Biol Blood Marrow Transplant 22 (2016) 462–469

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Allogeneic Stem Cell Transplantation Improves Survival in Patients with Acute Myeloid Leukemia Characterized by a High Allelic Ratio of Mutant *FLT3-ITD*

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Article history:
Received 21 April 2015
Accepted 28 October 2015

Key Words:
Allogeneic transplantation
Acute myeloid leukemia
FLT3-ITD
NPM1 mutation

A B S T R A C T

Allogeneic hematopoietic cell transplantation (alloHCT) as a postremission therapy in patients with *FLT3-ITD*–positive intermediate-risk acute myeloid leukemia (AML) remains controversial. *FLT3-ITD* mutations are heterogeneous with respect to allelic ratio, location, and length of the insertion, with a high mutant-to-wild-type ratio consistently associated with inferior prognosis. We retrospectively analyzed the role of alloHCT in first remission in relationship to the allelic ratio and presence or absence of nucleophosmin 1 mutations (*NPM1*) in the Study Alliance Leukemia AML2003 trial. *FLT3-ITD* mutations were detected in 209 patients and concomitant *NPM1* mutations in 148 patients. Applying a predefined cutoff ratio of .8, AML was grouped into high- and low-ratio *FLT3-ITD* AML ($HR^{FLT3\text{-}ITD}$ and $LR^{FLT3\text{-}ITD}$). Sixty-one patients (29%) were transplanted in first remission. Overall survival (OS) (HR, .3; 95% CI, .16 to .7; $P = .004$) and event-free survival (EFS) (HR, .4; 95% CI, .16 to .9; $P = .02$) were significantly increased in patients with $HR^{FLT3\text{-}ITD}$ AML who received alloHCT as consolidation treatment compared with patients who received consolidation chemotherapy. Patients with $LR^{FLT3\text{-}ITD}$ AML and wild-type *NPM1* who received alloHCT in first remission had increased OS (HR, .3; 95% CI, .1 to .8; $P = .02$) and EFS (HR, .2; 95% CI, .1 to .8; $P = .02$), whereas alloHCT in first remission did not have a significant impact on OS and EFS in patients with $LR^{FLT3\text{-}ITD}$ AML and concomitant *NPM1* mutation. In conclusion, our results provide additional evidence that alloHCT in first remission improves EFS and OS in patients with $HR^{FLT3\text{-}ITD}$ AML and in patients with $LR^{FLT3\text{-}ITD}$ AML and wild-type *NPM1*.

INTRODUCTION

Internal tandem duplication (ITD) mutations affecting the juxtamembrane domain of the tyrosine receptor kinase, fms-like tyrosine kinase 3 (FLT3), have been reported in

Financial disclosure: See Acknowledgments on page 468.

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http://dx.doi.org/10.1016/j.bbmt.2015.10.023
1083-8791/

approximately 20% to 30% of all patients with acute myeloid leukemia (AML) [1-5]. For AML with intermediate risk (ie, normal karyotype using cytogenetic analysis), *FLT3-ITD* mutations have been identified as the most significant prognostic factor in predicting relapse and poor overall survival (OS). Although most patients with *FLT3-ITD* positive (*FLT3-ITD*$^+$) AML might achieve a complete remission (CR) with conventional chemotherapy [2-4,6-9], the duration of CR is usually shorter, relapse rates are higher, and the median OS is shorter than in other intermediate risk AML subtypes.

The molecular mechanisms that lead to the poor outcome associated with *FLT3-ITD*$^+$ AML are still not completely understood. Several studies have shown that the relative mutant level might play a significant role [2,3,10,11]. Loss of *wt-FLT3* is seen in approximately 10% to 15% of patients at diagnosis [2,3]. This proportion increases significantly in relapse, indicating that loss of *wt-FLT3* is a mechanism of disease progression. Likewise, several studies reported a poor prognosis in patients with a high mutant-to-wild-type (mut/wt) allelic ratio of *FLT3*. In a retrospective quantitative analysis, our group has demonstrated that a high mut/wt ratio (>.78) was prognostic of poor OS and disease-free survival [3]. In a large cohort of young adult AML patients uniformly treated in 2 clinical trials within the United Kingdom Medical Research Council AML 10 and 12, Gale et al. [4] found a trend for higher relapse risk and lower OS with increasing *FLT3-ITD* mutant level. In addition to the allelic ratio, increasing size of the ITD (large, ≥40; small, <40 versus no ITD) and the location of the insertion (juxtamembrane versus first tyrosine kinase domain) have been associated with decreasing OS and relapse-free survival at 5 years [9,10,12].

Summarizing the results of the major studies focusing on patients with *FLT3-ITD*$^+$ AML, prevention of relapse by intensive postremission therapy remains a major challenge. Consolidation chemotherapy and autologous and allogeneic hematopoietic stem cell transplantation (HCT) have been adopted as postremission therapy for patients with poor risk AML. For patients with intermediate risk AML and high *FLT3* mut/wt ratio, no convincing evidence exists that consolidation chemotherapy with regimens containing high-dose cytosine arabinoside is able to improve the long-term outcome [5,7,11]. Although a few studies have suggested that autologous transplantation might improve event-free survival (EFS), randomized trials have thus far not demonstrated any long-lasting benefit for autologous HCT [6,13-16]. The role of allogeneic transplantation (alloHCT) is even less well defined, despite the favorable results reported in retrospective analyses [4,5,15,17,18].

In the randomized Study Alliance Leukemia AML2003 trial, patients were randomized between different postremission therapies, including allogeneic and autologous transplantations and chemotherapy. In addition to cytogenetic risk and early blast clearance, the patients were stratified by the ratio of mut/wt *FLT3* alleles. Here, we report the results of a planned subgroup analysis on the impact of alloHCT in patients with high versus low *FLT3-ITD* allelic ratio.

METHODS

Study Design

Between December 2003 and November 2009, 1179 patients between ages 16 and 60 years with de novo AML, secondary AML, or refractory anemia with excess blasts were enrolled into the randomized, multicenter AML2003 trial. The study (NCT00180102) was approved by all involved ethics committees and regulatory authorities and conducted in accordance with the Helsinki declaration.

AML treatment was risk-adapted and priority-based depending on donor availability. HLA typing of the patient and provisional unrelated donor search was performed at diagnosis. All patients received the classical "7+3" regimen with daunorubicin and cytarabine as induction therapy. In a 2-by-2 factorial design, 2 transplant strategies and 2 postremission chemotherapies were compared. Cytogenetic risk was classified according to modified Medical Research Council criteria as reported previously [19]. Here, we report on a subgroup analysis of patients with intermediate risk AML and *FLT3-ITD*.

Analysis of the FLT3-ITD mut/wt Ratio

Molecular analyses for *FLT3-ITD* mutations were performed by PCR in the reference laboratory in Dresden, as reported earlier [3]. Calculation of the *FLT3-ITD* mut/wt allelic ratio was based on high-resolution fragment analysis. The sensitivity of this method was 1%. Patients with an allelic ratio of mutant *FLT3-ITD* > .8 were denoted high ratio *FLT3-ITD* mutants ($HR^{FLT3\text{-}ITD}$), whereas the remaining *FLT3-ITD*$^+$ patients were denoted low ratio mutants ($LR^{FLT3\text{-}ITD}$).

Study Treatment

In the control arm all patients were scheduled for HLA-identical sibling transplantation in first CR (CR1) irrespective of the allelic ratio and day +15 response assessment. Patients without an HLA-identical sibling donor received consolidation chemotherapy.

In the experimental arm all patients were scheduled for alloHCT in aplasia from an HLA-compatible related or unrelated donor, except for patients with $LR^{FLT3\text{-}ITD}$ and ≤10% blasts in the day 15 marrow aspirate who were planned for alloHCT in CR1 from an HLA-identical sibling donor. Patients without an HLA-identical sibling or HLA-compatible unrelated donor were scheduled for further postremission chemotherapy as recently published [19].

Conditioning in aplasia after induction chemotherapy consisted of fludarabine 30 mg/m^2 on days −6 to −2 and melphalan 150 mg/m^2 on day −2. Graft-versus-host disease (GVHD) prophylaxis was performed with antithymocyte globulin (Fresenius) 10 mg/kg on days −5 to −2 in patients with unrelated donors and targeted cyclosporine starting on day −1 before transplantation.

Conditioning in CR1 consisted of 6 × 2 Gy total body irradiation administered on days −6 to −4 in combination with cyclophosphamide 60 mg/kg on days −3 and −2 or 4 × 2 Gy total body irradiation on days −5 and −4 in combination with fludarabine 30 mg/m^2 on days −3 to −1. GVHD prophylaxis consisted of a combination of cyclosporine and methotrexate. Patients with matched unrelated donors received antithymocyte globulin (Fresenius) 20 mg/kg on days −3 to −1.

Statistical Analysis

Data from the AML2003 study were used for a subgroup analysis on the impact of alloHCT in patients with high versus low ratio of mut/wt *FLT3-ITD*. OS and EFS were analyzed in multivariable Cox regression models. For the intention-to-treat analysis study, treatment by randomization was analyzed in patients with high versus low ratio of mut/wt *FLT3-ITD* AML, adjusting for the second randomization on consolidation chemotherapy. The study had not been powered for this subgroup analysis. For the as-treated analysis, the impact of alloHCT was analyzed as a time-dependent covariate adjusting for age, high versus low ratio of mut/wt *FLT3* alleles, absence of an *NPM1* mutation, the logarithm of the WBC count and the lactate dehydrogenase at diagnosis, and performance status. Multiple imputation was used to deal with missing information on single covariates. The effect of alloHCT as a time-dependent intervention was visualized with Simon Makuch plots [20]. These plots adjust for subjects under observation who switched from the control group (no transplantation in first remission) to the experimental group (alloHCT in first remission).

Demographic factors and disease characteristics were compared using the Mann-Whitney test for continuous and ordinal variables and the uncorrected chi-squared test for categorical variables. The CR rate was determined in the full analysis set by dividing all patients who achieved a CR with incomplete hematologic recovery (CR_i) or CR by all patients who entered the trial. CR rates were compared by means of a logistic regression model including indicators for treatment and high versus low *FLT3* ratio.

The Kaplan-Meier method was used to estimate OS, EFS, and leukemia-free survival. The cumulative incidence function for competing events was used to estimate nonrelapse mortality (NRM) and cumulative incidence of relapse (CIR). Comparisons of NRM and CIR in selected subgroups were performed with the Gray test. Point estimates for survival endpoints were reported together with 95% confidence intervals (CIs).

The study database was locked as of September 13, 2012 for this subgroup analysis. Statistical analyses were performed with SPSS version 19.0