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Pre-Emptive Immunotherapy for Clearance of Molecular Disease in Childhood Acute Lymphoblastic Leukemia after Transplantation

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Monitoring of minimal residual disease (MRD) or chimerism may help guide pre-emptive immunotherapy (IT) with a view to preventing relapse in childhood acute lymphoblastic leukemia (ALL) after transplantation. Patients with ALL who consecutively underwent transplantation in Frankfurt/Main, Germany between January 1, 2005 and July 1, 2014 were included in this retrospective study. Chimerism monitoring was performed in all, and MRD assessment was performed in 58 of 89 patients. IT was guided in 19 of 24 patients with mixed chimerism (MC) and MRD and by MRD only in another 4 patients with complete chimerism (CC). The 3-year probabilities of event-free survival (EFS) were .69 ± .06 for the cohort without IT and .69 ± .10 for IT patients. Incidences of relapse (CIR) and treatment-related mortality (CITRM) were equally distributed between both cohorts (without IT: 3-year CIR, .21 ± .05, 3-year CITRM, .10 ± .04; IT patients: 3-year CIR, .18 ± .09, 3-year CITRM .13 ± .07). Accordingly, 3-year EFS and 3-year CIR were similar in CC and MC patients with IT, whereas MC patients without IT experienced relapse. IT was neither associated with an enhanced immune recovery nor an increased risk for acute graft-versus-host disease. Relapse prevention by IT in patients at risk may lead to the same favorable outcome as found in CC and MRD-negative-patients. This underlines the importance of excellent MRD and chimerism monitoring after transplantation as the basis for IT to improve survival in childhood ALL.

INTRODUCTION

In recent years, several techniques have been developed to assess treatment response and risk stratification in patients with acute lymphoblastic leukemia (ALL) [1-7]. These efforts have led to the concept of minimal residual disease (MRD) assessment [8]. Beginning in the late 1990s, the clinical importance of MRD in childhood ALL could retrospectively be confirmed [1,2,6]. Thereafter, several studies were based mainly on clone-specific Ig and TCR gene rearrangements as real-time quantitative PCR targets, as well as on quantitative flow cytometry analysis for monitoring MRD in bone marrow samples [9-14].

Moreover, a special situation occurs in patients receiving allogeneic hematopoietic stem cell transplantation (HSCT) for treatment of ALL, where impending relapse can be assessed by chimerism analysis in both peripheral blood and bone marrow. Recurrent recipient signals, ie, the detection of mixed chimerism (MC) after transplantation in addition to the detection of MRD, strongly predict the risk for relapse in children with ALL after allogeneic HSCT [15-20].

Several approaches have been used to treat leukemia relapse after HSCT, such as reinduction chemotherapy, reapplied transplantation, and immunotherapy (IT), including the

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discontinuation of immunosuppression (IS) or donor lymphocyte infusion (DLI) [21,22]. IT is intended to induce graft-versus-leukemia reaction, but IT patients are also at risk of developing graft-versus-host disease (GVHD) or marrow aplasia. Particularly in the case of DLI, the efficacy and the risk of side effects depend on the type of leukemia and the dose of infused T lymphocytes, respectively [22-26]. Furthermore, because the response is generally limited in cases of florid relapse of ALL, IT is increasingly applied pre-emptively at the time of detectable MRD or MC [15]. Using serial MRD or chimerism analysis enables the detection of impending relapse in advance to allow pre-emptive IT [27,28].

Here, we retrospectively report our experience of pre-emptive IT guided by consistent MRD and chimerism monitoring for the detection of impending relapse in childhood ALL after transplantation. Outcomes of patients at risk for relapse with or without IT were compared with outcomes of patients not identified as being at risk for relapse after transplantation. Our follow-up analyses included molecular responses and the relation to outcome, as well as the impact on immune reconstitution and toxicity.

METHODS

Patients

Consecutive children older than 1 year of age were included in our study if they had received their first allogeneic HSCT for ALL in Frankfurt/Main, Germany, between January 1, 2005, and July 1, 2014 after written informed consent for retrospective data analysis. Written informed consent for retrospective studies was given at the time of transplantation. The institutional ethics committee approved this best practice approach at the center (number 529/15).

Molecular Target Identification and MRD Analysis

MRD analysis was performed in patients if a diagnostic sample of their leukemia cells was available. This analysis was possible in 58 of 89 (65%) patients because several patients were referred from abroad without a diagnostic sample taken at diagnosis or relapse. Cell sample isolation and identification of the markers for MRD evaluation have been previously reported [29,30]. Real-time quantitative PCR analysis was performed and interpreted according to the guidelines developed within the European Study Group for MRD detection in ALL (ESG-MRD-ALL) [3].

Risk Group Definition and Stratification According to MRD

If MRD levels differed between 2 MRD markers, the highest MRD level was chosen for MRD assessment, provided that the markers had a sensitivity of at least 10^{-4}. EuroMRD guidelines were used to reduce the risk for false-negative and false-positive results.

Chimerism Analysis

Chimerism determination started at the time of leucocyte recovery after transplantation. A previously described semi-quantitative PCR approach, based on the amplification of short-tandem-repeat (STR) markers, was used for chimerism analyses [15]. The sensitivity of our assay for detecting autologous cells was 1%. In this regard, singleplex STR-PCR approaches were run to avoid interference of the baseline, as observed frequently in multiplex PCR. Fragment analyses of fluorescence-labeled PCR products was performed by capillary electrophoreses using a 3100-avant device (Applied Biosystems, Darmstadt, Germany) and peak detection cut-off was defined as >50 relative fluorescence units (RFUs). For valid detection of 1% minority genotype, the majority peaks must exceed 5000 RFUs for STR-heterozygous or 2500 RFUs for STR-homozygous recipients.

In addition, if a patient showed 1% of autologous (recipient) cells in a peripheral blood or bone marrow sample, another sample was taken and assessed within 1 week. Patients with confirmed 1% autologous cells in 2 consecutive samples and patients with >1% of autologous cells in a single sample after transplantation were considered as having mixed chimeras.

Flow Cytometry

Flow cytometric analyses for immune monitoring were performed monthly as previously described [31]. In brief, immune reconstitution monitoring of $CD3^+CD56^-$ T cells, $CD3^-CD56^+$ natural killer (NK) cells, and $CD3^+CD56^+$ T-NK cells was performed in patients with or without IT treatment. As childhood blood values strongly depend on age, each patient's longitudinally determined measurement was calculated from its corresponded age-matched norm published by Huenecke et al. [32]. The age-adjusted values allowed for the comparison among the cell counts from patients of different ages.

Stratification

Serial and semi-quantitative analyses of post-transplantation hematopoietic chimerism in peripheral blood started at the time of leucocyte engraftment and were performed weekly until day 200 and monthly thereafter. For analyses of both hematopoietic chimerism and MRD, bone marrow was assessed at days 30 ± 15, 60 ± 15, 90 ± 15, 180 ± 30, and 365 ± 30 and also at 18 months $\pm$ 30 days after transplantation. Bone marrow punctures were not performed routinely at later time points, but they were performed in case of suspected relapse or other situations.

Patients with detectable MRD or MC (confirmed 1% autologous (recipient) signals in 2 consecutive samples or >1% of autologous signals in a single sample after transplantation) and with no or only mild signs of acute (aGVHD) (grade 1 aGVHD) were immediately offered pre-emptive IT. Regardless of the MRD status before transplantation and of the donor type or stem cell source, IT included discontinuation or tapering of IS for patients still receiving IS in the early post-transplantation period or administration of DLI as frontline therapy in patients without IS. DLI was also applied in patients without responses after stop of IS. The interval between both IT options as well as between respective DLI doses was 3 to 4 weeks. The recommended starting dose of DLI was 1×10^6 T cells/kg in cases of HLA-matched related donors, $.5 \times 10^6$ T cells/kg in cases of HLA-matched unrelated donors, and $.1 \times 10^6$ T cells/kg in cases of HLA-haploidentical donors. With pre-emptive IT, chimerism monitoring was performed weekly in peripheral blood samples and monthly in parallel with MRD monitoring in bone marrow samples. In case of persistence of MRD or MC 3 to 4 weeks after DLI, prudent dose escalation was considered for subsequent DLIs. In the matched transplantation setting, a doubling of the $CD3^+$ T cell numbers infused was contemplable, if no additional signs of GVHD had appeared.

Pre-emptive IT was stopped if MC converted to complete donor chimerism (CC) or in cases of clearance of MRD. Furthermore, pre-emptive IT was not applied if patients experienced aGVHD exceeding grade 1.

Statistical Analysis

The median follow-up time for all patients was obtained using the reversed Kaplan-Meier estimator. Fisher's exact test or the Wilcoxon-Mann-Whitney test were used to compare the patients' categorical data. Kaplan-Meier estimates were performed to predict the overall survival and event-free survival (EFS) probabilities. The log-rank test was used for comparisons. EFS was defined as survival without relapse and treatment-related mortality (TRM). TRM was defined as death in complete remission (CR) without pervious relapse. Cumulative incidence (CI) curves were calculated for the incidence of relapse (CIR) and TRM (CITRM) considering TRM as a competing risk for relapse. Gray's test was used for comparisons of CIs.

Cox regressions were performed to identify associations with patient or transplantation characteristics and MC (if applicable) and EFS, as well as CIR. Only factors that attained significance in the univariable regression were included in the multivariable analysis.

Mixed-effect regression with the linear spline model was fitted for the longitudinal analysis of each immune cell subpopulation after transplantation. Previously, the absolute cell values of T and NK cells were age adjusted and logarithmically transformed [32]. T-NK cells were only logarithmically transformed because of a lack of pediatric norm values. Furthermore, patients were classified into groups according the IT and GVHD grade.

Statistical tests were 2-sided with a significance level of 5% and 95% confidence interval. Data analysis was performed using the R software for statistical computing, Version 3.1.3. (R: A Language and Environment for Statistical Computing, http://www.R-project.org/). The survival package (Therneau, http://CRAN.R-project.org/package=survival), cmprsk package (Gray, http://CRAN.R-project.org/package=cmprsk), and nlme package (Pinheiro et al., http://CRAN.R-project.org/package=nlme) were used.

RESULTS

Patients

From January 1, 2005 to July 1, 2014, 89 consecutive patients with ALL received their first allogeneic HSCT in Frankfurt/Main, Germany.

Patients' ages ranged from 2.2 to 26.0 years (Table 1). Precursor B cell ALL was diagnosed in 63 patients, T cell ALL in 20 patients, and biphenotypic/bilinear ALL in 6 patients. Transplantation was performed as recommended by the respective treatment protocols (Cooperative study group for childhood acute lymphoblastic leukaemia or Associazione Italiana