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Monitoring and Prevention of Relapse after Allogeneic Hematopoietic Cell Transplantation for Myeloid Malignancies

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Acute myeloid leukemia and myelodysplastic syndromes are the most common indications for allogeneic hematopoietic cell transplantation. Although this treatment can be curative, even in advanced disease, treatment failure is commonly manifested by relapse of disease, for which treatment is successful in only a minority of patients. There is a necessity for new strategies for prevention of posttransplantation relapse through early disease detection and intervention in order to improve patient outcomes. Detection of minimal residual disease in posttransplantation surveillance is felt to be a necessary component of any strategy. In chronic myeloid leukemia, assessment of the *BCR-ABL1* load by quantitative real-time PCR provides an optimal guideline for posttransplantation therapeutic decisions, but in patients with acute myeloid leukemia or myelodysplastic syndromes, the situation is more complex because of the genetic heterogeneity of these disorders. Past strategies for relapse prevention have focused on use of donor lymphocyte infusions with variable success. Peritransplantation and maintenance therapies (eg, azacitidine) are under current investigation. This review summarizes the current status of minimal residual disease monitoring and prevention strategies for both pediatric and adult patients with myeloid malignancies in the transplantation setting and discusses perspectives for further improvement.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is often indicated as part of the initial treatment of patients with poor-risk acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), and it is the only curative option in those with advanced disease. Recent improvements in treatment plans and supportive care have reduced treatment-related mortality, and disease relapse has now emerged as the principle reason for treatment failure after transplantation. In patients with myeloid malignancies, therapeutic strategies aim to select poor-risk patients for allogeneic HCT [1]. For AML patients with intermediate and adverse cytogenetic risk, groups have a significant survival benefit from allogeneic HCT in first remission. In MDS, patients in intermediate-2 and high-risk International Prostate Symptom Score groups, immediate transplantation was associated with maximal life expectancy. Chronic myeloid leukemia (CML) became a domain of tyrosine kinase inhibitor (TKI) therapy, and allogeneic HCT has remained an up-front strategy only for patients whose disease is resistant to TKI treatment or advanced disease beyond first the chronic phase. Therefore, patients with AML or MDS who are candidates for allogeneic HCT have a higher relapse risk in the posttransplantation period. Indeed, reports from the Center for International Blood and Marrow Transplant Research demonstrated relapse rates following myeloablative conditioning regimens of over 60% for patients with active at the time of transplantation.

As reviewed in a previous workshop report [2], risk factors for relapse after transplantation vary with the diagnosis of the underlying malignancy, but patients who underwent transplantation while not in remission are at especially high risk for posttransplantation recurrence independent of diagnosis. Factors that influence the duration of survival after relapse include age, performance status, comorbidities, remission duration, tumor

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burden at relapse, and presence of mixed chimerism. With rare exception, however, posttransplantation relapse is ultimately fatal. Consequently, development of new strategies to prevent relapse is imperative if survival of patients who underwent transplantation is to improve.

STRATEGIES FOR MONITORING MINIMAL RESIDUAL DISEASE IN THE PRE- AND POSTTRANSPLANTATION PERIOD IN PATIENTS WITH MYELOID MALIGNANCIES

Traditionally, the detection of impending relapse in the posttransplantation period had been based on donor chimerism analysis, but the lack of specificity remains a problem. Therefore, interest focused on the introduction of minimal residual disease (MRD) diagnostics in the posttransplantation care of patients with myeloid malignancies aiming to detect an increase of the leukemic cells at the earliest possible time [3]. For patients with CML, MRD monitoring of the *BCR-ABL1* load is well established in the conservative [4] as in the transplantation context [5], but in AML and MDS, the development of posttransplantation MRD strategies is more complex because of the genetic heterogeneity of these disorders. Nevertheless, for some genetic subtypes, for example, reciprocal translocations [6] or *NPM1*-mutated AML [7,8], MRD monitoring in the posttransplantation period has already been realized. It further remains to be clarified whether the MRD status at the time of HCT is prognostically relevant in patients who achieved complete hematologic remission. This section summarizes the current status of strategies for MRD measurement (Table 1) for adult patients with AML, MDS, or CML in the pre- and posttransplantation period and discusses perspectives for further improvement.

Diagnostic Techniques

Mutation detection in hematologic malignancies relies on various modifications of the classical polymerase chain reaction (PCR) technique. Reciprocal gene fusions are detected by reverse transcription PCR (RT-PCR) following RNA extraction and cDNA synthesis. Real-time PCR enables the assessment of PCR amplification in the sample based on the detection of fluorescent dyes or fluorescent-tagged DNA probes (sensitivity: 10^{-4}-10^{-5}). Confirmation of the mutations and further analysis mostly relies on direct Sanger sequencing using dye terminators. This might change rapidly with the advent of high-throughput sequencing [9]. At present, quantitative real-time PCR (RQ-PCR) and nested PCR are the most useful methods for MRD assessment. Nested PCR is done by two consecutive PCR reactions with two different primer pairs targeting the region of interest, which results in high sensitivity ($\sim 10^{-6}$) and highest specificity. Immunophenotyping by multiparametric flow cytometry, which is able to combine up to 10 different fluorochromes, is less sensitive (10^{-3}-10^{-4}) compared with real-time PCR, but leukemia-associated immunophenotypes (which are characterized, eg, by cross-lineage expression of antigens) are detectable in virtually all AML patients. Interphase fluorescence in situ hybridization relies on fluorescence-tagged probes with specificity for distinct gene loci or for certain centromere regions. A total of 100 to 200 interphase nuclei can easily be evaluated for MRD purposes in patients with previously known cytogenetic alterations.

AML

Cytogenetic analysis detects prognostically relevant alterations in ~55% of de novo AML patients, and molecular methods reveal genetic alterations in the vast majority of normal karyotype cases. Mutations of the *NPM1* gene (in ~55% of normal karyotype AML cases) are prognostically favorable in case of isolated occurrence, but coincidence with the adverse *FLT3*-ITDs considerably worsens prognosis. Other alterations observed at lower frequencies in normal karyotype AML are the *FLT3*-TKD, the adverse *MLL*-PTDs, and *CEBPA* gene mutations, which are prognostically favorable in case of biallelic occurrence. The panel of known molecular markers in AML is becoming more complex: Mutations of the *TET2* ("tet oncogene family member 2") gene were identified in 12% to 25% of AML cases [10], and mutations of the isocitrate dehydrogenase genes (*IDH-1* and *IDH2*) occur in ~15% [11]. These markers seem to be prognostically relevant in distinct molecular subgroups of normal karyotype AML.

Pretransplantation MRD in AML

Walter et al. [12] investigated 99 AML patients receiving myeloablative HCT in first complete remission (CR) by 10-color flow cytometry before transplantation. MRD-positive patients had lower 2-year estimates of overall survival (OS) (30.2% versus 76.6%) and higher 2-year estimates of relapse (64.9% versus 17.6%), compared with MRD-negative patients. After adjustment for other prognostically relevant parameters, a positive MRD status pre-HCT was significantly associated with increased overall mortality and relapse relative to MRD-negative HCT. Studying 68 patients with AML/MDS receiving allogeneic HCT, Kebriaei et al. [13] found a trend toward improved outcomes in patients in cytogenetic remission compared with those with residual cytogenetic abnormalities at the time of HCT. Patients with cytomorphologic and cytogenetic remission had a median progression-free survival of 7.8 months compared