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Paroxysmal Nocturnal Hemoglobinuria (PNH)

Recommendations from the society for diagnosis and therapy of haematological and oncological diseases

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Paroxysmal Nocturnal Hemoglobinuria (PNH)

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Compliance rules:

- Guideline
- Conflict of interests

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1 Summary

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired hematologic disorder with variable clinical manifestations. The untreated disease is characterized by intravascular haemolysis, thrombophilia e.g. thrombosis at typical and atypical locations, In addition, the associated cytopenia can range from subclinically mild to severe pancytopenia (so-called aplastic anaemia/PNH syndrome). PNH phenotype is mainly caused by one (or more) acquired somatic mutation(s) in the phosphatidylinositol glycan class A (PIG-A) gene at the level of the pluripotent hematopoietic stem cell of the bone marrow.

Treatment is symptom-oriented. In asymptomatic patients, a wait-and-see approach could be performed. In these patients, a prophylactic anticoagulation may be considered. In symptomatic patients, the development of targeted drug inhibition of the terminal complement cascade has led to a significant improvement in clinical symptoms and prevention of disease-related complications. Compared to historical controls, the survival time of patients with hemolytic PNH is now significantly improved. The first terminal complement inhibitor, Eculizumab, was approved in 2007, followed by Ravulizumab in 2019 and Crovalimab in 2024. Pegcetacoplan, the first proximal complement inhibitor, became available in 2021. Danicopan and Iptacopan can also be used in 2024 following approval. Other newly developed complement inhibitors are still undergoing clinical trials.

2 Basics

2.1 Definition

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired disease of bone marrow hematopoietic stem cells that belongs to the group of bone marrow failure syndromes (BMFS). The disease has a variable clinical course and is characterized by intravascular hemolysis, thrombophilia with thrombosis in typical and atypical locations and a variable degree of cytopenia [1, 2].

2.2 Epidemiology

PNH is a very rare disease with an estimated prevalence of up to 16 cases/1 million and an incidence of around 1.3 cases/1 million individuals* (data from the UK / France). There are no reliable epidemiological data available for the prevalence and incidence of PNH in Germany. Due to its clinical heterogeneity, it can be assumed that this disease might be "underdiagnosed" [3].

2.3 Pathogenesis

The basis at the cellular level in paroxysmal nocturnal hemoglobinuria is a mutation in the X-linked PIG-A gene, which prevents the biosynthesis of the glycosylphosphatidylinositol (GPI) anchor molecule [4]. In single individual cases, GPI deficiency arises due to hereditary mutations in autosomal localized genes such as PIG-T, in which the second allele is switched off by an additional somatic mutation and thus the complete biosynthesis step is interrupted leading to a PNH phenotype in these cells [5, 6]. GPI deficiencies are found at the level of multipotent hematopoietic stem cells of the bone marrow [7, 8]. The acquired somatic mutation(s) do not affect all bone marrow stem cells, resulting in a so-called mosaic situation. Very low numbers of GPI-deficient cells with the corresponding mutation can also be detected in healthy subjects using high-resolution detection methods [9]. Other pathophysiological mechanisms discussed in the literature include autoimmune-mediated depletion of GPI+, non-mutated stem cells with secondary accumulation of GPI-deficient PNH stem cells [10]. In addition, previous observations show that at the stem cell level, GPI-deficient stem cells in individual PNH patients have an intrinsic growth advantage over normally expressing stem cells due to cytogenetic changes and thus exert clonal dominance in the bone marrow [11]. Recent studies using next generation sequencing (NGS) have shown that the mechanism of clonal expansion is much more complex in most PNH patients. In addition to the characteristic PIG-A mutations, other somatic mutations in myeloid genes (activating mutations) comparable to those in other myeloid diseases [12] as well as mutations in genes involved in immune recognition such as HLA molecules ('immune escape mutations') have been found in PNH patients. Time line studies on quantitative distribution of these individually mutated competing clones have shown that a complex clonal hierarchy - caused by the interaction of myeloid progenitor cells and the immune system - is usually present in the bone marrow of PNH patients [13].

The consequence of GPI deficiency on a significant proportion of peripheral blood cells is a lack of complement-regulating proteins. In particular, CD55, the so-called 'decay accelerating factor (DAF)' and CD59, the 'membrane inhibitor of reactive lysis (MIRL)' [14] are to be mentioned. In the case of complement activation, erythrocytes in particular are sensitive to terminal complement-mediated lysis due to the constitutive absence of transmembrane-anchored Complement-regulating surface molecules. Since almost all PNH-specific symptoms have also been described in a patient with an isolated CD59 defect, the CD59 molecule plays a central role in the clinical symptoms as a regulator of the membrane attack complex (MAC) [14].

4 Clinical characteristics

4.1 Symptoms

4.1.1 Hemolysis and hemoglobinuria

Although the classic manifestation of PNH is hemoglobinuria presenting with dark brown urine especially in the morning, only about 26% of PNH patients report this typical clinical sign at the time of initial diagnosis. Many PNH patients have no clinically apparent hemoglobinuria or at most intermittent episodes that bear no relation to the day/night rhythm. However, there is a correlation between the occurrence of hemoglobinuria and the size of the PNH clone, i.e. the proportion of GPI-deficient cells in the peripheral blood. The classic characteristics of chronic hemolytic anemia are weakness, fatigue and exertional dyspnea. The extent of fatigue is not strictly linearly correlated with the extent of anemia, but with the extent of hemolysis and the size of the PNH clone. An abnormal depletion of nitric oxide correlates with the intravascular release of hemoglobin, on the one hand due to NO consumption through the metabolization of hemoglobin to methemoglobin. On the other hand, there is also a release of erythrocytic arginase, which reduces arginine as a starting substance for de novo NO production [15]. This

is associated with endothelial cell dysfunction and platelet activation. The abnormal fatigue of PNH patients can also be linked to this depletion [15].

4.1.2 Thrombophilia

Thromboembolic complications are the most clinically relevant complication for patients with PNH and are the main cause of the increased morbidity and mortality of this disease [3]. The dysfunction caused by NO depletion is seen as part of thrombophilia, but coagulation-activating erythrocyte microvesicles and even inhibition of ADAMTS13 have also been identified. However, none of these factors alone can sufficiently explain the abnormal thrombophilia in patients with PNH [16, 17]. A significant proportion of patients with PNH without treatment with complement-inhibiting substances develop thromboses, which predominantly affect the venous system, but can also occur on the arterial side (e.g. myocardial infarction, apoplectic insult). The likelihood of suffering a thrombosis correlates with the presence of the classic symptoms, i.e. hemolysis and hemoglobinuria. Abdominal and thoracic pain, dyspnea and hemoglobinuria have been identified as early indicators of an impending thromboembolic event [18]. Venous thromboses in PNH patients occur in typical and atypical locations such as the abdominal veins, especially the splanchnic veins, the hepatic veins, cerebral veins or cutaneous veins.

In addition to venous thromboembolic events in typical and atypical localizations, these also occur in the arterial system and lead to vascular occlusions in cerebral, coronary, visceral and retinal vessels with corresponding clinical symptoms. The frequency of these arterial occlusions was estimated at 39% of all thromboembolic events in a Korean cohort [19]. However, based on observations from other cohorts worldwide, this figure appears to be extraordinarily high. Nevertheless, PNH should also be considered in the case of arterial events, particularly in the absence of pre-existing vascular disease.

4.1.3 Secondary aplastic syndromes or AA-PNH syndrome

Aplastic anemia (AA) and PNH are closely related in terms of pathophysiology and clinical manifestations:

1. Depending on the initial PNH clone size, the risk of developing clinical PNH in patients with acquired aplastic anemia is approx. 15-25% [20].
2. Even at the time of diagnosis, in >50% of patients with aplastic anemia a small or moderate GPI-deficient population can be detected by highly sensitive flow cytometry [21].
3. The presence of a PNH clone in patients with aplastic anemia is now considered as an indication of an immunologically induced bone marrow failure (acquired aplastic anemia) [21]
4. Depending on the study, 10-20% of patients with aplastic anemia develop manifest hemolytic PNH during the course of their disease, often many years after completing immunosuppressive therapy [22].
5. According to a retrospective study, approx. 20% of patients with PNH already have aberrant cytogenetic findings at the time of diagnosis which does not lead to an evolution to leukemia in these patients [23].
6. A distinction between PNH with limited bone marrow regeneration and PNH in the setting of MDS is not sufficiently done on just a purely morphological basis. Therefore, an existing MDS in hemolytic PNH is primarily diagnosed on a cytogenetic basis [23, 26].
7. Allogeneic stem cell transplantation can prevent the risk of secondary development of PNH in patients with aplastic anemia.

In conclusion, secondary clonal diseases such as PNH or MDS could develop from acquired aplastic anemia by natural course.

4.1.4 Renal manifestation

Impairment of renal function is found in two thirds of all patients with PNH, in 21% already in advanced stages [3]. Reversible renal function impairment as an expression of vasa afferentia vasospasm must be distinguished from renal parenchymal damage with tubule damage. Reversible renal function impairment is causally related to increased nitric oxide (NO) catabolism due to intravascular hemolysis, which leads to dysregulation of endothelial cells and smooth muscle cells in the vessel wall and resulting vasculopathy/vasospasm. Both hemosiderin deposition in the proximal tubules and microvascular thrombosis have been identified as correlates of renal parenchymal damage in patients with PNH. Clinically, impaired tubular function and a gradual decrease in creatinine clearance are predominant in the majority of patients [24].

4.1.5 Pulmonary manifestation

A frequent clinical symptom in patients with hemolytic PNH is dyspnea, which does not correlate solely with manifest anemia. Intravascular hemolysis results in a considerable NO consumption, which can lead to the development of pronounced pulmonary hypertension. On the one hand, this can be detected by a significant increase in the pro-BNP value, which was used here as a measure of right ventricular dysfunction [25]. In a second publication by the same working group, right ventricular dysfunction was measured by echocardiography or cardio-MRI in 8 out of 10 patients. In 4 of the 8 patients, perfusion scintigraphy measured perfusion defects as in thromboembolism, but the other 4 patients had no evidence of thromboembolic events [25]. Thus, Pathophysiology of pulmonary hypertension apart from anaemia is, on the one hand, a recurrent thromboembolic event and, on the other hand, a direct effect of hemolysis-induced NO consumption.

4.1.6 Nonspecific clinical manifestations

In addition, intermittent esophageal spasms, chest pain, nausea and swallowing difficulties occur, the latter particularly in connection with hemolytic episodes. Male patients sometimes also report erectile dysfunction. In addition, moderate to sometimes intense pain, especially back pain, headaches, muscle aches and abdominal pain occur in connection with hemolytic crises. These symptoms can be positively influenced by treatment with Complement-Inhibitors (see below) [26].

4.1.7 Fatigue

In hemolytic PNH, one of the central clinical features for patients is pronounced fatigue. In various cohorts, this is reported in over 80 % of patients [27]. Typically, the intensity of fatigue is not strictly proportional to the degree of anemia, but data on proximal complement inhibitors show that normalized Hb levels are associated with better fatigue scores than low Hb levels [28 - 31]. These symptoms can also occur under C5 inhibition, especially in patients who have developed clinically significant extravascular hemolysis under C5 inhibitor administration. The pathophysiological background of fatigue is currently not sufficiently understood. On the one hand, hemolysis activity itself is a possible explanation, as patients with high absolute reticulocyte counts in particular report such complaints. On the other hand, complement activation itself could also be responsible for the impressive occurrence of fatigue in these patients.

Fatigue manifests itself in patients as physical but also cognitive impairment. Many patients report a state of "shutting down", which is described by symptoms mentioned above and physical exhaustion. This can have a very negative impact on social live such as ability to work, starting a family well-beeing during leisure time and can also lead to additional psychological stress and a reduction in quality of life [32]. Thus, it requires special care and attention from the treating physicians. In particular, the extent of fatigue should also be included in the therapeutic decision for the patient.

Fatigue can develop gradually. Patients sometimes become accustomed to the reduced performance. It therefore makes sense to regularly record quality of life from the start of therapy using appropriate questionnaires. The FACIT-Fatigue Score and the AA/PNH QLQ-54 [33, 34], which has not yet been validated by the IPIG (International PNH Interest Group), are suitable for determining changes in fatigue and quality of life.

Overall, an associated disease burden in terms of the symptoms described above can be assumed for expanded PNH clones with a size of 10% [27].

5 Diagnostics

The diagnostic work-up for suspected PNH should include the following steps (Tables 1 and 2) [26].

Table 1: Basic diagnostics for suspected PNH

Investigations	Notes
Medical history	• Detailed family history and personal history to determine whether an acquired disorder is to be assumed or whether there are indications of congenital differential diagnoses, e.g. membranopathies, enzymopathies, etc. • Personal history including specific questioning about symptoms typical of PNH such as anemia symptoms, fatigue, dyspnea, urine discoloration, recurrent abdominal pain crises, dysphagia, headaches, erectile dysfunction, thromboembolic events, signs of bleeding
Physical examination	• Signs of anemia, icterus, evidence of acute or past thrombosis, signs of bleeding, constitutional abnormalities as in congenital aplastic anemia, splenomegaly
Laboratory tests [21]	• Differential blood count and reticulocyte count, erythrocyte morphology, with special respect **of fragmentocytes** to differentiate thrombotic microangiopathies (TMA). • Hemolysis parameters: • Mandatory: LDH, total bilirubin, direct bilirubin • Supplementary: haptoglobin, hemopexin, urine status with detection of hemoglobin. Optional: free haemoglobin in serum; haemosiderin in urine • Direct (monospecific) antiglobulin test (DAT), blood group. • Highly sensitive flow cytometry from peripheral blood: GPI-anchored proteins, see Table 3
Bone marrow diagnostics	• Cytology, cytogenetics, molecular genetics (NGS) and histology, if cytopenia is present to such an extent that PNH is suspected in the context of another hematological disease (especially aplastic anemia, MDS)
Sonography	• Upper abdominal sonography including color Doppler with special attention to the following aspects: Liver and spleen size, Doppler sonographic evidence of acute or past hepatic vein, portal vein, splenic vein or mesenteric vein thrombosis); if an acute thrombotic event is suspected, color Doppler and angiography of other flow areas (e.g. cerebral veins) may also be necessary.

Table 2: Extended diagnostics for suspected PNH

Investigation	Notes
Laboratory tests	• Creatinine, creatinine clearance • Ferritin, iron, transferrin, transferrin saturation, reticulocyte hemoglobin, soluble transferrin receptor • For ferritin values >1,000 ng/ml, further clarification of possible organ damage due to iron overload (echocardiography, blood sugar/blood sugar daily profile, thyroid values, TSH, multi-parameter MRI of liver and kidneys if necessary). • Plasma levels of folic acid and vitamin B_{12} • Pro-BNP in serum for the assessment of right ventricular function • For young patients with indication for stem cell transplantation: HLA typing of patient and siblings • If there is a positive family history of thromboembolic events: Thrombophilia screening (factor V Leiden, prothrombin mutations, protein C, protein S etc.). • Genetic analyses (PIG-A gene) are not required to confirm the diagnosis in routine diagnostics for typical constellations of findings. • In the case of atypical clinical manifestations/atypical flow cytometric findings, extended molecular defect diagnostics may be useful [35]

The standard method for detecting PNH-typical GPI anchor defects is the highly sensitive flow cytometric examination of blood cells. The previous 2x2 rule (2 cell series such as granulocytes and reticulocytes, each examined with 2 separable reagents) still applies today. Flow cytometric analysis is often performed as multiparameter analyses such as 8-color flow cytometry on granulocytes and monocytes [36]. Ideally, the highly sensitive FLAER (fluorescein-labeled aerolysin - a reagent that binds directly to the GPI anchor molecule) is also used [37- 39]. For the reliable detection of a PNH clone, a missing or reduced expression of at least two GPI-anchored markers should be shown on at least two cell lines.

The constellations listed in Table 3 should be the reason for a flow cytometric analysis of GPI-anchored proteins on blood cells.

Table 3: Indications for flow cytometric diagnostics of the expression of GPI-anchored proteins [23, 24]

Acquired, Coombs-negative hemolytic anemia (without signs of microangiopathic hemolytic anemia)
• Intravascular hemolysis (haptoglobin undetectable, hemoglobinuria, increased free plasma hemoglobin)
• Thrombosis if at least one of the following criteria is met: ◦ "Atypical" localization (sinus vein thrombosis, Budd-Chiari syndrome, mesenteric or portal vein or splenic vein thrombosis, dermal thrombosis) ◦ Thromboses (regardless of their location) in patients with signs of hemolytic anemia (LDH elevation) ◦ Thromboses (regardless of their localization) in conjunction with unclear cytopenia ◦ Thromboses (regardless of their location, including arterial) in the absence of risk factors
• Patients with unexplained iron deficiency anemia (after careful exclusion of other causes) in conjunction with signs of hemolytic anemia
• Diagnosis or strong suspicion of aplastic anemia
• Diagnosis or strong suspicion of low-risk MDS
• Recurrent abdominal pain crises of unknown origin or dysphagia, especially with concomitant signs of hemolysis

Follow-up checks of the flow cytometric analysis should be carried out depending on the current clinical situation. For example, if a significant GPI-deficient population is detected [40], the analysis should be repeated every 6 months, and then annually if the course is stable. Under complement inhibition therapy, diagnostics should also be repeated at 3 to 6-month intervals. If the proportion of GPI-deficient cells fluctuates or the clinical symptoms change, the examination intervals should be adjusted individually. After allogeneic transplantation, the flow cytometric analysis is recommended every three months if the proportion of recipients in the chimerism analysis is positive, until the GPI-deficient population is no longer detectable. If there is a clini-

cal suspicion of relapse after transplantation, a new analysis is also useful. In the follow-up after diagnosis of aplastic anemia, it is recommended to repeat the flow cytometric analysis at twelve-month intervals, provided there is no evidence of significant hemolysis.

Table 4: Flow cytometric analysis of GPI-anchored proteins [23, 24]

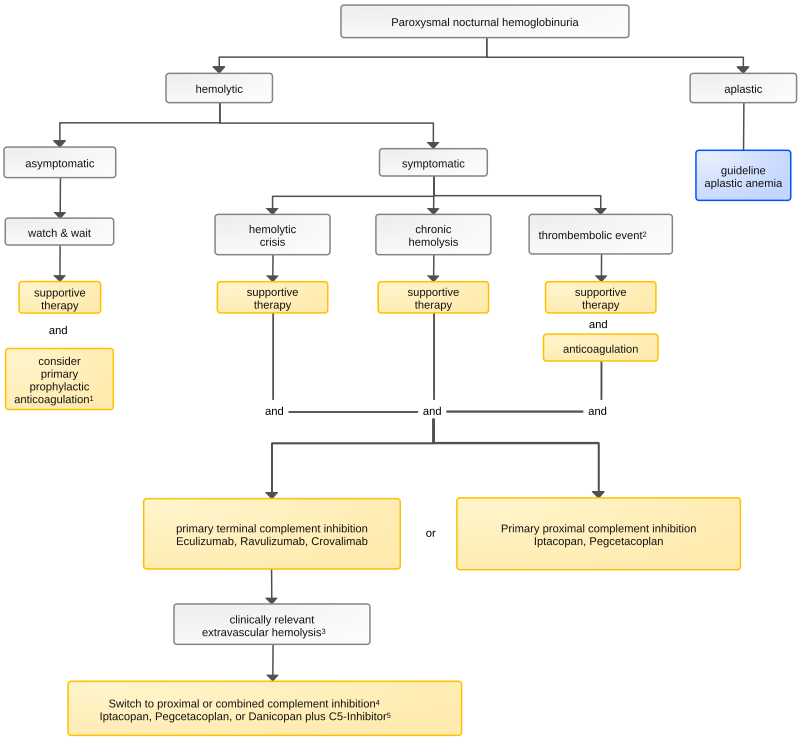
Criterion	Notes
Material	Peripheral blood (preferably EDTA-anticoagulated) (for routine diagnostics no examination of bone marrow (!), as the interpretation is very difficult due to physiological changes in the expression of GPI-anchored proteins in the context of hematopoietic differentiation).
Interval between sampling and examination	If possible < 48 hours For transport times > 24 hours, the sample should be cooled (+1 to +10^{o} C).
Cell series examined	Z. E.g. granulocytes and reticulocytes; At least 2 different markers (GPI-anchored proteins or GPI anchors themselves) should be examined per cell series. The investigated cell population should be identified with a non-GPI-anchored marker.
Findings	The findings should include quantitative data for the cell series examined with separate indication of the proportion of cells (PNH clone size) with completely absent expression of GPI-anchored proteins (PNH type III cells) and reduced expression (PNH type II cells).
Sensitivity	In routine examinations, it should be possible to detect a GPI-deficient population from a relative proportion of 1%. When examining patients with aplastic anemia, a reduction of the sensitivity limit to 0.001% should be considered.

For methodological aspects of performing the test, please refer to specialized literature [36, 38]. Participation in round robin tests for flow cytometric PNH diagnostics is recommended.

6 Therapy

6.1 Treatment structure

Figure 1: Algorithm for the treatment of paroxysmal nocturnal hemoglobinuria



Legend:
[1] Anticoagulation see chapter 6.2.1.2
[2] Venous thromboembolism or previous venous thromboembolism or increased risk (see chapter 4.1.2) In the event of a thromboembolic event as the first manifestation of PNH, immediate interruption of intravascular haemolysis (IVH) is necessary. According to current data, this is most reliably achieved by administering an effective terminal complement inhibitor (e.g. ravulizumab), as thrombin can directly activate C3 and C5 and plasmin also activates C5, making rapid C5 blockade useful [41, 42];
[3] Clinically relevant extravascular hemolysis is defined as symptomatic anemia with or without transfusion requirement for at least three months under therapy with C5 inhibitors with concomitant significant reticulocytosis and signs of hemolysis (see also chapter 6.2.3.4).
[4] Decisions on the choice of first-line therapy and also when switching therapy from a terminal to a proximal complement inhibitor are often difficult and confusing. Likewise, the evidence base for such a choice is often insufficient, while complications during treatment with the extended range of complement inhibitors have not become easier. The authors of the guideline therefore recommend a four-weekly virtual online conference on PNH, bone marrow failure diseases and aplastic anemia (Teams-based online conference. If you are interested, please contact Ms. Schifflers; Tel.: +49 241 80-38664; jschifflers@ukaachen.de). Patients with PNH should also be included in the International IPIG Registry and patients with acquired or hereditary AA and other adult-onset aplastic syndromes should be included in the newly established German AABMF Registry. This applies to all patients, but especially to those who are treated with the newly developed substances. See also chapter 6.2.3.7 and chapter 6.2.3.8.
5 Danicopan is only licenced as add-on for Eculizumab or Ravulizumab.

6.2 Treatment modalities

6.2.1 Supportive therapy

6.2.1.1 General information

The following recommendations essentially relate to the treatment of hemolytic PNH. With regard to the treatment of aplastic anemia with PNH clone, please refer to the Onkopedia guideline on aplastic anemia.

- Substitution of erythrocyte concentrates (washed erythrocyte concentrates are neither necessary nor useful) [43].
- Supplement of folic acid (1-5 mg/q.d. orally) and possibly also vitamin B_{12} (in the event of a deficiency) due to the compensatory significant increase in erythropoiesis in the bone marrow.
- Oral or, if necessary, intravenous substitution of iron in the event of a deficiency while monitoring iron stores (ferritin, transferrin saturation, reticulocyte haemoglobin). Due to the complement-blocking therapy, effective inhibition of intravascular hemolysis prevents chronic iron loss through hemoglobinuria or hemosiderinuria. Thus, no uncontrolled iron administration should take place during complement-blocking therapy and, if necessary, iron depletion should be initiated in the event of a possible iron overload (especially in the case of a residual transfusion requirement).
- Bacterial infections should be treated early and consistently with antibiotics, as these – as complement amplifying condition (CAC) - can lead to exacerbations of PNH with hemolytic crises and possible further complications [44].
- Sufficient hydration in the context of crisis hemolysis.

6.2.1.2 Anticoagulation

- Long-term or lifelong anticoagulation after a thrombosis is recommended. Despite effective anticoagulation, there is a risk of recurrence of thromboembolic events, which means that complement-blocking therapy is indicated for most patients [45]. In these patients, anticoagulation can also be discontinued with ongoing complement inhibition in accordance with standard recommendations for patients with thrombosis [46].
- In patients who are restricted in their mobility for a longer period of time, the initiation of complement-inhibiting therapy should be considered in order to avert the increased risk of thrombophilia despite sufficient anticoagulation.
- Treatment of thromboses in atypical locations, such as Budd-Chiari syndrome, should be carried out in a specialized center, if necessary with local or systemic lytic therapy and initiation of complement-blocking therapy [46].
- Primary prophylactic anticoagulation should be considered in patients who are not being treated with complement-inhibiting therapy.
- Both coumarins and heparins can be used therapeutically and prophylactically. Published data on the use of DOACs are only available for very small groups of patients, but their use, especially with simultaneous administration of complement inhibitors, seems to lead to comparable results based on experience in centers [47, 48].

6.2.1.3 Immunosuppressive therapy

- Immunosuppressive therapy is not indicated for the sole treatment of hemolytic activity.
- For treatment of aplastic anemia, refer to Onkopedia guideline on aplastic anemia. Before starting therapy with ATG, the necessity of continuing therapy with complement-blocking therapy should be discussed, as there is usually no longer an indication due to the existing aplasia (no or only low GPI-deficient reticulocytes/erythrocytes). If there is a relevant proportion of PNH reticulocytes/erythrocytes, complement-blocking therapy can be continued without any indication of a poorer response [49].

6.2.2 Curative therapy

The only potentially curative treatment approach for PNH is allogeneic stem cell transplantation. An **indication** for allogeneic stem cell transplantation arises in PNH in the context of severe aplastic anemia if there is already an indication for transplantation due to the aplastic anemia alone (refer to Onkopedia guideline on aplastic anemia).
Complications such as secondary bone marrow failure analogous to the procedure for severe aplastic anemia, transition to MDS or acute leukemia as well as recurrent thromboembolic complications despite thrombosis prophylaxis, and complement inhibition can be possible situations from which an indication for allogeneic stem cell transplantation (in the case of aplastic anemia preferentially with bone marrow analogous to the guideline on aplastic anemia) arises.

Allogeneic stem cell transplantation is still associated with significant transplant-associated morbidity and mortality due to the high rates of graft rejection, especially after conventional conditioning, infectious complications and GVHD (long-term survival rates approx. 50%-60%) [50- 52].

6.2.3 Drug therapy

If possible, untreated and previously treated PNH patients should be included in ongoing therapy studies. The mere detection of a PNH clone alone does not indicate the initiation of complement inhibition. Small PNH clones are frequently found, e.g. in patients with bone marrow failure or aplastic anemia. Clinically relevant hemolysis, which is typically found in larger PNH clone sizes, is relevant for the initiation of complement inhibition [40]. It should be emphasized that relevant hemolysis cannot be determined by a fixed parameter, but that the entire picture of clinical and laboratory parameters should be taken into account.

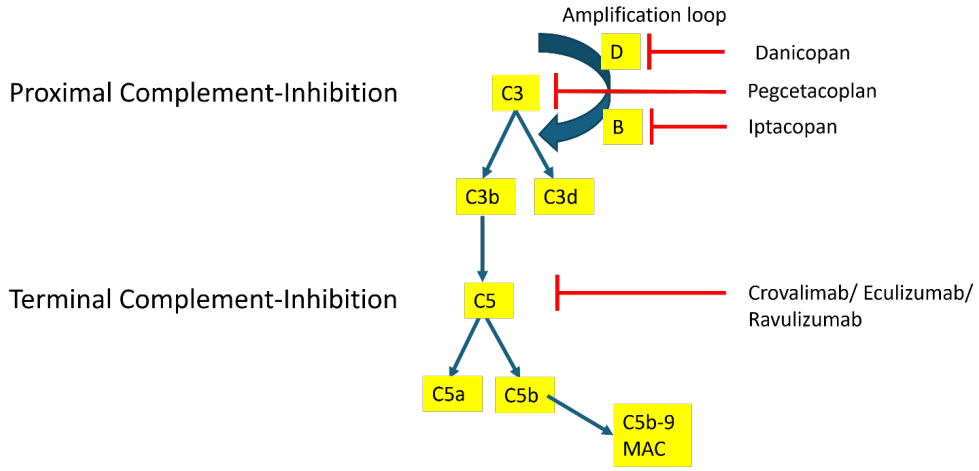
The expected benefit of complement inhibition in PNH depending on the clone size is shown under the following link [40]: https://doi.org/10.1182/hematology.2021000245

The following complement inhibitors are currently available in Europe:

Table 5: Overview of complement inhibitors available in Europe

Complement inhibition	Substance	Special features of the approval
Terminal complement cascade Anti-C5 agents		
	Crovalimab (Piasky®)	
	Eculizumab (Soliris®)	
	Eculizumab biosimilar (Bekemv®)	Treatment of hemolytic PNH
	Eculizumab biosimilar (Epysqli®)	
	Ravulizumab (Ultomiris®)	
Proximal complement cascade		
Anti-factor D	Danicopan (Voydeya®)	Add-on to terminal CI when clinically relevant EVH detectable
Anti-factor B	Iptacopan (Fabhalta®)	Switch due to EVH and for treatment of hemolysis (therapy naïve pts.) in PNH
Anti C3	Pegcetacoplan (Aspaveli®)	

Figure 2: Targets of currently approved and available complement inhibitors



6.2.3.1 Inhibition of complement component C5 by crovalimab, eculizumab or ravulizumab

Inhibition of the terminal complement system is a targeted therapeutic strategy. The humanized monoclonal antibodies eculizumab, ravulizumab, and crovalimab bind the complement factor C5, prevent its cleavage into the fragments C5a and C5b and thus block the subsequent formation of the terminal complement complex C5b-9 [53- 59]. Ravulizumab has been developed through molecular modifications of eculizumab. The amino acid exchanges in the region of the variable antigen binding site leads to a pH-dependent dissociation of C5 in the endosome. On the other hand, the exchange of two amino acids in the CH-3 domain of the severe chain results in pH-dependent binding to the neonatal Fc receptor. This allows the antibody to be recycled for extracellular re-use after dissociation of C5 in the endosome [58- 62]. This results in an extension of the clinical terminal half-life from 11.3 days for eculizumab to 49.7 days for ravulizumab.

Crovalimab is a C5 antibody that leads to increased uptake by pinocytosis by raising the isoelectric point of the immune complex. This further increases the clearance performance of the individual antibody molecule for the target molecule with an identical recycling mechanism via the neonatal Fc receptor, so that a significantly lower level of the antibody in the patient's serum leads to sufficient efficacy. The binding epitope of crovalimab is different from that of eculizumab and ravulizumab. Therefore, the substance is also effective in the rare variant of Arg885 in the C5 molecule in the treatment of hemolysis in PNH patients [63, 64].

Eculizumab therapy involves the administration of 600 mg eculizumab weekly for 4 weeks, followed by 900 mg every 2 weeks for approx. 30 minutes with a 60-minute follow-up (see Figure 3 and Eculizumab Information for healthcare professionals).

Therapy with ravulizumab is carried out with a weight-dependent dosage: Initially, saturation with 2700 mg ≥ 60 to < 100 kg bw (≥ 40 to < 60 kg bw = 2400 mg; ≥ 100 kg bw = 3000 mg) and after 2 weeks a maintenance therapy with 3300 mg ≥ 60 to < 100 kg bw (≥ 40 to < 60 kg bw = 3000 mg; ≥ 100 kg bw = 3600 mg) is carried out, which must then be repeated every 8 weeks. The rate and duration of the infusion is adjusted according to the amount of antibody according to the technical information, followed by a post-infusion of 100 ml NaCl for complete application (flushing) of the entire amount of ravulizumab in the tubing system (see Figure 4 and technical information of ravulizumab).

The dosage of Crovalimab is weight-dependent. A distinction is made between patients <100 kg and ≥100 kg. The initial dose in week 1 (1000 mg for bw <100 kg and 1,500 kg for bw ≥ 100 kg) is administered intravenously over 60 minutes on day 1. For dilution and administration, see the Information for healthcare professionals. On day 2, the dose of 340 mg is already administered subcutaneously, as well as weekly weeks 2 to 4. From week 5, the dose of 680 mg (= 2 phials per 340 mg) is administered subcutaneously at four-week intervals for patients <100 kg and 1020 mg (= 3 phials per 340 mg) for patients ≥100 kg.

An accompanying vaccination against meningococci is mandatory for treatment with complement inhibitors. According to the recommendation of the German Vaccination Commission as of 26.08.2021, in addition to the A-W-C-Y strain vaccination, vaccination against the meningococcal strain B prevalent in Central Europe with meningococcal group B vaccine (Bexsero® or Trumenba®) is also recommended.
Contrary to the original recommendation, it is recommended that this mandatory vaccination is not carried out before but on the day of the first infusion in new patients with complement inhibitors, as the application of these vaccines can increase haemolysis up to a haemolytic crisis and serious thromboses. Primary antibiotic prophylaxis with an antibiotic (Penicillin V 2x 1 Mega) can be carried out in accordance with the prescribing information until the vaccine is administered (see also Figures 3 to Figures 7).

Indications:

Eculizumab is indicated for the treatment of symptomatic patients with PNH [53]. The drug should be used primarily in cases of complications such as hemolysis-related transfusion requirements (hemolysis-related anemia should be differentiated from cytopenia-related anemia), after thromboembolic events, PNH-associated renal insufficiency, abdominal pain crises or other severe PNH-related symptoms. In some cases, it is difficult to assess whether the clinical situation is symptomatic or asymptomatic. The authors of the guidelines will be happy to clarify such a situation if questions arise. Treatment with eculizumab is usually given as a long-term therapy, as the underlying cell defect, measured by the clone size, is not causally affected by the administration of the antibody. Previously symptomatic patients (see chapter 4.1) can benefit from long-term eculizumab therapy by reducing disease-associated symptoms and complications such as renal dysfunction and pulmonary hypertension. Survival is also significantly improved in long-term observation according to real-world data compared to the age- and gender-adjusted expected value [24]. In addition to the original preparation (Soliris®), two biosimilar products (Bekemv® from Amgen and Epysqli® from Samsung-Bioepis) have now been approved for eculizumab with proof of comparable efficacy. All three products are also approved for the same indication in children and adolescents.

Ravulizumab has also been approved since 07/2019 for the treatment of hemolysis in adult and pediatric patients with PNH if one or more clinical symptoms are present as an indication of high disease activity, as well as in patients who are clinically stable after having been treated with eculizumab for at least the past 6 months (see Figure 4 and technical information on ravulizumab). Due to the significantly longer half-life of ravulizumab (50 vs. 11 days), maintenance therapy can only be given every 8 +/- 1 weeks [61].

Use of ravulizumab (see also Information for healthcare professionals):

- Weight-dependent dosage
- Dilution 1:1 with NaCl 0.9%
- Shelf life: 24 hours in the refrigerator
- Shelf life at room temperature 5 hours
- Intravenous infusion with 0.2 µm filter over 25 to 45 minutes, followed by "post-rinsing"
- Half-life 49.7 days
- Maintenance therapy every 8 weeks ± 7 days

There are no data on the use in pregnant women or in patients with a higher dose of eculizumab in the previous therapy.

Crovalimab was approved by the EMA for the treatment of PNH on August 22, 2024. Crovalimab is indicated in patients with symptomatic hemolysis and clinical signs indicating high disease activity, and in patients who are clinically stable after being treated with a complement C5 inhibitor for at least six months.

Crovalimab can therefore be applied subcutaneously in small volumes during the maintenance phase. Patients can do this themselves. Therapy is initiated with intravenous administration**.** There are no data on the use of crovalimab in pregnant women.

Use of Crovalimab (see also Information for healthcare professionals):

- Available in tubes 1 ml corresponding to 170 mg, 2 ml corresponding to 340 mg
- Weight-dependent dosage
- Day 1: 1,000 mg i.v. for body weight 40 to <100 kg; 1,500 mg for bw ≥100 kg

- Day 2, week 2,3,4: 340 mg s.c. for all equally)
- From week 5: 680 mg s.c. for bw 40 to < 100 kg, 1020 mg for bw ≥ 100 kg
- Serum concentration for complete C5 inhibition: 100 µg/ml
- Estimated half-life 53.1 days
- Bioavailability after subcutaneous application 83.0 %
- Dilution for intravenous administration with 100 or 200 ml NaCl 0.9%, rate 500 mg/30 minutes
- The use of an in-line filter is recommended for intravenous administration
- Shelf life: in the refrigerator
- Shelf life at room temperature temporarily up to 20 hours unproblematic
- Shelf life of the solution diluted for intravenous administration Temperature from 2 to 8°C 4 hours
- Intravenous infusion with 0.2 µm filter over 25 to 45 minutes, followed by "post-rinsing"

When switching therapy from eculizumab and ravulizumab to crovalimab, it should be noted that temporary formation of immune complexes (DTDC = drug-target-drug complexes) may occur due to the different binding epitopes. These immune complexes vary in size if the preceding antibodies (eculizumab/ravulizumab) are still present, with a maximum in the antigen-antibody equilibrium stage from around 1 week after the start of crovalimab, followed by a gradual decrease in manifestations [65]. The sites of manifestation are mostly skin vasculitis and, more rarely, arthralgias. In mild manifestations, temporary treatment with a steroid-containing skin cream is sufficient. Systemic steroids (prednisolone 20 to 30 mg) are also recommended for more severe symptoms.

The sub-study on switching from ravulizumab to crovalimab with special consideration of the development and effect of DTDC has not yet been conclusively evaluated. It should be taken into account that due to the long half-lives of both substances, the symptoms caused by DTDC may be more severe and longer lasting when switching a patient from ravulizumab to crovalimab.

6.2.3.2 Risks and problems associated with inhibition of complement component C5 (eculizumab, ravulizumab or crovalimab)

- Due to the inhibition of the terminal complement pathway, there is an increased risk of infection with capsule-forming bacteria, especially meningococci [66]; accordingly, a meningococcal vaccination should be given at the start of treatment (see chapter 6.2.3.6).
- If there are indications of an acute meningococcal infection (especially fever, but also headache with nausea or vomiting, neck stiffness, skin rash, confusion), seek immediate medical clarification as part of the risk plan (emergency card!). Furthermore, a stand-by therapy with 750 mg ciprofloxacin or amoxicillin/clavulanic acid is advisable if there are indications of a meningococcal infection (sudden fever, vigilance disorders, circulatory insufficiency, etc.).
- During treatment with eculizumab/ravulizumab/crovalimab, the blood count, reticulocytes, hemolysis parameters (especially LDH and bilirubin), iron parameters (especially ferritin), PNH clone size, folic acid, vitamin B_{12} and monospecific Coombs test to detect components of complement factor C3 on the erythrocyte surface should be checked regularly as an indication of extravascular hemolysis [67].

- Clinically relevant breakthrough hemolysis is characterized by a relevant increase in LDH levels and clinical symptoms. These may be due to underdosing (pharmacokinetic) or to complement-amplifying conditions (pharmacodynamic; e.g. infections, pregnancy) (see chapter 6.2.3.8).
- If hyper-immunoglobulins are administered at the same time, the induced hypercatabolism of both eculizumab and ravulizumab can lead to a drop in therapeutic antibody levels and thus to breakthrough hemolysis. The affected patients should therefore be closely monitored for breakthrough hemolysis or receive an additional dose of eculizumab or ravulizumab.
- If treatment with eculizumab or ravulizumab is discontinued, blood counts and hemolysis parameters should be monitored closely for early detection of any serious hemolysis or hemolysis-associated complications [68].

6.2.3.3 Assessment of the treatment response to the C5 inhibitors

Due to their mechanism of action, the anti-C5 antibodies eculizumab, ravulizumab and crovalimab can only influence the intravascular hemolysis of PNH. Extravascular hemolysis only occurs during therapy with C5 inhibitors and therefore remains unaffected and determines the clinical response under these therapies in addition to other factors (severe concomitant bone marrow failure, CR1 polymorphism, etc.). The assessment of the response under C5 complement inhibition can be evaluated on the basis of transfusion requirements, LDH, hemoglobin levels and the reticulocyte count (absolute value) [67]. The hematologic response to therapy in PNH patients was developed by the EBMT's severe aplastic anemia working party (SAAWP) and should be the basis for assessing both the individual response before a possible change in therapy and the benchmark within clinical trials.

Table 6: Possible classification of hematologic response under anti-complement therapy for PNH

Addressing	Transfusion requirements	Hemoglobin	LDH*	Reticulocytes
Complete response	None	≥12 g/dl	≤1.5x ULN	**and** ≤150/nl
Very good response	None	≥12 g/dl	>1.5x ULN	**or** >150/nl
Good response	None	≥10 g/dl - <12 g/dl	**A. ≤1**.5x ULN **B.** >1.5x ULN	Exclusion AA/BMF°
Partial response	None/occasionally (≤2 Eks every 6 months)	≥8 g/dl - <10 g/dl	**A. ≤1**.5x ULN **B.** >1.5x ULN	Exclusion AA/BMF°
Minor response^	None/occasionally (≤2 Eks every 6 months)	<8 g/dl	**A. ≤1**.5x ULN **B.** >1.5x ULN	Exclusion AA/BMF°
	Regularly (3-4 every 6 months)	<10 g/dl		
	Reduction by ≥50%	<10 g/dl		
No response^	Regularly (>6 every 6 months)	<10 g/dl	**A. ≤1**.5x ULN **B.** >1.5x ULN	Exclusion AA/BMF°

Legend:
ULN=Upper limit of normal range, AA=Aplastic anemia, BMF=Bone marrow failure syndrome
**A. and B. are subgroups without or with relevant intravascular hemolysis*
°Bone marrow diagnostics are particularly recommended for reticulocytes below 60/nl ^For patients who refuse transfusion of ECs: Minor response: Hemoglobin ≥6 g/dl - <8 g/dl, no response: Hemoglobin <6 g/dl
For assessment, the median value of hemoglobin, LDH and reticulocytes should be assessed over 6 months

6.2.3.4 Extravascular hemolysis under terminal complement inhibition

Following the introduction of terminal complement inhibition for the treatment of symptomatic hemolytic PNH, a significant improvement in patient survival was achieved. A major reason for this improvement is the favorable effect on abnormal thrombophilia, which is also life-terminating in many patients. Under effective terminal complement inhibition, patients experience a shift in hemolysis from intravascular to extravascular. This extravascular hemolysis (EVH) can become clinically relevant in some patients and might necessitate a switch to (or addition of) a proximal complement inhibitor.

The background for this shift of complement activity is the accumulation of products of the proximal cascade on the GPI-deficient erythrocytes - but protected by C5 inhibition - which lack the CD59 molecule as a terminal complement regulator and the CD55 molecule for regulation of the proximal cascade. When terminal complement inhibitors are used as medication, C3 fragmental products are found as C3d and C3c on the surface of the defective erythrocytes. This allows the opsonization and elimination of these cells by macrophages, which express the corresponding complement receptors and are mainly localized in the liver (Kupffer cells) [69, 70]. In contrast to intravascular hemolysis (IVH), this process largely takes place outside the blood vessels.

In about one third of patients, significant EVH leads to pronounced symptoms such as persistent anemia up to persistent transfusion dependency, reticulocytosis and an obviously associated significant impairment of the general condition with pronounced fatigue [40]. When considering persistent anemia under terminal complement inhibition, marrow insufficiency and the effects of possible persistent breakthrough hemolysis must be differentiated. Favoring factors for the degree of expression in carriers of the characteristics of extravascular hemolysis are variants in the genome of complement receptor 1 (CR1) on the erythrocytes and complement component 3 (C3) [71]. Overall, however, it must be noted that the manifestation of extravascular hemolysis is more of a quantitative difference. Minimal EVH under terminal complement inhibition can also occur below the detection level. For example, the products C3c and C3d can also be detected at a lower density by flow cytometry in less symptomatic patients under terminal complement inhibition.

6.2.3.5 Inhibitors of the proximal complement cascade

Danicopan is a recently approved substance. Danicopan is a small molecule with a high affinity for factor D as part of the amplification pathway of the proximal complement cascade. As an inhibitor of factor D, danicopan is administered orally TID as an add-on to ongoing therapy with the C5 inhibitors eculizumab or ravulizumab. In a group of patients who continued to achieve a hemoglobin level of less than or equal to 9.5 g/dL with significant reticulocytosis under terminal inhibition, this additional administration led to a significant improvement in hemoglobin levels and freedom from transfusion [72- 74]. The combined treatment also led to a significant improvement in general condition and fatigue. Due to the high molecular turnover of factor D in blood plasma, oral Danicopan must be taken three times a day. Danicopan has been approved since April 23, 2024 for the treatment of PNH patients with residual hemolytic anemia under terminal complement inhibition as an add-on to eculizumab and ravulizumab.

- In the clinical studies conducted, patients experienced a high improvement in quality of life.
- Due to the continued therapy with the terminal inhibitor (eculizumab or ravulizumab), this therapy variant is relatively safe with regard to potential breakthrough hemolysis. This means that even if the oral intake of danicopan is irregular, no severe breakthrough hemolysis is to be expected.

- Regular intake of Danicopan three times a day requires a lot of commitment from the patient.
- The persistent reticulocytosis without complete mean normalization in the alpha study suggests an incomplete overall effect on extravascular hemolysis in the patients. The same applies to the incomplete regression of the C3d load on the GPI-deficient erythrocytes.
- Danicopan is available as 50 mg and 100 mg film-coated tablets
- The recommended starting dose is 150 mg 3 x daily at intervals of 8 hours
- Depending on the clinical response, the dose can be increased to 200 mg TID (this dose increase was carried out in about 70% of patients in the Alpha study)
- Danicopan is a BCRP inhibitor (Breast Cancer Resistance Protein), which means that drug interactions occur when BRCP substrates such as rosuvastatin and sulfasalazine are administered at the same time. P-gp (P-glycoprotein) substrates such as fexofenadine, edoxaban, digoxin and dabigatran also interact.
- No dose adjustment is required for patients with mild renal impairment
- If GFR < 30 ml/min/1.73 m^2 , the starting dose should be reduced to 100 mg 3 times a day and the increased dose to 150 mg 3 times a day.
- If there is a clinically significant increase in ALT, a gradual reduction of the dose reached twice daily for 3 days or once daily for 3 days is recommended. If the increase continues or clinical symptoms persist, discontinuation of therapy should be considered.

Iptacopan as a small molecule, which binds and inhibits the active site of the serine protease factor B from the amplification pathway of the proximal complement cascade. It can achieve sufficient bioavailability by oral administration. Iptacopan was also originally introduced as an oral add-on in clinical trials for the treatment of extravascular hemolysis. Due to the pharmacokinetics of iptacopan and the very favorable pharmacokinetics, the phase III approval study APPLY was designed without the simultaneous administration of the terminal inhibitor. Patients with persistent anemia under terminal complement inhibition with a hemoglobin level below 10.0 g/dl and significant reticulocytosis were included. In this study too, there was a significant improvement in hemoglobin levels and a drop in reticulocytes to the normal range on average. It was also noted that the proportion of GPI-deficient erythrocytes had equalized to that of neutrophils and monocytes, which indicates that the ongoing hemolysis is controlled. The nearly normalization of fatigue values after switching to Iptacopan should also be emphasized. Iptacopan is also approved for the treatment of naive PNH patients (see chapter 6.2.3.7)

Iptacopan has been approved by the EMA for the treatment of hemolytic PNH since 01.06.2024.

- In all clinical studies conducted, patients experienced a high gain in quality of life within the observation periods after switching from terminal complement inhibition.
- The complete normalization of the mean reticulocyte count and the equalization of the proportion of GPI-deficient erythrocytes to that of neutrophils and monocytes during treatment with Iptacopan demonstrates the effective suppression of haemolysis.
- Particularly in view of the sometimes dramatic increase in GPI-deficient erythrocytes, there is a need for high patient adherence and compliance for regular use of the medication.
- Only a few breakthrough hemolyses have been reported in the clinical studies conducted to date [75] (11 events/100 patient-years in the APPLY study, 5.2 events/100 patient-years in the APPOINT study). These were described as mild in four cases, moderate in five cases and severe in one case (in the context of a severe corona infection)

- When limiting complement inhibition to the proximal side using Iptacopan, special adherence and compliance of PNH patients is required to avoid severe breakthrough hemolysis due to incorrect drug intake.
- The significant decrease in the mean reticulocyte values to normal values within the clinical studies indicates that hemolysis suppression has been fully achieved.
- Iptacopan is available as a 200 mg capsule to be taken twice daily at 12-hour intervals.
- In patients switching from eculizumab to iptacopan, treatment with iptacopan should not be initiated later than 1 week after the last dose of eculizumab.
- In patients switching from ravulizumab to iptacopan, treatment with iptacopan should be initiated no later than 6 weeks, but preferably 4 weeks after the last dose of ravulizumab.
- No dose adjustment is required for mild to moderate liver and kidney dysfunction. There are currently no data available on the use in severe renal dysfunction (dialysis) or liver dysfunction.
- The concomitant use of iptacopan with strong inducers of CYP2C8, UGT1A1, PgP, BCRP and OATP1B1/3 has not been clinically studied. Therefore, concomitant use is not recommended due to the possibility of reduced efficacy of Iptacopan.
- In vitro data showed that iptacopan has the potential for time-dependent inhibition of CYP2C8 and may increase the exposure of sensitive CYP2C8 substrates, such as repaglinide, dasabuvir or paclitaxel. The concomitant use of iptacopan and sensitive CYP2C8 substrates has not been clinically studied. Caution should be exercised when concomitant use of iptacopan with sensitive CYP2C8 substrates is required.
- When used concomitantly with clopidogrel (a moderate CYP2C8 inhibitor), the Cmax and AUC of iptacopan increased by 5% and 36% respectively. When used concomitantly with cyclosporine (a strong OATP1B1/1B3 inhibitor and a PgP and BCRP inhibitor), an increase in the Cmax and AUC of iptacopan of 41% and 50%, respectively, was observed. In the presence of iptacopan, the Cmax of digoxin (a P-gp substrate) increased by 8 %, while the AUC remained unchanged. The Cmax and AUC of rosuvastatin (an OATP substrate) did not change in the presence of iptacopan.
- When administered orally, peak plasma concentrations of iptacopan are reached approximately 2 hours after administration. When using the recommended dosing regimen of 200 mg twice daily, steady-state is reached within approximately 5 days, with low accumulation (1.4-fold). The pharmacokinetics of iptacopan are characterized by low to moderate inter- and intra-individual variability.
- The half-life ($t_{½}$) of Iptacopan after administration of 200 mg Iptacopan twice daily at steady-state is approximately 25 hours.
- A switch in the case of significant extravascular hemolysis under eculizumab or ravulizumab is best carried out in the middle of the last dosing interval (see above), i.e. one week after the last eculizumab and 4 weeks after the last ravulizumab administration.

Pegcetacoplan, a direct inhibitor of C3 and C3b, which together with the fragment Bb and properdin forms the C5 convertase from the alternative complement pathway, is the longest-approved substance from the series of proximal complement inhibitors. In a phase III study, in PNH patients with persistent anemia (hemoglobin < 10.5 g/dl) treated with eculizumab and significant reticulocytosis, the superiority of pegcetacoplan compared to eculizumab was shown in the improvement of anemia, freedom from transfusion and also in the quality of life of the patients [76- 79]. Pegcetacoplan has been approved since December 2021 for patients who have had symptomatic anemia due to extravascular hemolysis for at least three months under C5 blockade. However, newly occurring bone marrow failure should be excluded. In the meantime, approval has been extended to therapy naive patients (see chapter 6.2.3.7)

Treatment is initiated within the so-called four-week "run-in phase" [79]. The C5 inhibitors are given overlapping with pegcetacoplan for four weeks, as in the Pegasus study. Patients receive two further doses of eculizumab for two weeks each, while ravulizumab is given for the last four weeks of the last treatment interval. The dosage of pegcetacoplan is 1080 mg. This dose is administered subcutaneously by pump in a volume of 20 ml.

- As a pegylated polypeptide, the substance must be administered parenterally and has been developed for subcutaneous administration. The single dose is 1,080 mg, dissolved in 20 ml of liquid. The volume is administered subcutaneously by pump over 30 minutes. A video is available online to help with application (Aspaveli® application video | Sobi Austria). In the USA, application of the drug via the Enfuse system has already been approved, which significantly simplifies application. This form of application is still pending in Europe.
- Patients can administer the medication themselves. However, in order to achieve continuity and safety, a high level of patient adherence and reliability is required.
- After switching from the inhibitor of the terminal complement cascade to pegcetacoplan, a significant improvement in quality of life was achieved in the clinical studies relevant for the approval of the drug.
- In the Pegasus pivotal study, breakthrough hemolysis was observed in some patients, who therefore discontinued participation in the study in accordance with the protocol. Depending on the substance class, breakthrough hemolysis can be more intense with proximal inhibition. The rational approach to treating and avoiding breakthrough hemolysis has recently been published (see chapter 6.2.3.8).
- Pegcetacoplan is available in 20 ml vials containing 1,080 mg. Each vial also contains 820 mg sorbitol.
- Pegcetacoplan is administered twice weekly as a subcutaneous infusion of 1,080 mg. The twice-weekly dose must be administered on day 1 and day 4 of each treatment week.
- In the first 4 weeks, pegcetacoplan is administered to patients switching from the terminal complement inhibitor as twice-weekly subcutaneous doses of 1,080 mg in addition to the current dose of the C5 inhibitor in order to minimize the risk of hemolysis if treatment is discontinued abruptly. This is currently only tested for eculizumab, which is given twice in the joint run-in phase, and ravulizumab, for which the start of pegcetacoplan falls in the second half (4 weeks before the next dose). Patients must discontinue the C5 inhibitor 4 weeks after starting pegcetacoplan and then continue treatment as monotherapy with pegcetacoplan.
- The dosing regimen can be changed to 1,080 mg every three days if necessary (e.g. day 1, day 4, day 7, day 10, day 13, etc.) (see chapter 6.2.3.8).
- The typical infusion time is about 30 minutes (for a two-site infusion) or about 60 minutes (for a single-site infusion). The infusion must be started immediately after drawing up this medicine into the syringe. It must be administered within 2 hours of preparing the syringe.
- In healthy volunteers, the median time to reach maximum concentration (tmax) after a single subcutaneous dose is between 108 and 144 hours (4.5 to 6.0 days).
- The bioavailability of a subcutaneous dose of pegcetacoplan is estimated at 77% based on PK population analyses.
- The median effective elimination half-life (t1/2) is 8.0 days as estimated by population pharmacokinetic analysis.
- Severe renal impairment (creatinine clearance < 30 ml/min) had no effect on the pharmacokinetics (PK) of pegcetacoplan; therefore, no dose adjustment of pegcetacoplan is

required in patients with renal impairment. No data are available on the use of pegcetacoplan in patients with ESRD requiring dialysis.

- The safety and efficacy of pegcetacoplan in patients with hepatic impairment have not been studied; however, no dose adjustment is recommended as hepatic impairment is not expected to affect the clearance of pegcetacoplan.
- No drug-drug interaction studies have been conducted. Based on in vitro data, pegcetacoplan has a low potential for clinically relevant drug interactions.

6.2.3.6 Required vaccinations and procedure for initiating terminal and proximal complement inhibitors

Before initiating treatment with all inhibitors of the complement cascade, the patient's vaccination status must be checked. Vaccination against capsule-forming bacteria (Figure 3 to Figure 7), meningococci (both tetravalent vaccine against bacterial strains A, C, W and Y, as well as against strain B) is obligatory for therapy with the terminal inhibitors. In the case of proximal inhibitors, vaccinations against pneumococci (obligatory) and Haemophilus influenzae (recommended) are also required. Since the vaccines, especially those against meningococcus type B, are mandatory, an accompanying antibiotic prophylaxis is recommended at the beginning. Depending on the medication used, a time recommendation can be found in the following Figure 3 to Figure 7.

Figure 3: Therapy regimen for the use of eculizumab

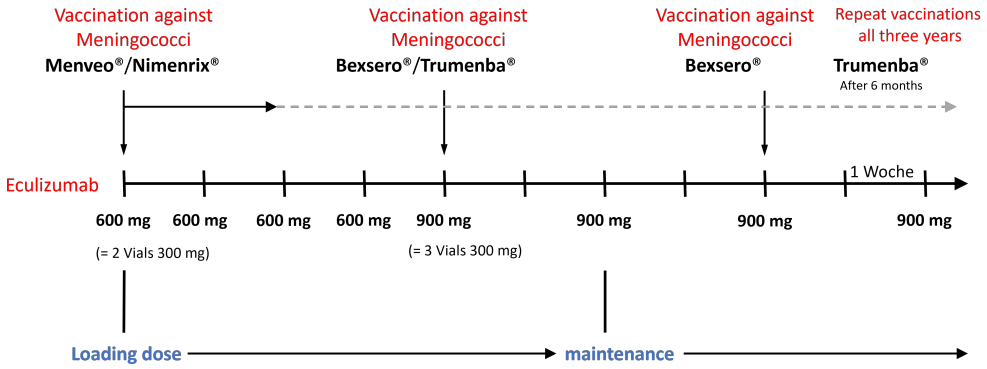


Figure 4: Therapy regimen for readjustment to ravulizumab

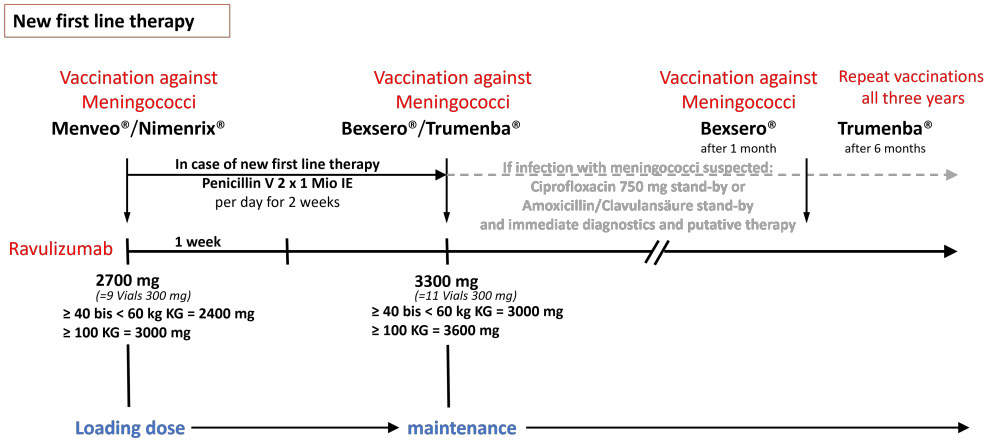


Figure 5: Therapy regimen for readjustment to crovalimab

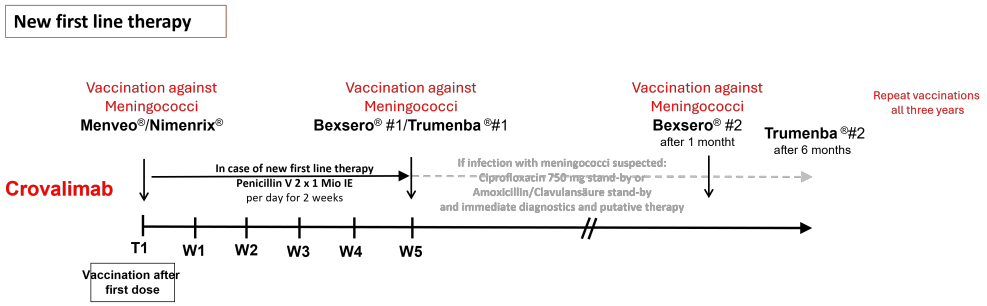


Figure 6: Therapy regimen for readjustment to pegcetacoplan

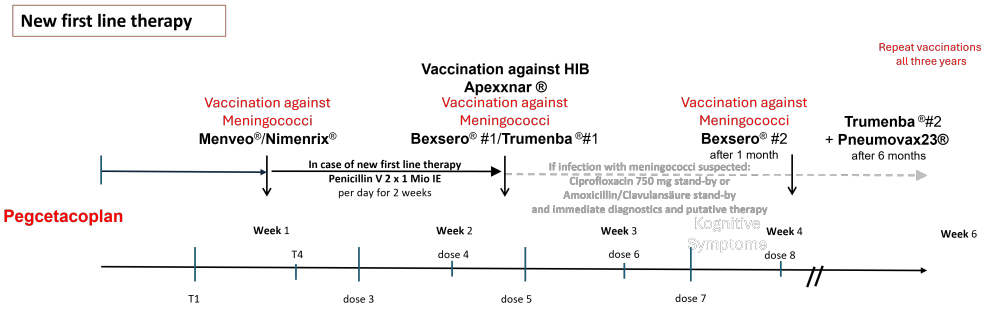
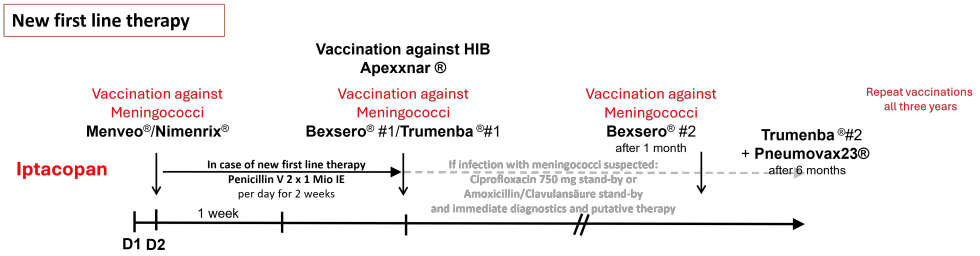


Figure 7: Therapy regimen for readjustment to Iptacopan



When using or switching PNH patients from a terminal to a proximal complement inhibitor, an additional vaccination against pneumococci (conjugate vaccine PVC20 Apexxnar/Prevenar 20) is mandatory. Vaccination against Haemophilus influenzae type b (Hib) is also recommended prior to switching (see respective information for healthcare professionals). This corresponds to the recommendations of the AGIHO (Working party for infectious diseases of the German Society of Hematology /Medical Oncology). The vaccine against Haemophilus (e.g. Hiberix®) outside of a multiple vaccination is approved in Germany, but must be imported. It is advisable to contact either the responsible health insurance company or a specialized vaccination center. Bridging the gap until the new inhibitor is saturated is not necessary for patients who have already been treated with the terminal inhibitor, as the saturated inhibition status is already given for these patients. Therefore, the vaccination can precede the switch and not trigger any hemolytic crises during ongoing terminal inhibition.

When vaccinating untreated PNH patients with a proximal complement inhibitor (Iptacopan or Pegcetacoplan) as part of a new treatment, vaccination can take place either immediately or, preferably, one day after the initial application. All vaccines except those against meningococci can be administered together. In addition, the recommendations of the Vaccination Commission on vaccination of immunocompromised patients also apply, with special consideration of influenza and other respiratory viral diseases.

For all patients under complement inhibition, it should always be considered that they carry a so-called stand-by medication with them (pill in the pocket) in order to be able to take an initial dose of antibiotics in the event of a sudden onset of fever and malaise. Amoxycillin/clavulanic acid 1,000/250 mg is suitable for this purpose. In the event of an allergy to aminopenicillins, ciprofloxacin 750 mg can also be used.

6.2.3.7 Proximal complement inhibition as first-line treatment

The approval of Iptacopan and the extension of the approval of Pegcetacoplan allow both substances to be used as first-line treatment for hemolytic PNH. For some patients, this treatment is certainly a very attractive therapeutic choice, which allows self-controlled intake and is also associated with a reliable improvement in quality of life.

However, there is insufficient evidence to decide which patients should be treated primarily with terminal complement inhibition and which with proximal inhibition in the first-line setting. Although the "PRINCE" approval study for pegcetacoplan was a randomized phase III study, the comparator arm was not the previous standard of care (SOC) in terms of terminal complement inhibition. The APPOINT study for Iptacopan was a non-randomized phase II study.

Genomic germline determination of the variants in the genes for complement receptor 1 (CR1) and complement component 3 (C3) is currently not generally available. Therefore, it is not clear which patients with hemolytic PNH will develop significant and impairing extravascular hemolysis under terminal complement inhibition, even before starting first-line therapy.

There is currently insufficient long-term data available on proximal complement inhibition, particularly with regard to infections and unexpected immunological effects. Particular caution is therefore advisable with regard to the new substances, especially in this line of therapy. Patients should be included in the International PNH Registry for early detection of adverse effects for all affected patients if possible. This can be done as part of a consultation with a specialized center with access to the registry.

6.2.3.8 Breakthrough hemolysis under proximal complement inhibition

Breakthrough hemolysis (BTH) refers to the recurrence of IVH under ongoing complement inhibition. To date, there is no globally standardized definition of BTH; the following definition was established as a possible definition at an expert consensus meeting [80]: A drop in hemoglobin ≥1.5 g/dL with a new increase in a previously stable LDH to more than 1.5 times the upper limit (or a significant increase from 2 x previously measured values) and the appearance of new or worsening of previous symptoms.

A basic distinction is made between pharmacokinetic (PK BTH) and pharmacodynamic BTH (PD BTH). PK BTH is caused by suboptimal dose levels of a complement inhibitor, while PD BTH is triggered by a so-called complement amplifying condition (CAC), even if this cannot always be precisely identified. A recent analysis of 182 patients from Italy and the UK shows that BTH is described in 75% of all PNH patients, with infections being the most common cause of BTH.

In principle, BTH can occur under any complement inhibition. With isolated proximal complement inhibition, however, the impressive effectiveness of proximal complement inhibition and the protection of GPI-deficient erythrocyte populations from IVH and EVH regularly lead to an expansion of the erythrocyte clone to values > 90%. On the other hand, the gap on the proximal complement side can lead to an amplifying activity of the C3/C5 convertase, so that the formation of membrane attack complexes can increase dramatically. Due to these two factors, the BTH can thus be significantly more impacting regards to the degree of hemolysis and the drop of hemoglobin [81].

The management of a BTH cannot be fully described in this guideline. In urgent cases, it is advisable to contact the authors of the guideline. In addition, consultation is possible as part of the four-weekly online conference for patients with AA or PNH (team-based online conference. If you are interested, please contact Ms. Schifflers; Tel.: +49 241 80-38664; jschifflers@ukaachen.de).

In principle, a distinction should be made between PK BTH and PD PTH, if clinically feasible. If the complement inhibitor concentration is suboptimal, e.g. the dose of eculizumab can be increased or the dose interval shortened. This is typically not necessary with ravulizumab and other reasons (e.g. genetic polymorphisms in complement regulators, CACs) should be taken into account in the case of recurrent BTH with ravulizumab.

Real-life data [82] and a post-hoc analysis of PEGASUS patients [83] are now available for the treatment of BTH with proximal complement inhibition with pegcetacoplan. In acute BTH with a significant LDH increase (> 2 x upper normal value) and Hb drop of more than 2 g/dl, in addition to treatment of the CAC (if possible), a dose intensification with doses of 1080 mg pegcetacoplan over three days (alternatively a single iv dose) followed by intensified maintenance with three doses per week should be carried out. However, this intensification above the administration every three days as well as the intravenous application is outside the existing approval. An RBC transfusion is also advisable in relevant clinical cases; in addition to optimizing haemoglobin, this also dilutes the PNH clone and thus reduces the number of unprotected erythrocytes. LDH, Hb, bilirubin and reticulocytes should be closely monitored in patients with BTH.

Therapeutic anticoagulation should also be considered in the case of relevant BTH and is clearly indicated in the event of a thromboembolic event.

In individual cases, additional administration of a terminal complement inhibitor may be necessary to treat massive BTH and clinical deterioration despite dose intensification (outside the approval); in patients with massive BTH, it is urgently recommended to contact a center experienced in the treatment of PNH patients.

There are still no standardized guidelines for BTH with iptacopan, but treatment of a possible CAC, transfusion, fluid administration and possibly the need for additional terminal complement inhibition (outside the approval) are potential treatment strategies here.

Since Danicopan is given in combination with eculizumab or ravulizumab, data to date show only a few self-limiting BTH events.

6.3 Special situations

6.3.1 Pregnancy

Pregnancies in PNH patients are associated with high maternal and fetal mortality with an increased risk of atypical thrombosis (11.6 % and 7.2 %, respectively [84]) and represent a high-risk pregnancy in any case [85, 86]. Successful pregnancies with eculizumab have been reported in case reports without evidence of teratogenicity, although the number of cases is small. Ongoing treatment with eculizumab should not be interrupted if a pregnancy is diagnosed. If PNH patients wish to have children, treatment with eculizumab should be considered on an individual basis after weighing up all risks and complications. A dose adjustment (up to 900 mg weekly) may be necessary due to breakthrough hemolysis during pregnancy. In contrast to eculizumab, there is insufficient data on the use of ravulizumab and all other newly approved complement inhibitors in pregnancy. Treatment with these substances should therefore be avoided for the time being if the patient is pregnant or already planning genetic counseling.

7 Psychosocial care and rehabilitation

The majority of patients affected are in employment, have a life expectancy of decades ahead of them and face the challenge of integrating this chronic illness into their lives. Hence, support should also include psychosocial measures. These include not only those directly affected by the disease (the patients), but also relatives and the patients' social environment. Professional discussions with psychologists make it easier to process the diagnosis and deal with the disease. Patients should be made aware of services offered by self-help groups/patient advocacy groups.

Patients should be informed at an early stage about the possibilities of outpatient and inpatient rehabilitation measures as well as other entitlements arising from social law. With regard to the choices of a rehabilitation clinic, the patient's wishes should be taken into account (§9 SGB IX).

Another challenge is the social and financial burden of being diagnosed with hemolytic PNH. Relief and restructuring in the workplace, hardship regulations, tax relief, etc. can help effectively. PNH or PNH/AA patients are entitled to a severely disabled person's pass. The effects of the disease and the current need for therapy are particularly relevant when determining the degree of disability.

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13 Links

A video on performing bone marrow puncture was produced by the Elisabethinen Hospital in Linz for training purposes and for patients (https://www.youtube.com/watch?v=3RgGmErO50g).

Ring tests: http://www.instandev.de/ringversuche/

Aplastic Anemia Association http://www.aplastische-anaemie.de/category/verein/

Light Cell Foundation: https://www.lichterzellen.de/

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15 Disclosure of Potential Conflicts of Interest

according to the rules of the responsible Medical Societies.