Relapse has become the major cause of treatment failure after allogeneic 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, 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 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 based on tumor-specific markers and donor 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 surveillance in individual diseases, which in turn, must be followed by studies to assess the potential impact of specific interventional strategies.
INTRODUCTION

Methodologic and technologic advances allow sensitive detection of minimal residual disease (MRD) and early recognition of recurrence of hematologic malignancies after allogeneic hematopoietic stem cell transplantation (alloHSCT). Importantly, intervention prior to florid relapse improves outcome for certain hematologic malignancies [1,2]. This manuscript by the Workshop Committee on Disease-Specific Methods and Strategies for Monitoring Relapse following Allogeneic Stem Cell Transplantation is divided 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 [3]. These are based on morphologic investigations of peripheral blood (PB) and/or bone marrow (BM), imaging, and/or specific laboratory findings. Given their critical importance to the goals of this Workshop, these criteria are summarized here. However, after alloHSCT, more sensitive methods such as molecular genetics, tumor-specific molecular primers, fluorescein in situ hybridization (FISH), multiparameter flow cytometry (MFC), and/or chimerism are commonly used to monitor patients with respect to relapse. Although some of these have clearly been shown to be predictive of outcome in specific diseases (eg, chronic myelogenous leukemia [CML]), and have become part of the standard criteria, assays for monitoring of disease status after alloHSCT have not yet been incorporated into the relapse definitions across all hematologic malignancies. Recommendations for the utilization of sensitive monitoring techniques to augment the standard definitions of posttransplant remission, persistence, progression, relapse, and the prediction of relapse are proposed, whenever possible, based on current evidence. 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. From the point of view of this Workshop, 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 (Committee on Disease-Specific Treatment of Relapse). Finally, major deficits and important questions for further clinical research will be addressed. In this first part, we focus on methods to detect and monitor disease response, persistence, progression, and relapse in acute leukemias and myelodysplastic syndrome (MDS), whereas in the forthcoming second part disease-specific monitoring for chronic leukemias, myeloproliferative neoplasms, and lymphoid malignancies will be reviewed.

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 hematopoietic chimerism. The latter characterizes the origin of posttransplant hematopoiesis, whereas MRD detection measures the malignant clone directly. For each approach, a variety of techniques is available, although in general there have been more studies looking directly at markers of residual malignancy than of chimerism. Issues of applicability, standardization, sensitivity, and specificity are discussed separately for each technique.

Chromosome Banding Analysis

Classical chromosome banding analysis still plays an essential role in the biologic characterization and for prognostic predictions in many hematologic malignancies. Whenever possible, cultivation of metaphases is performed from BM instead of peripheral blood because of the higher proliferation rates and higher proportions of malignant cells in the setting of leukemia. In vitro proliferation of cells is supported by specific cytokines. Application of colchicine leads to cell cycle arrest shortly before metaphase preparation. Staining of chromosomes is performed with Giemsa (G-), Quinacrin (Q-), or reverse (R-) banding techniques. Analysis of metaphases is improved by digital picture capture systems. The International System of Cytogenetic Nomenclature (ISCN) is used for the presentation of karyotypes. Analysis of 20 to 25 metaphases is required for valid results. With chromosome banding analysis, the whole karyotype can be illustrated within a single investigation. However,
chromosomal banding techniques are hampered by insufficient quality of BM samples (eg, myelofibrosis, recent history of total body irradiation [TBI]) as vital cells are required for metaphase culture. Moreover, chromosome banding analysis is limited to the detection of microscopically visible abnormalities. Sensitivity is further limited, as a maximum of 25 metaphases is usually evaluated within a single analysis. Further, full clarification of the chromosomal abnormalities often requires the combination with molecular cytogenetic methods using the FISH technique.

**FISH**

Diverse FISH techniques are available for the detection of submicroscopic alterations or for clarification of complex chromosomal changes including interphase FISH (IP-FISH), whole chromosome painting (WCP-FISH), 24-color-FISH (M-FISH or SKY), and comparative genomic hybridization (CGH). These techniques are all based on the hybridization of flourescence-tagged probes to a specific chromosomal region. WCP-FISH and 24-color FISH can only be performed on metaphases. In contrast, FISH with loci specific probes or with probes for the centromeric regions can be performed on metaphases or interphase nuclei and thus do not require dividing cells. As interphase-FISH provides a higher sensitivity (100-200 cells can easily be evaluated) [4], the technique can be used for MRD [5], albeit not at a sensitivity approached with other techniques. Moreover, analysis is limited to the probes that have been chosen for the individual case [6].

**MFC**

The principle of detection of MRD by MFC rests on the property that leukemic cells have phenotypic properties that, although broadly similar to those of normal cells, often show subtle differences in antigen expression. Considerable effort has been put into characterizing the patterns of antigen expression in normal and regenerating hematopoietic differentiation. Comparison of specimens of leukemia to these normal templates has shown that most cases have “leukemia-associated immunophenotypes” (LAIPs). Typically, antigens normally expressed at a particular stage of maturation are over- or underexpressed in leukemic cells. Thus, with design of appropriate combinations of antigens in MFC analysis, leukemic cells can be recognized because they occupy areas of “empty space” on correlated plots of antigen expression [7-10]. By analyzing large numbers of cells (500,000 or more) with modern high-speed flow cytometers, it is possible to identify clusters of as few as 10 to 20 leukemic cells in a background of normal cells, allowing for a theoretic sensitivity approaching \( 10^{-5} \) cells. In practice, however, sensitivities are often limited to about \( 10^{-3} \) and may not even reach that in some cases.

Sensitivity of detection of residual leukemic cells varies between subtypes and depends upon the magnitude of the differences seen between leukemic cells and normal hematopoietic cells. Moreover, the proportion of patients with informative phenotypes varies among different diseases. Studying larger panels of markers, either in higher order (more colors) flow cytometry or by adding additional tubes, will typically increase the ability to detect MRD. In B-lineage acute lymphoblastic leukemia (ALL), >90% of patients can be monitored with a relatively simple panel of antibodies [11,12], and similarly in B-chronic lymphocytic leukemia (CLL) a simple antibody combination is informative for detecting MRD at high sensitivity in nearly all cases [13]. By contrast, MRD detection in acute mylogenous leukemia (AML) is more complicated because leukemic signatures are often unique to an individual case, and custom panels are often designed to monitor particular patients [14-17]. Complicating detection of MRD by flow cytometry in acute leukemias is the fact that there may be phenotypic shifts following therapy, so looking only for those cells with the leukemia specific phenotype may underestimate, or even fail to identify residual leukemic cells [18-20]. Therefore, application of comprehensive antibody panels has been suggested for follow-up monitoring in AML patients. MRD in ALL can appear to undergo maturation following steroid therapy, and unless one is aware of this, MRD can be missed [21]. Phenotypic changes in AML are even more common, and the complexity of leukemic maturation patterns seen makes it difficult to clearly quantify leukemic populations even when some leukemic cells are identified. CLL and myeloma are clonal B

<table>
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<tr>
<th>Tumor Marker</th>
<th>Chimerism</th>
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<tr>
<td>Chromosome Banding</td>
<td>FISH</td>
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<td>Applicability</td>
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ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; PCR, polymerase chain reaction; qPCR, quantitative real-time PCR; STR, short tandem repeats.
cell diseases that show light chain restriction, but in the MRD setting, identifying changes in the kappa/lambda ratio, or using other markers to identify subsets of cells that express only 1 light chain is generally not as sensitive as methods that employ the same kinds of aberrant antigen markers that are useful in MRD monitoring of acute leukemias [13,22].

Flow cytometric detection of MRD has a number of advantages over other methods. It is often relatively inexpensive compared to highly sensitive molecular methods, and results are obtained rapidly, often within hours or at most a day or 2, allowing for the design of studies that have an early therapeutic intervention based on MRD results. The method is particularly suited not only to detect, but also to quantify disease burden. This is of major importance to evaluate disease kinetics. A major disadvantage to MRD studies by flow in all diseases except CLL and multiple myeloma (MM) is that no international consensus has been reached on standardized panel design and data interpretation. Different panels of antibodies are used by different investigators and in different studies, and highly specialized experience is necessary for accurate interpretation of MRD results. There is even variability in specimen processing, with some laboratories using ficell-sorted separated cells [12] and others using cell lysis techniques [11,23]; some but not all of the latter techniques express MRD results as a percentage of mononuclear cells [11] to better match results obtained with ficell-sorted samples. In contrast to molecular methods, there are not well-established protocols for demonstrating interlaboratory reproducibility. In B-lineage ALL, which is perhaps the best studied area in this regard, reproducibility is relatively high early in therapy when MRD burden is high and when there are the smallest number of contaminating normal B cell precursors. However, obtaining reproducibility at sensitivities of $10^{-5}$ or even $10^{-4}$ is very difficult [24]. Nevertheless, the clinical importance of MRD at such a low level has been well established in a variety of studies performed at 1 or few reference labs [11,23,25,26], suggesting that it is possible to measure clinically relevant MRD with that sensitivity.

Molecular Methods of Residual Disease Detection

The polymerase chain reaction (PCR) technique has proved to be an extremely robust method of high sensitivity and specificity with a wide range of applicability to residual disease detection depending upon the target chosen. PCR can be performed at the genomic level to look for DNA-based alterations, or alternatively reverse transcription PCR (RT-PCR) can be used to detect either structural or quantitative abnormalities in mRNA. Sensitivity and specificity of PCR can be improved by using “nested” PCR, in which a first reaction using primers directed at consensus sequences is followed by a second reaction using internal primers to further amplify DNA that was amplified in the first round. However, this “nested” PCR approach often yields unreliable quantitative results. Finally, PCR can either be performed using a qualitative or endpoint assay, or, now more commonly, quantitative real-time PCR (qPCR) can be used to quantify targets over a range of many orders of magnitude.

Minimal residual disease detection by antigen receptor PCR in lymphoid malignancies

Approaches for monitoring MRD in B- or T-lymphoid malignant diseases comprise techniques based on PCR to quantify tumor burden by disease-specific T cell receptor (TCR) or immunoglobulin (Ig) gene rearrangements [27]. Unique fingerprint-like sequences, assumed to be specific for each lymphoid cell, can be detected in the junctional regions of rearranged Ig and TCR genes. Cells of a lymphoid malignancy have a common clonal origin, with each cell having an identically rearranged Ig or TCR gene. Consequently, these gene rearrangements, located in the junctional regions of Ig and TCR genes, serve as specific targets for each leukemia and can be used for analysis of MRD [28,29]. At diagnosis, patient- and leukemia-specific clonal rearrangements are amplified by PCR. After this, the various disease specific rearrangements can be identified and selected [30]. After selection, PCR products are used for sequencing of the junctional regions. This specific sequence forms the basis for the design of junctional region specific oligonucleotides (allele-specific oligonucleotides [ASO]), required for leukemia, lymphoma, and myeloma-specific sensitive qPCR-based MRD analysis.

There are a number of potential pitfalls in the use of Ig and TCR clonality in MRD monitoring. Ig and TCR gene rearrangements in hematologic malignancies might be susceptible to subclone formation. Furthermore, secondary gene rearrangements could occur between diagnosis and relapse, which might cause false-negative MRD results. To reduce the number of false-negative MRD analyses in ALL, at least 2 Ig/TCR targets are used in clinical MRD studies [28,31]. Kreyenberg et al. [32] demonstrated that Ig and TCR gene rearrangements are stable markers for MRD in ALL after alloHSCT. Importantly, isolated false positive results in Ig-based qPCR systems have been observed at the time of intense B cell regeneration [33].

To assure comparability of MRD results between different laboratories, quality control and standardization are essential. A standardized approach for MRD analysis by qPCR has been implemented by the European Study Group on MRD detection in ALL (ESG-MRD-ALL), consisting of 30 laboratories worldwide. Furthermore, guidelines for the interpretation of qPCR-based MRD data have been developed.
Application of these guidelines allows identical interpretation of MRD data between different laboratories of the same MRD-based clinical protocol. Method and guidelines for its interpretation are precise to avoid both false-negative and false-positive results [31].

**Fusion gene-specific targets and mRNA expression**

Quantitative PCR can be used to monitor specific fusion gene transcripts as a sensitive indicator of MRD. Experience is most extensive in the setting of BCR-ABL1 transcript monitoring for individuals with CML. The methodology used for identifying BCR-ABL1 transcripts has evolved over the years. Initially to BCR-ABL1 transcripts were detected by single-step amplification or a 2-step “nested” amplification. In 1993, Cross et al. [34] developed a competitive semi-quantitative assay that expressed BCR-ABL1 transcripts as a ratio (%) compared with normal ABL (BCR-ABL1/ABL1 × 100). This method was adapted for qPCR when this technology became available [35,36]. Subsequently Hughes et al. [37], analyzing patients in the IRIS study, introduced the concept of logarithmic (log) reduction using as a baseline the laboratory-specific median BCR-ABL1 transcript level taken from 30 patients.

For valid PCR data, it is imperative to optimize each stage of the procedure, including sample collection, RNA extraction, and quantitative PCR. The quality of the RNA is extremely important for reproducible data, and consistency in sample collection, tissue type, transportation, and storage conditions will maximize the accuracy and reliability of analysis. Standard protocols for BCR-ABL1 PCR employ peripheral blood for MRD monitoring instead of BM aspirates.

It is highly desirable that a standardized international scale for measuring transcript levels be established, and it is probably preferable to move away from log reduction. An international collaborative group recently published recommendations whereby the standardized baseline would be determined using a reference material, and the absolute BCR-ABL1 value representing a major molecular response would be standardized at 0.1%. A value of 1.0% would be approximately equivalent to cytogenetic complete remission (CR) [38,39].

MRD monitoring by PCR techniques has also been utilized in the assessment of treatment response and for the early detection of relapse in other leukemias with sensitivities of 10⁻⁴ to 10⁻⁶ [40-48]. Specific reciprocal gene rearrangements (eg, PML/RARα, RUNX1-RUNX1T1 = AML1-ETO, CBFB-MYH11), intragenic duplications (eg, MLL-PTD, FLT3-ITD), and other mutation types (eg, alterations of the NPM1 gene) can be used for monitoring MRD in AML [49]. However, reciprocal gene fusions are applicable in a minority of AML patients only. NPM1 mutations represent ideal targets for MRD monitoring by qPCR in adults [50,51], but have a low prevalence in pediatric AML (8%-10%). Thus, MRD monitoring based on molecular markers has so far been realized only for subgroups of patients with AML. In 50% of patients with myelofibrosis and in 5%-10% of patients with MDS, the JAK2V617F mutation can be monitored by sensitive qPCR [52]. Furthermore, the MPLW515L/K mutation can be detected and monitored in about 5% of myelofibrosis patients [53].

The transcription factor WT1, originally described as a tumor suppressor gene, is a key molecule for neoplastic proliferation in a large number of hematologic malignancies, making it suitable both as an universal MRD marker and as a potential target for therapeutic strategies (eg, vaccination) [45,54-58]. WT1 has been shown to be expressed in the majority of adult and pediatric cases of acute leukemias, CML-blast phase, MDS, and both T- and B-lineage non-Hodgkin lymphoma (NHL), although the 3-4 log₁₀ overexpression necessary to make it a reasonable MRD marker is only seen in a subset of cases [59-65]. The frequency and degree of WT1 expression has been shown to correlate with MDS disease progression [66]. WT1 overexpression, quantified by qPCR, has been evaluated as a marker for risk stratification and for MRD detection in AML [44-48,66-69]. However, there are contradictory reports about the utility of WT1 overexpression to monitor MRD during frontline chemotherapy and after alloHSCT [45,46,56,57,70,71].

**Chimerism**

Analysis of chimerism allows monitoring of hematopoietic cells from donor and recipient origin after alloHSCT to determine engraftment as well as detection of imminent graft rejection and may also serve as an indicator for recurrence of the underlying malignancy. Because chimerism analyses were first performed, many different methods have been developed and implemented, all following the same basic principle using differences in polymorphic genetic markers and their products. These methods include cytogentic [72,73], red cell, or HLA phenotyping [74-76], restriction fragment-length polymorphism (RFLP) analysis [77-80], and FISH of sex chromosomes [81-86]. A major limitation of these different techniques is that they are time consuming and not applicable to all patients.

The breakthrough for clinical applicability came when the PCR technique was developed [87] and also utilized for investigation of chimerism [88-94]. During the 1990s, these analyses were mainly performed by the amplification of variable number of tandem repeats (VNTR) or by the characterization of short tandem repeat (STR) markers. Fluorescent labeling of the primers and resolution of PCR products by capillary electrophoresis allowed accurate
quantification of the degree of mixed chimerism. Semiautomated PCR analyses using the appropriate hardware permitted a high sample throughput [93,95-98] facilitating the study of chimerism in all patients in short time intervals starting early after transplantation. Thus, accurate monitoring of engraftment as well as surveillance of impending graft rejection and imminent relapse has become possible.

Furthermore, a qPCR assay for the analysis of the SRY gene on the Y chromosome has been established, which allows the identification of male DNA in the background of female DNA at very low levels [99]. This method is able to detect 1 male cell in the background of 100,000 female cells [100,101] increasing sensitivity enormously.

Over the past years, qPCR-based chimerism techniques aiming at the amplification of sequence polymorphisms (SPs) were established. SPs represent the most frequent genetic variation, occur on an average of 1.3 kilobases in the human genome, are mostly biallelic, and differ predominantly only in 1 single nucleotide, denoted by single nucleotide polymorphisms (SNPs) [102-104]. Alizadeh et al. [105] were the first to report a set of 11 biallelic polymorphisms using qPCR amplification for chimerism analysis. The limit of detection for the minor cell population (0.1%) was higher than in STR-PCR. This study was followed by others demonstrating the possibility of accurate characterization of chimerism by qPCR based on polymorphisms [106,107]. In contrast to STR-PCR, where virtually all recipient/donor pairs could be characterized, only 90% of donor/recipient pairs could be discriminated by this assay. Furthermore, this qPCR technique showed less quantitative accuracy with a variation coefficient of 30%-50% for higher autologous signals [105,106] compared to a variation coefficient of 5% when applying STR-PCR systems [95,97,108-111]. However, this did not hamper analysis when only low levels of mixed chimerism were needed to be quantified. Large prospective trials utilizing this qPCR method are needed to determine whether the clinical impact of chimerism analysis can be improved. For the time being, fluorescence-based PCR amplification of STR seems to be the gold standard method for posttransplant chimerism surveillance and the great majority of the major studies published to date have used this technique. Despite the increasing sensitivity of methods to determine chimerism, the clinical utility of this approach is limited. In most hematologic malignancies, chimerism is not a tumor-specific marker, and finding recipient cells does not necessarily indicate disease recurrence. The persistence or reappearance of recipient cells, detected by molecular methods, could reflect the survival of leukemic blasts, survival of normal host hematopoiesis, or a combination of both phenomena. Surviving host-derived hematopoietic cells could promote reemergence of the malignant clone by inhibiting immunocompetent donor-derived effectors.

Hematologic relapse was preceded by reappearing host-derived hematopoiesis in the mononuclear cell fraction in CML patients [112]. In consequence, the graft-versus-leukemia (GVL) effect could be weakened by a state of mixed chimerism [113]. In several early reports, it remained unclear if a state of mixed chimerism in patients with an acute leukemia was associated with an increased risk of relapse. The dynamic process of the evolution of chimerism was described in the 1990s, and it was concluded that monitoring of chimerism should be performed serially and in short intervals of time. The specificity in this regard is higher in diseases that originate from a stem- or progenitor cell (eg, CML), in contrast to malignancies that originate from a later cell stage of development (eg, CLL or myeloma), in which case the usefulness of chimerism to detect MRD or relapse is low. This lack of specificity might be overcome by performing chimerism on specific subsets of cells in some diseases (see later) [114,115]. Here, chimerism is performed on cell subtypes after isolation by cell sorting or enrichment (eg, CD138 for myeloma, CD34 for AML and MDS). Finding chimerism in a population enriched for tumor cells may increase the likelihood that it is a true marker of residual disease. Chimerism analysis may also have value, as it provides information about the alloreactivity and/or tolerance induction of the graft and thus may serve as a prognostic factor independent of its role as a marker for MRD.

Imaging

Imaging studies are not generally useful as MRD markers in leukemias or myeloproliferative neoplasms, but they play a role in lymphomas and MM. In MM, lytic lesions are generally diagnosed by radiographic analysis. One weakness of radiographic detection is that it may reveal lytic disease only when over 30% of the trabecular bone is lost [116]. Because of these limitations, computed tomography (CT) or magnetic resonance imaging (MRI) have been used to increase the sensitivity and specificity. CT is currently the most commonly used means for restaging patients with lymphoma [117,118]. However, CT lacks functional information, which impedes identification of disease in normal-sized organs. 18-F-fluoro-2-deoxyglucose (FDG) positron emission tomography (PET) may be an alternative to CT [119]. MRI is also utilized for bone and soft tissue reevaluation in specific situations.

CT

The mainstay of imaging studies for lymphoma has always been CT scans performed at various intervals. With current CT scanners, lymph nodes of 5 mm or
less in diameter can be detected. Extranodal involvement is typically demonstrated on a CT by organomegaly, abnormal masses, structural changes, or abnormal contrast enhancement. One problem with CT scans is that they have limited specificity, as many different processes can produce enlarged lymph nodes, even in patients with lymphoma [120], and a biopsy may be necessary to confirm residual disease. For use with MM patients, CT imaging is superior to that of standard radiography and reveals more lesions compared with conventional radiology [121].

**MRI**

MRI has high spatial resolution and excellent soft-tissue contrast that makes it an ideal tool for the detection of parenchymal and osseous lesions. However, because of long imaging time, limited availability, and extensive costs, MRI was previously used only as a tool to image limited anatomic areas of the body. Recent improvements in MRI technology have resulted in the availability of sufficiently fast and diagnostic sequences for whole-body (WB) MRI. However, there is no standard WB-MRI protocol for staging malignant lymphoma at this time. MRI provides superior soft-tissue contrast to CT. As with CT, assessment of the nodal involvement is based on size criteria, and biopsy is needed for final confirmation of relapse. The MRI sequences that are most informative for detection of bone lesions that may be observed in myeloma and lymphomas, are the T1-weighted, the T1-weighted with fat suppression and gadolinium contrast, the T2-weighted with fat suppression, and gadolinium contrast, and the short-time inversion recovery (STIR) images. The STIR images are particularly sensitive in detecting myelomatous lesions [122].

**PET**

PET is based on the use of positron-emitting radiopharmaceuticals and the detection in coincidence of the 2 nearly collinear 511-keV photons emitted following positron annihilation with an electron. The increased glycolytic rate of malignant cells is the rationale behind the common use of FDG as a radiotracer [123]. More recently FDG-PET and CT scans have been integrated, which provides both functional and anatomic information [124]. FDG-PET positivity is not restricted to malignancy. For example, uptake can be seen with inflammation and infection resulting in “false-positive” results in the setting of cancer evaluations. FDG-PET scanning has been integrated into standard staging criteria for lymphomas [125]. FDG-PET/CT can detect Richter’s transformation of CLL to diffuse large B cell lymphoma (DLBCL) with a high sensitivity and a high negative predictive value [126]. Standardized FDG-PET and PET/CT imaging procedure guidelines have been published to improve image quality, reporting, and common imaging standards, and may enable the use of semiquantitative techniques such as the SUV (standardized uptake value) in assessing response to therapy among centers worldwide [127]. PERCIST 1.0 is a proposed FDG-PET/CT response imaging criteria in solid tumors. This proposes imaging methodology and image analysis to assess response semiquantitatively or quantitatively. It is intended as a starting point for use FDG-PET in clinical trials and structured quantitative reporting, which may be particularly useful when assessing the activity of newer therapies that stabilize disease [128]. The standardization of imaging and response criteria could be a good step in establishing the use or lack of utility of FDG-PET/CT in early/interim (after 1-3 cycles of therapy) response monitoring or early PET response adapted therapy trials [129]. Multiple novel alternative clinical PET tracers have been developed, but the greatest experience outside of FDG-PET has been with 3'-deoxy-3'-[18F]fluorothymidine (FLT), which is an analog for thymidine used to image tumor proliferation. Early pilot studies using FLT-PET/CT appear to be very promising in early response evaluation in lymphoma [130].

**DISEASE-SPECIFIC DEFINITIONS AND MONITORING OF RELAPSE AFTER alloHSCT**

Standard diagnostic criteria have been established to define response and relapse for the hematologic 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 FDG-PET scans for NHL), and/or specific laboratory findings (eg, increased paraprotein by immunofixation and electrophoresis in 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.

**AML**

According to the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia, remission criteria in AML are not solely based on morphology, but also include cyto- and molecular genetic as well as flow cytometric data. Cytomorphology and hematologic parameters retain a central position (Table 2) [131]. However, very few follow-up
studies have been performed to specifically assess these criteria in the posttransplant period.

These criteria could also be integrated into the assessment of remission in the posttransplant period. Because of the limited sensitivity of chromosomal banding [132], interphase FISH seems to be a more suitable parameter for the assessment of the cytogenetic remission status. There are only a few studies that assess the prognostic value of interphase FISH after AML therapy [5], and even fewer studies addressing this issue in the posttransplant period. In pediatric cases with MDS or AML Fuehrer et al. [6] showed that clonal cytogenetic markers, as assessed by interphase FISH (eg, monosomy 7), reappeared at relapse after alloHSCT and could be followed after second transplant. Metaphase FISH proved useful for MRD diagnostics following standard chemotherapy or alloHSCT in an analysis of 22 AML patients performed by El-Rifai et al. [5]. After standard chemotherapy, the interval from the increase of fusion transcripts to morphological manifestation of relapse was 3-6 months. With respect to the posttransplant period, Elmaagacli et al. [138] followed 8 patients with inv(16)/CBFB-MYH11 with reverse transcription (RT-) PCR. The CBFB-MYH11 transcript was not detectable in 6 of 7 patients who remained in stable remission post-alloHSCT, whereas relapse occurred in 1 of 2 patients who had been positive 3 months after alloHSCT.

In patients with de novo AML, the prognostically favorable nucleophosmin (NPM1) mutations are detectable in approximately 35% of overall AML cases. In normal karyotype AML, frequencies about 55% were reported. qPCR or high melting resolution PCR ("HRM") can be used to detect the most frequent NPM1 mutation subtypes A, B, and D (Figure 1). Some studies demonstrated a correlation of the mutation load before and after chemotherapy with outcomes [139-141]. In most studies in the standard therapeutic setting, NPM1 mutations provided high stability during follow-up [50,139,140], whereas 1 study described loss of the mutation in 2 of 21 relapsing patients [142]. In an analysis involving 13 stem cell recipients with a history of NPM1 mutated AML, the achievement of PCR negativity after alloHSCT was a precondition for stable remission.

### Molecular genetics

**Molecular mutations.** A variety of genetic markers are available for MRD studies and have been investigated within a standard chemotherapy setting for their potential to guide therapeutic decisions. In this setting, MRD measurement is well established for the reciprocal rearrangements t(15;17)/PML-RARA, inv(16)/CBFB-MYH11, and t(8;21)/RUNX1-RUNXIT1. These are found in approximately 20% of de novo AML cases and confer a favorable prognosis for patients treated with standard chemotherapy. Ratios of aberrant gene expression after consolidation therapy and at diagnosis were demonstrated to correlate significantly with prognosis [137], and distinct thresholds of transcript copy numbers were determined to be associated with an increased relapse risk [42,41]. After standard chemotherapy, the interval from the increase of fusion transcripts to morphological manifestation of relapse was 3-6 months. With respect to the posttransplant period, Elmaagacli et al. [138] followed 8 patients with inv(16)/CBFB-MYH11 with reverse transcription (RT-) PCR. The CBFB-MYH11 transcript was not detectable in 6 of 7 patients who remained in stable remission post-alloHSCT, whereas relapse occurred in 1 of 2 patients who had been positive 3 months after alloHSCT.

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### Table 2. Response Criteria in AML According to an International Working Group [131]

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Complete Remission</th>
<th>Relapse</th>
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<tbody>
<tr>
<td>Morphologic and hematologic</td>
<td>BM blasts &lt;5%; thrombocytes ≥100 × 10⁹/L; neutrophils ≥1.0 × 10⁹/L</td>
<td>Reappearance of blasts after CR (BM: ≥5%; PB)</td>
</tr>
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<td>Cytogenetic</td>
<td>Disappearance of previous cytogenetic alteration</td>
<td>Reappearance of cytogenetic alteration</td>
</tr>
<tr>
<td>Molecular</td>
<td>Disappearance of molecular mutation</td>
<td>Reappearance of molecular mutation</td>
</tr>
<tr>
<td>Flow cytometric</td>
<td>Disappearance of cells with previously determined LAIP</td>
<td>Reappearance of cells with LAIP</td>
</tr>
</tbody>
</table>

AML indicates acute myeloid leukemia; BM, bone marrow; CR, complete remission; PB, peripheral blood; LAIP, leukemia associated immunophenotype.
whereas relapse after alloHSCT was preceded by an increase of the NPM1 mutation level with mean intervals of 24 days (range: 12 - 38 days) in all but 1 case. The short interval between MRD detection and relapse in NPM1 mutated AML implies that very frequent monitoring (eg, weekly intervals) would be necessary if this marker were to be used for early therapeutic intervention to prevent hematologic relapse. The more favorable prognosis of patients with isolated NPM1 mutations and a normal karyotype suggests that lower frequencies of this mutation might be expected in the alloHSCT setting. Posttransplant MRD studies for this marker would likely be more important in patients with coincident NPM1 mutations and the prognostically unfavorable internal tandem duplication of the FLT3 gene (FLT3-ITD) [143,144].

FLT3-ITDs are seen in 20%-25% of all AML cases and in approximately 40% of cases with normal karyotypes [145]. FLT3-ITD have variable lengths and different starting points; thus, qPCR monitoring requires the design of patient-specific primers [146]. Some studies have indicated that the FLT3-ITD mutation was unstable, with loss of the mutation in nearly a fifth of relapsed patients, limiting the validity of this marker for MRD monitoring [147]. However, others have reported instability rates of <5% [148]. Integration of FLT3-ITD in posttransplantation monitoring has been suggested [149], but is still controversial because of the potential for instability of the mutation [150]. Consequently, a combination with a second MRD strategy (eg, NPM1 mutations [49,151]), or with MFC, might be recommended for FLT3 mutated AML.

Mutations of the tyrosine kinase domain of the FLT3 gene (FLT3-TKD) are less frequent than the FLT3-ITD in AML. They occur in 6%-8% of all AML cases with normal karyotypes [152-156]. The impact on prognosis remains incompletely defined [157,156]. Scholl et al. [146] demonstrated that quantitative assessment during follow-up with qPCR is feasible as the FLT3-TKD mutations are represented by single nucleotide base exchanges. Quantitative follow-up of the FLT3-ITD and the FLT3-TKD was performed with qPCR in 4 stem cell recipients with a history of FLT3 mutated AML and showed a significant association of relapse to posttransplant PCR positivity, whereas disease-free survival (DFS) was associated with attainment of PCR negativity [157].

Partial tandem duplications of the MLL gene (MLL-PTD) represent another promising marker for

---

**Figure 1.** Proposed algorithm for follow-up diagnostics in the posttransplant period in AML.
posttransplant monitoring in AML. These prognostically unfavorable gain-of-function mutations [158] occur in 5%-10% of patients with a normal karyotype [159-162]. MRD studies with qPCR demonstrated that the amount of reduction of mutated cells was prognostically relevant in the standard therapeutic setting. Molecular relapse preceded clinical manifestations by several weeks [163]. To date, no study has been performed with this marker specifically in the posttransplant period.

Single gene expression. Monitoring of the expression of the WT1 gene represents another option for posttransplant MRD detection in patients with AML [46,150]. Using qPCR in patients with AML, ALL, or advanced phases of CML, Ogawa et al. [164] were able to determine thresholds above which relapse was predictable within 40 days after alloHSCT. There was a 100% relapse probability at an expression level of $1 \times 10^{-2}$, whereas the relapse risk was <1% with expression levels $<4 \times 10^{-4}$. Relapsed patients who responded to adoptive immunotherapy by donor lymphocyte infusion (DLI) or withdrawal of immunosuppression showed a significantly longer doubling time of WT1 expression compared to patients who were refractory to these approaches. Doubling time of WT1 transcripts $<13$ days was predictive for failure of treatment. Candoni et al. [165] described full concordance between WT1 expression levels and the remission status of AML before and after alloHSCT. Jacobsohn et al. [166] evaluated the prognostic significance of pretransplant WT1 gene expression as a marker of MRD in 36 pediatric patients with AML. Elevated WT1 gene expression before alloHSCT was predictive of posttransplant relapse and lower 5-year event-free survival (EFS). After alloHSCT, 76% of patients with high pretransplant WT1 expression relapsed, in contrast none of those with low WT1 expression.

Multicenter studies have been initiated to develop standardization of WT1 measurement (eg, by qRT-PCR normalized to the ABL gene) [58]. However, the physiologic background expression of WT1 in healthy individuals has to be considered. Barragan et al. [141] compared WT1 expression and monitoring of the NPM1 mutation load in 24 AML patients and found a strong correlation between both markers ($P < .001$), but with different disappearance kinetics. WT1 expression showed rapid decrease after induction therapy, but residual levels were maintained during CR, whereas NPM1 mutations showed a mild reduction after induction, but they were undetectable in long-term survivors. Thus, assessment by qPCR of the NPM1 mutation load was superior to the assay for WT1 quantification because of the specificity for leukemic cells and higher levels of expression at presentation.

Expression of other genes such as BAALC (brain and acute leukemia, cytoplasmic) [167] might be investigated for posttransplant monitoring in normal karyotype AML as well, although to date there are no data on their usefulness for this specific purpose. Thus, a variety of additional genetic markers should be investigated for their potential utility after alloHSCT, particularly those with an adverse impact on prognosis as they are likely to be overrepresented in the transplant setting. Efforts should continue to develop algorithms for monitoring patients with AML in the posttransplant period (Figure 1).

MFC

The value of immunophenotyping by MFC for the determination of the remission status in AML has been confirmed in many studies in the setting of standard therapy [131]. The decrease of cells with the specific LAIP from diagnosis to the end of induction therapy showed significant correlation with the remission rates and long-term prognosis [168-170], and the reduction of aberrant cells to a threshold of $<0.1\%$ after chemotherapy was associated with improved survival rates [171]. Only very few studies [172] have addressed the value of MFC specifically in the posttransplantation period, but it was shown that MFC was able to discriminate leukemic cells from regeneration blasts after alloHSCT [173]. In an individual patient with AML, withdrawal of immunosuppression was guided by the results of MFC, as there was an increase of cells with the LAIP 2 months posttransplant. This was followed by a subsequent reduction of aberrant cells [174]. Perez-Simon et al. [175] performed MFC in 13 patients with AML or MDS who had received RIC-alloHSCT. Persistence of cells with the LAIP within 21-56 days posttransplant was associated with a relapse risk close to 60%, whereas all patients with a negative MRD status in this period achieved stable remission. Diez-Campelo et al. [176] performed MRD monitoring by MFC in 41 alloHSCT recipients with AML or MDS. A cutoff level of $10^{-3}$ malignant cells at 100 days posttransplant was able to discriminate different risk populations. The 4-year overall survival was 73% in patients with a low MRD level versus 25% among patients with high MRD level at this time point, and EFS after 4 years was 74% versus 17% ($P = .01$) for the same 2 groups. The authors thus proposed that MRD monitoring with MFC has relevance for the therapeutic decisions in the early posttransplant period.

It should be emphasized that 1 advantage of MFC is the option to perform this technique in AML patients without evidence of a specific genetic marker. However, the frequency of changes of the LAIP in patients relapsing after alloHSCT is unknown, whereas relapse after standard therapy was associated with loss of the previous LAIP in approximately 25% of cases [177]. Thus, further evaluation is needed to evaluate the status of MFC for AML patients in the posttransplant period.
MDS

As in AML, standard criteria for the assessment of remission in MDS have been developed and revised by an international committee [178]. These new criteria integrate clinical, morphologic, cytogenetic, and hematologic parameters (Table 3). Few studies have evaluated MRD diagnostics in patients with MDS in the posttransplant period.

Molecular Markers

WT1 expression was shown to increase during leukemic transformation of MDS and to decrease following chemotherapy or alloHSCT [66]. In a study from Cilloni et al. [57] who included 131 patients with MDS, 65% of BM and 78% of PB samples from patients with refractory anemia (RA) showed WT1 overexpression, whereas 100% of samples from patients with either refractory anemia with excess blasts (RAEB) or secondary AML (sAML) samples WT1 transcript amounts greater than the level observed in healthy volunteers. Thus, there was significant correlation between the WT1 expression level and the IPSS score. Similar results were reported by Patmasiriwat et al. [179], who described increased WT1 levels in advanced MDS cases, but not in RA. Bader et al. [69] found significantly higher mean WT1 expression levels in pediatric patients with RAEB and juvenile myelomonocytic leukemia (JMML) when compared to expression levels of healthy donors (P < .001). However, single samples showed overlap between patients and healthy individuals.

Tamura et al. [180] reported on a patient of 14 years of age who had a diagnosis of RAEB with an increase of WT1 expression to 16,000 copies/μg RNA in peripheral blood after alloHSCT. Withdrawal of immunosuppression was followed by transient and self-limiting skin graft-versus-host disease (GVHD) and gradual decrease of WT1 expression during the next months. Sixteen months after alloHSCT, the patient was in CR, whereas WT1 expression was below the detection level. However, because it is difficult to differentiate clearly between physiologic and aberrant gene expression, the usefulness of WT1 expression for posttransplant monitoring of myeloid malignancies remains controversial.

Only a few studies have investigated molecular mutations in MDS. Most frequent are intragenic mutations of the RUNXI/AML1 gene [181,182]. FLT3-ITD occurs in 3%-5% of MDS patients [183,185]. Mutations of the NRAS protooncogene were identified in 6%-10% of all MDS cases. In contrast to AML, NPM1 mutations are rarely if ever seen in MDS [186]. Thus, the proportion of MDS patients who can be characterized with molecular markers is lower than in AML. However, as FLT3-ITD or NRAS mutations were demonstrated to increase during leukemic transformation [184,185], the selection of high-risk MDS cases for alloHSCT might be associated with an overrepresentation of these markers when compared to standard therapy cohorts. So far, no study has specifically determined the frequency of these molecular markers in the allo-transplant setting.

MFC

Several studies have demonstrated that MFC has potential diagnostic utility in MDS. Frequently, the side-scatter (SSC) signal is reduced because of lower granularity of granulocytes. Aberrant expression patterns of CD13 and CD16 on granulocytes or lack of CD71 on erythrocytes can also be characteristic [187,188]. The progression of MDS to higher stages was shown to be accompanied by an increase in the flow cytometric scores [189], which correlated with the International Prognostic Scoring System and prognosis [190]. At this time, no flow cytometric study has been performed specifically in the posttransplant period in MDS patients. Whether the frequent finding of bone marrow dysplasia in the posttransplant period will be an obstacle for this approach, remains to be clarified.

In conclusion, considering that molecular MRDs markers are available or under development for specific AML subgroups only, and the lack of MRD markers in most MDS patients, surveillance of chimerism represents the most broadly applicable molecular tool in patients with these disorders. Monitoring of the kinetics of chimerism in short intervals is clearly superior to the interpretation of a single measurement. In addition, the potential of CD34+ specific chimerism for myeloid disorders seems worthy of further evaluation.

ALL

Standard definitions for response and relapse are based on clinicopathologic evaluation of peripheral blood, BM, cerebrospinal fluid, and potential extramedullary sites of involvement. Response criteria rely most commonly on blood, marrow, and cerebral spinal fluid (CSF) cell counts and morphology (Table 4). From the standpoint of long-term outcome, CR has

Table 3. Revised Criteria for Assessment of Remission in MDS According to an International Working Group [180]

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete remission (CR)</td>
<td>without dysplasia, hemoglobin ≥11 g/dL, platelets ≥100 × 10^9/L, neutrophils ≥1.5 × 10^10/L</td>
</tr>
<tr>
<td>Partial remission (PR)</td>
<td>reduction of blasts by at least 50% or achievement of lower risk category than prior to treatment.</td>
</tr>
<tr>
<td>Major cytogenetic response</td>
<td>disappearance of a cytogenetic abnormality.</td>
</tr>
<tr>
<td>Minor cytogenetic response</td>
<td>≥50% reduction of abnormal metaphases.</td>
</tr>
</tbody>
</table>

MDS indicates myelodysplastic syndrome.
Progressive disease* induction and consolidation (MFC and PCR-based molecular methods) after has been well demonstrated that MRD positivity multivariate analyses most powerful prognostic factor for relapse in MRD, which in some studies was found to be the relapse appears to be proportional to the level of [208].

provide useful prognostic information in B-ALL [208,209]. However, measurement in PB may also levels of MRD in blood and marrow correlate closely so that PB may be utilized in MRD detection [208,209]. However, measurement in PB may also provide useful prognostic information in B-ALL [208], especially early in therapy during periods of BM aplasia (ie, day 8) [11]. Furthermore, the risk of relapse appears to be proportional to the level of MRD, which in some studies was found to be the most powerful prognostic factor for relapse in multivariate analyses [26,191-195,203,206,210].

Historically been considered the only meaningful goal and endpoint. However, criteria for CR with incomplete blood count recovery (CRI), partial response, and stable disease are sometimes utilized in the context of early phase clinical trials (Table 4).

Importantly, the standard definition for relapse in patients with CRI (>25% BM blasts) is insensitive for use after allogeneic haematopoietic stem cell transplantation, as detailed later.

### Prognostic Significance of MRD in Nontransplant Studies

Multiple studies support the independent prognostic value of MRD measurements in pediatric and adult patients with B- and T-lineage ALL [11,191-219]. It has been well demonstrated that MRD positivity (MFC and PCR-based molecular methods) after induction and consolidation [201,202,204] correlates with the risk of relapse. Most studies have employed BM and, in general, this is the preferred sample source; however, in T-ALL, in contrast to B-ALL, levels of MRD in blood and marrow correlate closely so that PB may be utilized in MRD detection [208,209]. However, measurement in PB may also provide useful prognostic information in B-ALL [208], especially early in therapy during periods of BM aplasia (ie, day 8) [11]. Furthermore, the risk of relapse appears to be proportional to the level of MRD, which in some studies was found to be the most powerful prognostic factor for relapse in multivariate analyses [26,191-195,203,206,210]. The kinetics of the decrease in MRD level has been shown to be more predictive than MRD analyzed at 1 time point [26,194]. Consequently, MRD detection has been utilized in the stratification for several ALL treatment protocols [196,197,206] and to evaluate the effectiveness of alloHSCT or alternative treatment approaches [194].

### Prognostic Significance of MRD at the Time of Transplant

#### Pediatric studies

Numerous studies have shown on a retrospective basis that the MRD status prior to conditioning was the strongest predictor for posttransplant relapse [211,212,213]. A British pediatric study in Bristol reported that an MRD load of greater than $10^{-3}$ pretransplant was associated with poor survival. In contrast, a portion of patients with low level disease burden ($<10^{-3}$) revealed a more favorable outcome, and MRD-negative children had the best chance of survival [210].

A retrospective study in Germany including 45 children reconfirmed these results, applying the same semiquantitative PCR approach. Those patients, entering transplantation with high-level MRD ($>10^{-3}$) were rarely cured. Interestingly, residual disease could be eradicated by posttransplant immunotherapy in some of these high-risk patients [206].

Recently Bader et al. [214] published prospective data on MRD analysis in 91 children with relapsed
ALL in second or higher remission prior to alloHSCT within the ALL-REZ BFM Study Group. The probability of EFS and cumulative incidence of relapse (CIR) in patients with MRD \( \geq 10^{-3} \) were 0.27 and 0.57, respectively, compared to 0.60 and 0.13 in 46 patients with MRD \(<10^{-4}\) (EFS: \( P = .004; \) CIR: \( P < .001 \)). Multivariate Cox regression analysis revealed MRD as the only independent parameter predictive for EFS. These findings proved the clinical significance of MRD analysis prior to alloHSCT as a predictor for postransplant outcome in a large prospective study. Based on this, new strategies with modified alloHSCT procedures will be evaluated within the ALL-BFM trials.

**Adult studies**

There are few studies examining the prognostic significance of MRD detection prior to and following alloHSCT for adults with standard risk B-cell ALL. In several older studies [198-201], semiquantitative PCR analysis failed to detect a strong association of MRD in CR1 prior to alloHSCT with postransplant DFS. In 1 of these studies [201], there was no significant difference in pretransplant MRD detection for the 14 patients who were in continuous CR after transplant; 8 were MRD positive and 6 were MRD negative prior to alloHSCT. However, there was an association with pretransplant MRD and risk of relapse for a cohort of patients who underwent autologous HSCT in CR. Twenty-one of 23 patients (91%) who were MRD-negative prior to autologous HSCT remained in long-term remission following transplant. In contrast, 6 of 7 patients (86%) who tested MRD-positive prior to autologous HSCT relapsed following the transplant (\( P = .005 \)). In this same study, postransplant MRD detection was strongly associated with relapse-free survival (RFS) for the 19 patients undergoing alloHSCT. There were no relapses in patients who remained MRD-negative following alloHSCT, whereas both patients with detectable MRD following transplant relapsed.

A more recent study using qPCR examined the clinical impact of MRD detection in 43 adults with high-risk ALL who underwent alloHSCT [215]. The group was heterogeneous in regard to disease subtype and status, conditioning, and donor type. Overall survival (OS) at 36 months was 48%. For patients who were PCR-negative prior to transplant, 80% were alive at 36 months in contrast to 49% survival for PCR-positive patients (\( P = .17 \)). For the same patients, the cumulative incidence of relapse was 0% for PCR-negative and 46% for PCR-positive patients (\( P = .03 \)). The relapse rate calculated according to the molecular results at 100 days following alloHSCT confirmed that the achievement or maintenance of a PCR-negative status within 3 months following transplantation was significantly associated with a lower incidence of relapse at 36 months postransplant (7% versus 80%, \( P = .0006 \)). In a multivariate analysis to investigate the role of clinical findings including disease status prior to transplant, cytogenetics, immunophenotype, age, and pretransplant MRD level, only molecular CR before conditioning proved to be a significant predictor of the achievement of MRD negativity at 100 days after transplantation.

Bassan et al. [206] recently demonstrated the utility of risk-adapted therapy based on MRD measurements for adults with ALL. MRD measurements taken at weeks 10, 16, and 22 of therapy using qPCR were used to stratify treatment for adults receiving intensive postremission chemotherapy for ALL in CR1. Of 280 patients registered to the trial, 112 were evaluable for MRD at the end of consolidation therapy. Of these, 58 had no measurable MRD and 54 were MRD-positive. Five-year OS was 75%, and DFS was 72% for the MRD-negative group in comparison with 33% OS and 14% DFS (\( P = .001 \)) for the MRD-positive group (regardless of pretreatment risk assignment based on clinical, immunophenotypic, and cytogenetic features). They further stratified postremission treatment based on MRD. Those patients who were MRD-positive at weeks 16 through 22 were assigned to receive alloHSCT in CR1 if an available donor was identified, or in the absence of a donor, to an intensification of postremission chemotherapy. Those who were MRD-negative went on to receive traditional maintenance therapy. Thirty-six of 54 (67%) MRD-positive cases proceeded to alloHSCT or to the intensified chemotherapy phase. There was a clear improvement in 4-year DFS for these 36 patients estimated at 33% in comparison to no survivors among the 18 MRD-positive patients who did not receive either intensified treatment approach. Notably, MRD status after the intensified treatment further defined prognosis for this group. For the patients who achieved a MRD-negative state following alloHSCT or intensified chemotherapy, the DFS was 51%; for those who remained MRD-positive, there were

### Table 4b. Bone Marrow Classification

<table>
<thead>
<tr>
<th>Bone Marrow Classification in ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blast Percentage (at least 200 nucleated cells counted)</td>
</tr>
<tr>
<td>M1</td>
</tr>
<tr>
<td>M2</td>
</tr>
<tr>
<td>M3</td>
</tr>
</tbody>
</table>

### Table 4c. Central Nervous System (CNS) Classification

<table>
<thead>
<tr>
<th>Cerebrospinal Fluid Cell Count and Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS1</td>
</tr>
<tr>
<td>CNS2</td>
</tr>
<tr>
<td>CNS3</td>
</tr>
</tbody>
</table>

Bone Marrow Classification in ALL.
Table 5. Published Studies on the Use of Chimerism and MRD in alloHSCT for ALL

### Chimerism

#### Late 1980s to mid 1990s

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients (n)</th>
<th>Graft (n)</th>
<th>T Cell Depleted</th>
<th>Interval of Investigations</th>
<th>Methods</th>
<th>State of Chimerism</th>
<th>Relapses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schattenberg et al., Blood 1989 [74]</td>
<td>29</td>
<td>29</td>
<td>yes</td>
<td>6, 12 months</td>
<td>Red cell phenotype</td>
<td>19/29</td>
<td>3/19</td>
</tr>
<tr>
<td>Van Leeuwen et al., Blood 1993 [245]</td>
<td>53</td>
<td>46</td>
<td>yes</td>
<td>2, 6, 12 months</td>
<td>VNTR</td>
<td>23/53, 30/53</td>
<td>8/23, 10/30</td>
</tr>
<tr>
<td>Roy et al., Blood 1990 [246]</td>
<td>43</td>
<td>43</td>
<td>yes</td>
<td>0.5, 1, 3, 6, 9, 12, 18, 24 months</td>
<td>RFLP</td>
<td>22/43, 21/43</td>
<td>11/22, 6/21</td>
</tr>
<tr>
<td>Lawler et al., Blood 1991 [89]</td>
<td>32</td>
<td>20</td>
<td>no</td>
<td>not given</td>
<td>VNTR and STR</td>
<td>10/12, 2/12</td>
<td>4/10, 0/2</td>
</tr>
<tr>
<td>Bader et al., BMT 1998 [247]</td>
<td>55</td>
<td>55</td>
<td>no</td>
<td>Weekly</td>
<td>VNTR and STR</td>
<td>18/36</td>
<td>Increasing MC: relapse</td>
</tr>
</tbody>
</table>

#### Chimerism with Intervention

##### 2003 to 2008

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients (n)</th>
<th>Diagnosis</th>
<th>Interval of Investigations</th>
<th>Methods</th>
<th>Relapses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formáková et al., Haematologica 2003 [229]</td>
<td>54</td>
<td>ALL, AML, CML, and MDS Children</td>
<td>weekly to +100; monthly</td>
<td>STR</td>
<td>MC associated with rejection and relapse, immunotherapy was possible</td>
</tr>
<tr>
<td>Gorczyńska et al., BMT 2004 [230]</td>
<td>14</td>
<td>ALL, AML Children</td>
<td>weekly to +100; monthly</td>
<td>STR</td>
<td>Increasing MC could be converted by immunotherapy to CC</td>
</tr>
<tr>
<td>Bader et al., JCO 2004 [235]</td>
<td>163</td>
<td>ALL Children</td>
<td>weekly to +100; monthly</td>
<td>STR</td>
<td>MC associated with rejection and relapse, immunotherapy was possible</td>
</tr>
<tr>
<td>Horn et al., BMT 2008 [248]</td>
<td>20</td>
<td>ALL, AML Children</td>
<td>1, 3, 6, 12 months; in MC bi-weekly</td>
<td>STR</td>
<td>MC associated with relapse, immunotherapy was not possible</td>
</tr>
</tbody>
</table>

### Pretransplant MRD

#### Retrospective Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients (n)</th>
<th>Diagnosis</th>
<th>Interval of Investigations</th>
<th>Methods</th>
<th>MRD ≥10⁻⁶; EFS 27%; CIR 57%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knechtli et al., Blood 1998 [213]</td>
<td>64</td>
<td>ALL</td>
<td>prior to conditioning</td>
<td>Ig/TCR PCR</td>
<td>EFS 27% CIR 57%</td>
</tr>
<tr>
<td>Bader et al., Leukemia 2002 [211]</td>
<td>41</td>
<td>ALL</td>
<td>prior to conditioning</td>
<td>Ig/TCR PCR</td>
<td>EFS 27% CIR 57%</td>
</tr>
<tr>
<td>Uzunel et al., Blood 2001 [249]</td>
<td>30</td>
<td>ALL</td>
<td>prior to conditioning</td>
<td>Ig/TCR PCR</td>
<td>EFS 27% CIR 57%</td>
</tr>
<tr>
<td>Smrzkova et al., Ped Blood Cancer 2007 [250]</td>
<td>25</td>
<td>ALL</td>
<td>prior to conditioning</td>
<td>Ig/TCR PCR</td>
<td>EFS 27% CIR 57%</td>
</tr>
</tbody>
</table>

#### Prospective Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients (n)</th>
<th>Diagnosis</th>
<th>Interval of Investigations</th>
<th>Methods</th>
<th>Survival According to MRD Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knechtli et al., BjH 1998 [237]</td>
<td>68</td>
<td>ALL</td>
<td>up to 24 months</td>
<td>Ig/TCR PCR</td>
<td>MRD ≥10⁻⁶; EFS 60%; CIR 13%</td>
</tr>
<tr>
<td>Uzunel et al., BjH 2003 [239]</td>
<td>23</td>
<td>ALL</td>
<td>24 months</td>
<td>Ig/TCR PCR</td>
<td>MRD ≥10⁻⁶; EFS 60%; CIR 13%</td>
</tr>
</tbody>
</table>

#### Posttransplant MRD

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients (n)</th>
<th>Diagnosis</th>
<th>Interval of Investigations</th>
<th>Methods</th>
<th>Survival According to MRD Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knechtli et al., BjH 2002 [238]</td>
<td>40</td>
<td>ALL</td>
<td>day 30, 60, 90, every 2 to 3 months</td>
<td>Flow cytometry</td>
<td>MRD ≥10⁻⁶; EFS 60%; CIR 13%</td>
</tr>
</tbody>
</table>

(Continued)
no long-term survivors at 4 years. These data suggest that allocation to standard maintenance therapy for patients with MRD-negative status following consolidation results in excellent DFS and that intensification therapy with alloHSCT or repeated cycles of intensification chemotherapy can salvage some very high-risk patients (MRD-positive at the end of consolidation) who would otherwise relapse with near certainty.

**Pretransplant MRD monitoring in Philadelphia chromosome-positive ALL**

MRD detection prior to transplant for Philadelphia chromosome positive (Ph+) ALL is a good predictor of RFS after transplant [216-220]. The addition of targeted tyrosine kinase inhibition with imatinib to frontline therapy of Ph+ ALL has resulted in the ability to significantly reduce or eradicate MRD during the first months of treatment using sensitive qRT-PCR assays [221-224]. A number of recent studies have demonstrated that achievement of a molecular CR as assessed by qPCR immediately prior to the time of transplant correlates with improved DFS and OS after alloHSCT. In 1 of the larger studies, when imatinib was added to frontline therapy, 82% of the patients became PCR negative and the majority was able to proceed to transplant [225]. With a median follow-up of 25 months following transplant, outcome of these patients appeared to be improved with a DFS of 78% in comparison to studies in the preimatinib era where DFS following alloHSCT for adults with Ph+ ALL was in the 30% to 50% range. The kinetics of MRD eradication prior to alloHSCT may also be an important prognostic feature. Lee et al. [226] studied MRD in 41 patients with Ph+ ALL who were treated with combination chemotherapy induction followed by 4 weeks of imatinib therapy. They found that 36 of 41 patients achieved at least a 3-log reduction of MRD following the first 4 weeks of treatment with imatinib. This early reduction in MRD was identified as the most powerful predictor of lower relapse rate following alloHSCT. For patients with at least a 3-log reduction in MRD, the relapse rate following alloHSCT was 12% in comparison to the other patients who had a relapse rate of 45% (P = .011) [227]. These data, similar to the pediatric data presented before, suggest that pretransplant MRD detection in adults with ALL may be an important guide to early posttransplant treatment to attempt to avert clinical relapse.

**Table 5. (Continued)**

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<thead>
<tr>
<th>Author</th>
<th>Patients (n)</th>
<th>Diagnosis</th>
<th>Interval of Investigations</th>
<th>Methods</th>
<th>Survival According to MRD Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bader et al. BMT 2009 [240]</td>
<td>92</td>
<td>ALL</td>
<td>day 30, 60, 100, 200, 365 posttransplant</td>
<td>Ig/TCR PCR</td>
<td>MRD $\geq 10^{-4}$: EFS 9%; RFS 11%; MRD $&lt; 10^{-4}$: EFS 48%; RFS 62%</td>
</tr>
<tr>
<td>Mortuza et al. JCO 2002 [201]</td>
<td>19</td>
<td>ALL (B-lineage)</td>
<td>1 to 20 months</td>
<td>Ig/TCR PCR (semiquant.)</td>
<td>positive – 0% negative – 100% positive $&gt; 10^{-4}$—20% negative—93%</td>
</tr>
<tr>
<td>Spinelli et al. Haematologica 2007 [215]</td>
<td>37</td>
<td>ALL</td>
<td>day 100</td>
<td>Ig/TCR or fusion gene PCR (quantitative)</td>
<td>positive $&gt; 10^{-4}$—0% negative—50%</td>
</tr>
<tr>
<td>Bassan et al. Blood 2009 [206]</td>
<td>18</td>
<td>ALL (All patients were PCR positive prior to transplant)</td>
<td>not defined</td>
<td>Ig/TCR PCR</td>
<td>EFS 48%; RFS 62%</td>
</tr>
</tbody>
</table>

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CIR, cumulative incidence of relapse; CC, complete chimerism; CML, chronic myelogenous leukemia; EFS, event-free survival; Ig, immunoglobulin; MC, mixed chimerism; MDS, myelodysplastic syndromes; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RFS, relapse-free survival; STR, short tandem repeats; TCR, T cell receptor; VNTR, variable number tandem repeats.

**Role of MRD Monitoring and Chimerism Assessment following alloHSCT for ALL**

**Pediatric and adult studies (Table 5)**

Assessment of chimerism using the semiquantitative STR-PCR approach, which analyzed microsatellite regions, has demonstrated that ALL patients with rapid increase of mixed chimerism were at the highest risk of relapse post-alloHSCT [113,228-231]. The analysis of cell subpopulations in acute leukemia patients revealed a potential difference between children and adults. Guimond et al. [232] demonstrated that mixed chimerism in T and natural killer (NK) cells was frequently detected in children with relapsed leukemia but not in pediatric patients, who remained in remission. Furthermore this phenomenon was not observed in relapsed adult patients.

The clinical relevance of a state of increasing mixed chimerism as a risk factor for relapse in patients with acute leukemia was demonstrated by Barrios et al. in...
2003 [233]. However, a group in Germany showed a more complicated relationship between chimerism and relapse [234]. They showed that the persistence of mixed chimerism in the early-phase posttransplant as predominantly caused by normal recipient-derived hematopoiesis. The reappearance of leukemic blasts was preceded by an increase of this recipient-derived normal hematopoiesis, denoted by increasing recipient chimerism. These results promoted the hypothesis that mixed chimerism weakened the clinical GVL effect, which was thought to be mediated by donor-derived alloreactive effectors in acute leukemia patients. These results formed the basis for several clinical trials to study the efficacy of relapse prevention by preemptive immunotherapeutic intervention based on the analysis of chimerism in acute leukemia patients [229,230].

By applying STR-based analysis of chimerism at defined regular intervals posttransplant, in 2004, Bader et al. [235] defined a subset of children with impending relapse, applying STR-based analysis of chimerism at defined regular intervals posttransplant. Beyond that, prevention of overt relapse was possible in a portion of patients by preemptive immunotherapeutic intervention (withdrawal of immunosuppression or administration of DLI), although impending relapse was not diagnosed in all patients because of the short time interval between the conversion of chimerism and the occurrence of relapse in some patients.

Importantly, analysis of chimerism by conventional STR-PCR does not incorporate the potential to detect MRD because of its limited sensitivity (approximately 1%). Thus, the decision to start immunotherapy should not be based exclusively on the state of chimerism, because this might result in a delay of onset of beneficial interventional therapy.

A German retrospective analysis including patients who had received DLI based on a state of increasing mixed chimerism also measured the MRD burden, applying qPCR (Ig/TCR rearrangements). It was demonstrated that those patients with an MRD burden of $>10^{-3}$ did not benefit from interventional therapy, whereas patients with an MRD load $<10^{-3}$ at initiation of immunotherapy showed a survival rate just below 40% at 2 years [236].

The significance of MRD analysis posttransplant has only been investigated in a few prospective studies. In 1998, Knechtli et al. [237] showed that persistent MRD positivity represented an unfavorable predictor for EFS. Here, MRD was assessed by a semiquantitative dot blot hybridization approach. These initial results could be confirmed later in a smaller cohort of patients by immunophenotyping of antigens associated with leukemia [238] and by molecular qPCR based approaches [175,215,239]. These trials all showed MRD to be a predictor for poor posttransplant outcome.

Recently, the ALL-REZ BFM Study Group monitored MRD in 92 pediatric ALL patients after alloHSCT to highlight patients with highest risk for relapse to whom preemptive treatment might be offered. The probability of EFS and RFS for MRD-negative patients was 0.55 and 0.77, respectively, compared to 0.48 and 0.62 in MRD-positive patients with $<10^{-4}$ and compared to 0.09 and 0.11 in MRD-positive patients with $\geq10^{-4}$, respectively (EFS: $P < .005$; RFS: $P < .001$). Patients who remained persistently MRD-negative showed a RFS of 0.78 compared to 0.5 in patients who became MRD-negative, and compared to only 0.1 in patients who developed an increase of MRD $\geq10^{-4}$ and of 0 in patients with MRD levels remaining permanently $>10^{-4}$ ($P < .001$). Consequently, patients with reappearing or persisting MRD load at a level of $\geq10^{-4}$ faced the highest risk for subsequent relapse and may therefore be candidates for further interventional therapy [240].

MRD by molecular Ig/TCR rearrangements is impossible in patients lacking a disease-specific marker. In these cases, the status of chimerism can be used as a surrogate marker for MRD if it is analyzed in specific cell subpopulations. Thiede et al. [241] demonstrated that a state of mixed chimerism in the CD34+ cell fraction predicted relapse in PB in ALL and AML patients. In 1998, Winiarski et al. [242] characterized chimerism in cell subsets, which carried the leukemic phenotype. A remarkable correlation between the presence of MRD and a state of mixed chimerism in the respective cell subpopulation was detected. However, large prospective trials proving the predictive value of mixed chimerism in cell subsets have not been published yet.

Taken together, posttransplant serial monitoring of chimerism by conventional STR-PCR in PB allowed for the identification of patients with impending relapse. Therefore, frequent chimerism analyses are indispensable within the first 200 posttransplant days, when most relapses occur.

The combination of characterization of chimerism and analysis of MRD ensures the documentation of engraftment and surveillance of posttransplant state of remission. These approaches provide a rational basis for individual immunotherapeutic intervention to prevent the recurrence of the underlying malignancy. However, it should be noted that the clinical importance of MRD in ALL patients after allogeneic stem cell transplantation could not be verified in some studies [210].

**Posttransplant MRD monitoring in Ph+ ALL**

Beginning in the late 1990s, studies to assess MRD status following alloHSCT have provided intriguing information about the risk of relapse in Ph+ ALL [216-219,243]. First, using qualitative RT-PCR testing with sensitivities reported in the $10^{-5}$ to $10^{-6}$ range, a number of studies found that patients who were consistently PCR negative following alloHSCT were unlikely to relapse. Conversely, patients in whom MRD was detected after transplant were at
very high risk of relapse. In the largest published series, Radich et al. [218,219] found that the relative risk (RR) of relapse was significantly higher for patients with detectable MRD following transplantation than for those without detectable \( \text{BCR-ABL1} \) transcripts (RR: 5.7; \( P = .025 \)). The prognostic significance of the PCR assay remained after controlling for other clinical variables (eg, stem cell source, GVHD) that could influence relapse risk. Interestingly, the genetic context of MRD may also be of relevance. In this study, Radich et al. noted that the risk of relapse was greatest for MRD-positive patients with a p190 \( \text{BCR-ABL1} \) transcript in comparison to patients who had detectable p210 \( \text{BCR-ABL1} \) transcripts after transplant.

Use of imatinib after alloHSCT appears to be useful for eradication of MRD in some patients. Wassmann et al. [244] showed that initiation of imatinib for MRD-positive patients after alloHSCT was associated with a rapid molecular CR and prolonged DFS in 14 of 27 treated patients. There have also been anecdotal reports of initiation of targeted tyrosine kinase inhibitor therapy at the time of clinical relapse with reinstatement of donor chimerism and achievement of prolonged remission with or without DLI.

**Summary and Future Directions: Risk-Adapted Therapy of ALL Based on MRD**

Taken together, all the studies suggest that MRD measurements in patients with ALL taken in the first 4-20 weeks of postremission therapy have strong prognostic significance and can identify patients with both a good and poor prognosis. Similarly, detection of pretransplant MRD in pediatric and adult studies is highly predictive of relapse following alloHSCT and, coupled with posttransplant MRD evaluation, may guide early posttransplant intervention such as early withdrawal of immunosuppression, administration of DLI, or addition of posttransplant maintenance therapy (eg, targeted tyrosine kinase inhibition for Ph+ ALL).

Thus, there are ample data demonstrating that MRD monitoring is a powerful prognostic tool in pediatric and adult ALL and may be used to risk-adapt treatment. The introduction of novel treatment strategies to eradicate MRD early during treatment should be a goal of future trials and may eventually obviate the need for alloHSCT. Characterization of MRD before transplantation defines those patients at highest risk for relapse after alloHSCT. Importantly, although it appears that MRD detection can facilitate the decision to advise an allogeneic transplant in CR1, the outcome for MRD+ positive patients, even with the most aggressive approaches currently available, is still relatively poor. After alloHSCT, MRD measurements can be used to guide novel therapeutic interventions and assess their efficacy. MRD monitoring allows the detection of impending relapse in a substantial

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**Table 6. Response and Relapse Definitions after alloHSCT — Application of Monitoring Methodologies**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Definition of Complete Remission</th>
<th>Definition of Relapse</th>
<th>Imaging</th>
<th>Flow Cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML/MDS</td>
<td>NCI First International Workshop</td>
<td>Applicable</td>
<td>Not applicable</td>
<td>4-color flow</td>
</tr>
<tr>
<td>ALL</td>
<td>Applicable</td>
<td>Applicable</td>
<td>Not applicable</td>
<td>4-color flow</td>
</tr>
</tbody>
</table>

**ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; Flow, multiparameter flow cytometry; MDS, myelodysplastic syndromes; qPCR, quantitative real-time PCR; STR, short tandem repeats.**

Further studies needed.
percentage of children undergoing transplantation for ALL. These analyses have recently served as the basis for treatment intervention to avoid graft rejection, maintain engraftment, and treat-imminent relapse.

**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, molecular methods, and new imaging modalities have been investigated in recent years and can substantially increase the sensitivity of disease detection up 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. Similar sensitivity can 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.

Critically important is the need for standardization of the different residual disease techniques. Table 6 summarizes the different methods of MRD detection in the monitoring of ALL, AML, and MDS, and the relative pros and cons of use of these methods after alloHSCT. 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, and the optimal timepoints to assess kinetic changes in these measurements remains to be determined across all hematologic malignancies. Subsequent studies must then be performed to 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. Standardize measurement of molecular markers for each hematologic malignancy for which alloHSCT is employed.
2. Determine the optimal frequency for monitoring MRD and chimerism after alloHSCT.
3. Define kinetic changes in MRD and chimerism that occur after alloHSCT and establish criteria for remission and relapse that incorporate measurement of these molecular markers in the definition of response and remission after alloHSCT.
4. Assess the efficacy of interventional strategies based changes in MRD and/or chimerism to prevent clinical relapse.

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**REFERENCES**


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