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

The treatment for the majority of patients with acquired aplastic anemia (AA) who are not suitable for hematopoietic stem cell transplant (HSCT) is immunosuppression with standard horse antithymocyte globulin (h-ATG) and cyclosporine (CSA) where hematologic responses are observed in 60-70%.[1] However, since 2007, h-ATG is no longer available in most Latin American, Asian and European countries, with rabbit ATG (r-ATG) being the only accessible formulation.[2,3] Hematologic response and overall survival when using r-ATG as first-line therapy for AA are significantly inferior as compared to h-ATG.[1,3,4] This led to many patients having need of salvage therapies following initial r-ATG. A repeat course of immunosuppression with h-ATG or alemtuzumab in this setting of initial r-ATG failure yields a response rate of about 20%,[5,6] but the outcomes of repeating a course with the same formulation of r-ATG in this scenario are not known.

To address this question, we conducted a retrospective analysis of 39 patients diagnosed with AA who failed or relapsed after initial r-ATG/CsA and were retreated with r-ATG/CsA as salvage between January 2005 and January 2014 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 (Hospital Juan P. Garrahan, Buenos Aires). The respective local institution review boards approved the study.

Aplastic anemia was diagnosed and disease severity classified according to established criteria[7,8] (for details see *Online Supplementary Methods*). Response to immunosuppression was assessed at three and six months following the commencement of therapy. For patients with severe AA (SAA) and very severe AA (vSAA), response was defined as no longer meeting severity criteria and achieving transfusion independence for 1 month or longer. For non-severe AA (NSAA) patients, response was defined as transfusion independence for 1 month or longer. A patient was considered refractory when they did not fulfill response criteria. Relapse was considered if the patient had a previous response following r-ATG/CsA and once more became transfusion dependent or met criteria for SAA or vSAA. For patients with NSAA, only transfusion-dependent patients were retreated. Of the 39 AA patients, 34 received salvage r-ATG/CsA according to respective institutional protocols in the three centers. The remaining five patients were enrolled in one of the institutions in a single-arm phase II study of salvage r-ATG/CsA associated with 2-5 weekly intravenous infusions of allogeneic marrow derived mesenchymal stromal cells (NCT01297972). This intervention was found not to alter immunosuppression response, thus their data were combined for the purpose of analysis. Patients with constitutional AA or who did not complete the five-day r-ATG schedule were excluded from analysis. All patients received r-ATG (Thymoglobulin®, Genzyme Corporation, Cambridge, MA, USA) at variable doses (median, 3.5 mg/kg/d; range, 1.65-5.0), for five consecutive days, depending on individual institutional protocols (see *Online Supplementary Methods*). CsA was started on day 1 at 5 mg/kg/d in two divided doses, and adjusted to maintain a serum trough level of 150–400 ng/ml for at least six months. For survival analysis, the Kaplan–Meier estimates were based on the survival days from the start of the salvage r-ATG therapy, and patients were censored at the time of last visit, death or HSCT. Differences in response rates between AA severity groups were evaluated by Fisher's exact test. Comparison of overall survival (OS) between responders and non-responders was performed by the log-rank test.

Thirty-nine patients received retreatment with r-ATG/CsA; two received r-ATG for only one day due to severe allergic reaction and were thus excluded from this analysis. Data from the remaining 37 patients are presented. After a median of 283 days from first r-ATG/CsA

Table 1. Patients' characteristics.

Characteristic	N=37
Age – years, median (range)	17 (3-63)
Male – no. (%)	21 (57)
Disease severity – no. (%)	
Non-severe	4 (11)
Severe	20 (54)
Very severe	13 (35)
Absolute neutrophil count – x10⁹/L, median (range)	0.45 (0-1.74)
GPI-negative clone – no. (%)*	
<1%	30 (81)
≥1%	2 (5)
Type of response to first rATG – no. (%)	
Refractory	32 (86)
Relapse	5 (14)
First rATG dose – mg/kg/day x 5 days, median (range)	3.1 (1.5-5.0)
Second rATG dose – mg/kg/day x 5 days, median (range)	3.5 (1.7-5.0)
Time from first rATG to second rATG – days, median (range)	283 (118-2379)

*Not performed in 5 cases. GPI: glycosylphosphatidylinositol; rATG: rabbit antithymocyte globulin.

Table 2. Hematologic response at 3 and 6 months to second course rabbit ATG plus cyclosporine.

	Refractory to first r-ATG/CsA (n=32)	95% CI	Relapsed to first r-ATG/CsA (n=5)	95% CI
Response at 3 months no. (%)	5 (16%)	3-28%	3 (60%)	17-100%
Response at 6 months no. (%)	7 (22%)	8-36%	3 (60%)	17-100%

(range, 118-2379), 37 patients (32 refractory and 5 relapsed) received a second five-day r-ATG course followed by CsA. Table 1 summarizes patients' characteristics. After a median follow-up of 24 months (range, 0.2-77), hematologic response was observed in 8 (22%) patients at 3 months and in 10 (27%) patients at 6 months for the entire cohort. Among those who were refractory to initial r-ATG, 7 out of 32 (22%) responded at 6 months (Table 2). Among those who had previously relapsed, 3 out of 5 (60%) responded (Table 2). None of the four patients classified as NSAA at diagnosis responded to the second r-ATG. Among those with more severe disease (vSAA or SAA) the hematologic response rate at 6 months was 10/33 (30%), with 7/20 (35%) patients with SAA and 3/13 (23%) with vSAA responding. Of these 10 responders, two patients relapsed at days 170 and 897 after retreatment, and were alive at last follow-up. These latter evolved to myelodysplastic syndrome (MDS) with normal karyotype. Among all non-responders (n=27), five clonal evolutions were observed: four to monosomy 7 and one to trisomies 8 and 21. In the entire cohort, three patients underwent HSCT due to refractory disease or evolution to MDS. In total, 12 patients died, all from among non-responders, and invasive fungal infection was the most frequent cause of death (3 out of 12). The overall survival at 4 years was 55% (95% CI, 33-72%; Figure 1A). Survival was superior in patients who responded to the second r-ATG/CsA as compared to non-responders (*P*=0.004; Figure 1B), and there was no statistical difference in survival for those who were refractory or relapsed (Figure 1C).

Thus, in the present study, we show that approximately only one in five AA patients who had not responded to first-line r-ATG/CsA may be rescued with a second course of r-ATG/CsA. Among those with SAA or vSAA (excluding NSAA), this rate was about 30% in our cohort. The salvage rate with h-ATG/CsA or alemtuzumab in this setting has been reported to be comparably low: only 8% for alemtuzumab and 21% for h-ATG.[5,6] Taking into account that only one-third of patients respond to initial r-ATG,1 it is reasonable to assume that at least half of AA patients will remain refractory to immunosuppression, even with repeat courses of r-ATG, h-ATG, or alemtuzumab when r-ATG is used in first-line therapy. The high success rate of initial h-ATG therapies cannot be recapitulated when r-ATG is administered first, given the low rate of hematologic responses with 1 or 2 courses after initial r-ATG, reinforcing the necessity for an effective "first shot" when treating AA with immunosuppression. With an initial response rate of 60-70% to h-ATG directly, and a salvage rate of about 30-40% (with repeat ATG or alemtuzumab), hematologic responses can be anticipated in 70-85% of cases when ISTs are administered in this order.[1,4,5,9-12]

We also show that achieving a response to a second course of r-ATG is strongly associated with excellent long-term survival.[3,9,12] The achievement of hematologic response has been a consistent and reliable surrogate for long-term survival in treatment-naïve SAA, and is confirmed in our cohort.[9,12,13]

In patients who had a previous response to r-ATG/CsA and relapsed, retreatment with r-ATG/CsA may associate with better results, with 3/5 (60%) patients responding. Despite the limited patient number, this result is comparable to that previously reported in the relapsed setting following h-ATG.[5,9] This difference in salvage rates between refractory and relapsed patients has been consistently shown, and may reflect distinct mechanisms for marrow failure. Relapsed patients have immunomodulatory responsive disease (exhibited with the initial response) and are probably more amenable to further immunomodulation. In contrast, refractoriness to first ATG may reflect alternative or additional mechanisms, such as severe hematopoietic stem cell depletion or a non-immune basis.

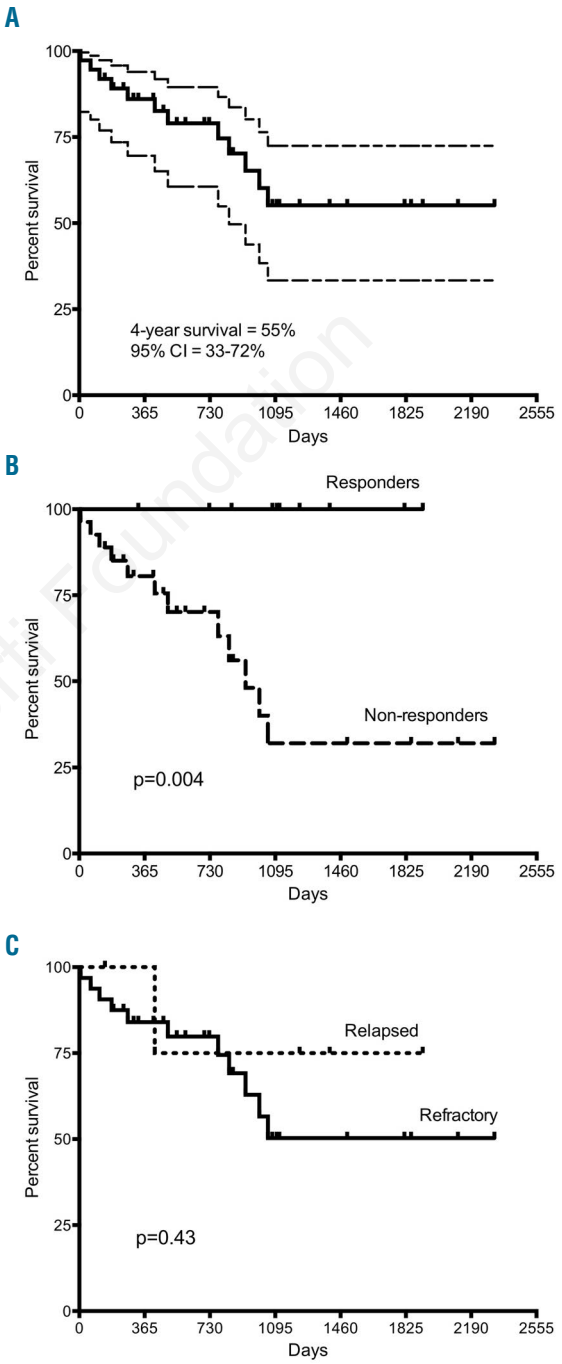

Figure 1. Overall survival curves. (A) Survival for the entire cohort of aplastic anemia patients who failed or relapsed after initial r-ATG/CsA and were retreated with r-ATG/CsA (n=37); dotted lines represent 95% confidence intervals (CI). (B) Survival of patients who responded (n=10) or did not respond (n=27) at 6 months after the second course of r-ATG/CsA. (C) Survival after second r-ATG/CSA of AA patients who were refractory (solid line) or relapsed (dotted line) after first r-ATG/CSA. Patients who underwent hematopoietic stem cell transplantation were censored at the time of transplant.

Given the low salvage following r-ATG, other rescue therapies could be considered that may include alternative donor HSCT, thrombopoietin receptor agonists, or alternative experimental therapies. It has recently been shown that eltrombopag induces hematologic response in up to 44% of refractory SAA patients, which included trilineage responses.14 This was expanded to a larger cohort with longer follow-up which confirmed the response rate of about 40%, and multilineage increments in blood counts were again observed.[15] One of the concerns related to rescue eltrombopag is the potential propensity to stimulate clonal evolution. However, the clonal evolution rate of 19%, as reported in initial eltrombopag studies, is similar both to our current analysis and to that reported in refractory SAA.[5,9,12,14,15] Ongoing studies employing eltrombopag in combination with h-ATG/CsA as first therapy will further elucidate the clonal evolution risk.

In the aggregate, the high success rate of initial h-ATG therapies cannot be recapitulated when r-ATG is administered first, given the relatively low salvage rate with alemtuzumab, h-ATG and now (*current data*) with r-ATG. This observation emphasizes the importance of initiating immunosuppressive therapy in SAA with h-ATG, which is associated with better outcomes overall. For those refractory to initial r-ATG a repeat course of IST can be effective and produce clinically meaningful hematologic responses in about 20-30% of cases. It is reasonable to consider alternative non-IST (transplant and non-transplant) therapies in this patient population. Those who relapse following initial r-ATG appear to fare better with a higher response rate. It seems unlikely that a more definitive prospective study will be conducted to better define the role of salvage r-ATG following initial r-ATG, thus leaving retrospective studies such as ours as the principal guide for hematologists who care for patients with marrow failure disorders.

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