Understanding pharmacological complement inhibition in paroxysmal nocturnal hemoglobinuria

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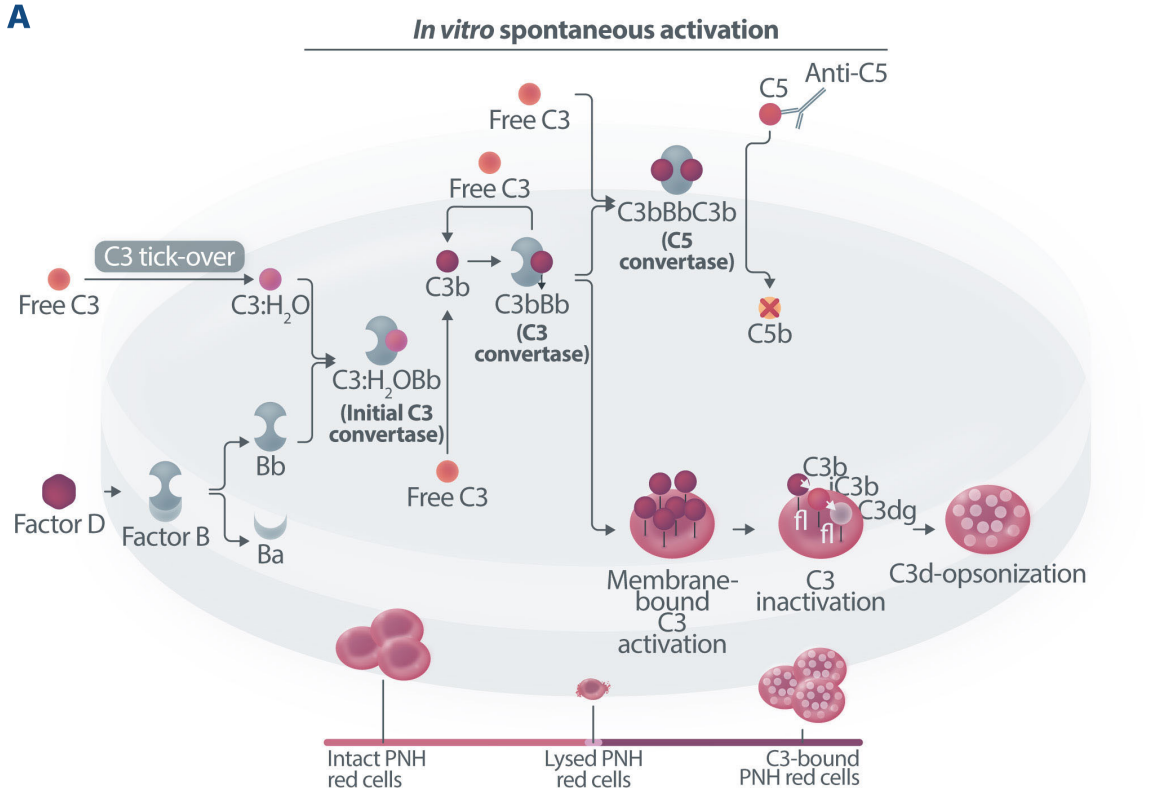
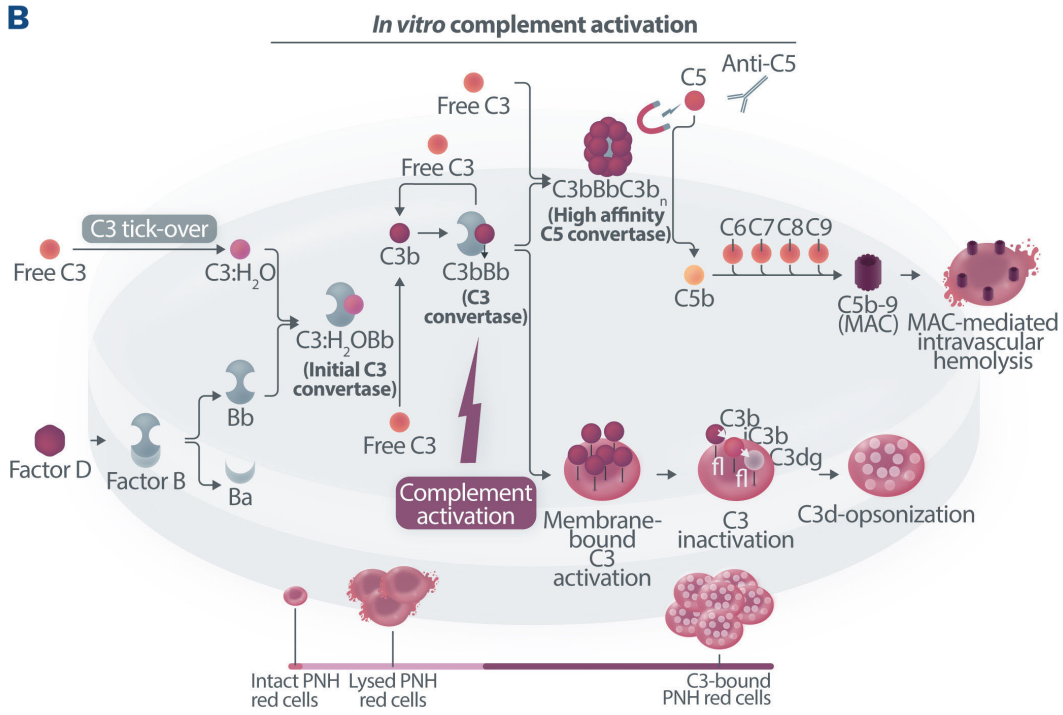
Following the description of the RAISE study,[1] in this issue of *Haematologica*, Kulasekararaj *et al.* report on the efficacy and safety of zilucoplan, a 15-amino acid macrocyclic peptide which blocks the terminal pathway of complement through its high affinity and specificity binding to C5.[1] The authors demonstrate that this small C5 inhibitor given subcutaneously as monotherapy efficiently controls intravascular hemolysis, as shown by lactate dehydrogenase (LDH) levels, in both eculizumab-naïve and eculizumab-treated patients with paroxysmal nocturnal hemoglobinuria (PNH), possibly leading to transfusion avoidance and hemoglobin stabilization.[2] However, this clinical benefit remained quite heterogeneous, with profound inter-patient variability and limited efficacy especially in patients switching from eculizumab to zilucoplan.[2]

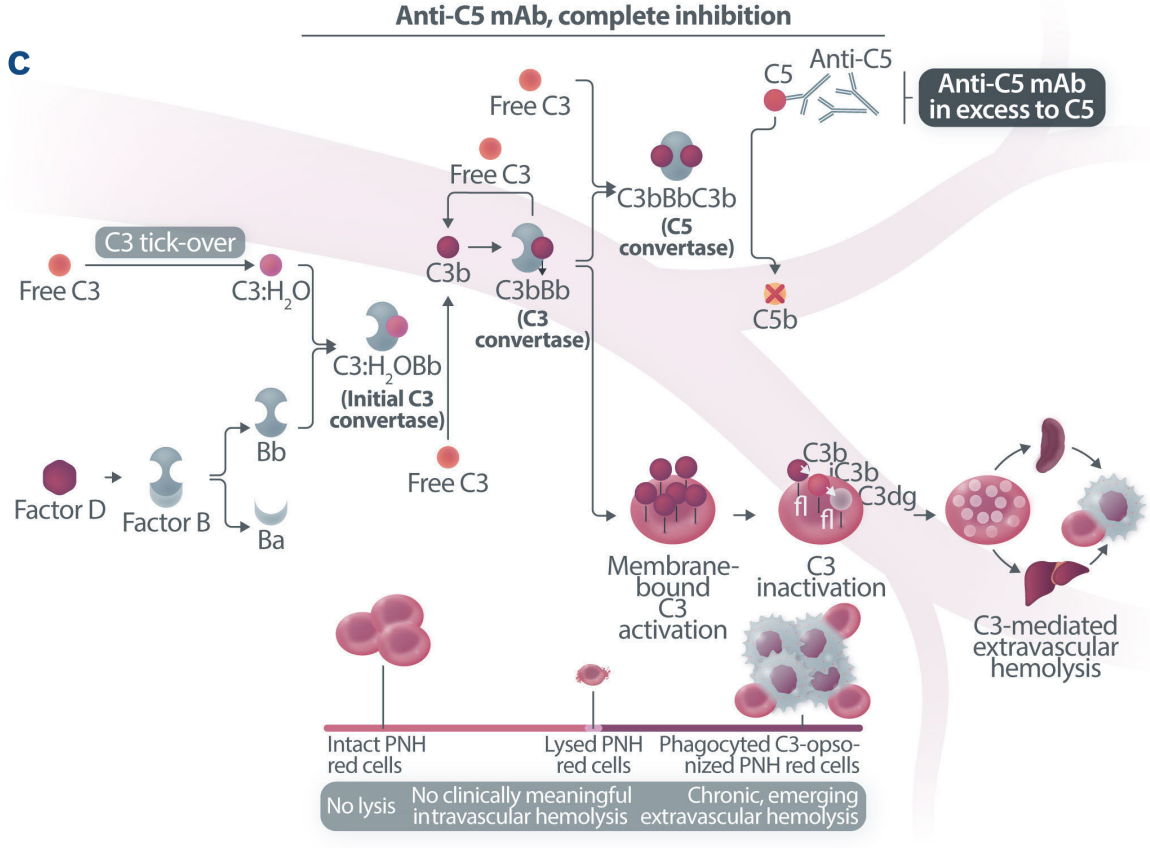
In recent years, a plethora of novel anti-complement agents have entered into preclinical and clinical development, especially for PNH,[3] the prototypic example of a purely complement-mediated hemolytic anemia. Even if, clinically speaking, the most promising results are coming from the so-called proximal inhibitors,[4] the development of novel terminal complement inhibitors is shedding light on our understanding of pharmacological complement inhibition. In this setting, well-conducted phase II studies are essential to investigate subtle differences among agents targeting even the same complement component, including pharmacokinetic and pharmacodynamic properties of individual inhibitors which ultimately influence their clinical efficacy and safety profile even more than their actual target. Therapeutic C5 inhibition has been well-established for more than 15 years, making the interpretation of novel observations much easier than that for novel proximal inhibitors.[4]

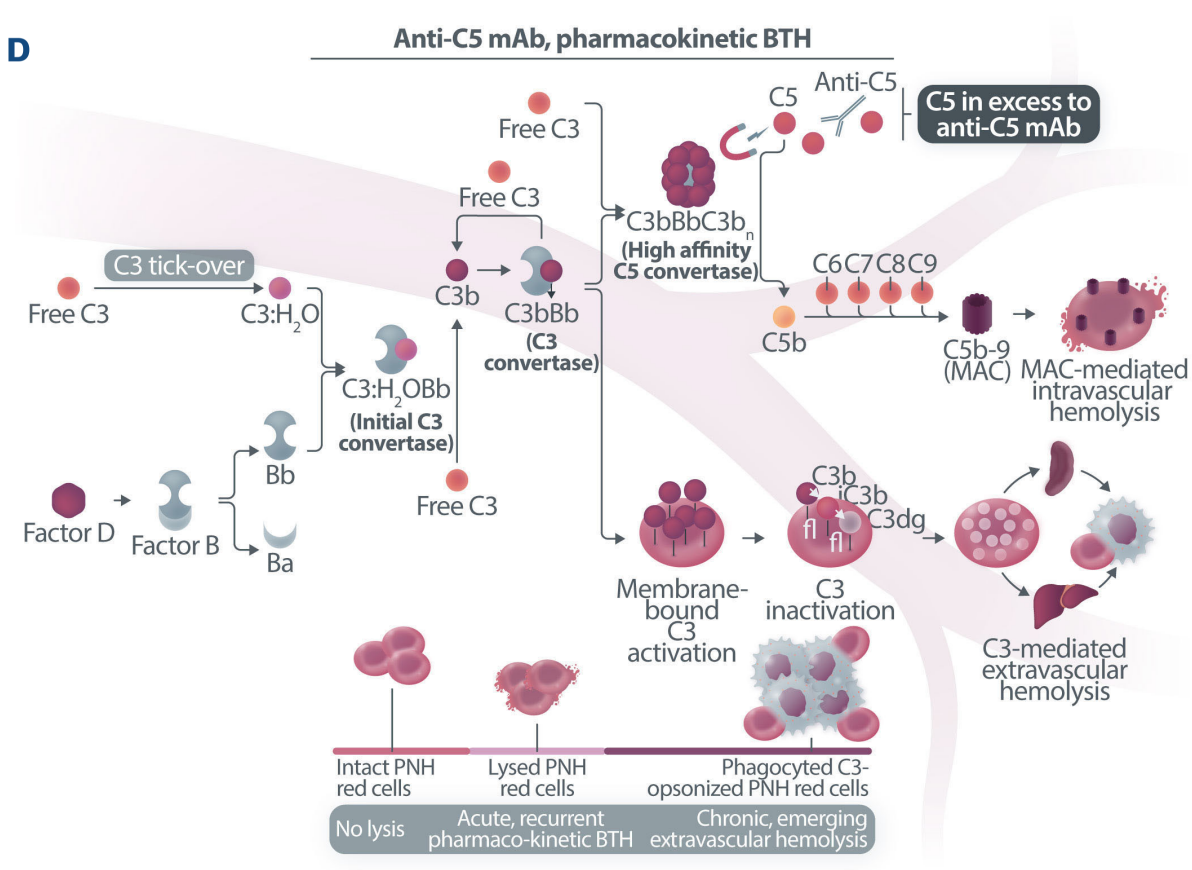
In the small phase II study by Kulasekararaj *et al.*, PNH patients who had not received previous treatment with eculizumab had significant benefits in terms of LDH decrease and transfusion avoidance when treated with zilucoplan, even if, according to the authors, changes in more meaningful clinical parameters (e.g., hemoglobin level) and other biomarkers of hemolysis were small or variable. Even more interestingly, PNH patients switching from eculizumab to zilucoplan consistently exhibited some increase in LDH level, with the largest increase seen in patients who were transfusion-dependent on eculizumab treatment (whose LDH levels were marginally increased at baseline). Collectively, these findings suggest that the C5 inhibition obtained with zilucoplan is obviously clinically meaningful (in comparison to no treatment, in eculizumab-naïve patients), but possibly less efficient than that of eculizumab (in patients switching from eculizumab). Notably, this somehow suboptimal inhibition was seen despite apparently complete complement inhibition (as assessed by functional assays measuring residual complement activity, suggesting that such assays are only partially informative as a pharmacodynamic measurement during anti-complement therapies), and despite the postulated dual mechanism of action of zilucoplan (likely because the effects on C5 cleavage and on subsequent C6 binding both rely on direct binding to C5, one being the effect of the other instead of two independent events). As a possible mechanism of reduced efficacy in patients switched from eculizumab, the authors propose the accumulation of high-density C3b on PNH erythrocytes, enabling non-enzymatic cleavage of C5 (i.e., conformational change[5]), claiming that this residual efficacy is a kind of iatrogenic effect due to a transiently combined effect of the two C5 inhibitors at the time of the switch.[2] The authors built their hypothesis on some *in vitro* data, which showed that combined exposure to eculizumab and zilucoplan results in a larger proportion of C3b-opsonized PNH erythrocytes.[2] However, their theory is not convincing for a number of reasons.

In 2009, we originally described C3 opsonization as an ineluctable phenomenon in PNH patients treated with eculizumab.[6] This phenomenon has been reproduced *in*









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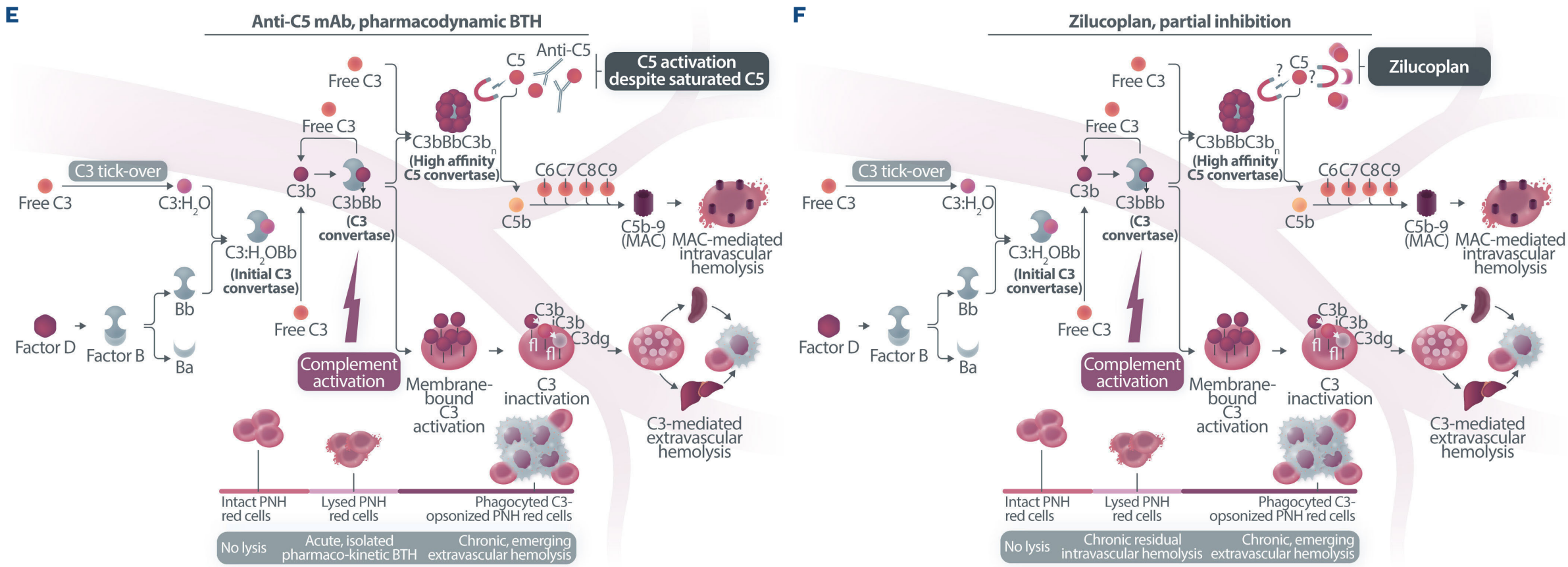

Figure 1. Mechanisms of residual hemolysis in the presence of C5 inhibitors *in vitro* and *in vivo*. (A) *Complement activation with C5 inhibitors in vitro, spontaneous activation*. Spontaneous, low-grade complement activation results in some degree of lysis on paroxysmal nocturnal hemoglobinura (PNH) erythrocytes *in vitro*; in the presence of anti-C5 monoclonal antibody (mAb) this lysis is almost completely inhibited, but surviving PNH erythrocytes accumulate C3 on their surface. (B) *Complement activation with C5 inhibitors in vitro, complement activation*. When PNH erythrocytes are exposed to complement activation (i.e., by lowering the pH) the inhibition seen with anti-C5 mAb is only partial, and C3 deposition is observed in all non-lysed PNH erythrocytes. More in detail, all PNH erythrocytes suffer from C3 activation; in some cells, the excess of surface-bound C3b leads to C5b-9 assembly and subsequent lysis (C3b remains detectable on erythrocyte ghosts), while on some other cells C3b is inactivated and its split fragment C3d remains the only detectable C3 fragment on non-lysed PNH red blood cells. (C) *Complement activation with C5 inhibitors in vivo, complete inhibition*. Ideally, anti-C5 mAb are in excess to C5, resulting in complete inhibition of C5 which prevents C5 cleavage and further membrane attack complex (MAC) formation; thus, intravascular hemolysis may be fully blocked *in vivo*, even if uncontrolled C3 activation accounts for continuous, low-grade C3 activation which clinically leads to C3-mediated extravascular hemolysis. However, partial inhibition of C5 may occur, possibly resulting in reappearance of intravascular hemolysis (acute or chronic), which in addition to C3-mediated extravascular hemolysis precludes the best hematologic benefit. (D) *Complement activation with C5 inhibitors in vivo, pharmacokinetic breakthrough hemolysis*. In the case of sub-therapeutic plasma levels of anti-C5 mAb, free C5 may become available to C5 convertase for cleavage, eventually resulting in acute hemolytic events that are defined pharmacokinetic breakthrough hemolysis (BTH). (E) *Complement activation with C5 inhibitors in vivo, pharmacodynamic breakthrough hemolysis*. Similar acute hemolytic events may occur even when C5 is fully saturated by the anti-C5 mAb, due to overt complement activation caused by specific triggers (i.e., complement amplifying conditions). In this case, an excess of C3b results in C3b-rich C5 convertases with enhanced affinity for C5 (eventually competing more efficiently with the anti-C5 mAb for their common target C5), or directly in a conformational change of C5 which may then start C5b-9 assembly; these acute hemolytic events are defined pharmacodynamic BTH. (F) *Complement activation with C5 inhibitors in vivo, zilucoplan*. Residual hemolysis is also seen with zilucoplan, resembling that seen with anti-C5 mAb in unfavorable pharmacokinetic or pharmacodynamic circumstances. It should be noted that residual hemolysis with zilucoplan seems rather chronic, in contrast to the acute BTH seen with anti-C5 mAb. Even if pharmacokinetic and pharmacodynamic information about zilucoplan is limited, this might suggest that the phenomenon of chronic, continuous residual intravascular hemolysis is associated with the specific pharmacodynamics of this compound, which may compete less efficiently with C5 convertase for their common substrate/target C5. Figure created with somersault18:24.

vitro, clearly documenting that uncontrolled complement activation on PNH erythrocytes generates initial membrane binding of C3b, which is then quickly converted into C3d, both *in vitro* and *in vivo*.[7,8] While C3d eventually accounts for C3-mediated extravascular hemolysis (which has fostered the development of proximal inhibitors), transient high-density C3b may account for more efficient C5 activation, either via conformational change of C5[5] or through the generation of C3-rich high-affinity C5 convertases.[9] This mechanism may justify the residual hemolysis documented *in vitro* in the presence of eculizumab upon complement activation,[7,8] which mirrors the so-called pharmacodynamic breakthrough hemolysis observed *in vivo* during eculizumab treatment[10] (Figure 1A, B).

However, this mechanism has nothing to do with the suboptimal efficacy of zilucoplan observed in some PNH patients *in vivo*. First of all, the *in vitro* finding of an increased proportion of C3-opsonised PNH erythrocytes after combined exposure to eculizumab and zilucoplan is simply the result of more efficient C5 inhibition (similar to that seen with coversin and eculizumab):[5] indeed, fewer C3-opsonised PNH erythrocytes proceed to be lysed due to the double C5 inhibition, eventually contributing to increase their final proportion. *In vivo*, C3 opsonization is mostly a very slow phenomenon resulting from progressive accumulation of C3d on PNH erythrocytes that stochastically suffer from a surface activation exceeding a given threshold (C3b is quickly converted into its inactive split fragments).[8] As a consequence, even a transient (from some days to a week) exposure to double C5 inhibition does not justify increased C3 deposition (which in any case was not proven in these patients). It must be highlighted that, in the presence of effective C5 blockade (such as that achieved with two concomitant inhibitors, which according to the authors would result in increased C3 opsonization), even the postulated increased C3 opsonization would lead to increased C3-mediated extravascular hemolysis and never to increased intravascular hemolysis, since C3d *per se* cannot contribute to overcome therapeutic C5 inhibition (Figure 1C-E). Taken together, these considerations suggest that the residual intravascular hemolysis seen in PNH patients switching from eculizumab to zilucoplan is actually due to a less favorable pharmacokinetic/pharmacodynamic profile of this small molecule C5 inhibitor (Figure 1F).

Unpredicted and somewhat disappointing results have been observed in different proof-of-concept trials investigating novel anti-complement therapies for PNH; for instance cemdisiran, an anti-C5 small interfering RNA, was found to be only partially effective in controlling hemolysis despite achieving a ≥95% silencing efficiency.[11] In the setting of proximal complement inhibitors, even subtle differences in pharmacokinetics and pharmacodynamics may account for meaningful clinical differences, eventually driving their use in monotherapy or in combination of different factor D inhibitors.[12,13] As acknowledged by Kulasekararaj *et al.*, all these data support the notion that in PNH any therapeutic complement blockade must be sustained and complete to result in meaningful clinical activity; to this aim, our deepest understanding of the pharmacokinetics and pharmacodynamics of any complement inhibitor is essential to optimize their best use either in monotherapy or in combination treatment.[14]

Disclosures

AMR has been serving in advisory board and/or speaker panels for Novartis, Apellis, SOBI, Roche, Pfizer and Alexion.

Contributions

The authors equally contributed to this work.

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