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

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

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1-8 Report for the Brazilian Marrow Failure Network (BONE).

Running head: Second rabbit ATG for acquired aplastic anemia
Correspondence

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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%. However, since 2007, h-ATG became unavailable in most Latin American, Asian and European countries, with rabbit ATG (r-ATG) the only accessible formulation. (2, 3) Hematologic response and overall survival for r-ATG as first therapy for AA are significantly inferior as compared to h-ATG. (1, 3, 4) This led many patients following initial r-ATG in need of salvage therapies. A repeat course of immunosuppression with h-ATG or alemtuzumab in this setting of initial r-ATG failure yields 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 supplement). Response to immunosuppression was assessed at three and six months after beginning 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. For non-severe AA (NSAA) patients, response was defined as transfusion independence for +1 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. 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 marrow derived mesenchymal stromal cells (NCT01297972). This intervention was found not to alter immunosuppression response and 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, 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 supplement). 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 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 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 only for one day due to severe allergic reaction and were excluded from this analysis. Data from the remainder 37 patients are presented. After a median of 283 days from first r-ATG/CsA (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%) 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 re-treatment and were alive at last follow-up. The 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 among nonresponders, and invasive fungal infection was the most frequent cause of death (three 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. 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 upfront 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 strongly associates 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 it 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 shown consistently and may reflect distinct mechanisms for marrow failure. Relapsed patients have immunomodulatory responsive disease (shown with the initial response) and probably are 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.

Given the low salvage following r-ATG, other rescue therapies could be considered that may include alternative donor HSCT, thrombopoietin receptor agonists, or other 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 in a larger cohort with longer follow-up confirming the response rate of about 40% and again multilineage increments in blood counts were observed.(15) A concern with rescue eltrombopag is the potential propensity to stimulate clonal evolution. However, the clonal evolution rate of 19% reported in the initial eltrombopag studies is similar 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 relative 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 associates 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. Alternative non-IST (transplant and non-transplant) therapies are reasonable to consider in this patient population. Those who relapse following initial r-ATG appear to fare better with a higher response rate. It is not likely that a more definitive prospective study will be conducted to better define the role of salvage r-ATG following initial r-ATG leaving
retrospective studies such as ours as the principal guide for hematologists who cares for patients with marrow failure disorders.

**Contributions and Disclosures**

DVC conceptualized the analysis, collected and organized the data, conducted the analysis, and drafted the manuscript. VC, EHA, DSPD, CBLL, MB and GS collected patient data and participated in interim discussions. DVC, RTC, and PS participated in study conceptualization, data analysis as well as interim discussions; oversaw the analysis; and wrote the paper. All authors approved the manuscript. The authors have no conflict of interest to report.
References


Figures and tables

Table 1: Patients' characteristics

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

* 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

<table>
<thead>
<tr>
<th></th>
<th>Refractory to first r-ATG/CsA (n=32)</th>
<th>95% CI</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>Response at 3 months no. (%)</td>
<td>5 (16%)</td>
<td>3-28%</td>
<td>3-17%</td>
</tr>
<tr>
<td></td>
<td>7 (22%)</td>
<td>8-36%</td>
<td>3-17%</td>
</tr>
<tr>
<td>Relapsed to first r-ATG/CsA</td>
<td>3 (60%)</td>
<td>17-100%</td>
<td>17-100%</td>
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</tbody>
</table>
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.
A

4-year survival = 55%
95% CI = 33-72%

B

Responders

Non-responders

p = 0.004

C

Relapsed

Refractory

p = 0.43
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 complete 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.

References