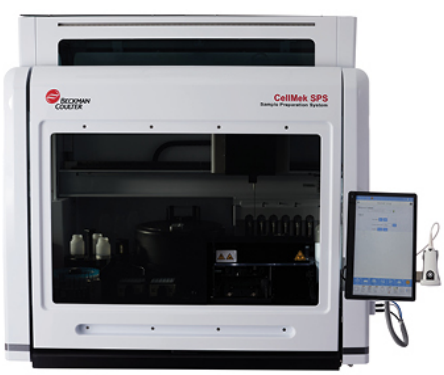

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Toward complement inhibition 2.0: Next generation anticomplement agents for paroxysmal nocturnal hemoglobinuria

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Abstract

Therapeutic complement inhibition by eculizumab has revolutionized the treatment of paroxysmal nocturnal hemoglobinuria (PNH) with a major impact on its natural history. Nevertheless, emerging unmet clinical needs may benefit from the development of novel complement inhibitors. Novel strategies of complement inhibition exploit different agents targeting C5, as well as compound intercepting the complement cascade at the level of its key component C3, or even upstream at the level of components involved in complement alternative pathway initiation. Many of these agents are already in their clinical development; preliminary data together with a deep understanding of PNH biology may help to anticipate their possible clinical effect. Novel anti-C5 agents include monoclonal antibodies (even long-lasting) as well as other small molecules bioavailable by subcutaneous administration; an anti-C5 small interfering RNA has been developed too. All these anti-C5 agents seem to recapitulate safety and efficacy of current eculizumab treatment; their main improvement pertains to better patient's convenience due to longer dosing interval and/or possible subcutaneous self-administration. The possibility of achieving a deeper C5 inhibition has been shown as well, but its actual clinical meaning remains to be elucidated. Upstream complement inhibitors include the anti-C3 small peptide compstatin (and its derivatives), and small inhibitors of complement factor D or complement factor B. This class of compounds anticipates a possible efficacy in prevention of C3-mediated extravascular hemolysis, in addition to inhibition of intravascular hemolysis, eventually leading to improved hematological responses. The availability of all these compounds will result soon in a substantial improvement of PNH management.

1 | INTRODUCTION

Therapeutic complement inhibition was first introduced in paroxysmal nocturnal hemoglobinuria (PNH) in 2002 with the first complement inhibitor eculizumab, a humanized anti-C5 monoclonal antibody (mAb)[1] registered in 2007. Ten years later, no additional anticomplement agent has been approved. The treatment of PNH has been revolutionized by eculizumab: since the first pioneering experience, therapeutic inhibition of C5 resulted in sustained inhibition of intravascular hemolysis typical of PNH, eventually resulting in a major clinical improvement.[2] Two subsequent phase III registration trials demonstrated a dramatic reduction of intravascular hemolysis, which led to hemoglobin stabilization and transfusion independency in about half of patients.[3,4] In addition to the control of all hemolysis-related symptoms and to improved quality of life, sustained inhibition of terminal complement resulted in a marked reduction of the thromboembolic events typical of PNH.[5] The actual mechanism of action of this effect has not been fully elucidated, since it is not clear whether it is simply due to an indirect effect which follows the inhibition of intravascular hemolysis, or it may also include a direct effect of complement inhibition on the pathogenic mechanisms of thrombosis in PNH (which appears to be multi-factorial). This remarkable clinical effect was obtained without major safety concerns, including the feared risk of infectious complications (all patients on eculizumab receive broad vaccinations against *Neisseria Meningitidis*).[3–5] The long-term use of eculizumab in PNH is more than a supportive treatment; two independent studies have clearly demonstrated that the survival of PNH patients on eculizumab exceeds 90% at 5 years,[6,7] which is by far higher than what known from previous natural history

data.[8,9] Thus, even if the only curative option for PNH remains hematopoietic stem cell transplantation,[10] eculizumab is the current gold standard for PNH patients presenting with hemolytic and/or thromboembolic disease. However, eculizumab (as any other future anti-complement treatment) has no effect on the bone marrow disorder underlying PNH, which mostly appears as bone marrow failure but may also lead to evolution to hematological malignancies.[7]

Nevertheless, some unmet clinical needs emerged in the era of eculizumab: (i) insufficient clinical benefit, seen as residual anemia; (ii) heaviness of life-long bi-monthly intravenous (IV) infusions; (iii) limited access to treatment due to elevated cost. The first point represents the main driver to develop novel strategies of therapeutic complement inhibition. True resistance to eculizumab has been documented only in a few patients, mostly of Japanese ethnicity, carrying an inherited polymorphism of C5.[11] However, even in patients with documented complement blockade, the hematological benefit is quite heterogeneous: indeed, residual anemia affects about most PNH patients on eculizumab, and about one third still requires red blood cell transfusions.[3,4,12–14] The most obvious cause of such residual anemia is inadequate compensatory erythropoiesis due to the well-established overlap between aplastic anemia (AA) and PNH[15]; these patients should receive the appropriate treatment according to the AA algorithm.[16–18] However, the vast majority of PNH patients on eculizumab remain anemic irrespective of increased reticulocyte count, eventually documenting residual hemolysis even during anti-C5 treatment. In 15%-20% of patients this is due to residual intravascular hemolysis which regularly occurs 1–2 days before the next dosing of eculizumab (the so-called "pharmacokinetic [PK] breakthrough")[14]; these patients may benefit from increased doses (1200 mg) or reduced dosing intervals (10 days) of eculizumab.[19] This modified treatment schedule has a limited effect to control the "pharmacodynamic [PD] breakthrough" (which rather is seen as low-grade continuous intravascular hemolysis) and possible hemolytic paroxysms in concomitance with massive complement activation.[20,21] However, the most common cause of residual anemia in PNH patients on eculizumab was identified in C3-mediated extravascular hemolysis.[12] This phenomenon mechanistically pertains to all PNH patients on eculizumab as a result of upper (upstream C5) complement activation, which remains uncontrolled irrespective of C5 blockade.[12] As a result, progressive deposition of C3 fragments on PNH erythrocytes sparing complement-mediated lysis eventually leads to chronic extravascular hemolysis.[12,22–24] While C3 opsonization has been confirmed by several groups,[12,14,25] its actual clinical relevance has not been fully acknowledged by all experts[7]; nevertheless, C3-mediated extravascular hemolysis impairs hematological response to eculizumab in 25%-50% of patients.[7,12,14,25] The reasons accounting for such heterogeneous clinical importance may include possible genetic variants of complement-related genes, as reported for the hypomorphic variant of complement receptor 1 (CR1) gene.[26] While the use of steroids is discouraged,[24] splenectomy has been anecdotically reported effective to treat C3-mediated extravascular hemolysis[27,28]; however, at the moment there is no reliable treatment option for this common event emerging during eculizumab treatment.[29]

2 | NOVEL STRATEGIES OF COMPLEMENT INHIBITION

A plethora of agents is currently in the pipeline of several pharmaceutical companies[30]; they can be divided according to their target in the complement cascade[31] (Figure 1; Table 1). Inhibitors of the effector pathway target C5, and include at least 6 novel mAb-derived compounds,[32–36] a small-interfering RNA (siRNA)[37] and 2 small molecules.[38–43] A second group of inhibitors intercept the complement cascade upstream C5; they include broad inhibitors of C3 and agents which specifically target complement activating pathways—classical (CCP), alternative (CAP), and mannose/lectine (CMP) ones (Figure 1; Table 1).[31] For PNH, candidate inhibitors target the CAP, inhibiting C3 convertase C3bBb,[50–55] or one of CAP key early components, ie, factor D (FD),[56–58] factor B (FB),[59,60] and properdin. These upstream inhibitors include mAb, small compounds, as well as engineered proteins based on endogenous regulators of complement activation.[50] Here, we summarize publically available information, describing the ongoing trials with their design (Table 2) and available data.

2.1 | ABP959 (Amgen)

The anti-C5 mAb ABP959 was initially tested in Australia in a phase I randomized trial investigating PK and PD of this compound as compared to eculizumab (ACTRN12616000509460).[61] Data are not publically available yet; nevertheless, a large phase III randomized trial is currently ongoing in Europe (EudraCT Number 2017-001418-27).[32] Since this compound has been announced as a biosimilar of eculizumab (even if the true meaning of this term may be questioned until details about drug manufacturing will be available), safety and efficacy profile should parallel those of eculizumab, and no further scientific discussion about ABP959 is provided.

2.2 | ALXN1210 (Alexion)

ALXN1210 is an anti-C5 mAb sharing with eculizumab the same target epitope. A phase I study of ALXN1210 in healthy volunteers demonstrated immediate, complete, and sustained C5 inhibition, with a terminal half-life about 4-fold longer than eculizumab.[32] Then, ALXN1210 was investigated in two phase I/II trials (NCT02598583 and NCT02605993) which enrolled 13 and 26 untreated PNH patients, aiming to evaluate safety, tolerability, and efficacy of different IV dosing regimens.[62,63] No death, serious adverse event, drug discontinuation, or adverse event (AE) leading to withdrawals were recorded.[64] ALXN1210 demonstrated rapid, complete, and sustained C5 inhibition, eventually leading to prompt and sustained LDH reduction and frequent hemoglobin stabilization in all treatment cohorts.[64,65] Patients receiving 600 and 900 mg as loading dose, followed by maintenance with 1800 mg every 4 weeks, showed the best outcome in terms of sustained control of intravascular hemolysis (LDH normalization in 6/7 and no breakthrough episode).[65] Notably, two patients on ALXN1210 experienced sepsis by *N. Meningiditis*[65]; both patients completely recovered after ceftriaxone treatment, and continued ALXN1210.[65]

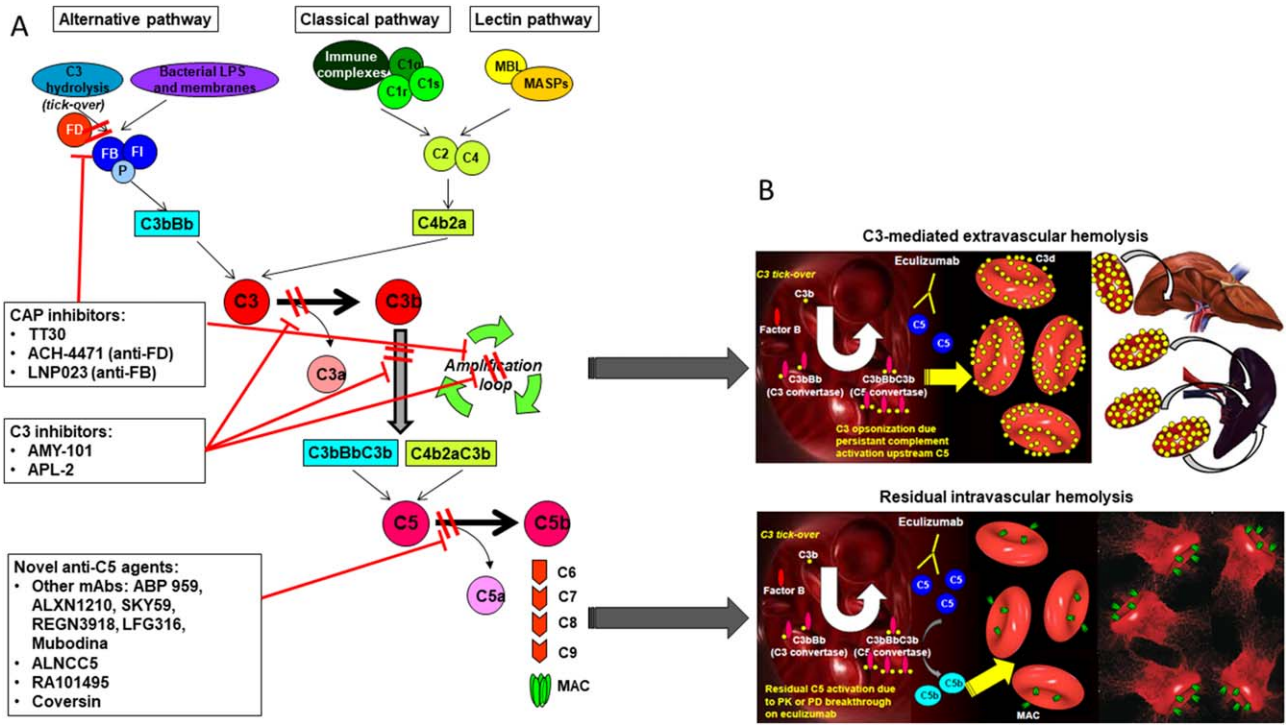

FIGURE 1 The complement cascade and its targeted modulation. (A) Overview of the complement cascade and of novel anticomplement agents. C3 cleavage by C3 convertases (C3bBb or C4b2a) is the key event in complement activation and may occur along one of the three different complement activating pathways—classical (CCP), alternative (CAP) and mannose/lectine (CMP) ones.[31] The three activating pathways are individually depicted, together with the alternative pathway amplification loop; they eventually lead to the C5 cleavage by C5 convertase (C3bBbC3b or C4b2aC3b), which arms the effector pathway of the complement cascade.[31] Candidate inhibitors are grouped according to their specific target; their modulatory effects are indicated by red lines intercepting specific steps of the complement cascade.[30] Therapeutic interception of the complement cascade may occur: i. at the level of its common terminal complement effector pathway (eg, C5); ii. at the level of its common key activation and amplification component (ie, C3); iii. at the level of pathway-specific initiating events. For the purpose of PNH, for this latter target only the CAP is considered, since the role of CCP and CMP in the pathophysiology of PNH is considered somehow limited (even if experimental evidences are lacking). (B) PNH pathogenic events eventually targeted by novel anticomplement agents. CAP inhibitors and C3 inhibitors both disable C3 activation, which results from *C3 tick-over* and in presence of therapeutic C5 inhibition leads to C3 opsonization of PNH erythrocytes. As a consequence, both CAP-inhibitors and C3 inhibitors anticipate therapeutic efficacy in preventing C3-mediated extravascular hemolysis. Mechanistically, this upstream inhibition should also prevent down-stream events of the terminal complement effector pathway; indeed, intravascular hemolysis should be prevented as well. Novel C5 inhibitors disable the terminal complement effector pathway, which usually result in intravascular hemolysis. In addition to represent an alternative to standard treatment eculizumab, these novel agents may result in a deeper C5 inhibition, eventually leading to the prevention of both pharmacokinetic and pharmacodynamics breakthrough possibly occurring during eculizumab treatment [Color figure can be viewed at wileyonlinelibrary.com]

Two large phase III trials are now comparing ALXN1210 used at 3300 mg every 8 weeks (maintenance dose, after a loading dose of 2700 mg) with the standard treatment eculizumab, both as initial treatment for PNH (NCT02946463),[66] and switch therapy for patients already on eculizumab (NCT03056040) with adequate control of hemolysis (LDH <1.5x ULN).[67] Notably, in both studies changes in LDH levels appear as primary endpoint, likely because of a deeper C5 inhibition initially seen with ALXN1210,[65] which may result in a better control of chronic intravascular hemolysis (eg, less PK and/or PD breakthrough). The clinical meaning of this more profound complement inhibition (with possible increased infectious risk)[65] will have to be investigated (low-level residual C5 activity seen with eculizumab[21] might be somehow protective from infectious agents). Nevertheless, the most obvious benefit from this long-lasting C5 inhibitor will pertain to patients' convenience in terms of treatment ease, as a result of the two-month dosing interval (even if still IV). The more profound C5 inhibition might also anticipate some benefit in patients experiencing frequent breakthrough, but at the moment this population was excluded from ongoing trials.

2.3 | SKY59/RO711268 (Roche)

SKY59/RO711268 is a recycling humanized anti-C5 mAb with long half-life, which was shown effective in suppressing C5 function and complement activity (even on the Japanese C5 variant p.Arg885His) *in vitro*.[34] This long-lasting anti-C5 mAb is currently under investigation within a phase I/II study (NCT03157635)[68] consisting of 3 sequential parts and an open-label extension; safety, tolerability, PK, and PD of single-doses of RO7112689 were initially evaluated in healthy volunteers during part 1. Then, part 2 and 3 include untreated and

TABLE 1 Candidate complement inhibitors in development for PNH

Pathway specificity	Target	Class of molecule	Name	Company	Main property	Preclinical data	Ref.
Effector pathway	C5	mAb	ABP959	Amgen	Biosimilar of ecu	n.a.	32
			ALXN1210	Alexion	Long-lasting, (SC?); same epitope of ecu	n.a.	33
			ALXN5500	Alexion	Long-lasting, SC	n.a.	30
			SKY59	Roche	Long-lasting, SC; different epitope *vs* ecu	Long-lasting C5 inhibition; *in vitro* C5 blockade (also C5 polymorphism)	34
			LFG 316	Novartis	Different epitope *vs* ecu	n.a.	35
		Minibody (mAb Fab fragments)	Mubodina®	Adienne	Different epitope *vs* ecu	n.a.	36
		si-RNA	ALNCC5	Alnylam	Targeting liver C5 production; SC	Long-lasting C5 silencing (>95%) *in vivo*	37
		Tick protein (recombinant)	Coversin (OmCI)	Akari	SC	*In vitro* efficacy in PNH	38–40
		Small molecule (unnatural peptide)	RA101348	Rapharma	Binds to the C5b region of C5, thereby preventing C5 cleavage and C5b interaction with C6; SC (possibly oral)	*In vitro* efficacy in PNH	41–43
CCP, CMP and CAP (including amplification loop)	C3/C3b	mAb	H17	EluSys	Selective targeting of membrane-bound C3b/iC3b	*In vitro* efficacy in PNH;[a]	30,44
		Compstatin and analogs	4(1MeW)/POT-4	Potentia	Broad inhibition	n.a.	45,46
			Cp40/AMY-101, PEG-Cp40	Amyndas	Broad inhibition, SC	*In vitro* efficacy in PNH	47,48
			4(1MeW)/APL-1, APL-2	Apellis	Broad inhibition, SC	*In vitro* efficacy in PNH	49
CAP (including amplification loop)	FD	mAb	Lampalizumab (FCFD4514S)	Genentech/ Roche	Possible rebound activity?	n.a.	30
		Small molecules (chemicals)	n.a.	Novartis	Potent and selective; oral	*In vitro* efficacy in PNH	56
			ACH-3856 ACH-4100 ACH-4471	Achillion	Potent and selective; oral	*In vitro* efficacy in PNH	57,58
	FB	mAb	TA106	Alexion	n.a.	n.a.	30
		Small molecules (chemicals)	LNP023	Novartis	Potent and selective; oral	*In vitro* efficacy in PNH	59
		si-RNA	Anti-FB siRNA	Alnylam	n.a.	n.a.	30
		Antisense	Ionis-FB-L_{Rx}	Ionis	Long-lasting FB silencing	Phase I in healthy volunteers	60
	Properdin	mAb	NM9401	Novelmed	n.a.	n.a.	30

(Continues)

TABLE 1 (Continued)

Pathway specificity	Target	Class of molecule	Name	Company	Main property	Preclinical data	Ref.
	C3 convertase (C3bBb)	FH-based protein	TT30 (CR2/CFH)	Alexion	Inhibition targeted at sites of complement activation	*In vitro* efficacy in PNH	51,52
			Mini-FH	Amyndas		*In vitro* efficacy in PNH	30,53
CCP and CAP	C3 convertase (C4bC3b and C3bBb)	CR1-based protein	Mirococept (APT070)	n.a.	Membrane-targeted version of CR1	In clinical development for kidney transplantation[b]	54,55

CAP: complement alternative pathway; CCP, complement classical pathway; CMP, complement mannose/lectine pathway; mAb, monoclonal antibody.
[a]Clinical development hampered by expected worsening of extravascular hemolysis due to Fc-FcR recognition.
[b]Rationale for a possible use in PNH supported pharmacogenetics (association between hypomorphic variant of CR1 and worse response to eculizumab).[26]

eculizumab-treated PNH patients, respectively, aiming to evaluate safety, tolerability, PK, PD and efficacy of RO7112689 in PNH.[68] Thus, this complex study merged in one trial different phases of clinical investigation, which could have been performed separately to better allow possible changes in treatment schedule and/or other protocol details. This program has completed the first two parts of the study; even if data are not yet publically available, they eventually supported the starting of part 3. Here, PNH patients receive a loading IV dose of RO7112689 on day 1, followed by SC administration of RO7112689 at different doses and schedules. This trial includes PNH patients on stable doses of eculizumab for at least three months; the primary endpoint is PD of RO7112689, as measured by LDH and complement activity assays. The anticipation is that RO7112689, once the right schedule is identified, should parallel excellent efficacy and safety of eculizumab, with possible advantage in terms of patients' convenience due to SC administration (also depending on dose interval). Detailed PD data will tell whether RO7112689 may also deliver a deeper C5 inhibition eventually preventing meaningful breakthrough. Since RO7112689 recognizes an epitope different from that bound by eculizumab, it should also address the problem of genetic resistance to eculizumab.[11,34]

2.4 | LFG316 (Novartis)

A proof-of-concept phase I study is currently investigating LFG316 in untreated PNH patients in Japan (NCT02534909); the primary endpoint of the study is biological efficacy, as measured by LDH change.[35] LFG316 may represent an option for patients carrying C5 polymorphisms associated with intrinsic resistance to eculizumab.[11]

2.5 | ALNCC5 (Alnylam)

ALNCC5 is a small interfering RNA (si-RNA) duplex specific for C5, which is able to completely silence C5 production from the liver in animal models.[37] ALNCC5 was proven safe and effective in healthy individuals ($n = 32$), resulting in >99% reduction of C5 plasma level and >95% inhibition of serum complement activity.[69,70] This phase I/II first-in-human trial (NCT02352493) also included an arm enrolling PNH patients[69]; data were presented at ASH 2016.[71] Six patients received ALNCC5 at weekly doses (200 or 400 mg, SC), 3 in monotherapy (untreated patients) and 3 on top of fortnightly eculizumab (one with persistent breakthrough). No severe AE was observed, nor AE requiring treatment discontinuation; one patient developed increased transaminases.[71] In untreated patients, ALNCC5 resulted in C5 knockdown >98%[70]; however, given the mechanism of action of this compound, the knockdown is established slowly and requires about two months to achieve therapeutic inhibition (which is definitely not acceptable for PNH patients requiring immediate intervention, eg, those with thromboembolic complications).[70] Nevertheless, LDH reduction (37% and 50%) was observed only in 2 of 3 patients with very high baseline LDH (>5x ULN); residual low-level hemolysis with LDH >1.5x ULN remained in all 3 patients.[71] This was consistent with data using C5-depleted sera in an *in vitro* model of PNH,[48,52] where the addition of recombinant C5 in amount as low as 0.9 μg/mL (equal to about 1% of normal C5 plasma level) restored >90% of the complement-mediated hemolytic activity (Risitano et al, unpublished observation). Nevertheless, these PNH patients treated in monotherapy with ALNCC5 then benefited from the addition of eculizumab, which resulted in full control of residual intravascular hemolysis at doses as low as about 25% of standard ones (600 mg monthly).[71] The potential efficacy of a combined treatment with ALNCC5 and eculizumab was also investigated in the 3 patients already on eculizumab; here, combined treatment resulted in LDH normalization, even in the patient experiencing breakthrough (who was able to reduce eculizumab at the standard schedule of 900 mg every other week).[71] Based on these data, the best setting for further development of ALNCC5 in PNH is in combination with eculizumab, as currently under investigation in an ongoing trial aiming to improve response in poor responders (EudraCT Number 2016–002943-40)[72]; this combination therapy may also represent an option to achieve deeper control of residual intravascular hemolysis, and/or reduce the dosing of eculizumab.

2.6 | Coversin (Akari)

Coversin is a 16 kDa protein derived from the tick *Ornithodoros moubata*[38] able to prevent C5 cleavage by its convertases[38]; its *in vitro*

TABLE 2 Ongoing clinical trials with novel complement inhibitors for PNH

Agent	Target	Clinical trial ID	Design	Patient population[a]	Study treatment	Primary endpoint
ALXN1210	C5	N.A.[32]	Phase I, randomized vs placebo	Healthy volunteers	SAD, IV infusions	Safety, PK and PD
		NCT02598583[62]	Phase I/II, open-label	Untreated PNH	Intra-patient DE by IV infusions	Safety and efficacy (by LDH)
		NCT02605993[63]	Phase I/II, open-label	Untreated PNH	MAD; IV infusions	Safety and efficacy (by LDH)
		NCT02946463[66]	Phase III, randomized vs Ecu	Untreated PNH	IV infusions (every 8 weeks)	Efficacy (by LDH)
		NCT03056040[67]	Phase III, randomized vs Ecu	Stable responders PNH	IV infusions (every 8 weeks)	Efficacy (by LDH)
ABP959	C5	ACTR-N126160005094-60[61]	Phase I, randomized vs Ecu	Untreated PNH	Single IV infusions	PK similarity
		EudraCT 2017-001418-27[31]	Phase III, randomized vs Ecu	Stable responders PNH	IV infusions	Efficacy (LDH)
SKY59	C5	NCT03157635[68]	Phase I/II, multi-part study	Healthy volunteers	SAD, IV infusions	Safety, tolerability, PK and PD
				Untreated PNH	Intra-patient DE by IV infusions, followed by SC injections	Safety, PD and efficacy
				Stable responders PNH		Safety, PD and efficacy
LFG316	C5	NCT02534909[34]	Phase II, open-label	Untreated PNH	IV infusions	Efficacy (by LDH)
ALNCC5	C5	NCT02352493[69]	Phase I/II, randomized vs Ecu, SAD and MAD	Healthy volunteers	SC injection (ALNCC5 or placebo)	Safety
				Untreated PNH	SC injections (ALNCC5 only)	Safety
		EudraCT 2016–002943-40[72]	Phase II, open-label	Poor responder PNH (by LDH >2x ULN)	SC injections	Efficacy (by LDH)
Coversin	C5	N.A.[38]	Phase I, SAD and MD	Healthy volunteers	SC injections	Safety, PK and PD
		NCT02591862[73]	Phase II, open-label	Poor responder PNH (by LDH >1.5x ULN)	SC injections; intra-patient DE	Efficacy (by LDH)
		EudraCT 2016–002067-33[74]	Phase II, open-label, fixed dose	Untreated PNH	SC injections	Efficacy (defined as LDH <1.5x ULN)
		EudraCT 2016–004129-18[76]	Phase II, open-label, extension	PNH patients exposed to coversin	SC injections	Safety
RA101495	C5	N.A.[78,79]	Phase I, SAD and MD	Healthy volunteers	Daily, SC injections	Safety, PK and PD
		NCT03078582[80]	Phase II, open label, fixed dose	Untreated PNH	Daily, SC injections	Efficacy (by LDH)
				Poor responders PNH (by LDH >2x ULN)		
		NCT03030183[81]	Phase II, open label, fixed dose	Poor responders PNH (by LDH >1.5x ULN)	Daily, SC injections	Efficacy (by LDH)
		NCT03225287[82]	Phase II, open-label, extension	PNH patients exposed to RA101495	Daily, SC injections	Safety
TT30	CAP	NCT01335165[83]	Phase I, SAD	Untreated PNH	SC injections and IV infusions	Safety, PK and PD

(Continues)

TABLE 2 (Continued)

Agent	Target	Clinical trial ID	Design	Patient population[a]	Study treatment	Primary endpoint
AMY-101	C3	NCT03316521[86]	Phase I, SAD and MD	Healthy volunteers	SC and IV infusions	PK and PD
		N.A.[85]	Phase II, open label, fixed dose	Untreated PNH	Daily, SC infusions	N.A.
		N.A.[85]	Phase II, open label, fixed dose	Poor responders PNH (moderate/severe anemia)	Daily, SC infusions	N.A.
APL-2	C3	N.A.[87]	Phase I, SAD and MD	Healthy volunteers	SC and IV infusions	Safety, PK and PD
		NCT02264639[89]	Phase Ib, open label, MAD, POC	Poor responders PNH (moderate/severe anemia)	Daily, SC infusions	Safety and tolerability
		NCT02588833[88]	Phase Ib, open label, MAD, POC	Untreated PNH	Daily, SC infusions	Safety and tolerability
		N.A.[90]	Phase II, open label, extension	PNH patients exposed to APL-2	Daily, SC infusions	N.A.
ACH-4471	FD	N.A.[91]	Phase I, SAD	Healthy volunteers	Orally, QD and BID	Safety and tolerability
		NCT03181633[92]	Phase Ib, open label, MD, POC	Untreated PNH	Orally, BID	Safety and efficacy (by LDH level)
		NCT03053102[93]	Phase II, open-label, extension	PNH patients exposed to ACH-4471	Orally, BID	Efficacy (by LDH level)
		EudraCT 2016–002652-25[95]	Phase II, open label, MD, POC	Untreated PNH	Orally, TID	Efficacy (by LDH level)
		EudraCT 2016–003526-16[94]	Phase II, open label, MD, POC	Poor responders PNH	Orally, TID	Efficacy (by hemoglobin gain)
LNP023	FB	EudraCT 2017-000888-33[96]	Phase II, open label	Poor responders PNH (by LDH >1.5x ULN)	Orally, BID	Efficacy (by LDH level)

[a]Stable or poor response is intended to standard eculizumab treatment; Abbreviations: DE, dose escalation; Ecu, eculizumab; LDH, lactate dehydrogenase; MAD, multiple ascending doses; MD, multiple doses; N.A, not available; SAD, single ascending dose; PK, pharmacokinetics; PD, pharmacodynamics; POC, proof-of-concept; SC, subcutaneous; IV, intravenous; QOD, *quaque die* (once a day); BID, *bis in die* (twice a day); TID, *ter in die* (thrice a day).

efficacy in PNH has been already documented,[39] even in patients carrying C5 polymorphisms.[40] In a phase I study in healthy volunteers coversin was shown bioavailable after SC injections, with excellent PK and PD in absence of immunogenicity and of other safety concerns.[39] The first proof of efficacy of coversin was shown in PNH patients resistant to eculizumab (NCT02591862).[73] A subsequent phase II study (COBALT; EudraCT Number 2016–002067-33) investigated coversin in untreated PNH patients, used SC bi-daily (15–30 mg, after a single 60 mg loading dose) for 28 days, and then daily (30 mg) for additional two months.[74] Out of the first 5 patients enrolled, four remained on coversin (one was withdrawn because of possible co-morbidity).[75] Coversin was well tolerated, with mild injection site reactions as only AE; no high-titer neutralizing antibodies were demonstrated. LDH reduction was observed in all patients, even if only two of them reached the primary endpoint of the study (LDH <1.8x ULN); irrespective of this residual hemolysis, no red cell transfusion was needed during the three-month period of the study.[75] According to authors' conclusions, this residual intravascular hemolysis is not related to sub-therapeutic plasma levels of coversin, since terminal complement activity, as assessed by CH50, remained fully inhibited along the study.[75] These data demonstrate that coversin has biological efficacy in PNH, with acceptable safety profile, and the benefit of possible self-administration; nevertheless, likely the treatment regimen needs to be optimized (residual intravascular hemolysis is quite frequent), and the concerns about possible neutralizing antibodies requires further investigations which are already ongoing in an extension trial (EudraCT Number 2016–004129-18).[76]

2.7 | RA101495 (Rapharma)

Rapharma is developing small synthetic, macrocyclic peptides (named cyclomimetic) with excellent bioavailability (even orally)[41,42,77] and *in vitro* efficacy in PNH.[42,43] Their lead compound RA101495[43] was safe and well tolerated as SC injections in healthy volunteers, with only mild local irritation[78]; a daily dosing allowed full (>95%) and sustained suppression of complement activity.[79] Two parallel phase II study were started: study NCT03078582 investigated a loading dose of 0.3 mg/kg followed by 0.1 mg/kg daily in both untreated and eculizumab-treated PNH patients with LDH >2x ULN (these latter switched to RA101495).[80] The second study NCT03030183 rather investigated the same regimen as add-on treatment in PNH patients with inadequate response to eculizumab, defined by LDH >1.5x ULN.[81] The

TABLE 3 Anticipated pros and cons of novel complement inhibitors in development for PNH

Company	Alexion	Novartis	Roche	Rapharma	Alnylam	Akari	Amyndas	Apellis	Achillion	Novartis
Name	ALXN1210	LFG316	SKY59	RA101495	ALNCC5	Coversin	AMY-101	APL-2	ACH-4471	LNP023
Target	C5	C5	C5	C5	C5	C5	C3	C3	FD	FB
Anticipated efficacy[$]										
Effect on C5-polymorphism	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Effect on breakthrough intravascular hemolysis on eculizumab[#]	Yes ++	N.A.	Yes +	Yes +^	Yes +++[§]	Yes +([§])	(Yes ++^)	(Yes ++^)	(Yes ++^)	(Yes ++^)
Effect on C3-mediated extravascular hemolysis	No	No	No	No	No	No	(Yes +++)	(Yes +++)	(Yes +++)	(Yes +++)
Effect on Marrow failure	No	No	No	No	No	No	No	No	No	No
Monotherapy*	Yes +++	Yes +++	Yes +++	Yes ++	No	Yes +	Yes ++	Yes ++	(Yes +)	(Yes +)
Safety										
Increased infectious risk[°]	Yes +	(No)	Yes +	(No)	Yes ++	(No)	(Yes ++)	(Yes ++)	(Yes ++)	(Yes ++)
Autoimmune complications	No	No	No	No	No	No	?	?	?	?
Breakthrough due to missed doses[#]	No	No	No	Yes +	No	Yes +	(Yes +)	(Yes +)	(Yes ++)	(Yes ++)
Breakthrough due to CCP activation[#]	No	No	No	No	No	No	No	No	Yes +++	Yes +++
Patients' compliance and convenience										
Adm. route	IV	IV	IV and SC	SC	SC	SC	SC (infusion)	SC (infusion)	Orally	Orally
Frequency[@]	8 weeks	2 weeks	1–2-4 weeks	Daily	4–8 weeks?	Daily	Daily	Daily	TID	BID
Self-administration	No	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes

Statements in parenthesis are based on preclinical data but still lack confirmation from clinical trials. The number of + indicates the strength of the evidence.
[$]: Efficacy on most typical reasons of eculizumab treatment failure.
[§]: Only in combination with eculizumab.
^: In combination with eculizumab and likely in monotherapy.
*: The possibility of monotherapy is based on available data and current trials rather than on theoretical assumptions.
[#]: Breakthrough intravascular hemolysis may occur because of suboptimal inhibition due to both PK reasons (agents with small therapeutic window or short half-life) or to PD reasons (transiently increased complement activation which may overcome the inhibitory effect of some agents, or activation through pathways which are not inhibited by selective agents)—the risk of breakthrough hemolysis is based on available clinical data and on the specific PK and PD of individual agents.
[°]: The infectious risk (as compared to eculizumab treatment) is based on the specific target (upstream inhibitors may carry an increased infectious risk), the deepness of inhibition (a deeper and more sustained inhibition may result in an increased infectious risk) and on the possibility to easily rescue complement activity by treatment stopping; IV: intravenous; SC: subcutaneous.
[@]: Frequency during maintenance treatment; BID: *bis in die* (twice a day); TID: *ter in die* (thrice a day).

primary endpoint of both study is LDH change; data from these trials are still pending. Likely, biological and even clinical efficacy in terms of control of intravascular hemolysis were seen, since an extension study for patients enrolled in any of the initial study has been launched (NCT03225287).[82]

2.8 | TT30 (Alexion)

TT30 is a 65 kDa engineered protein consisting of the functional domain of complement factor H (FH) merged with the iC3b/C3dg-binding domain of complement receptor 2 (CR2).[51] TT30 has shown complete abrogation of intravascular hemolysis and of C3 fragment

opsonization of PNH erythrocytes *in vitro*.[52] A single ascending dose, phase I study was started in 2011 (NCT01335165); a total of 10 untreated PNH patients were enrolled to investigate tolerability, PK, PD, and immunogenicity of two different formulations of TT30 given as single IV infusion or SC injection.[83] TT30 was safe and well tolerated, with no dose-related safety concern; no immunogenicity was observed.[84] Initial PK and PD data demonstrate that TT30 may achieve pharmacological levels able to inhibit the CAP; this inhibition resulted in transient LDH decrease, eventually proving the concept of therapeutic efficacy of CAP inhibitors in PNH.[84] However, the short half-life of the compound precludes further clinical development of TT30 in its current form, and this program was stopped.[84]

2.9 | AMY-101 (Amyndas)

Compstatin is a 13-residue disulfide-bridged peptide[45] which binds to human C3 and C3b, preventing C3 cleavage and C3b incorporation in C3/C5 convertase, eventually abrogating complement activation along all activating pathways.[46] AMY-101/Cp40 is a last generation compstatin analog with increased inhibitory potency and better PK profile,[47] which has been proven effective *in vitro* to inhibit lysis and C3 fragment opsonization of PNH erythrocytes[48]; thus, this is an excellent candidate which anticipates to prevent both intravascular and C3-mediated extravascular hemolysis of PNH.[48] After preclinical investigations in non-human primates,[85] a phase I single and multiple ascending dose study investigating safety, PK and PD of AMY-101 in healthy volunteers has been completed (NCT03316521).[86] Even if data have not been presented yet, Amyndas has announced a translation program for PNH which includes two separate phase II trials investigating the efficacy of a therapeutic regimen in untreated and poor responder PNH patients.[85]

2.10 | APL-2 (Apellis)

APL-2 is a pegylated version of the first-generation compstatin POT-4, with possible long-lasting action. Initial safety and PK of APL-2 was investigated in 40 healthy volunteers enrolled in two phase I studies[87]; two phase Ib studies for PNH patients are currently ongoing.[88,89] A first study (Pharoah; NCT02264639) investigates APL-2 as add-on therapy in PNH patients with inadequate response (defined as Hb level $<$10 g/L and/or need of red blood cell transfusion) to eculizumab.[88] The second study (Paddock; NCT02588833) rather exploits APL-2 as single agent for untreated PNH patients with meaningful hemolysis (LDH $>$2x ULN).[89] APL-2 was used as daily SC infusions, at doses escalating up to 270 mg per day. The primary endpoint of both studies is safety; however preliminary efficacy was investigated as well. Even if results are pending, Apellis has announced some data.[90] In all the 3 untreated PNH patients exploiting APL-2 as single agent, a marked decline in LDH level was observed[90]; in the 6 inadequate responders to eculizumab the add-on of APL-2 resulted in mild increase of hemoglobin and reduction of transfusion burden, with concomitant normalization of LDH.[90] These data seem to support the concept that upstream interception of the complement cascade may impact both intravascular and extravascular hemolysis; however, more detailed information are needed to understand the possible role and the best use of APL-2 in PNH (eg, monotherapy vs combined treatment, full vs subtotal C3 inhibition, treatment regimen, etc.). While an extension treatment is ongoing, Apellis also announced that a phase III program for PNH will start soon.[90]

2.11 | ACH-4471/ACH-0144471 (Achillion)

ACH-4471 is one of the anti-FD small molecules developed by Achillion with *in vitro* efficacy in PNH.[57,58] In a phase I study in healthy volunteers investigating single, oral, ascending doses of ACH-4471 no major safety issue was raised; doses of 200–600 mg resulted in peak plasma level within a few (1–2) hours, with terminal half-life of about 9 hours.[91] *Ex vivo* evaluation of plasma CAP activity showed that pharmacological levels (IC_{90} was identified at 230 ng/mL) were achieved even after a single dose, eventually anticipating a bi-daily dosing for therapeutic application.[91] Based on these single- and double-dose data (and on another 14-day multiple ascending dose trial in healthy volunteers), ACH-4471 started its clinical translation in PNH, initially as single agent in untreated patients. A first trial investigating ascending doses was performed in New Zealand (NCT03181633)[92]; data are not available, but an extension study is currently ongoing (NCT03053102),[93] eventually suggesting no major safety issue and possible efficacy.[94] Another similar phase II proof-of-concept study in untreated patients has been recently launched in Europe (EudraCT Number 2016–002652-25), exploiting a fixed dose of 150 mg (possibly increased to 200 mg) three times a day; again here the primary endpoint includes the inhibition of intravascular hemolysis, as assessed by LDH.[95] In parallel, Achillion has also announced a phase II trial for PNH patients with inadequate response to eculizumab (EudraCT Number 2016–003526-16); this approach is somehow supported by *in vitro* data showing a possible synergism between ACH-4471 and eculizumab.[94] As for other upstream complement inhibitors, the prediction is that ACH-4471 in the clinic should prevent both intravascular and extravascular hemolysis of PNH patients; whether the best approach is a monotherapy or a combination treatment will require further clinical investigations.

2.12 | LNP023 (Novartis)

Potent and selective orally bioavailable anti-FD and anti-FB agents have been developed by Novartis; all these compound *in vitro* prevent hemolysis and C3 opsonization of PNH erythrocytes.[56,59] A phase II trial with the anti-FB agent LNP023 for PNH has been announced (EudraCT Number 2017-000888-33), aiming to investigate safety, PK, PD and efficacy of this inhibitor as add-on therapy in PNH patients with residual hemolysis during eculizumab treatment.[96] The study population includes PNH patients who have LDH $>$1.5x ULN on stable doses of eculizumab.[96] LNP023 will be given orally 200 mg bi-daily, based on results (not publically available) from a previous phase I study in healthy volunteers. This investigational treatment will be added on top of standard of care, defined as stable doses of eculizumab. Notably, the primary endpoint of this study is the improvement of hemolysis, which will be assessed based on changes in LDH plasma level (C3 deposition will be studied as well).[96] Thus, both inclusion criteria and

primary endpoint address the issue of residual intravascular hemolysis during anti-C5 treatment, and may sound a little bit bizarre for an agent which has been designed to target C3-mediated extravascular hemolysis. Nevertheless, one may anticipate that, in case of therapeutic efficacy, the upstream inhibition of CAP will also prevent residual intravascular hemolysis, especially in combination with eculizumab. However, secondary endpoints may allow to explore the effect of LNP023 on possible C3-mediated extravascular hemolysis, hopefully to better understand the full potential of anti-FB agents as treatment for PNH, possibly even in mono-therapy.

3 | RECOMMENDATIONS FOR FUTURE INVESTIGATIONS

What is the best way to improve the current anticomplement treatment of PNH? There is no complete agreement among experts in the field, and different strategies are currently under investigation; some of them have been developed from robust scientific rationale, while other just derive from obvious competition between pharmaceutical companies. It has to be remarked that all the agents described in this manuscript may improve therapeutic complement inhibition for PNH patients, but none of them will cure PNH. Indeed, these agents are designed to treat only one of the clinical hallmark of PNH, namely complement-mediated intravascular hemolysis. Based on the long-term experience with eculizumab[5–7] we may anticipate that any effect on hemolysis (irrespective of the target in the complement cascade) may also translate into similar effect on thromboembolism, but no effect is expected on the underlying bone marrow disorder associated with PNH. Indeed, AA and possible evolution to malignant disease may appear during the course of PNH, and remain feared complications which require timely diagnosis for adequate treatment (other than complement inhibition). The preliminary data described here, together with a deep understanding of the pathophysiology of PNH (and of real medical needs), may already allow to make conclusions about what we can expect from each strategy, and which ones may prevail in the long term. The majority of novel anti-C5 agents address the possibility to improve patients' convenience with agents delivered with longer intervals, and/or in self-administration. Many of them also address the problem of intrinsic genetic resistance to eculizumab (even if we cannot exclude that we may see the emergence of different polymorphisms of C5 affecting regions targeted by other inhibitors). The next generation anti-C5 therapies aim to achieve a better control of intravascular hemolysis through a deeper inhibition of the terminal complement effector pathway; this might be achieved also combining two distinct anti-C5 agents, as shown *in vitro*.[23] Indeed, in some trials LDH normalization emerges as primary endpoint, even if the clinical meaning of this goal remains to be proven. After more than 10 years of experience with eculizumab it is obvious that residual complement activity exists due to either PD (eg, excess complement activation leading to massive C5 convertase generation, possibly favored by membrane-bound C3b)[21] and/or PK reasons.[19] Nevertheless, this quasi-complete C5 inhibition was sufficient to generate a great clinical benefit with minimal detrimental effect[2–7]; future data will have to prove that a deeper C5 inhibition may result in a better hematological response and in a meaningful clinical benefit without carrying increased safety risk (eg, infectious complications).[65] Based on current understanding of PNH biology during anti-C5 treatment, complete functional knock-down of C5 should limit PD and PK breakthrough, with further reduction of intravascular hemolysis[65]; however, no more than 15%-20% of patients show laboratory signs of intravascular hemolysis during eculizumab treatment and may benefit from these novel approaches.[19,97]

Conversely, C3-extravascular hemolysis seems the most important mechanism limiting hematological efficacy of eculizumab.[12,14,22–26] This pathogenic mechanism cannot be affected by any of the novel anti-C5 agents (even if used in combination), and rather requires therapeutic interference upstream in the complement cascade. The possible impact of such upstream inhibition on safety needs to be carefully investigated; at the moment there are no clinical data about the infectious risk associated with therapeutic inhibition of the upstream steps of complement activation. Some information may be inferred only from inherited complement deficiencies, which are rare and not always well characterized (ie, most data are based on a few patients selected for a severe phenotype).[98–100] About 20 families with inherited C3 deficiency have been described, with increased risk of infections (mostly by encapsulated bacteria, such as *Streptococcus pneumoniae*, *N. meningitidis*, and *Haemophilus influenzae*) which tend to be severe and recurrent.[99–101] Severe or recurrent infections have been associated also with the very rare deficiencies of all the key CAP component, ie, properdin,[102] FB,[103] and FD.[104] However, it has to be remarked that these severe infectious events mostly occur in the childhood, before that adaptative immunity has established, and most subjects carrying these deficiencies remain alive once the diagnosis is done and proper prophylaxis has been started. This observation, together with the lesson that therapeutic inhibition is not a phenocopy of inherited deficiency (as learnt by eculizumab), may support the feasibility of therapeutic upstream complement inhibition. At this stage the two main strategies of anti-C3 and anti-FD/FB inhibitors seem both equivalent in terms of possible efficacy. The obvious advantage of the oral administration of anti-FD/FB agents (which are CAP-selective) is somehow counterbalanced by the possible risk of hemolytic paroxysms due to activation through the CCP or the CMP, which instead would be prevented by broad C3 inhibitors; the preservation of CCP and CMP might also represent a benefit in terms of reduced infectious risk. All these upstream inhibitors promise to be the most active agents to ameliorate anemia in PNH, which, combined with a sustained control of intravascular hemolysis, should become the clinical goal for next generation complement inhibition. Of course, possible safety concerns, mostly infectious risk, may jeopardize the future of these agents. Likely, objective data will determine whether this strategy should be limited to patients with clinically meaningful C3-mediated extravascular hemolysis, or if it may have a broader use for all PNH patients. In this setting, the understanding of the complement cascade suggests that the complete inhibition of one key component of the cascade (eg, C5, or C3, or FD/FB) should be enough to get the therapeutic effect, eventually supporting monotherapy even for upstream inhibitors. However, it has

been also hypothesized that a sub-total inhibition of two key components (eg, C3 or FB/FD in addition to C5) may result in similar clinical results, while preserving a minimal complement activity which could somehow mitigate infectious risks.[20] Future clinical investigations will have to specifically address also this possibility.

4 | CONCLUSIONS

In summary, more than ten novel complement inhibitors are currently in development for PNH; different strategies have been exploited, and their specific pros and cons can be anticipated (Table 3). Novel anti-C5 agents promise to recapitulate the excellent results seen with eculizumab in terms of safety and efficacy, possibly improving the ease of treatment and subsequently patients' convenience; furthermore, hematological benefit may be anticipated in some patients not achieving adequate control of intravascular hemolysis. In contrast, upstream complement inhibitors targeting C3 or key molecules of the CAP (ie, FD and FB) have been conceived aiming to control C3-mediated extravascular hemolysis, eventually anticipating a broader improvement in terms of hematological efficacy. Likely, ongoing trials will not lead to the identification of a unique, perfect complement inhibitor recommended for all PNH patients. Nevertheless, if these trials will be adequately performed and coming data presented through standard peer-review process, these efforts may lead soon to the clinical availability of different novel complement inhibitors. In this future scenario, each agent may have a specific place in the treatment algorithm (possibly even tailored on individual patients), eventually leading to a substantial improvement of the management of PNH. Hopefully, this achievement won't be jeopardized by exaggerated pricing of these novel agents; indeed, a broader worldwide access to appropriate treatment remains a critical unmet need in the field of PNH.

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CONFLICT OF INTEREST

AMR received research support from Alexion, Alnylam, Rapharma and Novartis; AMR received lecture fees and serves as member of an investigator board for Alexion. AMR and SM are involved as investigators in clinical trials exploiting the following agents: TT30, ALXN1210, SKY59, ACH-4471, LNP023, and AMY-101.

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