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clinical practice. The authors professionally annotated a very large dataset and used a state-of-the-art computer vision algorithm to train and validate the model. The dataset can be used for future research to improve the model performance and to integrate it in the workflow of hematopathologists.

In summary, artificial intelligence and ML algorithms are changing our lives. These technologies will have significant impact on healthcare in the next decade. Although these algorithms may not replace physicians and researchers, it will definitely aid them in providing better care/research that can improve patient lives. As Oren Harari once said: "The electric light did not come from the continuous improvement of candles." If we really want to have a significant impact on healthcare in the future, we need to start embracing the impact of these technologies and learn how to use and integrate them into our daily workflow.

Conflict-of-interest disclosure: Speaker bureaus for Incyte Corporation and Novartis; data monitoring committee for MEI Pharma; advisory board/consulting (pharmaceutical/biotechnology) for AbbVie; and stock in Amazon. ■

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DOI 10.1182/blood.2021013259

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Kulasekararaj et al, page 1928

Control of hemolysis in patients with PNH

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Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by the triad of intravascular hemolysis (IVH), a tendency to thrombosis, and an element of bone marrow failure. With the introduction of the anti-C5 antibody eculizumab (ECU),[1] IVH and associated symptoms are abrogated, and the risk of thrombosis is greatly reduced; however, most patients remain anemic, and some remain transfusion dependent.[2] In this issue of *Blood*, Kulasekararaj et al[3] report that danicopan (DNC), an inhibitor of complement factor D, added to ECU, corrects or at least ameliorates the anemia.

This important clinical result is also of great interest with respect to pathophysiology (see figure panels A-B). When a patient with PNH is on ECU, red cells no longer suffer complement-mediated lysis, but unlysed red cells undergo opsonization by C3 fragments (see figure, panel C) and are thus marked for removal by macrophages. In patients who are on ECU, extravascular hemolysis (EVH) is a regular iatrogenic feature[4]; its clinical correlates are persistent anemia with reticulocytosis and hyperbiluribinemia, sometimes with visible jaundice.

Overcoming EVH has been a major stimulus for introducing agents that act upstream of C5 on the proximal complement pathway. There are now 3 such agents: pegcetacoplan (APL2), which is already approved by the US Food and Drug Administration,[5] as well as iptacopan (IPT)[6] and DNC, both of which are in phase 3 trials. In this context, the findings by Kulasekararaj et al are most relevant: DNC, in patients who are already on ECU, causes an increase in hemoglobin, a decrease in reticulocytes, and a decrease in bilirubin, which is exactly what one would expect when, with IVH already controlled by ECU, EVH is curbed as well. Of course the patients feel better, and Figure 3 in the article by Kulasekararaj et al illustrates a gratifying abolition of blood transfusion requirements in all but 1 patient. The decrease in the proportion of C3d-coated red cells is not as great as one might have hoped; perhaps plasma levels are not always optimal, and the dosage may need adjustments. Patient-to-patient variability in this parameter might also depend on the complement receptor 1 genotype,[7] which will require testing.

Because the complement system is part of innate immunity, tampering with it should not be taken lightly. Congenital C3 deficiency is associated with a risk for serious infection from capsulated and gram-negative bacteria; iatrogenic C3 deficiency may be less complete, but the same risk can be expected. We note, however, that with normally developed acquired immunity, the defensive function of complement may be less crucial in adults than in children.[8]

Inhibition of complement at the level of C3 can also largely preempt the distal pathway; therefore, in principle, any of the 3 agents mentioned above might control hemolysis in PNH on its own. This is not to say that the 3 are equivalent: APL2 targets C3 itself, a proenzyme and one of the most abundant plasma proteins (1.2 g/L); IPT targets factor B (plasma concentration of 200 mg/L), another proenzyme of the alternative pathway C3 convertase; and DNC targets factor D (plasma concentration 1 mg/L), the serine protease that cleaves-activates factor B (see figure). All of the drug-target interactions have been solved at the molecular level, but target concentrations and characteristics of the 3 drugs differ: the choice among them may not prove easy. Some of us believe that the price of a drug should not be dictated simply by market forces[9]; however, the benchmark will be the current

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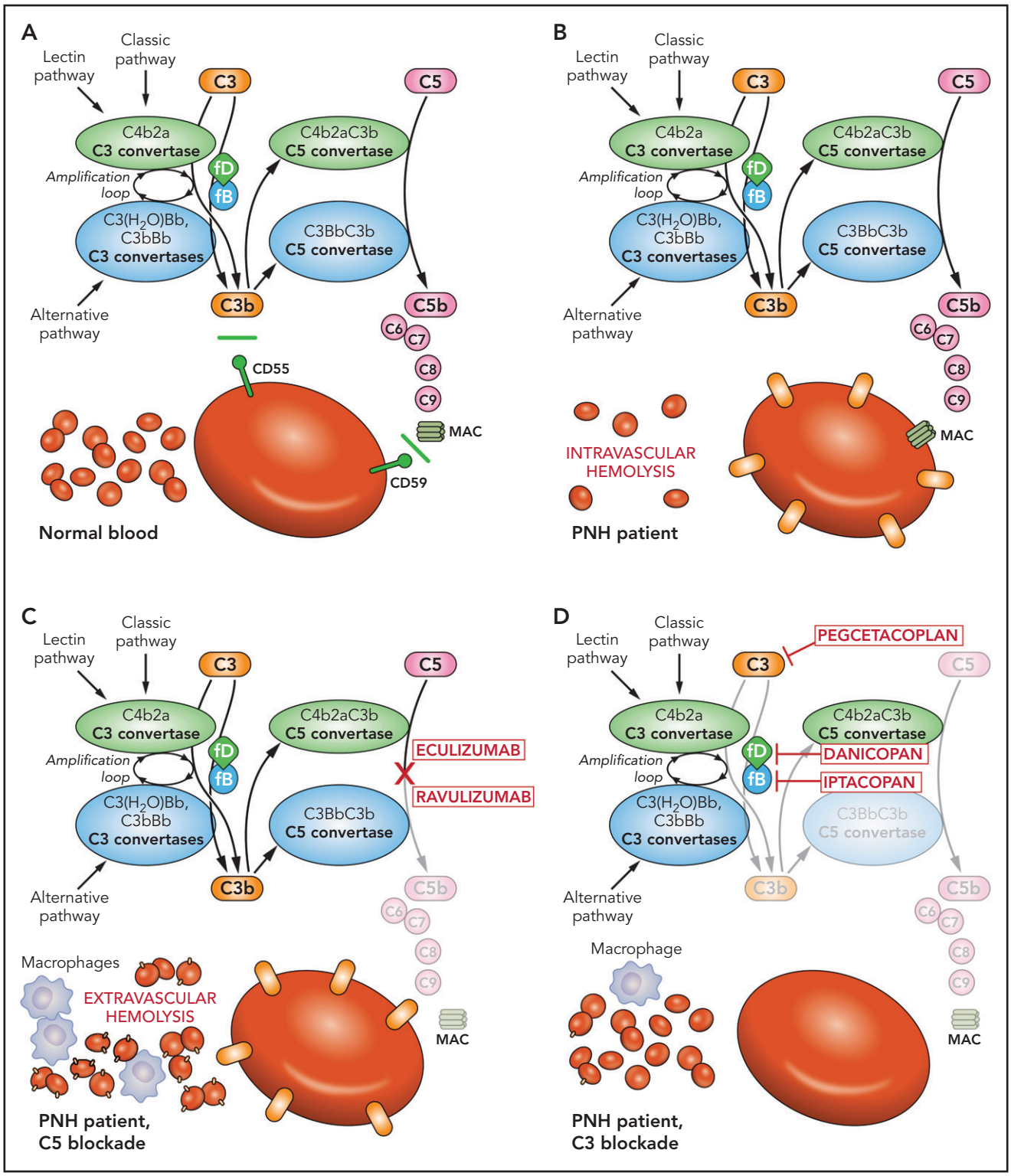

(A) In normal blood, when complement is activated, red cells are protected from lysis in several ways: primarily by the 2 glycosylphosphatidylinositol (GPI)-linked surface proteins CD55 (prevents binding of C3 fragments) and CD59 (prevents the membrane attack complex [MAC] from inserting into the membrane). (B) PNH red cells are deficient in CD55 and CD59 because the GPI biosynthetic pathway is blocked as a result of a PIGA mutation; therefore, C3 fragments, particularly C3d,[10] bind to their surface, and the red cells are rapidly lysed by the action of the MAC. (C) With drugs (monoclonal antibodies) that bind to C5 and prevent it splitting into C5a and C5b, the entire distal pathway from C5 onward is blocked, MAC is not formed, and IVH is abrogated. However, red cells opsonized by C3d will be destroyed in the spleen and elsewhere; this drug-induced EVH varies in severity between patients. The Coombs test, which is characteristically negative in PNH, becomes positive (provided that a "broad spectrum" or an anticomplement reagent is used). (D) With a drug that targets C3, C3b formation is inhibited, and the distal pathway is not triggered by C3b. Therefore, again, no MAC is formed (abrogating IVH), and, at the same time, opsonization of red cells by C3d is prevented, so that EVH is also curbed. The same is largely true for drugs that target factor B or factor D, although C3b can still be formed through the classical pathway. This figure does not do justice to the complexity of the complement system (eg, see Mastellos et al[8]). fB, factor B; fD, factor D. Professional illustration by Patrick Lane, ScEYEnce Studios.

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price of ECU, which is still unaffordable for more than half of the world. One might hope that, for once, competition will bring prices down.

With the advent of these new agents, the issue of monotherapy vs combination therapy is here with us. So far, most data are from patients who are already on ECU (or ravulizumab), but with DNC as a single agent, the distal pathway is already largely disabled (see figure, panel D). It seems reasonable that you do not need to use your foot brake when the handbrake is already on. The two brakes are not uniformly equivalent because with DNC and IPT the classic pathway C5 convertase can still be formed (see figure, panel D); however, the patients themselves will prefer one regular regimen rather than two.

A typical adverse event in the management of a patient with PNH is the so-called "breakthrough IVH"; on intravenous ECU, it can occur when massive complement activation (eg, from intercurrent infection) displaces ECU from C5. In a patient who is on an oral or subcutaneous drug (eg, ITP, DNC, APL2), it may occur simply because the patient has skipped a couple of doses. Having looked after patients with PNH for some decades before ECU,[2] when massive hemolytic attacks were not infrequent (and I have never lost a patient from one), I would not be too concerned by an occasional minor breakthrough IVH. However, the proportion of PNH red cells is usually <50% in untreated patients, in whom the total red cell mass is reduced as a result of anemia. In patients who are on complement-blockade therapy, instead, the red cell mass may be normal, and the proportion of PNH red cells may be >90%; thus, the complement-sensitive red cell population is much larger, and, therefore, the threat to life from a hemolytic attack is greater. Three things will be crucial: (1) dosage and pharmacokinetics, as it is not clear that they have been optimized for any of the upstream drugs; (2) very strict adherence to the schedule, demanding as it may be; and (3) one must have a protocol to deal with a sudden hemolytic contingency. An antibody, compared with most small molecules, has the advantage of a long in vivo half-life; for any patient on alternative pathway monotherapy, I recommend always having a dose of ECU on hand for emergency use.

Finally, I recall that when I saw my first patient with PNH I could only offer supportive treatment; I feel humbled because today we have the luxury of keenly debating which of several sophisticated targeted medicines is best. I am grateful for all I have learned from patients with PNH in 5 countries; we owe it to them to use each of these medicines optimally and safely, and we must make them available to all patients who may benefit.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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DOI 10.1182/blood.2021013169

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HEMATOPOIESIS AND STEM CELLS

Comment on Wang et al, page 1939

Upping the antizyme: AZIN1 directs stem cell fate

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In this issue of *Blood*, Wang et al[1] report that adenosine-to-inosine (A-to-I) RNA editing of antizyme inhibitor 1, *Azin1*, is a novel regulator of hematopoietic cell fate, capable of influencing self-renewal and differentiation at the stem cell level.

Hematopoietic stem cells have served as one of the main experimental models for decades.[2] The study of normal tissue-specific stem cells and the exploration of the capacity of cancer stem cells to self-renew and induce metastasis have been based on the biology of hematopoietic stem cells. Therefore, dissecting the regulatory networks that maintain stem cell function, such as quiescence, self-renewal, and differentiation, as Wang et al elegantly demonstrate, is both timely and impactful.

Enzymatic editing of RNA, and specifically A-to-I editing of *AZIN1*, has been implicated in the progression and therapeutic

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