Activity of alemtuzumab monotherapy in treatment-naïve, relapsed, and refractory severe acquired aplastic anemia

Phillip Scheinberg¹, Olga Nunez¹, Barbara Weinstein¹, Priscila Scheinberg¹, Colin O. Wu², Neal S. Young¹

Hematology Branch¹ and Office of Biostatistics Research², National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA.

Key Words: aplastic anemia, anti-thymocyte globulin, alemtuzumab, immunosuppression, pancytopenia

This research was supported by the Intramural Research Program of the NIH, National Heart, Lung and Blood Institute.

Correspondence to: Dr. Phillip Scheinberg, Hematology Branch, NHLBI, 10 Center Drive, Building 10 CRC, Room 3-5140, MSC 1202, Bethesda, MD 20892-1202
Phone: 301-496-5203
Fax: 301-402-3088
E-mail: scheinbp@mail.nih.gov
Abstract

Anti-thymocyte globulin (ATG) plus cyclosporine is effective in restoring hematopoiesis in severe aplastic anemia (SAA). We hypothesized that the humanized anti-CD52 monoclonal antibody, alemtuzumab, might be active in SAA due to its lymphocytotoxic properties. To test this hypothesis, we investigated alemtuzumab monotherapy from 2003–2010 in treatment-naïve, relapsed, and refractory SAA in three separate research protocols at the National Institutes of Health. Primary outcome was hematologic response at 6 months. For refractory disease, patients were randomized between rabbit ATG plus cyclosporine (n=27) vs. alemtuzumab (n=27); the response rate for alemtuzumab was 37% (95% confidence interval [CI], 18%-57%) and for rabbit ATG 33% (95% CI, 14%-52%; p=0.78). Three-year survival was 83% (95% CI, 68%-99%) for alemtuzumab and 60% (95% CI, 43%-85%) for rabbit ATG (p=0.16). For relapsed disease (n=25), alemtuzumab was administered in a single arm study; the response rate was 56% (95% CI, 35%-77%) and 3-year survival was 86% (95% CI, 72%-100%). In treatment-naïve patients (n=16), alemtuzumab was compared to horse and rabbit ATG in a 3-arm randomized study; the response rate was 19% (95% CI 0%-40%), and the alemtuzumab arm was discontinued early. Alemtuzumab is active in SAA but best results are obtained in the relapsed and refractory settings. The trials were registered at www.clinicaltrials.gov as NCT00195624, NCT00260689, and NCT00065260.
**Introduction**

Severe acquired aplastic anemia (SAA) is a hematologic disease characterized by pancytopenia and a hypoproliferative bone marrow. Although the ultimate etiology of aplastic anemia is not known, clinical experience and laboratory data implicate a proximate mechanism of immune-mediated destruction of hematopoietic progenitor and stem cells. Therapies directed to suppress the immune system are an alternative to hematopoietic stem cell transplantation (HSCT) in SAA. Horse anti-thymocyte globulin (ATG) plus cyclosporine, the most well studied regimen, produces hematologic response in 60-70% of patients when utilized as first therapy. However, relapse occurs in about 30% of responding patients, and clonal evolution occurs in about 10-15% of cases. Thus, unresponsiveness to initial immunosuppression, relapse, and clonal evolution have limited the success of horse ATG plus cyclosporine in SAA.

Although ATG plus cyclosporine can be administered to the majority of patients, associated toxicities are not minor. ATG administration causes: 1) infusion related toxicity, manifested as fevers, rigors, urticarial cutaneous eruption, and in some cases, hypotension and hypoxemia; 2) serum sickness 1-2 weeks after administration of ATG, characterized by fever, a cutaneous eruption, arthralgia, myalgia and non-specific gastrointestinal and neurologic symptoms; and 3) transient blood count depression, which may lead to a temporary increase in transfusion requirements. Hypertension and azotemia are serious toxicities of cyclosporine, and hirsutism, gingival hyperplasia, hypomagnesemia, and neurologic complaints are also common.
Horse ATG is considered moderately lymphocytotoxic through the action of polyclonal antibodies, which produce transient lymphodepletion (usually 1-2 weeks duration) and longer elimination of activated T-cells, assumed to contribute to the induction of tolerance.\textsuperscript{5-7} Rabbit ATG is more efficient in depleting peripheral blood lymphocytes \textit{in vivo} and is more cytotoxic on a weight basis \textit{in vitro}.\textsuperscript{8,9} In randomized studies, rabbit ATG has been reported to be more effective than horse ATG in preventing and reversing acute renal allograft rejection\textsuperscript{10,11} and in SAA, rabbit ATG plus cyclosporine has been shown effective in the relapse and refractory settings.\textsuperscript{12,13}

Lymphocytotoxic therapies that are better tolerated and not require concomitant cyclosporine use are an attractive alternative to ATG plus cyclosporine. As one successful example, daclizumab, a genetically engineered human IgG1 specific to the alpha subunit of the interleukin-2 receptor, has resulted in responses of about 40\% in patients with moderate aplastic anemia.\textsuperscript{14} In SAA, we hypothesized that alemtuzumab (Campath-1H\textsuperscript{®}), a humanized IgG1 monoclonal antibody directed against the CD52 protein, might have activity. Alemtuzumab produces more durable lymphopenia (compared to horse ATG), which has made it an attractive agent in a wide range of autoimmune diseases, lymphoid malignancies, and in transplantation.\textsuperscript{9,15-20} Furthermore, in a diverse population of 21 patients with severe autoimmune cytopenias resistant to standard therapies, alemtuzumab was used as salvage therapy with some success: responses were observed in 15 patients.\textsuperscript{21} Based on these experiences, we conducted prospective studies using alemtuzumab in various settings. Here we report the largest prospective experience of alemtuzumab in SAA patients in the treatment-naïve, relapsed and refractory settings.
Methods

Study patients

All consecutive patients who fulfilled protocol entry criteria were enrolled into 3 different treatment protocols from November 2003 to August 2010 at the Warren Grant Magnuson Clinical Center and the Mark O. Hatfield Clinical Research Center at the National Institutes of Health in Bethesda, MD. All adult patients or parents (or legal guardian) of children (< 18 years of age) signed informed consent following the Declaration of Helsinki, according to protocols approved by the Institutional Review Board of the National, Heart, Lung, and Blood Institute. In the randomized studies, assignment to treatment was in blocks, with the assignment probability fixed over the course of the trial. Construction of the randomization schedule was done using a table of random numbers and conducted by the Pharmacy Department at the Clinical Center of the National Institutes of Health. The trials were registered at www.clinicaltrials.gov as NCT00195624, NCT00260689, and NCT00065260.

All patients older than 2 years of age with SAA were eligible for these studies. For protocol entry purposes, SAA was defined as bone marrow cellularity of less than 30% and severe pancytopenia with at least two of the following peripheral blood count criteria: 1) absolute neutrophil count < 0.5 × 10^9/L; 2) absolute reticulocyte count < 60 × 10^9/L; 3) platelet count < 20 × 10^9/L. Exclusion criteria were: creatinine > 2.5 mg/dL, underlying carcinoma, a diagnosis of Fanconi anemia, prior history of immunosuppressive therapy with alemtuzumab, human immunodeficiency virus seropositivity, evidence of a clonal disorder on bone marrow
cytogenetics, pregnancy, inability to understand the investigational nature of the study or significant comorbidities such that imminent death was likely.

Bone marrow biopsy and aspiration, for morphology and cytogenetics, were performed before enrolment, 6 and 12 months after immunosuppressive therapy and then yearly thereafter. Children and young adults (< 40 years of age) had chromosomes assayed after in vitro exposure of peripheral blood lymphocytes to diepoxybutane and mitomycin C to exclude Fanconi anemia. All patients were tested for paroxysmal nocturnal hemoglobinuria by a flow cytometric assay; presence of a clone was defined as the absence of glycosylphosphatidylinositol-anchored surface proteins in greater than 1% of neutrophils or red cells.

Study design

Refractory SAA

For patients who were non-responders (or had a non-robust response) to initial horse ATG plus cyclosporine, a second course of immunosuppression was administered after randomization between rabbit ATG plus cyclosporine (the reference arm) or alemtuzumab (investigational arm; refractory study). Patients who did not respond to rabbit ATG were offered the option to “crossover” to the alemtuzumab arm in the same protocol and, conversely, those unresponsive to alemtuzumab could receive rabbit ATG plus cyclosporine (Supplemental Figure 1A).

Relapsed SAA

In patients who relapsed following horse or rabbit ATG-based immunosuppression, alemtuzumab was administered in a non-randomized single arm study (relapse study;
Supplemental Figure 1B). If relapse re-occurred after successful treatment with alemtuzumab, cyclosporine could be instituted in the same protocol.

Treatment-naïve SAA

Alemtuzumab also was administered to treatment-naïve patients as part of a 3-arm randomized study that was designed to compare horse ATG plus cyclosporine, rabbit ATG plus cyclosporine, and alemtuzumab as initial therapy (treatment-naïve study; Supplemental Figure 1C).

Immunosuppressive regimens

Rabbit ATG (Thymoglobulin®, Genzyme) was administered at a dose of 3.5 mg/kg/day for 5 consecutive days and cyclosporine at 10 mg/kg/day by mouth as previously described. Dosing was adjusted to maintain cyclosporine plasma levels between 200-400 ng/ml. After a 1 mg test dose, alemtuzumab (Campath®, Genzyme) was administered at 10 mg/dose/day intravenously for 10 days as an infusion over 2 hours. Children < 50 kg received alemtuzumab 0.2 mg/kg/day for 10 days (not to exceed 10 mg/day). In contrast to horse or rabbit ATG, cyclosporine was not administered with alemtuzumab. The alemtuzumab regimen was identical in all protocols.

As prophylaxis for Pneumocystis carinii pneumonia all patients received aerosolized pentamidine monthly for at least six months. Daily valacyclovir at a dose of 500 mg daily for at least 8 weeks was given for Herpes simplex virus prophylaxis. In the alemtuzumab treated patients, Pneumocystis carinii and antiviral prophylaxis were continued until CD4+ T-cells > 0.2 × 10^9/l, and ciprofloxacin 500 mg twice daily was given until ANC > 0.2 × 10^9/l. G-CSF and prophylactic antifungal therapy were not routinely administered with any of the immunosuppression regimens. Molecular monitoring for EBV and CMV was performed at
baseline, weekly for the first month after each treatment course, every two weeks in the second
month, and monthly thereafter for another 6 months. EBV and CMV quantitative real-time PCR
were performed as previously described. A positive PCR was defined as more than 250 EBV
copies/10⁶ mononuclear cells genome equivalents or more than 250 CMV copies/mL blood.
Because of reports of cardiotoxicity in alemtuzumab treated patients, a 2-D echocardiogram,
24-hour holter monitoring, and troponin levels were performed prior to and following
alemtuzumab infusion.

**Study endpoints**

Hematologic response was defined as no longer meeting criteria for SAA, and a robust
response was defined as platelet and absolute reticulocyte > 50 × 10⁹ /L at 3 months. Patients
who relapsed by definition required reinstition or augmentation of the dose of cyclosporine or
administration of another course of immunosuppression. The majority of relapsed patients again
met criteria for SAA but in some, pancytopenia was moderate when further immunosuppression
was deemed necessary for declining blood counts. In these cases, hematologic response was
defined by pre-specified improvements in blood counts (Supplemental Figure 2).

The primary endpoint for all studies was hematologic response at 6 months. Secondary endpoints
included hematologic response at 3 months and yearly, robustness of hematologic response,
relapse, clonal evolution to myelodysplasia or acute leukemia, and overall survival.

**Statistical methods**
For the *refractory* study, sample size was based on the primary endpoint, hematologic response at 6 months. At 5% significance level and 80% power in a two-sided test, 50 patients per treatment arm were required to detect a 30% difference for the response rate (our hypothesis). To allow for possible attrition such as early withdrawals, a total accrual of 120 patients was intended originally with a planned interim analysis when 25 patients per arm were accrued. For the *relapsed* study, sample size was calculated using a Two-Stage Minimax Design 27, based on the hypothesis that response at 6 months (primary endpoint) to alemtuzumab would be >70%. Sample size was determined by testing the null hypothesis (response rate <50%) versus the alternative (response rate >70%) at 5% significance level and 80% power in a two-sided test. For the two-stage design, 23 were accrued in the first stage and if 13 or more responded, a subsequent 13 patients would be entered into a second stage. For the *treatment-naïve* study, the sample size was computed based on the assumption that the response rate at 6 months (primary endpoint) for rabbit ATG plus cyclosporine and alemtuzumab be 25% higher than the historical response rate for horse ATG plus cyclosporine in this setting at our institution of 60%. Based on normal approximations for testing proportions and Bonferroni adjustment for two-way multiple comparisons, the study design required 60 subjects per arm in order to have a power of 80% (two-sided) at 5% significance. However, following recommendations from the Data Safety and Monitoring Board, the alemtuzumab arm of the study was terminated early due to a low response rate at 6 months, and thus comparison between alemtuzumab and other arms of the *treatment-naïve* study was not performed.

Patient characteristics were described using summary statistics including means, proportions, standard errors and 95% confidence intervals. P-values for comparing these patient
characteristics between the two treatment groups were calculated based on the T-tests. Survival analyses based on the Kaplan-Meier method and the Cox proportional hazard models were used to draw inferences about the distributions of the overall survival time between the two treatments. Cumulative event distributions were analyzed using the Kaplan-Meier method and the Cox proportional hazard models for time-to-relapse among patients who responded to the first IST treatments and time-to-evolution for all the patients. For the analyses of time-to-relapse and time-to-evolution distributions, patients who had died or who underwent stem cell transplant before these events were counted as censored. Log-rank p-values were based on the Cox proportional hazard models and used to compare the survival and cumulative event curves between the two treatments. Stopping rules for toxicity and futility were described in all protocols. Numerical results were computed using the S-PLUS software package (TIBCO Software Inc., Palo Alto, CA). Two-sided P-value was used throughout and considered statistically significant if <0.05. Analysis was intention to treat.

**Results**

A total of 90 patients ages 3-75 years received alemtuzumab in three different research protocols that investigated the role of alemtuzumab in SAA. The distribution of patients who received alemtuzumab were as follows: 27 were in the refractory study (which randomized between alemtuzumab and rabbit ATG plus cyclosporine), 25 were in the relapsed study (single arm), 16 in the treatment-naïve study, 12 patients received alemtuzumab (as salvage) after unresponsiveness to initial rabbit ATG plus cyclosporine, and 10 patients received alemtuzumab after failing both horse and rabbit ATG (Supplemental Figure 1). Patient characteristics for all studies are shown in Table 1, and a summary of the serious adverse events (requiring
hospitalization) is in Table 2. There were 7 cases of thyroid abnormalities in alemtuzumab treated patients; 5 developed hypothyroidism and 2 hyperthyroidism. All patients with thyroid toxicities were managed as outpatient and became euthyroid with conventional therapies. In all hypothyroid cases alemtuzumab was administered for refractory disease. Of the hyperthyroid cases, one had relapsed SAA and the other had received alemtuzumab after unresponsiveness to initial rabbit ATG plus cyclosporine.

Alemtuzumab generally was well tolerated. Labeled infusion related reactions were common and managed symptomatically. All patients completed the planned 10-day infusion of alemtuzumab. Increases in liver function tests with alemtuzumab infusion were common and in the randomized refractory study, comparable to those observed with rabbit ATG plus cyclosporine (Supplemental Figure 3). Liver function tests normalized overtime in both groups. Lymphodepletion was universal and prolonged (Figure 1). In the refractory randomized study, the degree of lymphopenia was similar between both arms (rabbit ATG and alemtuzumab; Figure 1), with lymphocyte count not yet reaching pre-treatment levels by 6 months. Subclinical EBV and CMV reactivations were common, as described previously. There were no cases of EBV or CMV disease and prophylactic or pre-emptive therapy was not employed in any case. An updated depiction of EBV and CMV reactivations for both the refractory and relapsed study is shown in Figure 2. There was no evidence of cardiotoxicity from alemtuzumab in any protocol. Troponin levels did not increase during or after the 10-day infusion, and the ejection fraction remained essentially unchanged pre-treatment, after the 10-day infusion and at 3 months following alemtuzumab (Supplemental Figure 4).
Alemtuzumab in patients unresponsive to initial horse ATG therapy (refractory study)

The activity of alemtuzumab in patients unresponsive to initial horse ATG plus cyclosporine was evaluated in a study conducted between 2003 and 2010, in which patients were randomized between rabbit ATG plus cyclosporine and alemtuzumab. Rabbit ATG was considered the reference arm. After a planned interim analysis conducted when 25 patients were evaluable for 6-month response, the study was closed by the Data Safety and Monitoring Board as there was no statistical difference in the primary outcomes between the two groups and the probability of detecting a significant difference with further accrual was remote. In total, 54 patients were enrolled; 27 were randomized to rabbit ATG plus cyclosporine and 27 to alemtuzumab (Supplemental Figure 5). All patients in the rabbit ATG arm were evaluable at 3 and 6 months and all but one patient was evaluable at 3 and 6 months in the alemtuzumab arm (due to a single early death from infectious complications). The overall response rates for alemtuzumab was 37% (95% CI, 18%-57%) and for rabbit ATG 33% (95% CI, 14%-52%; Table 3; log-rank p=0.78). One patient in the alemtuzumab arm responded between 6 and 12 months. The increment in blood counts among responders in both arms was comparable (Figure 3). Three patients in each group later relapsed. The cumulative incidence of relapse at 3 years for rabbit ATG was 19% (95% CI, 0%-36%) and for alemtuzumab 9% (95% CI, 0%-21%; Figure 4; p=0.76). Dispositions among non-responders in both arms are shown as Supplementary Figure 6.

Clonal evolution was observed in 5 patients, 4 in the rabbit ATG arm (two monosomy 7, one t(6;14), and one trisomy 6), and one in the alemtuzumab arm (monosomy 7). The 3-year cumulative incidence of clonal evolution for rabbit ATG was 16% (95% CI, 0%-32%) and for
Alematuzumab 5% (95% CI, 0%-14%; Figure 4; p=0.11). The 3-year survival of patients treated with rabbit ATG was 60% (95% CI, 43%-85%) and for those who received alematuzumab 83% (95% CI, 68%-99%; log-rank p=0.16; Figure 5). Median survival has not been reached in either group, at a median follow-up of 36 month (range, 3-87) for all patients and 63 months (range, 8-87) in surviving patients (Figure 5). In the rabbit ATG arm, causes of death were: clonal evolution (1), septicemia (1), fungal infection (2), heart failure (1), HSCT related (1), central nervous system hemorrhage (1), traffic accident (1), and unknown (1); and in the alematuzumab arm, HSCT-related (1), septicemia (1), pneumonia (1), and unknown (2). Thus, alematuzumab has activity in SAA and the hematologic response rate observed in those refractory to initial horse ATG plus cyclosporine is comparable to that in the reference arm, rabbit ATG plus cyclosporine.

Alemtuzumab in patients who relapsed after successful treatment with initial horse ATG regimens

The activity of alematuzumab in relapsed SAA was evaluated in a single arm, non-randomized study that began in 2005, in patients who had relapsed after successful treatment with initial ATG plus cyclosporine. A total of 25 patients, ages 8-75, were enrolled; 22 had relapsed after an initial horse ATG regimen and 3 had relapsed twice, once after initial horse and then again after rabbit ATG (administered for the first relapse). The overall response rate to alematuzumab was 56% (95% CI, 35%-77%; Table 3). The increment in blood counts among responders is shown in Figure 3. Of the 11 non-responders, 5 were re-treated with rabbit ATG plus cyclosporine, 2 underwent HSCT, and 4 are on supportive care. Four of the 14 responders later relapsed; further immunosuppression was pursued in 3 of which 2 have responded. The cumulative incidence of
relapse at 3 years was 23% (95% CI, 0-41; Figure 4). There were 2 clonal evolution events in the relapsed cohort, one to complex cytogenetics and one to deletion 13q (Figure 4). The cumulative incidence of clonal evolution at 3 years was 11% (95% CI, 0%-25%; Figure 4). The median follow-up was 39 months (range, 6 to 63) for all patients and for those surviving, 42 months (range, 6-63). The overall survival at 3 years was 86% (95% CI, 72%-100%; Figure 5). Thus, alemtuzumab is also active in relapsed SAA with a response rate comparable to reported experience with rabbit ATG plus cyclosporine in this setting.12

Alemtuzumab in treatment naïve patients

Based on these encouraging results in refractory and relapsed SAA, alemtuzumab was investigated in treatment-naïve patients in a three-arm study that began in 2006 which randomized among horse ATG plus cyclosporine, rabbit ATG plus cyclosporine, and alemtuzumab monotherapy. However, after 16 patients were randomized to alemtuzumab, this part of the study was discontinued at the recommendation of the Data Safety and Monitoring Board. Hematologic response had been observed in only three patients and there were three early deaths. Eight patients who were unresponsive to initial alemtuzumab then received rabbit ATG plus cyclosporine, and 5 have responded. At 3 years survival was 63% (95% CI, 43-91%; Figure 5). The causes of death were clonal evolution (1), fungal pneumonia (1), septicemia (2), necrotizing fasciitis post HSCT (1), and metastatic lung cancer (1). These data suggest that alemtuzumab is not as effective in treatment-naïve patients compared to standard treatment with horse ATG plus cyclosporine.
Alemtuzumab in other salvage settings

Alemtuzumab was also investigated in other settings in the context of these clinical trials (Supplemental Figure 1). In the treatment-naïve study, 13 patients unresponsive to initial rabbit ATG plus cyclosporine received salvage alemtuzumab, of which one responded. In total 10 patients refractory to both horse and rabbit ATG immunosuppression received alemtuzumab as a salvage third course; hematologic response was observed in 2 (both had shown small but incremental improvement with each prior ATG courses).

Discussion

The introduction of horse ATG plus cyclosporine for SAA about 20 years ago has led to hematologic recovery in 60-70% of patients and to long-term survival among responders greater than 80%.\(^1\) The majority of responses occur within 3 months, with occasional improvement observed between 3 and 6 months (about 5%) and even fewer between 6 and 12 months.\(^4\) Our and of others efforts have been to develop regimens that address the problems with standard horse ATG plus cyclosporine: toxicity, unresponsiveness, relapse, and clonal evolution. In refractory cases, the response rate to a second course of rabbit ATG plus cyclosporine has been reported to be 77%\(^\text{13}\) but in our experience was about 30% in the same setting.\(^\text{12}\) In relapsed SAA, reported response rates with re-treatment have been more consistent, about 50-60%.\(^\text{12,28,29}\) Unfortunately, the use of more potent immunosuppressive therapy has been controversial due to toxicity\(^\text{30,31}\) and the addition of a third drug to horse ATG plus cyclosporine has not led to a better hematologic recovery or a decrease in the rate of relapse or clonal evolutions.\(^\text{32,33}\)
Here we report that alemtuzumab is an effective treatment in SAA, with hematologic recovery in about 37% in those patients who were unresponsive to initial horse ATG plus cyclosporine, and about 55% in patients in relapse. The response rate in refractory patients was comparable to that observed in the reference arm (rabbit ATG plus cyclosporine), and the response rate in relapsed disease is comparable to that of a repeat course of rabbit ATG plus cyclosporine in this setting.12,28,29 Alemtuzumab, as administered in our protocols, has the significant advantage of not requiring cyclosporine, which is associated with significant morbidities, especially with chronic use and in older patients.

Overall alemtuzumab was well tolerated with serious adverse events primarily related to complications from cytopenias. There were 7 cases of thyroid abnormalities in alemtuzumab treated patients, which have been described with this agent in other settings.34,35 All were successfully managed as outpatient. There were no cases of clinically relevant EBV or CMV reactivations. However, one patient in the relapse study developed progressive multifocal leukencephalopathy one year after completing the alemtuzumab. This patient had a partial hematologic response and achieved transfusion-independence soon after completing the alemtuzumab therapy. The neurologic diagnosis one year later was based on clinical findings, imaging, and detection of JC virus in cerebrospinal fluid (by polymerase chain reaction). No brain biopsy was performed. The patient received an experimental oral formulation of cidofovir with significant improvement; she currently is followed as an outpatient, without need for transfusions. This occurrence in this patient appears to be a very rare event, as alemtuzumab has not been particularly associated to this infectious complication. In a recent survey of drug associations with progressive multifocal leukoencephalopathy, drug-induced cases were analysed.
in two spontaneous Adverse Drug Report (ADR) databases, US FDA Adverse Event Reporting System (AERS) and WHO Adverse Drug Reaction Database (VigiBase). The US FDA-AERS tracks post-marketing safety surveillance for all FDA-approved drugs, and the WHO-VigiBase tracks reported cases suspected of ADRs from 82 monitoring centers worldwide. In this study, the number of cases associated with alemtuzumab was 3 in FDA-AERS and 8 in WHO-VigiBase. The strength of the association cannot be determined by this reporting since the setting in which the encephalopathy occurred and associated drugs administered sequentially or concomitantly with alemtuzumab is not provided. In the same study, there were 78 cases of progressive multifocal leukoencephalopathy associated with rituximab and 20 with natalizumab consistent with prior reports associating these monoclonal antibodies to this infection.

Our conclusions regarding the activity of alemtuzumab in SAA is supported by recent small preliminary reports of investigators in South Korea, Europe, and Mexico. In a pilot dose-escalating study, Kim et al administered alemtuzumab intravenously (60 mg in 10 and 90 mg in 4 patients) plus cyclosporine to 14 patients with SAA, of which 11 were ATG-naïve: hematologic response was observed in three of the treatment-naïve patients, all at the lower dose of alemtuzumab. Risitano et al administered subcutaneous alemtuzumab (103 mg total) plus low dose cyclosporine in 6 treatment-naïve and 11 refractory patients with SAA; hematologic response was seen in 4 and 3 cases, respectively. More recently, in a Mexican pilot study, 14 treatment-naïve patients received 50 mg of alemtuzumab with low dose cyclosporine; hematologic response was observed in eight. The Korean experience in treatment-naïve patients (3/11 responses) resembles our data, of 3/16 responses in untreated SAA patients. The modest activity of alemtuzumab in treatment-naïve patients in our study was surprising and
possible explanations are currently being investigated. However, lower doses of alemtuzumab might be more effective in treatment-naïve patients, since half the alemtuzumab dose used in our study was employed in Mexico, where the response rate neared 60%. There are no obvious explanations in patient demographics or pre-treatment blood counts that could account for the lower response rate in treatment-naïve cases in our study. The reported experience of alemtuzumab in relapsed and refractory SAA is more limited to a handful of cases, but our data in 52 patients who received alemtuzumab for relapse or refractory disease suggests that this agent might be more effective in these settings. Furthermore, the role for low dose cyclosporine frequently used in the other pilot studies require definition, since a cyclosporine free regimen, as used in our study, has the advantage of improving tolerability of the immunosuppression. However, it is possible that cyclosporine might have a role in preventing relapse.

Alemtuzumab in patients who were refractory to both horse and rabbit ATG was not as effective, in accordance with the reported experience of lack of benefit of a third course of immunosuppression in refractory cases. In this setting, our experience with alemtuzumab in 10 patients yielded responded in 2, both of which had increments in the blood counts with previous courses of ATG that were not sufficient for a response. In the 3-arm randomized study, 13 patients unresponsive to an initial course of rabbit ATG plus cyclosporine received salvage therapy with alemtuzumab; response was observed in only one. This suggests that alemtuzumab may not be as effective in those who fail an initial course of rabbit ATG plus cyclosporine compared to those who are refractory to initial horse ATG plus cyclosporine.
Our study has limitations and strengths. The NIH Clinical Center is a federal research hospital and functions as a tertiary referral institution. Our patients may differ in important demographic features, such as socioeconomic status and ethnicity, and in clinical features, from other patient cohorts. The Hematology Branch specializes in the diagnosis and treatment of bone marrow failure syndromes, resulting in consistent and advanced care (such as the ready availability of histocompatible blood products, particularly granulocytes), which may influence outcomes. Aggregation of sufficient patients has made feasible serial interventional studies, but aplastic anemia’s low incidence limits the numbers of patients in specific disease categories, and protocols generally require years to accrue sufficient patients prospectively defined to answer important clinical questions.

A few reports on the use of alemtuzumab in aplastic anemia have been published. These were small pilot case series initiated after our protocols began which involved heterogeneous patient groups and treatment regimens that were not prospectively registered at clinicaltrials.gov. In contrast, the current reporting include trials that were prospectively designed and registered at the outset, with a much larger number of patients in well defined disease settings in SAA (treatment-naïve, relapse and refractory), which included randomization in patients with treatment-naïve (against two polyclonal antithymocyte globulins) and refractory disease (versus rabbit ATG). As a result, our studies allow for important inferences on the response rates of alemtuzumab in SAA. Trial registration minimizes the problem of selective or incomplete reporting and helps avoid publication bias in the reporting of mainly positive results (winners’ curse). Thus, our reporting is inclusive of consecutive patients who enrolled in research protocols, and all study parameters were defined at outset, and all patients were accounted for in
the analysis and final reporting. Because of the more robust nature of our study design, greater number of patients accrued in different disease settings, and longer follow-up, these results should allow the rational incorporation of this agent into clinical practice.

In summary, alemtuzumab is an active agent in SAA as monotherapy with the best results observed in the refractory and relapsed settings; either rabbit ATG or alemtuzumab can rescue about one-third of patients unresponsive to initial horse ATG plus CsA (direct comparison); the response rate of alemtuzumab in relapsed SAA is comparable to the reported response rate of 50%-60% in this setting; the salvage rate with alemtuzumab in those unresponsive to initial rabbit ATG plus cyclosporine appear low; and alemtuzumab cannot be recommended as first line therapy of SAA outside of a clinical research protocol. Alemtuzumab is an appealing alternative to ATG plus cyclosporine in the setting of relapsed or refractory disease especially in those who experienced significant toxicity with prior ATG courses, in older patients, and in those who require chronic use of cyclosporine to maintain adequate blood counts. The data also provide a basis for future research as well. Alemtuzumab should be as effective when administered subcutaneously as intravenously, and the former route would permit outpatient use. Avoidance of cyclosporine in our regimen may make it especially appealing in the treatment of patients with co-morbidities, especially renal disease, and in the elderly.

Acknowledgments

P.S. is the principal investigator for all studies and participated in primary conception, writing the protocols, execution, data collection, analysis and interpretation, and drafting of the manuscript; O.N, B.W. participated in data collection, execution, and attended to patients’ needs; Pr. S. tracked the toxicity data and attended to the protocols’ regulatory requirements; C.O.W
participated in primary conception, study design and did all the statistical analysis, and critical
revision of the manuscript; N.S.Y. participated in primary conception, protocol development,
interim discussions, data analysis and interpretation, and critical revision of the manuscript. The
authors have no conflicts to disclose. This research was supported by the Intramural Research
Program of the NIH, National Heart, Lung and Blood Institute.

References

1. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and
antilymphocyte globulin and methylprednisolone with or without cyclosporine. The German
3. Frickhofen N, Rosenfeld SJ. Immunosuppressive treatment of aplastic anemia with
4. Rosenfeld S, Follmann D, Nunez O, Young NS. Antithymocyte globulin and
cyclosporine for severe aplastic anemia: association between hematologic response and long-
5. Bonnefoy-Berard N, Verrier B, Vincent C, Revillard JP. Inhibition of CD25 (IL-2R
alpha) expression and T-cell proliferation by polyclonal anti-thymocyte globulins. *Immunology.*
6. Platanias L, Gascon P, Bielory L, Griffith P, Nienhuis A, Young N. Lymphocyte
phenotype and lymphokines following anti-thymocyte globulin therapy in patients with aplastic
7. Lopez-Karpovitch X, Zarzosa ME, Cardenas MR, Piedras J. Changes in peripheral blood
mononuclear cell subpopulations during antithymocyte globulin therapy for severe aplastic
8. Thomas FT, Griesedieck C, Thomas J. Differential effects of horse ATG and rabbit ATG
on T cell and T cell subset levels measured by monoclonal antibodies. *Transplantation
following antibody-based immunosuppressive regimens in patients with severe aplastic anemia.
thymoglobulin versus Atgam for induction immunosuppressive therapy in adult renal transplant
11. Gaber AO, First MR, Tesi RJ, et al. Results of the double-blind, randomized, multicenter,
phase III clinical trial of thymoglobulin versus Atgam in the treatment of acute graft rejection
12. Scheinberg P, Nunez O, Young NS. Retreatment with rabbit anti-thymocyte globulin and
cyclosporin for patients with relapsed or refractory severe aplastic anaemia. *Br J Haematol.*
2006;133(6):622-627.


**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Refractory study</th>
<th>P-value</th>
<th>Relapsed study</th>
<th>Treatment-naïve study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit ATG (N=27)</td>
<td>Alemtuzumab (N=27)</td>
<td></td>
<td>Alemtuzumab (N=25)</td>
<td>Alemtuzumab (N=16)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.1 ± 3.8</td>
<td>37.2 ± 4.5</td>
<td>39.9 ± 4.5</td>
<td>39.2 ± 6.1</td>
</tr>
<tr>
<td>Age &lt;18 yrs, number (%)</td>
<td>11.1 ± 6.2</td>
<td>25.9 ± 8.6</td>
<td>12 ± 6.6</td>
<td>25.0 ± 11.2</td>
</tr>
<tr>
<td>Male sex, number (%)</td>
<td>59.3 ± 9.6</td>
<td>51.9 ± 9.8</td>
<td>44 ± 10.1</td>
<td>75.0 ± 11.2</td>
</tr>
<tr>
<td>Etiology, number (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>100</td>
<td>92.6 ± 5.1</td>
<td>0.16</td>
<td>100</td>
</tr>
<tr>
<td>Post-hepatitis</td>
<td>0</td>
<td>7.4 ± 5.1</td>
<td>0.16</td>
<td>0</td>
</tr>
<tr>
<td>Blood counts (× 10^9 /L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARC</td>
<td>21.267 ± 4.064</td>
<td>27.048 ± 4.815</td>
<td>0.36</td>
<td>35.852 ± 6.452</td>
</tr>
<tr>
<td>ALC</td>
<td>1.078 ± 0.053</td>
<td>0.956 ± 0.086</td>
<td>0.24</td>
<td>1.329 ± 0.126</td>
</tr>
<tr>
<td>ANC</td>
<td>0.616 ± 0.137</td>
<td>0.717 ± 0.149</td>
<td>0.62</td>
<td>0.595 ± 0.079</td>
</tr>
<tr>
<td>ANC &lt; 0.2, number (%)</td>
<td>25.9 ± 8.6</td>
<td>11.1 ± 6.2</td>
<td>0.168</td>
<td>8.0 ± 5.5</td>
</tr>
<tr>
<td>Platelets</td>
<td>10.654 ± 1.482</td>
<td>14.037 ± 1.722</td>
<td>0.146</td>
<td>14.880 ± 1.820</td>
</tr>
<tr>
<td>PNH clone &lt; 1%</td>
<td>56.0 ± 9.7</td>
<td>77.7 ± 8.2</td>
<td>0.10</td>
<td>64.0 ± 9.8</td>
</tr>
<tr>
<td>PNH clone ≥ 1%</td>
<td>44.0 ± 9.7</td>
<td>22.3 ± 8.2</td>
<td>0.10</td>
<td>36.0 ± 9.8</td>
</tr>
</tbody>
</table>
Plus-minus values are mean ± SD. ARC, absolute reticulocyte count; ALC, absolute lymphocyte count; ANC, absolute neutrophil count; ATG, anti-thymocyte globulin; PNH, paroxysmal nocturnal hemoglobinuria. P-value refers to the comparison between rabbit ATG and alemtuzumab in the refractory study.
Table 2. Summary of serious adverse events

<table>
<thead>
<tr>
<th></th>
<th>Refractory study</th>
<th>Relapsed study</th>
<th>Treatment-naïve study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rabbit ATG (N=27)</td>
<td>Alemtuzumab (N=27)</td>
<td>Alemtuzumab (N=25)</td>
</tr>
<tr>
<td>Serum sickness</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Gingival</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenic fever, negative cultures</td>
<td>6</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Neutropenic fever, positive cultures</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>UTI</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Candidemia</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Septic shock</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Peri-rectal abscess</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Cervical abscess</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Skin abscess</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Cholecystitis</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Tonsillitis/pharyngitis</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>PML</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Viral exanthema</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Upper respiratory infection</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Parotitis</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>1</td>
<td>7</td>
</tr>
</tbody>
</table>

Serious adverse events depicted are those that resulted in prolonged hospitalization, hospital admission or death. Events shown are those that occurred after the initial cycle of immunosuppression for each study. Repeated hospitalizations in the same subject were counted as separate events. PML, progressive multifocal leukoencephalopathy.
Table 3. Response rate for alemtuzumab in refractory, relapsed, and treatment-naïve SAA

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Refractory study (n=54)</th>
<th>P-value</th>
<th>Relapse study (n=25)</th>
<th>Treatment-naïve study (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rabbit ATG (95% CI)</td>
<td></td>
<td>Alemtuzumab (95% CI)</td>
<td>Alemtuzumab (95% CI)</td>
</tr>
<tr>
<td>3 month response</td>
<td>19% (3, 34)</td>
<td>1.00</td>
<td>48% (27, 69)</td>
<td>19% (0, 40)</td>
</tr>
<tr>
<td>6 month response</td>
<td>33% (14, 52)</td>
<td>0.78</td>
<td>56% (35, 77)</td>
<td>19% (0, 40)</td>
</tr>
</tbody>
</table>

In the refractory study, patients initially unresponsive to horse ATG plus cyclosporine were randomized to rabbit ATG plus cyclosporine (n=27) and alemtuzumab (n=27). In patients with relapsed disease, alemtuzumab was administered in a single arm (non-randomized) study. CI, confidence interval. P value is for the comparison between rabbit ATG and alemtuzumab in the refractory study.
Figure Legends

**Figure 1. Absolute lymphocyte count (ALC) following rabbit ATG and alemtuzumab.** In the refractory study (left panel), the degree and duration of lymphopenia was similar between the rabbit ATG (red) and alemtuzumab arms (blue). The baseline ALC had not been reached for either group by 6 months. In the relapsed study (right panel), the pattern of lymphopenia was similar to that observed in the alemtuzumab arm in the refractory study. The mean ± SEM is shown.

**Figure 2. EBV and CMV reactivations following immunosuppression in the refractory and relapse studies.** Nearly all patients were seropositive for EBV (panel A) while CMV seropositivity ranged from 60-80% (panel B). Of the seropositive patients, EBV reactivation in the rabbit ATG arm was observed in about 80% with median peak copy numbers about 100,000 copies per 10⁶ mononuclear cell (MNCs) genome equivalents (panel C). In the alemtuzumab arm, EBV reactivations were less frequent and the median peak copy numbers much lower compared to rabbit ATG (panel C). There was no difference in the likelihood or degree of reactivation after alemtuzumab in the refractory and relapsed studies. CMV reactivations were less common for both rabbit ATG and alemtuzumab, with median peak copy numbers around 1,000 per ml. Only one patient in the relapsed study reactivated CMV after alemtuzumab. All reactivations were self-limited and subclinical with prophylactic or preemptive therapies not employed in any case. A positive PCR was defined as more than 250 EBV copies/ 10⁶ MNCs genome equivalents or more than 250 CMV copies/mL blood.

**Figure 3. Increase in blood counts after immunosuppression among responders in the refractory and relapsed studies.** In the refractory study, the increment in blood counts was similar in the rabbit ATG and alemtuzumab arms. In the relapsed study, the increment in blood counts following alemtuzumab was similar observed to the same regimen in the refractory study. Only one responder in the rabbit ATG arm is shown for 4 and 5-year follow-up. Blood counts after relapse are not shown as alternative therapies were sought. The mean ± SEM is shown.

**Figure 4. Cumulative incidence or relapse and clonal evolution.** In the refractory study (left panels), the rate of relapse was comparable between arms (A), while the rate of clonal evolution (C) was higher with rabbit ATG (red) compared to alemtuzumab (blue), but this difference was not statistically significant. In the relapsed study (right panels), the rate of a second relapse after alemtuzumab was 23% (B) and the clonal evolution rate 11% (D). For the relapse study panels, dotted lines represent 95% confidence interval.

**Figure 5. Overall survival.** In the treatment-naïve study (A), 3-year survival was 63%. In the relapsed study (B), 3-year survival was 86%. In refractory study (C), 3-year survival in the alemtuzumab arm (blue) was about 20% higher than rabbit ATG (red), but this difference was not statistically significant. The dotted lines in the relapse study panel represents 95% confidence interval. The median survival was not reached in any of the treatment arms in all studies.
Refractory study

Relapse study

Figure 1
Figure 2

(A) EBV frequency in different patient groups. (B) CMV frequency in different patient groups. (C) EBV copies per 10^6 MNCs. (D) CMV copies per ml.
Figure 3

- **Refractory study**
  - Rabbit ATG
  - Alemtuzumab

- **Relapsed study**
  - Alemtuzumab

- **Absolute reticulocyte count**
- **Hemoglobin**
- **Absolute neutrophils**
- **Platelets**

Graphs showing changes over time in months.
A. Alemtuzumab, 3-year surv = 63%

Treatment-naive study
(N=16)

B. Alemtuzumab, 3-year surv = 86%

Relapse study
(N=25)

C. Alemtuzumab, 3-year surv = 83%

R-ATG/CsA, 3-year surv = 60%

Log-rank p = 0.16

Refractory Study
(N=54)

Figure 5
Activity of alemtuzumab monotherapy in treatment-naïve, relapsed, and refractory severe acquired aplastic anemia

Phillip Scheinberg, Olga Nunez, Barbara Weinstein, Priscila Scheinberg, Colin O. Wu and Neal S. Young