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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

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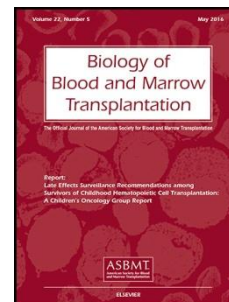
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1 **Achieving a molecular remission before allogeneic stem cell transplantation**
 2 **in adult patients with Philadelphia chromosome positive acute**
 3 **lymphoblastic leukemia: impact on relapse and long term outcome**

4
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35 **Short Title:** Impact of molecular remission before alloHSCT in Ph+ adult ALL

36 **Declaration of interests**

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

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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.

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

3

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

7

8

Introduction

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

1 evaluation of MRD values before alloHSCT is mandatory and should guide the clinical
2 decision making process. Therefore, we analyzed the prognostic value of the MRD level at
3 time of conditioning before alloHSCT in patients enrolled into 2 consecutive prospective
4 clinical trials conducted within the Northern Italy Leukemia Group (NILG).
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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 methods²⁰⁻²². 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 diagnosis²¹. 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).

1 *Study endpoints and statistical methods*

2 MRD evaluation was performed in all patients before alloHSCT. Patients who
3 achieved a complete or major molecular response at time of conditioning were defined as
4 MRD negative (MRD-), while patients carriers of any other positive MRD level in the bone
5 marrow derived mononuclear cells were defined as MRD positive (MRD+). The endpoints of
6 the study were defined according to the Statistical Guidelines for EBMT²³. The overall
7 survival (OS) was defined as the probability of survival irrespective of disease state at any
8 point in time from transplantation. Patients living at their last follow-up were censored.
9 Disease free survival (DFS) was measured from the time of alloHSCT until relapse or death.
10 The cumulative incidence of relapse (RI) was calculated as the time from transplantation to
11 the first evidence of recurrence or progression of disease, with death with no prior relapse or
12 progression as competing risk. Similarly, the non-relapse mortality (NRM) was defined as the
13 probability of dying without a previous occurrence of relapse or progression, considering
14 relapse or progression as competing risk. Relapse was defined by recurrence of more than 5%
15 of lymphoblasts in peripheral blood or in the bone marrow and/or by the presence of
16 extramedullary disease.

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

1 All reported P values are two-sided and statistical significance was set a P value less than
2 0.05. All analyses were performed with the use of R software, version 3.1.2

3 **Results**

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

16 Twenty-four patients (37%) were in complete or major molecular response
17 (undetectable disease or > 4 log MRD reduction) at time of conditioning (MRD- group),
18 while 41 (63%) remained carriers of any positive MRD level in the bone marrow (MRD+
19 group) ranging from 1.3×10^{-4} to 2×10^{-1} . Patients' characteristics were similar between MRD+
20 and MRD- groups, except for a higher hemoglobin levels and a predominance of male gender
21 in MRD- group (Table 1). Thirty-one patients received alloHSCT from a sibling and 34 from
22 unrelated donor. The conditioning regimen to transplant was myeloablative in 83% and
23 reduced intensity in 17% of patients. The stem cell source was the bone marrow in 18.5%, the
24 peripheral blood in 78.5% and cord blood in the remaining 3% of patients.

1 For the patient cohort analyzed for MRD before transplant (n=65), the 5 years OS was 52%
2 (95% CI: 41% - 66%). The MRD negativity at time of conditioning was associated with a
3 significant benefit in terms of risk of hematologic relapse at 5 years with a CIR of 8% (95%
4 CI: 2% - 33%) compared to 39% (95% CI: 26% - 58%) of patients with MRD positivity
5 (p=0.007) (Figure 3A). Nonetheless, the DFS and the OS probability at 5 years were not
6 significantly different in MRD- compared to MRD+ patients (58% [95% CI: 42% - 82%] vs
7 41% [95% CI: 29% - 60%], p=0.17 and 58% [95% CI: 42% - 82%] vs 49% [95% CI: 36% -
8 67%], p=0.55, respectively) (Figure 3B and 3C), probably as the consequence of an effective
9 post-transplant treatment with TKIs and/or DLI. Patients who received TKIs after transplant
10 were almost invariably MRD positive and about half of them converted to a stable MRD
11 negative condition. Patients, who did not achieve a molecular remission, invariably suffered a
12 subsequent clinical relapse. Among the 33 patients receiving TKIs post-transplant, imatinib
13 was the more frequently used: imatinib (n=22), dasatinib (n=8), imatinib followed by
14 dasatinib (n=2) and dasatinib followed by ponatinib (n=1). Not surprisingly, the need of post-
15 transplant treatment with TKIs and/or DLI was significantly higher among MRD+ group
16 compared with MRD- group (61% vs 33% p=0.034) (Figure 4). The cumulative incidence of
17 non relapse mortality at 5 years was similar in MRD- compared to that of MRD+ group (33%
18 [95% CI; 19% - 60%] vs 20% [95% CI: 10% - 37%], p=0.22). Different levels of MRD
19 positivity before transplant ($\leq 10^{-4}$ level, $>10^{-4}$ to $< 10^{-3}$, and $\geq 10^{-3}$) did not translate into a
20 significantly different clinical outcome.

Discussion

1
2 To our knowledge, this is one of the largest prospective analysis evaluating the long
3 term impact of pre-transplant MRD level on the clinical outcome of Ph+ ALL patients
4 undergoing alloHSCT. Our results confirm previous reports indicating that the addition of
5 imatinib improved the CR rate (94%) and the proportion of adult patients with Ph+ ALL to
6 whom an alloHSCT could be offered^{4,6,8,9}. The very long-term follow-up of this analysis
7 indicates that survival can be achieved close to 50% for patients with Ph+ ALL after
8 alloHSCT, a significant improvement compared to the pre-imatinib era¹⁻³. Our study
9 confirms that alloHSCT can be offered to a significant proportion of Ph+ALL patients and
10 remains the most active post-remissional treatment of choice. The main finding of our study
11 is that patients undergoing alloHSCT with measurable levels of MRD have a significant
12 higher risk of relapse after transplant. In agreement with our results, very recently the Japan
13 Society for Hematopoietic Cell Transplantation found in a retrospective analysis of data from
14 432 adult Ph+ ALL patients that the incidence of relapse in MRD negative patients at
15 transplant was significantly lower compared to patients transplanted in MRD positivity (19%
16 vs 29%, respectively)²⁴. Accordingly, any effort should be done to achieve a deep molecular
17 response before the beginning of the conditioning regimen. Although in our study the
18 significant reduction of relapse did not translate into a clear benefit in terms of DFS and OS,
19 this result is due to the remarkable NRM observed in patients undergoing transplantation
20 being MRD negative and to the effective response frequently obtained with the post-
21 transplant use of TKIs with or without DLI. In keeping with this interpretation, treatments
22 after transplant with TKIs and/or DLI were significantly more used among MRD+ group
23 compared with MRD- group. Moreover, patients who received a TKI based treatment had the
24 chance to convert to a stable MRD negative condition in half the cases, suggesting that TKIs
25 treatment, given as salvage option after transplantation, may be an effective rescue for a

1 remarkable proportion of patients. It is worth noting that previous experiences that explored
2 the use of prophylactic imatinib and nilotinib maintenance after transplantation^{25,26} showed a
3 high efficacy of treatment in preventing relapse, but this was hampered by a high rate of early
4 discontinuation due to poor tolerability^{25,26}. Unfortunately, our database did not contain
5 specific data about the characteristics and the complications of post-HSCT treatments (i.e.
6 DLI cell doses, cumulative incidence of chronic GvHD, TKIs related complications) nor
7 information about the presence of specific ABL mutations such as the T315I. Therefore, a
8 formal evaluation of their impact in terms of comorbidity, quality of life and treatment
9 response is limited. Treatment with TKIs after alloHSCT can be difficult to tolerate and may
10 be followed by a high rate of early discontinuation. Therefore, a careful risk assessment of
11 each patient with Ph+ ALL, may lead to timely initiation of an effective treatment with TKIs
12 and to avoid it, if not strictly necessary. In this context, the information of this study is
13 potentially very useful and suggests to limit TKIs prophylactic use to patients at high risk of
14 relapse, such as MRD positive patients before transplant and to apply a MRD-driven strategy
15 in MRD negative patients. Our results suggest that a deep molecular response before
16 transplant predict a better outcome, but in agreement with the EBMT results⁶, we have no
17 evidence that increasing levels of MRD positivity are associated with different risk of relapse,
18 even though a larger group of patients is requested to validate this result.

19 The concept that alloHSCT remains the standard of care for any patient suitable for
20 the procedure and with an available donor has been recently underlined by the French clinical
21 trial recently published by Chalandon et al⁹. However, patients achieving an early molecular
22 CR through the combination of specific targeted therapies plus chemotherapy may identify
23 patients who may be possibly cured without alloHSCT. In this regard, a recent study showed
24 that a negative MRD status after three months and beyond of a TKIs based chemotherapy
25 program was associated with a low risk of relapse and an OS above 60% at 3–5 years in

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

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

1 appropriate combination of the available therapies, with third generation TKIs with or
2 without new antibody or cellular therapies²⁸⁻³⁰.

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12 **Figure Legends**13 **Figure 1.** Study flow14 **Figure 2.** Overall survival according to alloHSCT in first remission15 **Figure 3 A-B-C.** Cumulative incidence of hematologic relapse, DFS and OS by MRD group16 **Figure 4.** Number of post-transplant treatments by MRD group

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18 **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	11 (16.9)	4 (16.7)	7 (17.1)	1.00
Myeloablative	54 (83.1)	20 (83.3)	34 (82.9)	
Donor type (%)				
Sibling	31 (47.7)	13 (54.2)	18 (43.9)	0.4240
Unrelated	34 (52.3)	11 (45.8)	23 (56.1)	

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

1 § MRD negative vs MRD positive

2 WBC: white blood cells; LDH: lactate dehydrogenase

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