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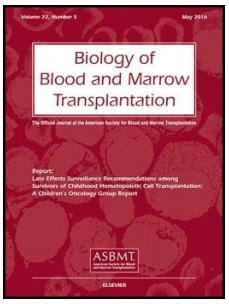

Title: Allogeneic Stem Cell Transplantation for Relapsed/Refractory B-Cell Lymphomas: Results of a Multicenter Phase II Prospective Trial Including Rituximab in the Reduced Intensity Conditioning Regimen

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Allogeneic stem cell transplantation for relapsed/refractory B-cell lymphomas: results of a multicenter phase II prospective trial including Rituximab in the reduced intensity conditioning regimen

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Highlights

- The graft versus host disease free, relapse free survival is 34% after transplantation in lymphomas;
- Reduced extensive chronic GVHD with a combination of ATG and Rituximab in recipients of unrelated graft
- One Rituximab Infusion prevents EBV-related lymphoproliferative disorders

Abstract

The treatment of patients with refractory/relapsed B-Cell non-Hodgkin lymphoma (NHL) is evolving due to the availability of novel drugs. Allogeneic stem cell transplantation (alloSCT) can be curative, but its morbidity and mortality remain a matter of concern. We conducted a multicentre prospective phase II trial to evaluate the benefit of including only one dose of Rituximab (R) in the conditioning regimen before alloSCT. The primary end-point was progression-free survival. The study enrolled 121 patients with relapsed/refractory B-cell lymphomas. The conditioning regimen consisted of thiotepa, cyclophosphamide, fludarabine and R (500 mg/ms). Rabbit anti-thymocyte globulin (ATG) was administered only in case of unrelated donors. Sixty-seven (55%) and fifty-four (45%) patients received grafts from related and unrelated donors, respectively. The crude cumulative incidence (CCI) of non-relapse mortality (NRM) was 21% at 3-years. The CCI of chronic GVHD at 3-years was 54% and 31% in recipients of matched sibling and unrelated grafts, respectively. At a median follow-up of 41 months, the estimated 3-years progression-free and overall survival were 50% and 61%, respectively. Long-term outcome was also evaluated with the composite end-point of graft-versus-host disease-free and relapse-free survival (GRFS). This is the first work evaluating the GRFS in a prospective trial of lymphomas patients: the 1 and 3-years GRFS were 40% and 34%, respectively. AlloSCT can cure a fraction of patients with rather low NRM and an encouraging PFS and GRFS.

Keywords: Rituximab,lymphomas, graft-versus-host disease-free/relapse-free survival

Introduction

Rituximab-based chemoimmunotherapy has improved the survival rate of patients with indolent and aggressive B-cell non-Hodgkin lymphoma (NHL) (1,2), but 50% of them fail to respond or relapse and only a fraction could be cured by autologous stem cell transplantation (autoSCT). Despite an increase of novel drugs and treatments (3), allogeneic stem cell transplantation (alloSCT) still represents the only chance of cure for patients relapsing after autoSCT or two lines of chemo-immunotherapy. In previous studies with reduced-intensity conditioning, we already reported a 5-year progression-free survival (PFS) of 57% and 54% in indolent and aggressive lymphomas, respectively (4,5).

Severe acute graft-versus-host disease (GVHD) and extensive chronic GVHD (6,7) are the two main complications associated to transplantation and may affect both non-relapse mortality (NRM) and quality of life. In the last years, a better understanding of GVHD biology prompted the design of novel GVHD prophylaxis regimens including the use of post-transplant cyclophosphamide or proteasome inhibitors, but the gold standard is still based on calcineurin inhibitors and methotrexate or mycophenolate mofetil (8-12).

B cells have a role in the pathogenesis of both acute GVHD (for their role as antigen-presenting cells) and chronic GVHD (for the production of autoantibodies from autoreactive B cells) (13). Rituximab (R) has been introduced during conditioning regimen in very few trials, however the results on disease control and prevention of acute or chronic GVHD are still unclear (14-16). The major experience derived from the pivotal studies of Khouri (14) that showed a rather good disease control with the unexpected finding of a limited incidence of acute and chronic extensive GVHD.

In the present multicenter prospective phase II study, we investigated the effect of a single dose of R (500mg/ms) in combination with a reduced-intensity conditioning (RIC) regimen on the progression-free survival of refractory/relapsed B-cell lymphomas. In addition also overall survival, incidence of acute and chronic GVHD, and the GVHD-free, relapse-free survival (GRFS) were evaluated.

Materials and Methods

Patient characteristics

Between December 2007 and December 2015, 121 patients were enrolled in a prospective study (EUDRACT 2007-003657- 87) involving 22 Italian Hematology Divisions. Inclusion criteria were as follows: i) patients were diagnosed with CD20+ B-cell NHL [i.e. Chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), follicular lymphoma (FL), mantle cell lymphoma (MCL), *de novo* or transformed diffuse large B-cell lymphoma (DLBCL)] relapsing or refractory after at least two lines of treatment or after failure of autoSCT; ii) MCL and DLBCL were

required to have chemosensitive disease; chemorefractory disease was allowed only for indolent lymphomas. Exclusion criteria were central nervous system localization, positive serologic markers for human immunodeficiency virus (HIV), active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, ejection fraction < 45% (or myocardial stroke in the last year), DLCO < 50%, no adequate renal and hepatic functions (clearance of creatinine < 50 ml/min, serum bilirubin levels > 2 the upper normal limit). All the patients received R-chemo as salvage before the transplant phase. The study was approved by the Institutional Review Board of all participating centers.

Donors and treatment plan

Donors age ranged between 18 and 65 years old in the case of identical sibling donors and 18 and 60 years old in the case of unrelated donors, The availability of human leukocyte antigen (HLA)-identical or one antigen mismatched (class I) sibling donors, or unrelated donors mismatched by one antigen or allele at HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1 loci, as identified by high or intermediate resolution typing.

Patients were registered at time of relapse. At registration patients were treated with salvage R-chemotherapy. The choice of the salvage regimen was left to center preference. All the patients included in this prospective trial received the following drugs intravenous: rituximab (500 mg/ms; day –6), thiotepa (6 mg/kg every 12 hours for 2 doses; day –5), cyclophosphamide (30 mg/kg; days –4 and –3) and fludarabine (30 mg/ms; days –4 and –3), which was administered 4 hours post-cyclophosphamide administration. The choice to administer only one dose of R was related to the fact that Rituximab was already administered with the previous salvage chemo-immunotherapy. In the case of matched related sibling donors, the GVHD prophylaxis consisted of intravenous or oral cyclosporine A, adjusted to maintain blood levels at 200–300 ng/ml, and a short course of intravenous methotrexate (10 mg/ms on day +1, and 8 mg/ms on days +3 and +6). Patients with a class I antigen mismatch (sibling donors) or with unrelated donors received intravenous rabbit anti-thymocyte globulin (Thymoglobuline, Genzyme-Sanofi; 0.5 mg/kg on day –4, 3 mg/kg on day –3, 3.5 mg/kg on day –2). On day 0 patients received stem cells > 3 $x10^8$/kg total nucleated stem cells in case of bone marrow and ≥$4x10^6$/kg CD34+ in case of peripheral blood stem cells. In absence of active acute GVHD, GVHD prophylaxis was administered until day 100 for siblings and day 150 for unrelated donors (details on immunesuppression tapering are given in Supplemental Material). Recommendations for supportive care were previously described (5).

Study endpoints

The primary endpoint of the study was progression-free survival (PFS). The secondary endpoints were non-relapse mortality (NRM), acute GVHD incidence within 100 days since alloSCT, chronic GVHD incidence after 100 days and

overall survival (OS). The study sample size was calculated to estimate the 1-year PFS and corresponding 90% one sided confidence interval. In a retrospective series of 115 patients with relapsed/refractory B-cell NHL, 1-year PFS was 70%, with 20% relapses (5). Assuming no treatment effect on NRM and a 35% relative improvement of relapse rate (from 20% to 13%), at a significance level of 10% (one-side test) a sample size of 190 assessable patients ensured a 80% probability of detecting a 70% to 77% increase in 1-year PFS. However, due to an expansion of trials incorporating novel agents and due to an increase of haploidentical donor use the accrual rate decreased in the last two years and the Data Safety and Monitoring committee suggested to stop enrollment also because a change in PFS was very unlikely. Therefore, the primary endpoint of improving PFS was not meet. We also analyzed our study, using the novel composite end point of GRFS that gives more informations regarding GVHD.

Response criteria and Statistical analysis

The response to therapy was evaluated at 1, 3, 6 months after alloSCT and every 6 months thereafter and was measured using the International Workshop NHL criteria, as previously described (17). Acute GVHD was evaluated using the criteria previously described by Glucksberg et al. (18). Chronic GVHD was diagnosed according to the Seattle criteria (19). Chronic GVHD was not evaluated by the NIH criteria, considering the study was designed before 2007. The occurrence of NRM, relapse, and acute and chronic GVHD were estimated in a competing risks setting using cumulative incidence estimates and the curves were compared by means of the Gray Test. In the estimation of GVHD, death without GVHD was evaluated as a competing event. NRM and relapse were competing events each other (20). We tested also the recent composite end point GRFS (21), introduced in the 2015, which reflects the survival free of major complications. GRFS events were defined as grade 3-4 acute GVHD or chronic GVHD requiring systemic immunosuppressive treatment at any time, disease relapse or death from any cause during the first 12 and 36 months after alloSCT. The OS, PFS, and GRFS curves were estimated using the Kaplan-Meier method and the curves were compared by means of the log-rank test. Univariable and multivariable analyses were performed using Fine and Gray (GVHD, NRM, relapse) or Cox regression models (OS, PFS, GRFS) to assess association between baseline characteristics and the end-points (22) The statistical association level was evaluated by means of Wald tests. In all the analysis, age at transplantation was evaluated as continuous variable using 3-knot restricted cubic spline. In the multivariable analysis for the evaluation of factors influencing acute and chronic GVHD, variables (age, donor type and donor gender) were chosen based on clinical considerations, and we did not apply any statistical procedure for variable selection. Statistical analyses were performed with SAS™ (SAS Institute, Cary, NC) and R software (R Development

Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/. Last access: July 7th 2016).

Results

Patient characteristics

One hundred and twenty-one relapsed/refractory lymphoma patients were enrolled in this multicenter prospective phase II trial (refer to Table 1 for baseline characteristics of patients). The median age was 52 years (range, 24-65 years). Diagnoses were *de novo* or transformed DLBCL (N=35 of which 2 transformed and 33 *de novo)*, MCL (N=22) and indolent lymphomas (FL, N=35; CLL/SLL, N=29). Sixty-seven out of 121 (55%) underwent transplantation from a related sibling (one mismatched siblings in only 2 cases) and fifty-four (45%) received a graft from an unrelated donor (34 matched, 20 mismatched). Twelve patients had mismatches in class I, the rest in class II, interestingly only 3 patients out of 20 patients (15%) had antigenic mismatches at locus C. It is important to say that most of the patients had a chemosensitive disease [CR, N=48 (39%); PR, N=61 (51%)] at transplant.

Cumulative Incidence of Non-relapse Mortality (NRM) and Graft-versus-Host disease (GVHD)

At a median follow-up of 41 months (range, 6-95 months), 24 patients died (20%) of treatment-related causes: acute GVHD (N=5), chronic GVHD (N=3), infections (N=11), thrombotic microangiopathy (N=1), cardiovascular complications (N=3), liver failure (N=1). The estimated 1-year and 3-years crude cumulative incidence (CCI) of NRM was 16% (95%CI, 11%-25%) and 21% (95%CI, 14%-30%), respectively (Figure 1). Non-relapse mortality, by univariate analysis (Table S1), was not affected by donor type [CCI at 1-year: 14% versus 19% in matched sibling donors and unrelated donors, respectively (p=0.325)] nor by lymphoma subtype [CCI at 1-year: 16% versus 17% in indolent and aggressive lymphomas, respectively (p=0.800)]. When we performed a multivariate analysis, NRM did not appear to be influenced either by age at transplant, or histotype, or by a previously failed autoSCT (Table 2). However, a higher hazard ratio was estimated for sex mismatch [female donor to male recipient versus other combinations: HR 2.5 (95%CI, 0.91-6.97), p=0.076], although results remained non-significant. Twenty-six patients (22%) were diagnosed with acute GVHD grade II-IV (N=16 grade II, N=9 grade III and only one subject grade IV) with an estimated CCI of 22% (95%CI, 15%-30%). Two patients experienced late onset acute GVHD beyond day 100

and were excluded from the CCI evaluation. The CCI of acute GVHD was not significantly different in patients receiving graft from sibling and from unrelated donors (21% versus 22%, respectively).

Ninety-four patients (77%) were evaluable for chronic GVHD whereas the others 27 were not included for early deaths (N=18), short follow-up (N=7), or an overlapping syndrome of acute and chronic GVHD (N=2). The CCI of chronic GVHD at 3 years was 44% (95%CI, 34%-56%) with a median time to onset of 353 days. Curves related to Acute and Chronic GVHD were reported on Figure 1. According to the modified Seattle criteria, the CCI of extensive GVHD was 17% (95%CI, 11%-27%) whereas the limited form was 27% (95%CI, 19%-39%) (Figure S1). In the univariable Fine and Gray model, the only factor that was protective against the occurrence of GVHD was the transplant from unrelated donors when rituximab and ATG were combined together [HR 0.50 (95%CI, 0.25-0.99), p=0.05]. The CCI of chronic GVHD at 3 years was 54% (95%CI,42%-71%) and 31% (95%CI, 19%-49%) for patients allografted from matched sibling and unrelated donors, respectively (p=0.04). The analysis of association between the occurrence of acute and chronic GVHD with different endpoints was performed by including each GVHD type as time-dependent covariate in univariable Cox Model. The occurrence of acute GVHD increased significantly the risk of NRM [HR. 3.33, (1.44-7.71, p=0.005). Non-relapse mortality, Acute and Chronic GVHD were not affected by donor type (matched sibling, matched and mismatched unrelated) (curves were reported on Supplemental Material). There were no cases of EBV lymphoproliferative disorders.

Relapse

Thirty patients relapsed (25%) and 18 of them died of disease (N=11 aggressive DLBCL, N=7 CLL). The CCI of relapse was 19% (95%CI, 13%-28%) at 12 months and 27% (95%CI, 19%-37%) at 3 years. In particular, the CCI of relapse at 3 years was 17% and 34% in indolent and aggressive histotypes, respectively (p=0.011). By multivariate analysis, a positive bone marrow infiltration at the time of transplant [adjusted HR 3.18, (95%CI, 1.16-8.66), p=0.024] and aggressive histotypes [adjusted HR , (95%CI, 3.03, (95%CI, 1.14-8.08), p=0.026] were associated to a higher relapse risk.

Progression-free survival (PFS), Overall survival (OS) and Graft-versus-host disease-free, relapse-free survival (GRFS)

Seventy-nine (65%) patients are currently alive, with a median follow-up for surviving patients of 41 months (range, 6-95 months). The estimated 3-years progression-free and overall survival were 50% and 61%, respectively. (Figure 2). At 3 years, the PFS and OS were as follows in the different subtypes: 70% (95%CI, 52%-82%) and 76% (95%CI, 59%-86%) in FL, 60%, (95%CI, 40%-75%) and 66% (95%CI, 45%-80%) in CLL/SLL, 40% (95%CI, 23%-57%) and 52% (95%CI, 32%-68%) in DLBCL, and 52% (95%CI, 28%-71%) and 66% (95%CI, 39%-83%) in MCL. In the two cohorts (indolent and aggressive) we have evaluated the impact of different prognostic factors on PFS and OS: 1) time from diagnosis to allogeneic transplantation, 2) previous autologous transplant; 3) disease status at time of allograft (CR versus others). Neither of these factors influenced significantly PFS or OS. In indolent subtype, patients in CR had a better PFS although not statistically significant [3 years: 73% (95%CI, 50%-86%) versus 52% (95%CI, 35%-67%), p=0.099] but the status of disease did not influence OS. In aggressive subtype, there was again a better survival for patients allografted in CR [3 years: 75% (95%CI, 49%-88%) versus 43% (95%CI,23%-61%),p=0.091] but was not statistically significant.

Univariate analysis for all the patients is shown in the Supplementary method (Table S2). The multivariate analysis for the PFS and OS showed that patients affected by an aggressive histotype had a worst outcome [PFS, HR 3.30 (p=0.003); OS, HR 3.73 (p=0.007)]. Bone marrow infiltration at time of transplant increased the risk of disease relapse and death [PFS, HR 4.78 (p<0.001); OS, HR 6.00 (p<0.001)]. The occurrence of acute GVHD was associated to shorter OS [(HR: 2.31 (1.20-4.44, p=0.012)].

For the entire cohort of patients, the 1-year and 3-year GRFS were 40% (95%CI, 32%-50%) and 34% (95%CI, 26%-44%), respectively. The GRFS at 3 years was significantly better in patients with indolent as opposed to aggressive lymphomas [43% (95%CI, 32%-58%) versus 22% (95%CI, 13%-38%), HR 1.69 (p=0.02)], mainly because of a lower relapse risk. These figures remained unchanged also at 5 years (data not shown). The 3-year GRFS was 41% and 30% in patients with complete remission (CR) and partial remission (PR) at the time of transplant, respectively (p=0.075). There was a trend for a better outcome in CR patients. The 3-year GRFS was 27% and 41% in patients allografted from matched sibling and unrelated donors, respectively (p=0.096). Distribution of individual components of GRFS is given in Supplementary Methods (Figure S5 and S6). Main clinical characteristics were not different between patients receiving sibling or MUD transplants: therefore the difference in GRFS was caused by a lower chronic GVHD requiring systemic therapy in patients allografted from unrelated donors. In multivariable model the same factors, affecting PFS and OS, influenced significantly GRFS [histotype, HR 2.02 (p=0.026); bone marrow infiltration, HR 2.70 (p<0.004)] (Table 3).

Discussion

In this large multicenter prospective phase II trial, we explored the effect of the inclusion of R in a RIC regimen for B-cell lymphomas. Our findings showed that: i) 3-year NRM was low (21%); ii) PFS was not improved compared to our previous data without R; iii) extensive chronic GVHD was low despite the high number of patients (45%) allografted from unrelated donors; iv) because of R inclusion there were non EBV-related post-transplant lymphoproliferative disorders; v) a lower incidence of chronic GVHD did not translate in a higher relapse rate.

In a previous trial we showed that alloSCT is an effective option for relapsed/refractory lymphomas. We conducted a clinical trial in 170 patients with different B and T-lymphoma subtypes allografted only from matched sibling donors with a RIC regimen and we obtained an encouraging NRM (14%), 56% PFS, but the trial was complicated by 14% CCI of severe acute GVHD (overall 35%) and 25% of chronic extensive form (overall 49%) (5). Most of literature reports show that approximately 50% of patients can be progression-free after alloSCT. In order to improve PFS we designed the present trial including R in the conditioning regimen, however here we show that there was no benefit in terms of disease control , but there was an advantage in terms of chronic GVHD occurrence. In general when chronic GVHD decrease there is an increased relapse rate, but interestingly this was not the case with the addition of R.

Despite several studies, conducted over the last decade, showed a significant decrease in NRM following alloSCT with RIC regimens (5), further progress is needed to improve GVHD prophylaxis since it is a major determinant of morbidity and mortality. To improve the above reported results (using also unrelated donors), we performed the present trial that included a single dose of R (500 mg/ms) administered during the conditioning. The rationale for a single pre-transplant R administration relies on the assumption that circulating CD20+ B cells of the recipient had already been depleted by R-supplemented salvage chemo-immunotherapy whereas pre-transplant R will probably work largely by depleting the donor's alloreactive B cells (and thus preventing also EBV reactivation) in the first 3 months after transplantation. In the current study, we had an incidence of acute GVHD (21% and 22% in transplant from related and unrelated donors, respectively) that was lower than in our previous study without R (35%) that included only patients allografted from HLA matched siblings and the same conditioning regimen and GVHD prophylaxis (5). The 3-year CCI of chronic GVHD was 31% versus 54% in patients allografted from unrelated and related donors, respectively. This finding is clinically relevant and could be related to the high dose ATG used or to a potential synergistic effect of R and ATG. This result substantially contributes in generating a 41% GRFS in patients allografted from unrelated donors. The dose of ATG in our trial was not associated to unacceptable risk of infection and derived from a previous italian trial (23) that evaluated two different GVHD prophylaxis strategies [ATG at 7.5 mg/kg versus Alemtuzmab] in patients

allografted from unrelated donors [8/8 or 7/8 (a allele mismatched was allowed)]. For all the patients the cumulative incidence of acute and chronic GVHD were 44% and 25%, respectively.

The impact of R on B-cell depletion has been previously evaluated in different trials with somehow conflicting results. First, the dose, the timing and the duration of antibody administration was variable. Second, these studies were not well comparable each other not only for the type of patients enrolled, but also for differences in the conditioning regimens as well as in the type and dose of the drugs used for GVHD prophylaxis (e.g., anti-thymocyte globulin) (14-15). Khouri et al. (14) were the first to explore the administration of high-dose R (before and after transplant) and reported results with a mature follow-up. They showed an impressive low incidence (10%) of grade III-IV acute GVHD and of extensive chronic GVHD (36%), which appeared to be not only related to patient selection (mainly matched sibling donors) but also to antibody administration. In that study also R naive patients were included. In a more recent paper (24), the same authors explored a new reduced-conditioning regimen (Bendamustine, Fludarabine and Rituximab), but they introduced also a low dose ATG for recipients of unrelated grafts. The 2-year cumulative incidence of chronic GVHD was 26% in patients allografted from unrelated donors that is comparable with our results. Recently, Laport et al. (25) performed a prospective trial using the FCR regimen in a population of 65 patients allografted from matched sibling and unrelated donors. The authors did not use antithymocyte globulin and the cumulative incidence of chronic GVHD for unrelated recipients was 66%. Despite that, the study was interesting because they found an association between higher R serum concentration and better outcome, and lower serum concentration and severe acute GVHD. Cutler et al. (15) explored different R infusions after day 100 demonstrating a 30% incidence of chronic GVHD requiring systemic corticosteroids (lower than 48% observed in the historical control) in patients transplanted from related and unrelated donors.

In contrast to the above studies, Glass et al. (16) evaluated the effect of post-transplant R in a randomized trial and did not observe any significant effect on either acute or chronic GVHD. However, the major limitation of this prospective trial was the use of ATG in a minority of patients allografted from unrelated donors and the discontinuation of R in a substantial number of patients (61%) allocated to the R group.

Recent randomized trials, performed mainly in myeloid malignancies, strongly supported the use of ATG as GVHD prophylaxis in patients receiving alloSCT not only from unrelated donors (26), but also from HLA-identical siblings (27). The benefit was mainly related to a reduction of the severe forms of chronic GVHD.

Epstain Barr virus reactivation is common (33%) after allogeneic transplantation from unrelated donors when the GVHD prophylaxis included anti-thymocyte globulin as recently reported also in the paper of Walker et al. (26). High levels of EBV DNAemia increase the risk of post-transplant lymphoproliferative disorders (PTLD). The use of

prophylactic Rituximab (200 mg on day +5) has been explored in a retrospective study (28) in recipients of transplant from alternative donor (the GVHD prophylaxis included rabbit anti-thymocyte globulin at dose of 6-10 mg/kg) and was associated to a lower rate of EBV DNAemia and to the absence of cases of PTLD as compared to control group. In our study the administration of Rituximab prevented efficiently the Epstain Barr virus reactivation and the PTLD.

Our trial expands the knowledge about the role of transplant in relapsed/refractory aggressive lymphomas. In fact, we demonstrated that at 3 years 54% of patients failing an autologous stem cell transplant and 43% of patients not in CR are alive. These data compare favourably with the results reported by Glass et al.(16) in patients with refractory disease at time of alloSCT (estimated 3 years OS of 38%) and with those described by Fenske T. et al. (29) in retrospective study enrolling patients progressing after autologous stem cell transplantation (estimated 3 years OS of 37%).

In the last years, a number of novel agents for lymphomas became available. Major advances occurred in relapsed/refractory CLL, MCL and FL using the Bruton tyrosine kinase inhibitors (ibrutinib), the inhibitor of BCL-2 venetoclax and phosphatidylinositol-3-kinase delta (PI3Kδ) inhibitor idelalisib (30-33). All these studies have intrinsic limitations mainly due to the very short follow-up and also the long-term efficacy and safety is lacking. The question of which strategy (RIC alloSCT or new drugs) is better for the treatment of transplant eligible patients is now open and appropriately designed prospective clinical trials will be required to challenge the 34% of patients that are alive and free of any complication after alloSCT.

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Author Contributions

A.D., P.C., A.R.: Conception and design of the study;

F.P., G.M., B.S., A.I, E.T., A.M., M. P., A.B., A.D., N.C., F.O., F.N., L.F.: collection and assembly of data;

A.D., R.M., F.B., A.R., P.C.: data analysis and interpretation;

All authors: manuscript writing.

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Figure Legends

Figure 1. A) Crude cumulative Incidence (CCI) of Non-Relapse Mortality. At 3 years the CCI of NRM was 21% (95%CI, 14%-29%); B) CCI of acute GVHD grade II-IV at 100 days: 21% (95%CI,15%-30%); C) CCI of limited and extensive chronic GVHD: 27% (95%CI, 19-38%) and 17% (95%CI, 11%-27%), respectively.

Figure 2. Kaplan-Meier estimates of Progression-free Survival and Overall Survival. A) Progression-Free Survival at 5 years was 49% (95%CI, 40%-61%); B) The Overall Survival at 5 years was 61% (95%CI, 51%-71%).

Figure 3. Adjusted Kaplan-Meier estimates of Graft-versus-Host Disease-free and Relapse-free survival (GRFS) at 3 years after allogeneic stem cell transplantation. A) GRFS for all the patients: 34% (95%CI, 26%-44%); B) GRFS according to donor type: 27% and 41% in patients allografted from matched sibling donors and unrelated donors, respectively (p=0.09).

Figure 4. Adjusted Kaplan-Meier estimates of Graft-versus-Host Disease-free and relapse-free survival (GRFS) at 3 years after allogeneic stem cell transplantation. A) GRFS upon hystotype: 43% indolent (95%CI, 32%-58%) versus 22% aggressive lymphomas (95%CI, 13%-38%), (p=0.02); B) GRFS upon pre-transplant disease status: 41% (95%CI, 28%-59%) and 30% (95%CI, 20%-44%) for patients in complete and partial remission, respectively (p=0.185).

Table 1. Patients characteristics

	N (121)	%
Age at AlloSCT, median (range)	52 (23-65)	
Gender		
Male	80	66%
Female	41	34%
Karnofsky performance status		
≤80	18	15%
>80	103	85%
Diagnosis*		
FL	35	28%
CLL	29	24%
DLBCL	35	28%
MCL	22	18%
Bone Marrow involvement		
Yes	30	25%
No	79	65%
Missing	12	10%
Extranodal involvement		
Yes	26	21%
No	78	64%
Missing	17	14%
PriorAutoSCT		
Yes	74	61%
No	47	39%
Disease Status at Transplant		
CR	48	40%
PR	64	53%
PD/SD	9	7%
Donor Type		
Matched/Mismatched Related **	67	55%
Matched Unrelated	34	28%
Mismatched Unrelated	20	17%
Sex mismatched		
Female donor-Male recipient	22	18%
Other combinations	90	74%
Missing	9	7%

Stem Cell Source		
PBSC	101	83%
BM	20	17%
Year of Allotransplant		
2007-2011	65	54%
2012-2015	56	46%

Abbreviations: AlloSCT, allogeneic stem cell transplantation; FCL, follicular cell lymphomas; CLL/SLL, chronic lymphocytic leukemia; SLL, small lymphocytic lymphomas; DLBCL, diffuse large B cell lymphomas; MCL; mantle cell lymphomas; CR, complete remission; PR, partial remission; PD, progressive diasese; SD, stable disease; autoSCT: autologous stem cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow.
*diagnosis were well balanced:
34 and 30 indolent lymphomas patients were transplanted from matched /mismatched related sibling, respectively;
33 and 20 patients with aggressive lymphomas were allografted from matched/mismatched unrelated donors;
**we had only two cases mismatched related ;

271 **Table 2. Multivariable analysis on Non-relapse mortality and Relapse**

	NRM		Relapse	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age at AlloSCT				
46 Vs 57*	1.45 (0.73-2.86)	0.55	---	---
Histotype subgroup				
Aggressive vs Indolent	1.07 (0.44-2.60)	0.891	3.03 (1.14-8.08)	**0.026**
Bone Marrow involvement				
Yes Vs No	---	---	3.18 (1.16-8.66)	**0.024**
Extranodal involvement				
Yes Vs No	---	---	0.95 (0.28-3.18)	0.930
Prior AutoSCT				
Yes vs No	1.82 (0.66-5.06)	0.250	1.11 (0.35-3.47)	0.860
Donor Type				
Unrelated Vs Related	1.75 (0.75-4.08)	0.197	0.80 (0.31-2.05)	0.640
Sex mismatched				
Female donor-Male recipient Vs Other combinations	2.52 (0.91-6.97)	**0.076**	0.12 (0.01-1.15)	**0.067**

Abbreviations: NRM, non-relapse mortality; HR: sub-distribution hazard ratio from Fine and Gray model; 95% CI, 95% confidence interval of HR; p value, Wald test p value; AlloSCT, allogeneic stem cell transplantation; * Age was evaluated as continuous variable. The two values are, respectively, the 3rd and 1st quartiles of the variable distribution.

Table 3. Multivariable analysis on PFS, OS, GRFS

	PFS		OS		GRFS	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Age at AlloSCT						
46 Vs 57*	1.63 (0.93-2.85)	0.234	1.70 (0.91-3.18)	0.22	1.62 (1.02-2.55)	0.113
Histotype subgroup						
Aggressive vs Indolent	3.30 (1.49-7.30)	**0.003**	3.73 (1.42-9.78)	**0.007**	2.02 (1.09-3.77)	**0.026**
Prior autoSCT						
Yes vs No	1.34 (0.60-3.02)	0.476	1.20 (0.48-3.00)	0.696	1.55 (0.78-3.05)	0.208
Bone Marrow involvement						
Yes Vs No	4.79 (2.12-10.82)	**<0.001**	6.00 (2.10-14.15)	**<0.001**	2.70 (1.37-5.32)	**0.004**
Extranodal involvement						
Yes Vs No	0.87 (0.40-1.90)	0.728	1.07 (0.45-2.53)	0.884	1.54 (0.82-2.89)	0.177
Donor Type						
Unrelated Vs Related	1.28 (0.59-2.76)	0.529	1.35 (0.56-3.3.30)	0.506	0.79 (0.41-1.51)	0.472
Sex mismatched						
Female donor-Male recipient Vs Other combinations	1.21 (0.48-3.04)	0.690	1.50 (0.53-4.26)	0.449	1.41 (0.68-2.91)	0.352

Abbreviations: PFS, progression-free survival; OS, overall survival; GRFS, graft-versus-host disease-free/relapse-free survival; HR: hazard ratio from Cox model; 95% CI, 95% confidence interval of HR; p value, Wald test p value; AlloSCT, allogeneic stem cell transplantation; autoSCT, autologous stem cell transplantation; * Age was evaluated as continuous variable. The two values are, respectively, the 3rd and 1st quartiles of the variable distribution.

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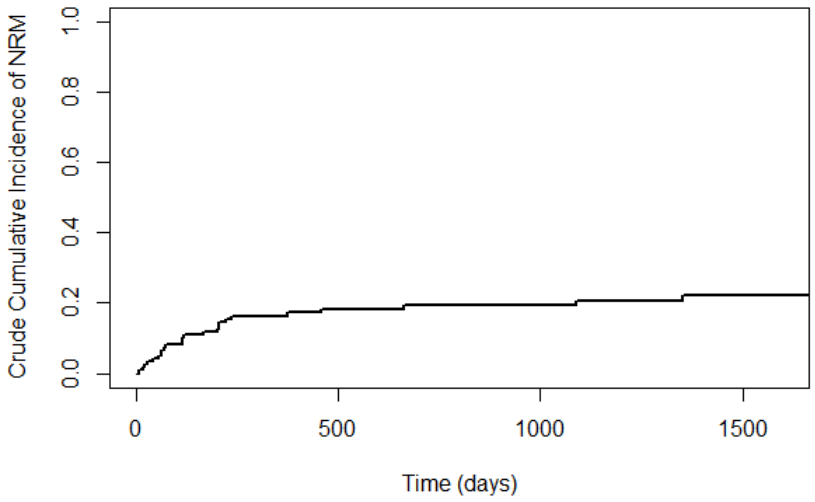
Figure 1.

A



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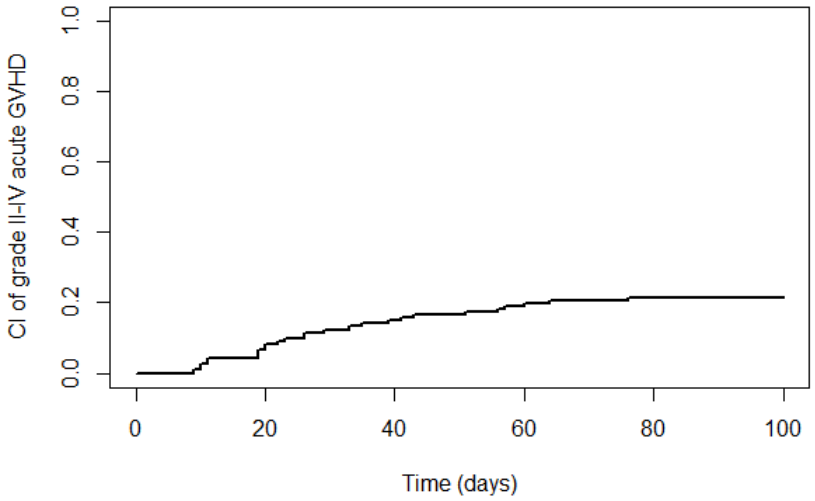

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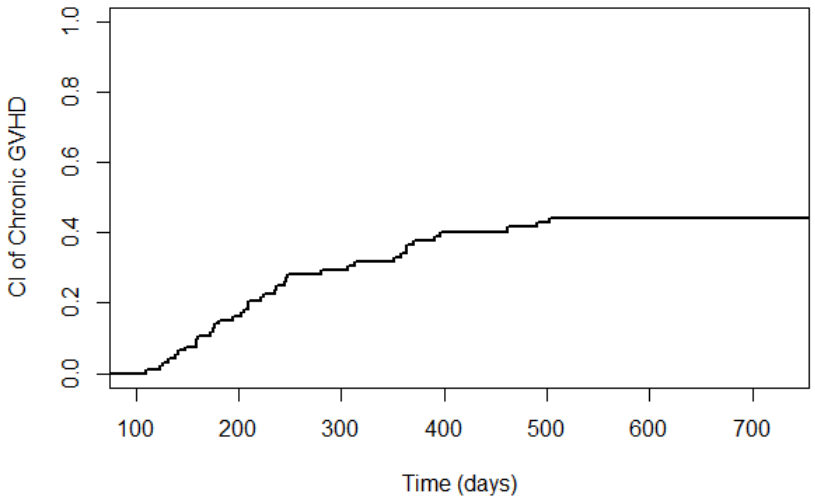

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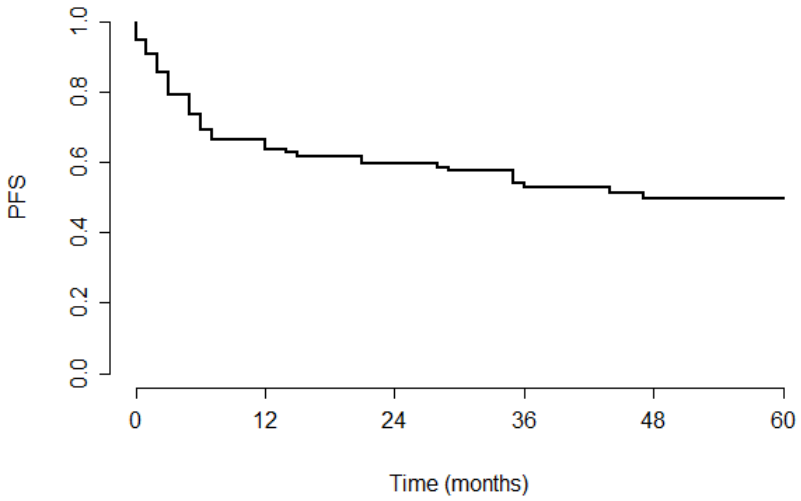
Figure 2.

A.



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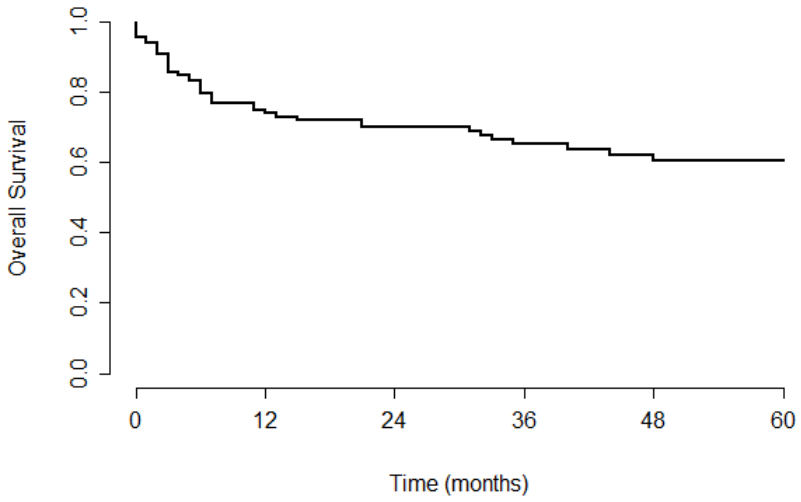
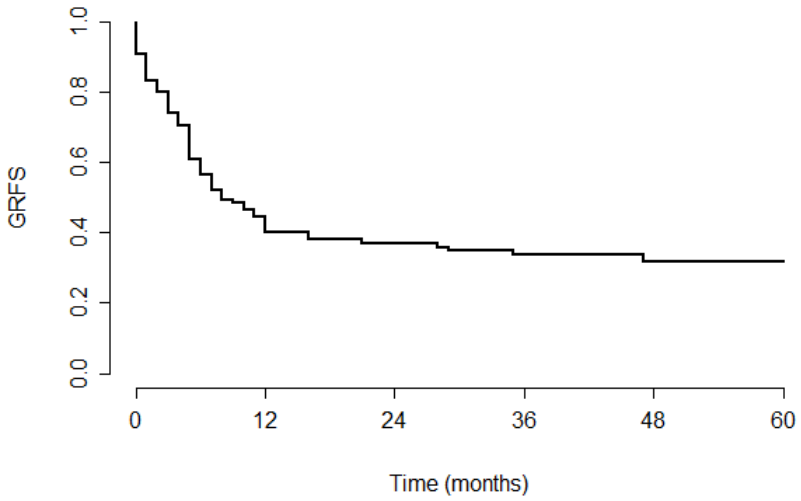


Figure 3

A.



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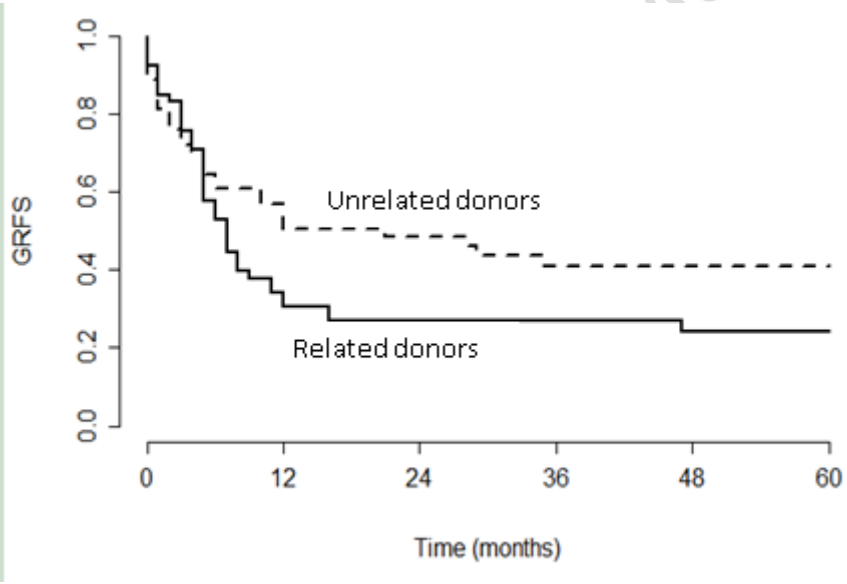
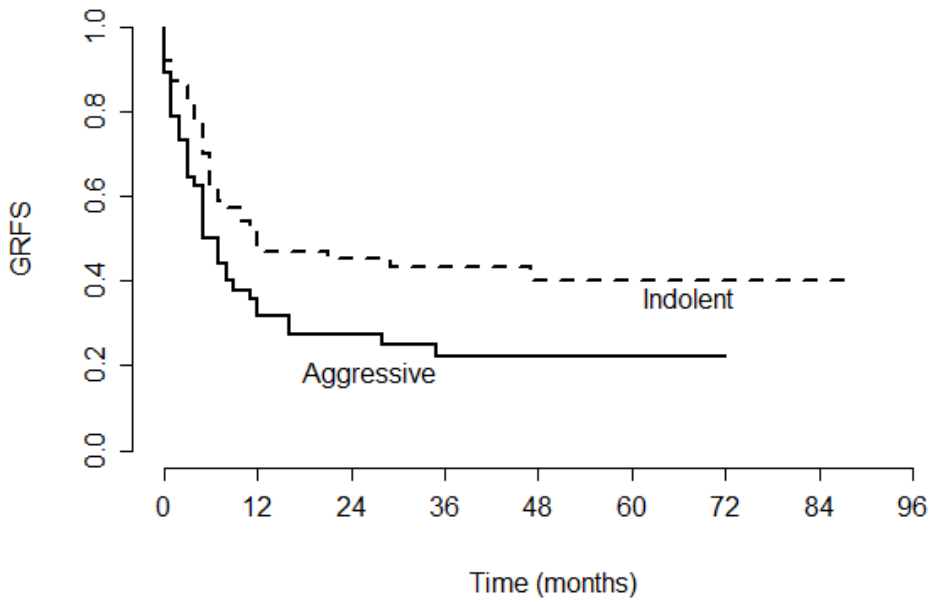


298 Figure 4.

299 A.



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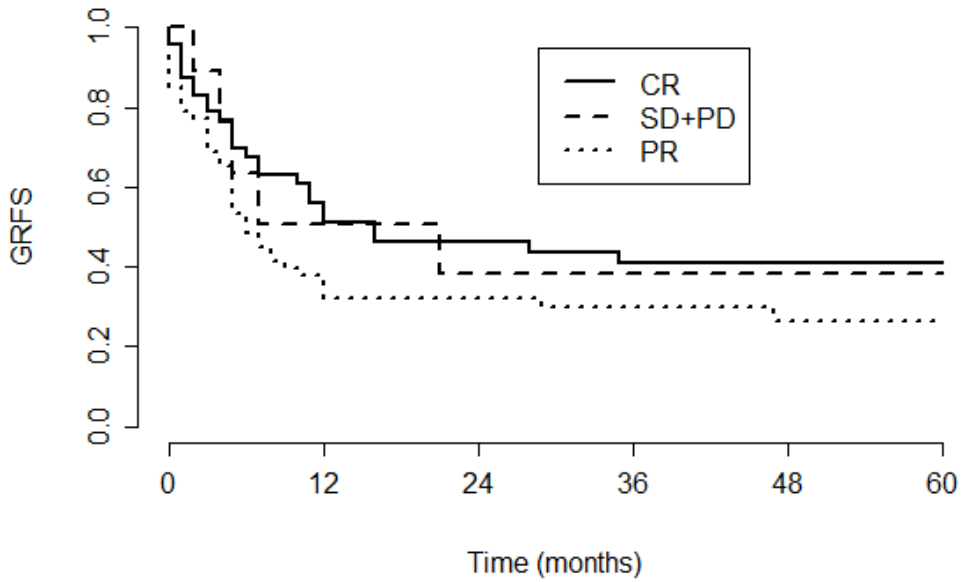
302 B.



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