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Hematopoietic Cell Transplantation for Paroxysmal Nocturnal Hemoglobinuria in the Age of Eculizumab

Jason P. Cooper[1,2], Rafic J. Farah[1,3,†], Philip A. Stevenson[1], Ted A. Gooley[1,4], Rainer Storb[1,3], Bart L. Scott[1,3,*]

[1] *Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington*
[2] *Division of Hematology, Department of Medicine, University of Washington School of Medicine, Seattle, Washington*
[3] *Division of Medical Oncology, Department of Medicine, University of Washington School of Medicine, Seattle, Washington*
[4] *Department of Biostatistics, University of Washington School of Medicine, Seattle, Washington*

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Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired clonal hematopoietic cell disease characterized by the destruction of hematopoietic cells through activation of the complement system with manifestations that can be life-threatening including hemolysis, thrombosis, and marrow failure. Allogeneic hematopoietic cell transplantation (HCT) remains the sole cure for PNH, but eculizumab, a terminal complement inhibitor of C5, has been used to prevent complement-mediated hemolysis in patients with PNH since its approval by the Food and Drug Administration in 2007. We examined outcomes of HCT in patients with PNH to evaluate the effects of disease subtype, conditioning intensity, and eculizumab use either pre-HCT or post-HCT. Fifty-five patients with a diagnosis of PNH underwent at least 1 HCT, with 4 patients requiring a second HCT for graft failure. The median age at the time of first HCT was 30.0 years (range, 4.2 to 66.9 years). Seventeen patients (30.9%) had classical PNH, and the remaining 38 patients had PNH associated with another marrow disorder (aplastic anemia in 26 of the 38). Indications for HCT included pancytopenia in 47.3% of the patients, myeloid malignancy (myelodysplastic syndrome, myeloproliferative neoplasm, or acute myelogenous leukemia) in 21.8%, recurrent hemolysis in 20.0%, and thrombosis in 10.9%. Of the 55 first HCTs, 26 were performed with myeloablative conditioning, 27 were performed with reduced-intensity conditioning, and 2 sets of identical twins underwent HCT without any conditioning. Donor types included HLA-matched related in 38.2%, HLA-matched unrelated in 34.5%, single HLA-allele mismatched unrelated in 16.4%, umbilical cord blood in 5.5%, syngeneic in 3.6%, and HLA-haploidentical in 1.8%. The median duration of follow-up in surviving patients was 6.1 years (range, 2.1 to 46.1 years) after first HCT. The median time to neutrophil and platelet engraftment was 17 days and 19 days, respectively; all but 2 patients (96.3%) had sustained engraftment. Overall survival was 70% at 5 years. Neither the choice of conditioning intensity nor PNH subtype affected survival. Nineteen patients died during follow-up, including 12 patients before day +365. Six patients received treatment with eculizumab before HCT, and 2 were treated after HCT. All patients treated with eculizumab were alive at a median follow-up of 2.3 years (range, .2 to 6.9 years). Both patients treated with eculizumab after HCT had minimal to no acute GVHD (aGVHD), with grade I skin aGVHD in 1 patient and no aGVHD in the other patient, and no chronic GVHD at 2.1 and 4.1 years post-HCT, respectively. With the approval of eculizumab, the indications for HCT include persistent hemolysis, persistent thrombosis, and associated marrow failure. Administration of eculizumab before and after HCT warrants further study, particularly considering our observation of minimal to no GVHD in 2 patients who received eculizumab after HCT.

INTRODUCTION

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal hematopoietic cell disorder characterized by intravascular hemolysis, thrombosis, and marrow failure [1]. PNH is rare, with an estimated prevalence of 1 to 10 per million, and has a well-defined pathophysiology in which abnormal hematopoietic cell clones carry mutations in the X-linked phosphatidylinositol glycan class A gene (*PIG-A*) [2–4]. These mutations result in defective synthesis of a glycosylphosphatidylinositol (GPI) anchor that is required for attachment of proteins to the cell surface. Absence

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CMV indicates cytomegalovirus.

* Correspondence and reprint requests: Bart L. Scott, MD, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, D1-100, PO Box 19024, Seattle, WA 98109-1024.
E-mail address: bscott@fredhutch.org (B.L. Scott).
† Current address: Hillman Cancer Center, University of Pittsburg Medical Center, Pittsburg, Pennsylvania.

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of 2 of these GPI-anchored proteins, CD55 (decay-accelerating factor) and CD59 (membrane inhibitor of reactive lysis), whose function is to protect red blood cells (RBCs) from complement-mediated destruction resulting in hemolysis, and are implicated in the pathogenesis of thrombosis [5–8]. Flow cytometry of peripheral blood using fluorescent-labeled aerolysin to detect the absence of *PIG-A* is the most sensitive method for diagnosing PNH [9]. Clinical manifestations of PNH range in severity from mild to life-threatening, and the median survival from diagnosis has improved from approximately 10 years in the 1990s to >20 years in the 2000s due to a combination of improved diagnostics and therapy [1,10]. Inferior survival is associated with thrombosis (relative risk [RR], 10.2), development of pancytopenia (RR, 5.5), evolution to myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML) (RR, 19.1), age >55 years at diagnosis (RR, 4.0), multiple treatments (RR, 2.1), and thrombocytopenia at diagnosis (RR, 2.2) [11].

For patients with classical PNH, allogeneic hematopoietic cell transplantation (HCT) and complement inhibition with eculizumab are the only disease-modifying therapies currently available [10]. Eculizumab, a monoclonal antibody that binds the terminal complement component C5 and prevents its cleavage via C5 convertase, is the sole Food and Drug Administration (FDA)-approved drug for treating PNH and been proven effective in reducing both intravascular hemolysis and thrombosis, as well as improving quality of life in patients with PNH [12–14]. However, eculizumab does not correct the underlying stem cell defect and thus must be administered indefinitely unless spontaneous remission occurs [10,15,16]. HCT was first performed in a patient with PNH in 1971 and remains the only curative therapy [17,18]. Current indications for HCT include life-threatening cytopenias, severe hemolysis or thrombosis refractory to eculizumab, and/or the concomitant presence of another marrow disorder, such as aplastic anemia (AA), MDS, or AML [18,19]. Both myeloablative conditioning (including busulfan-, treosulfan-, and ≥8 Gy total body irradiation [TBI]-based regimens) and reduced-intensity conditioning (including cyclophosphamide with or without antithymocyte globulin [ATG] and fludarabine with ≤6 Gy TBI-based regimens) have been successfully used for HCT in patients with PNH [20–25]. Documented cases of mixed chimerism and relapse of the PNH clone after HCT using syngeneic donors suggests that graft-versus-hematopoiesis effects make a significant contribution toward eliminating the PNH clone [25,26].

We have previously described our experience with HCT in PNH patients in small numbers. In 1992, we reported outcomes in 9 patients, of whom 3 received myeloablative conditioning (2 with busulfan and cyclophosphamide, 1 with 12 Gy TBI), 4 received reduced-intensity conditioning (all with cyclophosphamide), and 2 underwent transplantation from a syngeneic donor without conditioning [25]. In 2003, we described outcomes in 7 patients, all of whom received reduced-intensity conditioning with fludarabine and low-dose TBI [20]. We last updated the Seattle experience in 2015 with the outcomes of 28 patients who underwent HCT for PNH at our center [27]. Here we present the aggregate outcomes for 55 patients who underwent HCT for either classical PNH or PNH associated with another marrow disorder. Our results capture experience with both myeloablative and reduced-intensity conditioning regimens, run the gamut of donor types (related, unrelated, umbilical cord, and HLA-haploidentical), and include patients treated with eculizumab before and after HCT.

METHODS

Eligibility Criteria

We included 55 consecutive patients with either classical PNH or a PNH clone in conjunction with another marrow disorder (AA, MDS, AML, or a myeloproliferative neoplasm [MPN]) who underwent at least one HCT between November 15, 1971, and August 12, 2015. Patients were treated at 10 centers either on a study protocol or with a standard treatment plan. Institutional Review Boards at the participating institutions approved the individual study protocols, and written informed consent was obtained from all patients.

Patient Characteristics

Patient characteristics are summarized in Table 1. Fifty-five patients underwent a total of 59 HCTs. Fifty-one patients underwent a single HCT, and 4 patients required a second HCT for graft failure. The median age at first HCT was 32.1 years (range, 14.0 to 66.9 years), and 37 of the 55 patients (67.3%) were female.

Disease Characteristics

Disease characteristics are summarized in Table 1. The median interval between the diagnosis of PNH and first HCT was 1.3 years (range, .1 to 30.3 years). Of the 55 patients, 38 (69.1%) had a PNH clone associated with another marrow disorder, and 17 (30.9%) had classical PNH. For this analysis, in our 38 patients who had a PNH clone associated with another marrow disorder, we included 2 patients with a PNH clone size <10% (granulocyte clone sizes of .6% and .4%). These 2 patients would be classified as having subclinical PNH according to the most recent diagnostic update by Parker [2] but were included to complete our analysis of PNH associated with myeloid malignancies, as 1 patient had concurrent MDS and the other had AML. Of the 38 patients who had a PNH clone associated with another marrow disorder, the associated marrow disorder was AA in 26 patients (68.4%), MDS in 9 patients (23.7%), MPN in 2 patients (5.3%, both with myelofibrosis), and AML in 1 patient (2.6%). Four of the patients with MDS had records sufficient to determine Revised International Prognostic Scoring System scores, which

Table 1
Patient and Disease Characteristics

Characteristic	Value
Total patients, n	55
Total HCTs, n	59
Receipt of ≥2 HCTs, n (%)	4 (7.3)
Age at first HCT, yr, median (range)	32.1 (14.0-66.9)
Female sex, n (%)	37 (67.3)
Follow-up after first HCT for all 55 patients, median (range) years	3.8 (.03-46.1)
Follow-up after first HCT for 36 surviving patients, yr, median (range)	6.1 (2.1-46.1)
Time from PNH diagnosis to HCT, yr median (range)	1.3 (.1-30.3)
PNH type, n (%)	
Classical	17 (30.9)
Associated with a marrow disorder	38 (69.1)
Additional diagnoses, n (%)	
Aplastic anemia	26 (68.4)
MDS	9 (23.7)
MPN	2 (5.3)
AML	1 (2.6)
PNH clone size at HCT	
Granulocytes, n evaluable	32
%, median (range)	66.5 (.04-100.0)
Monocytes, n evaluable	30
%, median (range)	68.0 (.05-100.0)
Erythrocytes, n evaluable	30
%, median (range)	47.5 (.001-100.0)
Diagnoses via the Ham/Sucrose test, n	13
PNH-associated complications, n (%)	
Pancytopenia	30 (54.5)
Venous thrombosis	14 (25.5)
Budd-Chiari, arterial thrombosis	6 (10.9)
Indication for HCT, n (%)	
Pancytopenia	26 (47.3)
MDS/MPN/AML	12 (21.3)
Recurrent hemolysis	11 (20.0)
Refractory thrombosis	6 (10.9)
Treatment before HCT, n (%)	
Corticosteroids	36 (65.5)
Immunosuppression	20 (36.4)
Anticoagulation	17 (30.9)
Growth factors	13 (23.6)
Androgens	7 (12.7)
Eculizumab	6 (10.9)
Chelation	4 (7.3)

were low risk (score of 3) for 1 patient and high risk (scores of 5, 5.5, and 6) for 3 patients. The patient with AML had a complex karyotype with monosomy 7 and thus was considered adverse risk according to European Leukemia Network criteria [28]. Diagnoses of myelofibrosis were based on morphology, because driver mutation testing was not yet available.

Median PNH clone sizes measured by flow cytometry on peripheral blood at the time of first HCT were 66.5% (range, .6% to 100.0% in 32 assessed patients), 68.0% (range, .2% to 100.0% in 30 assessed patients), and 47.5% (range, .1% to 100.0% in 30 assessed patients) for neutrophils, monocytes, and erythrocytes, respectively. Thirteen patients were diagnosed using the acidified serum lysis test (Ham test) and/or sucrose serum lysis test. Indications for HCT included pancytopenia in 26 patients (47.3%); concurrent diagnosis of MDS, MPN, or AML in 12 patients (21.3%); recurrent hemolysis in 11 patients (20.0%); and refractory thrombosis in 6 patients (10.9%). Therapy provided before first HCT included corticosteroids in 65.5%, other immunosuppression (eg, ATG, calcineurin inhibitor) in 36.4%, growth factors in 23.6%, androgens in 12.7%, eculizumab in 10.9%, and chelation agents in 7.3%. Seventeen patients (30.9%) were anticoagulated before HCT. Three of these patients received anticoagulation for prophylaxis only, and the remaining 14 had a previously documented thrombosis. All 55 patients received multiple RBC and/or platelet transfusions before HCT.

One patient underwent HCT for PNH with concurrent AA after undergoing 2 previous HCTs for AA. She initially underwent HCT with cyclophosphamide and ATG conditioning from her HLA-identical brother (marrow source) and experienced delayed graft failure on day +918. She did not receive further conditioning but underwent a marrow stem cell infusion from the same HLA-identical brother as a second HCT. On day +1030 after the second HCT, subsequent to a viral upper respiratory infection, she developed recurrent pancytopenia and was first found to have a PNH clone (17% of granulocytes) in her peripheral blood. Cytogenetics of her marrow at the time of pancytopenia revealed a 46XY karyotype in 7 of 20 cells and host karyotype in the remaining 13 of 20 cells. She had a previously documented donor karyotype of 46XY. Testing on the PNH clone revealed that it was of host origin, and her brother (the previous donor) was tested and was found to not have a PNH clone. She underwent a third HCT from the same HLA-identical brother with fludarabine and 2 Gy TBI conditioning, engrafted fully, and was alive and disease-free at her date of last contact, 4.4 years after her third HCT.

HCT and Donor Characteristics

HCT characteristics are summarized in Table 2. In the first HCT for each patient, the graft source was an HLA-identical sibling in 20 patients (36.4%), an HLA-matched unrelated donor in 20 patients (36.4%), a single HLA allele level-mismatched unrelated donor in 7 patients (12.7%), umbilical cord blood (UCB) in 3 patients, a syngeneic donor in 2 patients, and a single HLA allele level-mismatched related donor, a 2 HLA allele level-mismatched unrelated donor, and an HLA-haploidentical donor in 1 patient each. Of the 7 single HLA allele level-mismatched donors, 5 were mismatched at HLA-C, 1 was mismatched at HLA-A, and 1 was mismatched at HLA-DQB1. The 1 HLA allele level-mismatched related donor was mismatched at HLA-A, and the 2 HLA allele level-mismatched unrelated donors were mismatched at HLA-B and HLA-DRB1.

Hematopoietic cell sources included bone marrow for 26 HCTs (47.2%), granulocyte colony-stimulating factor (GCSF)-mobilized peripheral blood stem cells for 25 HCTs (45.5%), UCB for 3 HCTs (5.5%), and both bone marrow and GCSF-mobilized peripheral blood stem cells for 1 HCT. The $CD34^+$ cell dose was available for 28 HCTs; the median dose was 5.8×10^6 $CD34^+$ cells/kg of body weight (range, 3.7×10^5 to 2.0×10^7 $CD34^+$ cells/kg). The lowest value in the range (3.7×10^5 $CD34^+$ cells/kg) was for the single UCB graft for which a $CD34^+$ cell count was available. The corrected dose of total nucleated cells (TNCs) was available for 38 HCTs; the median dose was 2.4×10^8 TNC/kg (range, 4.8×10^6 to 2.8×10^9 TNC/kg). These corrected TNC data include the 3 UCB grafts, with corrected TNC doses of 2.0×10^7, 2.5×10^7, and 3.0×10^7 TNC/kg. A single patient received a hematopoietic cell dose that was a combination of cells collected from bone marrow and from GCSF-mobilized peripheral blood; for this patient, the CD34+ cell doses were 1.1×10^6 and 1.7×10^6 $CD34^+$ cells/kg from bone marrow and peripheral blood, respectively; and the respective concomitant corrected TNC doses were 1.4×10^8 and 3.6×10^8 TNC/kg.

Considering only the first HCT for each patient, conditioning regimens were myeloablative for 26 patients (47.3%), reduced-intensity for 27 (49.1%), and no conditioning for 2 syngeneic donor graft recipients. A variety of conditioning protocols were used over the years as experience with HCT evolved. Myeloablative conditioning regimens comprised busulfan dosed to target a serum level of 600 to 950 ng/mL and given in daily doses for 4 to 5 days in 18 patients, treosulfan 42 g/m^2 and fludarabine 150 mg/kg with or without 2 Gy TBI and rabbit ATG 6 mg/kg in 4 patients, and cyclophosphamide 120 mg/kg with ATG 60 mg/kg and high-dose TBI (12.0 to 14.0 Gy) in 4 patients. Reduced-intensity conditioning regimens consisted of cyclophosphamide 200 mg/kg in 1 patient; cyclophosphamide 200 mg/kg with ATG 60 to 90 mg/kg in 3 patients; fludarabine 120 mg/m^2, cyclophosphamide 100 mg/kg, ATG 90 mg/kg, and low-dose TBI (2 Gy) in 2 patients; and fludarabine 90 to 150 mg/m^2 with low-dose TBI (2.0 to 4.0 Gy) in 21 patients.

GVHD prophylaxis also evolved during the period of this analysis [29]. For patients who were treated with myeloablative regimens, GVHD prophylaxis regimens consisted of calcineurin inhibitor (cyclosporine or tacrolimus) started on day -1 and continued at least through day +100, combined with 15 mg/m^2 of methotrexate given on day +1 and then 10 mg/m^2 of methotrexate given on days +3, +6, and +11 in 22 patients; cyclosporine from day -1 to day +100 combined with methylprednisolone .5 mg/kg given twice daily on days +1 to +4, followed by 1 mg/kg given twice daily on days +5 to +19, followed by a taper in 2 patients; cyclosporine alone from day -1 until day +100 in 1 patient; cyclosporine from day -1 to day +100 combined with mycophenolate mofetil from day +1 to day +28. For patients who were treated with a reduced-intensity conditioning regimen, GVHD prophylaxis regimens included a calcineurin inhibitor (cyclosporine or tacrolimus) started on day -1 and continued at least through day +100, combined with mycophenolate mofetil started on day +1 and continued through at least day +28 in 21 patients; a calcineurin inhibitor (cyclosporine or tacrolimus) started on day -1 and continued at least through day +100, combined with 15 mg/m^2 of methotrexate given on day +1, and then 10 mg/m^2 of methotrexate given on days +3, +6, and +11 in 5 patients; and one patient who received 15 mg/m^2 of methotrexate on day +1 and then 10 mg/m^2 mg of methotrexate on days +3,

Table 2
HCT Characteristics and Outcomes

Characteristic	Value
HCTs by decade, n	
1970-1979	2
1980-1989	4
1990-1999	12
2000-2009	28
2010-2015	9
Donor type, n (%)	
HLA-identical sibling	20 (36.4)
HLA-matched unrelated	20 (36.4)
1 HLA allele-mismatched unrelated	7 (12.7)
UCB	3 (5.5)
Syngeneic	2 (3.6)
1 HLA allele-mismatched related	1 (1.8)
2 HLA allele-mismatched unrelated	1 (1.8)
Haploidentical	1 (1.8)
Hematopoietic cell source, n (%)	
Bone marrow	26 (47.2)
Peripheral blood	25 (45.5)
Umbilical cord	3 (5.5)
Combined bone marrow + peripheral blood	1 (1.8)
Conditioning, n (%)	
Myeloablative	26 (47.3)
Reduced-intensity	27 (49.1)
None	2 (3.6)
CMV serostatus, donor/recipient, n (% of those with known CMV serostatus)	
Negative/negative	14 (34.1)
Negative/positive	11 (26.8)
Positive/positive	9 (22.0)
Positive/negative	7 (17.1)
Unknown	14 (34.1)
ABO group, n (% of those with known ABO group)	
Matched for ABO and Rh	18 (40.0)
Mismatched for ABO, matched for Rh	20 (44.4)
Matched for ABO, mismatched for Rh	3 (6.7)
Mismatched for ABO and Rh	4 (8.9)
Unknown	10
Days to engraftment, median (range)	
Neutrophils	17 (0-34)
Platelets	19 (0-99)
Delayed engraftment, n (%)	5 (9.1)
Graft failure, n (%)	4 (7.2)
GVHD, n (%)	
Acute, grade II-IV	34 (61.8)
Acute, grade III-IV	7 (12.7)
Chronic	26 (57.8)
Deaths after HCT, n (%)	
Total during follow-up	19 (34.5)
Before day +100	7
Between days +100 and +365	5

+6, +11, and then once weekly until day +100 (for our earliest HCT, performed on November 15, 1971) in 1 patient. The 2 patients who underwent syngeneic HCT did not receive GVHD prophylaxis.

Supportive care, including antimicrobial and cytomegalovirus prophylaxis, was administered as described previously [30,31]. The degrees of donor chimerism among T cells, granulocytes, and unsorted nucleated cells in the peripheral blood were assessed at days +28, +56, +84, +180, and/or+ 365 after HCT and, whenever possible, in the marrow. T cells and granulocytes were sorted via flow cytometry (fluorescence-activated cell sorting) and were analyzed by either fluorescence in situ hybridization to detect X and Y chromosomes for sex-mismatched HCTs or polymerase chain reaction-based analysis of polymorphic microsatellite regions for sex-matched HCTs. Fluorescence-activated cell sorting determination of CD55 and CD59 on granulocytes, monocytes, and erythrocytes in peripheral blood was performed at least once after HCT to document eradication of the PNH clone and as clinically indicated thereafter.

Regarding the management of anticoagulation around the time of HCT, information was available for 9 of the 17 patients who were treated with anticoagulation before HCT. Six of these patients remained on anticoagulation until they became thrombocytopenic either due to disease or after conditioning. The level of thrombocytopenia at which time anticoagulation was discontinued varied: $<30{,}000/\mu L$ in 4 patients, $<50{,}000/\mu L$ in 1 patient, and $<100{,}000/\mu L$ in 1 patient. In these 6 patients, anticoagulation was not restarted after HCT. One patient with PNH who was also heterozygous for the factor V Leiden mutation was treated with subcutaneous unfractionated heparin before HCT; anticoagulation for this patient was held once his platelets fell below $20{,}000/\mu L$ and was then restarted once the count recovered above this level after HCT. One patient who underwent HCT for an indication for refractory thrombosis had anticoagulation held once her platelets fell below $30{,}000/\mu L$ after conditioning, but then developed both abdominal thrombosis and variceal hemorrhage while off anticoagulation; she ultimately died on day +11 after HCT from uncontrolled variceal hemorrhage. Finally, in 1 patient who was treated with anticoagulation for prophylaxis early after her diagnosis of PNH, anticoagulation was discontinued after eculizumab was initiated, approximately 1.5 years before HCT.

Statistical Analysis

Overall survival was estimated by the Kaplan-Meier method, and univariate Cox proportional hazards models were created for comparison of overall survival by conditioning intensity (myeloablative versus reduced-intensity) and PNH subtype (classical versus associated with another marrow disorder).

RESULTS

Engraftment

The median time to neutrophil recovery (defined as an absolute neutrophil count [ANC] $\geq 500/\mu L$ for 3 consecutive days) was 17 days (range, 0 to 34 days). The median time to platelet recovery (defined as a platelet count $\geq 20{,}000/\mu L$ for 3 consecutive days) was 19 days (range, 0 to 99 days). In patients who had 0 days to neutrophil and platelet recovery, the post-HCT neutrophil count never fell below $500/\mu L$, and platelet count never fell below $20{,}000/\mu L$. Five patients experienced delayed engraftment, defined as an ANC $<500/\mu L$ at day +28 for HCTs using marrow or peripheral blood-collected stem cells or an ANC $<500/\mu L$ at day +55 for HCTs using UCB. Four of these 5 patients received bone marrow grafts, and 1 patient received a UCB graft. In these patients, the conditioning regimen was reduced-intensity in 2 patients (both of whom received cyclophosphamide and ATG) and a myeloablative regimen in 2 patients (1 with cyclophosphamide, ATG, and 13.5 Gy TBI and the other with 4-day busulfan, cyclophosphamide, and ATG). One patient who received a syngeneic graft received no conditioning.

Four patients experienced graft failure, including 2 who did not engraft after first HCT and underwent a second HCT on days +34 and +47 and 2 who experienced late graft failure and underwent a second HCT on days +236 and +1030. Conditioning regimens for the patients who experienced early graft failure included cyclophosphamide and 12 Gy TBI in 1 patient and a reduced-intensity regimen with fludarabine and 2 Gy TBI in the other patient. Both patients who experienced late graft failure were conditioned with fludarabine and 2 Gy TBI.

Survival, GVHD, and Causes of Death

The median duration of follow-up (measured starting on the day of first HCT) was 3.8 years (range, .03 to 46.1 years) for all 55 patients and 6.1 years (range, 2.1 to 46.1 years) for surviving patients. Overall survival was 70% at 5 years, and neither the choice of conditioning intensity (myeloablative versus reduced-intensity) nor PNH subtype (classical versus having a clone associated with another marrow disorder) affected survival (Figure 1). Although the change was not statistically significant, early survival after HCT for PNH appears to have improved over the years when comparing survival after HCT before 2000, between 2000 and 2006, and after 2006 (Figure 2).

All 55 patients were evaluable for acute GVHD (aGVHD); grade II-IV occurred in 61.8% and grade III-IV occurred in 12.7%. Chronic GVHD (cGVHD)developed in 57.8%. Nineteen patients (34.5%) died during follow-up, 7 died before day +100, and another 5 died after day +100 but before day +365. Causes of death are shown in Table 3; and major causes included infection in 9 patients and hemorrhage in 4 patients (2 of whom were receiving therapeutic anticoagulation).

PNH Clone Eradication

Post-HCT PNH clones in the granulocyte, monocyte, and erythrocyte lineages were monitored in 23 patients via flow cytometry of peripheral blood. Only 6 of the 23 patients had a low-level PNH clone (range, .02% to 3.30%) detectable in any lineage in peripheral blood at day +28; all others were undetectable. Of the 3 lineages, PNH clones were most likely to be detected in erythrocytes, as 4 of the 6 patients had a detectable PNH clone only in erythrocytes at day +28. In the 2 patients with PNH clones detected in multiple lineages, the PNH clone in the erythrocyte lineage persisted the longest on sequential analysis of peripheral blood; that is, the erythrocyte PNH clone continued to be detectable at lower levels at days +56 and +84 before disappearing.

Eculizumab Treatment Pre-HCT and Peri-HCT

Six of the 55 patients were treated with eculizumab before undergoing HCT (Table 4). One of these 6 patients had classical PNH, and the other 5 patients had a concomitantly associated marrow disorder. The median time from diagnosis of PNH to HCT was 2.9 years (range, 1.0 to 30.3 years). One patient continued to receive eculizumab after HCT (see below), 3 patients discontinued eculizumab at the time of HCT, and in 2 patients the timing of eculizumab discontinuation is unknown. The conditioning regimen was myeloablative in 3 patients and reduced-intensity in the other 3. All 6 patients who received eculizumab pre-HCT were still alive at the date of last contact, with a median follow-up of 2.1 years (range, .2 to 6.9 years).

Two patients received eculizumab peri-HCT (Table 4). The first patient, an 18-year-old male with classical PNH, received eculizumab before HCT for hemolysis and had an incomplete response; he was given eculizumab at a dose of 900 mg on days -1, +12, +26, and +40 to decrease his level of hemolysis until engraftment. The second patient, a 14-year-old female with PNH and AA, initiated eculizumab at a dose of 600 mg on day -9 before HCT and continued to receive 600 mg on days -1, +5, +12, and +19 owing to mild hemolysis and to prevent thrombosis. Both patients received myeloablative conditioning and GVHD prophylaxis with tacrolimus and methotrexate. Both patients engrafted before day +28 without evidence of hemolysis or thrombosis. One patient had grade I aGVHD of the skin, and the other patient did not develop aGVHD. Neither

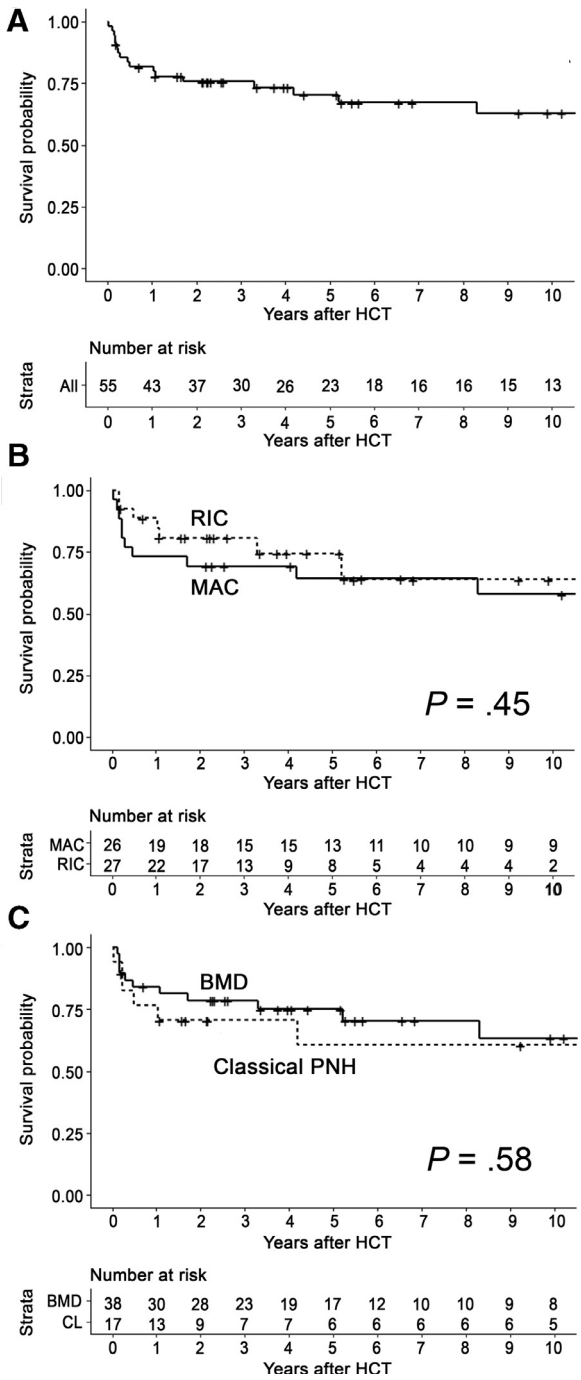

Figure 1. Survival after HCT for PNH. (A) Overall survival for 55 patients who underwent HCT for PNH. (B) Comparison of overall survival in patients who underwent HCT with myeloablative conditioning (MAC, solid line; n = 26) versus those who received reduced-intensity conditioning (RIC, dashed line; n = 27) regimens. (C) Comparison of overall survival in patients who underwent HCT for PNH with an associated marrow disorder (BMD, solid line; n = 38) versus those with classical PNH (CL, dashed line; n = 17).

patient developed cGVHD after 2.1 and 4.1 years of follow-up, respectively.

DISCUSSION

Therapy for PNH changed radically in 2007 with the FDA's approval of eculizumab. However, despite the success of eculizumab as treatment for unregulated activity of the complement system, allogeneic HCT remains the sole potential cure

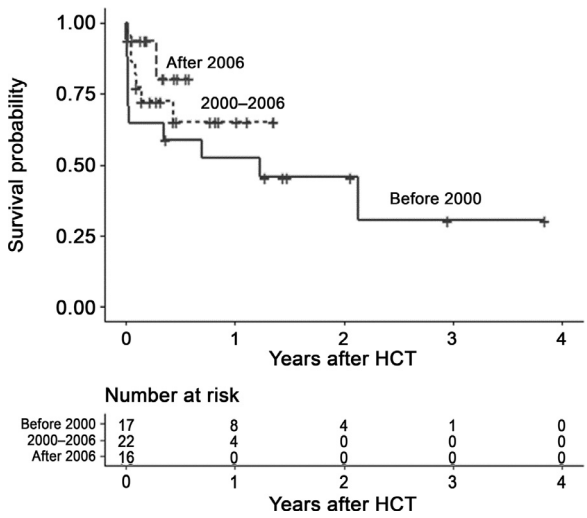

Figure 2. Survival trends by year of HCT for PNH. Shown is overall survival by year of HCT: before 2000 (before our current RIC conditioning regimen of fludarabine and low-dose TBI), 2000 through 2006 (before FDA approval of eculizumab), and after 2006 (after FDA approval of eculizumab). *P* values for the differences between individual survival curves were .32 for the comparison of "before 2000" with "2000 to 2006" and .10 for the comparison of "before 2000" with "after 2006."

for PNH through elimination of the defective clone and replacement with a donor hematopoietic system. The earliest HCT for PNH was performed in 1971 for a 24-year-old male who presented with AA; he received a bone marrow graft from a sibling who was matched at the HLA-A locus and by nonreactivity in mixed leukocyte coculture after receiving a nonmyeloablative conditioning regimen of cyclophosphamide 50 mg/kg/day for 4 days. At the time of this report, he was alive, in good health, and disease-free for almost 47 years [17].

Various centers besides ours have described their experience with HCT for PNH. The largest of these reports, from the European Group for Blood and Marrow Transplantation in 2012, examined outcomes from 211 patients with PNH who underwent HCT between 1978 and 2007 and compared them with outcomes in a cohort of 402 controls, patients with PNH who did not undergo HCT between 1950 and 2005 [21]. In the patients, graft failure occurred in 7%, and overall survival at 5 years post-HCT was 68%; in the controls, estimated overall survival of 76.3% at 10 years [32]. They also found worse survival post-HCT in patients with PNH and thromboembolic complications and those with other complications (recurrent hemolysis or aplastic anemia) compared with a matched cohort of patients with thromboembolic complications who did not undergo HCT.

Pantin et al [33] at the National Institutes of Health reported their experience with a reduced-intensity

Table 3
Causes of Death

Cause	n (%)	Day(s) post-HCT
Infection	9 (47.4)	58-9208
Hemorrhage	4 (21.1)	11-390
Complications of GVHD	1 (5.3)	1188
Myocardial infarction	1	615
Multiorgan failure secondary to pancreatitis	1	60
Cirrhosis secondary to hepatitis C virus	1	5315
Post-HCT lymphoproliferative disorder	1	169
Unknown	1	2989

Table 4
Characteristics of Individual Patients Who Received Pre-HCT or Peri-HCT Eculizumab

Patient No.	Sex	PNH Type	Marrow disorder	PNH Clone Size, %*	Pre-HCT Treatment	Indication for pre-HCT Eculizumab	Pre-HCT Eculizumab Dosing	Indication for HCT	Age at HCT, yr	Conditioning	Donor Type	CMV Serostatus, Donor/ Recipient	Blood Group, D/R	Stem Cell Source	GVHD Prophylaxis	Peri-HCT Eculizumab Dosing	Survival, yr	aGVHD, Grade (Site)	cGVHD
1	Female	Marrow disorder	AA	82/79/6	ATG, Csp	Mild hemolysis, thrombosis prevention	600 mg starting on day -9	Marrow failure	17.6	Treo + Flu + ATG	MMUD	Pos/neg	O^+/O^+	PB	FK/MTX	600 mg on days -1, +5, +12, and +19	>4.1	I (skin)	No
2	Male	Classical	None	23/78/54	Eculizumab	Hemolysis	1200 mg every 2 wk until HCT	Hemolysis	14.9	Treo + Flu + ATG	MRD	Pos/neg	O^+/O^-	BM	FK/MTX	900 mg on days -1, +12, +26, and +40	>2.1	No	No
3	Male	Marrow disorder	AA	97/98/8	Deferasirox, eculizumab	Hemolysis	600 mg every 7 d for 2 mo without response	Marrow failure	49.9	Flu + LD -TBI	MUD	Pos/pos	A^+/O^+	BM, PB	Csp/MMF		>6.9	III (skin)	Yes
4	Female	Marrow disorder	MDS, monosomy 7	48/62/21	Deferasirox, eculizumab	Hemolysis	900 mg every 2 wk until HCT	MDS	54.9	Treo + Flu + LD-TBI	MUD	Neg/neg	O^+/O^+	BM	FK/MTX		>2.3	I (skin)	Yes
5	Female	Marrow disorder	MDS, del7p	100/100/100	ATG, Csp, danazol, prednisone, leuprolide, eculizumab	Hemolysis	900 mg every 2 wk until HCT	MDS	27.8	Flu + Cy + HD-TBI	UCB	Neg/pos	O^+/B^-	UCB	CspP/MMF		>5.7	II (skin, gut)	Yes
6	Female	Marrow disorder	MDS		Prednisone, eculizumab			MDS	28.0	Flu + LD -TBI	MUD			PB	Csp/MMF		>.7	No	Yes
7	Female	Marrow disorder	AA		ATG, Csp, IVIG, prednisone, eculizumab			Marrow failure	21.0	Flu + LD -TBI	MMUD			PB	Csp/MMF		>.2	I (skin)	

BM, bone marrow; Csp, cyclosporine, Cy, cyclophosphamide; FK, tacrolimus; Flu, fludarabine; HD-TBI, high-dose total body irradiation; IVIG, intravenous immunoglobulin; LD-TBI, low-dose total body irradiation; MMUD, mismatched unrelated donor; MRD, matched related donor; MUD, matched unrelated donor; PB, peripheral blood; Treo, treosulfan.

* Numbers listed are percentages for granulocytes/monocytes/erythrocytes as determined via flow cytometry on peripheral blood samples.

conditioning regimen of cyclophosphamide and fludarabine with or without ATG followed by peripheral blood stem cell HCT in 17 patients with either classical PNH or a PNH clone with another concurrent marrow disorder. None of the patients received treatment with eculizumab before HCT, and 2 patients experienced cytogenetic evolution to MDS. No patients experienced graft rejection or late failure, and sequential post-HCT peripheral blood flow cytometry showed declining populations of GPI-deficient neutrophils, with a median time to disappearance of +100 days. Fifteen of the 17 patients (87.8%) were alive and without evidence of PNH at a median follow-up of 6 years.

More recently, Tian et al. [34] presented their experience of HCT in 18 patients with either classical PNH or a PNH clone with another concurrent marrow disorder. HCT was performed with an HLA-haploidentical donor in 10 patients, an HLA-matched sibling donor in 5 patients, and an HLA-matched unrelated donor in 3 patients. The graft source was peripheral blood stem cells for all patients. Conditioning regimens consisted of cytarabine, busulfan, cyclophosphamide, and simustine, with or without ATG. One patient developed graft failure and ultimately died of cytomegalovirus infection after a second HLA-haploidentical HCT, but the remaining 17 patients were alive without evidence of PNH at a median follow-up of 20 months.

Our cohort of 55 patients with either classical PNH or a PNH clone associated with another marrow disorder who underwent HCT had excellent overall survival, estimated at 70% after 5 years, and 51 of 55 patients experienced durable engraftment. We observed no differences in survival after HCT performed with myeloablative conditioning regimens, which varied significantly over the years, compared with HCT performed with reduced-intensity regimens, which were more uniform, with 78% of patients receiving fludarabine and low-dose TBI. We also observed no difference in long-term survival after HCT between patients with classical PNH and those who had a PNH clone associated with another marrow disorder. We acknowledge that the conditioning regimens, donor sources, and GVHD prophylaxis regimens were variable for these 55 patients, and thus our data should be interpreted with caution.

Although our population contained a higher number of patients with a concurrent myeloid malignancy compared with the larger analysis reported by Peffault de Latour (20% versus 7%) [21], it is particularly notable that 75% of our 12 patients with a concurrent diagnosis of MDS, AML, or myelofibrosis remained alive at a median follow-up of 4.5 years (range, .7 to 12.2 years) after HCT. Of the patients with PNH with concurrent MDS, 3 of the 4 with sufficient data were considered high risk according to the Revised International Prognostic Scoring System. This skewing toward higher risk categorization may simply be a consequence of patient selection; however, the PNH clone size in these patients was significant (granulocytes: median, 47.7% [range, .4% to 99.9%]; monocytes: median, 55.5% [range, .2% to 99.9%]; erythrocytes: median, 66.6% [range, .1% to 99.9%]), likely due to the high-risk classification owing to concurrent cytopenias.

Serial measurements of PNH clone size post-HCT that were available for a small number of our more recent patients showed that although the PNH clone in both the granulocyte and monocyte lineages disappeared by day +28 after HCT, the PNH clone in the erythrocyte lineage could be detected, albeit at diminishing levels, at days +56 and +84. Physiologically, this finding was not unexpected, given the differences in circulating lifespan of granulocytes (6 to 8 hours) and monocytes (20 to 24 hours) versus erythrocytes (90 to 120 days). It is also consistent with previous observations of erythrocytes completely lacking CD55 and CD59 that were found circulating in peripheral blood for up to 60 days [10].

To date, treatment with eculizumab has never been directly compared with HCT in a randomized trial of patients with PNH. In 2013, Hillmen et al [35] reported the long-term outcomes from 3 prospective trials of eculizumab in 195 patients with PNH, including a 97.6% overall survival at 3 years, an 81.8% decrease in thromboembolic events, and a 54.7% decrease in the number of RBCs transfused. The majority of patients carried a diagnosis of classical PNH, but 28.7% had a history of AA, and 1.5% had a history of MDS. Serious adverse events were reported in 38.5% of patients, and 40 patients (20.5%) reported infection-related adverse events.

With a half-life of 11.3 $\pm$ 3.4 days, eculizumab is typically dosed every 2 weeks and is required indefinitely. Eculizumab is expensive; in 2014, based on predicted gains of 1.13 life-years and 2.45 quality-adjusted life-years, Coyle et al [36] estimated the cost of eculizumab to be $4.62 million per life year and $2.13 million per quality-adjusted life-year (both in Canadian dollars). A longer-acting anti-C5 monoclonal antibody (ravulizumab; Alexion Pharmaceuticals) that can be dosed up to every 12 weeks is currently in clinical development [37].

HCT remains the sole curative therapy for PNH based on its ability to eliminate the PNH clone through conditioning intensity and/or the graft-versus-hematopoiesis effect. Indications include recurrent hemolysis and/or thromboembolic complications despite treatment with eculizumab or a concurrent marrow disorder, such as AA or MDS, accompanying the PNH clone. The risks of HCT are well known and include treatment-related mortality and cGVHD, which occurred in 22% and 57.8% of our cohort, respectively. Because of these risks and the efficacy of eculizumab, the use of HCT as treatment for PNH has become more limited. We performed 28 HCTs for PNH between 2000 and 2009 (2.8 per year), but only 9 between 2010 and 2015 (1.8 per year). However, 4 of the 9 HCTs performed after 2010 were in patients who had an incomplete response to eculizumab with persistent hemolysis (n = 2), thrombosis (n = 1), or pancytopenia (n = 1). Thus, even in patients in whom eculizumab is indicated, HCT remains a viable therapeutic option in cases of treatment failure.

Although the number of patients is small and the differences are not significant, early survival after HCT for PNH appears to have improved over time. This is likely a result of lower rates of TRM with reduced-intensity conditioning (the fludarabine + low-dose TBI regimen was introduced in late-1990s) and improved management of infectious and GVHD complications. Moreover, the use of eculizumab as the primary treatment for classical PNH precludes known pretransplantation complications such as thrombosis, which has been associated with inferior post-transplantation outcomes [27]. It also can be postulated that pre-HCT eculizumab therapy reduces morbidity in patients with classical PNH who ultimately require HCT for refractory disease.

One may wonder whether eculizumab and HCT could be useful therapies together, and we were surprised by the finding of minimal to no GVHD in the 2 patients who were treated with eculizumab peri-HCT. Relevant caveats to this observation include the fact that the 2 patients were young (age 14 years and 18 years at the time of HCT) and received a treosulfan-based conditioning regimen (which has been associated with low rates of aGVHD and cGVHD), and that eculizumab was not given specifically for GVHD prophylaxis [38]. As a result, no conclusion can be drawn from that observation in only 2 patients, but there may be relevant biological

mechanisms behind this finding. Kwan et al [39] reported that in irradiated murine models of GVHD, the development of GVHD was minimized when donor T cells were deficient in the C3a and/or C5a receptor. In addition, they showed that the pharmacologic blockade of the C5a receptor with a small-molecule inhibitor reduced GVHD morbidity. More recently, Nguyen et al [40] used a similar murine model of GVHD to show that a deficiency of host C3a and C5a receptors (particularly on host antigen-presenting cells) ameliorated aGVHD and improved survival. Despite these in vivo studies, however, the role of complement activation after HCT in humans is largely unknown. However, Cherry et al [41] documented increased deposition of C4d in skin and colon biopsy specimens from solid organ transplantation recipients who developed GVHD of those organs. Taken together, the foregoing in vivo studies, the observations in patients with GVHD after solid organ transplantation, and our own observation of minimal to no GVHD in 2 patients after myeloablative HCT with peri-HCT eculizumab might support the hypothesis that complement activation plays a role in the development of GVHD after HCT. Complement inhibitors such as eculizumab and the longer-acting anti-C5 monoclonal antibody ravulizumab may merit clinical study for the prevention of GVHD. Our 2 patients received eculizumab after HCT for a limited time (up to 6 weeks); thus, the required duration of treatment with a complement inhibitor, if it were tested for the prevention of GVHD, may be short.

In summary, even in the age of eculizumab, HCT remains a viable treatment for PNH, with indications including hemolysis or thrombosis refractory to eculizumab therapy, accompanying marrow failure, or another concurrent marrow disorder, including MDS, MPN, or AML. Overall survival for our cohort of 55 patients was 70% at 5 years, and the most frequent causes of death were infection and hemorrhage. Although the numbers are small in this study, outcomes after HCT for PNH appear to have improved over the years, and potential contributors to this improvement include reduced TRM and the influence of eculizumab therapy on the PNH population that ultimately needs HCT. Additional clinical studies and observations are needed to further elucidate the influence of complement inhibition on HCT.

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