Repeat course of rabbit antithymocyte globulin as salvage following initial therapy with rabbit antithymocyte globulin in acquired aplastic anemia

Diego V. Clé,[1] Elias H. Atta,[2] Danielle S. P. Dias,[3] Carlos B. L. Lima,[3] Mariana Bonduel,[9] Gabriela Sciuccati,[9] Larissa A. Medeiros,[4] Michel M. de Oliveira,[4] Marco A. Salvino,[5] Marlene Garanito,[6] Sara T. Ollala Saad,[7] Rodrigo T. Calado,[1] and Phillip Scheinberg[1,8]

[1]Division of Hematology, University of São Paulo at Ribeirão Preto School of Medicine, Ribeirão Preto, Brasil; [2]CEMO, National Cancer Institute, Rio de Janeiro, Brasil; [3]Hematopoietic Stem Cell Program, Hemorio, Rio de Janeiro, Brasil; [4]Bone Marrow Transplantation unit, Federal University of Paraná, Curitiba, Brasil; [5]Bone Marrow Transplantation unit, Federal University of Bahia, Salvador; [6]Child's Institute, University of São Paulo, Brasil; [7]National Institute of Science and Technology of Blood, University of Campinas, Brasil; [8]Clinical Hematology, Antônio Ermírio de Moraes Cancer Center, Hospital São José e Beneficência Portuguesa, São Paulo, Brasil; [9]Servicio de Hematología-Oncología, Hospital de Pediatría Prof. Dr. Juan P. Garrahan, Buenos Aires, Argentina; and [1-8]Report for the Brazilian Marrow Failure Network (BONE)

Correspondence: dvcle@hcrp.usp.br
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Supplemental file

Methods

Patients

Between January 2005 and January 2014, 39 patients were diagnosed with AA and failed or relapsed after initial r-ATG/CsA and were retreated with r-ATG/CsA as salvage at two marrow failure centers in Brazil (University of São Paulo at Ribeirão Preto School of Medicine, Ribeirão Preto, SP and Hemorio, Rio de Janeiro, RJ) and one in Argentina (Servicio de Hematología-Oncología, Hospital de Pediatría Prof. Dr. Juan P. Garrahan, Buenos Aires). The respective local institution review boards approved the study.

Aplastic anemia was defined as a bone marrow cellularity of less than 30% and pancytopenia with at least two of the following peripheral blood count criteria: 1) hemoglobin <100 g/L; 2) absolute neutrophil count (ANC) $<1.5x10^9$/L; or 3) platelet count $<50x10^9$/L.(1) Severe AA (SAA) was considered if two of the following three criteria were fulfilled: 1) ANC $<0.5x10^9$/L; 2) absolute reticulocyte count (ARC) $<60x10^9$/L; or 3) platelet count $<20x10^9$/L;(2) and for very severe AA (vSAA) the same as for SAA but also ANC $<0.2x10^9$/L.(3) AA was classified as non-severe AA (NSAA) in patients not fulfilling the criteria for SAA.

Response to immunosuppression was assessed at three and six months after beginning therapy. For patients with SAA and vSAA, response was defined as no longer meeting severity criteria and achieving transfusion independence for at least one month. For NSAA patients, response was defined as transfusion independence for at least one month. Refractoriness was considered when patient did not fulfill response criteria. Relapse was considered if the patient had a previous response following r-ATG/CsA and again became transfusion dependent or met criteria for SAA or vSAA. For patients with NSAA, only transfusion-dependent patients were retreated.

Patients with constitutional aplastic anemia (Fanconi anemia with a positive chromosome breakage test or clinical diagnosis of dyskeratosis congenita) and those AA patients who did not completed the five-day r-ATG schedule were excluded from analysis.

Treatment regimen

Of the 39 AA patients, 34 received salvage r-ATG/CsA according to respective institutional protocols in three centers. The remaining five patients were enrolled in a single-arm phase II study in one institution of salvage r-ATG/CsA associated with 2-5 weekly intravenous infusions of allogeneic unrelated non-HLA-matched bone marrow derived mesenchymal stromal cells (registered at clinicaltrials.gov, NCT01297972). This intervention was found not to alter immunosuppression response and thus their data were combined for the purpose of analysis.

All patients were hospitalized for r-ATG (Thymoglobulin®, Genzyme, Cambridge, MA, USA) administration at variable doses (median, 3.5 mg/kg/d; range, 1.65-5.0), for five consecutive days, depending on individual institutional protocols. CsA was started on day 1 at 5 mg/kg/d in two divided doses, and adjusted to maintain a serum through level of 150–400 ng/ml, for at least six months. Serum sickness prophylaxis, usually methylprednisolone at 1mg/kg/d, was given for at least 2 weeks to prevent serum sickness and trimethoprim–sulfamethoxazole 400/80 mg q12h was administered two to three times a week for *Pneumocystis jiroveci* infection prophylaxis. Additional antimicrobial prophylaxis was provided in some cases per treating physician. Granulocyte colony-stimulating factor (G-CSF) was administered if clinically indicated. Red blood cells were transfused in patients with symptomatic anemia and platelets were prophylactically transfused to maintain the platelet level above $10x10^9$/L.

Statistical analysis

For this analysis the primary endpoint was hematologic response at 3 and 6 months after salvage r-ATG. Secondary endpoints included relapse, clonal evolution, and overall

survival (OS). For survival analysis, the Kaplan–Meier estimates were based on the survival days from the start of salvage r-ATG therapy. Differences in response rates between AA severity groups were evaluated by Fisher's exact test. For OS, patients were censored at the time of last visit, death or HSCT and analyzed by the Kaplan–Meier method. Comparison of OS between responders and non-responders was performed by the log-rank test. Absolute neutrophil count at the start of salvage r-ATG therapy was measured and the difference between responders and non-responders was analyzed by Mann Whitney nonparametric test. All statistical analyses were performed using Graphpad Prism 6.0 software.

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