and hypoxia inducible factor-1a (HIF-1a), are downregulated with PU-H71 treatment.

Another study that examined samples from patients with de novo and relapsed/refractory primary AML also reported a high level of epichaperomes in nearly 50% of the samples.[6] Furthermore, there was a recent report of a patient with a novel *PML-SYK* fusion AML who achieved durable complete remission after PU-H71 treatment.[7] Phase 1 clinical trials evaluating PU-H71 in metastatic breast cancer, lymphoma, and myeloproliferative neoplasms have been completed in recent years (clinicaltrials.gov identifiers NCT03166085 and NCT01393509). The findings by Carter et al suggest that AML with mutant *TP53* should also be added to the list of target malignant cancers for exploration with PU-H71 given alone or in combination with venetoclax. However, further work is needed to optimize the dosage for combination therapy, as PU-H71/venetoclax did not extend the survival of nonobese diabetic severe combined immunodeficiency gamma SGM3 (NSGS) mice in the PDX model, despite effectively reducing disease burden. In addition, combination therapy needs to be explored in the laboratory in the 17% of patient samples with AML that are resistant to PU-H71.[6] Last, evaluating the abundance of the epichaperome in each patient before treatment would be recommended for effective personalized treatment.

Overall, the exciting study by Carter et al presents compelling evidence that combining the epichaperome inhibitor PU-H71 with the BCL-2 inhibitor venetoclax is a potent and synergistic drug combination. This discovery opens up new avenues for therapeutic intervention in AML, particularly for patients with *TP53* mutations.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

## REFERENCES


1. Carter BZ, Mak PY, Muftuoglu M, et al. Epichaperome inhibition targets *TP53*-mutant AML and AML stem/progenitor cells. *Blood*. 2023;142(12):1056-1070.
2. DiNardo CD, Jonas BA, Pullarkat V, et al. Azacitidine and venetoclax in previously untreated acute myeloid leukemia. *N Engl J Med*. 2020;383(7):617-629.
3. DiNardo CD, Tiong IS, Quaglieri A, et al. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. *Blood*. 2020;135(11):791-803.
4. Pollyea DA, Pratz KW, Wei AH, et al. Outcomes in patients with poor-risk cytogenetics with or without TP53 mutations treated with venetoclax and azacitidine. *Clin Cancer Res*. 2022;28(24):5272-5279.
5. Rodina A, Wang T, Yan P, et al. The epichaperome is an integrated chaperome network that facilitates tumour survival. *Nature*. 2016;538(7625):397-401.
6. Tavakkoli M, Kaner JD, Sahil S, et al. Epichaperome abundance predicts response to the epichaperome inhibitor, Puh-71, in acute myeloid leukemia. *Blood*. 2019;134(suppl 1):5145.
7. Sugita M, Wilkes DC, Bareja R, et al. Targeting the epichaperome as an effective precision medicine approach in a novel PML-SYK fusion acute myeloid leukemia. *NPJ Precis Oncol*. 2021;5(1):44.


https://doi.org/10.1182/blood.2023021386

© 2023 by The American Society of Hematology

THROMBOSIS AND HEMOSTASIS

*Comment on* *Jiang et al*, *page 1071*

# New targeted therapy for hemophilia

**Roger J. S. Preston** | Royal College of Surgeons in Ireland University of Medicine and Health Sciences and Children's Health Ireland Crumlin

**In this issue of *Blood*, Jiang et al[1] describe the development of a new monoclonal antibody that binds with very high affinity to activated protein C (APC) and selectively inhibits its anticoagulant activity to restore hemostasis in preclinical models of hemophilia.**

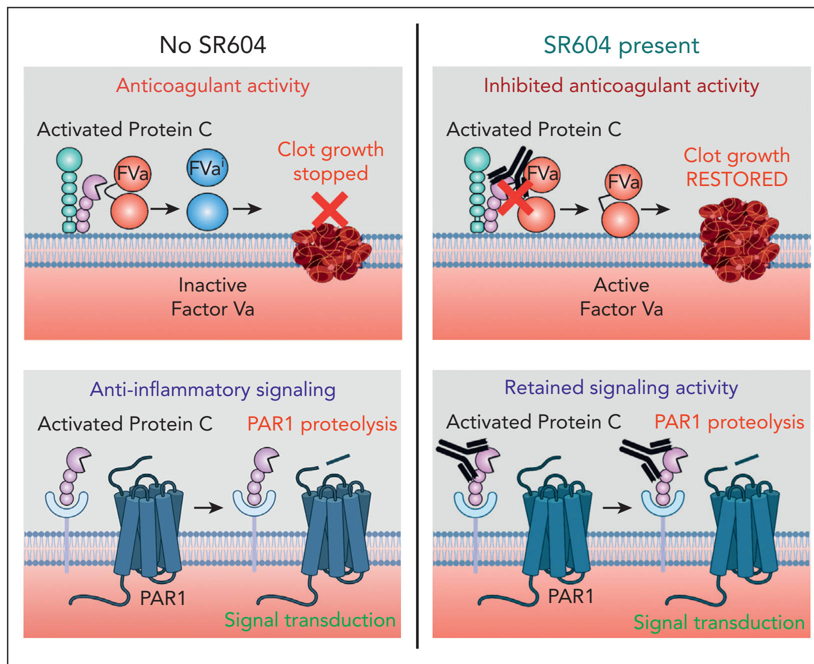
Maintenance of hemostasis is central to the prevention of thrombosis and bleeding. Diminished thrombin generation and clot formation arising from clotting factor deficiencies, such as hemophilia, disrupt this delicate balance and promote bleeding. Factor replacement is the standard of care for people with hemophilia in wealthy countries, but alternative hemostatic products are required if inhibitors to replacement factors develop. Although the factor VIII (FVIII) mimetic bispecific antibody emicizumab has enhanced the treatment of patients with hemophilia A with inhibitors, the need for alternative therapies for individuals with hemophilia B and other rare bleeding disorders has stimulated the generation of a plethora of new prohemostatic agents that target different facets of the coagulation system. Of these, novel therapies that can safely attenuate endogenous anticoagulant pathways may represent a solution to "rebalance" hemostasis in individuals with inherited or acquired bleeding disorders. To this end, antibody-mediated inhibition of tissue factor pathway inhibitor or aptamer-mediated suppression of antithrombin has already demonstrated positive outcomes in clinical trials with patients with hemophilia.

Inhibition of the protein C pathway also represents an attractive target based on its central role in the dynamic regulation of thrombin generation.[2] Plasma protein C is activated by thrombin bound to its anticoagulant receptor, thrombomodulin, which is abundantly expressed on the vessel wall. This complex, in turn, converts protein C into its activated form, APC, in a process accelerated by protein C binding to the endothelial protein C receptor. APC with its cofactor protein S then degrades activated procoagulant cofactors FVa and FVIIIa to restrict further thrombin generation (see figure). APC also exists in minute quantities in plasma (~40 pM), and attenuation of its anticoagulant properties may help promote thrombin generation in individuals with bleeding disorders. Several creative approaches have already been developed to achieve APC inhibition. SerpinPC, a recombinant α1-antitrypsin variant with enhanced specificity for APC, promotes hemostasis by inhibiting APC anticoagulant activity and is currently being evaluated in phase II



Downloaded from http://ashpublications.net/blood/article-pdf/142/12/1032/2077516/blood_bld-2023-021385-c-main.pdf by guest on 21 May 2024

Check for updates




SR604 restores hemostasis by selectively blocking APC anticoagulant activity. The anti-APC mAb SR604 binds with ultra-high affinity to APC at an epitope important for binding to its substrate, FVa. In doing so, it potently blocks APC anticoagulant activity to restore thrombin generation in preclinical hemophilia models. In contrast, SR604 does not impede endogenous APC anti-inflammatory activity via PAR1.

clinical trials in patients with hemophilia B.[3] In addition, SuperVa, an APC-resistant FVa variant, effectively promotes thrombin generation in preclinical bleeding models.[4] Furthermore, inhibition of the APC anticoagulant cofactor protein S promotes hemostasis in hemophilia plasma and preclinical hemophilia models.[5] Most recently, monoclonal antibodies (mAbs) targeting APC have shown significant promise in directly blocking FVa proteolysis by APC to promote hemostasis.[6,7]

A critical safety concern for therapies that inhibit anticoagulant pathways is that they do not "overbalance" hemostasis to promote thrombosis. Therapeutic approaches that inhibit APC may have an additional safety consideration, namely, the inadvertent inhibition of important APC properties that are distinct from its role in regulating hemostasis. APC possesses complex anti-inflammatory signaling functions that are principally mediated by proteolysis of protease-activated receptor 1 (PAR1) on both endothelial and peripheral immune cells (see figure). Importantly, genetic deficiency or antibody inhibition of APC can exacerbate both acute and chronic inflammatory disease in preclinical models. Although not evaluated to date, it is reasonable to assume that such a possibility could arise in patients with hemophilia receiving APC-blocking therapies that inhibit all proteolytic activity. The latest iterations of APC-targeting monoclonal antibodies have therefore sought to selectively attenuate APC anticoagulant properties without influencing the capacity to promote APC signaling.[6,7] Helpfully, exosites on the APC surface necessary for isolating specific APC functions have already been extensively characterized.

In this issue, Jiang et al describe the development and characterization of an engineered humanized mAb (SR604) designed to target an APC exosite located within the APC protease domain that is critical for anticoagulant activity but dispensable for PAR1-dependent anti-inflammatory signaling. SR604 was based on a previous murine mAb (HAPC1573) that bound APC but required high doses to restore hemostasis in animal hemophilia models. Evaluation of mAb-APC binding affinity by surface plasmon resonance showed that SR604 possessed a 60-fold higher affinity for APC than its parent antibody. This translated into enhanced hemostatic efficacy in ex vivo plasma experiments, in which SR604 dose-dependently shortened clotting times in various coagulation factor-deficient plasmas with APC generation. In vivo, SR604 limited tail bleeding from hemophilic mice to a similar extent as recombinant FVIII and reduced joint bleeding and arthropathy in a mouse knee injury model performed in hemophilic mice. Importantly, the authors confirmed that SR604 did not exert a similar inhibition of APC signaling activity on endothelial cells, showing that SR604 did not affect PAR1-mediated APC protection of endothelial cell barrier function from thrombin-induced permeability. Intriguingly, SR604 accelerated APC proteolysis of cytotoxic extracellular histones, another crucial anti-inflammatory APC function during acute infection, suggesting SR604 may also represent a useful tool for better understanding the mechanistic basis of this distinct anti-inflammatory activity. To further demonstrate the lack of SR604-mediated interference in endogenous APC anti-inflammatory properties, the authors administered SR604 to hemophilic mice that had been exposed to a sublethal lipopolysaccharide (LPS) dose and showed that, in contrast to an anti-protein C monoclonal antibody that blocks APC generation, SR604 binding to endogenous APC did not provoke increased mortality in response to LPS challenge. These data support the reduced impact of SR604 on APC anti-inflammatory activity compared with its potent inhibition of anticoagulant activity. However, further studies will be required for more granular assessment of whether SR604 modulates other noncanonical APC signaling functions[8,9] and to determine whether SR604 impacts APC signaling in chronic inflammatory disease settings in which endogenous APC is also protective.[10]

Although pending evaluation in clinical trials, the prohemostatic properties, limited off-targeted toxicities, and favorable pharmacokinetics described in this study suggest SR604 represents an exciting addition to the growing range of prohemostatic nonfactor therapies and underscore the potential of precision therapies that target selected protein C pathway functions. This study also provides a salient example of the continued refinement of approaches to rebalance hemostasis safely, which is anticipated to expand further the therapeutic options available to individuals with hemophilia and other rare bleeding disorders.

Downloaded from http://ashpublications.net/blood/article-pdf/142/12/1032/2077516/blood_bld-2023-021385-c-main.pdf by guest on 21 May 2024

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


## REFERENCES

1. Jiang M, Yang F, Jiang Y, et al. Safety and efficacy of an anti–human APC antibody for prophylaxis of congenital factor deficiencies in preclinical models. *Blood*. 2023;142(12):1071-1081.
2. Leon G, Rehill AM, Preston RJS. The protein C pathways. *Curr Opin Hematol*. 2022;29(5):251-258.
3. Polderdijk SGI, Adams TE, Ivanciu L, Camire RM, Baglin TP, Huntington JA. Design and characterization of an APC-specific serpin for the treatment of hemophilia. *Blood*. 2017;129(1):105-113.
4. Chumappumkal Joseph B, Miyazawa BY, Esmon C, et al. An engineered activated factor V for the prevention and treatment of acute traumatic coagulopathy and bleeding in mice. *Blood Adv*. 2022;6(3):959-969.
5. Prince R, Bologna L, Manetti M, et al. Targeting anticoagulant protein S to improve hemostasis in hemophilia. *Blood*. 2018;131(12):1360-1371.
6. Zhao X-Y, Wilmen A, Wang D, et al. Targeted inhibition of activated protein C by a non-active-site inhibitory antibody to treat hemophilia. *Nat Commun*. 2020;11(1):2992.
7. Jiang M, Yang F, Jiang Y, et al. Blocking human protein C anticoagulant activity improves clotting defects of hemophilia mice expressing human protein C. *Blood Adv*. 2022;6(11):3304-3314.
8. Ranjan S, Goihl A, Kohli S, et al. Activated protein C protects from GvHD via PAR2/PAR3 signalling in regulatory T-cells. *Nat Commun*. 2017;8(1):311.
9. Yang XV, Banerjee Y, Fernández JA, et al. Activated protein C ligation of ApoER2 (LRP8) causes Dab1-dependent signaling in U937 cells. *Proc Natl Acad Sci U S A*. 2009;106(1):274-279.
10. Bock F, Shahzad K, Vergnolle N, Isermann B. Activated protein C based therapeutic strategies in chronic diseases. *Thromb Haemost*. 2014;111(4):610-617.


https://doi.org/10.1182/blood.2023021385

© 2023 by The American Society of Hematology

**TRANSFUSION MEDICINE**

*Comment on* Jajosky et al, *page 1082*

# Removing antigens but not cells: a key to AMIS?

**Rick Kapur** | Sanquin Research and Landsteiner Laboratory

**In this issue of *Blood*, Jajosky et al demonstrate that passive immunization with red blood cell (RBC) antibodies that have the ability to remove target antigens from RBCs, without affecting RBC clearance or CD4$^+$ T-cell proliferation, can convert an augmented RBC alloimmune response to antibody-mediated immunosuppression (AMIS) in an established murine model of RBC alloimmunization.[1] These findings shed light on the mechanisms of AMIS and have the potential to facilitate development of new strategies for AMIS against non-RhD RBC alloantigens to expand the prevention of hemolytic disease of the fetus and newborn (HDFN).**

Development of maternal alloantibodies against paternally inherited fetal RBC antigens can lead to HDFN.[2] These maternal antibodies, most commonly anti-RhD, are actively transported across the placenta, where they bind to fetal RBCs carrying the corresponding antigen, which can result in RBC clearance via phagocytic Fcγ receptors (FcγRs).[3] If untreated, this can result in perinatal mortality and morbidity, with the occurrence of fetal anemia, jaundice, hydrops, and stillbirth.[2] Polyclonal anti-RhD immunoprophylaxis administered to women at risk has immensely decreased the rate of alloimmunization and reduced the risk of HDFN,[4] and it is widely considered to be among the major medical breakthroughs of the 20th century. Despite this major success, anti-RhD immunoprophylaxis targets a single RBC antigen and is the only clinical example of AMIS. Notably, alloimmunization toward non-RhD and non-ABO alloantigens accounts for 0.1% to 1.1% of live births,[5] and for these alloantigens, no immunoprophylactic approaches exist.

The mechanisms involved in AMIS remain incompletely understood. RBC antibodies can elicit various immune responses that impact the development of alloantibodies or induction of AMIS, dependent on the target antigen and the antibody used.[6] AMIS has previously been attributed to increased RBC clearance; however, murine studies have suggested that AMIS can also occur independent of RBC clearance or epitope masking[7] and may result from RBC antigen loss unrelated to FcγRs.[8] RBC challenge dosages may also differentially influence the outcomes[9]; however, different RBC challenge dosages have not been investigated in parallel and in relation to antibodies with different targets in AMIS studies.

In the current study, Jajosky et al employ a highly systematic approach examining the effects of RBC challenge dosages and antibody combinations on the outcome of AMIS, with evaluation of RBC alloantibody formation, RBC clearance, and RBC antigen loss and induction of CD4$^+$ T-cell proliferation. They use an established murine model of HOD RBC alloimmunization, which consists of a chimeric triple-fusion protein containing hen egg lysozyme (HEL; contains B-cell epitopes), a portion of ovalbumin (contains CD4$^+$ T-cell epitopes), and the Duffy antigen (anchors the protein using its transmembrane domain) expressed on murine RBCs. To investigate the occurrence of AMIS, different dosages of HOD RBCs ($10^7$, $10^8$, and $10^9$) were transfused in the presence of anti-Duffy. A low dose of $10^7$ RBCs induced a detectable anti-HEL IgG response, which was blunted by passive immunization with anti-Duffy. In contrast, a high dose of $10^8$ or $10^9$ RBCs resulted in an opposite outcome, reflected by an increase in anti-HEL IgG on passive immunization with anti-Duffy. This illustrates that the HOD RBC dose can determine the occurrence of RBC alloimmunization in the presence of anti-Duffy. The anti-Duffy responses were shown to be related to induction of antigen loss on the RBC surface and increased RBC clearance at low and high RBC dosages. This suggests that at a high RBC dose, antibody-mediated RBC clearance may enhance de novo alloimmunization. Using FcγR-knockout mice as HOD RBC transfusion recipients, FcγRs were found to be required for both anti–Duffy-mediated RBC clearance and antigen removal. Similarly, FcγRs were found to be required for the anti–Duffy-induced suppression (low-dose $10^7$ RBCs) or augmentation (high-dose $10^9$



Downloaded from http://ashpublications.net/blood/article-pdf/142/12/1032/2077516/blood_bld-2023-021385-c-main.pdf by guest on 21 May 2024