bladé, Durie et al. [93, 103]

Complete Remission (CR) requires all of the following:
- Absence of the original monoclonal paraprotein in serum and urine by immunofixation, maintained for a minimum of 6 weeks. The presence of oligoclonal bands consistent with oligoclonal immune reconstitution does not exclude CR.
- <5% plasma cells in a bone marrow aspirate and also on trephine bone biopsy, if biopsy is performed. If absence of monoclonal protein is sustained for 6 weeks, it is not necessary to repeat the bone marrow, except in patients with nonsecretory myeloma where the marrow examination must be repeated after an interval of at least 6 weeks to confirm CR.
- No increase in size or number of lytic bone lesions (development of a compression fracture does not exclude response).
- Disappearance of soft tissue plasmacytomas.

IWG Criteria

Complete Remission (CR)
- Negative immunofixation on the serum and urine
- Disappearance of any soft tissue plasmacytomas.
- ≤5% plasma cells in bone marrow

Stringent Complete Remission (sCR)
- CR as defined above plus
- Normal free light chain ratio and
- Absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence

Relapse

EBMT Criteria requires at least 1 of the following:
- Reappearance of serum or urinary paraprotein on immunofixation or routine electrophoresis, confirmed by at least one further investigation and excluding oligoclonal immune reconstitution.
- ≥5% plasma cells in a bone marrow aspirate or on trephine bone biopsy.
- Development of new lytic bone lesions or soft tissue plasmacytomas or definite increase in the size of residual bone lesions (development of a compression fracture does not exclude continued response and may not indicate progression).
- Development of hypercalcemia (corrected serum calcium >11.5 mg/dL or 2.8 mmol/L) not attributable to any other cause.

IWG Criteria

Relapse from CR requires at least 1 of the following:
- Reappearance of serum or urinary M-protein by immunofixation or electrophoresis
- ≥5% plasma cells in a bone marrow.
- Appearance of any other sign of progression (i.e, new lytic bone lesions or soft tissue plasmacytomas or hypercalcemia).

EBMT indicates European Blood and Marrow Transplant; IWG, International Working Group.

The incidence of relapse in patients with MM after alloHSCT is higher than in other hematologic diseases. One reason might be that about 50% of the patients will not achieve CR (defined as negative immunofixation) after alloHSCT. Therefore, in those patients, "relapse" should be better classified as "progressive disease" rather than relapse. But even a substantial percentage of the patients who achieve CR according to the EBMT-criteria (Table 7) will relapse, demonstrating the low sensitivity of immunofixation to detect residual disease. The following methods can be used to detect either relapse/progressive disease or persistence of residual disease after alloHSCT but these, in general, are not included in the aforementioned common criteria of relapse or CR: (1) imaging methods, (2) chimerism: nonlineage-specific or lineage-specific (plasma cells), (3) cytogenetic analysis/

FISH, (4) PCR with patient-specific primers (IgH rearrangements), (5) flow cytometry, (6) BM histology with immunohistochemistry, and (7) other methods, such as free light-chain assay.

Imaging Methods

MM is characterized by the presence of lytic bone lesions and >80% of the patients develop osteolytic bone lesions [104]. Beside osteolytic bone lesions almost 10% of MM patients present with diffuse osteopenia at diagnosis. The hallmark of myeloma bone disease is an increased osteoclastic bone resorption and an exhausted osteoblast function resulting in reduced bone formation even in patients in CR [105,106]. Therefore, bone scan offers less information in follow-up of bone disease in myeloma patients. Imaging methods to monitor patients with MM should: (1) detect skeletal complications, (2) determine intramedullary bone disease, and (3) detect extramedullary disease. Currently, standardized recommendations for imaging in MM have not been established for newly diagnosed patients or for follow-up to determine disease progression [107].

Conventional X-ray

Conventional radiologic skeletal survey that includes the cervical, thoracic, and lumbar spine, skull, chest, pelvis, humeri, and femora is still the standard for newly diagnosed MM patients and is repeated in progressing or relapsing patients as part of the restaging process. Conventional X-ray may also reveal diffuse osteoporosis. There are a number of major disadvantages of conventional radiology: some areas of the spine are not well visualized; the sensitivity for detection of osteolytic lesions is rather low; it fails to distinguish myeloma-related osteoporosis from osteoporosis because of other causes; and it cannot be used for assessment of response to therapy as lytic bone lesions do not show "healing" and new fractures do not always indicate disease progression [106,108].

CT

CT scanning is superior to conventional radiology with respect to sensitivity and allows detection of small osteolytic lesions that are not detected by conventional X-ray. This holds true especially for areas such as the scapula, ribs, and sternum, which cannot be visualized accurately by conventional radiology [109]. Furthermore, CT scan has been proven to be superior in estimating fracture risk [110]. CT scan can further depict the extent of soft tissue masses, which are not detectable by conventional radiology. A new CT technology, the multidetector row computed tomography (MDCT), has been found to be very sensitive in detecting osteolytic lesions less than 5 mm in the spine compared with MRI and PET [111]. In comparison to conventional radiology, CT scanning is much faster, but the amount of radiation dose delivered to the patient is up to three times higher [112].

MRI

MRI allows visualization of the medullary cavity, and therefore, the degree of myeloma cell infiltration can be assessed [113]. The sensitivity of MRI is higher than conventional radiology in detecting osteolytic bone disease. For suspected cord compression MRI is the technique of choice [114]. MRI can also distinguish between malignant compression fractures and other causes such as osteoporotic fracture.

Different MRI techniques have been developed to assess BM involvement [115]. The most informative sequences are the T1-weighted, the T2-weighted with fat suppression, the short time inversion recovery (STIR), and the gadolinium T1-weighted with fat suppression. Myeloma lesions usually have low signal intensity on T1-weighted images and high signal intensity on T2-weighted and STIR images and enhancement on gadolinium images [116]. BM involvement can be recognized by 5 MRI patterns [117]: (1) focal involvement, (2) diffuse infiltration, (3) combined diffuse and focal infiltration, (4) "salt and pepper" pattern, and (5) normal appearance despite plasma cell infiltration. Low myeloma cell infiltration is usually associated with normal MRI pattern, whereas high myeloma cell burden is suspected if there is a diffuse hypointense change on T1-weighted images, diffuse hyperintensity on T2-weighted images, and enhancement with gadolinium injection.

MRI is more sensitive than conventional radiology in detecting osteolytic lesions in the pelvis (75% versus 46%) and spine (76% versus 42%) [118]. In another large study, a focal lesion could be detected by MRI in 52% of the patients with normal skeletal survey [119]. This advantage was mainly observed for the spine, pelvis, and sternum, whereas a higher number of lesions were detected by conventional radiology for ribs and long bones (humeri and femora) [119]. Another advantage of MRI is the detection of solitary bone plasmacytoma (SBP). MRI was able to detect abnormal lesions in 4 of 12 patients with SBP that had not been detected by conventional radiology [120].

MRI can also be used to assess response to therapy. CR was associated with complete resolution of the preceding BM abnormalities and partial response was demonstrated by conversion of a diffuse to a focal pattern [120]. MRI might also help to assess remission in non-secretory myeloma. Focal lesions detected by MRI were seen in 27 of 30 patients with nonsecretory myeloma. After treatment, CR by BM examination occurred in 81%, but MRI-based CR was only seen in 41% of patients [119]. Furthermore, the number of focal lesions detected by MRI serves as an independent prognostic factor. After intensive chemotherapy,

resolution of MRI lesions was seen in 60% of patients, which was associated with an improved survival. Relapse after CR was associated with focal lesions by MRI in 70%, including 26% new focal lesions [119].

Nuclear medicine imaging

Technetium bone scintigraphy is able to detect osteolytic bone lesions in up to 60% of myeloma patients, but its specificity and sensitivity to detect or follow bone lesions is lower compared to conventional radiography [121,122], mainly because of the osteoblast dysfunction in myeloma. To improve the sensitivity ^{99m} Technetium-labeled hexakis-2-methexyiso-butylisonitrile ($^{99\ m}$Tc-sestamibi or MIBI) was introduced, which favors accumulation in tissues with high cell density and mitochondrial activation. MIBI has been shown to be highly sensitive (92%) and specific (96%) in MM [123], with localization inside the myeloma cell infiltrating BM [124]. MIBI can detect soft tissue and skeleton lesions with a higher sensitivity than conventional radiology [125]. In comparison to FDG-PET scan, the sensitivity of MIBI is lower and in comparison to MRI the extent of myeloma infiltration in BM is underestimated [126]. PET using FDG cannot detect small osteolytic lesions seen by conventional radiology [127]. To overcome these limitations PET and CT can be combined (PET-CT). Several studies have shown that PET-CT is a reliable method to detect osteolytic lesions in MM of at least 1 cm [128] and can be used to monitor nonsecretary myeloma patients as well as patients in CR without measurable M-component [129]. PET-CT has been included as an option in the diagnosis and monitoring of myeloma patients within the NCCN guidelines (http://www.nccn.org/professional/physiciangls/PDF/myeloma.pdf). Regarding extramedullary disease, PET-CT is more sensitive than other imaging modalities, showing in up to 30% additional lesions in patients who have been diagnosed with solitary plasmacytoma by MRI [130,131]. Small studies have demonstrated superiority of PET-CT in comparison to conventional radiography [132]. 18F-FDG PET-CT is comparable to MRI in the detection of focal lesions in the spine and pelvis, but it is superior for an accurate whole-body evaluation [133], and MRI is superior to PET-CT in detecting BM involvement [134]. In summary, new imaging methods allow the detection of small osteolytic lesions and extramedullary disease. For monitoring myeloma patients, MRI or CT can be used for response evaluation of soft tissue masses to therapy and to monitor patients for relapse during post-treatment follow-up. The roles of PET-CT and/or MIBI need to be investigated.

Chimerism

Accurate quantitative analysis of donor-recipient cell chimerism has been reported to permit detection of residual disease as well as early relapse after alloHSCT [28,135]. However, this methodology is only useful in that regard if the underlying disease originates at an early hematopoietic stem cell level such as acute leukemia or CML. In MM, which originates from a late stage of B cell development, no correlation between donor chimerism and relapse could be found [136]. This problem of monitoring relapse by donor chimerism in patients with MM after an allograft may be overcome by using lineage-specific chimerism. In a small study, chimerism of plasma cells was monitored after CD138$^+$ cell enrichment [137]. In this trial, sequential monitoring of donor plasma cell chimerism showed that increasing or stable chimerism was associated with ongoing remission in 93% of the patients, whereas decreasing donor plasma cell chimerism predicted clinical relapse in 5 of 6 patients. By using qPCR the sensitivity of the method is 10^{-4} to 10^{-5}. The disadvantage of this method is the lack of specificity. In patients with acute leukemia after alloHSCT it could be shown that full conversion to complete donor plasma cell chimerism is delayed in comparison to other hematopoietic cells. Donor plasma cell chimerism was only 98.6% at 6 months and 99.8% at 1 year after transplant, whereas 100% donor T cell chimerism was almost always achieved at day 100 after transplantation [137].

Cytogenetic Analysis and FISH

Conventional cytogenetic analysis in myeloma is difficult to obtain and to date this method has been reported only after autoHSCT where suppression of abnormal karyotype is associated with improved survival [138]. Because of the low proliferation of malignant plasma cells, only about 30% of patients with MM have detectable chromosomal abnormalities [139]. To resolve the problem of conventional cytogenetic analysis, interphase FISH has been introduced, which enables assessment regardless of the proliferation potential. The most frequent abnormalities are del(13q14), t(4;14), del 17p, and t(14;16), which have been shown to be prognostically relevant [139-143]. The sensitivity of FISH is about 1%, but this method has not been used so far to detect residual disease or relapse. A major disadvantage is that the known specific abnormality is not detectable in all myeloma cells within individual cases.

PCR Using Patient-Specific IgH Primers

The most sensitive method is based on clonal markers derived from the rearrangement of IgH genes, which have to be generated from each patient at diagnosis or relapse. Depending on the number of malignant plasma cells and the pretreatment, these primers can be generated in 60% to 80% of the patients [144-146]. Using these patient-specific primers, residual myeloma cells can be detected by PCR with a sensitivity

of 10^{-4} to 10^{-6} [144,145]. Molecular remissions are seen more often after allogeneic than after autoHSCT. In patients who achieved clinical CR, 9 of 14 allograft-patients, but only 2 of 15 autograft-patients entered molecular remission. It is of interest that molecular remission after allografting occurred in some patients more than 3 years after transplantation [145].

The importance of achieving molecular remission for long-term disease freedom has been shown for MA alloHSCT in a retrospective EBMT study [146]. Using highly sensitive patient-specific primers to monitor residual disease, it could be shown that durable PCR-negativity after allografting had a cumulative risk of relapse at 5 years of 0%, in comparison to 33% for PCR-mixed patients and 100% for patients who never achieved PCR negativity [146]. More recently, Kröger et al. investigated posttransplant immunotherapy with escalating DLI and novel agents (thalidomide, bortezomib, and lenalidomide) to target CR in 32 patients with MM who achieved only PR after alloHSCT. CR defined either by EBMT criteria, or flow-cytometry, or either patient-specific IgH or plasma cell chimerism as defined by qPCR was accomplished in 59%, 63%, and 50% of patients, respectively. Achievement of CR resulted in an improved 5-year PFS and OS according to EBMT criteria (53% versus 35%; $P = .03$ and 90% versus 62%; $P = .06$), flow-cytometry (74% versus 15%; $P = .001$ and 100% versus 52%; $P = .1$), or molecular methods (84% versus 38%; $P = .001$ and 100% versus 71%; $P = .03$) [147]. These findings demonstrate the clinical relevance of the depths of remission after allografting for long-term survival in myeloma patients and that these methods should be implemented in clinical trials of alloHSCT in MM.

Flow Cytometry

Flow cytometry has become an easily applied method to detect residual myeloma cells. The European Myeloma Network recommends a minimal panel including CD19 and CD56. A preferred panel would also include CD20, CD117, CD28, and CD27. Plasma cell gating should be based on CD38 versus CD138 expression [148]. This method can achieve sensitivities of 10^{-4}, but it is less sensitive than patient-specific IgH primers [149,150].

Recent studies have shown that achieving remission by flow cytometry after autologous or alloHSCT resulted in improved survival in comparison to patients who achieved only negative immunofixation [147,151, 152] illustrating the need to include this method for further definition of remission and relapse.

Free Light Chains and Other Assays

The qualitative assay for free light chains has been reported to be sensitive and specific for detecting and monitoring diseases caused by monoclonal gammopathies such as MM [153]. The IWG definition of stringent CR (Table 7) requires normalization of the free light chain ratio in serum [103]. More recently, the IWG also published guidelines for serum free light-chain analysis in MM and related disorders [154]. To determine stringent CR it was recommended to perform a serum-free light-chain assay in all patients who achieved a CR with negative immunofixation. In 52 patients who achieved CR according to the EBMT criteria with negative immunofixation for at least 3 months after alloHSCT, the free light-chain kappa/lambda ratio was also normal in 51 patients, which does not support an additional value of free light-chain ratio to determine the depth of remission in immunofixation negative patients [155]. However, this assay may detect CR and relapse earlier than immunofixation in serial measurements because of the short half-life of free light chain compared to intact immunoglobulin. As evidence of this, 26 patients with negative imunofixation after alloHSCT were monitored sequentially by both the serum-free light-chain assay and immunofixation. The authors observed that normalization of the free light chain ratio preceded the occurrence of immunofixation negativity by about 3 months. Furthermore, in 10 patients who relapsed during follow-up from CR the free light-chain ratio became abnormal at a median of 90 days before immunofixation became positive [156]. These preliminary data suggest that the free light-chain ratio does not help to determine the depths of remission after alloHSCT, but is a useful marker for earlier detection of remission or progression in myeloma patients. The proposed definition of stringent CR also requires a normal kappa/lambda ratio in bone marrow by immunohistochemistry, but so far no data on immunohistochemistry as a method to detect MRD are available.

The detection of tumor-specific antigens such as cancer testis antigens on myeloma cells have raised the question whether monitoring of cancer testis antigens by PCR is helpful to detect relapse. Few data are available so far, but the applicability of this approach will likely be limited by the fact that cancer testis antigens are not expressed in all myeloma within individual cases [157].

SUMMARY, CONCLUSIONS, AND FUTURE RESEARCH

Because the intention of alloHSCT is to cure the underlying hematologic malignancy, and because there is increasing evidence that minimal disease after alloHSCT may be eradicated by immunotherapeutic approaches such as DLI, monitoring of disease is of great importance. The current definitions of remission and relapse utilized to evaluate most hematologic malignancies during upfront therapy lack sufficient sensitivity for use after alloHSCT. Flow cytometry,

Table 8. Response and Relapse Definitions after AlloHSCT—Application of Monitoring Methodologies

Disease	Definition of Complete Remission	Definition of Relapse	Molecular Markers	Cytogenetics	Chimerism	Imaging	Flow Cytometry	Other Methods
Multiple myeloma	1) EBMT 2) IWG	1) EBMT 2) IWG	ASO-primer (IgH)	Chromosome banding analysis, FISH	PCR or VNTR/STR	MRI PET-CT	4-8 color flow	Free light chain assay
Applicable	All patients	All patients	40%-80%	subgroups	All patients	All patients	All patients	Subgroups
Comment	Accepted but less sensitive.	Accepted but less sensitive.	Important, but not included in EBMT and IWG definition.	May be useful.*	Mononuclear cell donor chimerism not useful. Lineage-specific donor chimerism ($CD138^+$ plasma cells) predicts relapse.*	Not established, but useful for extramedullary disease.*	More sensitive than EBMT/IWG in predicting relapse.*	Proposed by IWG, but no valid data.*
Lymphoma	Cheson criteria	Cheson criteria	ASO-primer (IgH) for B-cell NHL	Chromosome banding analysis, FISH	PCR or VNTR/STR	CT/PET	4-6 color flow	
Applicable	All patients	All Patients	Subgroups	Subgroups	All Patients	All Patients	Subgroups	
Comment	Well established for all lymphomas.	Well established for all lymphomas.	Bcl-2 for FL. Bcl-1for about 30% of MCL. Clonal TCR rearrangements for T-NHL.	t(14;18) for FL. t(11,14) for MCL.	Monitoring T cell by PCR useful in NHL. Role not established in HD.	Well established in all lymphomas.	Could be helpful for FL and MCL.*	
CML	Hematologic Cytogenetic Molecular	Hematologic Cytogenetic Molecular	*BCR-ABL1* RT-PCR	Chromosome banding analysis, FISH	PCR or VNTR/STR		4-6 color flow	
Applicable	All patients	All patients	All patients	All patients	All patients	Not applicable	Subgroups	
Comment			qPCR identifies relapse risk groups.	Not as sensitive as qPCR for MRD detection.			Only helpful in identifying aberrant blasts in advanced phase disease.	
Myelofibrosis	IWG-MRT	IWG-MRT	*JAK2/MPL*	Chromosome banding analysis, FISH	PCR or VNTR/STR	MRI	Flow cytometry	
Applicable	All patients	All patients	Subgroups	Subgroups	All patients	All patients	All patients	
Comment	Not fully applicable.	Not fully applicable.	High sensitivity and predictive for relapse.*	Not investigated.*	Correlates with molecular marker, but less specific.*	Correlates with fibrosis regression.*	Circulating CD34+ cells may be useful.*	
CLL	IW-CLL/NCI	IW-CLL/NCI	ASO-primer *IGH* qPCR	Chromosome banding analysis, FISH	PCR or VNTR/STR	CT	MRD flow	
Applicable	All patients	All patients	~90%	Subgroups	All patients	All patients	>95%	
Comment	iwCLL definition of MRD negativity: MRD $<10^{-4}$ by qPCR or flow.		Predictive for sustained remission if $<10^{-4}$ 1 year post-SCT. More sensitive than flow ($<10^{-4}$).	No role in relapse monitoring.	Complete donor chimerism usually prerequisite for MRD negativity, but not suitable as MRD marker.	Only to be used if CR by clinical methods or in clinical trials.	Predictive for sustained remission if $< 10^{-4}$ 1 year post-alloHSCT. Equally sensitive and specific as qPCR up to10^{-4}.	

FL indicates follicular lymphoma; flow, multiparameter flow cytometry; MCL, mantle cell lymphoma; MRD, minimal residual disease; NHL, non-Hodgkin lymphoma; qPCR, quantitative real-time PCR; RT-PCR, reverse-transcription PCR; TCR, T cell receptor; VNTR, variable number tandem repeats; PET, positron emission tomography; CLL, chronic lymphocytic leukemia; CT, computed tomography; MRI, magnetic resonance imaging; FISH, fluorescence in situ hybridization; EBMT, European Blood and Marrow Transplant; IWG, International Working Group; IWG-MRT, International Working Group for Myelofibrosis Research and Treatment.
*Further studies needed

molecular methods, and new imaging modalities have been investigated in recent years and can substiantially increase the sensitivity of disease detection to 10^{-4} to 10^{-6}. The highest sensitivity and specificity can be achieved by molecular monitoring of tumor- or patient-specific markers measured by PCR, but not all diseases have such targets for monitoring. Flow cytometry, although generally not as sensitive as PCR, is a valuable and even preferable method in some diseases, but is not suitable in others. Very high sensitivity can also be achieved by determination of donor chimerism, but its specificity regarding detection of relapse is low and differs substantially among diseases. A higher specificity might be obtained by lineage-specific donor chimerism, but there are only a few such studies with limited number of patients. Table 8 summarizes the different methods of MRD detection in the monitoring of CML, MPN, CLL, lymphoma, and MM, and the relative pros and cons of the use of these methods after alloHSCT.

Critically important is the need for standardization of the different residual disease techniques. Further clinical trials to assess the utility of these techniques in each disease entity are also mandatory. The predictive value of posttransplant MRD and chimerism remains to be determined across all hematologic malignancies, and we also do not understand how to exploit fully the kinetics of MRD and chimerism in the prediction of clinical relapse. Subsequent studies should evaluate the efficacy of MRD- and chimerism-guided therapeutic interventions designed to prevent overt relapse. Thus, critical objectives for future studies in this area should include the following:

1. Standardization of measurement of molecular markers for each hematologic malignancy for which alloHSCT is employed.
2. Define the kinetics of molecular remission and molecular relapse and the optimal frequency of MRD and chimerism monitoring after alloHSCT.
3. Define the utility of molecular markers in regard to the natural history of posttransplant relapse, and incorporate MRD markers in the definition of response and remission after alloHSCT.
4. Assess the efficacy of interventional strategies based on changes in MRD and/or chimerism to prevent clinical relapse.

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