NOVEL ANTI-COMPLEMENT AGENTS FOR PNH: FROM ECULIZUMAB TO COMPLEMENT INHIBITION 2.0

Serena Marotta

Hematology, Department of Clinical Medicine and Surgery; Federico II University, Naples (Italy).

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, irrespective of the enthusiastic efforts from academicians and pharmaceutical companies aiming to develop novel complement inhibitors, no additional anti-complement agent has been approved. Indeed, the treatment of PNH has been revolutionized by eculizumab: since the pioneering experience, it became evident that therapeutic inhibition of C5 resulted in sustained inhibition of intravascular hemolysis typical of PNH, eventually resulting in a major clinical improvement.[2] The 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] 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 *N. Meningitidis*).[3-5] Furthermore, 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 long-term use of eculizumab in PNH is more than a supportive treatment, since it has been demonstrated that it largely affects the natural history of the disease. Indeed, 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, eculizumab is the current gold standard for PNH patients presenting with hemolytic and/or thromboembolic disease (while those with meaningful bone marrow failure should follow treatment algorithm of aplastic anemia).[10,11]

Nevertheless, PNH is not cured by eculizumab, since the only curative option for PNH remains hematopoietic stem cell transplantation;[12] and, irrespective of the remarkable impact of anti-C5 treatment on survival and disease manifestations, some unmet clinical needs emerged in the era of eculizumab. Three major unmet needs can be identified: i. limited access to current anti-complement treatment due to elevated cost (eculizumab is not approved in vast areas of the world, and even where it is approved the indications to treatment are somehow restricted to more ill patients); ii. heaviness of life-long bi-monthly intravenous infusion; iii. insufficient clinical benefit, usually in terms of residual anemia. While the first two points may have a limited scientific interest, the discussion of suboptimal clinical hematological response to eculizumab represents the main driver to develop novel strategies of therapeutic complement inhibition. True resistance to eculizumab treatment has been documented only in a few patients, mostly of Japanese ethnicity, carrying an inherited polymorphism of C5;[13] for these patients at the moment there is no available treatment. However, even in patients with clear evidence of effective complement blockade (as documented by lactate dehydrogenase [LDH] level), the hematological benefit is quite heterogeneous, and residual anemia pertain to about two thirds of PNH patients on eculizumab, with about one third still requiring red blood cell transfusions.[3,4,14-16] The most obvious cause of residual anemia during eculizumab is inadequate compensatory erythropoiesis due to

concomitant aplastic anemia. Indeed, even in patients with hemolytic PNH, bone marrow failure is possible at any time during the disease course, due to the well-established overlap between AA and PNH;[17-19] these patients should receive the appropriate treatment according to the AA algorithm.[10,11,20,21] 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. It is now well accepted that 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 breakthrough").[16] Furthermore, a "pharmacodynamic breakthrough" has been described, which accounts for lack of LDH normalization in most patients (i.e., as a marker of low-grade continuous intravascular hemolysis), as well as for possible hemolytic paroxysms in concomitance with infectious episodes (or any other event) triggering a massive complement activation.[22,23] For patients with pharmacokinetic breakthrough either the increase of eculizumab dose to 1200 mg or the reduction of the dosing interval to 10 days have been proven somehow effective.[24] However, the most common cause of residual anemia in PNH patients on eculizumab has been identified in C3-mediated extravascular hemolysis. This phenomenon, which is never seen in untreated PNH patients and unmasked by eculizumab treatment, mechanistically pertains to most PNH patients on eculizumab as a result of upper (upstream C5) complement activation, which remains uncontrolled irrespective of C5 blockade.[25] As a result, progressive deposition of C3 fragments on PNH erythrocytes sparing terminal complement-mediated lysis eventually leads to chronic extravascular hemolysis.[25-28] While C3 opsonization has been confirmed by several groups,[25,29,30] the actual clinical relevance of extravascular hemolysis has not been fully acknowledged by all experts;[7] nevertheless, C3-mediated extravascular hemolysis impairs hematological response to eculizumab in 25-50% of patients.[7,25,29,30] The reasons accounting for such heterogeneous clinical weight remain to be elucidated, even if a possible role for genetic variants of complement-related genes has been postulated and already reported, for instance, for the hypomorphic variant of complement receptor 1 (CR1) gene.[31] While the use of steroids is discouraged,[27] splenectomy has been anecdotically reported effective to treat C3-mediated extravascular hemolysis;[32,33] however, at the moment there is no reliable treatment option for this common event emerging during eculizumab treatment.[34]

Novel strategies of complement inhibition

The unmet clinical needs described above, together with a growing financial interest driven by the high price of current anti-complement treatment, have triggered the development of novel complement inhibitors. A plethora of agents is currently in the pipeline of several pharmaceutical companies (Table 1);[35] they can be divided in two major categories, according to their mechanism of action. The first category exploits novel agents targeting C5 (similarly to eculizumab), while the second one pioneers the possibility of intercepting the complement cascade upstream, at the level of its initiating activation steps.

Novel inhibitors of C5

Giving the excellent safety and efficacy of eculizumab, C5 remains a good target for developing alternative agents blocking the terminal complement effector pathway. Novel C5 inhibitors include anti-C5 mAb, as well as different classes of compounds. In addition to the eculizumab biosimilar ABP 959 developed by AMGEN,[36] there are at least five additional anti-C5 mAb in clinical or preclinical development. ALXN1210 and ALXN5500 are two anti-C5 mAb with longer half-life, potentially available even for subcutaneous injections, developed by Alexion; they share with eculizumab the same C5 target epitope. The leading compound ALXN1210 has been granted with orphan designation by FDA; a phase I study in healthy volunteers demonstrated immediate, complete, and sustained C5 inhibition, with a terminal half-life about four-fold longer than eculizumab.[37] Another humanized anti-C5 mAb with long half-life is named SKY59 (also known as RO7112689), and it is in development by Roche. SKY59 has a pH-dependent binding to a different epitope of C5, which leads to a continuous recycling eventually accounting for its very long half-life.[38] In a preclinical model, SKY59 was shown effective in suppressing C5 function and complement activity (even on the Japanese C5 variant p.Arg885His) for a significantly longer duration compared to conventional mAb.[38] Novartis is also developing a fully human anti-C5 mAb named LFG316; this agent, initially developed for ophthalmic indications, is now under investigation for PNH within a proof of concept study enrolling patients in Japan.[39] The list of mAb-based C5 inhibitors also includes an engineered derivative exploiting the Ab fragment of an anti-C5 mAb; this minibody called Mubodina is in development by Adienne Pharma & Biotech,[40,41] but so far no trial for PNH has been started.

Therapeutic C5 inhibition is not limited to anti-C5 mAb; indeed, different strategies exploiting non-Ab molecules have been exploited. The most elegant and novel strategy exploits a small interfering RNA (siRNA) duplex specific for C5, which is able to completely silence C5 production from the liver in an animal model.[42] This compound, developed under the name of ALNCC5 by Alnylam, has been proven safe and effective in a phase I trial in healthy individuals (n=32), resulting in >99% reduction of C5 plasma level and >95% inhibition of serum complement activity.[43] ALNCC5 has been already investigated in proof of concept studies for PNH, as discussed below. Two additional non-Ab anti-C5 compounds are currently under investigation in PNH. Coversin (also known as OmCI) is a 16 kDa protein of the lipocalin family derived from the tick Ornithodoros moubata.[44,45] Coversin is able to prevent C5 cleavage by its convertases,[44,45] and its *in vitro* efficacy in PNH has been already documented,[46] even in patients carrying C5 polymorphisms.[47] In a phase I study in healthy volunteers, coversin was shown bioavailable after subcutaneous injections, with excellent PK and PD in absence of immunogenicity and of other safety concerns.[45] Akari is currently performing clinical studies investigating this agent in PNH patients. Finally, a small macrocyclic peptide named RA101495 has been developed by RApharma.[48] This is a small, cyclic, peptide-like polymer of the class of cyclomimetics which is produced by ribosomal synthesis of unnatural peptides, and harbors excellent cell permeability, stability, potency, and bioavailability.[48,49] RA101495 has been shown to inhibit hemolysis in an *in vitro* model of PNH,[49,50] and a clinical translation program for PNH is ongoing. A

number of alternative technologies have been exploited to develop newer classes of compounds (e.g., aptamers, SOMAmers, affibodies)[35] with high specificity for C5; however, since they are in early stage of clinical development, they are not discussed in this manuscript.

Inhibitors of early steps of complement activation

The concept that the inhibition of early steps of complement activation might be beneficial in PNH rose from the initial description of C3-mediated extravascular hemolysis,[15] and took advantage from preclinical researches done in other fields of medicine.[51-53] C3 cleavage by C3 convertases 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. Indeed, any initial complement activation merges at the level of C3, and eventually leads to C5 cleavage and to the activation of the harmful terminal effector mechanisms. Given that each complement pathway exploits different proteins, inhibitors of early complement activation may be divided in two groups: i. broad inhibitors of C3 and ii. pathway-specific inhibitors of initiating events (see Table 2 and Figure 1). Here we'll focus only on agents potentially effective for PNH, which includes C3 inhibitors and CAP inhibitors (the role of CCP and CMP in the pathophysiology of PNH is considered somehow limited, even if experimental evidences are lacking).

Direct inhibition of C3 has been postulated, but it is challenging because of the very high plasma level of C3 (~1 mg/mL). Indeed, mAb specific for native C3 have not been developed; a mAbselectively targeting the activated C3 fragments C3b and iC3b (3E7, and its de-immunized derivative H17) has been investigated in PNH *in vitro*, showing prevention of both hemolysis and C3 opsonization of PNH erythrocytes.[54] However, these anti-C3b/iC3b mAb (which eventually work as selective CAP inhibitors) cannot be used in PNH as they are due to expected worsening of extravascular hemolysis through the Fc receptor; further derivatives of these mAb exploiting Fab fragments for therapeutic purpose are still in preclinical investigation.[55] Thus, therapeutic inhibition of C3 is mostly exploited using small peptide inhibitors, namely the 13-residue disulfide-bridged peptidecompstatin and its derivatives.[56] Compstatin is a broad complement inhibitor which binds to human (and all primate) C3 and C3b, preventing C3 cleavage and C3b incorporation in C3/C5 convertase.[57,58] Thus, compstatin and its derivatives completely abrogate complement activation along all the three activating pathways CCP, CAP and CMP; these molecules also disable the CAP-mediated amplification loop.[57,58] The spectrum of compstatin analogs is growing, with improved PK and PD properties which may facilitate therapeutic application;[59,60] both first and second generation compstatin derivatives have been proven effective in PNH *in vitro*, showing excellent control of hemolysis and of C3 opsonization.[61,62]

In addition to molecules targeting C3, therapeutic complement inhibition in PNH may aim to intercept the activation through the CAP; the two main strategies of CAP inhibition exploit engineered proteins based on endogenous regulators of complement activation, as well as small inhibitors of key components of CAP.[35] CR1 and CFH are endogenous negative regulators of complement which both

act as: i. co-factor of complement factor I for C3b inactivation;[63] ii. and prevention of formation and acceleration of decay of C3 convertase (for CFH, only CAP convertase).[64,65] Given their pleiotropic effects on complement regulation, these endogenous inhibitors have been exploited for therapeutic use through the generation of engineered proteins with targeted inhibitory properties.[66] The first agent of this class is TT30, a 65 kDa engineered protein consisting of the functional domain of CFH merged with the iC3b/C3dg-binding domain of complement receptor 2 (CR2).[67] The activity of TT30 in PNH has been investigated *in vitro,* showing complete abrogation of intravascular hemolysis and C3 fragment deposition of PNH erythrocytes.[68] Based on this data, TT30 has entered its clinical translation in PNH.[69] CFH-based inhibitors have been developed also through the generation of miniaturized versions fusing the inhibitory domain and the C3-binding domain of CFH;[70] as TT30, mini-FH prevents hemolysis and C3 opsonization of PNH erythrocytes *in vitro*.[70] Inhibitors based on CR1 have been developed too;[35] mirococept (APT070) is an engineered version of CR1 specifically designed for incorporation in cell membranes,[71,72] which is now in clinical development for kidney transplantation.[73,74] The possible role of CR1-based inhibitors in PNH is somehow supported by the clinical observation that PNH patients carrying the hypomorphic variant of CR1 have a worse clinical response to eculizumab *in vivo,* and exhibit more pronounced C3 deposition both *in vitro* and *in vivo.*[75]

The latest strategy of selective inhibition of CAP is based on inhibitors of complement factor B (FB) and complement factor D (FD), the two key molecules of the complement activation along the alternative pathway; inhibitors of properdin have been postulated as well.[76] Even if antibody-based inhibitors have been considered, the most recent class of compound include small molecules specific for either FD or FB. Potent and selective anti-FD agents have been developed by Novartis;[77] all these compound *in vitro* prevent hemolysis and C3 opsonization of PNH erythrocytes.[78] Anti-FD small molecules have been developed also by Achillion (ACH-3856, ACH-4100, ACH-4471), with similar potential activity in PNH *in vitro*.[79,80] Notably, all these anti-FD small molecules are bioavailable after oral administration.[78,79] Furthermore, CAP inhibitors developed by Novartis also include small molecules targeting FB;[81] these orally bioavailable anti-FB small molecules share with anti-FD agents the *in vitro* activity on PNH erytnrocytes.[82] Both anti-FD and anti-FB have entered their clinical development, and clinical trials for PNH patients have started.

Clinical translation: status of art and anticipated results

Many of the agents described above have started their clinical development for the treatment of PNH; here we'll summarize publically available information, describing the ongoing trials with their design and preliminary data (when available), or results anticipated by their mechanism of action.

ABP 959 (Amgen)

The anti-C5 mAb ABP 959 has been initially tested in Australia in a phase I randomized trial investigating PK and PD of this compound as compared to eculizumab (ACTRN12616000509460).[83]

Data are not publically available yet; nevertheless, a large phase III randomized trial is currently ongoing in Europe (EudraCT Number 2017-001418-27).[36] 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 ABP 959 is provided.

ALXN1210 (Alexion)

ALXN1210 has been already tested in two phase I/II trials enrolling untreated PNH patients (NCT02598583 and NCT02605993) aiming to evaluate safety, tolerability, and efficacy of two different intravenous (IV) dosing regimens of ALXN1210.[84,85] No deaths, serious adverse events, drug discontinuations, or adverse event (AE) leading to withdrawals were recorded.[86] The interim analysis of these studies demonstrated rapid, complete, and sustained C5 inhibition, eventually leading to prompt and sustained LDH reduction and frequent hemoglobin stabilization in all treatment cohorts;[86] these results have just been updated.[87] The two studies enrolled 13 and 26 patients, respectively. 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, as demonstrated by LDH reduction (with normalization in 6/7) and no breakthrough episode.[87] Notably, 2 out of 39 patients on ALXN1210 experienced *N. Meningiditis* infections presenting as sepsis;[87] both patients completely recovered after ceftriaxone treatment, and continued the ALXN1210 treatment within the study.[87] These results represented the background to design the two large phase III trials which 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,[88] as well as switch therapy for patients already on eculizumab with adequate control of hemolysis (LDH <1.5x ULN).[89] Notably, in both studies changes in LDH levels appear as primary endpoint, likely because of a possible deeper inhibition of terminal effector complement with ALXN1210 (as suggested by larger reduction of LDH),[87] which may result in a better control of chronic intravascular hemolysis (e.g., less PK and/or PD breakthrough). Whether this more profound complement inhibition may affect the risk of infectious complications (i.e., the actual incidence of *N. Meningiditis* infections needs to be evaluated carefully;[87] low-level residual C5 activity demonstrated with eculizumab[23] might be somehow protective from infections), as well as the clinical meaning of this better control of intravascular hemolysis will be answered by experimental data expected by late 2018. Nevertheless, the most obvious benefit from this long-lasting C5 inhibitor will pertain to patients' compliance, 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 the ongoing trials.

SKY59/RO711268 (Roche)

This other long-lasting anti-C5 mAb is currently under investigation within a phase I/II study (NCT03157635)[90] 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 I. Then, part 2 and 3 included untreated and eculizumab-treated PNH patients, respectively, aiming to evaluate safety, tolerability, PK, PD and efficacy of RO7112689 in PNH.[90] 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. Nevertheless, 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 of the trial. In this part PNH patients will receive a loading IV dose of RO7112689 on Days 1, followed by SC administration of RO7112689 at different doses and schedule. To be included in the trial PNH patients must be on stable doses of eculizumab for at least three months; the primary endpoint of the study is PD of RO7112689, as measured by LDH and complement activity assays. Here the anticipation is that this molecule, once the right schedule is identified, should parallel excellent efficacy and safety of eculizumab, with possible advantage in terms of patients' compliance due to SC administration (also depending on dose interval). Detailed PD data will tell whether, as anticipated for ALXN1210, 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 address the problem of genetic resistance to eculizumab.[13,38]

LFG316 (Novartis)

A proof of concept phase I study is currently investigating LFG316 in untreated PNH patients; the primary endpoint of the study is biological efficacy, as measured by LDH change.[39] This study, which is performed mostly in Japanese centers, will likely represent an option for patients carrying C5 polymorphisms associated with intrinsic resistance to eculizumab.[13]

ALNCC5 (Alnylam)

The phase I/II first-in-human trial with ALNCC5 also included an arm enrolling PNH patients;[91] preliminary data were presented at ASH 2015[92] and updated at ASH 2016.[93] 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 irrespective of increased dose up to 1200 mg). No severe AE was observed, nor AE requiring treatment discontinuation; one patient developed increased transaminases, possibly related to the drug (or to liver damage and/or hemolysis secondary to concomitant infection).[92,93] In untreated patients, ALNCC5 resulted in C5 knockdown >98% (as previously seen in healthy individuals).[43] 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.[92,93] This was consistent with data using C5-depleted sera in an in vitro model of PNH,[61,68] 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).[92,93] The potential efficacy of a combined treatment with ALNCC5 and eculizumab was also investigated in the 3 patients already on eculizumab; indeed, combined treatment resulted in LDH normalization, even in the patients experiencing breakthrough (who was able to reduce eculizumab at the standard schedule of 900 mg every other week).[92,93] Based on these data, the best setting for further development of ALNCC5 in PNH is in combination with eculizumab, to improve response in poor responders;[94] this combination therapy may also represent an option to achieve deeper control of residual intravascular hemolysis, and/or reduce the dosing of eculizumab (cost-effectiveness of this strategy can be drawn only knowing the price of ALNCC5).

Coversin (Akari)

Coversin is currently under investigation in PNH within different phase II trials;[95,96] the first proof of efficacy of coversin was shown in PNH patients resistant to eculizumab.[95] Subsequently, coversin was investigated in a global phase II study (COBALT) which exploited SC coversin at a fixed initial dose of 60 mg followed by bi-daily doses (15-30 mg) for 28 days and then 30 mg daily for additional two months.[96] Data are available for the first 5 untreated PNH patients enrolled;[97] four of them remained on coversin (one was withdrawn because of possible co-morbidity). 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.[97] 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.[97] 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 may be optimized (with the current schedule residual intravascular hemolysis is quite frequent), and the concerns about possible neutralizing antibody requires further investigations.

RA101348 (RApharma)

Rapharma is developing a groups of anti-C5 small macrocyclic peptides,[49] including some orally bioavailable agents;[98] the lead compound RA101495[50] was investigated in a phase I study in healthy volunteers.[99] RA101495 was safe and well tolerated as SC injections in healthy volunteers, with only mild local irritation;[99] a daily dosing allowed full and sustained suppression of complement activity (>95%).[100] Based on these data, 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 (these latter switched to RA101495).[101] The second study NCT03030183 rather investigated the same treatment regimen in PNH patients with inadequate response to eculizumab, defined as LDH >1.5x ULN.[102] The primary endpoint of both study is LDH change; data from these trials are still pending. Based on preclinical work, it is expected that biological

and even clinical efficacy in terms of control of intravascular hemolysis are seen, since an extension study for patients enrolled in any of the initial study has been launched.[103]

TT30 (Alexion)

TT30 was the first second-generation complement inhibitor tested *in vivo* in PNH patients; indeed, a single ascending dose, phase I study was started in 2011 and a total of 10 untreated PNH patients were enrolled to investigate tolerability, PK, PD, and immunogenicity of two different formulations of TT30 (ALXN1102 and ALXN1103) given as single IV infusion or SC injection (NCT01335165).[69] Preliminary results from this study ws presented at ASH 2015:[104] TT30 was safe and well tolerated, with no dose-related safety concern; no immunogenicity was observed. Initial PK and PD data demonstrate that TT30 may achieve pharmacological levels able to inhibit the CAP; this inhibition may result in transient LDH decrease, eventually proving the concept of possible therapeutic efficacy of CAP inhibitors in PNH.[104] However, the short half-life of the compound in its current form precluded further clinical development of TT30 in its current form, and this program was stopped.[104]

AMY-101 (Amyndas)

AMY-101/Cp40 is a last generation compstatin analog with increased inhibitory potency and better PK profile,[60] which has been proven effective *in vitro* to inhibit lysis and C3 fragment opsonization of PNH erythrocytes;[61] thus, this is an excellent candidate which anticipates to prevent both intravascular and C3-mediated extravascular hemolysis of PNH.[61] The potential therapeutic benefit of AMY-101 over standard anti-C5 treatment has been recognized by medicines agencies from both Europe (EMA) and US (FDA), who recently granted Orphan Drug designation to AMY-101 for the treatment of PNH. After pre-clinical investigations in non-human primates,[105] a phase I single and multiple ascending dose study investigating safety, PK and PD of AMY-101 in healthy volunteers has been completed.[106] 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 (as identified in phase I) in untreated and poor responder PNH patients.[105]

APL-2 (Apellis)

Another compstatin analog is in development by 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;[107] two phase Ib studies for PNH patients are currently ongoing.[108,109] 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.[108] The second study (Paddock; NCT02588833) rather exploits APL-2 as single agent for untreated PNH patients with meaningful hemolysis (LDH >2x ULN).[109] 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, and even if full results are pending Apellis has disclosed some data.[110] In all the 3 untreated PNH patients exploiting APL-2 as

single agent, a marked decline in LDH level was observed;[110] in the 6 inadequate responders to eculizumab the add-on of APL-2 resulted in mild increase in hemoglobin and reduction in transfusion burden, with concomitant normalization of LDH.[109] 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 (e.g., monotherapy vs combined treatment, full vs subtotal C3 inhibition, possible bi-weekly dosing, etc.). While an extension treatment is ongoing, Apellis also announced that a phase III program for PNH will start soon.[110]

ACH-0144471 (Achillion)

ACH-0144471 (also known as ACH-4471) was initially investigated in a Phase I study in healthy volunteers;[111] ACH-4471 was given orally at single ascending doses. 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.[111] *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.[111] Based on these single- and double-dose data (and on another 14-day multiple ascending dose trial in healthy volunteers), ACH-4471 has started its clinical translation in PNH, initially as single agent in untreated patients. A first trial investigating ascending doses was performed in New Zealand;[112] data are not available, but an extension study is currently ongoing,[113] eventually suggesting no major safety issue and possible efficacy.[114] Another similar phase II proof-of-concept study in untreated patients has been recently launched in Europe, 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 changes in LDH levels.[115] In parallel, Achillion has also announced a phase II trial for PNH patients with inadequate response to eculizumab; this approach is somehow supported by *in vitro* data showing a possible synergism between ACH-4471 and eculizumab.[114] As for other upstream complement inhibitors, the anticipation 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.

LNP023 (Novartis)

A phase II trial with the anti-FB agent LNP023 for PNH has been announced by Novartis;[116] this study aims to investigate safety, PK, PD and efficacy of this FB inhibitor as add-on therapy in PNH patients showing residual hemolysis during eculizumab treatment.[116] The study population includes PNH patients who have LDH >1.5x ULN (a marker of intravascular hemolysis) on stable doses of eculizumab.[116] 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, in PNH patients. Notably, the primary endpoint of this study is improvement of hemolysis, which will be assessed based on changes in LDH plasma level (C3 deposition will be studied as well).[116] 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, even in mono-therapy.

Recommendations for future investigations

What is the best way to improve the current anti-complement treatment of PNH? There is no complete agreement among experts in the field, and different strategies have been investigated; some of them seem to be driven also from financial interest of pharmaceutical companies rather than from robust scientific arguments. Nevertheless, 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' compliance 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 different polymorphisms involving other C5 regions targeted by novel agents may eventually emerge). The next generation anti-C5 therapies aim to achieve a better control of intravascular hemolysis through a deeper inhibition of the terminal effector complement; this might be achieved also combining two distinct anti-C5 agents, as also shown *in vitro*.[23] Indeed, in some trials LDH normalization emerges as a primary endpoint, even if the clinical meaning of this biomarker remains to be proven. After more than 10 years of experience with eculizumab it is obvious that residual complement activity exist due to either PD (e.g., excess complement activation leading to massive C5 convertase generation, possibly favored by membrane-bound C3b)[23] and/or PK reasons.[24] 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 (e.g., infectious complications).[87] 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;[87] however, no more than 15-20% of patients show laboratory signs of intravascular hemolysis which can benefit from this approach.[24,117]

On the other hand, C3-extravascular hemolysis seems the most important factor limiting hematological efficacy of eculizumab, since even after LDH normalization on anti-C5 therapy most (if not all) PNH patients continue to show increased reticulocyte counts with a various degree of anemia.[25-30] 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 complement inhibition on safety needs to be carefully investigated; however, even if the theoretically increased risk of infectious complications requires specific attention, clinical observations from the few families carrying inherited deficiencies of complement genes[118] anticipate the feasibility of this approach. 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 is somehow counterbalanced by the possible risk of hemolytic paroxysms due to activation through the CCP or the CMP (these are CAP-specific inhibitors), which would be prevented by broad C3 inhibitors. Furthermore, the preservation of some of the complement pathways may also represent a benefit in terms of reduced infectious risk. Even if these upstream inhibitors are in an earlier stage of development as compared to some novel anti-C5 agents (e.g., the long-acting ALXN1210 and SKY59), they anticipate to be the most active agents to ameliorate anemia in PNH. Indeed, such an endpoint, combined with sustained control of hemolysis, should become the clinical goal for next generation complement inhibitors. Of course, possible safety concerns, mostly infectious risk, might 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 (e.g., C5, or C3, or FD/FB) should be enough to get the therapeutic effect. However, it has been also hypothesized that a sub-total inhibition of two key components (e.g., C3 or FB/FD in addition to C5) may result in similar clinical results, while preserving a minimal complement activity which could somehow mitigate safety concerns.[119] Future clinical investigations will have to specifically address this possibility as well.

Conclusions

In summary, more than ten novel complement inhibitors are currently in development for PNH; different strategies have been exploited, each one with specific pros and cons (Table 2). Novel anti-C5 agents promise to recapitulate the excellent results seen with eculizumab in terms of safety and efficacy, possibly improving the compliance to the treatment; furthermore, hematological benefit may be anticipated in some patients not achieving adequate control of intravascular hemolysis. On the other hand, upstream complement inhibitors targeting C3 or key molecules of the CAP (i.e., FD and FB) are developed aiming to control C3-mediated extravascular hemolysis, eventually anticipating a broader improvement in terms of hematological efficacy. Likely, these 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 (rather than as pharmaceutical company announcements), hopefully these efforts will lead soon to the clinical availability of different novel complement inhibitors. In this future scenario, each agent might 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 of these novel agents; indeed, a broader worldwide access to appropriate treatment remains the most critical unmet need in the field of PNH.

References (100)

1. Rother RP, Rollins SA, Mojcik CF, et al. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. Nat Biotechnol. 2007; 25:1256-64.
2. Hillmen P, Hall C, Marsh JC, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. N Engl J Med. 2004 Feb 5;350(6):552-9.
3. Hillmen P, Young NS, Schubert J, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. N Engl J Med. 2006; 355:1233-43.
4. Brodsky RA, Young NS, Antonioli E, et al. Multicenter phase III study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. Blood. 2008; 114:1840-47.
5. Hillmen P, Muus P, Duhrsen U, et al. Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. Blood. 2007; 110:4123-8.
6. Kelly RJ, Hill A, Arnold LM, et al. (2011) Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. Blood.117:6786-92.
7. Loschi M, Porcher R, Barraco et al. Impact of eculizumab treatment on paroxysmal nocturnal hemoglobinuria: A treatment versus no-treatment study. Am J Hematol. 2016;91(4):366-70. doi: 10.1002/ajh.24278.
8. Hillmen P, Lewis SM, Bessler M, et al. Natural history of paroxysmal nocturnal hemoglobinuria. N Engl J Med. 1995;333(19):1253-1258.
9. de Latour RP, Mary JY, Salanoubat C, Terriou L, Etienne G, Mohty M, Roth S, de Guibert S, Maury S, Cahn JY, Socié G; French Society of Hematology; French Association of Young Hematologists. Paroxysmal nocturnal hemoglobinuria: natural history of disease subcategories. Blood. 2008;112(8):3099-106.
10. Bacigalupo A. How I treat acquired aplastic anemia. Blood. 2017;129(11):1428-1436. doi: 10.1182/blood-2016-08-693481.
11. Killick SB, Bown N, Cavenagh J, et al. Guidelines for the diagnosis and management of adult aplastic anaemia. Br J Haematol. 2016;172(2):187-207. doi: 10.1111/bjh.13853.
12. Peffault de Latour R, Schrezenmeier H, Bacigalupo A, et al. Allogeneic stem cell transplantation in paroxysmal nocturnal hemoglobinuria. Haematologica. 2012;97(11):1666-73. doi: 10.3324/haematol.2012.062828.
13. Nishimura J, Yamamoto M, Hayashi S et al. Genetic variants in C5 and poor response to eculizumab. N Engl J Med. 2014;370(7), 632-639.
14. Hillmen P, Muus P, Roth A, et al. Long-term safety and efficacy of sustained eculizumab treatment in patients with paroxysmal nocturnal haemoglobinuria. Br J Haematol. 2013;162(1):62-73.

15. Risitano AM, Notaro R, Marando L, et al. Complement fraction 3 binding on erythrocytes as additional mechanism of disease in paroxysmal nocturnal hemoglobinuria patients treated by eculizumab. Blood. 2009;113:4094-100.

16. Hill A, Rother RP, Arnold L, et al. Eculizumab prevents intravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria and unmasks low-level extravascular hemolysis occurring through C3 opsonization. Haematologica. 2010;95,567-573.

17. Dameshek W. Riddle: what do aplastic anemia, paroxysmal nocturnal hemoglobinuria (PNH) and "hypoplastic" leukemia have in common? Blood. 1967;30(2):251-4.

18. Rotoli B, Luzzatto L. Paroxysmal nocturnal haemoglobinuria. Baillieres Clin Haematol. 1989;2(1):113-38.

19. Luzzatto L & Notaro R. Paroxysmal nocturnal hemoglobinuria. In Handin RI, Lux SE, Stossel TP (eds): Blood, principles and practice of hematology (2nd ed.). Philadelphia, PA (USA), Lippincot Williams & Wilkins; 2003:319-34.

20. Marotta S, Giagnuolo G, Basile S, Pagliuca S, Grimaldi F, Pane F, Risitano AM. Excellent outcome of concomitant intensive immunosuppression and eculizumab in aplastic anemia/paroxysmal nocturnal hemoglobinuria syndrome. J Hematol Thromb Dis. 2014;2(1):128.

21. Pagliuca S, Risitano AM, Sicre de Fontbrune F, et al. Combined intensive immunosuppression and eculizumab for aplastic anemia in the context of hemolytic paroxysmal nocturnal hemoglobinuria: a retrospective analysis. Bone Marrow Transplant. 2017;in press.

22. Sica M, Rondelli T, Ricci P, et al. Eculizumab treatment: stochastic occurrence of C3 binding to individual PNH erythrocytes. J Hematol Oncol. 2017;10(1):126. doi: 10.1186/s13045-017-0496-x.

23. Harder MJ, Kuhn N, Schrezenmeier H, et al. Incomplete inhibition by eculizumab: mechanistic evidence for residual C5 activity during strong complement activation. Blood. 2017;129(8):970-980. doi: 10.1182/blood-2016-08-732800.

24. ASH abstract on eculizumab 1200 (vedi computer al policlinico, documenti per farmacia)

25. Risitano AM, Notaro R, Marando L, et al. Complement fraction 3 binding on erythrocytes as additional mechanism of disease in paroxysmal nocturnal hemoglobinuria patients treated by eculizumab. Blood. 2009;113:4094-100.

26. Lin Z, Schmidt CQ, Koutsogiannaki S, Ricci P, Risitano AM, Lambris JD, Ricklin D. Complement C3dg-mediated erythrophagocytosis: implications for paroxysmal nocturnal hemoglobinuria. Blood. 2015;126(7):891-4. doi: 10.1182/blood-2015-02-625871.

27. Luzzatto L, Risitano AM, Notaro R. Paroxysmal nocturnal hemoglobinuria and eculizumab. Haematologica. 2010;95(4):523-526.

28. Risitano AM, Notaro R, Luzzatto L, et al. Paroxysmal Nocturnal Hemoglobinuria: Hemolysis Before and After Eculizumab. New Engl J Med. 2010;363:2270-2.

29. Hill A, Rother RP, Arnold L, et al. Eculizumab prevents intravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria and unmasks low-level extravascular hemolysis occurring through C3 opsonization. Haematologica. 2010;95,567-573.

30. Höchsmann B, Leichtle R, von Zabern I, et al. Paroxysmal nocturnal haemoglobinuria treatment with eculizumab is associated with a positive direct antiglobulin test. Vox Sang. 2012 Feb;102(2):159-66. doi: 10.1111/j.1423-0410.2011.01530.x.

31. Rondelli T, Risitano AM, Peffault de Latour LR et al. Polymorphism of the complement receptor 1 gene correlates with the hematologic response to eculizumab in patients with paroxysmal nocturnal hemoglobinuria. Haematologica 2014;99(2), 262-266.

32. Risitano AM, Marando L, Seneca E, et al. Hemoglobin normalization after splenectomy in a paroxysmal nocturnal hemoglobinuria patient treated by eculizumab. Blood. 2008; 112:449-51.

33. Araten DJ, Iori AP, Brown K, et al. Selective splenic artery embolization for the treatment of thrombocytopenia and hypersplenism in paroxysmal nocturnal hemoglobinuria. J Hematol Oncol. 2014;7:27. doi: 10.1186/1756-8722-7-27.

34. Brodsky RA How I treat paroxysmal nocturnal hemoglobinuria. Blood 2009;113:6522-6527.

35. Risitano AM, Marotta S. Therapeutic complement inhibition in complement-mediated hemolytic anemias: Past, present and future. Semin Immunol. 2016;28(3):223-40. doi: 10.1016/j.smim.2016.05.001.

36. EU Clinical Trials Register. https://www.clinicaltrialsregister.eu/ctr-search/search?query=2017-001418-27.

37. Sahelijo L, Mujeebuddin A, Mitchell D, et al. First in human single-ascending dose study: safety, biomarker, pharmacokinetics and exposure-response relationships of ALXN1210, a humanized monoclonal antibody to C5, with marked half-life extension and potential for significantly longer dosing intervals. Blood. 2015;126(23):4777.

38. Fukuzawa T, Sampei Z, Haraya K, et al. Long lasting neutralization of C5 by SKY59, a novel recycling antibody, is a potential therapy for complement-mediated diseases. Sci Rep. 2017;7(1):1080. doi: 10.1038/s41598-017-01087-7.

39. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT02534909

40. Woodruff TM, Nandakumar KS, Tedesco F. Inhibiting the C5-C5a receptor axis. Mol Immunol. 2011;48(14):1631-42.

41. Adienne Pharma & Biotech. 2014. http://www.adienne.com/en/rd/pipeline/mubodina®.html

42. Borodovsky A, Yucius K, K Sprague A, et al. Development Of RNAi Therapeutics Targeting The Complement Pathway. Blood. 2013;122(21):2471.

43. Borodovsky A, Yucius K, Sprague A, et al. Aln-CC5, an Investigational RNAi Therapeutic Targeting C5 for Complement Inhibition. Blood. 2014;124:1606.

44. Nunn MA, Sharma A, Paesen GC, et al. Complement inhibitor of C5 activation from the soft tick Ornithodoros moubata. J.Immunol. 2005;174(4):2084-2091.

45. Roversi P, Lissina O, Johnson S, et al. The structure of OMCI, a novel lipocalin inhibitor of the complement system. J Mol Biol. 2007;369(3), 784-793.

46. Weston-Davies WH, Nunn MA, Pinto FO, et al. Clinical and Immunological Characterisation of Coversin, a Novel Small Protein Inhibitor of Complement C5 with Potential As a Therapeutic Agent in PNH and Other Complement Mediated Disorders. Blood. 2014;124:4280.

47. Ueda Y, Osato M, Weston-Davies W, et al. Coversin Blocked in Vitro Hemolysis in an Eculizumab-Resistant PNH Patient with the C5 Polymorphism (c.2654G>A). Blood. 2015;126:2138.

48. Josephson K, Ricardo A, Szostak JW. mRNA display: from basic principles to macrocycle drug discovery. Drug Discov Today. 2014;19(4):388-99.

49. Ricardo A, Arata M, DeMarco S, et al. Development of RA101348, a Potent Cyclic Peptide Inhibitor of C5 for Complement-Mediated Diseases. Blood. 2014;124:2936.

50. Ricardo A, Arata M, DeMarco S, et al. Preclinical Evaluation of RA101495, a Potent Cyclic Peptide Inhibitor of C5 for the Treatment of Paroxysmal Nocturnal Hemoglobinuria. Blood. 2015;126:939.

51. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol. 2010;11:785-97.

52. Varela JC, Tomlinson S. Complement: an overview for the clinician. Hematol Oncol Clin North Am. 2015;29(3):409-27. doi: 10.1016/j.hoc.2015.02.001.

53. Holers VM. The spectrum of complement alternative pathway-mediated diseases. Immunol Rev. 2008;223:300-16.

54. Lindorfer MA, Pawluczkowycz AW, Peek EM, et al. A novel approach to preventing the hemolysis of paroxysmal nocturnal hemoglobinuria: both complement-mediated cytolysis and C3 deposition are blocked by a monoclonal antibody specific for the alternative pathway of complement. Blood. 2010;115(11):2283-2291.

55. Paixao-Cavalcante D, Torreira E, Lindorfer MA et al. A humanized antibody that regulates the alternative pathway convertase: potential for therapy of renal disease associated with nephritic factors. J.Immunol. 2014;192(10), 4844-4851.

56. Sahu A, Kay BK, Lambris JD. Inhibition of human complement by a C3-binding peptide isolated from a phage-displayed random peptide library. J Immunol. 1996;157(2):884-891.

57. Ricklin D, Lambris JD. Compstatin: a complement inhibitor on its way to clinical application. Adv Exp Med Biol. 2008;632:273-292.

58. Mastellos DC, Yancopoulou D, Kokkinos P, et al. Compstatin: a C3-targeted complement inhibitor reaching its prime for bedside intervention. Eur J Clin Invest. 2015;45(4):423-40. doi: 10.1111/eci.12419.

59. Qu H, Ricklin D, Bai H, et al. New analogs of the clinical complement inhibitor compstatin with subnanomolar affinity and enhanced pharmacokinetic properties. Immunobiology. 2013;218(4):496-505.

60. Ricklin D, Lambris JD. Complement in immune and inflammatory disorders: therapeutic interventions. J Immunol. 2013;190(8):3839-3847.

61. Risitano, AM, Ricklin, D, Huang, Y et al. Peptide inhibitors of C3 activation as a novel strategy of complement inhibition for the treatment of paroxysmal nocturnal hemoglobinuria. Blood. 2014;123(13):2094-101.

62. Kaudlay P, Hua H, He G, et al. Red Cell Complement Loading In PNH Patients On Eculizumab Is Associated With a C3 Polymorphism Which Influences C3 Function, Predicts For Increased Extravascular Hemolysis and Provides a Rationale For C3 Inhibition. Blood. 2013;122:21):2466.

63. Ross GD, Lambris JD, Cain JA, et al. Generation of three different fragments of bound C3 with purified factor I or serum. I. Requirements for factor H vs CR1 cofactor activity. J Immunol. 1982;129(5):2051–60 .

64. Whaley K and Ruddy S. Modulation of the alternative complement pathway by β1H globulin. J Exp Med. 1976;144:1147-63.

65. Iida K, Nussenzweig V. Complement receptor is an inhibitor of the complement cascade. J Exp Med. 1981;153(5):1138–50.

66. Holers VM, Rohrer B, Tomlinson S. CR2-mediated targeting of complement inhibitors: bench-to-bedside using a novel strategy for site-specific complement modulation. Adv Exp Med Biol. 2013;735:137-54.

67. Fridkis-Hareli M, Storek M, Mazsaroff I, et al. Design and development of TT30, a novel C3d-targeted C3/C5 convertase inhibitor for treatment of human complement alternative pathway-mediated diseases. Blood. 2011;118(17):4705-13.

68. Risitano AM, Notaro R, Pascariello C et al. The complement receptor 2/factor H fusion protein TT30 protects paroxysmal nocturnal hemoglobinuria erythrocytes from complement-mediated hemolysis and C3 fragment. Blood 2012;119(26), 6307-6316 (2012).

69. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. http://clinicaltrials.gov/ct2/show/NCT01335165

70. Schmidt CQ, Bai H, Lin Z et al. Rational engineering of a minimized immune inhibitor with unique triple-targeting properties. J. Immunol. 2013;190(11), 5712-5721.

71. Souza DG, Esser D, Bradford R, et al. APT070 (Mirococept), a membrane-localised complement inhibitor, inhibits inflammatory responses that follow intestinal ischaemia and reperfusion injury. Br J Pharmacol. 2005;145(8):1027-34.

72. Smith RA. Membrane-targeted complement inhibitors. Mol. Immunol. 2012;38, 249–255.

73. Sacks S, Karegli J, Farrar CA, et al. Targeting complement at time of transplantation. AdvExp Med Biol. 2013;735:247-55.

74. International Standard Randomised Controlled Trial Number Register. (2104) http://www.controlled-trials.com/ISRCTN49958194.

75. Rondelli T, Risitano AM, Peffault de Latour LR et al. Polymorphism of the complement receptor 1 gene correlates with the hematologic response to eculizumab in patients with paroxysmal nocturnal hemoglobinuria. Haematologica. 2014;99(2), 262-266.

76. Bansal R. Novelmed Therapeutics. (2013) Anti-properdin antibodies. US Patent 8435512, 5 July 2013. http://www.freepatentsonline.com/8435512.htmlhttps://www.google.com/patents/US20140186348

77. AltmannE, Hommel U, Lorthiois ELJ, et al. Novartis Ag. Indole compounds or analogs thereof as complement factor D inhibitors useful for the treatment of age-related macular degeneration and their preparation. 2012. From PCT Int. Appl., WO 2012093101 http://www.google.com/patents/WO2012093101A1?cl=en&hl=it July 12, 2012

78. Maibaum J, Liao SM, Vulpetti A, et al. Small-molecule factor D inhibitors targeting the alternative complement pathway. Nat Chem Biol. 2016;12(12):1105-1110. doi: 10.1038/nchembio.2208.

79. Morgan BP, Thanassi J, Podos S, Phadke A, Ghadachanda V, Pais G, Hashimoto A, Whang W, Chen D, Wang X, Argawal A, Deshpande M, Huang Y, Wiles J, Huang M. Novel Small-Molecule Inhibitors Targeting Complement Factor D for the Treatment of Paroxysmal Nocturnal Hemoglobinuria. Blood 2014;124:4817.

80. Yuan X, Gavriilaki E, Thanassi JA, et al. Small-molecule factor D inhibitors selectively block the alternative pathway of complement in paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. Haematologica. 2017;102(3):466-475. doi: 10.3324/haematol.2016.153312.

81. Dechantsreiter MA, Grob JE, Mac Sweeney A, et al. Novartis Ag. Preparation of quinazoline compounds as modulators of the complement alternative pathway for treating macular degeneration, diabetic retinopathy, and other diseases. 2013. From PCT Int. Appl., WO 2013192345 http://www.google.com.ar/patents/WO2013192345A1?cl=en&hl=it December 27, 2013

82. Schubart A, Maibaum J, Anderson K, et al. Small-molecule Factor B inhibitors for oral treatment of alternative pathway driven diseases. Paper presented at: 9th International Conference on Complement Therapeutics, Rhodes, June 28 - July 3, 2016. Aegean Conferences 100, abs 12.

83. Australia New Zealand Clinical Trial Registry. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=369851

84. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT02598583

85. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT02605993

86. Lee JW, Bachman E, Aguzzi R, et al. Immediate, Complete, and Sustained Inhibition of C5 with ALXN1210 Reduces Complement-Mediated Hemolysis in Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH): Interim Analysis of a Dose-Escalation Study. Blood 2016;128(22):2428.

87. Roeth A, Rottinghaus ST, Hill A, et al. Optimization of Dose Regimen for ALXN1210, a Novel Complement C5 Inhibitor, in Patients with Paroxysmal Nocturnal Hemoglobinuria: Results from 2 Phase I/II Studies. Blood. 2017; in press. ASH 59th Annual Meeting & Exposition.

88. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT02946463

89. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT03056040

90. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT03157635

91. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT02352493

92. Hill A, Taubel J, Bush J, et al. A Subcutaneously Administered Investigational RNAi Therapeutic (ALN-CC5) Targeting Complement C5 for Treatment of PNH and Complement-Mediated Diseases: Interim Phase 1 Study Results. Blood. 2015;126:2413.

93. Hill A, Valls AG, Griffin M, et al. A Subcutaneously Administered Investigational RNAi Therapeutic (ALN-CC5) Targeting Complement C5 for Treatment of PNH and Complement-

Mediated Diseases: Preliminary Phase 1/2 Study Results in Patients with PNH. Blood. 2016;128:3891.

94. EU Clinical Trials Register. https://www.clinicaltrialsregister.eu/ctr-search/search?query=2016-002943-40

95. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT02591862

96. EU Clinical Trials Register. https://www.clinicaltrialsregister.eu/ctr-search/search?query=2016-002067-33

97. Hill A, Weston-Davies W, Nunn M, et al. Coversin, a novel C5 complement inhibitor for the treatment of PNH: results from a phase 2 clinical trial. Haematologica. 2017;102(s2):64.

98. Ricardo A, Hoarty MD, Blain JC. Discovery of orally bioavailable small molecules for inhibition of complement C5. Haematologica. 2017;102(s2):189.

99. Johnston J, Ricardo A, Arata M, et al. A phase I single-ascending-dose clinical study of RA101495, a subcutaneously administered macrocyclic peptide inhibitor of complement C5 for treatment of paroxysmal nocturnal hemoglobinuria. Haematologica. 2016;101(s1):347.

100. Johnston J, Ricardo A, Arata M, et al. A phase I multiple-dose clinical study of RA101495, a subcutaneously administered macrocyclic peptide inhibitor of complement C5 for treatment of paroxysmal nocturnal hemoglobinuria. Haematologica. 2016;101(s1):415.

101. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT03078582

102. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT03030183

103. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT03225287

104. Risitano AM, Storek M, Sahelijo L, Doyle M, Dai Y, Weitz IC, Marsh JCW, Elebute M, O'Connell CL, Kulasekararaj AG, Ramsingh G, Marotta S, Hellmann A, Lundberg AS. Safety and Pharmacokinetics of the Complement Inhibitor TT30 in a Phase I Trial for Untreated PNH Patients. Blood. 2015;126:2137.

105. Lambris JD, Reis E, Ricklin D, et al. Compstatin CP40 (AMY-101): a C3-targeted complement inhibitor reaching its prime for bedside intervention. Paper presented at: 10th International Conference on Complement Therapeutics, Heraklion, June 24-29, 2017. Aegean Conferences 110, abs 33.

106. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT03316521

107. Grossi FV, Bedwell P, Deschatelets P, et al. APL-2, a Complement C3 Inhibitor for the Potential Treatment of Paroxysmal Nocturnal Hemoglobinuria (PNH): Phase I Data from Two Completed Studies in Healthy Volunteers. Blood 2016;128:1251.

108. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT02588833

109. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT02264639

110. http://www.apellis.com/pdfs/PressReleaseAPL-2ClinicalUpdateFINALv6-170629

111. Ellius-Pegler JB, Schwabe C, Ajari I, et al. An orally administered small molecule factor D inhibitor (ACH-4471) for treatment of PNH and complement diseases: preliminary phase I results in healthy volunteers. Haematologica. 2016;101(s1):416.

112. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT03181633

113. ClinicalTrials.gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ct2/show/NCT03053102

114. Patel D, Thanassi J, Yang G, et al. In Vitro Combination Studies of ACH-4471 with Eculizumab to Assess a Potential "Switch" Treatment Approach for Paroxysmal Nocturnal Hemoglobinuria. Blood. 2017; in press. ASH 59th Annual Meeting & Exposition.

115. EU Clinical Trials Register. https://www.clinicaltrialsregister.eu/ctr-search/search?query=2017-000665-79

116. EU Clinical Trials Register. https://www.clinicaltrialsregister.eu/ctr-search/search?query=2017-000888-33

117. Newton DJ, McKinley CE, Ricardo A, et al. Assessment of Eculizumab and C5 Levels in PNH Treatment: Molar Ratio of Eculizumab to C5 Level Effectively Predicts Ongoing Dosage Changes in a Cohort of 50 Patients Treated with Eculizumab. Blood. 2017; in press. ASH 59th Annual Meeting & Exposition.

118. Mayilyan KR. Complement genetics, deficiencies, and disease associations. Protein Cell. 2012 Jul;3(7):487-96. doi: 10.1007/s13238-012-2924-6.

119. Sica M, Rondelli T, Ricci P, De Angioletti M, et al. Eculizumab treatment: stochastic occurrence of C3 binding to individual PNH erythrocytes. J Hematol Oncol. 2017;10(1):126. doi: 10.1186/s13045-017-0496-x.

FIGURE LEGEND

Figure 1. The complement cascade and its targeted modulation.

A. Overview of the complement cascade and of novel anti-complement agents.

The three activating pathways (alternative, classical, and mannose/lectin) are individually depicted, together with the alternative pathway amplification loop.[44] Candidate inhibitors are grouped according to their specific target; their modulatory effects are indicated by red lines intercepting specific steps of the complement cascade.[31] Therapeutic interception of the complement cascade may occur: i. at the level of its common terminal effector pathway (e.g., C5); ii. at the level of its common key activation and amplification component (i.e., 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 anti-complement 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 effector complement; indeed, intravascular hemolysis should be prevented as well.

Novel C5 inhibitors disable the terminal effector complement, 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.

Figure 2. Novel complement therapeutics towards their clinical translation.

Graphical overview of the status of art of clinical translation for most relevant novel candidate complement therapeutics (from reference 35). The colors of the target indicate the phase of development (from preclinical work to marketing authorization): in white preclinical work (laboratory work or animal models); in light blue phase I trials (in healthy individuals or in PNH); in red phase II trials (in oterh indication or in PNH); in yellow phase III trial or marketing authorization. The target is divided in 4 quadrants which indicate the four most relevant classes of counds (based on their targets): upper left, inhibitors of the classical complement pathway (CCP); lower left, inhibitors of the classical alternative pathway (CAP); upper right, inhibitors of the complement component 5 (C5); lower right, inhibitors of the complement component 3 (C3). Each arrow indicates a specific agent with its stage of development (see main manuscript for detailed description).

Table 1. Candidate complement inhibitors in development for PNH and other complement-mediated diseases.

Target	Name	Company	Class of molecule	Status of development	Reff.
C5	ALXN1210	Alexion	Monoclonal antibody	Active. Clinical (Phase III, PNH)	84-89
C5	ALXN5500	Alexion	Monoclonal antibody	Active. Preclinical (PNH, unknown)	35
C5	SKY59	Roche	Monoclonal antibody	Active. Clinical (Phase II, PNH)	38,90
C5	ABP 959	Amgen	Monoclonal antibody	Active. Clinical (Phase III, PNH)	36,83
C5	LFG 316	Novartis/Morphosys	Monoclonal antibody	Active. Clinical (Phase II, AMD and PNH)	39
C5	Mubodina®	Adienne	Monoclonal antibody (minibody)	Active. Preclinical (non-PNH, TMAs)	40,41
C5	Anti-C5 siRNA	Alnylam	si-RNA	Active. Preclinical (non-PNH and PNH); clinical (Phase II, healthy volunteer and PNH)	42,43,91-93
C5	Coversin (OmCI)	Akari	Small animal protein (recombinant)	Active. Preclinical (PNH); clinical (Phase II, healthy volunteers and PNH)	44-47, 95-97
C5	RA101348	Rapharma	Small molecule (unnatural peptide)	Active. Preclinical (unknown); clinical (PNH, phase II)	48-50, 98-102
C5	ARC1005	Novo Nordisk	Aptamers	Active. Preclinical (non-PNH); clinical (Phase I, AMD)	35
C5	SOMAmers	SomaLogic	Aptamers (SELEX)	Active. Preclinical (non-PNH)	35
C5	SOBI002	Swedish Orphan Biovitrum (Affibody)	Affibody (fused with albumin-binding domain)	Not active. Preclinical (non-PNH); clinical (Phase I, healthy volunteers)	35
C3 (C3b/iC3b)	H17	EluSys Therapeutics	Monoclonal antibody	Not active. Preclinical (PNH and non-PNH)	55
C3/C3b	4(1MeW)/POT-4	Potentia	Compstatin family	Not active. Preclinical (non-PNH); clinical (Phase I and II, AMD)	56-58
C3/C3b	Cp40/AMY-101, PEG-Cp40	Amyndas	Compstatin family	Active. Preclinical (PNH and non-PNH); clinical (Phase I, healthy volunteers and Phase II PNH, planned)	56-61,105,106
C3/C3b	4(1MeW)/APL-1, APL-2	Apellis	Compstatin family	Active. Preclinical (PNH and non-PNH); clinical (Phase II, healthy volunteers and PNH)	62,107-110

CFB	TA106	Alexion Pharmaceuticals	Monoclonal antibody	Not active. Preclinical (unknown)	35
CFD	Lampalizumab (FCFD4514S)	Genentech/Roche	Monoclonal antibody	Active. Preclinical (non-PNH); clinical (Phase II, AMD)	35
CFB	Anti-FB siRNA	Alnylam	Si-RNA	Not active. Preclinical (non-PNH)	35
CFB and CFD	SOMAmers	SomaLogic	Aptamers (SELEX)	Not active. Preclinical (non-PNH)	35
CFB	LNP023	Novartis	Small molecules (chemicals)	Active. Clinical (PNH phase II)	81,82,116
CFD	n.a.	Novartis	Small molecules (chemicals)	Active. Preclinical (non-PNH)	77,78
CFD	ACH-3856 ACH-4100 ACH-4471	Achillion	Small molecules (chemicals)	Active. Preclinical (PNH); clinical (Phase I, healthy volunteers and phase II PNH)	79,80,111-115
Properdin	NM9401	Novelmed	Monoclonal antibody (and mAb derivatives)	Not active. Preclinical (unknown)	35,76
C1s	TNT003	True North Therapeutics	Monoclonal antibody	Active. Preclinical (CAD); clinical (Phase I, healthy volunteers and CAD)	35
MASP-1, MASP-2, MASP-3	OMS721 (anti-MASP-2)	Omeros	Monoclonal antibody	Active. Preclinical (PNH and non-PNH); clinical (TMAs and kidney disorders)	35
C1r/C1s	Cinryze®	Shire	Human purified protein (C1-INH)	Active. Clinical (approved for Hereditary Angio-Edema)	35
CAP C3 convertase	TT30 (CR2/CFH)	Alexion	CFH-based protein	Active. Preclinical (PNH and non-PNH); clinical (Phase I, PNH)	69,104
CAP C3 convertase	Mini-FH	Amyndas	CFH-based protein	Active. Preclinical (PNH and non-PNH)	70
CAP C3 convertase	Mini-FH	n.a.	CFH-based protein	Not active. Preclinical (non-PNH)	35
CAP C3 convertase	CRIg/CFH	n.a.	CFH-based protein	Not active. Preclinical	35
CAP and CP C3 convertase	sCR1 (CDX-1135)	Celldex	CR1-based protein	Not active. Preclinical (non-PNH); clinical (Phase I, DDD)	35
CAP and CCP C3 convertase	Mirococept (APT070)	n.a.	CR1-based protein	Active. Preclinical (non-PNH); clinical (Phase III, renal transplant)	71-74
CAP and CCP C3 convertase	TT32 (CR2/CR1)	Alexion	CR1-based protein	Not active. Preclinical (non-PNH)	35

Table 2. Ongoing clinical trials with novel complement inhibitors for PNH.

Agent	Target	Clinical trial ID	Design	Patient population*	Study treatment	Primary endpoint
ALXN1210	C5	N.A.[33]	Phase I, randomized vs placebo	Healthy volunteers	SAD, IV infusions	Safety, PK and PD
		NCT02598583[63]	Phase I/II, open-label	Untreated PNH	Intra-patient DE by IV infusions	Safety and efficacy (by LDH)
		NCT02605993[64]	Phase I/II, open-label	Untreated PNH	MAD; IV infusions	Safety and efficacy (by LDH)
		NCT02946463[67]	Phase III, randomized vs Ecu	Untreated PNH	IV infusions (every 8 weeks)	Efficacy (by LDH)
		NCT03056040[68]	Phase III, randomized vs Ecu	Stable responders PNH	IV infusions (every 8 weeks)	Efficacy (by LDH)
ABP 959	C5	ACTRN12616000509460[62]	Phase I, randomized vs Ecu	Untreated PNH	Single IV infusions	PK similarity
		EudraCT 2017-001418-27[32]	Phase III, randomized vs Ecu	Stable responders PNH	IV infusions	Efficacy (LDH)
SKY59	C5	NCT03157635[69]	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[35]	Phase II, open-label	Untreated PNH	IV infusions	Efficacy (by LDH)
ALNCC5	C5	NCT02352493[70]	Phase I/II, randomized vs Ecu, SAD and MAD	Healthy volunteers	SC injection	Safety
				Untreated PNH	SC injections	Safety
		EudraCT 2016-002943-40[73]	Phase II, open-label	Poor responder PNH (by LDH >2x ULN)	SC injections	Efficacy (by LDH)
Coversin	C5	N.A.[39]	Phase I, SAD and MD	Healthy volunteers	SC injections	Safety, PK and PD
		NCT02591862[74]	Phase II, open-label	Poor responder PNH (by LDH >1.5x ULN)	SC injections; intra-patient DE	Efficacy (by LDH)
		EudraCT 2016-002067-33[75]	Phase II, open-label, fixed dose	Untreated PNH	SC injections	Efficacy (defined as LDH <1.5x ULN)
		EudraCT 2016-004129-18[77]	Phase II, open-label, extension	PNH patients exposed to coversin	SC injections	Safety
RA101495	C5	N.A.[79,80]	Phase I, SAD and MD	Healthy volunteers	Daily, SC injections	Safety, PK and PD
		NCT03078582[81]	Phase II, open label, fixed dose	Untreated PNH	Daily, SC injections	Efficacy (by LDH)
				Poor responders PNH (by LDH >2x ULN)		
		NCT03030183[82]	Phase II, open label, fixed dose	Poor responders PNH (by LDH >1.5x ULN)	Daily, SC injections	Efficacy (by LDH)
		NCT03225287[83]	Phase II, open-label, extension	PNH patients exposed to RA101495	Daily, SC injections	Safety
TT30	CAP	NCT01335165[84]	Phase I, SAD	Untreated PNH	SC injections and IV infusions	Safety, PK and PD
AMY-101	C3	NCT03316521[87]	Phase I, SAD and MD	Healthy volunteers	SC and IV infusions	PK and PD
		N.A.[86]	Phase II, open label, fixed dose	Untreated PNH	Daily, SC infusions	N.A.

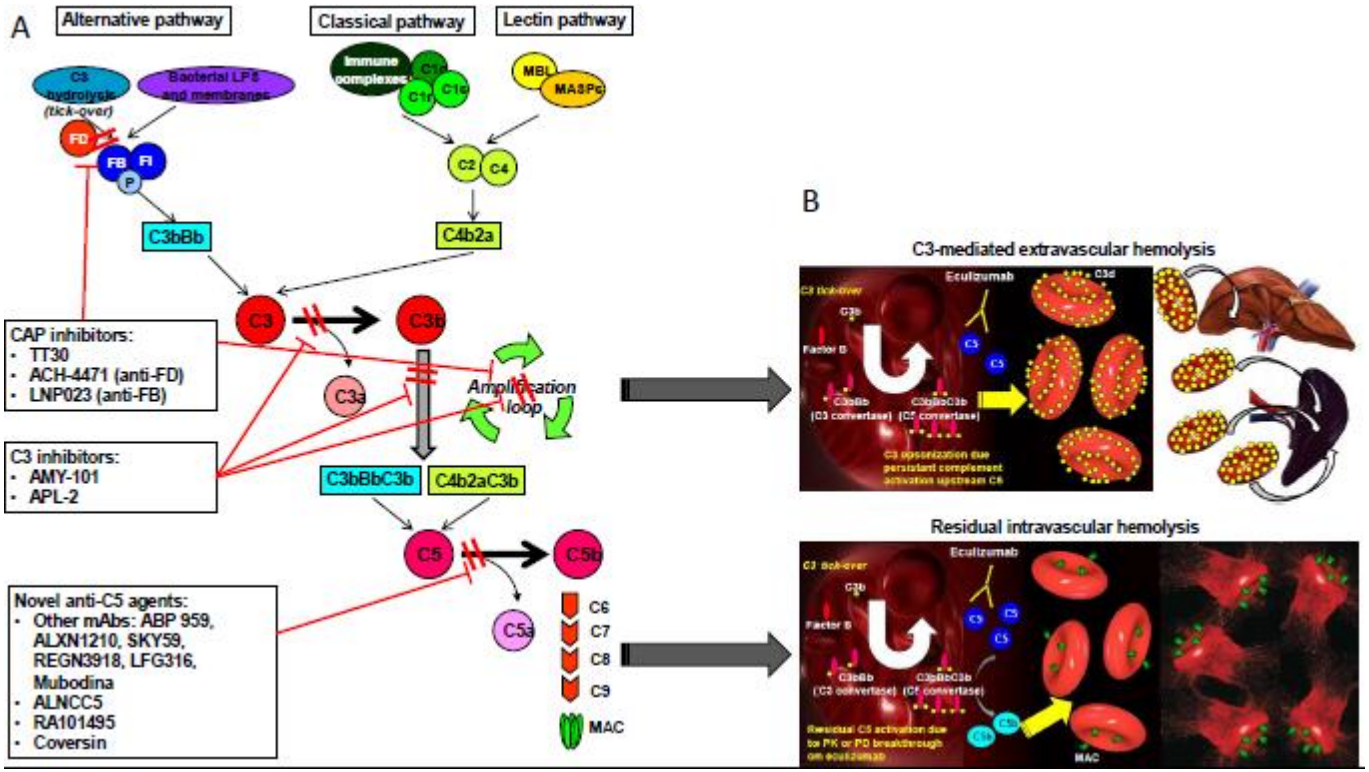
		N.A.[86]	Phase II, open label, fixed dose	Poor responders PNH (moderate/severe anemia)	Daily, SC infusions	N.A.
APL-2	C3	N.A.[88]	Phase I, SAD and MD	Healthy volunteers	SC and IV infusions	Safety, PK and PD
		NCT02264639[90]	Phase Ib, open label, MAD, POC	Poor responders PNH (moderate/severe anemia)	Daily, SC infusions	Safety and tolerability
		NCT02588833[90]	Phase Ib, open label, MAD, POC	Untreated PNH	Daily, SC infusions	Safety and tolerability
		N.A.[91]	Phase II, open label, extension	PNH patients exposed to APL-2	Daily, SC infusions	N.A.
ACH-4471	FD	N.A.[92]	Phase I, SAD	Healthy volunteers	Orally, QD and BID	Safety and tolerability
		NCT03181633[93]	Phase Ib, open label, MD, POC	Untreated PNH	Orally, BID	Safety and efficacy (by LDH level)
		NCT03053102[94]	Phase II, open-label, extension	PNH patients exposed to ACH-4471	Orally, BID	Efficacy (by LDH level)
		EudraCT 2016-002652-25[96]	Phase II, open label, MD, POC	Untreated PNH	Orally, TID	Efficacy (by LDH level)
		N.A.[95]	N.A.	Poor responders PNH	N.A.	N.A.
LNP023	FB	EudraCT 2017-000888-33[97]	Phase II, open label	Poor responders PNH (by LDH >1.5x ULN)	Orally, BID	Efficacy (by LDH level)

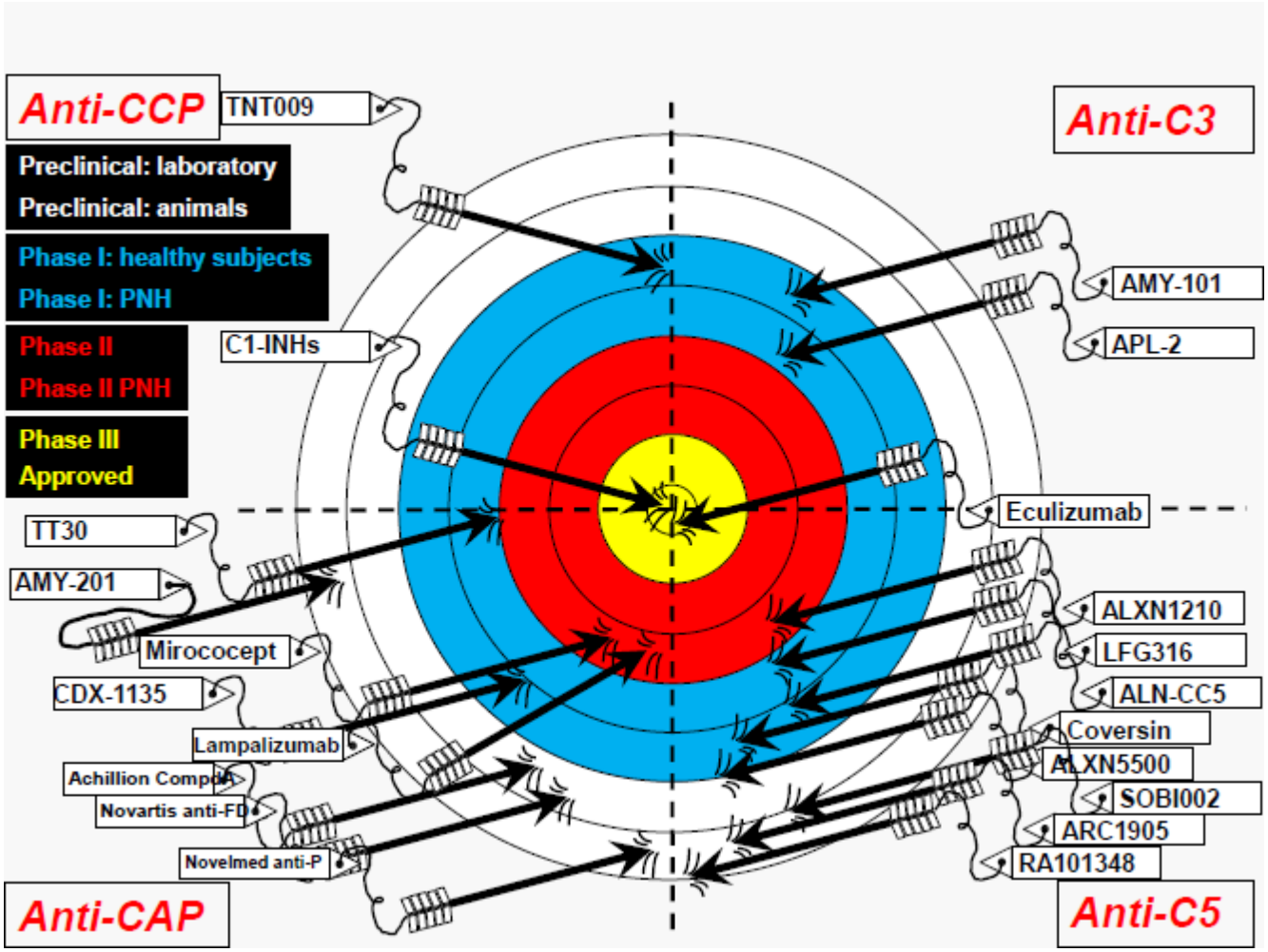
*: stable or poor response is intended to standard eculizumab treatment; Abbreviations: N.A:: not available; Ecu: eculizumab; SAD: single ascending dose; MAD: multiple ascending doses; MD: multiple doses; POC: proof-of-concept; DE: dose escalation; SC: subcutaneous; IV: intravenous; QOD: *quaque die*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

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

Company	ALEXION	NOVARTIS	Roche	Ra Pharma	Alnylam	AKARI THERAPEUTICS	AMYNDAS	Apellis	ACHILLION	NOVARTIS
Name	ALXN1210	LFG316	SKY59	RA101495	ALNCC5	Coversin	AMY-101	APL-2	ACH-4471	LNP023
EFFICACY										
C5-polymorphism	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Breakthrough intravascular hemolysis	Yes ++	N.A.	Yes +	Yes +(§)	Yes +++§	Yes +(§)	(Yes ++[§])	(Yes ++[§])	(Yes ++[§])	(Yes ++[§])
C3-mediated extravascular hemolysis	No	No	No	No	No	No	(Yes +++)	(Yes +++)	(Yes +++)	(Yes +++)
Marrow failure	No	No	No	No	No	No	No	No	No	No
Monotherapy*	Yes +++	Yes +++	Yes +++	Yes ++	No	Yes +	Yes ++	Yes ++	(Yes +)	(Yes +)
SAFETY										
Off-targets	No	No	No	No	No	No	(No)	(No)	(No)	(No)
Immunogenicity	No	No	No	(No)	No	Yes +	(No)	(No)	(No)	(No)
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 +++
COMPLIANCE										
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	TID
Self-administration	No	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes
COST-EFFECTIVENESS										
Possible price	+++ ?	+ ?	+++ ?	++ ?	+++ ?	++ ?	+ ?	+++ ?	++ ?	++ ?

Statements in parenthesis are based preclinical data but still lack full confirmation from clinical trials; [§]: in combination with eculizumab; *: the possibility of monotherapy is based on available data and current trials rather than on theoretical assumptions; °: the infectious risk (as compared to eculizumab treatment) is based on the specific target, the deepness of inhibition 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).


A
Alternative pathway
Classical pathway
Lectin pathway
C3 hydrolysis (tick-over)
Bacterial LPS and membranes
Immune complexes
C1q
C1r
C1s
MBL
MASPs
FD
FB
FI
P
C2
C4
C3bBb
C4b2a
C3
C3b
C3a
Amplification loop
C3bBbC3b
C4b2aC3b
C5
C5b
C5a
C6
C7
C8
C9
MAC
CAP inhibitors:
• TT30
• ACH-4471 (anti-FD)
• LNP023 (anti-FB)
C3 inhibitors:
• AMY-101
• APL-2
Novel anti-C5 agents:
• Other mAbs: ABP 959, ALXN1210, SKY59, REGN3918, LFG316, Mubodina
• ALNCC5
• RA101495
• Coversin
B
C3-mediated extravascular hemolysis
Eculizumab
C3d
C3b
Factor B
C3bBb (C3 convertase)
C3bBbC3b (C5 convertase)
C3 opsonization due persistent complement activation upstream C5
Residual intravascular hemolysis
Residual C5 activation due to PK or PD breakthrough on eculizumab
MAC


Anti-CCP
TNT009
Preclinical: laboratory
Preclinical: animals
Phase I: healthy subjects
Phase I: PNH
Phase II
Phase II PNH
Phase III
Approved
C1-INHs
TT30
AMY-201
Mirococept
CDX-1135
Lampalizumab
Achillion Compd A
Novartis anti-FD
Novelmed anti-P
Anti-CAP
Anti-C3
AMY-101
APL-2
Eculizumab
ALXN1210
LFG316
ALN-CC5
Coversin
ALXN5500
SOBI002
ARC1905
RA101348
Anti-C5