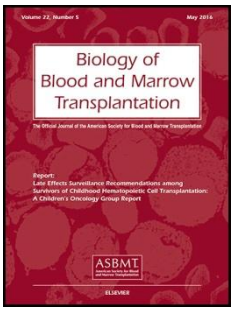
Accepted Manuscript



Title: Achieving a Molecular Remission before Allogeneic Stem Cell Transplantation in Adult Patients with Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia: Impact on Relapse and Long Term Outcome

Author: Federico Lussana, Tamara Intermesoli, Francesca Gianni, Cristina Boschini, Arianna Masciulli, Orietta Spinelli, Elena Oldani, Manuela Tosi, Anna Grassi, Margherita Parolini, Ernesta Audisio, Chiara Cattaneo, Roberto Raimondi, Emanuele Angelucci, Irene Maria Cavattoni, Anna Maria Scattolin, Agostino Cortelezzi, Francesco Mannelli, Fabio Ciceri, Daniele Mattei, Erika Borlenghi, Elisabetta Terruzzi, Claudio Romani, Renato Bassan, Alessandro Rambaldi

PII: S1083-8791(16)30257-9
DOI: http://dx.doi.org/doi: 10.1016/j.bbmt.2016.07.021
Reference: YBBMT 54352

To appear in: *Biology of Blood and Marrow Transplantation*

Received date: 26-4-2016
Accepted date: 22-7-2016

Please cite this article as: Federico Lussana, Tamara Intermesoli, Francesca Gianni, Cristina Boschini, Arianna Masciulli, Orietta Spinelli, Elena Oldani, Manuela Tosi, Anna Grassi, Margherita Parolini, Ernesta Audisio, Chiara Cattaneo, Roberto Raimondi, Emanuele Angelucci, Irene Maria Cavattoni, Anna Maria Scattolin, Agostino Cortelezzi, Francesco Mannelli, Fabio Ciceri, Daniele Mattei, Erika Borlenghi, Elisabetta Terruzzi, Claudio Romani, Renato Bassan, Alessandro Rambaldi, Achieving a Molecular Remission before Allogeneic Stem Cell Transplantation in Adult Patients with Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia: Impact on Relapse and Long Term Outcome, *Biology of Blood and Marrow Transplantation* (2016), http://dx.doi.org/doi: 10.1016/j.bbmt.2016.07.021.

Achieving a molecular remission before allogeneic stem cell transplantation in adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia: impact on relapse and long term outcome

Federico Lussana[1], Tamara Intermesoli[1], Francesca Gianni[1], Cristina Boschini[1], Arianna Masciulli[1], Orietta Spinelli[1], Elena Oldani[1], Manuela Tosi[1], Anna Grassi[1], Margherita Parolini[1], Ernesta Audisio[2], Chiara Cattaneo[3], Roberto Raimondi[4], Emanuele Angelucci[5], Irene Maria Cavattoni[6], Anna Maria Scattolin[7], Agostino Cortelezzi[8], Francesco Mannelli[9], Fabio Ciceri[10], Daniele Mattei[11], Erika Borlenghi[3], Elisabetta Terruzzi[12], Claudio Romani[5], Renato Bassan[7] and Alessandro Rambaldi[1,13]

[1]Hematology and Bone Marrow Transplant Unit, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy; [2]Hematology Department, AOU Città della Salute e della Scienza di Torino, Torino, Italy; [3]Hematology, Spedali Civili di Brescia, Brescia, Italy; [4]Hematology and BMT Unit, Ospedale San Bortolo, Vicenza, Italy; [5] Ematologia e Centro Trapianti, Ospedale Oncologico di Riferimento Regionale 'Armando Businco', Azienda Ospedaliera "Brotzu", Cagliari, Italy; [6]Hematology and BMT Unit, Central Hospital of Bolzano, Bolzano, Italy; [7]Hematology, Ospedale dell'Angelo & Ospedale SS. Giovanni e Paolo, Mestre-Venezia, Italy; [8]Oncohematology Unit, University of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [9]Hematology Department, University of Florence, , AOU Careggi , Firenze, Italy; [10]Hematology and Bone Marrow Transplantation Unit, San Raffaele Hospital Scientific Institute, Milan, Italy; [11]Department of Hematology, Ospedale S. Croce, Cuneo, Italy; [12]Hematology Division and BMT Unit, Ospedale San Gerardo, Monza, Italy; [13] Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano, Milano, Italy

Address for correspondence:

Federico Lussana, MD
Hematology and Bone Marrow Transplant Unit
Ospedale Papa Giovanni XXIII Bergamo
Piazza OMS, 1
24127 Bergamo
ITALY
Telephone: +39 035 2673684
Fax:+39 035 2674968
Email: flussana@asst-pg23.it

Short Title: Impact of molecular remission before alloHSCT in Ph+ adult ALL

Declaration of interests

The authors do not have any commercial association, which might pose a conflict of interest.

None funding.

Highlights:

- MRD positivity in ALL is a major risk factor for relapse and poor outcomes
- We evaluated the impact of MRD levels before alloHSCT on outcomes of Ph+ ALL
- Patients with measurable levels of MRD have a higher risk of relapse
- Achieving a MRD negativity should be a prerequisite for successful alloHSCT

Abstract

Background. Allogeneic stem cell transplantation (alloHSCT) in first complete remission (CR1) remains the consolidation therapy of choice in Philadelphia positive acute lymphoblastic leukemia (Ph+ ALL). The prognostic value of measurable levels of minimal residual disease (MRD) at time of conditioning is a matter of debate.

Methods. We analyzed the predictive relevance of MRD levels before transplant on the clinical outcome of Ph+ALL patients treated with chemotherapy and imatinib in 2 consecutive prospective clinical trials. MRD evaluation before transplant was available for 65 of the 73 patients who underwent an alloHSCT in CR1.

Results. A complete or major molecular response at time of conditioning was achieved in 24 patients (37%) while 41 (63%) remained carriers of any other positive MRD level in the bone marrow. The MRD negativity at time of conditioning was associated with a significant benefit in terms of risk of relapse at 5 years with a relapse incidence of 8% compared to 39% of patients with MRD positivity (p=0.007). However, thanks to the post-transplant use of tyrosine kinase inhibitors (TKIs), the disease free survival probability was 58% vs 41% (p=0.17) and the overall survival was 58% vs 49% (p=0.55) in MRD negative compared to MRD positive patients, respectively. The cumulative incidence of non relapse mortality was similar in the 2 groups.

Conclusions. Achieving a complete molecular remission before transplant reduces the risk of leukemia relapse even though TKIs may still rescue some patients relapsing after transplant.

Keywords: Acute Lymphoblastic Leukemia
Allogeneic transplantation
Minimal Residual Disease (MRD)

Introduction

In the pre-tyrosine kinase inhibitors (TKIs) era, Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL) marked the most unfavorable subgroup of adult ALL and the overall survival observed in unselected series of patients was less than 20%, even when allogeneic hematopoietic stem cell transplantation (alloHSCT) was offered [1,2]. The incorporation of TKIs, most commonly imatinib, into the standard ALL chemotherapy has substantially improved the outcomes mainly as a consequence of an improved rate of complete responses [3-6], a longer duration of remission [3,5,7,8] and an increased proportion of patients to whom an alloHSCT transplantation could be offered in first complete remission (CR1) [3,4,9]. To date alloHSCT remains the only treatment for which a definitive curative potential has been demonstrated in Ph+ ALL, even though promising durable remission have been demonstrated also for some patients treated with TKI based treatments [7,10,11]. The outcome of alloHSCT is largely dependent on the non-relapse mortality (NRM) and post-transplant relapse. While some improvement in reduction of NRM has been achieved, relapse remains a major cause of treatment failure [6]. In childhood and adult ALL patients, recent studies have provided reasonable support to suggest an inferior outcome of patients undergoing alloHSCT with measurable level of MRD at time of conditioning [12-14]. Since in Ph+ ALL a deeper molecular response is potentially achievable with innovative targeted therapies, such as second and third-generation TKIs or immunotherapy [9-11,15-18], an accurate

evaluation of MRD values before alloHSCT is mandatory and should guide the clinical decision making process. Therefore, we analyzed the prognostic value of the MRD level at time of conditioning before alloHSCT in patients enrolled into 2 consecutive prospective clinical trials conducted within the Northern Italy Leukemia Group (NILG).

Patients and methods

Patients, Diagnoses, and Minimal Residual Disease evaluations

One hundred and six consecutive Ph+ ALL adult patients (≥ 18 years), treated front-line with chemotherapy and imatinib (target dose 800-600 mg daily) were analysed. Detailed treatment descriptions of the 2 trials were reported previously [5,19] and according to the study design all patients in first complete remission (CR1) were eligible to perform an alloHSCT provided that a HLA identical related or unrelated donor could be identified. Individual levels of MRD were determined in the bone marrow and peripheral blood by quantitative polymerase chain reaction (RQ-PCR) according to validated methods [20-22]. Briefly, BCR-ABL level was expressed as ratio between BCR-ABL copy number and ABL copy number, both calculated on a plasmid dilution standard curve (Normalized Copy Number). Sensitivity and Quantitative Range (QR) of RQ-PCR method were defined according to European guidelines [20,22]. Sensitivity was 10 copies in all the experiments and QR varied from 100 and 10 copies. MRD level was expressed as logarithmic reduction of BCR-ABL level detected at fixed time point respect to diagnosis and was obtained by dividing BCR-ABL level at the selected time point by BCR-ABL level at diagnosis [21]. Complete molecular response was defined as undetectable disease and major molecular response was defined as > 4 log MRD reduction in bone marrow derived mononuclear cells. The post-transplant treatment with TKIs and/or donor lymphocyte infusions (DLI) was left free to each individual physician discretion.

The studies were approved by the institutional review board of all participating institutions, conducted in accordance with the declaration of Helsinki and registered with the National Clinical Trial Identifier numbers NCT00358072 (trial 09/00) and NCT00358072 (trial 10/07).

Study endpoints and statistical methods

MRD evaluation was performed in all patients before alloHSCT. Patients who achieved a complete or major molecular response at time of conditioning were defined as MRD negative (MRD-), while patients carriers of any other positive MRD level in the bone marrow derived mononuclear cells were defined as MRD positive (MRD+). The endpoints of the study were defined according to the Statistical Guidelines for EBMT [23]. The overall survival (OS) was defined as the probability of survival irrespective of disease state at any point in time from transplantation. Patients living at their last follow-up were censored. Disease free survival (DFS) was measured from the time of alloHSCT until relapse or death. The cumulative incidence of relapse (RI) was calculated as the time from transplantation to the first evidence of recurrence or progression of disease, with death with no prior relapse or progression as competing risk. Similarly, the non-relapse mortality (NRM) was defined as the probability of dying without a previous occurrence of relapse or progression, considering relapse or progression as competing risk. Relapse was defined by recurrence of more than 5% of lymphoblasts in peripheral blood or in the bone marrow and/or by the presence of extramedullary disease.

Baseline continuous characteristics were presented as median with range, and were compared among the two MRD groups using T-test or Mann- Whitney as appropriate. Categorical variables were reported with absolute and percentage frequencies and compared with Chi-squared test or Fisher's exact test. OS and DFS were estimated by the Kaplan-Meier method and any differences in MRD groups were evaluated with log-rank test. Competitive risk approach was applied to estimate cumulative incidence of both relapse and non-relapse mortality; Gray's test was used to compare the study groups. To evaluate transplant effect on OS, a Cox proportional hazard model with time dependent variable has been estimate on patients in CR1.

All reported P values are two-sided and statistical significance was set a P value less than 0.05. All analyses were performed with the use of R software, version 3.1.2

Results

Among 106 adult patients (median age 44.1, range 18.5-66.1) with newly diagnosed Ph+ ALL enrolled into 2 consecutive clinical trials, 100 patients (94%) achieved CR after induction, and 73 of these patients underwent an alloHSCT in CR1. In the remaining 27 patients, the reasons for not undergoing alloHSCT were: absence of donor (n=8), early death (n=4), poor performance status (n=1), early relapse (n=12), patient refusal (n=1) and unknown (n=1). Among these 73 patients, the MRD status measured at time of conditioning was available for 65 patients (89%) who are the subject of this report (Figure 1). The overall survival of patients who received or not an alloHSCT in first remission is shown in Figure 2. However, to overcome the bias of time to transplant, the therapeutic efficacy of transplant was tested by a Cox proportional hazard model with transplant as a time-dependent variable. When considering the 100 patients in CR1, the alloHSCT does not reduce significantly the risk of death (Hazard Ratio=0.68 [95%CI: 0.39-1.20], P=0.1840).

Twenty-four patients (37%) were in complete or major molecular response (undetectable disease or > 4 log MRD reduction) at time of conditioning (MRD- group), while 41 (63%) remained carriers of any positive MRD level in the bone marrow (MRD+ group) ranging from $1.3x10^{-4}$ to $2x10^{-1}$. Patients' characteristics were similar between MRD+ and MRD- groups, except for a higher hemoglobin levels and a predominance of male gender in MRD- group (Table 1). Thirty-one patients received alloHSCT from a sibling and 34 from unrelated donor. The conditioning regimen to transplant was myeloablative in 83% and reduced intensity in 17% of patients. The stem cell source was the bone marrow in 18.5%, the peripheral blood in 78.5% and cord blood in the remaining 3% of patients.

For the patient cohort analyzed for MRD before transplant (n=65), the 5 years OS was 52% (95% CI: 41% - 66%). The MRD negativity at time of conditioning was associated with a significant benefit in terms of risk of hematologic relapse at 5 years with a CIR of 8% (95% CI: 2% - 33%) compared to 39% (95% CI: 26% - 58%) of patients with MRD positivity (p=0.007) (Figure 3A). Nonetheless, the DFS and the OS probability at 5 years were not significantly different in MRD- compared to MRD+ patients (58% [95% CI: 42% - 82%] vs 41% [95% CI: 29% - 60%], p=0.17 and 58% [95% CI: 42% - 82%] vs 49% [95% CI: 36% - 67%], p=0.55, respectively) (Figure3B and 3C), probably as the consequence of an effective post-transplant treatment with TKIs and/or DLI. Patients who received TKIs after transplant were almost invariably MRD positive and about half of them converted to a stable MRD negative condition. Patients, who did not achieve a molecular remission, invariably suffered a subsequent clinical relapse. Among the 33 patients receiving TKIs post-transplant, imatinib was the more frequently used: imatinb (n=22), dasatinib (n=8), imatinib followed by dasatinib (n=2) and dasatinib followed by ponatinib (n=1). Not surprisingly, the need of post-transplant treatment with TKIs and/or DLI was significantly higher among MRD+ group compared with MRD- group (61% vs 33% p=0.034) (Figure 4). The cumulative incidence of non relapse mortality at 5 years was similar in MRD- compared to that of MRD+ group (33% [95% CI; 19% - 60%] vs 20% [95% CI: 10% - 37%], p=0.22). Different levels of MRD positivity before transplant ($\leq 10^{-4}$ level, $>10^{-4}$ to $< 10^{-3}$, and $\geq 10^{-3}$) did not translate into a significantly different clinical outcome.

Discussion

To our knowledge, this is one of the largest prospective analysis evaluating the long term impact of pre-transplant MRD level on the clinical outcome of Ph+ ALL patients undergoing alloHSCT. Our results confirm previous reports indicating that the addition of imatinib improved the CR rate (94%) and the proportion of adult patients with Ph+ ALL to whom an alloHSCT could be offered [4,6,8,9]. The very long-term follow-up of this analysis indicates that survival can be achieved close to 50% for patients with Ph+ ALL after alloHSCT, a significant improvement compared to the pre-imatinib era [1-3]. Our study confirms that alloHSCT can be offered to a significant proportion of Ph+ALL patients and remains the most active post-remissional treatment of choice. The main finding of our study is that patients undergoing alloHSCT with measurable levels of MRD have a significant higher risk of relapse after transplant. In agreement with our results, very recently the Japan Society for Hematopoietic Cell Transplantation found in a retrospective analysis of data from 432 adult Ph+ ALL patients that the incidence of relapse in MRD negative patients at transplant was significantly lower compared to patients transplanted in MRD positivity (19% vs 29%, respectively) [24]. Accordingly, any effort should be done to achieve a deep molecular response before the beginning of the conditioning regimen. Although in our study the significant reduction of relapse did not translate into a clear benefit in terms of DFS and OS, this result is due to the remarkable NRM observed in patients undergoing transplantation being MRD negative and to the effective response frequently obtained with the post-transplant use of TKIs with or without DLI. In keeping with this interpretation, treatments after transplant with TKIs and/or DLI were significantly more used among MRD+ group compared with MRD- group. Moreover, patients who received a TKI based treatment had the chance to convert to a stable MRD negative condition in half the cases, suggesting that TKIs treatment, given as salvage option after transplantation, may be an effective rescue for a

remarkable proportion of patients. It is worth noting that previous experiences that explored the use of prophylactic imatinib and nilotinib maintenance after transplantation [25,26] showed a high efficacy of treatment in preventing relapse, but this was hampered by a high rate of early discontinuation due to poor tolerability [25,26]. Unfortunately, our database did not contain specific data about the characteristics and the complications of post-HSCT treatments (i.e. DLI cell doses, cumulative incidence of chronic GvHD, TKIs related complications) nor information about the presence of specific ABL mutations such as the T315I. Therefore, a formal evaluation of their impact in terms of comorbidity, quality of life and treatment response is limited. Treatment with TKIs after alloHSCT can be difficult to tolerate and may be followed by a high rate of early discontinuation. Therefore, a careful risk assessment of each patient with Ph+ ALL, may lead to timely initiation of an effective treatment with TKIs and to avoid it, if not strictly necessary. In this context, the information of this study is potentially very useful and suggests to limit TKIs prophylactic use to patients at high risk of relapse, such as MRD positive patients before transplant and to apply a MRD-driven strategy in MRD negative patients. Our results suggest that a deep molecular response before transplant predict a better outcome, but in agreement with the EBMT results [6], we have no evidence that increasing levels of MRD positivity are associated with different risk of relapse, even though a larger group of patients is requested to validate this result.

The concept that alloHSCT remains the standard of care for any patient suitable for the procedure and with an available donor has been recently underlined by the French clinical trial recently published by Chalandon et al [9]. However, patients achieving an early molecular CR through the combination of specific targeted therapies plus chemotherapy may identify patients who may be possibly cured without alloHSCT. In this regard, a recent study showed that a negative MRD status after three months and beyond of a TKIs based chemotherapy program was associated with a low risk of relapse and an OS above 60% at 3–5 years in

patients excluded from HSCT as first-line therapy [7]. Moreover, a more recent phase 2 trial, that examined the combination of chemotherapy with ponatinib, showed that long-term DFS was not affected by alloHSCT in patients achieving MRD negative status [11]. These interesting results confirm the importance of achieving a complete molecular remission, even suggesting that the paradigm of myeloablative alloHSCT, as indispensable post-remission therapy in adult Ph+ ALL, may be subject to revision. Moreover, these latter results [11] strongly emphasized the absolute need to reduce the early and late NRM to an acceptable level and this may lead to dispute the need of a myeloablative conditioning (MAC) regimen in most of these patients. In this respect, Buchanova et al. recently showed that reduced intensity conditioning (RIC) alloHSCT may represent a viable alternative to MAC alloHSCT for patients with Ph+ ALL that have achieved a MRD negative status, mainly for older patients, who were at a greater risk of NRM [27]. In our study, only 14 patients received a bone marrow (n=12) or a cord blood (n= 2) graft, while a non myeloablative conditioning regimen was given only to 11 patients. These small numbers prevent drawing any meaningful interpretation from our results. Yet, further studies specifically designed to test prospectively whether or not different stem cell source or conditioning regimens at lower intensity may prove equally effective and safe for patients achieving a negative MRD status need to be developed.

In conclusion, our results reinforce evidence that patients undergoing alloHSCT with measurable level of MRD clearly show an inferior outcome after transplant in terms of relapse incidence and this information is certainly useful for an effective HSCT planning. Indeed, an effort to achieve a convincing molecular CR should be pursued and considered an essential prerequisite for successful alloHSCT along with the reduction of the NRM. In this respect, the future challenge will be to achieve a deep molecular response thanks to an

appropriate combination of the available therapies, with third generation TKIs with or without new antibody or cellular therapies [28-30].

Acknowledgments

The authors do not have any commercial association, which might pose a conflict of interest.

None funding.

References

1. Bassan R, Hoelzer D. Modern therapy of acute lymphoblastic leukemia. J Clin Oncol 2011;29:532-543.

2. Fielding AK, Rowe JM, Richards SM, et al. Prospective outcome data on 267 unselected adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia confirms superiority of allogeneic transplantation over chemotherapy in the pre-imatinib era: results from the International ALL Trial MRC UKALLXII/ECOG2993. Blood 2009;113:4489-4496.

3. Thomas DA, Faderl S, Cortes J, et al. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. Blood 2004;103:4396-4407.

4. de Labarthe A, Rousselot P, Huguet-Rigal F, et al. Imatinib combined with induction or consolidation chemotherapy in patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: results of the GRAAPH-2003 study. Blood 2007;109:1408-1413.

5. Bassan R, Rossi G, Pogliani EM, et al. Chemotherapy-phased imatinib pulses improve long-term outcome of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Northern Italy Leukemia Group protocol 09/00. J Clin Oncol 2010;28:3644-3652.

6. Brissot E, Labopin M, Beckers MM, et al. Tyrosine kinase inhibitors improve long-term outcome of allogeneic hematopoietic stem cell transplantation for adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia. Haematologica 2015;100:392-399.

7. Ravandi F, Jorgensen JL, Thomas DA, et al. Detection of MRD may predict the outcome of patients with Philadelphia chromosome-positive ALL treated with tyrosine kinase inhibitors plus chemotherapy. Blood 2013;122:1214-1221.

8. Fielding AK, Rowe JM, Buck G, et al. UKALLXII/ECOG2993: addition of imatinib to a standard treatment regimen enhances long-term outcomes in Philadelphia positive acute lymphoblastic leukemia. Blood 2014;123:843-850.

9. Chalandon Y, Thomas X, Hayette S, et al. Randomized study of reduced-intensity chemotherapy combined with imatinib in adults with Ph-positive acute lymphoblastic leukemia. Blood 2015;125:3711-3719.

10. Foa R, Vitale A, Vignetti M, et al. Dasatinib as first-line treatment for adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood 2011;118:6521-6528.

11. Jabbour E, Kantarjian H, Ravandi F, et al. Combination of hyper-CVAD with ponatinib as first-line therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia: a single-centre, phase 2 study. Lancet Oncol 2015;16:1547-1555.

12. Balduzzi A, Di Maio L, Silvestri D, et al. Minimal residual disease before and after transplantation for childhood acute lymphoblastic leukaemia: is there any room for intervention? Br J Haematol 2014;164:396-408.

13. Spinelli O, Peruta B, Tosi M, et al. Clearance of minimal residual disease after allogeneic stem cell transplantation and the prediction of the clinical outcome of adult patients with high-risk acute lymphoblastic leukemia. Haematologica 2007;92:612-618.

14. Bar M, Wood BL, Radich JP, et al. Impact of Minimal Residual Disease, Detected by Flow Cytometry, on Outcome of Myeloablative Hematopoietic Cell Transplantation

for Acute Lymphoblastic Leukemia. Leukemia Research and Treatment 2014;2014: 421723 .

15. Vignetti M, Fazi P, Cimino G, et al. Imatinib plus steroids induces complete remissions and prolonged survival in elderly Philadelphia chromosome-positive patients with acute lymphoblastic leukemia without additional chemotherapy: results of the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) LAL0201-B protocol. Blood 2007;109:3676-3678.

16. Pfeifer H, Wassmann B, Pavlova A, et al. Kinase domain mutations of BCR-ABL frequently precede imatinib-based therapy and give rise to relapse in patients with de novo Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). Blood 2007;110:727-734.

17. Topp MS, Gokbuget N, Zugmaier G, et al. Long-term follow-up of hematologic relapse-free survival in a phase 2 study of blinatumomab in patients with MRD in B-lineage ALL. Blood 2012;120:5185-5187.

18. Martinelli G, Dombret H, Chevallier P, et al. Complete Molecular and Hematologic Response in Adult Patients with Relapsed/Refractory (R/R) Philadelphia Chromosome-Positive B-Precursor Acute Lymphoblastic Leukemia (ALL) Following Treatment with Blinatumomab: Results from a Phase 2 Single-Arm, Multicenter Study (ALCANTARA). Blood 2015;126:679-679.

19. Bassan R, Masciulli A, Intermesoli T, et al. Randomized trial of radiation-free central nervous system prophylaxis comparing intrathecal triple therapy with liposomal cytarabine in acute lymphoblastic leukemia. Haematologica 2015;100(6):786-793.

20. Gabert J, Beillard E, van der Velden VH, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion

gene transcripts for residual disease detection in leukemia - a Europe Against Cancer program. Leukemia 2003;17:2318-2357.

21. Beillard E, Pallisgaard N, van der Velden VH, et al. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) - a Europe against cancer program. Leukemia 2003;17:2474-2486.

22. van der Velden VH, Panzer-Grumayer ER, Cazzaniga G, et al. Optimization of PCR-based minimal residual disease diagnostics for childhood acute lymphoblastic leukemia in a multi-center setting. Leukemia 2007;21:706-713.

23. Iacobelli S. Suggestions on the use of statistical methodologies in studies of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant 2013;48 Suppl 1:S1-37.

24. Nishiwaki S, Imai K, Mizuta S, et al. Impact of MRD and TKI on allogeneic hematopoietic cell transplantation for Ph+ALL: a study from the adult ALL WG of the JSHCT. Bone Marrow Transplant 2016;51:43-50.

25. Pfeifer H, Wassmann B, Bethge W, et al. Randomized comparison of prophylactic and minimal residual disease-triggered imatinib after allogeneic stem cell transplantation for BCR-ABL1-positive acute lymphoblastic leukemia. Leukemia 2013;27:1254-1262.

26. Shimoni A, Volchek Y, Koren-Michowitz M, et al. Phase 1/2 study of nilotinib prophylaxis after allogeneic stem cell transplantation in patients with advanced chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia. Cancer 2015;121:863-871.

27. Bachanova V, Marks DI, Zhang MJ, et al. Ph+ ALL patients in first complete remission have similar survival after reduced intensity and myeloablative allogeneic

transplantation: impact of tyrosine kinase inhibitor and minimal residual disease. Leukemia 2014;28:658-65.

28. Topp MS, Gokbuget N, Stein AS, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. Lancet Oncol 2015;16:57-66.

29. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014;371:1507-1517.

30. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet 2014;385:517-528.

Figure Legends

Figure 1. Study flow
Figure 2. Overall survival according to alloHSCT in first remission
Figure 3 A-B-C. Cumulative incidence of hematologic relapse, DFS and OS by MRD group
Figure 4. Number of post-transplant treatments by MRD group

Table 1. Patients' characteristics according to MRD group

Characteristics	All patients (N=65)	MRD negative (N=24)	MRD positive (N=41)	P
Age years , median (range)	43.2 (18.5-62.4)	45 (21.4-58.2)	42.7 (18.5-62.4)	0.8340
Male sex (%)	31 (47.7)	16 (66.7)	15 (36.6)	0.0191
WBC, X 10^9/L, median (range)	15 (0.9-680)	27.7 (0.9-350)	12 (1.1-680)	0.1238
Hemoglobin, g/dL, median (range)	9.6 (3.7-16.5)	11.4 (5.4-14.6)	9 (3.7-16.5)	0.0191
Platelets, X 10^9/L, median (range)	37 (3-336)	41 (4-336)	34 (3-325)	0.4374
LDH, U/L median (range)	795 (65-8104)	1231 (353-8104)	715 (65-6194)	0.1147
Conditioning regimen (%) Reduced intensity Myeloablative	 11 (16.9) 54 (83.1)	 4 (16.7) 20 (83.3)	 7 (17.1) 34 (82.9)	1.00
Donor type (%) Sibling Unrelated	 31 (47.7) 34 (52.3)	 13 (54.2) 11 (45.8)	 18 (43.9) 23 (56.1)	0.4240

Graft type (%) Bone marrow Peripheral blood Cord blood	12 (18.5) 51 (78.5) 2 (3.1)	3 (12.5) 20 (83.3) 1 (4.2)	9 (22) 31 (75.6) 1 (2.4)	0.5749

§ MRD negative vs MRD positive

WBC: white blood cells; LDH: lactate dehydrogenase