# Stem cell transplantation for paroxysmal nocturnal hemoglobinuria

**Robert A. Brodsky**

*Division of Hematology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, USA.*
*E-mail: brodsro@jhmi.edu* *doi:10.3324/haematol.2010.023176*

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Paroxysmal nocturnal hemoglobinuria (PNH) is a rare blood disease that results from clonal expansion of a multipotent hematolopoietic stem cell harboring a *PIG-A* mutation.[1] The *PIG-A* gene product is required for the biosynthesis of glycophosphatidylinositol anchors, a glycolipid moiety that tethers dozens of proteins to lipid bilayer of cell membranes. Consequently, the PNH stem cell and its progeny have a reduction or absence of all GPI-anchored proteins. Two of these proteins, CD55 and CD59, are complement regulatory proteins and are fundamental to the pathophysiology of paroxysmal nocturnal hemoglobinuria.[2,3] CD55 inhibits C3 convertases and CD59 blocks formation of the membrane attack complex (MAC). The loss of complement regulatory proteins renders PNH erythrocytes susceptible to both intravascular (due to CD59 deficiency) and extravascular (due to CD55 deficiency) hemolysis, but it is the intravascular hemolysis that contributes to much of the morbidity and mortality from the disease.[4] Intravascular hemolysis leads to the release of free hemoglobin into the plasma. Free plasma hemoglobin scavenges nitric oxide and contributes to numerous paroxysmal nocturnal hemoglobinuria manifestations, including esophageal spasm, male erectile dysfunction, renal insufficiency and thrombosis. The natural history of paroxysmal nocturnal hemoglobinuria is highly variable.[5-8] The median survival without effective therapy is roughly 15 years. Thrombosis is the leading cause of death, but patients may succumb to complications of bone marrow failure, renal failure, myelodysplastic syndromes, and leukemia.

Paroxysmal nocturnal hemoglobinuria can arise *de novo*, or in the setting of acquired aplastic anemia.[9] Patients with the classical form of paroxysmal nocturnal hemoglobinuria tend to have a large percentage of PNH cells by flow cytometry, an elevated reticulocyte count, a normocellular bone marrow, and markedly elevated levels of lactate dehydrogenase. It is important to recognize that patients with acquired aplastic anemia often have small populations of PNH cells. These patients represent an overlap between aplastic anemia and paroxysmal nocturnal hemoglobinuria (AA/PNH); they often present with more severe peripheral cytopenias, a hypocellular bone marrow, a low reticulocyte count, normal to mildly elevated levels of lactate dehydrogenase. Currently, the only effective therapies for paroxysmal nocturnal hemoglobinuria are allogeneic bone marrow transplantation and inhibition of terminal complement with eculizumab, a humanized monoclonal antibody that binds to C5 and blocks formation of the MAC.[10] Eculizumab is highly effective in reducing intravascular hemolysis in paroxysmal nocturnal hemoglobinuria and markedly reduces the risk for thrombosis;[11-13] it does not improve bone marrow function and is not very effective for AA/PNH. Moreover, eculizumab is expensive, does not eradicate the PNH clone, and must be given lifelong; thus, it is best reserved for patients with classical paroxysmal nocturnal hemoglobinuria.

Bone marrow transplantation is the only curative therapy for paroxysmal nocturnal hemoglobinuria and has been shown to eradicate the PNH clone in patients with classical paroxysmal nocturnal hemoglobinuria and AA/PNH; however, it is associated with significant morbidity and mortality. The International Bone Marrow Transplant Registry (IBMTR) reported a 2-year survival probability of 56% in 48 recipients of HLA-identical sibling transplants between 1978 and 1995.[14] The median age was 28 years. The majority of the deaths in this study occurred within one year of transplantation. One of 7 recipients of alternative donor allogeneic transplants reported to the IBMTR during this period was alive five years after transplant. The European Blood and Marrow Transplant group reported a 5-year survival rate of 70% following allogeneic bone marrow transplantation for paroxysmal nocturnal hemoglobinuria; however, only 54% met criteria for classical PNH.[6] The median age in that study was 30 years. Graft failure occurred in 6% of patients and acute and chronic graft-versus-host disease occurred in 15% and 20% of patients, respectively. Both non-myeloablative syngeneic bone marrow transplantation and non-myeloablative stem cell transplants from HLA-matched or HLA-haploidentical donors have been successfully performed in paroxysmal nocturnal hemoglobinuria patients.[15-18] Interestingly, the latter approach, but not the former, appears to cure the disease, suggesting that there is an important "graft-versus PNH" effect with bone marrow transplantation. Now that an effective, non-transplant therapy is available, the use of allogeneic bone marrow transplantation to treat paroxysmal nocturnal hemoglobinuria has decreased. Before the introduction of eculizumab, paroxysmal nocturnal hemoglobinuria patients with severe symptoms (e.g, thrombosis, intractable pain paroxysms, etc.) from classical PNH and patients with AA/PNH with peripheral cytopenias meeting criteria for severe aplastic anemia were considered good candidates for allogeneic bone marrow transplantation, especially if a matched sibling donor was available. Now that there is a highly effective drug therapy for paroxysmal nocturnal hemoglobinuria, the indications for allogeneic bone marrow transplantation in this setting have changed.

In this issue of the journal, Santarone *et al.*, on behalf of the Gruppo Italiano Trapianto Midollo Osseo (GITMO), report on a retrospective study of 26 patients (median age, 32 years) who received a bone marrow transplantation for paroxysmal nocturnal hemoglobinuria (4 AA/PNH) in Italy between 1988 and 2006.[19] HLA-matched sibling donors were used as a stem cell source for 22 patients; one patient received stem cells from a matched unrelated donor and 3 received stem cells from mismatched donors (2 related and one unrelated). A myeloablative conditioning regimen (busulfan + cyclophosphamide) was used for 15 of the patients. The remaining 11 received a variety of different reduced intensity conditioning regimens, most being cyclophosphamide or fludaribine

based. Graft-versus-host disease prophylaxis was highly variable, but largely cyclosporine-based. The 10-year probability of survival was 57% for all patients, with a median follow-up of 131 months. There was one primary graft failure in a patient receiving a myeloablative conditioning regimen and one secondary graft failure in a patient who received a reduced intensity conditioning bone marrow transplantation; both patients eventually died from complications of a second transplant. Acute graft-versus-host disease greater than stage 2 occurred in 3 of the 26 patients; chronic graft-versus-host disease occurred in 10 of 20 evaluable patients with 4 (16%) experiencing extensive chronic graft-versus-host disease. The transplant related mortality at one year was 26% in patients receiving a myeloablative conditioning regimen and 63% in the group that received a reduced intensity conditioning regimen; however, this was likely due to the fact that all 3 patients in the reduced intensity conditioning group who received a bone marrow transplantation from a non-identical donor died of multiorgan failure. There was just one patient in the myeloablative group who received a bone marrow transplantation from an unrelated or mismatched donor. None of the patients who achieved stable engraftment experienced relapse of their paroxysmal nocturnal hemoglobinuria, confirming that allogeneic bone marrow transplantation following a reduced intensity conditioning regimen can extinguish the PNH clone.

What should we conclude from these studies? First, bone marrow transplantation should not be offered as initial therapy for most patients with classical paroxysmal nocturnal hemoglobinuria given the high transplant related mortality, especially when using unrelated or mismatched donors. Exceptions are paroxysmal nocturnal hemoglobinuria patients in countries where eculizumab is not available. Bone marrow transplantation is also a reasonable option for patients who do not have a good response to eculizumab therapy. Second, AA/PNH patients continue to be reasonable candidates for bone marrow transplantation if they have life-threatening cytopenias. Similar to other bone marrow transplantation patients, younger age and the availability of a fully matched sibling donor are favorable prognostic factors. Third, a myeloablative conditioning regimen is not required to eradicate the PNH clone. Allogeneic bone marrow transplantation following non-myeloablative conditioning regimen can cure paroxysmal nocturnal hemoglobinuria. Whether or not there is an advantage to one approach over the other will require further study; however, non-myeloablative regimens may be preferable in young patients seeking to maintain fertility or patients with moderate organ dysfunction who may not tolerate a myeloablative regimen. Lastly, since bone marrow transplantation is the only curative therapy available for paroxysmal nocturnal hemoglobinuria, continued use and investigation of this approach in selected patients is reasonable. Recent advances in mitigating graft-versus-host disease such as post-transplant high-dose cyclophosphamide may be particularly effective in non-malignant hematopoietic diseases such as paroxysmal nocturnal hemoglobinuria, aplastic anemia and hemoglobinopathies.[18,20]

*Dr. Robert A. Brodsky is The Edythe Harris Lucas and Clara Lucas Lynn Professor of Hematology and Director of the Division of Adult Hematology at The Johns Hopkins University School of Medicine.*

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