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Understanding pharmacological complement inhibition in paroxysmal nocturnal hemoglobinuria

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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 in monotherapy efficiently controls intravascular hemolysis in both eculizumab-naïve and eculizumab-treated Paroxysmal Nocturnal Hemoglobinuria (PNH) patients, as shown by LDH levels, 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 the most recent years, a plethora of novel anti-complement agents have entered in their 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 2 studies are essential to investigate subtle differences among agents targeting even the same complement component, including pharmacokinetic and pharmacodynamic properties of individual inhibitors which finally drive their clinical efficacy and safety profile even more than their actual target. Therapeutic C5 inhibition is well-established since more than 15 years, making the interpretations of novel observations much easier, as compared to that of novel proximal inhibitors (4).

In this small phase 2 study, PNH patients with no previous treatment with eculizumab exhibited a significant benefit in terms of LDH decrease and transfusion avoidance once treated with zilucoplan, even if according to the authors the changes in more meaningful clinical parameters (e.g., hemoglobin level) and other biomarkers of hemolysis were small or variable. Even more interesting, 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). Altogether, these findings suggest that 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 of apparently complete complement inhibition (as assessed by functional assays measuring residual complement activity, suggesting that they are only partially informative as pharmacodynamic measurement during anti-complement therapies), and despite of 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, being one the effect of the other instead of two independent events). The authors propose as a possible mechanism of reduced efficacy in patients switched from eculizumab 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 larger proportion of C3b-opsonised PNH erythrocytes (2). However, their theory is not convincing for a number of reasons.
In 2009, we have originally described C3 opsonization as an ineluctable phenomenon in PNH patients treated with eculizumab (6). This phenomenon has been reproduced in vitro, clearly documenting that uncontrolled complement activation on PNH erythrocytes generates initial membrane binding of C3b, which then is 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 for the residual hemolysis documented in vitro in 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 increased proportion of C3-opsonised PNH erythrocytes after combined exposure to eculizumab and zilucoplan is simply the result of a 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 stocastically suffered 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 for increased C3 deposition (which in any case was not proven in these patients). It has to be highlighted that, in presence of an effective C5 blockade (as the one achieved with two concomitant inhibitors, which accordingly to the authors would result in increased C3 opsonization), even the postulated increased C3 opsonization would lead to an increased C3-mediated extravascular hemolysis and never to an increased intravascular hemolysis, since C3d \textit{per se} cannot contribute to overcome therapeutic C5 inhibition (Figure 1C,D,E). Taken together, these considerations suggest that the residual intravascular hemolysis seen in PNH patients switching from eculizumab to zilucoplan is rather due to a less favorable pharmacokinetic/pharmacodynamic profile of this small molecule C5 inhibitor (Figure 1F).

Unpredicted and somehow 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 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 the use in monotherapy or in combination of different factor D inhibitors (12,13). As acknowledged by Kulasekhararaj 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 pharmacokinetics and pharmacodynamics of any complement inhibitor is essential to optimize their best use either in monotherapy or in combination treatment (14).
REFERENCES


**Figure**

Figure 1. Mechanisms of residual hemolysis in presence of C5 inhibitors *in vitro* and *in vivo* *(created by somersault18:24).*

A. **Complement activation with C5 inhibitors in vitro, spontaneous activation.**
   Spontaneous, low-grade complement activation results in some degree of lysis on PNH erythrocytes in vitro; in presence of anti-C5 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 erythrocyte.

C. **Complement activation with C5 inhibitors in vivo, complete inhibition.** Ideally, anti-C5 monoclonal antibodies (mAb) are in excess to C5, resulting in complete inhibition of C5 which prevents C5 cleavage and further 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 hematological benefit.

D. **Complement activation with C5 inhibitors in vivo, pharmacokinetic breakthrough hemolysis (BTH).** In case of sub-therapeutic plasma level of anti-C5 mAb, free C5 may become available to C5 convertase for cleavage, eventually resulting in acute hemolytic events which are defined pharmacokinetic (PK) BTH.

E. **Complement activation with C5 inhibitors in vivo, pharmacodynamic breakthrough hemolysis (BTH).** 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 then may start C5b-9 assembly; these acute hemolytic events are defined pharmacodynamic (PD) BTH.

F. **Complement activation with C5 inhibitors in vivo, zilucoplan.** Residual hemolysis is also seen with zilucoplan, resembling what seen with anti-C5 mAbs at time of unfavorable PK or PD circumstances. It has to be remarked that residual hemolysis with zilucoplan seems rather chronic, in contrast to the acute BTH events seen with anti-C5 mAb. Even if PK and PD information with zilucoplan are limited, this might suggest that this phenomenon of chronic, continuous residual intravascular hemolysis is associated with the specific PD of this compound, which may less efficiently compete with C5 convertase for their common substrate/target C5.