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REVIEW ARTICLE

Pharmacological Therapies in Paroxysmal Nocturnal Haemoglobinuria: Focus on Complement Inhibition

Richard J. Kelly[1,2] · **Matthew Holt**[1,2] · **Jeff Szer**[3,4]

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Abstract

Paroxysmal nocturnal haemoglobinuria (PNH) is an ultra-rare acquired genetic stem cell disorder based on a mutation in the PIGA gene that results in susceptibility of resulting blood cells to complement-mediated intravascular haemolysis (IVH). In countries where anti-complement therapy is available, pharmacological treatments have transformed this disease from a highly morbid and sometimes lethal disorder. The first treatment developed was the terminal complement (C5) monoclonal antibody inhibitor eculizumab, in 2002. This has been largely supplanted by a longer-acting antibody, ravulizumab, targeting the same binding site on C5. These agents significantly modify the natural history of the disease by reducing the risk of thrombosis, the most lethal complication of PNH, as well as reducing transfusion dependence and improving renal function, quality of life and probably, survival. Other terminal inhibitors available include eculizumab biosimilars, crovalimab, pozelimab and cemdisiran (combination). Despite this, a proportion of patients develop extravascular haemolysis (EVH) based on the accumulation of C3 components on these PNH blood cells, which no longer undergo IVH because of C5 inhibition. This has led to the development of proximal complement inhibitors, which have been generally successful at reducing this iatrogenic complication, improving haemoglobin concentrations, reducing transfusion dependency and improving quality of life. Currently available proximal inhibitors (and their targets) are pegcetacoplan (C3), danicopan (Factor D) and iptacopan (Factor B). While effective, as with all other complement inhibitors, there is a risk of breakthrough IVH with their use and approaches to manage this complication are being developed.

Key Points

The introduction of complement inhibitors, initially targeting C5, transformed the clinical landscape of patients with paroxysmal nocturnal haemoglobinuria (PNH) and improved symptoms, burden of care and survival.

The later introduction of inhibitors of proximal complement has further improved disease control in many patients and alternative routes of therapy, other than intravenous infusion, have been developed.

Original and newer therapies have generally been very safe and well tolerated and there is ongoing development of newer approaches to the control of PNH.

✉ Jeff Szer
jeff.szer@mh.org.au

[1] St James's University Hospital, Leeds, Yorkshire, UK

[2] University of Leeds, Leeds, Yorkshire, UK

[3] Department of Clinical Haematology, Peter MacCallum Cancer Centre and The Royal Melbourne Hospital, Melbourne, VIC, Australia

[4] University of Melbourne, Melbourne, VIC, Australia

1 Introduction

Paroxysmal nocturnal haemoglobinuria (PNH) is an ultra-rare, acquired blood disorder with an estimated disease incidence of 0.35 cases per 100,000 people per year and an estimated prevalence of 3.81 per 100,000 people [1]. Paroxysmal nocturnal haemoglobinuria occurs on a background of bone marrow failure, usually aplastic anaemia (AA), with affected individuals experiencing chronic intravascular haemolysis (IVH) and an increased risk of thrombosis [2].

Symptoms experienced in PNH are due to uncontrolled complement activation. The complement system is part of the innate immune system and is an important part of the body's defence against pathogens. The complement cascade is composed of multiple, small molecules which, when activated, cause a chain reaction of protein activation leading to the formation of a C5 convertase on the surface of the invading pathogen. This in turn, leads to the generation of the membrane attack complex (MAC), the end product of complement activation, which forms pores in the cell surface resulting in cell death [3, 4]. Complement can be activated by antibody complexes binding directly to invading pathogens (classical pathway), mannose-binding lectin and ficolins, binding to the bacterial surface (lectin pathway), or the hydrolysis of the complement protein C3 due to direct contact with cell surfaces (alternative pathway) [5]. Whilst complement activation is a vital component of the immune system, it is important that it is regulated to prevent damage to host tissues.

This paper describes the underlying pathophysiology of the disease and the rationale for pharmacological therapies in PNH with the focus on complement inhibition.

2 Acquired Genetic Defect

The majority of cases of PNH are caused by a mutation of the PIGA gene [6–8]. PIGA is required for the formation of the glycophosphatidyl (GPI) anchor in the endoplasmic reticulum, before the GPI anchor is transported to the cell surface where it is used by many proteins for their expression on the cell surface [9]. Two of these GPI-dependent proteins, decay accelerating factor (DAF, CD55) and membrane inhibitor of reactive lysis (MIRL, CD59), prevent cells from attack by complement. The CD55 regulates complement on the cell surface by preventing the formation of C3 convertases, limiting amplification of the complement pathway [10]. In the absence of CD55, uncontrolled C3 convertase formation occurs via the alternative pathway. Alternate pathway C3 convertases cause terminal complement activation by the formation of C5 convertases and cleavage of further C3 proteins, leading to further C3 convertases causing a rapid upregulation of complement activation to fight infections, the amplification loop [11]. CD59 prevents the formation of the MAC, thereby preventing IVH [3, 4].

In PNH, typically but not always, a reduction or an absence of these complement regulatory proteins leads to IVH. The PNH erythrocytes have either a partial deficiency of GPI-anchored proteins (type II cells) or a complete deficiency (type III cells) [12]. Type III cells are 15–25 times more sensitive to lysis and type II cells 3–5 times more sensitive than normal type I erythrocytes [13]. Typically, the degree of IVH experienced is due to the proportions of type II and type III erythrocytes present with individuals with larger type III clones more susceptible to severe IVH.

2.1 Rare Mutations Detected in PNH

Rare cases of PNH have been identified due to homozygous mutations in other PIG genes, such as in PIGB, PIGM, PIGT and PIGV mutations [14–19], but these mutations need to be present in both alleles (although not for PIGB) in the same cell to affect GPI production. However, the majority of PNH cases occur due to PIGA mutations. The PIGA gene is located on the X chromosome and is mono-allelically expressed due to lyonization in women, a somatic mutation in one PIGA gene is sufficient to disrupt GPI assembly leading to complete loss of function [17].

3 Disease Pathogenesis

The PIGA mutations do not provide PNH cells with an intrinsic survival advantage when compared with normal cells [20] and in isolation are not enough to cause PNH, as PNH cells can be identified in healthy individuals at very low levels [21]. It is likely that PNH only develops on a background of bone marrow failure, usually AA, where there is an environment that allows the PNH clone to expand. Small clones of PNH cells are present in approximately one-third of cases of AA [22], but the mechanism underlying the expansion of PNH clones remains unclear. It is believed that the immune attack on the bone marrow in AA by autoreactive CD8+ T-lymphocytes requires a GPI-linked protein and that the absence of one of these proteins protects PNH cells from this attack [23]. This "escape model" allows for haematopoiesis to be rescued, albeit with PIGA-deficient haemopoietic cells. Other possible explanations for clonal expansion in PNH include a GPI-linked protein working to suppress cell growth or to provide PNH cells with resistance to apoptosis, or clonal selection and clonal expansion

occurring by two separate events [24, 25]. Further research is required into the pathogenesis of PNH to understand why the proportion of PNH cells increases in some individuals and not others.

4 Natural History and Supportive Measures

Paroxysmal nocturnal haemoglobinuria is panethnic, seen equally in males and females and can occur at any age with the most common age of diagnosis being in the fourth decade of life [26, 27]. Misdiagnoses or long delays in making a diagnosis are common, with a recent study of 509 patients reporting one-third of cases where a diagnosis was made after a period greater than 12 months [28]. Early detection of the disease is vital to allow patients to receive targeted therapy at the appropriate time and reduce morbidity and mortality. It should be noted that not all patients with a diagnosis of PNH require anti-complement therapy even where it is available and these patients require regular evaluation for progression of the disease. Prior to the availability of anti-complement therapy, life expectancy was greatly reduced, being reported as a median survival time of between 10 and 22 years from diagnosis [2, 28]. Treatment was supportive in nature, including transfusions and oral folic acid, which is recommended for all causes of haemolytic anaemia. Assessment of iron status was, and still remains, important with some patients requiring iron replacement due to persistent haemoglobinuria, whereas those receiving red cell transfusions may need consideration of iron chelation or venesection—although the latter is not possible in transfusion-dependent patients.

5 Diagnosis and Disease Classification

Flow cytometry is the preferred technique to diagnose PNH and is routinely performed to evaluate the size of erythrocyte and neutrophil and monocyte clones [29]. The PNH neutrophil clone size is believed to be the best marker for evaluating the proportion of bone marrow cells affected, as the erythrocyte clone can be reduced due to both IVH and red cell transfusions [30]. Due to variations in PNH diagnostic methods, guidance on a standardised method of flow cytometric analysis was proposed by the International Clinical Cytometry Society [29].

Lactate dehydrogenase (LDH) is found in almost all animal tissues and plays an important role in metabolic pathways. In PNH, elevated LDH levels can be caused by IVH—and LDH is an important biomarker of disease activity. An LDH level of $> 1.5 \times$ the upper limit of normal (ULN) in untreated patients, has been shown to be predictive of both an increased risk of thrombosis and of mortality in PNH [31].

In 2005, a classification of PNH with 3 categories was proposed [32]. This incorporated classical PNH (symptoms of haemolytic PNH with IVH but no signs of any other bone marrow pathology), PNH in the setting of another specified bone marrow disorder (symptoms of IVH but with, or previously had, an underlying bone marrow failure, AA or myelodysplasia [MDS]) and subclinical PNH (small PNH clones with no clinical or laboratory evidence of ongoing haemolysis).

6 Clinical Features

In general, the higher the proportion of PNH cells present, the greater the likelihood that an individual will have symptoms of the disease. Patients experience chronic IVH with episodes or "paroxysms" of more severe haemolysis and symptoms [33]. Lysis of erythrocytes in the circulation leads to release of free haemoglobin, which is avidly bound to nitric oxide, causing smooth muscle dysfunction [34]. This may lead to dysphagia abdominal pain and erectile dysfunction in men. Dyspnoea occurs in part due to anaemia but may also be a symptom of pulmonary hypertension, caused by increased pulmonary vascular resistance, as a consequence of smooth muscle dysfunction [35]. Symptoms experienced due to chronic IVH vary between individuals, but tiredness and fatigue are commonly experienced. This leads to a reduced ability to work and to perform everyday activities [36].

Impaired kidney function is evident in around two-thirds of patients and has been shown to contribute to up to 18% of PNH-related deaths [26, 37]. This may be in part due to ciclosporin when it is used to treat underlying AA. The kidneys develop tubulointerstitial inflammation and haemosiderin deposition in the renal tubules. Other potential causes of renal dysfunction in PNH include the occurrence of microvascular thromboses and decreased vascular tone in renal arterioles due to reduced nitric oxide levels [38]. The classical presentation in PNH is of haemoglobinuria present in the morning, with the urine colour becoming lighter as the day goes on. Haemoglobinuria may be present all the time or just periodically.

Prior to anti-complement therapy, thrombosis was the leading cause of death in PNH, described in 40–67% of deaths [39]. Thromboses are generally venous in nature, especially affecting hepatic veins (Budd-Chiari syndrome), although arterial thromboses have been documented to occur in around 15% of experienced events [2].

Thrombosis is the presenting feature in PNH in around 12.5% of patients [28] and in over one-fifth of cases, thromboses occurring at multiple sites in the one patient

are described [39]. Symptoms experienced due to thromboses, depend on the thrombosis site as well as the extent of the thrombosis. The aetiology of thrombosis in PNH is believed to be multifactorial and potential causes include platelet activation and subsequent release of procoagulant microparticles, IVH and nitric oxide depletion, endothelial activation and dysfunction, reduced fibrinolysis and increased inflammatory cytokines [39]. In patients not receiving anti-complement therapy, prevention of thrombosis with anticoagulation can be considered as a primary preventive measure. In these patients, a PNH neutrophil clone of > 50% is strongly predictive of thrombotic risk, and anticoagulation has been recommended to reduce this risk [40]. Careful selection and counselling of patients considered for anticoagulation is important to weigh up the potential benefit and the risk of major haemorrhage, especially as many patients are also thrombocytopenic.

7 Treatment Strategies Targeting Complement

Dysregulation or increased activation of complement leads to a wide variety of inflammatory and autoimmune diseases including PNH, systemic lupus erythematosus, atypical haemolytic uraemic syndrome and acute organ rejection [41, 42]. The concept of targeting complement as a treatment for PNH is therefore attractive. Replacing CD59 on the surface of PNH cells was considered [43]. This method would theoretically protect PNH cells from complement without negatively impacting on complement function. In the mouse model of PNH, administration of recombinant CD59 to mice resulted in sufficient CD59 on the mouse erythrocytes to prevent complement-mediated damage [44, 45]. Soluble recombinant CD59 therapy was not pursued due to the clinical success of terminal complement inhibition.

7.1 Complement Protein C5 Inhibition

The complement protein C5 was thought to be an ideal point in the complement system to target in PNH as all 3 activation pathways come together to cleave C5 into its active components, C5a and C5b (Fig. 1). By targeting C5 and preventing terminal complement activation, the function of the proximal components of complement was expected to remain intact. This was felt to be protective against most infective organisms, as inherited deficiencies of terminal complement are only associated with an increased frequency of infection with encapsulated microorganisms [46]. Figure 1 and Table 1 show where the different available complement inhibitors act on the complement pathway.

7.1.1 Eculizumab

Eculizumab is a 148-kilodalton molecule composed of murine complementary determining regions within human germline framework regions and immunoglobulin (Ig)G2 and IgG4 heavy chain constant regions [47]. Eculizumab binds to the complement protein C5, preventing its cleavage to C5a and C5b. It therefore stops the assembly of the MAC and by doing so prevents IVH. Eculizumab is administered as an intravenous 600-mg infusion weekly for the first 4 weeks followed by 900-mg eculizumab from Week 5 and then every 14 days thereafter.

A pilot study evaluating eculizumab in 11 patients was undertaken in 2002 [48]. This was a 12-week open-label study, which showed a reduction in IVH and transfusion requirements. The mean transfusion rate reduced from 2.1 to 0.6 units per patient per month and there was a 96% reduction in the number of haemolytic episodes that occurred. This led to further studies on the benefit of eculizumab in patients with PNH.

The pivotal phase 3 TRIUMPH study was a multinational, multicentre, double-blinded study, comparing eculizumab to placebo over a 26-week period in 87 patients with PNH who had received four or more transfusions in the previous year and had a platelet count of at least 100×10^9/L [49] (Tables 1, 2). The primary endpoints of stabilisation of haemoglobin levels in the absence of transfusions and the number of transfusions required were met. Stabilisation of haemoglobin levels was observed in 49% of the eculizumab-treated group but none in the placebo group. Fifty-one percent of patients on eculizumab remained transfusion independent for the duration of the study, whereas transfusions were needed in all of the placebo group population. Lactate dehydrogenase levels reduced and remained at near normal levels in the eculizumab group and both fatigue and quality-of-life scores were significantly improved in those receiving eculizumab.

A further study, SHEPHERD, allowed for eculizumab to be assessed in patients with PNH with lower platelet counts (down to 30×10^9/L) and those that were transfused less frequently [50] (Tables 1, 2).

These trials led to the approval of eculizumab for treating patients by the Food and Drug Administration (FDA) in 2007.

The patients enrolled in these clinical studies were evaluated as a common group to try to determine the effect of eculizumab on the occurrence of thrombosis [51]. The thrombotic event (TE) rate from diagnosis to starting eculizumab was compared with the TE rate since starting eculizumab. The TE rate fell from 7.37 events/100 patient-years prior to

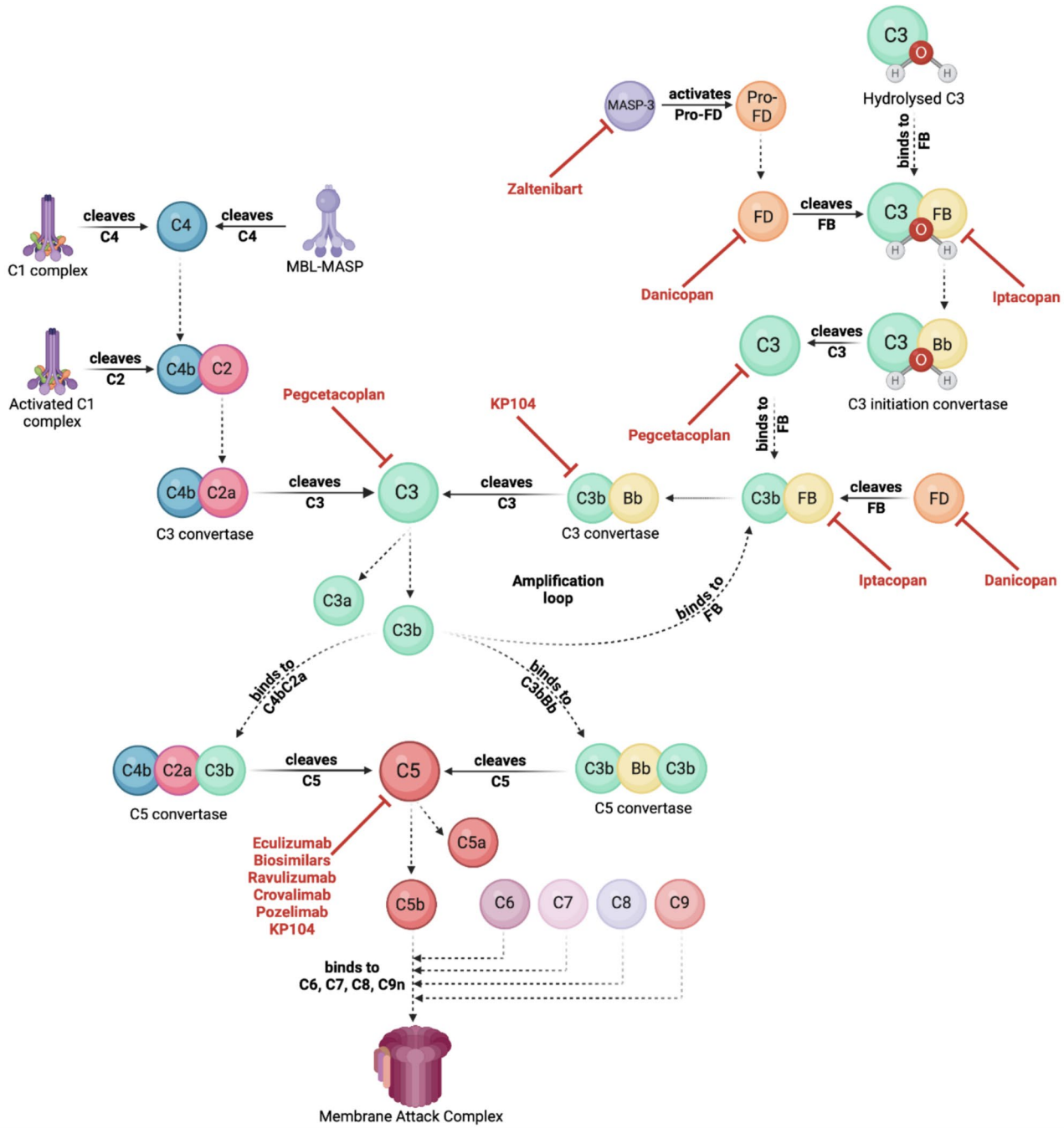

Fig. 1 Illustration of the complement cascade, highlighting the targets of available complement inhibitors (pictured in red). *FB* factor B, *FD* factor D, *MASP* MBL-associated proteases, *MBL* mannose-binding lectin

eculizumab to 1.07 events/100 patient-years on eculizumab therapy. With the reduction in TEs experienced, the subsequent impact of eculizumab on mortality has been evaluated.

Eculizumab is the only therapy with long-term data—other therapies have been approved for use in patients with PNH only since 2018. A study of 79 consecutive patients treated with eculizumab showed dramatically improved survival, with no difference in mortality between patients on eculizumab and the normal population over an 8-year period [52]. A further analysis of 509 patients with PNH treated over a 20-year period, mainly with eculizumab but also with ravulizumab, has confirmed improvement in patient outcomes [28].

Table 1 List of complement inhibitors in paroxysmal nocturnal haemoglobinuria

Complement inhibitor and its target	FDA/EMA status	Administration	Half-life	Standard or weight-based dosing in adults	Loading dose	Standard maintenance dosing
Eculizumab and its biosimilars SB12 and ABP959 (C5)	Approved	IV	11.3 ± 3.4 days	Standard	600 mg weekly for 4 weeks	From Week 5: 900 mg Q2W
Ravulizumab (C5)	Approved	IV	49.6 days	Weight-based	2400 mg for 40–60 kg 2700 mg for 60–100 kg 3000 mg for ≥100 kg	2 weeks after loading: 3000 mg for 40–60 kg 3300 mg for 60–100 kg 3600 mg for >100 kg Q8W
Crovalimab (C5)	Approved	IV loading SC maintenance	53.1 days	Weight-based	Day 1 (IV): 1000 mg for 40–100 kg 1500 mg for ≥100 kg Days 2, 8, 15, 22 (SC): 340 mg for all weights Day 29: 680 mg for 40–100 kg 1020 mg for ≥100 kg	680 mg for 40–100 kg 1020 mg for ≥100 kg Q4W
Pegcetacoplan (C3)	Approved	SC	8 d	Standard	Not required	1080 mg twice per week
Iptacopan (Factor B)	Approved	Oral	25 h	Standard	Not required	200 mg twice per day
Danicopan (Factor D)	Approved	Oral	9 h	Standard	Not required	150–200 mg three times per day
Pozelimab (C5)	Investigational	IV loading SC maintenance	13.5 to 14.1 days	Weight-based loading Standard maintenance	30 mg/kg (IV)	400 mg Q4W
Cemdisiran (C5)	Investigational	SC	Unavailable	Standard	Not required	200 mg Q4W
Zaltenibart (MASP-3)	Investigational	IV	Unavailable	Weight-based	Not required	Under investigation Dosing Q8W
KP104 (C5 and C3 convertase)	Investigational	IV loading SC maintenance	15.7 days (1)	Under investigation	1200 mg (IV)	Under investigation Dosing Q2–4W

C3 3rd component of complement, *C5* 5th component of complement, *IV* intravenous, *MASP* mannan-binding lectin serine protease, *Q2W* every 2 weeks, *Q4W* every 4 weeks, *Q8W* every 8 weeks, *SC* subcutaneous

7.1.2 Ravulizumab

Ravulizumab is a humanised monoclonal IgG antibody inhibiting C5, which was bioengineered from eculizumab. Ravulizumab differs from eculizumab by 4 amino acid substitutions. These changes in amino acid sequence allow ravulizumab both an increased affinity for the neonatal Fc receptor (FcRn) and endosomal dissociation of the ravulizumab-C5 structure, increasing its half-life to 4 times that of eculizumab [53]. Ravulizumab is administered as an intravenous infusion every 8 weeks using a weight-based regimen. Ravulizumab has been evaluated in patients naïve to complement inhibition (301 study) and in those who were clinically stable on eculizumab (302 study) [53, 54] (Tables 1, 2).

In patients naïve to complement inhibition (301 study), 246 patients were enrolled in a 2:1 randomisation between ravulizumab and eculizumab for a 26-week period and met its two primary endpoints: transfusion avoidance and normalisation of LDH with ravulizumab being non-inferior to eculizumab for both endpoints. In patients who were stable on eculizumab therapy (302 study), the primary endpoint was percentage change in LDH and again ravulizumab achieved non-inferiority to eculizumab. Data on mortality

Table 2 Clinical trial data of complement inhibitors in paroxysmal nocturnal haemoglobinuria

Complement inhibitor	Pivotal clinical trial (year of published data)	Main eligibility criteria (abridged)	Study design	Population numbers	Key outcomes
Phase 3 clinical trials					
Eculizumab [49]	TRIUMPH NCT00122330 (2006)	Age ≥18 LDH ≥1.5× ULN ≥4 RBC transfusions in the prior 12 months Platelets ≥100 $\times 10^9$/L	Phase 3 Multicentre Randomised Double-blind Placebo-control	87 randomised (43 eculizumab, 44 placebo)	49% achieved haemoglobin stabilisation without transfusion; median 0 vs 10× RBC units transfused (eculizumab vs placebo) over 26 weeks
	SHEPHERD NCT00130000 (2008)	Age ≥18 LDH ≥1.5× ULN ≥1 RBC transfusions in the prior 2 years or anaemia-related symptoms Platelets ≥30 $\times 10^9$/L	Phase 3 Multicentre Open-label Single-arm	97 enrolle (single-arm eculizumab)	Eculizumab appeare safe and well tolerated and associated with a substantial reduction in haemolysis in all patients
Ravulizumab [54, 55]	Study 301 NCT02946463 (2019)	Age ≥18 Treatment-naïve LDH ≥1.5× ULN ≥1 PNH-related sign/symptom	Phase 3 Multicentre Randomised Open-label Active-control	246 randomised (125 ravulizumab, 121 eculizumab)	Ravulizumab was non-inferior to eculizumab in both transfusion avoidance and LDH normalisation
	Study 302 NCT03056040 (2019)	Age ≥18 Stable on eculizumab for ≥6 months LDH ≤1.5× ULN	Phase 3 Multicentre Randomised Open-label Active-control	195 randomised (97 ravulizumab, 98 eculizumab)	
Crovalimab [56–58]	COMMODORE 1: NCT04432584 (2024)	COMMODORE 1: Age ≥18 Stable on eculizumab for ≥24 weeks LDH ≤1.5× ULN	COMMODORE 1 & 2: Phase 3 Multicentre Randomised Open-label Active-control	COMMODORE 1: 89 randomised (45 crovalimab, 44 eculizumab)	COMMODORE 1: Crovalimab was well tolerated but type III hypersensitivity reactions occurred in 16%
	COMMODORE 2: NCT04434092 (2024)	COMMODORE 2: Age ≥18 Treatment-naïve LDH ≥2× ULN, ≥1 RBC transfusion in the prior 12 months		COMMODORE 2: 204 randomised (135 crovalimab, 69 eculizumab)	COMMODORE 2: Crovalimab was non-inferior to eculizumab in both transfusion avoidance and LDH normalisation
	COMMODORE 3: NCT04654468 (2023)	COMMODORE 3: Age ≥12 Weight ≥40 kg Treatment-naïve LDH ≥2× ULN ≥4 RBC transfusions in the prior 12 months	COMMODORE 3: Phase 3 Multicentre Open-label Single-arm	COMMODORE 3: 51 enrolled (single-arm crovalimab)	COMMODORE 3: Haemolysis control in 78.7% and transfusion avoidance in 51%

Table 2 (continued)

Complement inhibitor	Pivotal clinical trial (year of published data)	Main eligibility criteria (abridged)	Study design	Population numbers	Key outcomes
Pegcetacoplan [63–65]	PRINCE NCT04085601 (2023)	Age ≥18 Treatment-naïve LDH ≥1.5× ULN Hb below LLN	Phase 3 Multicentre Randomised Open-label Active-control	53 randomised (35 pegcetacoplan, 18 control)	Pegcetacoplan was superior to control for haemoglobin stabilisation and change from baseline in LDH
	PEGASUS NCT03500549 (2021)	Age ≥18 Stable on eculizumab for ≥3 months Hb <10.5 g/dL	Phase 3 Multicentre Randomised Open-label Active-control	80 randomised (41 pegcetacoplan, 39 eculizumab)	Pegcetacoplan was superior to eculizumab in improving haemoglobin levels
Iptacopan [70–72]	APPOINT: NCT04820530 (2024)	Age ≥18 Treatment-naïve LDH ≥1.5× ULN Hb <10 g/dL	Phase 3 Multicentre Open-label Single-arm	40 enrolled (single-arm iptacopan)	A ≥2 g/dL haemoglobin increase from baseline was met in 92% patients
	APPLY: NCT04558918 (2024)	Age >18 Stable on eculizumab or ravulizumab for ≥6 months Hb <10 g/dL	Phase 3 Multicentre Randomised Open-label Active-control	97 randomised (62 iptacopan, 35 continued existing C5i)	Iptacopan was superior to C5i for both ≥2 g/dL haemoglobin increase from baseline, and haemoglobin ≥12 g/dL without transfusion
Danicopan [75]	ALPHA trial NCT04469465 (2023)	Age ≥18 Stable on eculizumab or ravulizumab for ≥6 months Hb <9.5 g/dL ARC ≥120 $\times 10^9$/L	Phase 3 Multicentre Randomised Double-blind Placebo-control	73 randomised (49 danicopan and C5i, 24 placebo and C5i)	Danicopan add-on therapy to C5i was superior to placebo for increase in haemoglobin from baseline (2.94 g/dL vs 0.5 g/dL)
Pozelimab & cemdisiran [59]	ACCESS-1 NCT05133531 (2024) ACCESS-2 NCT05131204 (2024)	Age ≥18 Treatment-naïve LDH ≥2× ULN ≥1 PNH-related sign/symptom	Phase 3 Multicentre Randomised Open-label Active-control	48 randomised (25 pozelimab and cemdisiran, 23 ravulizumab)	By week 26, pozelimab and cemdesiran combination controlled LDH ≤1.5× ULN in 96–100%
SB12 (eculizumab biosimilar) [61]	NCT04058158	Age ≥18 LDH ≥1.5× ULN ≥1 RBC transfusion in prior 12 months or ≥1 PNH-related symptom	Phase 3 Multicentre Randomised Double-blind Active-control Crossover	50 randomised (25 SB12 then eculizumab, 25 eculizumab then SB12)	All primary, secondary, other efficacy endpoints, PK, PD, safety, and immunogenicity profiles were comparable between SB12 and eculizumab
ABP 959 (eculizumab biosimilar) [60]	NCT03818607	Age ≥18 y Stable on standard dose eculizumab for ≥6 months LDH <1.5× ULN Hb ≥9 g/dL	Phase 3 Multicentre Randomised Double-blind Active-control Crossover	42 randomised (20 ABP 959 then eculizumab, 22 eculizumab then ABP 959)	ABP 959 has comparative efficacy and safety compared with eculizumab

Table 2 (continued)

Complement inhibitor	Pivotal clinical trial (year of published data)	Main eligibility criteria (abridged)	Study design	Population numbers	Key outcomes
Phase 2 clinical trials					
Zaltenibart [78]	NCT05972967	Age ≥18 Stable on ravulizumab for ≥4 months Hb <10.5 g/dL	Phase 2 Multicentre Open-label Single-arm	13 enrolled (single-arm zaltenibart)	Zaltenibart monotherapy was well tolerated and resulted in mean haemoglobin level increase from baseline of 3.27 g/dL
KP104 [79]	NCT05476887	Age ≥18 LDH ≥2× ULN ≥1 PNH-related sign/symptom Hb ≤10 g/dL	Phase 2 Multicentre Open-label Dose-escalation arms	18 enrolled (6 patients per three dose-escalation cohorts)	KP104 was well tolerated with all patients achieving ≥2 g/dL haemoglobin increase from baseline

This table presents pivotal Phase 3 data where available and available data for investigational drugs. *ARC* absolute reticulocyte count, *C5i* C5 inhibitor, *Hb* haemoglobin, *LDH* lactate dehydrogenase, *LLN* lower limit of normal, *RBC* red blood cell *ULN* upper limit of normal

rates on ravulizumab are encouraging but are limited to 4 years of follow-up with 86% survival reported [55].

7.1.3 Crovalimab

Crovalimab is a humanised C5 inhibitor, which binds to a different epitope to that of eculizumab and ravulizumab. It is administered subcutaneously every 4 weeks. The phase 1/2 study, COMPOSER, evaluated crovalimab in healthy volunteers, complement treatment-naïve patients and patients previously treated with C5 inhibition [49]. Forty-three patients with PNH were evaluated in COMPOSER in an open-label extension period, and over a 3-year median period, crovalimab demonstrated control of IVH, haemoglobin stabilisation and reduced transfusion requirements. This led to phase 3 trials of crovalimab [56, 57].

Commodore 1 was a multicentre, randomised trial comparing crovalimab and eculizumab in C5 inhibitor treated patients [56] (Tables 1, 2). The primary endpoint was safety.

Drug-target-drug complexes causing type 3 hypersensitivity reactions occurred in 16% of patients switching from eculizumab to crovalimab. These reactions occurred because crovalimab binds to a different C5 epitope than eculizumab, resulting in both drugs binding to the same C5 proteins and forming immune complexes. These complexes can cause transient mild or moderate vasculitic skin reactions until the complexes have been removed from the circulation. The reactions all resolved, and most were mild or moderate in nature [56].

Commodore 2 was a multicentre, randomised, non-inferiority trial comparing crovalimab and eculizumab in patients with PNH who had not been treated with anti-complement therapy [57] (Tables 1, 2). The primary trial endpoints were the control of IVH (the LDH being ≤1.5 ULN) and the proportion of patients not requiring transfusion during the 24-week study. Crovalimab was non-inferior to eculizumab for both primary endpoints.

Commodore 3 was a single-arm trial that evaluated crovalimab in C5 inhibitor-naive patients with PNH [58] (Tables 1, 2). The primary trial endpoints, the mean proportion of patients with control of IVH and the difference in the proportion of patients not requiring transfusion during the 24-week study and the 24 weeks just prior to study entry, were met.

Overall, crovalimab has been shown to be a potential alternative to eculizumab and ravulizumab and is especially beneficial in patients with poor venous access or in countries where crovalimab can be self-administered rather than attending a hospital for an intravenous therapy. In cases where a patient changes therapy from either eculizumab or ravulizumab to crovalimab, or from crovalimab to eculizumab or ravulizumab, counselling regarding the risk of

immune complex formation, or a strategy to prevent this by temporarily using a complement inhibitor that doesn't target C5, could be considered.

7.1.4 Pozelimab and Cemdisiran

Pozelimab is a human immunoglobulin monoclonal antibody, which also binds to C5 preventing its cleavage to C5a and C5b. Cemdisiran is a N-acetylgalactosamine-conjugated small interfering RNA targeting C5 messenger RNA, which reduces the production of C5 in the liver. The combination of pozelimab and cemdisiran is currently being evaluated as a combination therapy for patients with PNH in clinical trials (NCT05133531 and NCT05744921) (Tables 1, 2) [59]. The rationale for this combination is to provide better control of IVH by reducing levels of C5 available so if a complement-activating event occurred (such as an infection) the aim would be that IVH would be less likely to occur.

7.1.5 Eculizumab Biosimilars

SB12 and ABP 959 are biosimilar to eculizumab. ABP 959 is a recombinant, humanised, monoclonal IgG antibody which, like eculizumab, binds to the complement protein C5 preventing its cleavage, and thereby preventing IVH. It was evaluated in a randomised, multicentre, double-blinded, two-period crossover study on patients with PNH who were on a stable dose of eculizumab [60] (Tables 1, 2). To evaluate the efficacy of ABP 959 compared to eculizumab, the study had 2 primary endpoints based on control of IVH, a comparison of the LDH at Week 27 in the study and the time-adjusted area under the effect curve (AUEC) of LDH from Week 13 to Week 27, Week 39 to Week 53, and Week 65 to Week 79. Lactate dehydrogenase levels at Week 27 showed a similarity of efficacy of ABP 959 when compared to eculizumab. The second primary efficacy endpoint for the crossover comparison was haemolysis, measured by the AUEC of LDH, which confirmed ABP 959 as being non-inferior to eculizumab [60].

SB12 is another humanised monoclonal antibody against the complement protein C5 which was assessed in a randomised, double-blind cross-over phase 3 study in complement inhibitor-naïve PNH patients to determine equivalency to eculizumab (Tables 1, 2). The two primary endpoints were LDH at Week 26 and the AUEC of LDH from Week 14 to Week 26 and Week 40 to Week 52. Lactate dehydrogenase levels at Week 26 and the AUEC of LDH showed SB12 and eculizumab to be equivalent [61].

7.1.6 Benefit and Unmet Needs in Targeting the Complement Pathway at C5

Targeting C5 has been the main treatment for patients with PNH since eculizumab was first administered in 2002. There are long-term data using eculizumab or ravulizumab showing an improvement in symptoms, [64, 65] a reduction in PNH-related complications and improved mortality. Vaccination against *Neisseria meningitidis* is important as there is a small but significant risk of overwhelming and potentially fatal meningococcal sepsis (around 0.35 events per 100 patient years) [28]. Vaccination against MenACWY and MenB vaccines are recommended at commencement of anti-complement therapy with booster doses periodically whilst on therapy. Patients must remain vigilant for signs and symptoms of infection so that they know to seek urgent medical attention. Antibiotics that cover meningococcal infection are essential if they develop a fever. The role of antibiotic prophylaxis is controversial. It has been adopted in some countries but not universally due to concerns regarding antibiotic resistance [62].

Crovalimab provides a subcutaneous alternative, which patients and clinicians may prefer, especially if venous access is difficult. Eculizumab biosimilars have shown equivalence to eculizumab and can prove a more cost-effective option in place of eculizumab for patients with PNH.

Anaemia can be due in part to co-existent bone marrow failure but is often due to extravascular haemolysis (EVH). Extravascular haemolysis is a phenomenon of treatment with C5 inhibition and was first described in 2009 [64]. Whilst terminal complement activation is inhibited, the earlier aspects of the complement cascade are not. Extravascular haemolysis occurs due to opsonisation of PNH erythrocytes by C3b, which mark the blood cells for removal from the circulation and destruction in the reticuloendothelial system (Fig. 2).

7.2 Proximal Complement Inhibition

To improve upon C5 inhibition, new therapies targeting earlier components of the complement pathway were developed. By inhibiting earlier components of complement activation, therapies could prevent IVH without causing EVH. The initial concern with this approach was related to the risk of infection and whether more infective complications would be observed by inhibiting the complement pathway earlier. Post-marketing studies of proximal inhibitors will be important to ensure both their efficacy and safety in a real-world setting. There have been no reported cases of *N. meningitidis* but longer-term data are still needed [67]. Patients are routinely vaccinated against *Haemophilus influenza B* and *Streptococcus pneumoniae* to help mitigate this potential

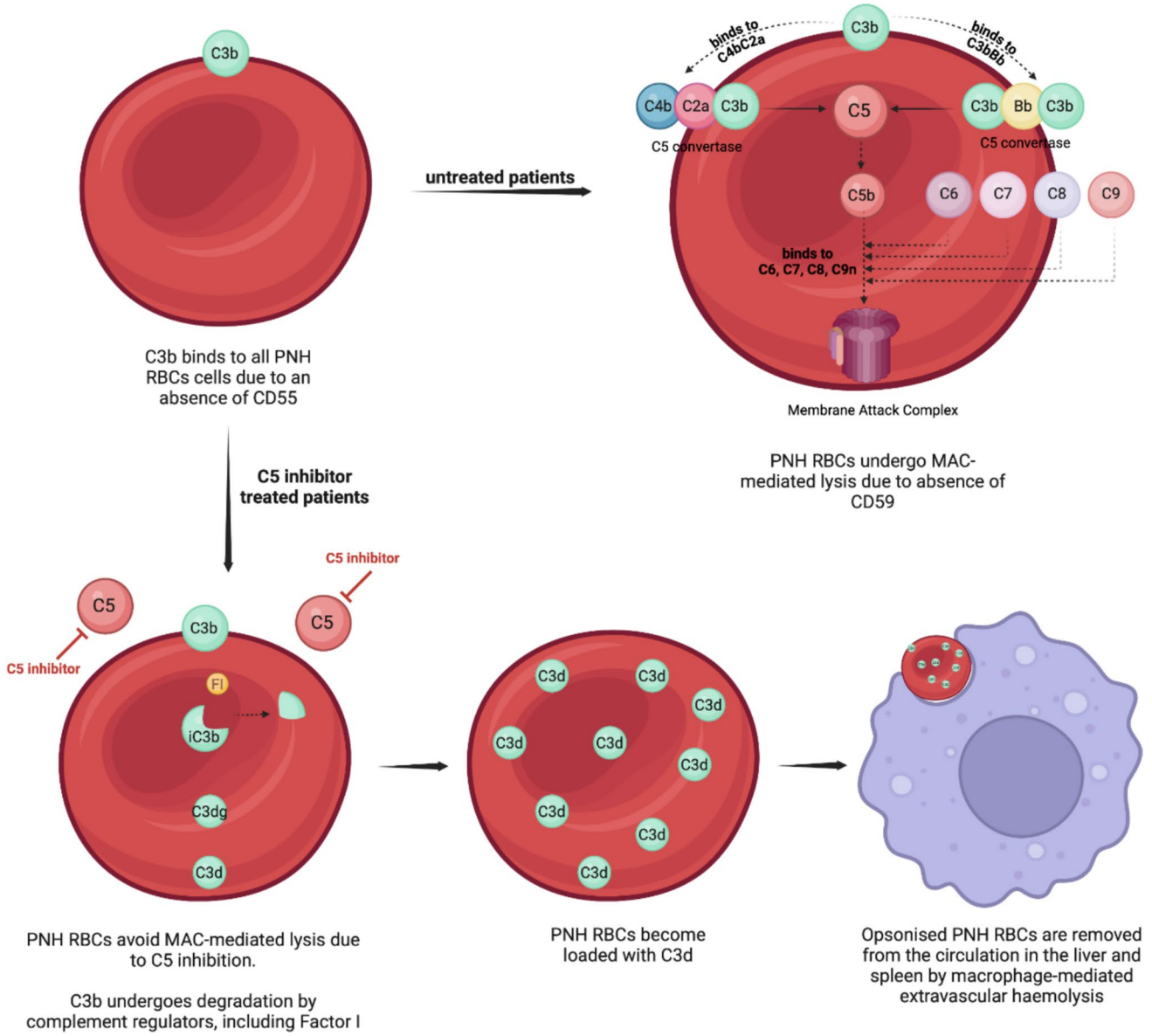

Fig. 2 Illustration of the mechanism of extravascular haemolysis in patients with PNH receiving C5 inhibitors. PNH red blood cells are deficient in complement regulators CD55 and CD59. In untreated patients, complement activation on PNH red blood cells leads to MAC-mediated intravascular haemolysis. C5 inhibitors prevent MAC formation, protecting PNH RBCs from intravascular lysis, but instead these cells become coated with C3b which is degraded to C3d through the action of factor I. C3d-loaded red cells are removed by macrophages in the liver and spleen via extravascular haemolysis. *FI* factor I, *MAC* membrane attack complex, *PNH* paroxysmal nocturnal haemoglobinuria, *RBC* red blood cells

risk. There are currently 3 proximal complement inhibitors which have undergone phase 3 clinical trials: pegcetacoplan, iptacopan and danicopan.

7.2.1 Pegcetacoplan

Pegcetacoplan is made up of two 15 amino acid cyclic peptides conjugated to a linear, polyethylene glycol molecule. It has two mechanisms of action; it binds to the complement protein C3 preventing it from cleaving to C3a and C3b, thereby preventing opsonisation of PNH erythrocytes by C3b, and it binds to C3b stopping C3b from being incorporated into C5 convertase complexes, preventing IVH. It is administered as a subcutaneous infusion at a dose of 1080 mg twice a week.

The PADDOCK trial, a phase 1b open-label pilot trial, and the PALOMINO trial, a phase 2a, open-label trial, assessed the safety and efficacy of pegcetacoplan in adult patients with PNH [67]. In PADDOCK, patients were given daily subcutaneous injections of pegcetacoplan 180

mg in cohort 1 (3 patients) and pegcetacoplan 270 mg in cohort 2 (20 patients), which could be increased to 360 mg if there was a suboptimal response. In PALOMINO (4 patients), dosing was as per cohort 2 of PADDOCK. The primary endpoints were mean change from baseline in haemoglobin, LDH, haptoglobin, and the number and severity of treatment-emergent adverse events. These early trials showed that pegcetacoplan improved haematologic parameters by controlling IVH in patients with PNH and led to further evaluation in phase 3 studies.

PRINCE was a phase 3, randomised, multicentre, open-label, controlled study to evaluate the efficacy and safety of pegcetacoplan against supportive care in complement inhibitor-naive patients with PNH in countries where complement inhibition was either not generally available or not approved for use [67] (Tables 1, 2). The coprimary end points, haemoglobin stabilisation (the avoidance of a >1 g/dL decrease in haemoglobin from baseline to Week 26) and change from baseline in LDH levels at Week 26, were met.

PEGASUS was a phase 3, open-label, controlled trial assessing the efficacy and safety of pegcetacoplan compared to eculizumab in adults with PNH with haemoglobin levels < 10.5 g/dL despite being on a stable dose of eculizumab for at least 3 months [68] (Tables 1, 2). The primary end point was the change in haemoglobin from baseline to Week 16 during the study with the study showing pegcetacoplan was superior to eculizumab in improving haemoglobin levels with a mean change from baseline of 2.37 g/dL with pegcetacoplan and a −1.47 g/dL with eculizumab, making a mean difference between treatments of 3.84 g/dL after 16 weeks of monotherapy. Reports of the real-world experience in treating patients with pegcetacoplan from the USA, UK and France are consistent with the clinical trial data, confirming its efficacy and safety profile [69, 70].

A trial comparing pegcetacoplan with eculizumab (the standard of care at the time) in complement inhibitor-naïve patients has not been performed.

7.2.2 Iptacopan

Iptacopan is an oral, proximal complement inhibitor. It is taken at a dose of 200 mg twice a day and it selectively inhibits factor B in the alternative pathway and the subsequent amplification of the terminal pathway.

An open-label phase 2 study in patients with PNH naïve to anti-complement therapies was carried out in 13 adult patients with a median age of 35 years (range 20–62). Patients with an LDH >1.5 ULN and a haemoglobin level of < 10.5 g/dL were enrolled. Patients received 25 mg iptacopan twice per day for 4 weeks followed by 100 mg twice a day for up to 2 years (Cohort 1) or 50 mg twice a day for 4 weeks followed by 200 mg twice a day for up to 2 years (Cohort 2). The primary endpoint was a reduction in LDH levels by 60% by Week 12. At the interim analysis, the 12 evaluated patients had a reduction in LDH > 60% compared to baseline and both patient cohorts showed clinically significant improvement in haemoglobin levels, and reduction in markers of haemolysis, with 11 patients remaining transfusion independent during the 12-week period [71].

A phase 2 trial evaluating the addition of iptacopan to eculizumab in patients with PNH with active haemolysis was also undertaken in 10 adult patients. Patients with an LDH >1.5 ULN were enrolled, with the mean LDH level being 2.15 × ULN. The primary endpoint was the change from baseline to Week 13 in LDH, with a significant reduction in mean LDH from 539 to 235 IU/L observed. A significant improvement in haemoglobin also occurred with the mean haemoglobin increasing by 3.19 g/dL over the 13-week study [72].

The phase 3 studies evaluating iptacopan in patients with PNH were APPOINT and APPLY (Tables 1, 2). APPOINT evaluated iptacopan in 40 adult, complement inhibitor-naïve patients over 24 weeks in patients with a mean haemoglobin < 10 g/dL and an LDH >1.5 × ULN. The primary endpoint of a ≥ 2 g/dL haemoglobin increase from baseline was met in 92% of patients. The mean change in haemoglobin levels from baseline was 4.3 g/dL [73]. It would have been more informative to have compared iptacopan with the standard of care therapy, C5 inhibition, but no such study has been performed.

The APPLY study assessed patients on eculizumab or ravulizumab with a mean haemoglobin < 10g/dL, with patients either continuing their C5 inhibitor or switching to iptacopan for a 24-week period. Primary endpoints were a ≥ 2 g/dL haemoglobin increase from baseline and a haemoglobin ≥ 12 g/dL without transfusion support. Iptacopan was superior to C5 inhibition for both primary endpoints with 51 of the 60 patients receiving iptacopan having a ≥ 2 g/dL haemoglobin increase from baseline and 42 of the 60 patients attaining a haemoglobin of ≥ 12 g/dL from baseline without red cell transfusions. In both studies, iptacopan was safe and well tolerated with minimal side effects observed [74].

The 48-week follow-up data have shown increases in total cholesterol and LDL cholesterol in patients taking iptacopan, with the mean increase from baseline to Week 48 being 0.3–0.9 mmol/L [74] in all patients. Despite this, mean cholesterol levels remained in the normal range and no direct link between iptacopan and cholesterol was found.

7.2.3 Danicopan

Danicopan is an oral factor D inhibitor. Factor D catalyses the cleavage of factor B to Ba and Bb in the alternative pathway. A phase 2 dose finding study of 10 adults with PNH naïve to anti-complement therapy was undertaken using danicopan as a single agent [75]. The primary endpoint was the

change in LDH from the start of the study to Day 28. The study showed a reduction in LDH levels but IVH was still observed. Danicopan was then evaluated in a phase 2 study of danicopan as an additional therapy in 12 adult patients with PNH on eculizumab with EVH [76]. Inclusion criteria included a haemoglobin of < 10 g/dL, receiving a red cell transfusion within the prior 12 weeks and being on a stable dose of eculizumab. The primary endpoint was the change in haemoglobin from the start of the study to Week 24. Eleven patients completed the 24-week treatment period with a mean haemoglobin increase of 2.4 g/dL observed. The positive results from this study led to danicopan being evaluated as an addition to eculizumab or ravulizumab in a phase 3 study, the ALPHA trial [77].

ALPHA was a phase 3, randomised, double-blind, placebo-controlled trial comparing danicopan with placebo in adult patients with PNH and EVH on eculizumab or ravulizumab (Tables 1, 2). The pre-specified interim analysis evaluated 63 patients, 42 on danicopan and 21 on placebo. The primary endpoint was met (change in haemoglobin from baseline at 12 weeks) with a 2.44 g/dL improvement in haemoglobin when compared with the placebo group. Transient elevations of aspartate aminotransferase and/or alanine aminotransferase were seen in 6/42 patients in the danicopan group. There were no safety concerns and the addition of danicopan to C5 inhibition was superior to placebo in this study [77].

7.2.4 Unanswered Questions Regarding Proximal Inhibition

No trial has directly compared the benefit of pegcetacoplan, iptacopan and danicopan add-on therapy in patients with PNH, and the entry criteria for the phase 3 trials in these therapies were different, making a direct comparison impossible. All 3 drugs have merit and have been well tolerated, with good safety profiles and have shown improvement in haemoglobin levels in patients with EVH. Pegcetacoplan has the longest duration of safety data available. Danicopan has the benefit of being combined with a C5 inhibitor, so if there is an issue with adherence to danicopan, there should be no increased risk of IVH and its consequences. However, it does mean patients receiving both oral and intravenous treatments. Iptacopan is the only oral monotherapy, which is an attractive option to both patients and clinicians. The importance of compliance with this medication and how to manage episodes when an oral treatment might not be possible, such as with vomiting or surgery, should be considered when using iptacopan.

7.2.5 Breakthrough Haemolysis

Breakthrough haemolysis (BTH) is the recurrence of IVH and symptoms of PNH and occurs either due to suboptimal complement inhibition or increased complement activity such as with infections or surgery. Breakthrough haemolysis can occur in patients on any complement inhibitor and it is important to identify early and treat the underlying cause. As EVH does not occur in patients on proximal inhibitors, recognition of BTH is especially important because the proportion of circulating PNH erythrocytes is higher and if BTH then occurs, the symptoms experienced can be more severe.

7.3 Other Treatments in Development

Zaltenibart is a selective IgG4 monoclonal antibody that binds to and inhibits mannan-binding lectin-associated serine protease-3 (MASP-3). Mannan-binding lectin-associated serine protease-3 is an upstream activator of factor D so inhibiting it may provide alternative pathway inhibition. The phase 2 trial evaluated 13 adult patients with PNH with a suboptimal response to ravulizumab [78]. One patient discontinued zaltenibart due to a treatment-emergent serious adverse event. Ten of the 12 patients received zaltenibart monotherapy and there was a mean increase in haemoglobin of 3.27 g/dL from baseline at Day 169 of the study. Zaltenibart monotherapy was well tolerated and resulted in sustained clinically meaningful improvements in haemoglobin levels.

KP104 is a bifunctional fusion protein combining a humanised anti-C5 monoclonal antibody with complement regulator factor H, thereby inhibiting both the alternative and the terminal complement pathways. Phase 2 results of 18 adult complement inhibitor-naïve patients demonstrated a haemoglobin increase of ≥ 2 g/dL from baseline in all 18 patients with 15 patients achieving a haemoglobin > 12 g/dL [79]. KP104 was well tolerated with a favourable safety profile with no serious adverse events or treatment-emergent adverse events described.

Phase 3 trials of both zaltenibart and KP104 are needed to confirm the benefit of these therapies for patients with PNH.

8 Conclusions

Eculizumab therapy revolutionised treatment for patients with PNH when it was approved in 2006. Since then, complement inhibition at C5 has been the "gold standard" therapy with long-term data supporting its safety and effectiveness in reducing morbidity and mortality. However, anti-complement therapy is expensive and is currently only available in some wealthy countries. More needs to be done to allow patients with PNH access to life-changing treatment,

irrespective of where they are in the world. Further research into the mechanism of clonal expansion in PNH is also important. If clonal expansion is inhibited, PNH could be prevented rather than treating the symptoms from IVH with complement inhibition.

Newer therapies have focused on improving haemoglobin levels and the convenience of treatment; longer-acting, intravenous treatments and subcutaneous and oral therapies empower patients to oversee treatment for their own illness. Earlier inhibition of the complement pathway prevents EVH occurring, allowing for improved haemoglobin levels whilst preventing IVH and its consequences. Additional data are needed on proximal complement inhibitors to confirm their long-term safety and efficacy in a real-world setting, as well as defining the appropriate management of BTH. To date, the only definite increased infection risk is that of meningococcal sepsis. The evolution of treatment in PNH should allow for individualised treatment to suit the needs of patients.

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Declarations

Conflict of interest RK: Alexion, Astra-Zeneca Rare Diseases: Speaker bureau, advisory boards and honoraria; Florio: honoraria; Novartis: research funding (to institute), speaker bureau, advisory boards and honoraria; Omeros: honoraria; Otsuka: honoraria; Roche: advisory boards and honoraria; Sobi: research funding (to institute), speaker bureau, advisory boards and honoraria. MH: Sobi: Research funding and honoraria; Novartis: honoraria; Alexion, Astra-Zeneca Rare Diseases: travel support. JS: Sobi: Speaker bureau, advisory board, honoraria and travel support; Alexion, Astra-Zeneca Rare Diseases: honoraria and speaker bureau; Novartis: advisory board, speaker, honoraria and travel support; Eli Lilly, honoraria; Takeda, honoraria.

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Consent to participate Not applicable.

Code availability Not applicable.

Consent to publish Not applicable.

Availability of data and materials Not applicable: data used to prepare this manuscript were all available in the published literature and references to data form the reference list.

Author contributions All authors participated equally in sourcing the data, writing the manuscript and correcting the manuscript. MH developed the figures.

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