Efficacy of rabbit antithymocyte globulin in severe aplastic anemia

by Manuel G. Afable, Mohammed Shaik, Yuka Sugimoto, Paul Elson, Michael J. Clemente, Hideki Makishima, Mikkael A. Sekeres, Alan Lichtin, Anjali Advani, Matt Kalaycio, Ramon V. Tiu, Christine O'Keefe, and Jaroslaw Maciejewski

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Efficacy of rabbit antithymocyte globulin in severe aplastic anemia

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Key word: aplastic anemia, cytokine gene polymorphism, anti-thymocyte globulin

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Abstract

**Background.** A combination of horse anti-thymocyte globulin and cyclosporine produces responses in 60-70% of patients with severe aplastic anemia. We performed a phase II study of rabbit anti-thymocyte globulin and cyclosporine as first line therapy for severe aplastic anemia.

**Design and Methods.** 20 severe aplastic anemia patients treated with rabbit anti-thymocyte globulin were compared to 67 historical control cases with matched clinical characteristics treated with horse anti-thymocyte globulin.

**Results.** Response rates were similar for patients treated with rATG compared to horse anti-thymocyte globulin at 3, 6 and 12 months: 40% vs. 55% (p=0.43), 45% vs. 58% (p=0.44) and 50% vs 58% (p=0.61), respectively. No differences in early mortality rates and overall survival were observed. We then performed multivariable analyses of response at 6 months and overall survival and we identified the presence of a paroxysmal nocturnal hemoglobinuria clone (p=0.01) and a pretreatment absolute reticulocyte count >30x10^9/L (p=0.007) as independent predictors of response and younger age (p=0.003), higher pretreatment absolute neutrophil (p=0.02) and absolute lymphocyte counts (p=0.03) as independent predictors of overall survival. None of the immunogenetic polymorphisms studied were predictive of response to immunosuppressive therapy.

**Conclusions.** Despite reports suggesting differences in biologic activity of different anti-thymocyte globulin preparations, rabbit and horse anti-thymocyte globulin appear to have a similar efficacy for upfront treatment of severe aplastic anemia.

*The registration number for this trial at clinicaltrials.gov is NCT01231841.*
Introduction

Aplastic anemia (AA) is a hematopoietic stem cell disorder characterized by pancytopenia and hypocellular bone marrow. The pathogenesis of most cases of idiopathic AA involves T cell-mediated destruction of hematopoietic progenitors and stem cells(1); consequently, therapeutic immunosuppression (IST) has been effective in a large proportion of patients. The most effective IST regimen includes horse antithymocyte globulin (hATG) in combination with cyclosporine (CsA), a treatment which shows superiority to either ATG or CsA alone(2;3). When used as a first-line treatment for severe AA (sAA), hematologic response occurs in 60-70% of patients(4-6). In one representative study, the actuarial 10-year survival for patients treated with IST was 68%(5).

ATG can exert diverse effects on the immune system, including T-cell depletion in the blood and peripheral tissues, down-modulation of adhesion molecules and chemokine receptors(7), and possibly a direct effect on hematopoietic stem cells(8-10). While most studies have been performed with hATG, more recently, rabbit-derived ATG (rATG) has been introduced into clinical practice, particularly in organ transplantation. Although both types of ATG share similar mechanisms of action, rATG may be more potent than hATG at equivalent concentration based on its capacity to produce more profound and prolonged lymphocytopenia. Thus, it is not surprising that EBV viral loads have been found to be higher in patients receiving rATG(6). In addition, rATG may selectively promote the expansion of regulatory T cells (Tregs)(11), possibly inducing by this mechanism immune tolerance in vivo(7).

While hATG has been the primary treatment used for sAA, relapsing or refractory AA patients after a first course of hATG often receive rATG. Although initial reported response rates in this setting approached 77%(12), responses in subsequent studies were more modest, at 30% in hATG refractory patients and 65% in relapsed primary responders(13). Only limited efficacy data are available for rATG used as initial therapy in sAA. In a single arm phase II study, 62% (13/21) of AA patients responded when treated with rATG/CsA upfront(14). When patients were treated with either hALG (lymphoglobulin) or rATG, similar response rates for both were obtained (48% vs. 46%)(15). Similarly, a prospective study in China enrolled AA patients into 4 different ATG-based treatment regimens, including hATG and rATG in combination with CsA and growth factors(16). The response rate was 73% (22/30) versus 53% (17/32) (p=.039), respectively. Recently, a retrospective study showed that the response rate at 6 months was significantly higher in patients receiving hATG (59%) compared to rATG (34%)(17). Of note, the median rATG dose used in this study was only 2.5 mg/kg/day x 5 days. In another cohort of AA patients, 13/14 (93%) AA patients treated with hATG responded, while only 8/17 (47%) responded to rATG(18).

We conducted a Phase 2 study of the rATG/CsA combination as an initial treatment for sAA, and compared responses to those of a historical cohort of sAA patients treated with hATG. We also investigated the activity of hATG and rATG as salvage therapy, and analyzed factors associated with therapy responsiveness.
Design and Methods

Patients
We analyzed 89 patients with sAA who underwent initial therapy with rATG from 2005 to 2009 (N=22) as part of a prospective phase 2 study and compared to a retrospective series of well-matched hATG treated cohort from 1996 to 2010 (N=67). Since the characteristics of the patients treated with each therapy were similar, both groups were then pooled to assess prognostic factors. Informed consent was obtained in accordance with protocols approved by the Institutional Review Board of Cleveland Clinic (Cleveland, OH). For this study, sAA was defined as a bone marrow cellularity of less than 30% associated with at least two of the following peripheral blood count criteria: 1) absolute neutrophil count (ANC) <0.500x10^9/L; 2) absolute reticulocyte count (ARC) <60x10^9/L (for manual counting <20x10^9/L) and 3) platelet count <20x10^9/L(4;19). Baseline laboratory values were defined as the lowest value during the 4 weeks prior to IST and were determined prior to transfusions and therefore the prognostic algorithm is free of the transfusion artifacts.

We also studied 23 AA patients with relapsed (N=12) or refractory disease (N=11) fulfilling severity criteria. Out of those who were initially treated with hATG, 13 patients were re-treated with rATG and 4 with repeat hATG while in patients who received rATG initially, 6 patients were re-treated with hATG. Patients were administered hATG (Pfizer, Kalamazoo, MI) at a dose of 40 mg/kg per day intravenously on days 1-4, or rATG (Genzyme, Cambridge, MA) at a dose of 3.5 mg/kg per day intravenously on days 1-5. CsA was given at a dose between 200 to 400 mg per day following the last day of hATG or rATG infusion and adjusted to maintain a serum level of 200-400 ng/ml or based on renal toxicity or tolerability. At our center, patients typically received corticosteroids, usually methylprednisolone, at 1 mg/kg per day, from day 1 until day 14 followed by a taper to prevent serum sickness. All patients received supportive care whenever indicated as previously described(4).

Chromosome breakage test was done in patients younger than 30 years old to rule out Fanconi anemia.

Response criteria
Patients were considered responders if they no longer met the criteria for sAA in the absence of recent transfusions and administration of granulocyte colony-stimulating factor(4;19). Response was evaluated at 3 months, 6 months and 12 months post-IST. We defined relapse as a need for retreatment with ATG following an initial response4. Patients who underwent stem cell transplantation (SCT) prior to 6 months and patients who died <90 days of therapy were considered as non-responders. Median follow up time was 792 days (37 -1800) and 1174 (8-4492) days for rATG and hATG, respectively.

Auxilliary studies
Screening for a paroxysmal nocturnal hemoglobinuria (PNH) clone was done using the flow cytometric analysis of CD55 and CD59 expression on neutrophils or red blood cells as previously reported(20). Patients were considered to have positive clone in the absence of glycophasphatidylinositol
(GPI) anchored surface proteins >1% (19). V beta (Vb) flow cytometric analysis was also done to quantitate the percentage of each Vb family in the CD4 and CD8 lymphocyte populations as previously described (21). In this study, a significant clonal expansion was defined as one that was greater than the mean plus 3 SD of healthy controls (for details see (22)). HLA typing was performed for all patients.

**Genotyping of cytokine polymorphisms**

Cytokine single nucleotide polymorphisms (SNPs) were determined by sequencing the following: IFN-γ intron (+874 A/T), TGF-β codons (10 C/T and 25 C/G), IL-4 (-1098 G/T, -590 C/T) and IL-4Ra (+1902 A/G), TNF-α promoter position (-308 A/G), IL-1α (-889 C/T), IL-1β (-511 C/T and +3962 C/T), IL-1 receptor (+1970 C/T), IL-1Ra (+11100 C/T), IL-2 (-330 G/T, +1662 G/T), and IL-6 (-174 C/G, nt565 A/G).

**Statistical analysis**

Response at 6 months and overall survival, measured from the date treatment started to the date of death or last follow-up, were the primary outcomes analyzed; however other outcomes including response at 3 and 12 months, relapse and early death were also examined. Categorical data were summarized as frequency counts and proportions, measured data were summarized as medians and ranges and duration of response and overall survival was summarized using the method of Kaplan and Meier.

Univariable analyses were performed using Fisher’s exact test and the Cochran-Armitage trend test (categorical data), the Wilcoxon rank sum test (measured data), and the Cox proportional hazards model (overall survival and duration of response). Logistic regression and Cox proportional hazards models were used to simultaneously assess multiple factors. In multivariable analyses a step-up selection algorithm with p=.10 as the criterion for entry into the model was used to identify independent predictors of six month response and overall survival. For convenience and simplicity a recursive partitioning algorithm was used to identify optimal cut points for measured data such as age at diagnosis and pretreatment blood counts. A bootstrap re-sampling algorithm that employed 1000 simulations was used to provide internal validation of the final response and survival models. All data analyses were performed using SAS version 8.0 (SAS Inc., Cary, NC, USA).
Results

**Patient characteristics**

Eighty nine patients were initially considered for inclusion in the analysis of de novo treated patients; however 2 patients treated off-protocol with rATG were excluded for analysis. Patient characteristics of the analyzed cases are summarized in Table 1; 67 patients (77%) were treated with hATG (historical control group) and 20 (23%) were treated with rATG. With the possible exception of pretreatment absolute reticulocyte count (ARC; median 28 x 10^9/L for hATG vs. 20 x 10^9/L for rATG, p=.09) there were no statistically significant differences in baseline characteristics between the two groups (Table 1). There were 10 refractory patients who underwent SCT in the hATG and 4 in the rATG group. No excess toxicity was observed in patients who received rATG that resulted to premature termination of the protocol. The difference in the efficacy of rATG and hATG was the primary focus of this study; however because the characteristics of the patients treated with each therapy were similar both groups were pooled to assess prognostic factors.

**Hematologic response following IST**

Therapy response was analyzed at 3, 6, and 12 months following the course of IST (Table 2). At 3 months, the response rate for patients in the hATG group was 55% (CR=5%, PR=50%), while for patients receiving rATG was 40% (PR=40%) (p=0.43). At 6 months, the response rate was 58% (CR=6%, PR=52%) vs. 45% (PR=45%) (p=0.44) and at 12 months, 58% (CR=8%, PR=50%) vs. 50% (PR=50%) (p=0.61), respectively. Six (9%) patients died early (<90 days from initiation of IST) in the hATG group: 4 due to infectious complications, 1 due to renal failure and subsequently pulmonary edema, and 1 undetermined. In the rATG group, 2 (10%) patients died early, both due to neutropenic sepsis.

**Refractory/Relapse following IST**

The relapse rate for patients treated with hATG was 16% and 5% for rATG (p=0.28). In our center, we maintain patients on CsA as long as they are responding or not developing renal complications. In the hATG cohort, 6 primary non-responders patients were retreated with rATG and none responded at 6 months. There were 11 patients who relapsed, 7 were retreated with rATG and 4 with repeat hATG. Of the 7 patients retreated with rATG, 4 responded at 6 months, 1 patient was non-responder and 2 were not evaluable for respone. Of the 4 patients retreated with hATG, 2 responded at 6 months and 2 patients did not.

In the rATG group, 5 primary non-responders were retreated with h-ATG; 1 patient responded at 6 months while another patient responded at 12 months. 2 were non-responders and 1 patient underwent BMT at 2 months after retreatment. 1 patient relapsed and was not a responder at 6 months after hATG treatment. Because of very limited number of patients, caution should be exercised in interpreting this data.
Evolution

In our cohort, a total of 15/87 patients (17%) experienced clonal evolution. Median follow up time was 792 days (37-1800) and 1174 (8-4492) days for rATG and hATG, respectively. In the h-ATG cohort, 11 patients progressed (9 myelodysplastic syndromes (MDS), 3 acute myeloid leukemia (AML), 1 clinically manifest PNH) while 2 patients progressed in the r-ATG group (1 MDS, 1 AML). Of these 15 patients, 10 had del(7) or del(7q). No factors were found to predict clonal evolution, nor was there a difference in this complication between rATG and hATG groups.

Correlation of clinical parameters and response

Considering the patients’ baseline factors described in Table 1, higher pretreatment platelet count (>12 x 10^9/L vs. <12 x 10^9/L, p=0.003) ARC (>30 x 10^9/L vs. <30 x 10^9/L, p=0.01), and ANC (>0.500 x 10^9/L vs. 0.100-0.500 x 10^9/L vs. <0.100 x 10^9/L, p=0.03) were all associated with an increased likelihood of response at 6 months. In addition, there was some suggestion that higher baseline absolute lymphocyte count (ALC) (>0.750 x 10^9/L vs. 0.750 x 10^9/L, p=0.07) may also be associated with response. In multivariable analyses, which initially considered all the factors in Table 1 (gender, age, HLA DR15, PNH clone, hemoglobin, platelet, ALC, ANC, and ARC), reticulocyte count was again seen to be prognostic for response (p=.007). The presence of a PNH clone, which was not statistically significant in univariable analysis, was also seen to be an independent predictor of response (p=0.01, Table 3). Adjusting for these two factors, the difference in response rates between rATG and hATG remained non-significant, p=0.51.

As the point estimates in multivariable analyses of response for the presence of a PNH clone and ARC were similar in magnitude (1.97 and 2.30, respectively), a simple prognostic score could be created by simply counting the number of poor risk features present, where the absence of a PNH clone and pretreatment ARC <30,000/µL each count as one poor feature. Based on this scoring, three distinct prognostic groups could be defined: 23% (10/44) of patients had the most favorable profile (i.e. no poor risk features - PNH clone and pretreatment ARC >30 x 10^9/L) and all 10 patients responded. In contrast, in patients with 1 poor risk feature (i.e. no PNH clone or pretreatment ARC<30 x 10^9/L), 11/20 patients (55%) responded and among those patients with an unfavorable profile (i.e. no PNH clone and pretreatment ARC>30 x 10^9/L), only 3/14 patients (21%) responded (p=<0.001) (Table 4). When in addition to the clinical parameters, the impact of TCR repertoire at presentation on the quality of subsequent response was analyzed, flow cytometric VB typing showed that patients with significantly expanded CD4 T cell clones at baseline were more likely to respond at 3 months. Overall, 15/57 had positive CD4 VB expansion by our criteria; responder 38% (12/32) vs non responder 12% (3/25) (p=0.03). CD8 expansions had no relationship with response, 35/57 (61%) overall had a CD8 expansion with 15/25 (60%) non-responders and 20/32 (63%), p=1.00.
**Impact of immunogenetic polymorphisms**

Among a large number of immunogenetic polymorphisms studied (see Materials and Methods), only TGF-β codons 10/25, IFN-γ, IL-4 codons 590/1098 and IL4-ra 1902 were shown to have a higher frequency in our internal AA cohort (data not shown). When these parameters were analyzed within the initial cohort of sAA patients (both rATG as well as hATG) with regard to the contribution to refractoriness / responsiveness to IST, none of these factors were predictive.

**Survival analyses**

Overall 36% of patients have died (h-ATG=36% and r-ATG=35%, p=.54); median follow-up for all the patients alive at the time of analysis was 43.5 months (range 3.6-147.3). Median survival has not been reached; however 2- and 5-year estimated survival are 76+5% and 64+6%, respectively. Considering the factors in Table 1, younger age (<18 vs 18-70 vs >70, p<0.001), female gender (p=0.05), pretreatment ALC (>0.750 x 10^9/L vs <0.750x10^9/L, p<.001), ANC (>500x10^9/L vs .100-.500 x 10^9/L vs <.100x10^9/L , p<0.001), and platelet count (>12x10^9/L vs <12x10^9/L , p=0.03) were all individually associated with improved survival. In multivariable analyses, age (p=0.003), pretreatment ANC (p=0.02), and pretreatment ALC (p=0.03) were the only factors found to be independent predictors for survival (Table 5). After adjusting for these three factors, the difference in survival between rATG and hATG remained non-significant, p=0.73.

Similar to the multivariable analysis of response at six months, the point estimates associated with age, and pretreatment ANC and ALC were similar enough in magnitude (1.25, 0.83, and 1.04, respectively) to allow prognostic groups to be determined based solely on the number of poor risk features present; where age 19-70, ANC .100-.500 x 10^9/L , and ALC <.750 x 10^9/L each count as one poor-risk feature, and age>70 and ANC<.100 x 10^9/L each count as two. Using this scoring system, two distinct prognostic groups could be defined (Table 6): patients with <2 points and those with >2 points. Most (44/62) patients (71%) who fell into the favorable prognostic group and had estimated 2- and 5-year survival rates of 85%+6% and 71% +9%, respectively; 29% of patients (18/61) had an unfavorable profile, with an estimated 2- and 5-year survival of 37+12% and 16%+10%, respectively; p<.001 (Fig.1).
DISCUSSION

The introduction of a new preparation of ATG derived through immunization of rabbits prompted the clinically practical question as to whether this new and likely more potent IST would result in improved response rates when used as first line treatment in patients with sAA. This question is the main focus of the current study. Certainly, when used in the salvage setting, rATG shows comparable efficacy to that seen with hATG. We conducted a pilot phase II study of rATG in a primary setting with comparison to historical controls who received hATG. The dose of 3.5 mg/kg x 5 days has been adopted from previous reports of rATG in the refractory setting(12;13), although smaller doses could also be effective, and in one study 2.5 mg/kg was used in older patients(23).

Our results suggest that rATG given as 3.5 mg/kg over 5 days may be similar to 40 mg/kg of hATG administered over 4 days with respect to response, overall survival or the risk of early death. While both prospectively studied patients and the historical group had similar baseline clinical characteristics, reliance on historical control cases treated with hATG is a clear limitation of our study. Nevertheless, results are comparable to previously published findings in a Spanish AA cohort(15). However, preliminary data from the NIH randomized trial showed rATG to be inferior to hATG at 3 month post IST(24). The final result of this study will decisively settle the issue of clinical differences between rATG and hATG. Other groups also reported superiority of hATG-based regimen over rATG in AA(16;17;17). In MDS, the results may be more confusing as single center studies, mostly using hATG, yielded diverse responses from 16%-50%(25-27). A prospective study comparing rATG versus hATG in MDS yielded similar response rates(28).

Of note is that previous clinical observations have suggested that rATG may be more potent than hATG based on more profound and prolonged lymphocytopenia(6). However, this stronger lympholytic activity may not necessarily mean that it is more immunosuppressive. In our study, there were no excess infectious complications leading to early death in patients treated with rATG and overall, the toxicity profile was similar to what has been reported for rATG.

In the present study multivariable analyses showed that the presence of a PNH clone and higher pretreatment ARC were independent predictors of response at 6 months. Similarly, the NIH group(19) showed higher baseline ARC but not a PNH clone as highly predictive for response at 6 months. However, a previous paper reported that PNH positive cells are reliable markers for response to IST(29). In general, biomarkers of response are likely to predict the survival as refractory AA patients were shown to have poor prognosis(19). Importantly, in the present study combining the effects of PNH clonality and baseline ARC defined three distinct prognostic groups that had 6-month response rates that ranged from 21% to 100%. Other clinical parameters have been shown to be predictive of responsiveness to IST. For example, in another recent study, higher ANC was also shown to be predictive for response(30). Baseline ANC was also correlated with response in our study in univariable analysis, but was not seen to be an independent predictor in multivariable analysis once the impact of PNH clone and baseline ARC were taken into account (p=.64).
Our multivariate analysis of overall survival showed that younger age and higher pretreatment ALC and ANC were independent positive predictors of survival. Higher ALC and ARC were previously correlated with a higher survival rate(19) while in another report, only higher ARC was predictive(30). As with response, distinct prognostic groups could be defined by using a simple scoring system to combine the effects of age and pretreatment ANC and ALC. The survival rate at 5 years for patients with ≤2 poor survival features was 71±9% compared to 16±10% for those with >2 poor survival features, p<.001. Identification of distinct prognostic groups such as those defined here for response and overall survival could have a major clinical impact in terms of therapeutic decision making by helping to identify patients who may benefit from early SCT rather than repeated cycles of ATG or other forms of IST. Additional prospective studies in larger patient groups however will be needed to validate these prognostic groups.

HLA-DR15 can serve as an example of an immunogenetic polymorphism that has been linked to AA and responsiveness to IST(31;32). Similarly, allelic polymorphisms within the regulatory regions of cytokine gene promoters can affect their transcription and thereby influence pathogenesis of immune-mediated diseases. For example, IFN-γ and TNF-α suppress the growth of hematopoietic progenitor and stem cells(33) and thus “high secretor or high affinity” genotypes could contribute to the pathogenesis of AA. Indeed, TNF-α promoter/enhancer polymorphism was associated with increased responsiveness after IST(34) and various other cytokine gene polymorphism were found to be overrepresented in patients with AA or PNH(35;36). However, in our study, none of the wide variety of immunogenetic polymorphisms studied (TGF-β codons 10 and 25, IFN-γ, IL-4 codons 590 and 1098, IL-4Ra) were significantly associated with response to IST or survival.

In sum, we conclude that despite reports suggesting differences in biologic activity of different ATG preparations, rATG appears to have a similar efficacy to hATG for upfront treatment of sAA. Irrespective of the ATG preparation used, presence of PNH clone and a higher pretreatment ARC appear to be predictive of response to IST while higher pretreatment ANC and ALC correlated with better survival. However, the historical comparison of rATG to established hATG therapy is an obvious limitation of this study and until results of a randomized study is reported, these conclusions have to be approached with proper caution.
Authorship and Disclosures

MGA was responsible for overall design, data collection, data analysis, data interpretation, manuscript preparation, writing and completion and final approval of manuscript; MS was responsible for data gathering, data analysis, data interpretation, and final approval of manuscript; YS performed the experiment, and approved the final manuscript; PE analyzed statistical data and approved the final manuscript; MC and HM gathered data, performed the experiment and approved the final manuscript; MS, AL, AA, MK, and RT provided patient samples, edited the manuscript, and approved the final manuscript; CLO analyzed data, interpreted data, and approved the final manuscript and JPM was responsible for overall design, patient samples, financial support, manuscript preparation, writing and completion, and final approval of manuscript. MGA: lecture honorarium from Genzyme; MS: consulting fee/honorarium from Celgene; MJP: research support from Genzyme, grants from NIH, AA/MDS International Foundation.

The rest of the authors reported no potential conflict of interests.
Reference List


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<th>Baseline Characteristics</th>
<th>Rabbit ATG</th>
<th>Horse ATG</th>
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ATG: anti-thymocyte globulin; ALC: absolute lymphocyte count; ANC: absolute neutrophil count; HLA: human leucocyte antigen.
Table 2. Response after first ATG treatment.

<table>
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<tr>
<th>Type of ATG</th>
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<th>Response (%)</th>
<th>Relapse (%)</th>
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<td>6 months</td>
</tr>
<tr>
<td>h-ATG</td>
<td>67</td>
<td>6</td>
<td>55</td>
<td>58</td>
</tr>
<tr>
<td>r-ATG</td>
<td>20</td>
<td>2</td>
<td>40</td>
<td>45</td>
</tr>
</tbody>
</table>

*p value

|            | 1.0  | 0.43  | 0.44  | 0.61  | 0.28  |

*death occurring within the first 90 days.

Table 3. Multivariable Analysis for response at 6 months (n=45).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds Ratio (95% Conf. Interval)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNH clone (No vs Yes)</td>
<td>9.35 (1.98-43.48)</td>
<td>0.005</td>
</tr>
<tr>
<td>Reticulocytes (&lt;0.03 vs &gt;0.03 M/µL)</td>
<td>9.09 (1.69-50.00)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 4. Prognostic grouping for response at 6 Months (n=45)

<table>
<thead>
<tr>
<th>No. of Poor Prognostic Features Present</th>
<th>N</th>
<th>No. (%) Responders</th>
<th>p'</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11</td>
<td>11 (100)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>12 (60)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>3 (21)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 based on reticulocyte count and presence of a PNH clone; reticulocyte count <0.03 M/µL and absence of a PNH clone each counts as 1 poor prognostic feature.

2 Cochran-Armitage trend test
Table 5. Multivariable Analysis for survival (n=61).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Hazard Ratio (95% Conf. Interval)</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (70 vs 19-70 vs &lt;18)</td>
<td>3.48 (1.53-7.90)</td>
<td>.003</td>
</tr>
<tr>
<td>ANC (&lt;0.1 vs 0.1-&lt;0.5 vs &gt;0.5 K/µL)</td>
<td>2.30 (1.16-4.57)</td>
<td>.02</td>
</tr>
<tr>
<td>ALC (&lt;0.75 vs &gt;0.75 K/µL)</td>
<td>2.82 (1.10-7.19)</td>
<td>.03</td>
</tr>
</tbody>
</table>

1the group(s) with the poorer outcome is (are) listed first; 2Wald test.

Table 6. Prognostic grouping for survival (N=61)

<table>
<thead>
<tr>
<th>Poor Prognostic Features¹</th>
<th>N</th>
<th>#Deaths (%</th>
<th>2 Year Survival</th>
<th>3 Year Survival</th>
<th>5 Year Survival</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,1,2</td>
<td>43</td>
<td>10 (22)</td>
<td>85%±6%</td>
<td>79%±7%</td>
<td>71%±9%</td>
<td></td>
</tr>
<tr>
<td>&gt;2</td>
<td>18</td>
<td>15 (83)</td>
<td>37%±12%</td>
<td>24%±11%</td>
<td>16%±10%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

¹based on age, lymphocyte count, and neutrophil count; age 19-70, ANC 0.1-0.5 K/µL, and ALC <0.75 K/µL each count as 1 poor prognostic feature; age >70 and ANC <0.1 count as 2 poor prognostic features; ²Wald test from Cox proportional hazards model.
Figure 1. Survival by number of poor prognostic features present. At 5 years, pts with >2 poor prognostic features (i.e. higher age, lower ANC and ALC) had a 16%±10% survival rate compared to 71%±9% for patients with ≤2 poor prognostic features (p<.001).