Prospective study of rabbit antithymocyte globulin and ciclosporin for aplastic anemia from the EBMT Severe Aplastic Anemia Working Party

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**Running title:** Rabbit ATG in aplastic anemia

**Key words:** Aplastic anemia, ATG, antithymocyte globulin, immune suppression

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Summary

Rabbit ATG (Thymoglobuline, Genzyme) in combination with ciclosporin (CSA), as first
line immunosuppressive therapy (IST), was evaluated prospectively in a multicentre,
European phase II pilot study, in 35 patients with aplastic anemia (AA). Results were
compared to 105 age- and disease severity- matched patients from the EBMT registry, treated
with hATG (Lymphoglobuline) and CSA. Primary endpoint was response at 6 months. At 3
months, no patients had achieved a complete response (CR) to rATG. Partial response (PR)
occurred in 11 (34%). At 6 months, CR rate was 3% and PR rate 37%. There were 10 deaths
after rATG (28.5%) and one following subsequent HSCT. Infections were the main cause of
death in 9/10 patients. The best response rate was 60% for rATG and 67% for hATG. For
rATG, overall survival at 2 yr was 68%, compared to 86% for hATG, P = 0.009. Transplant
free survival was 52% for rATG and 76% for hATG, P = 0.002. On multivariate analysis,
rATG (HR = 3.9, P = 0.003) and age > 37 years (HR = 4.7, P = 0.0008), were independent
adverse risk factors for survival. This study was registered at www.clinicaltrials.gov as
NCT00471848.

Word count = 193
Introduction

Historically, horse antithymocyte globulin (ATG) has been the preferred animal source of ATG as first line treatment for acquired aplastic anemia (AA) patients who are ineligible for hematopoietic stem cell transplantation (HSCT). For severe AA (SAA) the combination of ATG and ciclosporin (CSA) results in a response rate of 60-75% of patients and the response is superior to using either agent alone \(^1\)\(^-\)\(^5\). The addition of granulocyte colony stimulating factor (G-CSF) to the combination of ATG and CSA has shown no significant benefit either in terms of response or survival \(^6\)\(^-\)\(^8\), although it may reduce infectious complications and duration of hospital admission \(^6\). For patients with non-severe AA (NSAA) who are transfusion dependent, the combination of ATG and CSA is superior to CSA alone, with a higher response rate, higher blood counts and improved disease free survival \(^9\).

Rabbit ATG (rATG) is more commonly used for a second course following relapse or lack of response to a first course of horse ATG (hATG). Response to a second course for non-response to a first course varies from 30-77\% \(^10\)\(^-\)\(^11\), and only 11\% in children \(^12\). In contrast, in patients relapsing after a first course, the response to a second course is 65\% \(^11\)\(^-\)\(^13\).

Until 2007, there were two preparations of hATG, namely Lymphoglobuline (Genzyme) and ATGAM (Pfizer). The most commonly used preparation of rATG (Thymoglobuline, Genzyme) uses the same immunogen as Lymphoglobuline; horses or rabbits are immunised with human thymocytes obtained at time of cardiac surgery from newborn infants. Rabbit ATG is more immunosuppressive than hATG; it results in more prolonged lymphopenia \(^14\), and it is more effective at preventing and treating acute renal allograft rejection \(^15\). This may be related to differences in CD4+CD25+FOXP3+ Tregs numbers seen in vitro after treatment with rATG, which produces expansion of functional Tregs from normal PB MNC in contrast to reduction in numbers following hATG (ATGAM) \(^16\).

Horse ATG (Lymphoglobuline) was withdrawn in 2007, resulting in the non-availability of hATG in Europe and other countries worldwide. In contrast, hATG (ATGAM) is manufactured in the USA but is almost exclusively available in the USA. Subsequently, the use of rATG (Thymoglobuline) as first line IST has been evaluated in prospective and retrospective studies. Most of the recent studies indicate a significantly worse response rate and survival for AA
patients treated with rATG in Europe and USA 17-21 but studies from Spain 22 and Cleveland Clinic 23 and two retrospective studies from Asia indicate similar response to hATG 24, 25.

We undertook a European study conducted by the European Blood and Marrow Transplant (EBMT) Group to assess the efficacy of rATG. The objectives of this study were, firstly, to assess the tolerability and efficacy of rATG (Thymoglobuline) with CSA in the first line treatment of patients with acquired SAA, and patients with NSAA who are transfusion dependent. The second objective was to compare the response rate of the combination of rATG and CSA from this pilot study with the response rate observed in a series of matched AA patients, treated after 1994 with the combination of hATG (Lymphoglobuline) and CSA. We also examined CD4 T-cell subsets in a subset of patients in order to understand further the mechanism of action of rATG in AA. (This study was registered at www.clinicaltrials.gov as NCT00471848).

Patients and methods

Study design

This was a phase II, non-randomized, prospective, open-label multi-centre trial of rATG (Thymoglobuline®) with CSA in patients with acquired AA, conducted by the EBMT Severe Aplastic Anaemia Working Party (EBMT SAAWP), sponsored by the EBMT. EUdraCT number: 2007-000902-55. The study aimed to accrue 35 patients with AA from specified EBMT centers in the UK, Germany, France, Italy, Saudi Arabia and Switzerland. AA was defined by at least two of the following: haemoglobin < 10g/dl, platelet count < 50 x 10⁹/l, neutrophil count < 1.5 x 10⁹/l, and a hypocellular bone marrow on bone marrow biopsy. 2 SAA and NSAA were defined by standard criteria 2. Patients enrolled were ineligible for HLA identical sibling donor BMT, had not received prior IST with ATG or CSA and included both SAA and transfusion dependent NSAA. Other inclusion criteria were time from diagnosis to study registration ≤ 6 months and no prior treatment except for hematopoietic growth factors and intravenous immunoglobulin (IVIg) with or without corticosteroids (as shown in Table 1) given for no more than 4 weeks and androgens. Patients enrolled were ≥ 16yrs of age (≥ 18yrs in Germany and Switzerland in accordance with German and Swiss law) and there was no upper age limit. Exclusion criteria were (1) eligibility for an HLA-matched sibling donor transplant, (2) prior therapy with ATG or CSA (3) prior therapy with hematopoietic growth factors more than 4
weeks before study enrollment (4) diagnosis of Fanconi anemia, dyskeratosis congenita or congenital bone marrow failure syndrome (5) evidence of myelodysplastic disease (MDS) (6) paroxysmal nocturnal hemoglobinuria (PNH) with evidence of significant haemolysis, history of PNH associated thrombosis or a PNH clone > 50% by flow cytometry (7) diagnosis or previous history of carcinoma (except local cervical, basal cell, squamous cells, or melanoma) (8) pregnancy (e.g. positive HCG test) or breast feeding (9) severe uncontrolled infection or unexplained fever > 38°C (10) hepatic, renal, cardiac, metabolic or other concurrent diseases of such severity that life expectancy was less than 3 months. Primary endpoint was response at 6 months post ATG treatment and secondary endpoints were failure free- and overall survival at 2 years post ATG treatment. An independent data monitoring committee (IDMC) was established to perform an interim evaluation of side effects of rATG and response after 15 patients had been enrolled.

Patients were compared retrospectively with matched AA patients previously treated since 1994 with horse ATG and CSA and reported to the EBMT. Patients were matched for age (categorised as <20, 21 to 60 and 60+ years) and disease severity (non-severe and severe). Assuming a total response of 60% with hATG and CSA, a ratio of 1:3 between the two groups (35 patients treated with rATG and CSA and 105 historical controls), would demonstrate a 25% difference in total response for rATG and CSA, with 80% power at a 5% level of significance.

This study was approved by the local ethics committee at each center and all patients gave informed written consent in accordance with the Declaration of Helsinki.

**Treatment protocol**
Rabbit ATG dose was 1.5 vials/10kg (3.75mg/kg) daily for 5 days, given as an intravenous infusion over 12-18 hours. CSA dose was 5mg/kg/day orally from day +1 for a minimum of 6 months, with later tailing according to individual patient response. The aim was to maintain trough whole blood CSA levels between 150 and 250 ng/ml. For prevention of serum sickness, methylprednisolone (or prednisolone) 1-2mg/kg/day was given (according to individual centre preference) from day 1-5, then halved every 5 days. An antihistamine was given prior to each ATG infusion (dephenhydramine or dexchlorpheniramine) as well as oral antipyretics, such as paracetamol. Red cell and platelet transfusions were given to maintain safe blood counts according to the local centre’s policy. Patients received prophylactic antifungal agent, antibiotic such as ciprofloxacin or oral non-absorbable antibiotics and antiviral drug. Response was defined according to established criteria ².
In vitro studies
PBMCs from 7 patients were analysed at diagnosis and following rATG (see Supplementary Data).

Statistical analysis
Length of survival in the two treatment groups was investigated using the Kaplan-Meier method; treatment groups were compared using the log rank test. Patients receiving rATG and CSA or hATG and CSA, were compared for total response at six months. The number of patients treated with rATG and CSA was 35. For the comparative study, patients were compared to historical cases treated with hATG with CSA on the EBMT Register. Patient data were analysed with the NCSS package. Comparisons between treatment groups were performed using chi-square test for categorical variables and non-parametric Mann-Whitney U test for variables where distributional assumptions could not be made. End point for survival analysis was death due to any cause. Cox Proportional Hazards model was used to test the independent effects of a series of explanatory variables after the assumption of proportional hazards had been verified. Critical level of significance was set at 0.05 (5%).

Results
1. Phase II pilot study
Patient characteristics
A total of 35 patients were recruited between 4th August 2008 and 31st September 2010, from 10 centres in Europe and Saudi Arabia. Patients’ characteristics are shown in Table 1. Median age was 36 years (range 17-75). Numbers with VSAA, SAA and NSAA were 6 (17%), 20 (57%) and 9 (26%), respectively. At diagnosis, a PNH clone was detected in 8 (23%) and an abnormal cytogenetic clone (trisomy 6 in 3/19 metaphases) in one of 15 evaluable patients. Previous therapies were haemopoietic growth factors in 3 (19%), intravenous immunoglobulin (IVIg) with corticosteroids in one and IVIg alone in one patient.

Response
Median follow up for all patients was 397 days (range 6-805). Response is summarised in Table 2. At 3 months, out of 32 evaluable patients, no patient had achieved a complete response (CR) and partial response (PR) was seen in 11 (34%) patients. At 6 months, out of 30 evaluable patients, one patient had achieved a CR (3%) and 11 (37%) had a PR, giving a total response rate of 40%. One patient relapsed at day + 59 from start of ATG and died on day 390. A second
patient relapsed at day + 750. Subsequent events are summarised in Table 2. At last follow up at 24 months after treatment with rATG, with or without HSCT, 2 patients were in CR, 3 in PR, one with NR, one was in relapse, 8 were transplanted and 10 patients had died.

For patients with SAA (n=26), no patient had a CR by 3 months and 8 (35%) had a PR. At 6 months, there was one CR (5%) and 29% PR. At 24 month follow up, two patients had a CR, 3 PR, one patient had relapsed, 4 were transplanted and 8 had died (see Table 2).

**Further treatment**

Eight patients underwent unrelated donor (UD) HSCT for non-response to ATG, at 84, 153, 168, 282, 321, 322, 352 and 460 days post ATG. At time of last follow up (18th October 2011), three patients had received a second course of ATG, at 139, 287 and 377 days post ATG, respectively.

**Survival analyses**

Overall survival (OS) was 68% at 2 years, and transplant free survival was 52%. For patients with SAA (n=26), OS was 73% and transplant free survival 64% (see Figure 1).

**Side effects and cause of death**

Complications and toxicity are listed in Table 3. Infections occurred in 22 (63%), elevated liver function in 10 (29%), rash in 8 (23%), haemorrhage in 7 (20%), hypertension in 6 (17%), abnormal renal function in 6 (17%), arthralgia in 5 (14%) and one case of avascular bone necrosis. Renal impairment occurred in 6 patients. Maximum median serum creatinine was 291μmol/l (range 155-430). At last follow up, median serum creatinine was 130 (range 99-347). Renal impairment was considered to be secondary to sepsis in 3, CSA toxicity in 2 and unknown in one patient. Recovery of renal function occurred in 4/6 patients. There were 10 deaths after rATG, and one patient died following UD HSCT. Infection was the main cause of death (see Table 3).

**In vitro studies of CD4+ T-cell subsets**

To further understand the mechanism of action of rATG, CD4+ T-cell subsets were analysed in 7 patients pre and post rATG. The results, summarised in Supplementary Data, show that pre ATG, the number of Tregs was significantly lower in AA compared to healthy age-matched controls while Th1 cells and Th2 cells were higher in AA patients. Post ATG, the frequency of Th2 cells
was significantly reduced whereas there was no significant change in the frequency of Tregs, Th1 and Th17 cells.

2. Comparison of rATG with hATG: matched pair analysis

Patient characteristics

Patients receiving rATG were matched for age and disease severity with patients receiving hATG as first line therapy; all patients also received CSA (see Table 4). There were no significant differences between the two groups in terms of age, disease severity and time interval from diagnosis to ATG treatment. Median follow up was significantly longer in the historical horse ATG group.

Response rate

Response to hATG and CSA is recorded in the EBMT database at a given time, so response rates at specific time points of 3, 6, 12 and 24 months are not available. Comparison of best response rates for rATG with the 105 age- and disease-matched patients treated with hATG are shown in Table 5. The best CR rate was 23% and 44% for rATG and hATG, respectively, and PR rate 37% and 23%, respectively. The best total response for rabbit ATG was 60% compared to 67% for horse ATG.

Survival and mortality

Median follow up of all patients was 397 days (range 6 – 805). Two year overall survival after rATG was 68% compared to 86% for horse ATG, (P = 0.009), see Figure 1a. The transplant free survival after rATG was 52% compared to 76% for hATG (P = 0.002), see Figure 1b. For patients with SAA, the OS was 91% and 73% for horse and rabbit ATG, respectively (P 0.01), see Figure 1c, and the transplant free survival 80% and 64% for horse and rabbit ATG, respectively (P 0.04), see Figure 1d. On multivariate analysis, the use of rabbit ATG (HR 3.9 [1.5-10.1], P 0.003) and age >37 years (HR 4.7 [1.9-11.9], P 0.0008), were independent adverse factors for survival. There were 11/35 (31%) deaths in the rATG study compared with 19/105 (18%) in the hATG group.

Discussion

Horse ATG has been used in the treatment of AA since the late 1970s, with the later addition of CSA, resulting in response in two thirds of patients\(^2\). Since then there has been a progressive improvement in survival, to around 80% at 5 years, although patients with severe disease and older patients (> 60 years of age) show inferior response and survival\(^6\). Following the
withdrawal of hATG (Lymphoglobuline), rATG has been used instead as first line IST. It was hoped and expected that similar, or even better, response might be seen with rATG as it is more immunosuppressive than hATG, producing a lower and more prolonged lymphopenia (especially CD4+ T-cells). However, our study shows inferior outcomes with rATG. A prospective, randomised study from NIH, USA, compared horse ATG (ATGAM) and CSA with rATG and CSA in 120 patients. Response at 6 months for the hATG arm was 68% compared to 37% for the rATG arm ($P=0.001$). Overall survival at 3 years was 96% versus 76% ($P=0.04$), respectively. A phase II study from the Cleveland Clinic, USA, of 20 SAA patients treated with rATG and CSA did not show a significant difference in response (45%) at 6 months compared to 58% among historical controls treated with hATG (ATGAM) and CSA ($p=0.44$). Of seven recent retrospective studies, four have shown worse outcomes with rATG (Thymoglobuline) compared with hATG and three similar results. Our study supports the conclusion that outcomes are worse with rATG in USA and Europe. Although we report a similarly low response rate to rATG as in the NIH study, with longer follow up, comparable best response of around 60% was seen comparing rATG with hATG (Lymphoglobuline) in the retrospective part of the study. Total response to rATG at 6 months was 40% but a further two patients responded by 12 months, indicating that a later evaluation of response may be appropriate for some patients, and this may parallel the late responses seen after cyclophosphamide treatment. Two recent retrospective studies from Japan and South Korea, respectively, showing similar response with rATG and hATG suggest that differences in ethnicity may play a contributing role in determining outcomes after ATG. In the Japanese study, the presence of GPI-deficient clones predicted response to rATG. Finally, the observed inferiority of rATG may pertain to the disease of AA itself, and probably not to other indications for using the drug, such as solid organ transplantation, HSCT conditioning regimens, treatment of graft versus host disease.

We observed a higher number of deaths due to infection after rATG compared to the NIH study. We also report a lower transplant free survival rate of 52% compared to 76% in the NIH study. The difference between the survival curves for rATG and hATG in this study start to separate after 6 months, indicating that late mortality may be related to non-response rather than direct toxicity of rATG. However, similar non-response rate was seen between the rATG and hATG groups. Another difference between the two studies is patient age. The mean age of patients treated with rATG in the NIH study was 37.4 +/- 2.7, and 31.2 +/- 2.6 for those treated with hATG (ATGAM). In this study of rATG, 31% of patients were aged > 50 years, and 20% > 60 years of age. However, a similar proportion (30% and 21%, respectively) of patients aged > 50
and > 60 years received hATG, Lymphoglobuline, but with a lower number of deaths. Therefore, older age was not a contributory factor for the differences seen between Thymoglobuline and Lymphoglobuline. A further possibility may be the more intense degree of immunosuppression caused by rATG which might be responsible for the high incidence of severe infections. Patients who received hATG were also matched for disease severity (see Table 5). The NIH study enrolled only patients with SAA, whereas our study also included patients with non-severe disease, in whom we would expect a lower risk of infections. The IDMC reviewed response to ATG and side effects after the first 15 patients were enrolled. The study investigators requested a further IDMC review after patient number 27 died on day + 9 after ATG. On both occasions, the IDMC advised there was no reason to stop the study. We also observed renal impairment in 6 (17%) patients after treatment with rATG associated with sepsis in 3 patients and which was not reversible in two patients.

The difference in response rates between rATG and hATG (Lymphoglobuline) suggests a different mechanism of action in AA. The NIH group have shown that following ATG treatment in AA, compared to hATG (ATGAM), rATG induced a significantly lower frequency and number of CD4+ cells and a higher frequency but lower number of Tregs. Tregs are known to be reduced in AA 27 and Th17 increased 28. We have recently reported that AA is characterised by clonally restricted expansion of CD4+ Th1 cells in AA 29. In this study, Tregs were decreased, whereas the Th1 and Th2 cells are increased in some AA patients compared to healthy controls. Following rATG treatment, the frequency of Th2 cells is significantly reduced (Supplementary data) Further studies on larger numbers of patients are now indicated to confirm these preliminary results.

At time of last follow up, 8 of 13 patients who had not responded to rATG received an UD HSCT. The indication for considering UD HSCT is failure to respond to one course of IST. We observed a high mortality in patients between 6 to 12 months who had not responded to rATG, in support of early HSCT in non-responding patients. Moreover, because outcomes after UD HSCT for children with SAA are now similar to outcomes after matched sibling donor HSCT 30, UD HSCT is being considered as first line treatment for SAA patients who lack a matched sibling donor, instead of prior treatment with a course of ATG 31. In the light of the poor survival after treatment with rATG shown by this and the NIH study 17, first line UD HSCT may also be considered in young adults, as overall survival after UD HSCT is around 75-83% 32,33 which is superior to survival after IST using rATG. Another alternative therapeutic option is to consider
using alemtuzumab. Responses have been reported in a number of small series for untreated
patients and non-responders to ATG. A large prospective study from NIH showed 37%
response for refractory SAA and 56% for relapsed SAA. Patients with untreated SAA were
randomised to alemtuzumab as part of the third arm of the prospective randomised study from
NIH, the other two arms being horse ATG and rabbit ATG, respectively, as reported by
Scheinberg and colleagues. The third arm using alemtuzumab was closed prematurely due to 3
early deaths and a response rate of only 19%.

In conclusion, this study shows that the combination of rATG and CSA results in low response at
3 and 6 months, similar later response compared to hATG, and worse survival compared to
hATG and CSA. Due to the high risk of infections, and the results of a recent randomized trial,
GCSF may be considered, when using rabbit ATG as first line therapy with CSA. Unrelated
donor transplantation for young adults would also be a clinical option.

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data acquisition. The Cambridge NIHR Biomedical Research Centre supported the
study at Addenbrooke’s Hospital. We also acknowledge the help of Britta Hochsmann
for helping to activate the study and recruit patients in Germany, and Louise Wallis at
the Royal Bournemouth Hospital, UK.

Author contribution
JM was the principal investigator for this study and wrote the paper;
JM, JP, AT, HS, GS, AB contributed to the study design as were study investigators; JM,
AB, HS, AT, AMR, JP, MA, CD, SBK, AB, TB, AW, AF, PS, RO, AR, SK, GM, GS
contributed to data analysis and writing the paper. RO, AB, PS, contributed to data
analysis; SK preformed the in vitro studies; JM, AB, AT, JRP, SBK, AR, AF, AW,
MA, HA, TF, MO, GJM, SG contributed to patient recruitment; PS, AB, RO, AB
contributed to the statistical analysis.

Conflict of interest
JM held a consultancy with Genzyme from May 2008-May 2009 and from June 2009
to July 2009, and received research funding in 2010 from Genzyme. AB had been on
the speakers’ bureau of Genzyme from 2000 to 2011, and has received research funding from Pfizer. CD received research funding in 2010 from Pfizer. AMR received honoraria from Genzyme in 2010. HS received research funding in 2010/2011 from Genzyme and was a speaker in Genzyme-sponsored symposium in 2010. GM has received research funding in 2010 from Genzyme. The remaining authors declare no competing financial interests.
References


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<th>Table 1: Patient characteristics</th>
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<td><strong>Disease severity (VSAA:SAA:NSAA)</strong></td>
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<td><strong>Median disease duration (range)</strong></td>
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Table 2: Response to rabbit ATG

(a) All patients

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(b) Severe AA

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Legend to Table 2: Shown in table is status of (a) all patients, and (b) those with severe AA, after ATG treatment at the time of each evaluation.
Table 3: Side effects and cause of death after rabbit ATG

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<tr>
<th>Side effects</th>
<th>Number of events, n=70</th>
<th>% events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>22 (31%)</td>
<td>31</td>
</tr>
<tr>
<td>Elevated liver function tests</td>
<td>10 (14%)</td>
<td>14</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>7 (10%)</td>
<td>10</td>
</tr>
<tr>
<td>Renal impairment</td>
<td>6 (9%)</td>
<td>9</td>
</tr>
<tr>
<td>Rash</td>
<td>8 (11%)</td>
<td>11</td>
</tr>
<tr>
<td>Hypertension</td>
<td>6 (9%)</td>
<td>9</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>5 (7%)</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (4%)</td>
<td></td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ventricular impairment</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Avascular bone necrosis</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cause of death (n=11)</th>
<th>Time of death</th>
<th>Patient age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis (n = 5)</td>
<td>9/210/243/326/370</td>
<td>75/65/28/23/21</td>
</tr>
<tr>
<td>Pneumonia (n = 2)</td>
<td>207/209</td>
<td>65/56</td>
</tr>
<tr>
<td>Sepsis, renal failure and multi-organ failure</td>
<td>6</td>
<td>36</td>
</tr>
<tr>
<td>Sepsis and cardiac arrest</td>
<td>148</td>
<td>56</td>
</tr>
<tr>
<td>Intracranial hemorrhage</td>
<td>288</td>
<td>71</td>
</tr>
<tr>
<td>Post HSCT invasive fungal infection</td>
<td>390</td>
<td>39</td>
</tr>
</tbody>
</table>
Table 4: Comparison of rabbit ATG with horse ATG: matched pair analysis

<table>
<thead>
<tr>
<th></th>
<th>Rabbit ATG (n = 35)</th>
<th>Horse ATG (n = 105)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-20</td>
<td>4 (11%)</td>
<td>12 (11%)</td>
</tr>
<tr>
<td>21-60</td>
<td>24 (69%)</td>
<td>72 (69%)</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>7 (20%)</td>
<td>21 (20%)</td>
</tr>
<tr>
<td>Disease severity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 0.5 x 10⁹/l neutrophils</td>
<td>26 (74%)</td>
<td>78 (74%)</td>
</tr>
<tr>
<td>&gt; 0.5 x 10⁹/l neutrophils</td>
<td>9 (26%)</td>
<td>27 (26%)</td>
</tr>
<tr>
<td>VSAA</td>
<td>6 (17%)</td>
<td>26%</td>
</tr>
<tr>
<td>Median interval diagnosis to treatment (days)</td>
<td>53</td>
<td>75 (P=0.3)</td>
</tr>
<tr>
<td>Median follow up (range) in days</td>
<td>285 (6 – 781)</td>
<td>1241 (21 – 4802) (P &lt;0.00001)</td>
</tr>
</tbody>
</table>
Table 5: Comparison of response to rabbit and horse ATG

<table>
<thead>
<tr>
<th>Rabbit ATG</th>
<th>Alive</th>
<th>Dead</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>PR</td>
<td>13</td>
<td>0</td>
<td>13</td>
<td>37</td>
</tr>
<tr>
<td>NR</td>
<td>3</td>
<td>9</td>
<td>12</td>
<td>34</td>
</tr>
<tr>
<td>Not evaluable*</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>11</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Horse ATG</th>
<th>Alive</th>
<th>Dead</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>43</td>
<td>3</td>
<td>46</td>
<td>44</td>
</tr>
<tr>
<td>PR</td>
<td>22</td>
<td>2</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>NR</td>
<td>21</td>
<td>14</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>19</td>
<td>105</td>
<td></td>
</tr>
</tbody>
</table>

* 2 patients not evaluable for response as they died on day 6 and 9 after ATG.
Legends for Figure 1

Figure 1a: Overall survival for all patients treated with rabbit ATG and CSA compared to horse ATG and CSA, including patients who later received an HSCT for non-response to ATG.

Figure 1b: Transplant free survival for patients treated with rabbit ATG and CSA compared to horse ATG and CSA: transplant is considered an event

Figure 1c: Overall survival for patients with severe aplastic anemia treated with rabbit ATG and CSA compared to horse ATG and CSA, including patients who later received an HSCT for non-response to ATG

Figure 1d: Transplant free survival for patients with severe aplastic anemia treated with rabbit ATG and CSA compared to horse ATG and CSA: transplant is considered an event
Figure 1a: Overall survival for all patients

- Horse ATG; n=105
- Rabbit ATG; n=35

Survival rates:
- Horse ATG: 86%
- Rabbit ATG: 68%

Statistical significance: P=0.009
Figure 1b: Transplant free survival for all patients: transplant is considered an event.

**Graph Description**
- **x-axis**: Days from ATG
- **y-axis**: Surviving
- **Lines**: h-ATG and r-ATG
- **Survival Rates**:
  - h-ATG: 76%
  - r-ATG: 52%
- **Statistical Significance**: P = 0.002
Figure 1c: Overall survival for severe aplastic anaemia

Horse ATG; n=67
Surviving 91%

Rabbit ATG; n=26
Surviving 73%

P=0.01
Figure 1d: Transplant free survival for severe aplastic anaemia: transplant is considered an event

- Horse ATG; n=67
  - 80%

- Rabbit ATG; n=26
  - 64%

P = 0.04
Prospective study of rabbit antithymocyte globulin and ciclosporin for aplastic anemia from the EBMT Severe Aplastic Anemia Working Party.