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Highlights

- Different serotherapy strategies did not provide a survival advantage in children affected by non-malignant disorders receiving PBSC transplantation.
- A higher rate of severe acute GvHD occurred in patients receiving ATG.
- Alemtuzumab negatively impacted on T cell recovery in the first three months after transplant.

Impact of *in vivo* lymphodepletion on outcome in children with non-malignant disorders receiving peripheral blood stem cell transplantation

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Abstract

Introduction: Peripheral blood stem cell (PBSC) transplantation with *in vivo* lymphodepletion can provide faster neutrophil recovery with limited risk of severe GvHD in children with non-malignant disorders (NMDs). We aimed to provide an historical comparison between these two strategies for prevalence of GvHD, viral reactivation, timing of immune reconstitution and final outcome.

Methods: Data on 98 children receiving PBSC were collected in five European pediatric transplant centers. Only patients with NMDs, receiving treosulfan or myeloablative busulfan conditioning and 9-10/10 HLA-matched transplant were included and divided in two groups according to *in vivo* lymphodepletion (ATG or alemtuzumab). We compared acute and chronic GvHD, EBV, CMV and ADV reactivations, chimerism, lymphocytes recovery, overall and event free survival.

Results: Rate of severe acute GvHD (grade III-IV) was significantly higher in patients receiving ATG (26%; alemtuzumab 10%, $p<.05$), while viral reactivations occurred with a similar rate in both groups (alemtuzumab 56%, ATG 57%). Alemtuzumab was the major risk factor for delayed T cell immune reconstitution in the first 3 months after transplant (OR 6.0, 95%CI 1.8-19, $p<.005$). Extended chronic GvHD, adenovirus reactivation, slower $CD3^+$ cells recovery and HLA-mismatch reduced the probability of survival. Infections were the main cause of mortality in our cohort and delayed T cell recovery was significantly associated with mortality in multivariate analysis (OR 12, 95%CI 1.2-114, $p<.05$). Ultimately, no difference was noted in overall survival and event free survival between ATG and alemtuzumab.

Conclusion: Both ATG and Alemtuzumab showed a similar impact on outcome of children receiving PBSC for NMDs. Strategies of *in vivo* lymphodepletion showed specific drawbacks that were counter-balanced by benefits that ultimately lead to a comparable survival rate. A patient-centered planning of lymphodepleting strategy can be advised in children receiving PBSC for NMDs, by favouring T cell recovery in presence of invasive infections or GvHD prevention in high risk mismatched-transplant.

Keywords: peripheral blood stem cell transplantation; lymphodepletion; ATG; alemtuzumab; GvHD; immune reconstitution.

1. Introduction

Allogeneic haematopoietic stem cell transplantation (HSCT) represents the only curative strategy for several non-malignant disorders (NMD). Very good outcomes are reported with the combination of *in vivo* T cell depletion and peripheral blood stem cells (PBSC), with the former preventing graft versus host disease (GvHD) and the latter yielding rapid granulocytes recovery. Two main strategies are usually adopted for *in vivo* lymphodepletion: anti-thymocyte globulin (ATG) or alemtuzumab (anti-CD52 monoclonal antibody). Comparisons of efficacy between ATG and alemtuzumab as GvHD prophylaxis in children are variably reported: data suggest a greater protective effect against severe acute GvHD (aGvHD grade III-IV) with alemtuzumab, but raise concerns on significantly slower lymphocyte recovery and reduced survival[1,2]. With the aim to provide an historical data comparison, we report the results of a retrospective multicenter study on PBSC transplants in children with NMD assessing the impact of two strategies of *in vivo* lymphodepletion.

2. Methods

We retrospectively collected data on consecutive HSCT in children with NMD using PB as stem cell source in five European reference centers. Transplants performed between December 2007 and December 2017 using myeloablative busulfan or treosulfan-containing reduced toxicity conditioning[3,4] were included. All procedures in this study performed in accordance with the ethical standards of the institutional research committees and with the 1964 Helsinki declaration and its later amendments. HLA-matching was evaluated by high-resolution molecular typing for HLA-A, -B, -C, -DR, -DQ alleles. The diagnosis of GvHD was made clinically, confirmed pathologically with skin, mucosal or liver biopsy whenever possible and grading was performed according to internationally accepted criteria[5,6]. Immune reconstitution was evaluated through absolute count lymphocyte subsets by means of flow cytometry at 1, 3, 6 and 12 months after transplant. Patients

underwent weekly polymerase chain reaction (PCR) analysis of blood for adenovirus (ADV), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) monitoring. Treatment for blood viral infection was administered as per institutional guidelines. Chimerism was evaluated on mononucleated cells in peripheral blood at +1 and +2 years after transplant or at the last available follow-up. Medians of continuous variables were compared using the Mann-Whitney test, while the Log rank test was used to compare Kaplan-Meyer survival curves. To estimate event-free survival (EFS), events were defined as either transplant rejection, death or disease recurrence. Risk factors analyses were performed using the Fisher's exact test for univariate analyses and logistic regression for multivariate analyses (including variables associated in univariate analysis with $p<0.2$). Thresholds for statistical significance were determined as $p<.05$.

3. Results

Data from ninety-eight consecutive patients were retrieved. Patients were divided into two groups ATG (Grafalon or Thymoglobulin, n=35) versus alemtuzumab (n=63). Seven patients received ATG Grafalon at a cumulative planned dose ranging from 30 to 60 mg/kg from day -3 to day -1, while 28 patients received Thymoglobulin with a cumulative planned dose ranging from 6 to 10 mg/kg from day -4 to day -2. Higher doses were used in patients with robust T cell immunity or receiving a 9/10 HLA mismatched transplant, according to local policies. Alemtuzumab was administered from day -5 to day -1 for a cumulative dose of 1 mg/kg. No significant differences were noted for age at the time of transplant, underlying disease, HLA-matching, and donor type. A significantly higher proportion of patients of the alemtuzumab group received a treosulfan-based conditioning and did not receive methotrexate as part of the GvHD prophylaxis (**Table S1**). With a median follow up of 29 months (range 1.3-128) the 2-years overall survival (OS) and event free survival (EFS) of the whole cohort are 85% (95%CI ± 7.4%) and 80% (95%CI ± 8%), respectively. No significant differences in OS (ATG 86% *vs* alemtuzumab 85%) and EFS (ATG 83% *vs*

alemtuzumab 79%) were observed, and primary graft rejection rarely occurred in both groups (ATG: 1/35, 3%; alemtuzumab 1/62, 2%). In the alemtuzumab group the most common causes of death were viral infections (4/8: 2 ADV pneumonia, 1 influenza pneumonia and 1 JC virus infection), with pneumonitis of unknown etiology (n=1), treatment related toxicity (n=2, one before stem cell infusion) and vasculopathy (n=1) in the remainders. In the ATG group two patients died of sepsis, one of CMV pneumonia, one due to vasculopathy, one from severe aGvHD.

Similar frequencies of post-transplant cumulative ADV, EBV and CMV viraemia requiring treatment as per institutional guidelines were observed in the two groups (57% in ATG *vs* 56% in alemtuzumab, *p= 0.9*). Similarly, each single viraemia occurred in both groups with no significantly different prevalence (CMV: alemtuzumab 27% *vs* ATG 26%, *p=0.9*; ADV: alemtuzumab 23% *vs* ATG 20%, *p=0.9*; EBV alemtuzumab 24% *vs* ATG 23%, *p=0.9*). Severe aGvHD (grade III-IV) occurred more frequently with the use of ATG (9/35, 26%) over alemtuzumab (6/62, 10%) (*p<.05*), and was not associated with other transplant-related variables (HLA-matching, conditioning, GvHD prophylaxis, data not shown). Among patients evaluable at 100 days of follow-up, fourteen (14/94, 15%) showed signs of cGvHD, that progressed from aGvHD in 11 patients. No statistically significant differences were noted in the prevalence of extensive cGvHD between the two serotherapy strategies (ATG 12%; alemtuzumab 5%, *p=0.2*). At last follow-up a slightly higher proportion of mixed chimerism (donor cells < 95% in whole blood) was observed in patients receiving alemtuzumab (20/63, 32%) than in the ATG group (6/35, 17%), but this difference was not statistically significant (*p=0.1*). Similarly, in a sub-analysis of PID patients comparable proportions of mixed chimerism were found in both groups (ATG 4/16 *vs* alemtuzumab 14/43, 25% *vs* 33%). Overall, among patients with mixed chimerism after transplant, only 3 treated with alemtuzumab presented with disease recurrence: one Wiskott-Aldrich syndrome patient with low platelet counts and developing IBD; one patient with congenital neutropenia still requiring G-CSF; one LRBA deficiency patient with recurrent infections and refractory diarrhea.

Univariate analysis investigating risk factors for mortality detected that survival was negatively impacted by HLA mismatch, ADV reactivation, extensive chronic GvHD (cGvHD) and delayed T cell recovery ($CD3^+$ < 300/mmc at 3 months after transplant) (**Fig. 1)**. Of note, in the multivariate analysis delayed T cell recovery was the only independent variable significantly associated with an increased rate of mortality (OR: 12; 95%CI: 1.2-114; *p<.05*) (**Table S2**). Medians' comparison of absolute lymphocytes and subsets showed that total lymphocytes and $CD3^+$ were significantly higher in patients receiving ATG up to three months after transplant (+90d $CD3^+$ cells: alemtuzumab 150/mmc *vs* ATG 680/mmc, *p <.0001*), whereas $CD3^+$ counts were comparable at 6 and 12 months. A more striking difference was noted in $CD8^+$ subset, with a faster and higher expansion in the ATG group, regardless of viral reactivations. Conversely, kinetics of recovery of $CD4^+$, $CD19^+$, $CD16^+/56^+$ cells were comparable between the two groups (**Fig. 2**). Logistic regression showed that only the use of alemtuzumab displayed a significant negative impact on $CD3^+$ cell recovery at three months after transplant (**Table S3**).

4. Conclusion

To the best of our knowledge, this is the largest study on children receiving PBSC for NMD comparing two strategies of *in vivo* lymphodepletion. The higher $CD34^+$ cell dose infused with PBSC can facilitate engraftment in non-malignant transplant recipients[7]. However, previous reports showing a significantly higher rate of GvHD and TRM do not allow to consider PBSC equivalent to or preferable over bone marrow stem cells in children[8]. *In vivo* lymphodepletion plays a pivotal role in avoiding transplant rejection but also provides efficient prevention of GvHD, and the higher doses of $CD34^+$ and $CD3^+$ cells infused with PBSC transplant promote earlier haematological recovery. The first important result of this analysis is that both lymphodepleting strategies resulted in a limited TRM and in a very low rate of transplant rejections. This is reassuring given previous reports documenting higher mortality rate in alemtuzumab-based conditioning[2]. Overall, we showed that poor $CD3^+$ recovery discerned patients with lower survival rate in both groups and this

was the most significant predictor of mortality. Our data support the advantage of alemtuzumab in effectively mitigating severe aGvHD in children receiving PBSC for NMD, for which no benefits from allo-immune reactivity is expected, unlike in malignant diseases. A detrimental effect on early T cell recovery was observed in the alemtuzumab group, which in our cohort did not translate into worse survival, possibly due to the limited size of the cohort. Although we are not able to draw definitive conclusions, mainly due to the retrospective nature of the study and the limited number of patients, especially in the ATG group, it is possible to speculate that advantages and disadvantages associated with ATG and alemtuzumab could counter-balance negative and positive effects leading to a similar survival rate in the context of PBSC transplants. Therefore, a patient-tailored decision on the optimal *in vivo* lymphodepletion in this setting might be warranted. In children with active infections at the time of transplant, the higher risk of mortality associated with delayed immune reconstitution and the negative impact on $CD3^+$ counts of alemtuzumab could support clinicians' decision to use ATG as lymphodepleting agent. By contrast, in patients with additional risk factors for GvHD (e.g. highly mismatched transplants) and low burden/limited risk of infections the use of alemtuzumab could confer a protective effect against the occurrence of severe aGvHD, and ultimately lead to a better outcome. Nevertheless, it is increasingly clear that drug exposure, more than the type of serotherapy, have the greatest impact on immune reconstitution and GvHD: adjustment of ATG and alemtuzumab exposure, investigated by pharmacokinetics/pharmacodynamics studies, could potentially address patient-tailored dose targeting, aiming to achieve rapid T cell recovery and reduction of TRM in the setting of PBSC transplantation[2,9,10].

In spite of the evident limitations of a retrospective analysis, our data prompt prospective studies to investigate the best approach to lymphodepletion in PBSC transplants in children. Although PB is not currently preferred as stem cell source in pediatrics, broadening the knowledge in this setting is warranted to guide transplant planning in those patients who might benefit from a higher $CD34^+$

cell dose or when only PBSC are available due to donor choice or barriers to the logistic of bone marrow harvesting.

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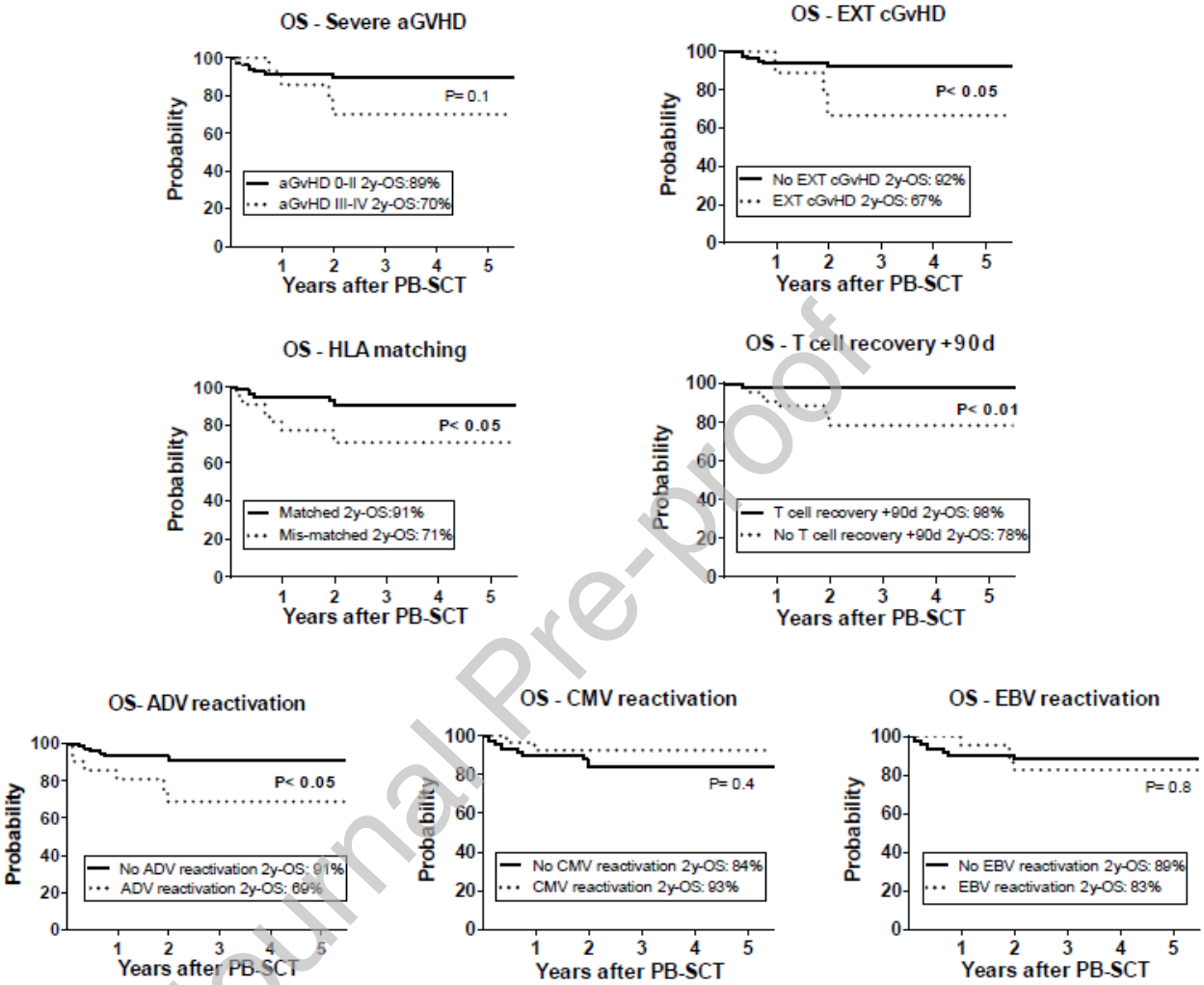

Fig. 1: Overall survival of 98 patients receiving PBSC and in vivo T cell depletion stratified according to (**a**) occurrence of severe aGVHD; (**b**) occurrence of extensive cGvHD; (**c**) HLA-matching; (**d**) T cell recovery at 3 months after transplant; (**e**) ADV reactivation; (**f**) CMV reactivation; (**g**) EBV reactivation. PB-SCT: Peripheral blood stem cell transplantation.

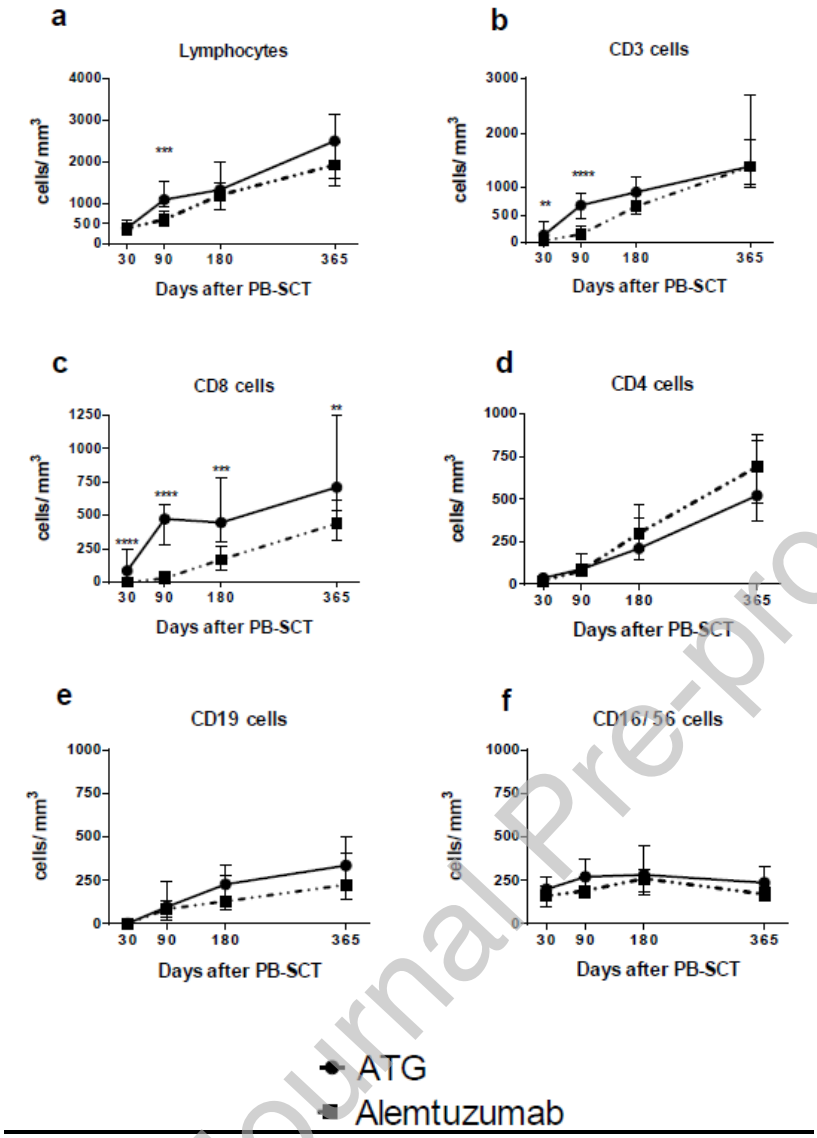

Fig. 2: Median values (Alemtuzumab= square; ATG=dot) and 95% confidence intervals (whiskers) of lymphocyte count (panel **a**) and lymphocyte subsets (**b**=$CD3^+$, **c**=$CD4^+$, **d**=$CD8^+$, **e**=$CD19^+$, **f**=$CD16/56^+$) according to two strategies of in vivo lymphodepletion. P<.05= *; p<.01=**; p<.001=***; p<.0001=****.