Guidance for flow cytometric testing for GPI-deficient populations and Paroxysmal Nocturnal Haemoglobinuria (PNH)

This guidance has been developed by the Birmingham Heartlands Hospital.

Introduction

The name Paroxysmal Nocturnal Haemoglobinuria (PNH) itself is a misnomer, as it is not paroxysmal or nocturnal, haemolysis is constant and only around a quarter of patients present with haemoglobinuria.[1]

PNH is an acquired haematopoietic stem cell disorder in which somatic mutation of the X-linked phosphatidylinositol glycan complementation class A (PIG-A) gene results in a partial or absolute deficiency of all proteins normally linked to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor.[1,2] Clonal expansion of this cell population also frequently occurs in patients with aplastic or hypoplastic anaemia in which normal haematopoiesis has failed.

Classical clinical features of this condition are intravascular haemolysis, bone marrow failure and a thrombotic tendency, though patients show a wide spectrum of clinical presentation,[3,4] including abdominal pain, dysphagia, erectile dysfunction, renal failure and extreme fatigue or lethargy which is disproportionate to their anaemia and patient presentation is highly variable. The disease is rare with a reported incidence of 1.3/million/year and prevalence of 15.9/million.[5] Median age of diagnosis is around 30 years and median survival is around 10 years.

As PNH is a rare disease patients may go undiagnosed for many months. Consequently, the availability of a diagnostic flow cytometry assay means that in patients with suspected PNH, a definitive diagnosis can be rapidly established resulting in improved patient management and prognosis.

Requests for testing should be sent to:

Flow Cytometry Laboratory, Haematology Department,
Laboratory Medicine, Birmingham Heartlands Hospital,
Bordesley Green East, Birmingham, B9 5SS. Tel. 0121 424 0704

Recommended sample requirements: 3mL of EDTA blood received within 24 hours of collection[6]

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Soliris prescribing information can be found on the back page.

ALEXION

Test for GPI-deficient populations in the following groups:[6]

Coombs-negative haemolytic anaemia[1,6]	Haemoglobinuria and/or haemosiderinuria	Unexplained thrombosis (venous or arterial)	Unexplained cytopenias[6]	Hypoplastic or difficult diagnosis MDS	Aplastic anaemia	Renal dysfunction
Non-schistocytic, non-infectious haemolytic anaemias[6] Or haemolytic anaemias with concomitant iron deficiency[1] Absence of other RBC abnormalities (sickle cells, spherocytes etc.)	26% of patients reported haemoglobinuria at diagnosis[1]	Venous or arterial thrombosis accounts for >40% of deaths[7,8] Common sites - Deep vein thrombosis (DVT)[8] Unusual sites - hepatic veins (Budd-Chiari syndrome) other intra-abdominal veins, cerebral sinuses, dermal veins[6]	Consider PNH after adequate work-up	IPIG and NCCN guidelines recommend PNH testing in MDS[1,9]	British Committee for Standards in Haematology (BCSH) recommend screening at diagnosis and annually[10]	With signs of haemolysis (↑ LDH or ↑ reticulocyte count or ↓ haptoglobin)[1,11,12]

Also, suspect PNH in patients with the following:

Laboratory analysis	Clinical features
	Unexplained:
Indicators of haemolysis: ↑ LDH,[1,13] ↓ Haptoglobin,[1,13] ↑ Indirect bilirubin[1]	Thrombosis[6]
Indicators of renal dysfunction: ↑ Creatinine,[14] ↓ eGFR,[14] ↑ BUN[13]	Blood transfusions[6]
Granulocytopenia and/or thrombocytopenia[6]	Renal dysfunction[6]
Abnormal reticulocyte count[1]	Abdominal pain[6]
↑ D-Dimers[15]	Oesophageal spasm[6]
↓ Haemoglobin[1]	Pulmonary hypertension[6,16]
N-terminal pro-B-type natriuretic peptide (NT-proBNP) ≥160pg/ml[16] predictive of pulmonary hypertension and independent mortality risk	Erectile dysfunction[6]
	Severe fatigue[8]

Patients with any clone size detected are eligible for referral to the national PNH Service at St James, Leeds, or Kings College Hospital, London, for further assessment/evaluation. Outreach clinics are also available at the Peterborough City Hospital, the Queen Elizabeth Hospital Birmingham and the Oxford Churchill Hospital.

The National PNH Service Indications for Treatment with Soliris® (eculizumab)

Patients fulfilling any of the following categories:

1. Complications associated with haemolysis:
   - Renal failure
   - Pulmonary hypertension
2. Thrombosis related to PNH
3. Haemolytic (LDH >1.5xULN) symptomatic PNH
   - With anaemia (Hb <12g/L)
   - With agreement with Joint Service colleagues at MDT
4. Pregnancy (and for at least 3 months post-partum)
5. Transfusion dependent (four or more transfusions in 12 months)

Exceptional cases in whom Soliris is considered appropriate (not fulfilling the above criteria) will be approved through discussion between the two Nationally Commissioned PNH Services and the National Commissioners.

Prescribing Information

SOLIRIS® **Eculizumab** 300 mg concentrate for solution for infusion prescribing information. **Presentation:** 30 ml vial containing 300 mg eculizumab (10 mg/ml). **Indication:** Treatment of paroxysmal nocturnal haemoglobinuria (PNH) in adults and children. Evidence of clinical benefit is demonstrated in patients with haemolysis with clinical symptom(s) indicative of high disease activity, regardless of transfusion history.Treatment of atypical haemolytic uraemic syndrome (aHUS) in adults and children. **Dosage and method of administration:** *Adult (≥ 18 years of age) PNH initial phase:* 600 mg weekly x 4 weeks. *Adult (≥18) PNH maintenance phase:* 900 mg week 5, followed by 900 mg every 14 (± 2) days. *Adult (≥ 18 years of age) aHUS initial phase:* 900 mg weekly for 4 weeks. *Adult (≥18 years of age) aHUS maintenance phase:* 1200 mg week 5, followed by 1200 mg every 14 (± 2) days. *aHUS paediatric patients (<18 years of age) ≥40 kg initial phase:* 900 mg weekly x 4. *aHUS paediatric patients (<18 years of age) ≥ 40 k g maintenance phase:* 1200 mg week 5; then 1200 mg every 2 weeks. *PNH paediatric patients (<18 years of age); ≥40 kg initial phase:* 600 mg weekly x 4. *≥ 40 kg maintenance phase:* 900 mg week 5; then 900 mg every 2 weeks. *aHUS and PNH paediatric patients 30 - <40 kg initial phase:* 600 mg weekly x 2. *aHUS and PNH paediatric patients 30 - <40 kg maintenance phase:* 900 mg week 3; then 900 mg every 2 weeks. *aHUS and PNH paediatric patients 20 - <30 kg initial phase:* 600 mg weekly x 2. *aHUS and PNH paediatric patients 20 - <30 kg maintenance phase:* 600 mg week 3; then 600 mg every 2 weeks. *aHUS and PNH paediatric patients 10 - <20 kg initial phase:* 600 mg weekly x 1. *aHUS and PNH paediatric patients 10 - <20 kg maintenance phase:* 300 mg week 2; then 300 mg every 2 weeks. *aHUS and PNH paediatric patients 5 - <10 kg initial phase:* 300 mg week x 1. *aHUS and PNH paediatric patients 5 - <10 kg maintenance phase:* 300 mg week 2; then 300 mg every 3 weeks. Supplemental dosing is required with concomitant plasma treatment, please refer to the SmPC. *Administration:* Dilute to a concentration of 5 mg/ml. Administer via an intravenous infusion over 25 - 45 minutes in adults and 1-4 hours in paediatric patients via gravity feed, a syringe-type pump, or an infusion pump. Patients should be monitored for 1 hour post infusion. *Elderly:* No evidence to suggest that special precautions are required, although experience is limited. *Renal impairment:* No dose adjustment. *Hepatic impairment:* Not studied. *Monitoring:* aHUS patients should be monitored for thrombotic microangiopathy (TMA). Recommended to continue Soliris treatment unless discontinuation is medically justified. **Contraindications:** Hypersensitivity to active ingredient, murine proteins or other excipients. Must not initiate in patients with unresolved *Neisseria meningitidis* infection or patients who are not currently vaccinated against *Neisseria meningitides* (unless they receive prophylactic treatment with appropriate antibiotics until 2 weeks after vaccination). **Special warnings and precautions:** *Meningococcal infection, immunisation and other systemic infections:* Increased risk of meningococcal infection. All patients must be vaccinated against meningococcal infection ≥2 weeks prior to starting Soliris unless the risk of delaying Soliris therapy outweigh the risks of developing a meningococcal infection. Patients treated with Soliris <2 weeks after receiving a meningococcal vaccine must receive treatment with appropriate prophylactic antibiotics until 2 weeks after vaccination. Vaccines against serotypes A, C, Y, W135 and B where available are recommended in preventing the commonly pathogenic meningococcal serotypes. Patients must be vaccinated or revaccinated according to national vaccination guidelines. Vaccination may not be sufficient to prevent meningococcal infection. Cases of serious or fatal meningococcal infection have been reported. All patients must be monitored for early signs of meningococcal infection and evaluated immediately if infection is suspected and treated with appropriate antibiotics if necessary. Patients should be informed of signs and symptoms of infection and steps taken to seek medical care immediately. Physicians must discuss the benefits and risks with patients and provide them with a patient information brochure and a patient safety card. Use with caution in patients with other active systemic infections. Patients may have increased susceptibility to infections, especially with encapsulated bacteria. Patients <18 years must be vaccinated against *Haemophilus influenzae* and pneumococcal infections. *Infusion reactions:* Administration may result in infusion reactions or immunogenicity that could cause allergic or hypersensitivity reactions, including anaphylaxis. Treatment should be interrupted in patients who experience severe infusion reactions and appropriate medical therapy administered. *Anticoagulant therapy:* Should not be altered. *Laboratory Monitoring:* PNH patients should be monitored for signs and symptoms of intravascular haemolysis, including serum LDH levels. During treatment, patients who show signs and symptoms of intravascular haemolysis may require dose adjustment within the recommended 14 (± 2) day dosing schedule during the maintenance phase. aHUS patients should be monitored for TMA by measuring platelet counts, serum LDH and serum creatinine and may require dose adjustment within the recommended 14 (± 2) day dosing schedule during the maintenance phase. *Discontinuation:* PNH patients who discontinue should be monitored for serious intravascular haemolysis for ≥8 weeks. If serious haemolysis occurs, consider: blood transfusion, or exchange transfusion if the PNH red blood cells are >50% of the total red blood cells by flow cytometry; anticoagulation; corticosteroids or reinstitution of Soliris. aHUS patients who discontinue should be monitored closely for severe TMA complications. Monitoring may be insufficient to predict or prevent severe TMA complications in patients with aHUS after discontinuation of Soliris. If severe TMA complications occur consider: reinstitution of Soliris treatment, supportive care with PE/PI, or appropriate organ-specific supportive measures including renal support with dialysis, respiratory support with mechanical ventilation or anticoagulation. TMA complications were observed as early as 4 weeks and up to 127 weeks following discontinuation in aHUS clinical studies. *Excipients:* 5 mmol sodium per vial. **Interactions with other medicinal products:** No studies. **Pregnancy:** Should be given only if clearly needed. Women of childbearing potential have to use effective contraception during treatment and for 5 months after treatment. **Breast-feeding:** Unknown whether Soliris is excreted into human milk. Should be discontinued during treatment and for 5 months after treatment. **Undesirable effects:** The most common adverse reaction was headache and the most serious adverse reaction was meningococcal sepsis. Very common side effects (≥1/1 0): Headache. Common side effects (≥1/100 to <1/10): Meningococcal sepsis, aspergillus infection, arthritis bacterial, upper respiratory tract infection, nasopharyngitis, bronchitis, oral herpes, urinary tract infection, viral infection, thrombocytopenia, leukopenia, haemolysis, anaphylactic reaction, decreased appetite, dizziness, dysgeusia, hypotension, dyspnoea, cough, nasal congestion, pharyngolaryngeal pain, rhinorrhoea, diarrhoea, vomiting, nausea, abdominal pain, constipation, dyspepsia, rash, alopecia, pruritus, arthralgia, myalgia, muscle spasms, bone pain, back pain, neck pain, pain in extremity, oedema, pyrexia, chest discomfort, chills, fatigue, asthenia, Coombs test positive, influenza like illness. Please refer to the SmPC for a full list of adverse events. In PNH and aHUS clinical trials the most serious adverse event was meningococcal septicaemia. Antibodies to Soliris were detected in 2% of PNH pat i ent s using an ELISA assay and 3% of patients with aHUS using the ECL bridging format assay. Cases of haemolysis have been reported with missed or delayed Soliris dose in PNH clinical trials. Cases of TMA complication have been reported in the setting of missed or delayed Soliris dose in aHUS clinical trials. The most common adverse reaction in paediatric PNH patients was headache. The safety profile in the different paediatric subsets of age appears similar. **Overdose:** No cases reported. **MA number:** EU/1/07/393/001 **MAH:** Alexion Europe SAS, 25 1-15, avenue Edouard Belin 92500 Rueil-Malmaison France. Further information available from Alexion Pharma UK, 3 Furzeground Way, Stockley Park, Uxbridge, Middlesex, UB11 1EZ. Tel 0800 321 3902. **Date of Authorisation:** 20-06-2007. **Legal Category:** POM. **UK Cost:** £3,150.00 per vial. **Irish Cost:** €4557.50 per vial. **Preparation date:** September 2015.

Adverse events should be reported. Information on reporting adverse events can be found at **www.hpra.ie**. Adverse events should also be reported to Alexion Pharma UK Ltd on 1800 936 544 **or** uk.adverseevents@alxn.com

Adverse events should be reported. Reporting forms and information can be found at **www.mhra.gov.uk/yellowcard**. Adverse events should also be reported to Alexion Pharma UK Ltd on 0800 321 3902 **or** uk.adverseevents@alxn.com.

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