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Brief communication

T cell costimulation blockade promotes transplantation tolerance in combination with sirolimus and post-transplantation cyclophosphamide for haploidentical transplantation in children with severe aplastic anemia☆

Sarita Rani Jaiswal[a,b,*], Prakash Bhakuni[b], Shamsuz Zaman[b], Satish Bansal[b], Priyanka Bharadwaj[b], Sneh Bhargava[b], Suparno Chakrabarti[a,b]

[a] Manashi Chakrabarti Foundation, Kolkata, India
[b] Department of Blood and Marrow Transplantation, Dharamshila Narayana Superspeciality Hospital and Research Centre, New Delhi, India

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ABSTRACT

We conducted a pilot study employing extended T cell costimulation blockade (COSBL) with Abatacept along with sirolimus and post-transplantation cyclophosphamide (PTCy) in 10 patients (median age 12) with severe aplastic anemia (SAA). Nine patients engrafted in the COSBL group, compared to all 10 patients (median 14 vs 13 days) treated on PTCy protocols without abatacept (CONTROL group). The incidence of acute graft-versus-host disease (GVHD) was 10.5% in the COSBL group compared to 50% in the CONTROL group ($p = 0.04$). Chronic GVHD (12.5% vs 56%, $p = 0.02$) and CMV reactivation (30% vs 80%, $p = 0.03$) were also reduced in the COSBL group. T and NK cell subset analysis revealed higher $CD56^{bright}CD16^{-}$ NK cells in the CONTROL group ($p = 0.004$), but similar $CD56^{dim}CD16^{+}$ NK cells in both groups at day + 30. Tregs ($CD4^{+}$ $CD25^{+}$ $CD127^{dim/-}$ FoxP3 +) were markedly higher in the COSBL group at day + 30 (8.4% vs 1.1%) and the trend was maintained through day + 90 ($p < 0.01$). The GVHD and Disease-free survival at one year in the COSBL group was 80% vs. 30% in the CONTROL group ($p = 0.05$). Our preliminary findings suggest that COSBL in combination with PTCy and sirolimus might augment transplantation tolerance in children with SAA, probably due to synergistic effect on early recovery of Tregs.

1. Introduction

Haploidentical family donor hematopoietic stem cell transplantation (HSCT) assumes primacy due to its immediate availability in patients with severe aplastic anemia (SAA). The urge for developing a successful haploidentical transplant protocol in this condition has been widely recognised [1]. We and others have previously described our experience with high dose post-transplantation cyclophosphamide (PTCy) based approach in patients with SAA in both children and adults [2–4]. In our hands, a very high incidence of early alloreactivity in the form of acute graft-versus-host disease (GVHD) or post-transplant hemophagocytic syndrome (PTHPS) was noted in younger children with SAA [4,5] using peripheral blood stem cell graft. The introduction of Sirolimus on day −8 pre-transplant reduced this complication in adults, but not so much in children [4,6].

Costimulation blockade (COSBL) with CTLA4Ig has been effective in inducing transplantation tolerance in preclinical studies [7]. It has been found to be safe and effective in clinical trials when co-incubated with the graft [8] or employed as GVHD prophylaxis or treatment [9,10]. However, a minority of naïve and majority of memory T cells have the potential of being activated without engaging CD28-B7 pathway [11]. We hypothesised that COSBL with Abatacept prior to graft infusion would take care of the predominant alloreactive T cell population and a minority of COSBL resistant T cells which might be activated during the 72 h window could be effectively eliminated by PTCy. In addition, a combination of sirolimus and Abatacept might enhance transplantation tolerance through the regulatory T cell pathway, which are unaffected by PTCy due to increased expression of aldehyde dehydrogenase

Abbreviations: CMV, cytomegalovirus; COSBL, T cell costimulation blockade; CSA, cyclosporine A; EBV, Epstein Barr Virus; G-CSF, granulocyte-colony stimulating factor; GVHD, graft-versus-host disease; HCT-CI, Hematopoietic Cell Transplantation-Comorbidity Index; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; MMF, mycophenolate mofetil; NK, natural killer cell; PRBC, packed red cells; PTCy, post-transplantation cyclophosphamide; PTHPS, post-transplant hemophagocytic syndrome; SAA, severe aplastic anemia; SDP, single donor platelets; Tregs, regulatory T cells

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* Corresponding author at: Department of Blood and Marrow Transplantation & Hematology, Dharamshila Narayana Superspecialty Hospital, Vasundhara Enclave, New Delhi 110096, India.
E-mail address: drsaritaranij@dhrc.net.in (S.R. Jaiswal).

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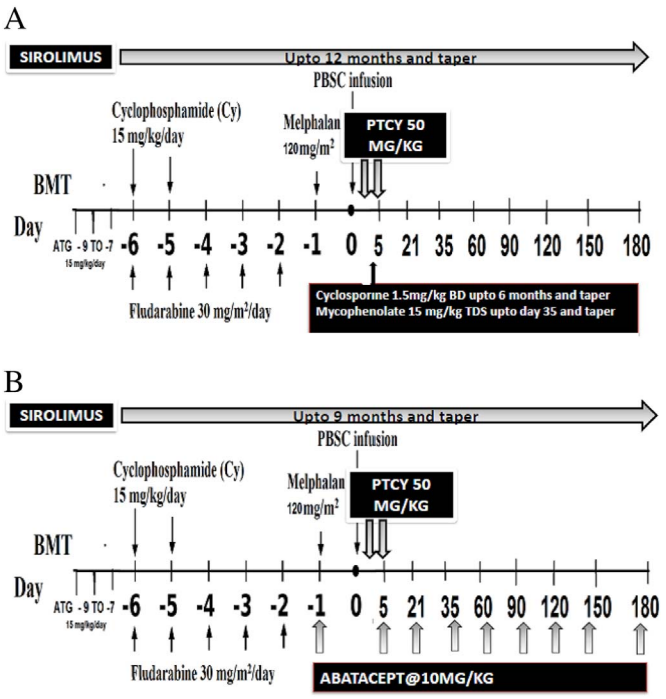

Fig. 1. Conditioning Regimens and GVHD prophylaxis employed in the CONTROL group (A) and COSBL group (B).

[12,13].

2. Patients and methods

Ten patients between the age of 4 and 21 years received a Haploidentical HSCT from January 2015 to May 2016 in the study protocol (COSBL group). A retrospective cohort of 10 patients who were treated on PTCy based protocols [4,6] was analysed for comparison (CONTROL group). Written informed consent and institutional approval were obtained for all of the patients in accordance with the Declaration of Helsinki.

2.1. Conditioning regimen and GVHD prophylaxis

The conditioning protocol for SAA has been described previously [4] and illustrated in Fig. 1A. This comprised of Fludarabine, low dose Cy and Melphalan along with Antithymocyte Globulin (ATG). GVHD prophylaxis consisted of PTCy at 50 mg/kg on days + 3 and 4 with sirolimus from day − 7 (with trough levels of 8–14 ng/ml on Day 0) until nine months. GVHD prophylaxis in the CONTROL group comprised of Cyclosporine (CSA) and Mycophenolate Mofetil (MMF) [4]. In the study group (Fig. 1B), CSA and MMF were replaced with Abatacept. Abatacept was administered at 10 mg/kg on days − 1, + 5, + 20, + 35 and then every 4 weeks until day + 180. A dosage of 250 mg or less of Abatacept was dissolved in 30 ml normal saline and infused over 30 min.

2.2. Stem cell source and harvest

Mother or Non-inherited maternal antigen mismatched sibling was the preferred donor. Donor-specific antibodies were quantitated and tissue cross-matching was done to rule out clinically significant anti-donor HLA antibodies. Donors were treated with G-CSF 12 μg/kg/day in divided doses for 4 days before initiation of harvest on the fifth day. On an average, 3 times the blood volume was processed. The target dose of CD34 + cells was 5–10 × 10^6/kg with the minimum cell dose required being 3 × 10^6/kg.

2.3. Supportive care

Antimicrobial prophylaxis was instituted as per the departmental guidelines. CMV prophylaxis was guided by pre-emptive monitoring of viral CMV load by quantitative PCR twice a week until day 100. Viral loads for adenovirus and EBV were also monitored weekly.

2.4. Assessment of immune reconstitution [10,14]

This was carried out at days + 30, + 60 and + 90 following the HSCT on peripheral blood mononuclear cells. Cell surface staining procedure was performed in 5 ml propylene tube containing 1.5 × 10^6 cells in 100 μl of the peripheral blood. The NK cell and T cell immunophenotypes were carried out by six colour flow Cytometry in Navios (Beckman Coulter Inc., USA) using the following mouse anti-human mAbs from Beckman Coulter, Immunotech, Marseille, France: CD45 (J33), CD3 (UCHT1), CD4 (13B8.2), CD8 (B9.11), CD56 (N901), CD16 (3G8). The samples were incubated with antibodies was for 20 min at room temperature in the dark followed by incubation with RBC lyses buffer Optimise C (Beckman Coulter) for 10 and two washes and resuspension of the pellet in 500 μl of PBS buffer. Data were analysed in Kaluza ver 1.3 (Beckman Coulter) in analysis software.

T regulatory cells (Tregs) were analysed from peripheral blood using mouse anti-human mAbs from BD Biosciences; CD4 (SK3;), CD25 (2A3), CD127 (HIL-7R-M21). Tregs were defined as the population of lymphocytes expressing $CD4^+$ $CD25^+$ $CD127^{dim/-}$ phenotype with expression of FoxP3. Intracellular staining for FoxP3 was carried out with Mouse anti-human FoxP3 (259D/C7; BD Biosciences). The gating strategies for T cell subsets, NK cell subsets and Tregs are illustrated in supplementary data.

2.5. Statistics

Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined as per modified Glucksberg criteria and NIH consensus guidelines respectively. Primary graft failure was defined as lack of neutrophil engraftment by 28 days. The primary endpoints of the study were neutrophil and platelet engraftment, acute GVHD and transplant-related mortality (TRM). Secondary end points included secondary graft failure, cGVHD and 'GVHD and disease free survival' at one year. Probabilities of survival were estimated using the Kaplan-Meier product-limit method. The cumulative incidence rates of TRM, relapse, aGVHD and cGVHD were computed to take account of the presence of competing risks. A survival outcome was determined to be significantly different if the observed p value was < 0.05. Binary variables were compared between the groups using chi-square test, and the continuous variables were analysed using independent sample t-test taking into account Levene's test for equality of variances. All Analyses were performed using statistical software IBM SPSS Statistics Version 22.

3. Results

The pre-transplantation patient characteristics are described in Table 1.

3.1. Engraftment

Nine patients in the COSBL group achieved neutrophil and platelet engraftments at 14 and 15 days respectively. All engrafted in the CONTROL group with similar engraftment kinetics (Table 1). There were no secondary graft failures in either group. Donor chimerism was a median of 96.3% at day + 30 in the CONTROL group compared to 95% in the COSBLgroup.