

## Accepted Manuscript

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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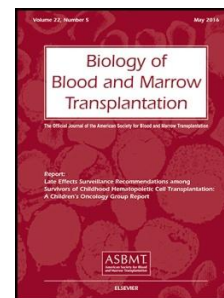
PII: S1083-8791(17)30382-8  
DOI: <http://dx.doi.org/doi: 10.1016/j.bbmt.2017.03.031>  
Reference: YBBMT 54626

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

Received date: 8-12-2016  
Accepted date: 23-3-2017

Please cite this article as: Anna Dodero, Francesca Patriarca, Giuseppe Milone, Barbara Sarina, Rosalba Miceli, Anna Iori, Francesco Barretta, Elisabetta Terruzzi, Alberto Mussetti, Massimo Pini, Alberto Bosi, Alida Dominiotto, Nicola Cascavilla, Francesco Onida, Franco Narni, Lucia Farina, Alessandro Rambaldi, Paolo Corradini, 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, *Biology of Blood and Marrow Transplantation* (2017), <http://dx.doi.org/doi: 10.1016/j.bbmt.2017.03.031>.

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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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Total Word count: 3838.

## 1 Highlights

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

## 7 Abstract

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

22  
23 **Keywords:** Rituximab, lymphomas, graft-versus-host disease-free/relapse-free survival

24

## 25 **Introduction**

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

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

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

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

46

47

## 48 **Materials and Methods**

### 49 *Patient characteristics*

50 Between December 2007 and December 2015, 121 patients were enrolled in a prospective study (EUDRACT 2007-  
51 003657- 87) involving 22 Italian Hematology Divisions. Inclusion criteria were as follows: i) patients were diagnosed  
52 with CD20+ B-cell NHL [i.e. Chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), follicular  
53 lymphoma (FL), mantle cell lymphoma (MCL), *de novo* or transformed diffuse large B-cell lymphoma (DLBCL)]  
54 relapsing or refractory after at least two lines of treatment or after failure of autoSCT; ii) MCL and DLBCL were

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

61

### 62 *Donors and treatment plan*

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

67 Patients were registered at time of relapse. At registration patients were treated with salvage R-chemotherapy. The  
68 choice of the salvage regimen was left to center preference. All the patients included in this prospective trial received  
69 the following drugs intravenous: rituximab (500 mg/ms; day -6), thiotepa (6 mg/kg every 12 hours for 2 doses; day -5),  
70 cyclophosphamide (30 mg/kg; days -4 and -3) and fludarabine (30 mg/ms; days -4 and -3), which was administered 4  
71 hours post-cyclophosphamide administration. The choice to administer only one dose of R was related to the fact that  
72 Rituximab was already administered with the previous salvage chemo-immunotherapy. In the case of matched related  
73 sibling donors, the GVHD prophylaxis consisted of intravenous or oral cyclosporine A, adjusted to maintain blood  
74 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  
75 and +6). Patients with a class I antigen mismatch (sibling donors) or with unrelated donors received intravenous rabbit  
76 anti-thymocyte globulin (Thymoglobuline, Genzyme-Sanofi; 0.5 mg/kg on day -4, 3 mg/kg on day -3, 3.5 mg/kg on  
77 day -2). On day 0 patients received stem cells > 3 x10<sup>8</sup>/kg total nucleated stem cells in case of bone marrow and  
78 ≥4x10<sup>6</sup>/kg CD34+ in case of peripheral blood stem cells. In absence of active acute GVHD, GVHD prophylaxis was  
79 administered until day 100 for siblings and day 150 for unrelated donors (details on immunosuppression tapering are  
80 given in Supplemental Material). Recommendations for supportive care were previously described (5).

### 81 *Study endpoints*

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

84 overall survival (OS). The study sample size was calculated to estimate the 1-year PFS and corresponding 90% one  
85 sided confidence interval. In a retrospective series of 115 patients with relapsed/refractory B-cell NHL, 1-year PFS was  
86 70%, with 20% relapses (5). Assuming no treatment effect on NRM and a 35% relative improvement of relapse rate  
87 (from 20% to 13%), at a significance level of 10% (one-side test) a sample size of 190 assessable patients ensured a  
88 80% probability of detecting a 70% to 77% increase in 1-year PFS. However, due to an expansion of trials  
89 incorporating novel agents and due to an increase of haploidentical donor use the accrual rate decreased in the last two  
90 years and the Data Safety and Monitoring committee suggested to stop enrollment also because a change in PFS was  
91 very unlikely. Therefore, the primary endpoint of improving PFS was not meet. We also analyzed our study, using the  
92 novel composite end point of GRFS that gives more informations regarding GVHD.

93

#### 94 *Response criteria and Statistical analysis*

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

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

115

## 116 **Results**

### 117 *Patient characteristics*

118 One hundred and twenty-one relapsed/refractory lymphoma patients were enrolled in this multicenter prospective  
119 phase II trial (refer to Table 1 for baseline characteristics of patients). The median age was 52 years (range, 24-65  
120 years). Diagnoses were *de novo* or transformed DLBCL (N=35 of which 2 transformed and 33 *de novo*), MCL (N=22)  
121 and indolent lymphomas (FL, N=35; CLL/SLL, N=29). Sixty-seven out of 121 (55%) underwent transplantation from a  
122 related sibling (one mismatched siblings in only 2 cases) and fifty-four (45%) received a graft from an unrelated donor  
123 (34 matched, 20 mismatched). Twelve patients had mismatches in class I, the rest in class II, interestingly only 3  
124 patients out of 20 patients (15%) had antigenic mismatches at locus C. It is important to say that most of the patients  
125 had a chemosensitive disease [CR, N=48 (39%); PR, N=61 (51%)] at transplant.

126

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

128 At a median follow-up of 41 months (range, 6-95 months), 24 patients died (20%) of treatment-related causes: acute  
129 GVHD (N=5), chronic GVHD (N=3), infections (N=11), thrombotic microangiopathy (N=1), cardiovascular  
130 complications (N=3), liver failure (N=1). The estimated 1-year and 3-years crude cumulative incidence (CCI) of NRM  
131 was 16% (95%CI, 11%-25%) and 21% (95%CI, 14%-30%), respectively (Figure 1). Non-relapse mortality, by  
132 univariate analysis (Table S1), was not affected by donor type [CCI at 1-year: 14% versus 19% in matched sibling  
133 donors and unrelated donors, respectively (p=0.325)] nor by lymphoma subtype [CCI at 1-year: 16% versus 17% in  
134 indolent and aggressive lymphomas, respectively (p=0.800)]. When we performed a multivariate analysis, NRM did  
135 not appear to be influenced either by age at transplant, or histotype, or by a previously failed autoSCT (Table 2).  
136 However, a higher hazard ratio was estimated for sex mismatch [female donor to male recipient versus other  
137 combinations: HR 2.5 (95%CI, 0.91-6.97), p=0.076], although results remained non-significant. Twenty-six patients  
138 (22%) were diagnosed with acute GVHD grade II-IV (N=16 grade II, N=9 grade III and only one subject grade IV)  
139 with an estimated CCI of 22% (95%CI, 15%-30%). Two patients experienced late onset acute GVHD beyond day 100

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

142 Ninety-four patients (77%) were evaluable for chronic GVHD whereas the others 27 were not included for early deaths  
143 (N=18), short follow-up (N=7), or an overlapping syndrome of acute and chronic GVHD (N=2). The CCI of chronic  
144 GVHD at 3 years was 44% (95%CI, 34%-56%) with a median time to onset of 353 days. Curves related to Acute and  
145 Chronic GVHD were reported on Figure 1. According to the modified Seattle criteria, the CCI of extensive GVHD was  
146 17% (95%CI, 11%-27%) whereas the limited form was 27% (95%CI, 19%-39%) (Figure S1). In the univariable Fine  
147 and Gray model, the only factor that was protective against the occurrence of GVHD was the transplant from unrelated  
148 donors when rituximab and ATG were combined together [HR 0.50 (95%CI, 0.25-0.99), p=0.05]. The CCI of chronic  
149 GVHD at 3 years was 54% (95%CI, 42%-71%) and 31% (95%CI, 19%-49%) for patients allografted from matched  
150 sibling and unrelated donors, respectively (p=0.04). The analysis of association between the occurrence of acute and  
151 chronic GVHD with different endpoints was performed by including each GVHD type as time-dependent covariate in  
152 univariable Cox Model. The occurrence of acute GVHD increased significantly the risk of NRM [HR. 3.33, (1.44-7.71,  
153 p=0.005). Non-relapse mortality, Acute and Chronic GVHD were not affected by donor type (matched sibling,  
154 matched and mismatched unrelated) (curves were reported on Supplemental Material). There were no cases of EBV  
155 lymphoproliferative disorders.

156

### 157 *Relapse*

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

164

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



167 Seventy-nine (65%) patients are currently alive, with a median follow-up for surviving patients of 41 months (range, 6-  
168 95 months). The estimated 3-years progression-free and overall survival were 50% and 61%, respectively. (Figure 2).  
169 At 3 years, the PFS and OS were as follows in the different subtypes: 70% (95%CI, 52%-82%) and 76% (95%CI, 59%-  
170 86%) in FL, 60%, (95%CI, 40%-75%) and 66% (95%CI, 45%-80%) in CLL/SLL, 40% (95%CI, 23%-57%) and 52%  
171 (95%CI, 32%-68%) in DLBCL, and 52% (95%CI, 28%-71%) and 66% (95%CI, 39%-83%) in MCL. In the two cohorts  
172 (indolent and aggressive) we have evaluated the impact of different prognostic factors on PFS and OS: 1) time from  
173 diagnosis to allogeneic transplantation, 2) previous autologous transplant; 3) disease status at time of allograft (CR  
174 versus others). Neither of these factors influenced significantly PFS or OS. In indolent subtype, patients in CR had a  
175 better PFS although not statistically significant [3 years: 73% (95%CI, 50%-86%) versus 52% (95%CI, 35%-67%),  
176  $p=0.099$ ] but the status of disease did not influence OS. In aggressive subtype, there was again a better survival for  
177 patients allografted in CR [3 years: 75% (95%CI, 49%-88%) versus 43% (95%CI, 23%-61%),  $p=0.091$ ] but was not  
178 statistically significant.

179 Univariate analysis for all the patients is shown in the Supplementary method (Table S2). The multivariate analysis for  
180 the PFS and OS showed that patients affected by an aggressive histotype had a worst outcome [PFS, HR 3.30  
181 ( $p=0.003$ ); OS, HR 3.73 ( $p=0.007$ )]. Bone marrow infiltration at time of transplant increased the risk of disease relapse  
182 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  
183 OS [(HR: 2.31 (1.20-4.44,  $p=0.012$ )).

184 For the entire cohort of patients, the 1-year and 3-year GRFS were 40% (95%CI, 32%-50%) and 34% (95%CI, 26%-  
185 44%), respectively. The GRFS at 3 years was significantly better in patients with indolent as opposed to aggressive  
186 lymphomas [43% (95%CI, 32%-58%) versus 22% (95%CI, 13%-38%), HR 1.69 ( $p=0.02$ )], mainly because of a lower  
187 relapse risk. These figures remained unchanged also at 5 years (data not shown). The 3-year GRFS was 41% and 30%  
188 in patients with complete remission (CR) and partial remission (PR) at the time of transplant, respectively ( $p=0.075$ ).  
189 There was a trend for a better outcome in CR patients. The 3-year GRFS was 27% and 41% in patients allografted from  
190 matched sibling and unrelated donors, respectively ( $p=0.096$ ). Distribution of individual components of GRFS is given  
191 in Supplementary Methods (Figure S5 and S6). Main clinical characteristics were not different between patients  
192 receiving sibling or MUD transplants: therefore the difference in GRFS was caused by a lower chronic GVHD requiring  
193 systemic therapy in patients allografted from unrelated donors. In multivariable model the same factors, affecting PFS  
194 and OS, influenced significantly GRFS [histotype, HR 2.02 ( $p=0.026$ ); bone marrow infiltration, HR 2.70 ( $p<0.004$ )]  
195 (Table 3).

196 **Discussion**

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

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

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

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

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

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

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

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

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

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

263 In the last years, a number of novel agents for lymphomas became available. Major advances occurred in  
264 relapsed/refractory CLL, MCL and FL using the Bruton tyrosine kinase inhibitors (ibrutinib), the inhibitor of BCL-2  
265 venetoclax and phosphatidylinositol-3-kinase delta (PI3K $\delta$ ) inhibitor idelalisib (30-33). All these studies have intrinsic  
266 limitations mainly due to the very short follow-up and also the long-term efficacy and safety is lacking. The question of  
267 which strategy (RIC alloSCT or new drugs) is better for the treatment of transplant eligible patients is now open and  
268 appropriately designed prospective clinical trials will be required to challenge the 34% of patients that are alive and free  
269 of any complication after alloSCT.

### Acknowledgments

This work was supported by a AIRC and AIL grants. The authors have no potential conflict of interest to disclose  
Thanks to patients, families, nurses and GITMO (GRUPPO ITALIANO TRAPIANTO MIDOLLO OSSEO) for its  
participation in this Study and in particular to:

- Attilio Olivieri MD, Ospedali Riuniti di Ancona, Italia;
- Benedetto Bruno MD, Ospedale le Molinette di Torino, Italia;
- Monica Bocchia MD, Ospedale Policlinico di Siena, Italia;
- Paolo Di Bartolomeo Prof, Azienda Sanitaria di Pescara, Italia;
- Andrea Bacigalupo Prof, Policlinico Gemelli, Roma, Italia;
- Nicola Mordini MD, Azienda Ospedaliera S.Croce e Carle, Cuneo, Italia;
- Russo Domenico Prof, Ospedali Civili di Brescia, Brescia, Italia,
- Fabio Benedetti MD, Policlinico GB Rossi, Verona, Italia.

Thanks to the Clinical Trial Office Fondazione IRCCS Istituto Nazionale dei Tumori (Debora Deglinnocenti).

Thanks to Carniti Cristiana PhD (Hematology Laboratory, Fondazione IRCCS Istituto Nazionale dei Tumori, Italia)

**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 histotype: 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)	%
<b>Age at AlloSCT, median (range)</b>	52 (23-65)	
<b>Gender</b>		
Male	80	66%
Female	41	34%
<b>Karnofsky performance status</b>		
≤80	18	15%
>80	103	85%
<b>Diagnosis*</b>		
FL	35	28%
CLL	29	24%
DLBCL	35	28%
MCL	22	18%
<b>Bone Marrow involvement</b>		
Yes	30	25%
No	79	65%
Missing	12	10%
<b>Extranodal involvement</b>		
Yes	26	21%
No	78	64%
Missing	17	14%
<b>Prior AutoSCT</b>		
Yes	74	61%
No	47	39%
<b>Disease Status at Transplant</b>		
CR	48	40%
PR	64	53%
PD/SD	9	7%
<b>Donor Type</b>		
Matched/Mismatched Related **	67	55%
Matched Unrelated	34	28%
Mismatched Unrelated	20	17%
<b>Sex mismatched</b>		
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 disease; 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
<b>Age at AlloSCT</b>				
46 Vs 57*	1.45 (0.73-2.86)	0.55	---	---
<b>Histotype subgroup</b>				
Aggressive vs Indolent	1.07 (0.44-2.60)	0.891	3.03 (1.14-8.08)	<b>0.026</b>
<b>Bone Marrow involvement</b>				
Yes Vs No	---	---	3.18 (1.16-8.66)	<b>0.024</b>
<b>Extranodal involvement</b>				
Yes Vs No	---	---	0.95 (0.28-3.18)	0.930
<b>Prior AutoSCT</b>				
Yes vs No	1.82 (0.66-5.06)	0.250	1.11 (0.35-3.47)	0.860
<b>Donor Type</b>				
Unrelated Vs Related	1.75 (0.75-4.08)	0.197	0.80 (0.31-2.05)	0.640
<b>Sex mismatched</b>				
Female donor-Male recipient Vs Other combinations	2.52 (0.91-6.97)	<b>0.076</b>	0.12 (0.01-1.15)	<b>0.067</b>

**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 3<sup>rd</sup> and 1<sup>st</sup> quartiles of the variable distribution.

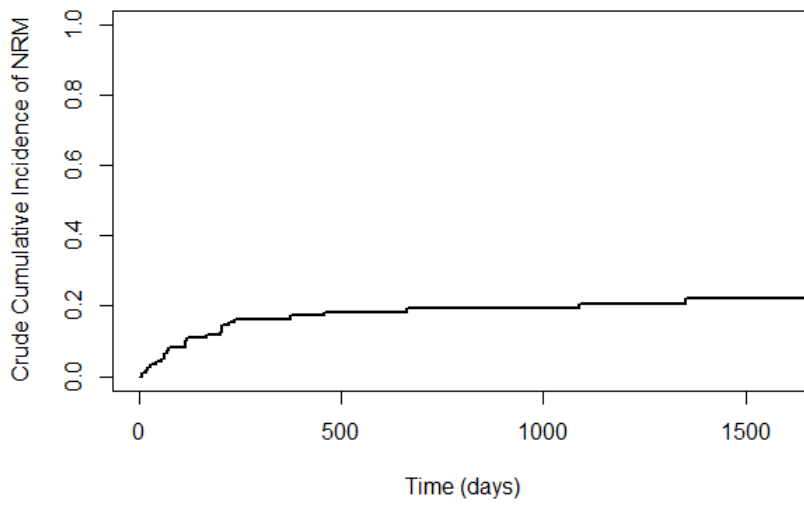
**Table 3. Multivariable analysis on PFS, OS, GRFS**

	PFS		OS		GRFS	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
<b>Age at AlloSCT</b>						
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
<b>Histotype subgroup</b>						
Aggressive vs Indolent	3.30 (1.49-7.30)	<b>0.003</b>	3.73 (1.42-9.78)	<b>0.007</b>	2.02 (1.09-3.77)	<b>0.026</b>
<b>Prior autoSCT</b>						
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
<b>Bone Marrow involvement</b>						
Yes Vs No	4.79 (2.12-10.82)	<b>&lt;0.001</b>	6.00 (2.10-14.15)	<b>&lt;0.001</b>	2.70 (1.37-5.32)	<b>0.004</b>
<b>Extranodal involvement</b>						
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
<b>Donor Type</b>						
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
<b>Sex mismatched</b>						
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.

273 Figure 1.

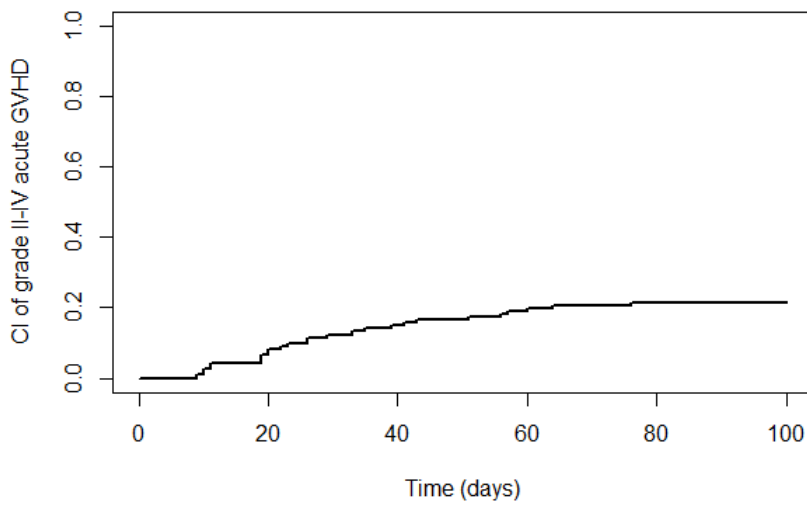
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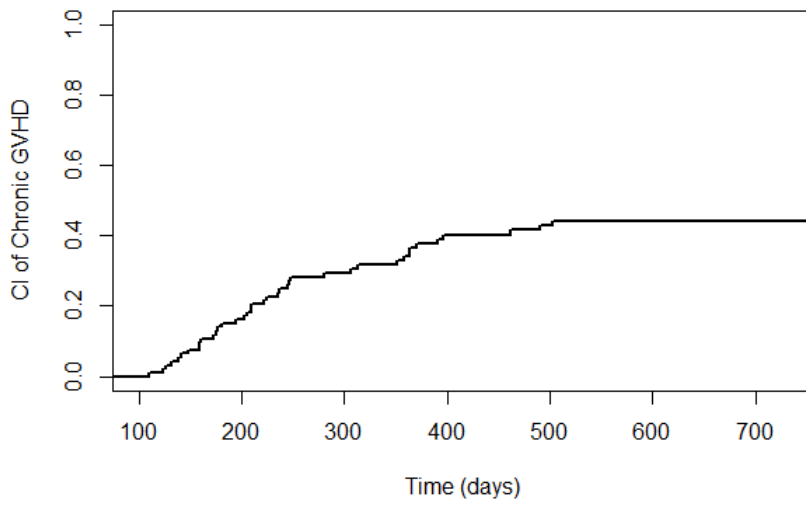
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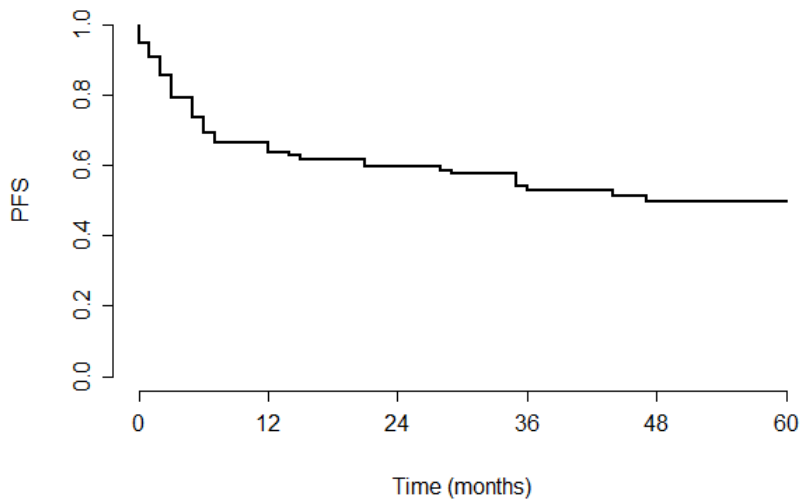
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284 Figure 2.

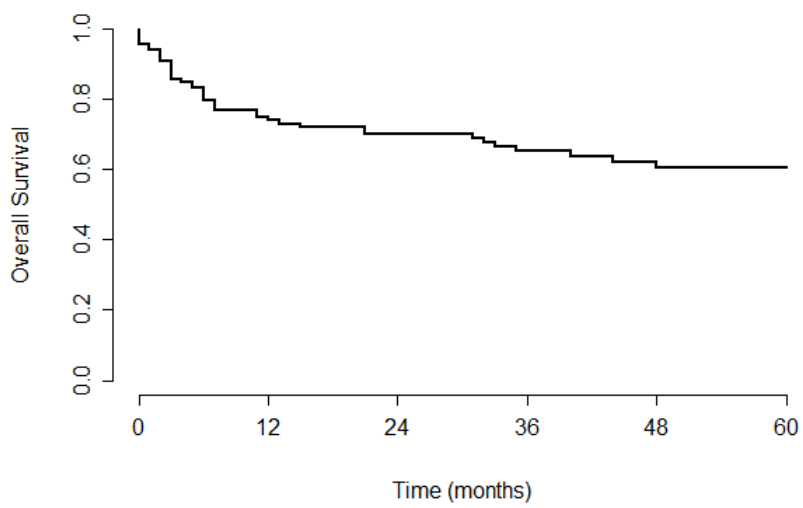
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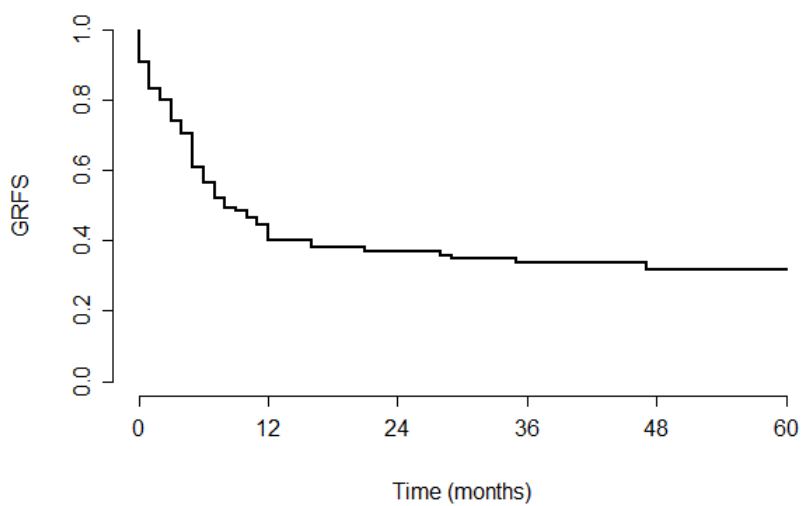
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291 Figure 3

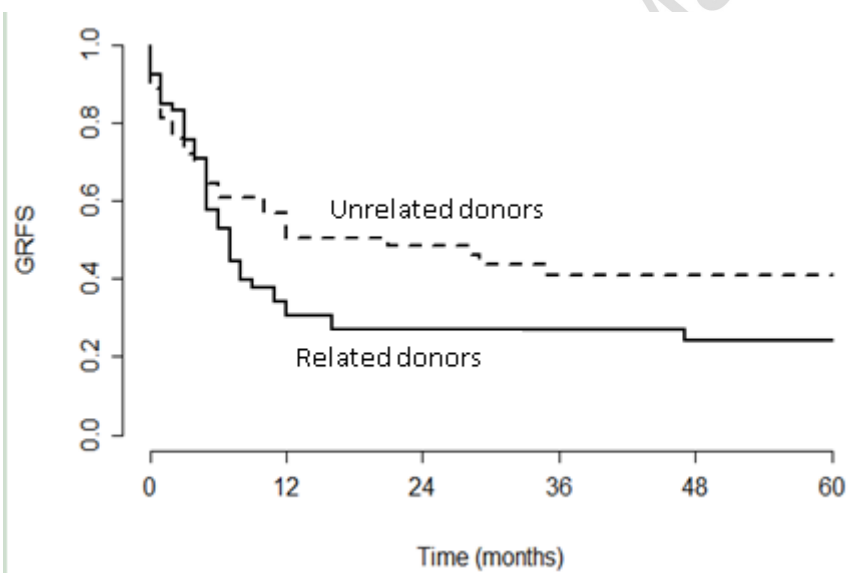
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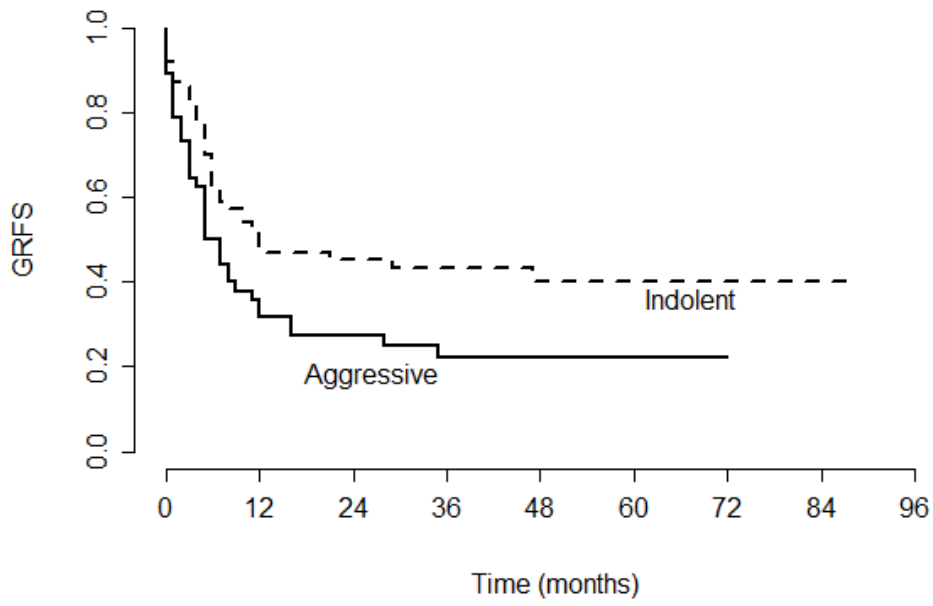


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298 Figure 4.

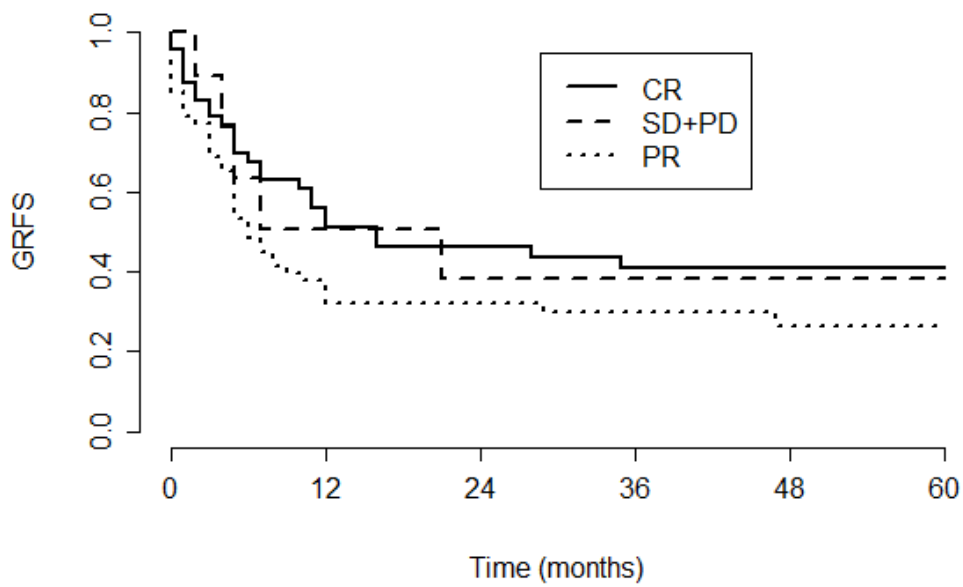
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