Comparing Clinical Efficacy of C5, C3 and Factor B Inhibition in Paroxysmal Nocturnal Hemoglobinuria

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ABSTRACT

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, deadly, hematological disease that arises from the body's inability to regulate the complement system's attack on PNH RBCs which lack the ability to regulate ongoing complement activity resulting in RBC hemolysis, persistent anemia, extreme lethargy, and elevated risk for thrombosis. Among the treatment options available to patients and their physicians, three therapeutics specifically targeting the complement system are available on the market: ravulizumab, pegcetacoplan, and iptacopan. Each of these three treatments blocks a different portion of the complement cascade, C5, C3 and factor B respectively, resulting in variable efficacy. This paper explores and assesses the clinical efficacy of these three therapeutics through a collective analysis of each asset's pivotal phase 3 trials through: 1) a collection of primary and key secondary endpoints from each trial and 2) a table-based comparison of common reported PNH biomarkers drawn from the collective analysis. Both points of methodology are conducted in patients who had never received complement inhibition therapy previously and patients who had been switched from one form of complement inhibition to another. Based on the alternative pathway's ability to amplify convertase production, reasoning is provided as to why blocking certain nodes of the complement pathway displays higher efficacy than other nodes. Recommendations are put forward to further pursue research within these nodes for development of future therapeutics. Lastly, development of new complement-based therapeutics for PNH, such as Voydeya, is discussed.

Introduction

The Complement System

The complement system is one of the oldest, most innate immunological defense systems present within the human body. It contains more than 50 proteins that are responsible for initiating and mediating systemic cascades that regulate inflammation, total-body surveillance, and clearance of pathogens and debris via phagocytosis (Mastellos et al., 2023). There are three main pathways in which complement fulfills its function within the human body: 1) the classical pathway, 2) the lectin pathway and 3) the alternative pathway. The classical pathway functions through the detection of bound antibodies to pathogens, and subsequent binding of C1q complement to said antibodies at their fragment, crystallizable (Fc) portion (Mastellos et al., 2023). The lectin pathway becomes activated through the binding of soluble complement components known as mannose-binding lectins and ficolins (Mastellos et al., 2023). There are two categories of molecular markers that lectins and ficolins can bind to: 1. pathogen-associated molecular patterns (PAMPs) and 2. damage-associated molecular pathway (DAMPs). PAMPs are typically based on carbohydrate-based chemical identifiers found in bacteria and viruses that ficolins and lectin can bind to, eliciting a 'tagging' or opsonization of the pathogen for later recognition by phagocytic cells such as macrophages. DAMPs function in the same carbohydrate-based manner but differ from PAMPs. DAMPs differ because they originate from dying self-cells rather than external pathogens. Thus, DAMPs signal to the immune system that a cleanup of debris must take place and thus can also be used to tag dying cells via ficolin and lectin binding (Mastellos et al., 2023).

The alternative pathway (AP) not only may be triggered by the end-products of both the classical and the lectin pathways, but also through the steady state hydrolysis of C3 into C3H2O, which act as a platform for convertase production and thus lead to a rapid amplification of the complement cascade if a trigger is presented to the immune system (e.g. infection, genetic mutations, etc.) (Mastellos et al., 2023). Notably, the AP is an incredibly essential area of research that must be well understood to address complement disorders. Within the AP, molecules known as factors, such as factor B and factor D, play a vital role in complement-mediated elimination of pathogens, i.e. opsonization. Factor B can bind to C3b that has already been cleaved through the classical and/or lectin pathway. This will result in the creation of C3bB. Following the creation of this construct, factor D, a protease, will cleave factor B into two fragments, resulting in the creation of a C3bBb, a C3 convertase. This will result in an amplification loop, also known as spill over, as more C3 is cleaved, and thus more C3b is created, amplifying the process of C3 cleavage.

Crucially, all three pathways will converge to perform two distinct functions: 1) the cleavage of C3 and 2) the cleavage of C5. Each pathway's end product results in the creation of C3 and C5 convertases, which act as molecular scissors that snip central complement molecules, like C3, into smaller pieces such as C3a, C3b, C5a and C5b, which in turn contribute to further the immune response (Mastellos et al., 2023). Both C3a and C5a act as anaphylatoxins, which induce increased inflammation and vascular dilation within the area of infection, thereby eliciting greater recruitment of adaptive immune cells such as macrophages and neutrophils. C3b binds to pathogens based on their individual molecular signatures, thereby opsonizing, or tagging the pathogen for phagocytosis by C3a and C5a's recruited phagocytes. In a similar manner to C3b, C5b will attach to the surface of pathogens but elicit a different outcome. Instead of opsonization, C5b will create an anchor point for other complement molecules, such as C6, C7, C8 and C9, which will eventually come together to form the membrane attack complex (MAC). The MAC will create a pore within the wall of a pathogen damaged cell or susceptible cell, effectively breaching the pathogen's protective membrane. This will result in essential fluid component leakage and ultimately result in pathogen lysis (Mastellos et al., 2023).

Each component of the complement system is uniquely designed to identify, eliminate, and protect the host body from pathogenic invasion. However, while the complement system is extremely efficient at eliminating pathogenic threats, in some cases it can turn against the host body, such as in the disease paroxysmal nocturnal hemoglobinuria.

Paroxysmal nocturnal hemoglobinuria (PNH)

Paroxysmal nocturnal hemoglobinuria, or PNH, is a rare hematological disease that involves a defective gene known as phosphatidylinositol glycan class A or *PIGA* (Cançado et al., 2021). This mutation inhibits the ability of the body to produce phosphatidylinositol N-acetyl glucosaminyltransferase, subunit A, thereby impeding the production of GPI anchors. As a result of the lack of GPI anchors, two important complement regulators, CD59 or protectin and CD55 or decay accelerating factor (DAF), can no longer attach to and remain on the surface of RBCs (Peffault de Latour et al., 2024; Stern & Connell, 2019). CD59 prevents the formation of the MAC complex on RBC surfaces by inhibiting C5b binding, while CD55 prevents C3b-mediated opsonization of RBCs by inactivating C3 convertases (Sarmoko et al., 2023; Spendlove et al., 1999). Without the ability to anchor themselves to host cells using GPI anchors, CD59 and CD55 cannot regulate the attachment of complement to RBCs. As a result, C3b and C5b can then stick to healthy RBCs, leading to both RBC opsonization via C3b and the creation of the MAC complex on the surface of the RBC via C5b, which ultimately leads to both intravascular hemolysis (IVH) and extravascular hemolysis (EVH) in patients with PNH. Intravascular hemolysis is any kind of RBC destruction that occurs directly within the blood, leading to hemoglobinemia and hemoglobinuria. In IVH, acute release of free hemoglobin, normally protected by a cell membrane, results in platelet activation, smooth muscle contraction (depletion of nitric oxide or NO), and overall activation of the coagulation cascade. Extravascular hemolysis is a slower process of the destruction of RBCs outside of the vasculature, such as blood-filtering organs like the spleen or liver, by macrophages. When a macrophage identifies C3b/d coated RBCs, or dying or fragmented RBCs at these organ locations, they will take them up and destroy them, resulting in EVH. Patients with PNH also harbor a varied level of bone marrow insufficiency, increased risk of thrombosis due to

platelet attachment to fragmentary particles of lysed RBCs (schistocytes), severe anemia because of the complement-mediated hemolysis of their RBCs, and multifactorial (including inflammation) fatigue (Mastellos et al., 2023).

PNH Biomarkers

When assessing a patient for PNH, clinicians look for specific biomarkers or molecular signals within the body, as indicators of PNH. Within their initial diagnostic evaluation, clinicians will assess a patient's complete blood count (CBC), reticulocyte count, elevated hemoglobin levels, elevated levels of lactate dehydrogenase (LDH) and bilirubin in the blood and will typically perform peripheral blood flow cytometry to further characterize a patient's blood composition (Cançado et al., 2021; Brodsky 2024). Such indicators determine the health of a patient's blood. For example, LDH is a fundamental glycolytic enzyme that is found in all cells including RBCs. If it is found circulating in open blood, this is an indication to clinicians that RBCs are being ruptured. Similarly, hemoglobin is only found on RBCs, and thus if there is a decreased level of hemoglobin circulating within the blood, which is another indication that RBCs could be lysing. Such decrease in circulating hemoglobin could also be compounded further by patient anemia that lacks an environmental cause, pointing to hindered ability to carry oxygen due to possible RBC damage. Other signs of non-antibody mediated intravascular hemolysis that clinicians will look for include: an increased reticulocyte count greater than 1%, decreased haptoglobin, hemoglobinuria with pink and/or red urine, negative direct antiglobulin via the Coombs test or the DAT test, and evidence of organ damage that is caused by hemolysis and/or thrombosis (Cançado et al., 2021).

Clinical trials may use other biomarkers to further characterize improvement of PNH signs, including assays measuring the presence of factor Bb fragments in the blood, the Wieslab® alternative complement pathway activity assay data, and C3 fragment deposition on RBCs, in order to measure the efficacy of the exposure-response relationship between the treatment under investigation and the patient (Risitano et al., 2022). Furthermore, increased transfusion requirements and the presence of hemolysis (indicated by LDH levels greater than 1.5 times the upper limit of normal or ULN) are also important measures of the severity and progression of PNH in those participating in clinical trials (Lee et al., 2019).

Treatment of PNH

Since PNH is a complement-mediated disease, therapeutics have been engineered and put out onto the market that target and inhibit the complement cascade. Prior to the introduction of causal biology (complement) directed therapy, patients with PNH had high mortality that was mostly due to a highly thrombophilic state. There are three main categories that make up the field of complement-inhibitor therapeutics: 1) C5 inhibitors 2) C3 inhibitors and 3) AP inhibitors (factor B, factor D inhibition). Below is a summary of each kind of therapeutic and how they function to combat PNH.

C5 Inhibitors

Eculizumab (Soliris®)

Since the launch of complement inhibitors, many forms of complement-inhibiting treatment have entered the pharmaceutical market. Eculizumab, a recombinant humanized monoclonal IgG2/4K antibody, was developed as a first-in-class complement-inhibitor by Alexion Pharmaceuticals (Xiao et al., 2021). Eculizumab works by binding to free C5 in the blood, halting the creation of the terminal complement C5 by-products C5a and C5b, thereby preventing the formation of the MAC complex and RBC lysis (hemolysis). However, C5 inhibition comes with a risk of increased infection, especially meningococcal infection. As a result, patients that are set to be prescribed complement inhibitors must be vaccinated against infection before beginning any medical regiment relating to complement inhibition.

Eculizumab has been shown to reduce intravascular hemolysis in PNH patients (Peffault de Latour et al., 2020). Eculizumab requires intravenous dosing every two weeks due to endosomal degradation which shortens its half-life. Eculizumab's successor, ravulizumab, was developed with this issue in mind (Peffault de Latour et al., 2020).

Ravulizumab (Ultomiris®)

Ravulizumab, also an intravenous C5 inhibitor developed by Alexion Pharmaceuticals, was derived from eculizumab via a substitution of four amino acids in the heavy chain (effector) region. This substitution maintained high affinity C5 binding, and created a greater complex dissociation in the endosome, effectively leaving behind C5 inside of the endosome, while simultaneously increasing ravulizumab recycling by improving its affinity to the neonatal FC receptor (FcRn), allowing ravulizumab to re-enter the vasculature through the FcRn pathway. This increased the dosing interval to every eight weeks, as compared to eculizumab's previous dosing regimen of every two weeks. When comparing efficacy between ravulizumab and eculizumab in patients with PNH who had not received complement-inhibitor therapy previously, ravulizumab was found to be noninferior over eculizumab (Lee et al., 2019). While a longer half-life in dosing was achieved through ravulizumab, the C5 inhibition mechanism that both therapeutics operated on remained intact. As a result of ravulizumab's extended dosing interval, the treatment burden on those with such a life-threatening disease as PNH has significantly decreased. Furthermore, an expansion of care access and the reduction in healthcare resource use may have also resulted due to said increase in interval of required dosing (Lee et al., 2019).

Despite the demonstrated efficacy of anti-C5 therapeutics, such as eculizumab and ravulizumab, for the treatment of PNH and improvement of related patient outcomes, some patients will experience clinically significant EVH due to persistent C3b deposition on their RBCs. This occurs effect becomes evident because PNH RBCs have longer lifespan, a larger clone size, yet still lack protection from upstream, ongoing C3 deposition. Therefore, modulation upstream complement cascade, specifically the generation of C3 via the AP, could have more overall therapeutic benefits in such patients to better manage intra and extravascular health (Peffault de Latour et al., 2024).

C3 Inhibitors

Pegcetacoplan (Empaveli®)

Pegcetacoplan is a twice-weekly, intravenous and/or subcutaneously injected, C3 inhibitor developed by Apellis Pharmaceuticals. It is a pegylated pentadecapeptide, which specifically binds to C3, preventing its cleavage and thus its activation in the complement cascade (Hillmen et al., 2021). Pegylation is a method by which polyethylene glycol (PEG) chains are attached to a specific candidate molecule. PEG molecules associate tightly with water, thereby increasing their ability to take up space so that they may function as if they are five to ten times larger than any protein of a similar molecular mass. Due to this ability, PEG molecules can form a shield around the attached drug candidate, protecting it from a variety of the body's clearance mechanisms such as enzymes, renal filtration, and cell surface proteins. This allows for the improvement of therapeutic delivery within the body, especially the administration of encapsulated drugs (Harris & Chess, 2003). In comparison to C5 inhibitors, C3 inhibitors target the earliest branches of the complement cascade, preventing the formation of C3 split products such as C3a and C3b, thereby allowing for the prevention of extravascular hemolysis as well as intravascular hemolysis since C5 convertase cannot be created in the absence of C3 convertases. Clinical trial data has demonstrated that targeting C3 instead of C5 was associated with a greater increase in return to baseline hemoglobin level as compared to eculizumab. This may be because targeting upstream complement, such as C3, prevents the amplification of downstream complement signaling thereby limiting PNH symptomology to a greater extent than terminal complement inhibition (Hillmen et al., 2021).

However, as is the case for C5 inhibition, there are both scientific and clinical concerns regarding an increased risk of encapsulated bacterial infection in the case of C3 inhibition (Hillmen et al., 2021). Since C3 inhibition address an earlier component of the complement cascade, a more comprehensive blocking of complement could lower the immune defenses of a patient. This occurs because C3 is the initial catalyst as well as the basis of essential convertases

that are needed to further cleave other components of the complement system for the cascade to both initiate and continue. By blocking C3, patients on C3 inhibitors would be unable to fight off preliminary infections via the complement cascade as a result. Since the complement cascade is an essential part of the innate immune system or the initial, non-specific defense against pathogens, this serves as the underlying reasoning as to why patients on C3 inhibitors are at risk for more serious infection. Yet, even with C3 inhibitors' advantages in relation to both intravascular and extravascular hemolytic control, C3 inhibitors may display a greater risk of breakthrough hemolysis (Notaro & Luzzatto, 2022). This is because if one dose of a C3 inhibitor is missed, C3 will be readily created within the complement system, thereby kickstarting a volatile chain reaction that creates C3 convertases and subsequently C5 convertases, which can be expected to resume the lysing of a patient's RBCs.

Factor B Inhibitors

Iptacopan (Fabhalta®)

Iptacopan is a first-in-class, oral, twice-daily, small molecule factor B inhibitor developed by Novartis Pharmaceuticals. In a similar manner to pegcetacoplan, iptacopan targets the proximal portion of the complement cascade. However, iptacopan addresses the amplification loop that occurs in the AP pathway by binding to factor B. Iptacopan inhibits factor B's ability to attach to hydrolyzed C3 created by spill over, as well as any present C3b already created by either the classical or lectin pathways. As a result of this mechanism of action, iptacopan has been shown to control both intravascular hemolysis as well as extravascular hemolysis as well as has demonstrated a clinically significant improvement in hemoglobin levels, even in patients on C5 inhibitors who displayed persistent anemia while using such inhibitors (Peffault de Latour et al., 2024). Iptacopan has also been shown to demonstrate a lower rate and less severe clinical presentation of breakthrough hemolysis, or hemolysis triggered by complement system reactivation due to incomplete inhibition, in patients with PNH as compared to those prescribed pegcetacoplan (Hillmen et al., 2021). Furthermore, since ravulizumab and pegcetacoplan both require IV and subcutaneous infusion, iptacopan stands outs as it attends to a market of unmet need for orally administered complement inhibitor treatment (Jang et al., 2022). However, twice-daily oral medication may be a drawback for some, as it can be difficult to keep up with daily administration and consistently comply with the prescribed regiment. This is one of the larger concerns with oral-based administration, especially when the consequences of missing one dose are so dire, such as breakthrough hemolysis. Similarly to ravulizumab and pegcetacoplan, there is a higher risk of encapsulated bacterial infection when taking iptacopan due to its complement inhibitory properties.

Methods

Comparing Therapeutics

This paper focuses on comparing three of the four complement-inhibition therapeutics that were mentioned and categorized: ravulizumab, pegcetacoplan and iptacopan. To assess and compare each kind of therapeutic, two main approaches were taken. First, a review of each therapeutic's pivotal phase 3 trials was undertaken, focusing on both naïve (patients who had never been prescribed a complement inhibitor for PNH) and switch patients (those who were previously on a complement inhibitor and were switched onto a different asset). This resulted in the analysis of six total trials, two per asset. In this first review of published data, both primary and secondary endpoints from each trial were collected and tabled. For ravulizumab, naïve patient data was drawn from the 301 study, registered with www.clinicaltrials.gov as NCT02946463, while switch patient data was drawn from the 302 study, registered with www.clinicaltrials.gov as NCT03056040 (Lee et al., 2019; Kulasekararaj et al., 2019). For pegcetacoplan, naïve patient data was drawn from the PRINCE study, registered with www.clinicaltrials.gov as NCT04085601, while switch patient data was drawn from the PEGASUS study, registered with www.clinicaltrials.gov as NCT03500549 (Wong et al., 2023; Hillmen et al.,

2021). Lastly, for iptacopan, naïve patient data was drawn from the APPOINT study, registered with www.clinicaltrials.gov as NCT04820530, while switch patient data was drawn from the APPLY study, registered with www.clinicaltrials.gov as NCT04558918 (Peffault de Latour et al., 2024). In this first review of published data, both primary and secondary endpoints from each trial were collected and tabled.

Table 1: Study Name, Trial Number and Patient Population of the Pivotal Phase 3 Trials of Ravulizumab, Pegcetacoplan, and Iptacopan Assessed

	Ravulizumab		Pegcetacoplan		Iptacopan	
Study Name	301	302	PRINCE	PEGASUS	APPOINT	APPLY
Trial Number	NCT02946463	NCT03056040	NCT04085601	NCT03500549	NCT04820530	NCT04558918
Patient Population	Naïve	Switch	Naïve	Switch	Naïve	Switch

Table 2: End Point Assessment of Ravulizumab, 301 and 302 Studies*

	Patient Population	Primary Endpoints	Key Secondary Endpoints
Ravulizumab (Alexion)	Study 301: 1) LDH level of ≥1.5 x ULN at screening† 2) within three months of screening, presenting with one or more PNH-signs or symptoms present‡ 3) patients that had not been exposed to complement inhibitors currently or previously Study 302: 1) Clinically stable patients who had received eculizumab treatment six months or more at labeled dose before trial entry 2) LDH level of 1.5 or less x ULN† Study 301 and Study 302: 1) enrolled adult patients (18 or older) diagnosed with PNH via high-sensitivity flow cytometry of red and white blood cells with granulocyte clone size of 5% or more	Study 301: 1) transfusion avoidance, defined as representation of patients who remained transfusion-free up until day 183 2) hemolysis, measured by LDH normalization from days 29 to 183 Study 302: 1) hemolysis measurement, based on percentage change in LDH levels (from baseline to day 183)	Study 301: 1) Percentage change in LDH (from baseline to day 183) Study 302: 1) Transfusion avoidance§ Study 301 and Study 302: 1) breakthrough hemolysis in proportion of patients enrolled¶ 2) FACIT-Fatigue score assessment of change in quality of life (from baseline to day 183) 3) Stabilized hemoglobin in proportion of enrolled patients‖

* Adapted from definitions provided by Study 301 and 302 in published trial data (Lee et al., 2019; Kulasekararaj et al., 2019).
† ULN = Upper Limit of Normal (246 U/L)
‡ Defined by: fatigue, hemoglobinuria, abdominal pain, shortness of breath (dyspnea), anemia (ie, hemoglobin level ,10 g/dL), or history of MAVEs (including thrombosis), dysphagia, erectile dysfunction, or history of packed RBC transfusion because of PNH.
§ Defined by: the proportion of patients who remained transfusion free and did not require a transfusion per protocol-specified guidelines.
¶ Defined by: at least 1 new or worsening symptom or sign of intravascular hemolysis (fatigue, hemoglobinuria, abdominal pain, shortness of breath [dyspnea], anemia [hemoglobin ,10 g/dL], major adverse vascular event [including thrombosis], dysphagia, or erectile dysfunction) in the presence of elevated LDH ≥2 x the ULN after prior reduction of LDH to <1.5 x the ULN on treatment.
‖ Defined by: avoidance of a ≥2-g/dL decrease in hemoglobin level from baseline in the absence of transfusion.

Table 3: End Point Assessment of Pegcetacoplan, PRINCE and PEGASUS Studies*

	Patient Population	Primary Endpoints	Key Secondary Endpoints
Pegcetacoplan (Apellis)	PRINCE: 1) hemoglobin level below the LLN (for males: <13.6 g/dL; for females: <12.0g/dL) 2) lactate dehydrogenase (LDH) level ≥1.5 times the upper limit of normal (ULN ≥339 U/L) 3) vaccinated against Streptococcus pneumoniae, Neisseria meningitidis (types A, C, W, Y, and B), and Haemophilus influenzae (type B) PEGASUS: Patients had hemoglobin levels of less than 10.5 g/dL and received eculizumab for at least three months PRINCE and PEGASUS: Enrollment of adults patients 18 or older with diagnosed PNH (via high-sensitivity flow cytometry)	PRINCE: 1) hemoglobin stabilization† 2) change from baseline in LDH levels at week 26 PEGASUS; 1) Change of hemoglobin level from baseline to week 16 check-in	PRINCE: 1) hemoglobin response‡ 2) change from baseline in hemoglobin level 3) patients (in percentage) who received transfusion and/or had a >2-g/dL decrease from baseline in hemoglobin level 4) number of packed red blood cell (PBRC) units transfused during the 26-week randomized control period 5) change from baseline in global health status/QoL scores using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) instrument 6) Absolute reticulocyte normalization§ PRINCE AND PEGASUS: 1) Transfusion avoidance 2) FACIT-F Score change from baseline 3) Absolute reticulocyte count change from baseline

* Adapted from definitions provided by PRINCE and PEGASUS in published trial data (Wong et al., 2023; Hillmen et al., 2021).
† Defined by: avoidance of a >1-g/dL decrease in hemoglobin levels from baseline to week 26.
‡ Defined by: hemoglobin increase ≥1 g/dL from baseline.
§ Defined by: ARC (absolute reticulocyte count) < ULN (upper limit of normal) [male: from 10 × 109 to 140 × 109 cells per L; and female: from 10 × 109 to 120 × 109 cells per L]).

Table 4: End Point Assessment of Iptacopan, APPOINT and APPLY Studies*

	Patient Population	Primary Endpoints	Key Secondary Endpoints
Iptacopan (Novartis)	APPOINT-PNH: patients that had not received C5 inhibitor therapy, that had LDH levels more than 1.5 times the most upper limit of the normal designated range APPLY-PNH patients had received either eculizumab or ravulizumab for at least six months APPOINT-PNH and APPLY-PNH: 1) enrolled adult patients (18 or older) diagnosed with PNH via flow cytometry 2) Patients with PNH and mean hemoglobin levels less than 10 g/dL	APPOINT-PNH: 1) increase hemoglobin levels at least 2 g per dL from baseline w/o RBC transfusion APPLY-PNH: 1) increase hemoglobin levels at least 2 g per dL from baseline w/o RBC transfusion 2) increase hemoglobin level of at least 12 g per dL w/o RBC transfusion	APPOINT-PNH: 1) increase hemoglobin level of at least 12 g per dL w/o RBC transfusion APPOINT-PNH AND APPLY-PNH: 1) Transfusion avoidance† 2) Changes in hemoglobin level from baseline 3) FACIT-Fatigue survey scores 4) Absolute reticulocyte count 5) Change percentage of LDH level from measured from baseline 6) Occurrences of clinical breakthrough hemolysis‡

* Adapted from definitions provided by APPOINT-PNH and APPLY-PNH in published trial data (Peffault de Latour et al., 2024).
† Defined by: not receiving red cell transfusions and not meeting the protocol specified criteria for transfusion between days 14 and 168.
‡ Defined by: meeting one of the two clinical criteria [decrease in hemoglobin level ≥2 g per deciliter or PNH symptoms of gross hemoglobinuria, hemolytic crisis, dysphagia, or any other clinically significant sign or symptom associated with PNH] in addition to elevated LDH level [>1.5 times the ULN]).

Second, based on the stated primary and secondary endpoints of each trial, commonly reported biomarker data across all pivotal phase 3 studies analyzed were collected and separately tabled into five key categories across each asset: 1) LDH level (LDH normalization, percentage of patients reaching LDH ≤1.5 x upper limit of normal, etc.), 2) breakthrough hemolysis (BTH) rate in the percentage of patients enrolled, 3) FACIT-Fatigue Score (change from baseline), 4) hemoglobin level (change from baseline, mean improvement) and 5) transfusion avoidance in the percentage of patients enrolled. From these designated categories and the tabled data that was assigned to each category, a proper basis of comparison across all three assets was established, allowing for complete analysis of efficacy. Data collected from the two approaches described above are shown below.

Results

When looking at the data presented in Table 5, a key trend can be observed. As one moves from ravulizumab to pegcetacoplan and then iptacopan within each biomarker of PNH, values tend to increase from each therapy to the next. For example, in the case of transfusion avoidance in naïve patients in Table 5, ravulizumab's 301 study reported percentage of patients who avoided transfusion was 73.6%, as compared to pegcetacoplan's PRINCE study which reported 91.4% and iptacopan's APPOINT study which reported 98%. Furthermore, within naïve patients, those on

ravulizumab displayed a mean point change from baseline of 7.07 in FACIT-Fatigue score, while those on pegcetacoplan demonstrated a mean score increase of 7.78 and those on iptacopan demonstrated a mean score increase of 10.80 (Lee et al., 2019; Wong et al., 2023; Peffault de Latour et al., 2024). Comparatively against ravulizumab, this represents a 10% increase in FACIT-Fatigue score for patients on pegcetacoplan and a 53% increase in FACIT-Fatigue score for patients on iptacopan. This is important to note, as the general population FACIT-fatigue score is 43 out of 52 (Cella et al., 2023). In a strict analysis of ravulizumab and pegcetacoplan or pegcetacoplan and iptacopan, the same trend is seen. When comparing percentage of naïve patients who reached LDH normalization between those on ravulizumab and pegcetacoplan, ravulizumab reports 53.6% of patients reached normalization, while on pegcetacoplan 65.7% reached normalization (Lee et al., 2019; Wong et al., 2023). The same trend is also visible when comparing pegcetacoplan to iptacopan in the category of naive patient mean hemoglobin change from baseline. For pegcetacoplan, mean change in hemoglobin was reported as 2.9 g/dL, while for iptacopan, the reported mean change in hemoglobin was 4.3 g/dL (Wong et al., 2023; Peffault de Latour et al., 2024).

Table 5: Naïve Patient Data Outcomes in Ravulizumab, Pegcetacoplan and Iptacopan Studies*

	Ravulizumab (Study 301)	Pegcetacoplan (PRINCE)	Iptacopan (APPOINT-PNH)
Percent of patients who reached LDH normalization (<ULN)	53.6%	65.7%	-
Percent of patients who reached LDH ≤1.5	-	-	95%
BTH rate (%)	4.0% (5 out of 125 patients)	-	0.0% (0 patients out of 40 patients)
FACIT-Fatigue (mean) point score change from baseline	7.07	7.78	10.80
Hemoglobin level (mean) change from baseline (g/dL)	-	2.9	4.3
Change from initial hemoglobin level to end mean level (g/dL)	-	9.4 to 12.8	8.2 to 12.6
Percentage of Patients who reached Transfusion Avoidance	73.6%	91.4%	98%

* Data adapted from 301, PRINCE, and APPOINT-PNH studies (Peffault de Latour et al., 2024; Lee et al., 2019; Wong et al., 2023).

Interestingly, the pattern that is seen above in naïve patients is not necessarily seen in switch patients. In ravulizumab's 301 study, there was a reported rate of breakthrough hemolysis of 4.0%, while in iptacopan's APPOINT study, no breakthrough hemolysis was reported (Lee et al., 2019; Peffault de Latour et al., 2024). In the case of ravulizumab's 302 study however, no breakthrough hemolysis was reported for switch patients, while in iptacopan's APPLY study, 3.0% of patients experienced breakthrough hemolysis and in pegcetacoplan's PEGASUS study, 10% of patients experienced breakthrough hemolysis (Kulasekararaj et al., 2019; Peffault de Latour et al., 2024; Hillmen et al., 2021). Other categories such as transfusion avoidance and FACIT-Fatigue score mirror this trend, as seen in Table 6. However, switch patients still display an increasing mean change in hemoglobin, with pegcetacoplan reporting a 2.4 g/dL mean increase, while iptacopan reports a 3.6 g/dL mean increase (Hillmen et al., 2021; Peffault de Latour et al., 2024).

Table 6: Switch Patient Data Outcomes from Ravulizumab, Pegcetacoplan and Iptacopan Studies*

	Ravulizumab (Study 302)	Pegcetacoplan (PEGASUS)	Iptacopan (APPLY-PNH)
Percent of patients who reached LDH normalization (<ULN)	66.0%	71.0%	-
Percent of patients who reached LDH ≤1.5	-	-	-
BTH rate (%)	0.0% (0 out of 97 patients)	10% (4 out of 41 patients)	3.0% (2 out 62 patients)
FACIT-Fatigue (mean) point score change from baseline	2.0	9.2	8.6
Hemoglobin level (mean) change from baseline (g/dL)	-	2.4	3.6
Change from initial hemoglobin level to end of treatment mean level (g/dL)	-	8.7 to 11.5	8.9 to 12.6
Percentage of Patients who reached Transfusion Avoidance	87.6%	85%	95%

* Data adapted from 302, PEGASUS, and APPLY-PNH studies (Peffault de Latour et al., 2024; Hillmen et al., 2021; Kulasekararaj et al., 2019).

Discussion

Inherently, the trends above arise from the difference in not only inhibition, but more specifically whether a given therapeutic is blocking a portion of the terminal or the proximal complement pathway. For naïve patients, an increase in transfusion avoidance, FACIT-Fatigue score and LDH normalization as markers of efficacy exists between ravulizumab, a terminal complement inhibitor, and pegcetacoplan and iptacopan, which are both proximal complement inhibitors. This indicates that as a therapeutic moves away from a terminal complement target and towards a proximal complement target, the efficacy of said therapeutic on PNH symptomology increases. To note, this trend may not be as clear with switch patients, as due to prolonged exposure to C5 inhibitors such as eculizumab or ravulizumab, switching onto another complement inhibitor may not display an improvement in PNH symptomology as profoundly as patients who had never received complement inhibition therapeutics previously. Furthermore, this trend is amplified when both the proximal complement pathway and the AP are targeted for inhibition, as is seen in the case of iptacopan. While both pegcetacoplan and iptacopan target proximal complement, out of the two, iptacopan seems to display more efficacious control of PNH, due to greater transfusion avoidance, a smaller rate of breakthrough hemolysis and a higher mean hemoglobin level as displayed by the individual results of each tabled biomarker. Why may this be the case?
Within the AP, there is instance of an amplification loop or spill over, i.e. the hydrolysis of C3 into C3H2O, which can act as a platform for the binding of factor B and subsequently factor D, creating C3H2OBb, a C3 convertase. As a result, the AP is always on and can produce C3 convertases at a constant, low level. Furthermore, the amplification loop within the AP can be triggered easily by infection. This is because infection will subsequently trigger both the classical and lectin pathways, which will produce C3 convertase byproducts that will cause a spike in C3 convertase via the AP. As a result, an acceleration of the complement defense system will take place, further perpetuating self-on-

self attack in diseases such as PNH. Thus, controlling the AP may be the key to regulating spill over and limiting its amplified activation through the classical and lectin pathways. By regulating the AP and keeping both the classical and the lectin complement pathways intact, this may allow a patient with PNH to readily combat their symptoms, while maintaining their immunological barriers against infection. However, to truly understand why factor B inhibition displays an increased clinical efficacy in patients with PNH, further research must be conducted, including potentially the use of quantitative systems pharmacology (QSP) model to mathematically represent the complement system's reaction to certain programmed therapeutic inhibitions.

New Developments in the Therapeutic Space

Due to the newly demonstrated research that targeting the AP may lead to more efficacious outcomes in patients with PNH, development of new therapeutics addressing blockage of this specific pathway has increased. For example, danicopan, a factor D inhibitor, was recently approved as an add-on to ravulizumab, for patients with clinically significant EVH, under the name of Voydeya Alexion Pharmaceuticals. This combination therapy addresses the AP in a similar mechanism to iptacopan, as both Voydeya and iptacopan act upon factors D and B respectively within the AP, but also maintains complete blockade of IVH. When PNH RBC clones expand under effective treatment, catastrophic IVH has been reported with proximal-only therapy, and long-term safety and efficacy remain to be established.[14] As mentioned previously, these factors are responsible for the creation of spill over or the amplification loop that allows the AP to continuously create C3 convertases at a low level. While on Voydeya, those experiencing persistent anemia as well as continued extravascular hemolysis had demonstrated improved symptoms as compared to symptomatology when only on a C5 inhibitor. Thus, by adding factor D inhibition to ravulizumab, a previously established safe and efficacious C5 inhibitor, Voydeya has the potential to address PNH in an equal or even more efficacious way (Lee et al., 2023).

Conclusion

Through the comparison of ravulizumab, pegcetacoplan, and iptacopan and their individual inhibition of different portions of the complement cascade (C5, C3 and factor B), a variety of clinical efficacy has been shown. Ultimately, blocking pathways that make up the proximal complement pathway demonstrate a greater effectiveness, as opposed to therapeutics that address the terminal complement pathway through C5 inhibition. This is demonstrated through pegcetacoplan's and iptacopan's ability to block both intravascular and extravascular hemolysis in patients with PNH, as opposed to ravulizumab which only blocks intravascular hemolysis. Furthermore, therapeutics that address the amplification loop of the AP seem to mitigate the complement-mediated symptomology of PNH to a more efficacious degree as opposed to strict C3 inhibition. This is shown through iptacopan's ability to maintain a higher average increase in hemoglobin level, a greater rate of transfusion avoidance and a smaller breakthrough hemolysis rate as compared to pegcetacoplan in both naïve and switch patient populations. Further research must be conducted to firmly understand why factor B inhibition displays a greater therapeutic efficacy in PNH as compared to C3 and/or C5 inhibition.

Limitations

It must be noted that when assessing and compiling data from pivotal phase 3 trials, there will always be slight variations in specific baseline of the PNH patient population screened and chosen to participate within a given study, how the study is conducted, what given primary and key secondary endpoints are deemed to be the cornerstone of the study and what specific biomarkers are reported at the end of data collection. This may make it slightly difficult to compare efficacy truly and objectively across all asset trials presented in this paper. However, it also must be noted that since

PNH is a strictly complement-driven disease, these limitations may not affect data assessment to the same detrimental level as other diseases. Thus, it is important to consider such limitations in future therapeutic-based comparative works like the one that is presented here.

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Conflict of Interest

The author (T.G.) has received employment from Alexion Pharmaceuticals, Inc.

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