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Aplastic Anemia Patients with a Response Two Years After ATGAM-Based Treatment Remain at Risk for Relapse and Clinically Relevant Clonal Evolution: Real-World Data from a National Registry

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Abstract

Background: Immunosuppressive therapy (IST) is highly effective in acquired aplastic anemia (AA), but relapse and clonal evolution to MDS/AML or clinically relevant paroxysmal nocturnal hemoglobinuria (PNH) are potential long-term complications. International guidelines therefore recommend lifelong annual screening for PNH clones and blood counts. **Methods:** Using the Dutch national AA registry, we performed a landmark study to evaluate the long-term risk of relapse, clonal evolution to MDS/AML, or clinically relevant PNH in AA patients with a response 2 years after ATGAM-based IST. Overall survival (OS), event-free survival (EFS), and cumulative incidences of these events were assessed and stratified by response and presence of a PNH clone at the landmark. **Results:** Analysis was performed in 106 responders 2 years after ATGAM-based IST. Five-year OS was 88% and EFS was 69%. Five-year cumulative incidences were 18% for relapse and 7% for MDS/AML, without significant differences between PR and CR patients. The cumulative incidence of developing a clinically relevant PNH requiring complement-inhibitor treatment was 5% and was associated with the presence of a PNH clone >1% up to the landmark ($p = 0.01$). **Conclusions:** In AA patients with a response at 2 years after ATGAM-based IST, the combined long-term risk of relapse, MDS/AML, and clinically relevant PNH after ATGAM-based IST was 24%. These findings support continued hematological follow-up after successful ATGAM-based IST, regardless of response depth at the landmark.

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Keywords: aplastic anemia; long-term outcomes; PNH; MDS/AML; immune suppressive treatment; anti-thymocyte globulin; bone marrow failure

1. Introduction

Acquired aplastic anemia (AA) is a rare disease in which immune-mediated destruction of hematopoietic progenitor cells causes bone marrow failure. If left untreated, complications of pancytopenia, e.g., infections, bleeding, and anemia-related symptoms such as heart failure, could be ultimately fatal. First-line immune suppressive therapy (IST) consisting of horse-derived anti-thymocyte globulin (ATGAM) and ciclosporin A (CsA), with or without the addition of Eltrombopag is used in the majority of adult patients, with hematological response rates at 6 months of up to 80% in clinical trials [1–3]. Responses may continue to improve well beyond 6 months. Jain et al. reported a time to stable response of up to 18 months [4]. Relapsing disease is also common and can be observed in 24–39% of patients with an initial response to IST [5,6]. Patel et al. reported that two-thirds of the observed relapses in their study occurred within 2 years after ATGAM treatment, mostly concurrent with tapering or discontinuation of CsA. Hematopoiesis could be restored by reintroducing oral medication (CsA and/or eltrombopag) or increasing the dose in 67% of these relapsing patients [6]. If the relapse is refractory to reintroduction of CsA and/or eltrombopag, second-line IST or allogeneic stem cell transplantation (alloSCT) is needed.

In a bone marrow that is targeted by autoreactive T-cells, clonal evolution of aberrant hematopoietic stem cells carrying a survival advantage may occur. Clonal evolution does not always translate into clinically overt disease, but well-recognized late complications of clonal evolution after IST are myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) and paroxysmal nocturnal hemoglobinuria (PNH). The reported cumulative incidence of MDS/AML is 5–20% [6–8]. The cumulative incidence of the development of clinically relevant PNH is not known, but in a cohort of 207 patients treated with IST, 7 developed PNH-associated disease after IST [9].

MDS/AML following IST is considered to result from the expansion of cell clones carrying myeloid driver mutations or cytogenetic abnormalities. The timing of progression to myeloid malignancies may depend on the characteristics of the pathogenic clone. Guttierez-Rodriguez et al. reported that chromosome 7 abnormalities were generally associated with early development of myeloid malignancies (within 1 year), whereas later development of myeloid malignancies (4–5 years after IST) was associated with ASXL-1 or U2AF1-mutated clones [10]. PNH, on the other hand, is the result of PIGA mutations in hematopoietic stem cells. In AA, PIGA-mutant clones are thought to have a selective advantage due to a possible immune escape mechanism resulting from the loss of PIGA-dependent glycosylphosphatidylinositol (GPI)-anchored proteins [11,12]. At the same time, loss of GPI-anchored proteins renders PNH erythrocytes susceptible to complement-mediated hemolysis and an associated increased thrombosis risk. Clinically overt hemolysis is generally associated with larger PNH clones, often exceeding 50% of circulating cells [9,13]. Clinically relevant PNH manifesting as hemolysis and/or thrombosis is an indication for treatment with a complement inhibitor such as eculizumab. In 40–50% of AA patients, a (small) PNH clone is present at diagnosis [9,13]. Although these PNH clones most often do not cause clinically relevant hemolysis or thrombosis, they might increase and become clinically relevant at a later timepoint.

Because of the long-term risk of the development of MDS/AML and PNH-associated disease, international guidelines recommend long-term screening for PNH clones and blood counts at least annually in AA patients treated with ATGAM-based IST [14,15]. Although

the need for long-term follow-up in non-responding AA patients seems clear, the necessity of continued monitoring in patients with a stable hematological response and no detectable PNH clone after IST remains uncertain.

In this study, we investigated the long-term risk of the development of MDS/AML or clinically relevant PNH in AA patients with a hematological response after ATGAM-based IST. Therefore, we performed a landmark analysis in patients from the Dutch Aplastic Anemia Registry who had a hematological response at two years after ATGAM.

2. Methods

2.1. Data Source and Study Design

The Dutch national AA registry, established in 2012, is a population-based registry in which data is collected from all consecutive patients in the Netherlands who received ATGAM in combination with CsA as first-line treatment for AA according to the national guideline [12]. Treatment is given in a limited number of Dutch hospitals that all contribute data to the registry. Completeness of patient cohorts per hospital was checked using delivery data of ATGAM to the hospital pharmacies. Clinical data and laboratory results were collected by the treating hematologists or local data managers at baseline and at regular intervals after the start of ATGAM and checked by a central data manager. The Institutional review board of the Leiden University Medical Centre approved data collection and analysis (protocol nr. C13.014). The dataset was closed for this analysis on 1 February 2026.

To specifically assess the incidence of long-term relapse and clonal evolution in responders after IST, a landmark design was used. The 2-year mark was chosen to allow sufficient time for hematological responses to develop and to identify patients who had reached a relatively stable phase [4,16]. We screened all patients who were alive at least until the two-year landmark. If patients had developed MDS/AML, undergone an allogeneic stem cell transplantation (alloSCT) or received complement inhibition before this 2-year landmark, they were excluded from analysis. The final cohort consisted of patients who had achieved a response to treatment (PR or CR) at the 2-year landmark. Follow-up for subsequent relapse and clonal evolution was assessed from the 2-year landmark onward.

2.2. Definitions and Endpoints

Disease severity was classified according to the criteria of the International Aplastic Anemia Study Group. Severe aplastic anemia (SAA) was defined as bone marrow cellularity <25% and at least two of the following three peripheral blood criteria: neutrophil count $<0.5 \times 10^9$/L, platelet count $<20 \times 10^9$/L, or reticulocyte count <1%. Very severe aplastic anemia (VSAA) was defined as SAA with a neutrophil count $<0.2 \times 10^9$/L. Patients with a hypocellular bone marrow and cytopenias who did not fulfill the criteria for SAA were classified as having non-severe aplastic anemia (NSAA).

Response to treatment was classified as complete response (CR), partial response (PR), or non-response (NR). CR was defined as normalization of all peripheral blood counts. PR was defined as transfusion independence and a neutrophil count $>0.5 \times 10^9$/L without complete normalization of blood counts. Patients who did not meet the criteria for CR or PR were classified as having NR.

Relapse was defined as loss of a previously achieved hematological response requiring additional therapy and/or transfusion support. Clonal evolution to MDS/AML was defined as the development of MDS or AML meeting established diagnostic criteria and resulting in an indication for disease-directed treatment. PNH-clones were assessed by flow cytometry based on deficient expression of glycosylphosphatidylinositol (GPI)-anchored proteins on granulocytes. The flow cytometry assay had a detection sensitivity of 0.01%. The PNH-clone size up to the landmark was dichotomized into ≥1% versus <1%, based on the

highest value measured between diagnosis and the landmark. PNH clones were considered clinically relevant if treatment with a complement inhibitor was initiated. Time to clinically relevant PNH was defined as the time from the 2-year landmark assessment to initiation of complement inhibitor therapy.

2.3. Statistical Analysis

Overall survival (OS) and event-free survival (EFS) were assessed using the Kaplan-Meier method. Event-free survival (EFS) was defined as the time from landmark to the first occurrence of death, relapse, clonal evolution to MDS/AML, or development of a clinically relevant PNH clone, whichever occurred first. Patients without an event were censored at the date of last follow-up. Differences in OS and EFS according to hematological response were assessed using the log-rank test. To further explore the individual contribution of each event to EFS, the cumulative incidence of relapse, clonal evolution to MDS/AML, and development of a clinically relevant PNH clone were evaluated separately, using death as a competing risk. Differences in cumulative incidence according to hematological response and the presence of a PNH clone up to landmark were assessed using Gray's test. All statistical analyses were conducted using R version 4.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Landmark Cohort Characteristics

At data extraction on 1 February 2026, the registry contained 171 patients who had received ATGAM-based IST up to 1 February 2024, allowing a potential follow-up duration of at least 2 years. Of these 171, 22 patients died, 2 developed MDS/AML, 27 underwent alloSCT, and 6 were lost to follow-up within 2 years after IST, leaving 114 patients evaluable at the 2-year landmark. Eight patients did not meet criteria for PR or CR at that timepoint and were excluded, resulting in a landmark cohort of 106 patients (Figure 1). In this landmark cohort, 75 (71%) had a PR and 31 (29%) had a CR. Follow-up duration from the 2-year landmark onwards ranged from 0 until 121 months, with a median of 46 months.

The median age at diagnosis of the landmark cohort was 51 (range 18–79), with 47 females (44%). Non-severe AA (NSAA) was diagnosed in 33 (31%), severe AA (SAA) in 48 (45%), and very severe AA (VSAA) in 25 (24%) patients. All patients were transfusion-dependent at the initiation of ATGAM-based IST.

PNH clone status was assessed in 93 patients (88%) at diagnosis and in 75 patients (71%) at the 2-year landmark. Among the 99 patients with at least one PNH clone assessment between diagnosis and landmark, 53 (54%) had a PNH clone size >1% detected at least once during this interval (Table 1). PNH clone assessment data were missing altogether in 7 patients. Over the entire follow-up period, patients with at least one assessment had a median of six PNH clone assessments (range 1–34).

Table 1. Baseline characteristics of landmark cohort.

Characteristics	Whole Cohort (N = 106)
Age at diagnosis (years)	
- Median	51
- Range	18–79
Gender	
- Women	47 (44%)
- Men	59 (56%)

Table 1. *Cont.*

Characteristics	Whole Cohort (N = 106)
Severity at diagnosis	
- Non severe	33 (31%)
- Severe	48 (45%)
- Very severe	25 (24%)
Response at landmark	
- PR	75 (71%)
- CR	31 (29%)
PNH clone size up to landmark	
- <1%	46 (43%)
- 1–10%	29 (27%)
- 10–50%	16 (15%)
- >50%	8 (8%)
- Missing	7 (7%)

Characteristics of 106 responders, 2 years after ATGAM-based IST. Absolute numbers of patients are reported for gender, severity of diagnosis, response at landmark, and PNH-clone size up to landmark.

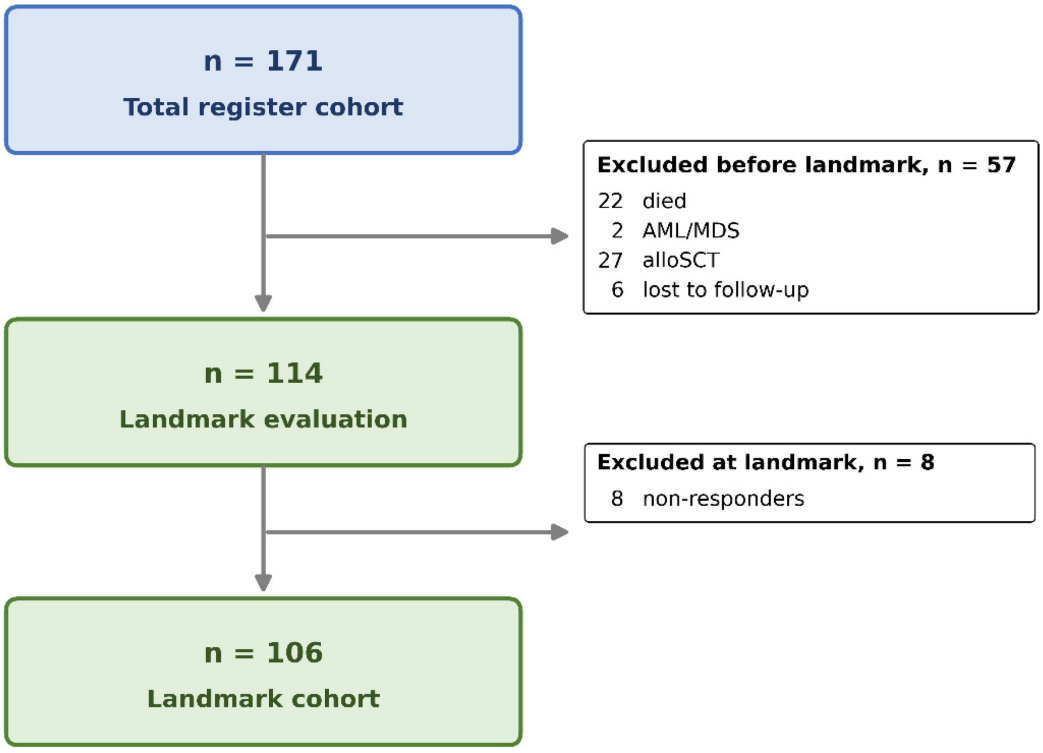


Figure 1. Inclusion flowchart. At data extraction on 1 February 2026, 171 patients in the registry received ATGAM-based IST up until 1 February 2024 and had a potential follow-up time of 2-years. Fifty-seven patients were excluded from analysis because they either died, developed MDS/AML, received allogeneic stem cell transplantation or were lost to follow-up before the 2-year landmark. Eight patients did not meet criteria for partial or complete response at the 2-year landmark, resulting in a landmark cohort of 106 patients.

3.2. Overall Survival and Event-Free Survival in Patients with a Hematological Response at 2 Years

The estimated overall survival (OS) rates at 1, 2, and 5 years from landmark were 98% (95% CI: 95–100%), 96% (95% CI: 92–100%), and 88% (95% CI: 80–96%), respectively. Nine patients died during follow-up: 1 patient died due to relapse AA, 3 due to MDS/AML, 2 due to a secondary solid malignancy, and 1 from pneumonia, and in 2 patients, the cause of death was unknown. Log-rank tests showed no significant associations between OS and treatment response at landmark (PR or CR, $p = 0.88$; Figure 2A).

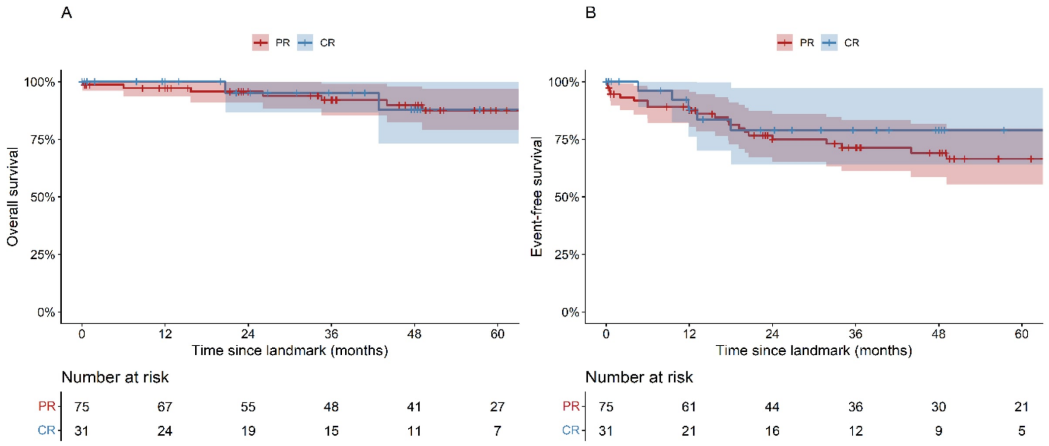


Figure 2. Overall survival (**A**) and event-free survival (**B**) categorized by response at landmark. Overall survival (**A**) and event-free survival (**B**) are defined as time from landmark to the first occurrence of either death, relapse, MDS/AML, or clinically relevant PNH, stratified for depth of response at landmark, with shadowed 95% confidence intervals.

Estimated event-free survival (EFS), defined as time to the first occurrence of either death, relapse, MDS/AML, or clinically relevant PNH, at 1, 2 and 5 years from landmark was 88% (95% CI: 82–94%), 76% (95% CI: 68–85%), and 69% (95% CI: 60–80%), respectively. Treatment response at landmark was not significantly associated with EFS using Log-rank tests ($p = 0.60$; Figure 2B).

3.3. Relapse

From the 2-year landmark, in 17 patients a relapse of AA occurred after a median of 12 months (range 0–32). The cumulative incidence of relapse with death as a competing risk was 8% at 1 year, 15% at 2 years, and 18% at 5 years. Most relapsing patients re-initiated or increased the dose of CsA. Eltrombopag was restarted or added as second-line treatment in 9 patients. Four patients eventually underwent alloSCT for bone marrow failure. The response at the last follow-up of the 13 patients treated without alloSCT was CR in 1, PR in 5, and NR in 5 (1 died). MDS/AML developed in 2 patients (1 died). Gray's test showed no significant differences between the cumulative incidence curves based on the 2-year response status ($p = 0.70$; Figure 3A).

3.4. MDS/AML

Six patients developed MDS/AML after a median of 20 months (range: 4–34 months). At diagnosis, a normal karyotype was reported in all patients developing MDS/AML, except for one patient with 46, XX, -13q. In this patient, AML developed 12 months after the landmark assessment. Two patients died before they could receive an alloSCT. Of the remaining 4 who underwent alloSCT, 1 died of severe graft-versus-host-disease and AML relapse. Cumulative incidence of MDS/AML with death as a competing risk was 2% at 1 year, 5% at 2 years, and reached a plateau of 7% at 4 years after the landmark. Gray's

test showed no significant association between the development of MDS/AML and disease response at landmark ($p = 0.60$; Figure 3B).

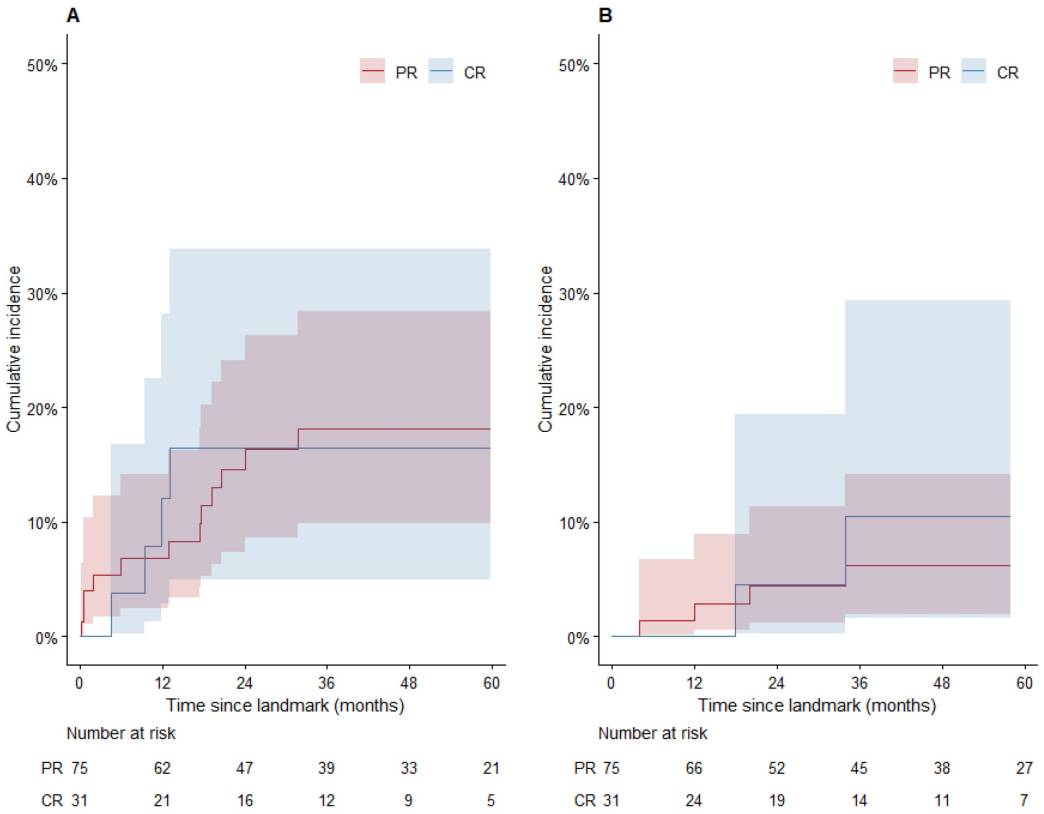


Figure 3. Cumulative incidence of relapse (**A**) and MDS/AML (**B**) with death as competing risk, stratified for depth of response at landmark. Cumulative incidence of relapse AA (**A**) and clonal evolution to MDS/AML (**B**) with death as competing risk, stratified for depth of response at landmark with shadowed 95% confidence intervals.

3.5. PNH

After the landmark, 7 patients developed a clinically relevant PNH clone and were treated with a complement inhibitor after a median of 43 months (range 4–89 months). In all 7 patients, relevant PNH clone expansion occurred concurrently with an AA relapse. The indication for complement inhibitor initiation was hemolytic PNH in 6 patients, and thrombotic PNH in 1 patient (Table 2). The cumulative incidence of developing a clinically relevant PNH clone with death as a competing risk was 1% at 1 year, 2% at 2 years, and 5% at 5 years after landmark (Figure 4A). Treatment response at landmark was not associated with the development of a clinically relevant PNH clone (Gray's test $p = 0.30$).

Table 2. Characteristics of patients who developed clinically relevant PNH.

ID	Age at Diagnosis (Years)	Severity at Diagnosis	Response at Landmark	Present PNH Clone (>1%) up to Landmark	PNH Clone Size at Diagnosis (%)	PNH Clone at Landmark (%)	Complement Inhibitor Initiation (Months After Landmark)	Treatment Indication
1	19	NSAA	CR	No	0	-	89	Hemolytic PNH
2	56	NSAA	PR	Yes	63	71	24	Thrombotic PNH

Table 2. *Cont.*

ID	Age at Diagnosis (Years)	Severity at Diagnosis	Response at Landmark	Present PNH Clone (>1%) up to Landmark	PNH Clone Size at Diagnosis (%)	PNH Clone at Landmark (%)	Complement Inhibitor Initiation (Months After Landmark)	Treatment Indication
3	24	SAA	PR	Yes	57	16	43	Hemolytic PNH
4	29	SAA	PR	Yes	32	44	26	Hemolytic PNH
5	29	VSAA	PR	Yes	52	35	4	Hemolytic PNH
6	18	SAA	CR	Yes	1	4	78	Hemolytic PNH
7	58	SAA	CR	Yes	5	-	68	Hemolytic PNH

Characteristics of patients who developed clinically relevant PNH for which complement inhibitor treatment was initiated. In 2 patients, PNH clone status was not assessed at landmark. Treatment indication was most often hemolytic PNH, and time from landmark until initiating complement inhibitor treatment ranged from 4 to 89 months. All 7 patients had concurrent AA relapse when developing clinically relevant PNH.

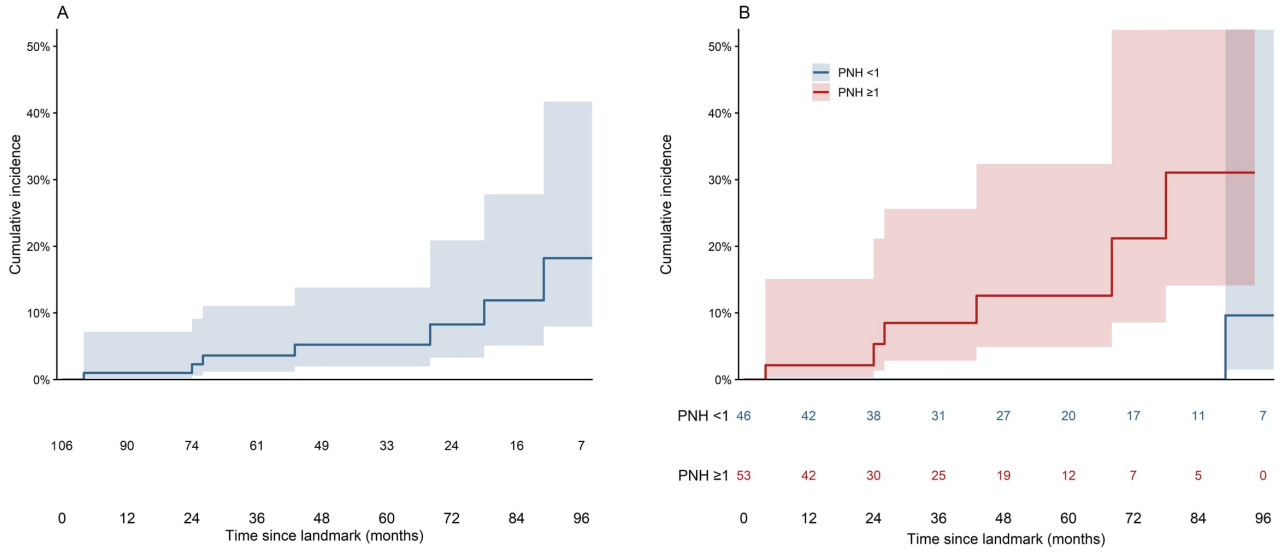


Figure 4. Cumulative incidence of clinically relevant PNH in total cohort (**A**) and stratified by the presence of PNH clone >1% up to landmark (**B**). Cumulative incidence of clinically relevant PNH for which treatment with complement inhibitor was started with death as competing risk in total cohort (**A**) and stratified by the presence of a PNH clone >1% up to landmark (**B**), with shadowed 95% confidence intervals. Seven patients were included in the cumulative incidence of the whole cohort, but not on the cumulative incidence curves stratified by PNH-clone presence up to landmark, because no data on PNH-clone evaluation was available from diagnosis up to landmark.

The presence of a PNH clone up to a landmark correlated strongly with the development of a clinically relevant PNH with an indication for treatment with a complement inhibitor (Gray's test $p = 0.01$; Figure 4B). Of the 53 patients with a PNH clone >1% up to landmark, 6 developed a clinically relevant PNH clone (Table 2). Only 1 of 46 patients with a PNH clone <1% up to landmark ultimately needed treatment with eculizumab 89 months after landmark.

3.6. Combined Event Relapse, MDS/AML, and Clinically Relevant PNH

The cumulative incidence of relapse, MDS/AML, or clinically relevant PNH as a combined event, with death as competing risk, was 10% at 1 year, 21% at 2 years, and 24% at 5 years after landmark (Figure 5A). Cumulative incidence curves of the combined events did not significantly differ between patients in PR and patients in CR (Gray's test $p = 0.87$; Figure 5B).

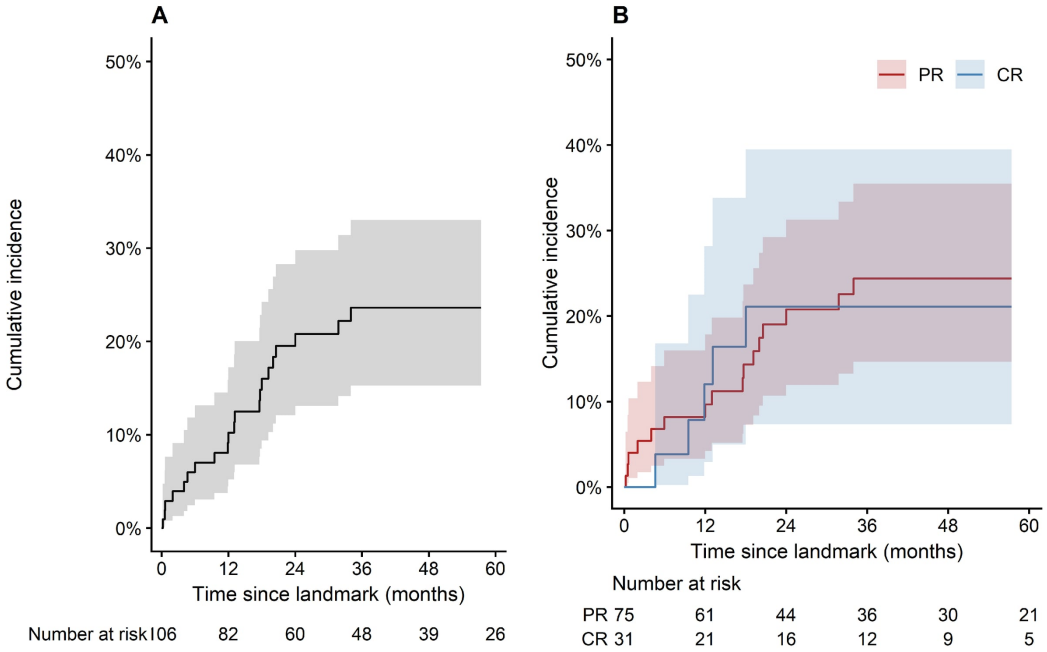


Figure 5. Cumulative incidence of combined events in total cohort (**A**) and stratified for depth of response at landmark (**B**). Cumulative incidence of combined events, defined as time from landmark to the first occurrence of either relapse, MDS/AML, or clinically relevant PNH with death as competing factor in whole cohort (**A**) and stratified for depth of response at landmark (**B**), with shadowed 95% confidence intervals.

4. Discussion

Most studies report cumulative incidences of clonal evolution to MDS/AML and PNH after IST from the start of this treatment. To specifically assess whether patients with a long-term response to IST are still at risk for the development of these hematological diseases, we performed a landmark analysis at 2 years after ATGAM-based IST and found that even among the patients with a response at the 2-year landmark, 24% still developed bone marrow disease thereafter, with no significant difference between patients in PR or CR.

In the Dutch aplastic anemia registry, 106 out of 171 patients (62%) had a hematological response 2 years after landmark, without receiving alloSCT as second-line treatment. We chose to exclude patients who were treated with alloSCT, as transplantation with a new hematopoietic stem cell compartment substantially reduces the risk of relapse of AA and clonal evolution. Because the registry was initiated in 2012 when Eltrombopag was not available in the Netherlands for treatment of AA, Eltrombopag was not added to ATGAM as first-line treatment in a proportion of patients. Still, the 2-year response rate in our cohort is comparable with the 2-year response rate as reported in the RACE trial [17], possibly caused by the use of Eltrombopag or rabbit-derived ATG as second-line treatment. OS in this real-life cohort was comparable to OS reported in historical cohorts of IST-treated AA patients [6,10,17]. Therefore, we believe that the results of this study are representative of AA patients treated with ATGAM-based IST in general and may contribute to improved patient counselling and shared decision-making.

The 5-year EFS, defined as time from landmark to the first occurrence of either death, relapse, MDS/AML, or clinically relevant PNH, was 69% in the whole cohort. The EFS curves for patients in PR and CR appeared to diverge from 2 years after landmark onward, with lower EFS among patients in PR and no further events among patients in CR. However, this difference did not reach statistical significance. Longer follow-up and larger patient numbers are needed to determine whether this difference persists over time and if patients who are in CR at 4 years after ATGAM are at lower risk for the development of bone marrow disease.

The cumulative incidence of MDS/AML in our study is lower than reported from diagnosis in historical cohorts [6–8]. However, this difference is expected given the landmark design of our study, which evaluated only the events occurring after the 2-year landmark in patients with a durable response. Our study showed that both patients in PR and CR 2 years after ATGAM-based IST experienced relapse or clonal evolution to MDS/AML, but landmark and cumulative incidences did not differ significantly based on depth of response. Interestingly, the cumulative incidence of developing MDS/AML appeared to plateau approximately 4 years from landmark, although longer follow-up is needed to determine whether this represents a true plateau.

As PNH clone-size is not always directly associated with the observed symptoms of hemolysis or thrombosis, we used the initiation of complement inhibitors as a proxy for the development of a clinically relevant PNH clone. Patel et al. [18] and Fatizzio [19] et al. reported comparable cumulative incidences of complement inhibitor use in AA patient cohorts. In most patients who developed a clinically relevant PNH clone, a PNH clone >1% was already present between diagnosis and landmark, supporting continued monitoring of patients with present PNH clones. However, in 1 patient no PNH clone was detectable at diagnosis (<0.01%), and the clone remained below 1% (0.7%) 4 years after treatment. Clinically relevant PNH in this patient only developed at the time of AA relapse, 84 months after landmark, demonstrating that even if during the first years after IST no PNH clone was detected, patients with AA still can develop clinically relevant PNH at a later timepoint.

Overall, we could not identify a specific subgroup of patients with a response at two years after ATGAM-based IST in whom follow-up of blood counts could be reduced due to a lower risk of long-term complications. On the contrary, we found that even complete responders and patients without a detectable PNH clone up to the 2-year landmark were not protected from developing new bone marrow complications. Whether continued PNH clone testing is necessary in all patients can be questioned since the growth of a clinically relevant PNH clone was invariably concurrent with a relapse of AA. Evaluating PNH clones only when blood counts change might be considered in patients without earlier PNH clones.

Based on our real-life data, we advocate that continuous follow-up of AA patients with a response at two years after IST remains essential even if a patient appears ‘cured’ with a complete response.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request. However, due to privacy concerns and the potential risk of re-identification of individuals with this rare condition, the dataset is not publicly available.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. de Latour, R.P.; Kulasekararaj, A.; Iacobelli, S.; Terwel, S.R.; Cook, R.; Griffin, M.; Halkes, C.J.; Recher, C.; Barraco, F.; Forcade, E.; et al. Eltrombopag Added to Immunosuppression in Severe Aplastic Anemia. *N. Engl. J. Med.* **2022**, *386*, 11–23. [CrossRef] [PubMed]
2. Townsley, D.M.; Scheinberg, P.; Winkler, T.; Desmond, R.; Dumitriu, B.; Rios, O.; Weinstein, B.; Valdez, J.; Lotter, J.; Feng, X.; et al. Eltrombopag Added to Standard Immunosuppression for Aplastic Anemia. *N. Engl. J. Med.* **2017**, *376*, 1540–1550. [CrossRef] [PubMed]
3. Scheinberg, P.; Nunez, O.; Weinstein, B.; Scheinberg, P.; Biancotto, A.; Wu, C.O.; Young, N.S. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N. Engl. J. Med.* **2011**, *365*, 430–438. [CrossRef] [PubMed]
4. Jain, A.; Jandial, A.; Mani, T.; Kishore, K.; Singh, C.; Lad, D.; Prakash, G.; Khadwal, A.; Das, R.; Varma, N.; et al. Comparable outcomes with low-dose and standard-dose horse anti-thymocyte globulin in the treatment of severe aplastic anemia. *Blood Res.* **2024**, *59*, 6. [CrossRef] [PubMed]
5. Drexler, B.; Zurbriggen, F.; Diesch, T.; Viollier, R.; Halter, J.P.; Heim, D.; Holbro, A.; Infanti, L.; Buser, A.; Gerull, S.; et al. Very long-term follow-up of aplastic anemia treated with immunosuppressive therapy or allogeneic hematopoietic cell transplantation. *Ann. Hematol.* **2020**, *99*, 2529–2538. [CrossRef] [PubMed]
6. Patel, B.A.; Groarke, E.M.; Lotter, J.; Shalhoub, R.; Gutierrez-Rodrigues, F.; Rios, O.; Raffo, D.Q.; Wu, C.O.; Young, N.S. Long-term outcomes in patients with severe aplastic anemia treated with immunosuppression and eltrombopag: A phase 2 study. *Blood* **2022**, *139*, 34–43. [CrossRef] [PubMed]
7. Groarke, E.M.; Patel, B.A.; Shalhoub, R.; Gutierrez-Rodrigues, F.; Desai, P.; Leuva, H.; Zaimoku, Y.; Paton, C.; Spitofsky, N.; Lotter, J.; et al. Predictors of clonal evolution and myeloid neoplasia following immunosuppressive therapy in severe aplastic anemia. *Leukemia* **2022**, *36*, 2328–2337. [CrossRef] [PubMed]
8. Gurnari, C.; Pagliuca, S.; Prata, P.H.; Galimard, J.-E.; Catto, L.F.B.; Larcher, L.; Sebert, M.; Allain, V.; Patel, B.J.; Durmaz, A.; et al. Clinical and Molecular Determinants of Clonal Evolution in Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria. *J. Clin. Oncol.* **2023**, *41*, 132–142. [CrossRef] [PubMed]
9. Scheinberg, P.; Marte, M.; Nunez, O.; Young, N.S. Paroxysmal nocturnal hemoglobinuria clones in severe aplastic anemia patients treated with horse anti-thymocyte globulin plus cyclosporine. *Haematologica* **2010**, *95*, 1075–1080. [CrossRef] [PubMed]
10. Gutierrez-Rodrigues, F.; Groarke, E.M.; Miao, R.; Shalhoub, R.; Alemu, L.; Mizumaki, H.; Rios, O.; Glass, J.; Durrani, J.; Catto, L.F.B.; et al. Clonal Selection and Evolution after Treatment of Severe Aplastic Anemia. *NEJM Evid.* **2005**, *5*, EVIDoa2500171.
11. Ogawa, S. Clonal hematopoiesis in acquired aplastic anemia. *Blood* **2016**, *128*, 337–347. [CrossRef] [PubMed]
12. Mizumaki, H.; Gao, S.; Wu, Z.; Gutierrez-Rodrigues, F.; Alemu, L.; Raffo, D.Q.; Darden, I.; Rios, O.; Lotter, J.; Kajigaya, S.; et al. Imputing causality and clonal dynamics from single-cell transcriptomics in paroxysmal nocturnal hemoglobinuria. *Leukemia* **2026**, *40*, 1368–1380. [CrossRef] [PubMed]
13. Neal, S.Y. Paroxysmal nocturnal hemoglobinuria and myelodysplastic sydromes: Clonal expansion of PIG-A-mutant hematopoietic cells in bone marrow failure. *Haematologica* **2008**, *94*, 3–7. [CrossRef] [PubMed]
14. Kulasekararaj, A.; Cavenagh, J.; Dokal, I.; Foukaneli, T.; Gandhi, S.; Garg, M.; Griffin, M.; Hillmen, P.; Ireland, R.; Killick, S.; et al. Guidelines for the diagnosis and management of adult aplastic anaemia: A British Society for Haematology Guideline. *Br. J. Haematol.* **2024**, *204*, 784–804. [CrossRef] [PubMed]
15. Tjon, J.M.-L.D.; Heubel-Moenen, L.G.M.; Nur, F.C.J.I.; Klein, E.; Hazenberg, S.K.; Snijders, C.L.E.; Langemeijer, T.J.F.; Broers, S.M.; Raaijmakers, A.E.C.; Halkes, M.H.G. Verworven aplastische anemie bij volwassenen. [Richtlijn]. 2024. Available online: https://publicatie.hematologienederland.nl/richtlijnen/verworven-aplastische-anemie-bij-volwassenen-2/ (accessed on 21 September 2026).
16. Halkes, C.J.M.; Koster, E.A.S.; Bogers, E.J.M.; Heubel-Moenen, F.C.J.I.; Daenen, L.G.M.; Klein, S.K.; Langemeijer, S.M.C.; Nur, E.; Raaijmakers, M.H.G.; Snijders, T.J.F.; et al. Anti-thymocyte globulin-based treatment frequently leads to enduring treatment success in both old and young adult patients with aplastic anaemia: A real-world analysis from the Dutch aplastic anaemia registry. *Ann. Hematol.* **2026**, *105*, 51. [CrossRef] [PubMed]
17. Risitano, A.M.; Iacobelli, S.; Kulasekararaj, A.; van Os, M.; Terwel, S.R.; Tuffnell, J.; Piepenbroek, B.; Griffin, M.; Halkes, C.J.M.; Recher, C.; et al. Eltrombopag Added to Standard Immunosuppressive Treatment as Front-Line Therapy for Severe Aplastic Anemia: Long-Term Outcomes of the Phase-3 Randomized Superiority EBMT-SAAWP RACE Study. *Am. J. Hematol.* **2026**, *101*, 2520–2532. [CrossRef] [PubMed]

18. Patel, B.J.; Barot, S.V.; Kuzmanovic, T.; Kerr, C.; Przychodzen, B.P.; Thota, S.; Lee, S.; Patel, S.; Radivoyevitch, T.; Lichtin, A.; et al. Distinctive and common features of moderate aplastic anaemia. *Br. J. Haematol.* **2020**, *189*, 967–975. [CrossRef] [PubMed]
19. Fattizzo, B.; Gurnari, C.; Giammarco, S.; Ricchiuti, A.; Awada, H.; Bortolotti, M.; Galli, N.; Pedone, G.L.; Versino, F.; Consonni, D.; et al. Elderly Patients With Aplastic Anemia: Treatment Patterns and Outcomes in the Real World. *Am. J. Hematol.* **2025**, *100*, 584–591. [CrossRef] [PubMed]

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