



## CLINICAL TRIALS AND OBSERVATIONS

Comment on Röth et al, page 980

# A virtuosic CADENZA played by sutimlimab

Satheesh Chonat<sup>1,2</sup> and Sean R. Stowell<sup>3</sup> | <sup>1</sup>Children's Healthcare of Atlanta; <sup>2</sup>Emory University School of Medicine; and <sup>3</sup>Harvard Medical School

**In this issue of *Blood*, Röth et al<sup>1</sup> report on the results from a phase 3 placebo-controlled study to assess the efficacy and safety of sutimlimab in participants with primary cold agglutinin disease (CAD) without a recent history of blood transfusion (CADENZA). The results of this study demonstrate the efficacy of sutimlimab in treating nontransfusion-dependent patients with CAD.**

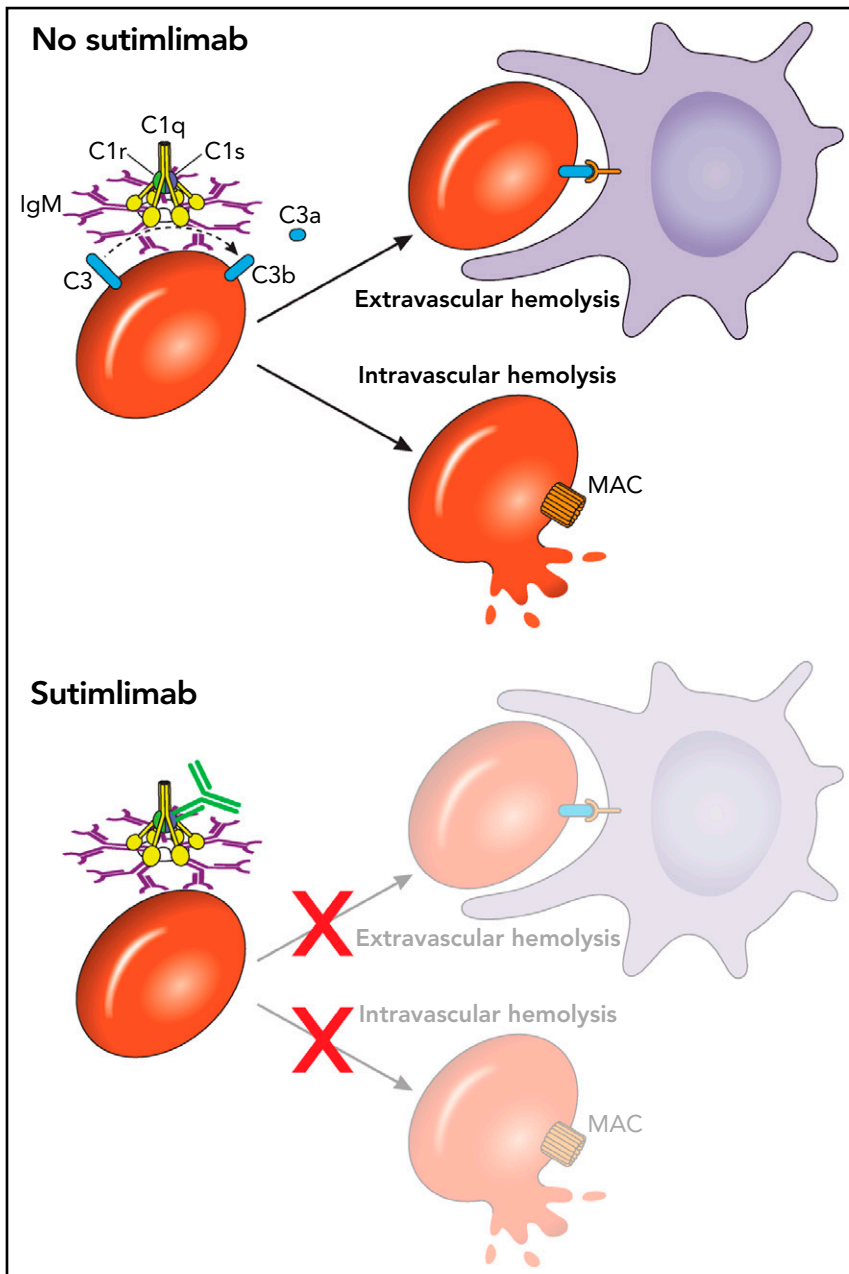
CAD is a rare autoimmune hemolytic anemia first described nearly 70 years ago. However, despite its early discovery, treatment options for CAD have often been indirect and of limited efficacy. CAD is initiated by binding immunoglobulin M (IgM) autoantibodies to the I antigen on erythrocytes at  $\leq 37^\circ\text{C}$  temperature (cold agglutinins), resulting in agglutination.<sup>2</sup> This antibody-antigen complex triggers the activation of the classical complement pathway, leading to marked C3b-mediated extravascular hemolysis (EVH) and, to a lesser degree, C5b-9-mediated intravascular hemolysis (IVH), mostly during acute exacerbations (see figure). Clonal lymphocytes produce these predominantly monoclonal IgM antibodies in the bone marrow of patients with indolent clonal B-cell lymphoproliferative disorder.<sup>3</sup> Patients with CAD can present with chronic anemia requiring blood transfusions and periodic acute hemolytic crisis, chronic fatigue, and cold-induced circulatory symptoms such as acrocyanosis, Raynaud-like symptoms, and even gangrene/ulcerations. In addition, some of these patients are at risk of thrombosis.<sup>3</sup> It is paramount to distinguish this from transient cold agglutinin syndrome, which occurs secondary to infections (*Mycoplasma pneumoniae*

and Epstein-Barr virus) or cancer (aggressive lymphoma).

Immunomodulators have been the first-line agents to reduce clonal lymphoproliferation and monoclonal IgM antibody production. Rituximab to deplete B cells alone induced remission in approximately 50% of patients with a median response time of 1.5 months and a duration of 11 months.<sup>4</sup> The addition of fludarabine, a nucleoside analog, to rituximab, as tried in other diseases such as Waldenström macroglobulinemia and lymphoma, was used in CAD with improved overall response rates to 76%, including an increased median response duration of 5.5 years. Unfortunately, this regimen was met with grade 3 or 4 hematological toxicity in about 40% of patients.<sup>5</sup> A second combination therapy using bendamustine, an alkylating agent, with rituximab showed a similar overall response rate of about 70% with a slightly higher remission rate (40%) with a median response duration of just over 2.5 years. In addition, the rates of neutropenia and infections were slightly lower when compared with the rituximab-fludarabine combination.<sup>6</sup> While these approaches can reduce antibody concentrations, none of these approaches directly target the ability of antibodies to induce hemolysis.

Although a key driver in CAD is antibody-mediated complement activation, very few therapeutics have historically been designed to directly modulate complement. This completely changed in recent years. Driven in part by the success of the monoclonal antibody against complement factor C5, eculizumab, an entire array of complement inhibitors is under investigation. The importance of targeting distinct components of the complement cascade is evident by an inadequate treatment response of CAD with eculizumab. A non-randomized phase 2 study of terminal complement inhibition using eculizumab to block IVH in CAD therapy of chronic cold agglutinin disease with eculizumab (DECADE) demonstrated that C5 inhibition could reduce lactate dehydrogenase concentrations and transfusion dependency in 13 individuals but only resulted in modest improvement in hemoglobin (Hb), likely as a result of ongoing EVH.<sup>7</sup>

The inability of eculizumab to target proximal complement activation and, therefore, C3b-mediated EVH suggested that proximal complement inhibition may be necessary to effectively treat CAD. As a proof-of-concept study that the proximal classical pathway of complement inhibition may reduce EVH, Shi and colleagues reported that treating plasma samples from patients with CAD using a monoclonal antibody (TNT003) that targeted C1s inhibited the cold agglutinin-mediated C3 fragment deposition on normal erythrocytes, prevented subsequent complement anaphylatoxin (C4a, C3a, and C5a) production and downstream phagocytosis.<sup>8</sup> Then sutimlimab, a humanized anti-C1s IgG monoclonal antibody, was studied in a phase 1b and phase 3 open-label, single-arm CARDINAL study.<sup>9,10</sup> Both of these studies showed the benefit of C1s inhibition in improving Hb by halting EVH and reducing fatigue. However, the CARDINAL study only included patients who had received transfusion within 6 months before enrollment.



Sutimlimab reduces intravascular and extravascular hemolysis in CAD by inhibiting C1s. CAD results from monoclonal IgM antibodies that target carbohydrate antigens on the surface of red blood cells, leading to complement fixation. Early IgM-induced complement activation can lead to extravascular hemolysis by facilitating macrophage engagement or can lead to intravascular hemolysis by proceeding to terminal complement activation and membrane attack complex (MAC) formation. Sutimlimab inhibits early complement activation by targeting C1s, thereby protecting cells from complement-mediated extravascular and intravascular hemolysis. Professional illustration by Patrick Lane, ScEYence Studios.

In this paper, Roth and colleagues provide further evidence of the benefit of sutimlimab in CAD. A rigorously performed phase 3 randomized, placebo-controlled clinical trial was conducted at 27 sites across 13 countries and involved 42 patients with a confirmed diagnosis of CAD with Hb  $\leq 10$  g/dL and excluded patients with a history of blood transfusion within 6 months of screening or a history

of  $>1$  transfusion within 12 months. Sutimlimab was shown to meet the study-defined composite endpoint in 73% compared with 15% in the placebo group. This result is significant as all the responders to the drug demonstrated sustained rapid increase in their Hb concentrations by  $\geq 2$  g/dL associated with a reduction in hemolysis and improved fatigue scores. This is important as the study protocol

prohibited immunomodulator therapies. There was no difference in adverse events between the groups. Of note, 3 patients in the sutimlimab group discontinued the study drug prematurely because of progression of underlying disease, a new diagnosis of lymphoma, and infusion-related reaction.

These findings give insight into the mechanisms of proximal complement pathway inhibition and how early or prompt initiation of complement inhibiting therapy can potentially be beneficial in such diseases. Furthermore, like studies using terminal complement inhibition, complement blockade in this study resulted in the improvement in fatigue, which along with amelioration of chronic anemia, could reflect inhibition of complement-mediated inflammation. While there was no difference in the infections between the groups, treating providers need to remain cautious. Additional real-world studies are needed to more fully define the benefits and risks associated with sutimlimab treatment. Equally important, future clinical trials should identify the patients who would likely benefit from such complement inhibition and how baseline patient characteristics affect the response. However, given the unique ability of sutimlimab to block proximal complement activation, this approach holds promise in providing another avenue of complement inhibition that may be useful not only for CAD but other disease states with complement involvement that are less responsive to more terminal complement inhibitors.

*Conflict-of-interest disclosure:* S.R.S. is a consultant for Alexion, Novartis, Cellics, and Argenx and receives honoraria for speaking engagements for Grifols. S.C. is a scientific advisor to Agios, Alexion, Daichi Sankyo, Forma Therapeutics, Novartis, and Takeda Pharmaceuticals and receives research funds from Novartis and Global Blood Therapeutics. ■

## REFERENCES

- Röth A, Berentsen S, Barcellini W, et al. Sutimlimab in patients with cold agglutinin disease: results of the randomized placebo-controlled phase 3 CADENZA trial. *Blood*. 2022;140(9):980-991.
- Sokol RJ, Hewitt S, Stamps BK. Autoimmune haemolysis: an 18-year study of 865 cases referred to a regional transfusion centre. *Br Med J (Clin Res Ed)*. 1981;282(6281):2023-2027.

- Berentsen S, Barcellini W, D'Sa S, et al. Cold agglutinin disease revisited: a multinational, observational study of 232 patients. *Blood*. 2020;136(4):480-488.
- Berentsen S, Ulvestad E, Gjertsen BT, et al. Rituximab for primary chronic cold agglutinin disease: a prospective study of 37 courses of therapy in 27 patients. *Blood*. 2004;103(8):2925-2928.
- Berentsen S, Randen U, Vågan AM, et al. High response rate and durable remissions following fludarabine and rituximab combination therapy for chronic cold agglutinin disease. *Blood*. 2010;116(17):3180-3184.
- Berentsen S, Randen U, Oksman M, et al. Bendamustine plus rituximab for chronic cold agglutinin disease: results of a Nordic prospective multicenter trial. *Blood*. 2017;130(4):537-541.
- Röth A, Bommer M, Hüttmann A, et al. Eculizumab in cold agglutinin disease

(DECADE): an open-label, prospective, bicentric, nonrandomized phase 2 trial. *Blood Adv*. 2018;2(19):2543-2549.

- Shi J, Rose EL, Singh A, et al. TNT003, an inhibitor of the serine protease C1s, prevents complement activation induced by cold agglutinins. *Blood*. 2014;123(26):4015-4022.
- Jäger U, D'Sa S, Schörghenhofer C, et al. Inhibition of complement C1s improves severe hemolytic anemia in cold agglutinin disease: a first-in-human trial. *Blood*. 2019;133(9):893-901.
- Röth A, Barcellini W, D'Sa S, et al. Sutimlimab in cold agglutinin disease. *N Engl J Med*. 2021;384(14):1323-1334.

DOI 10.1182/blood.2022017284

© 2022 by The American Society of Hematology

## HEMATOPOIESIS AND STEM CELLS

Comment on Keyvani Chahi et al, page 992

# HSCs: slow me down with PLAG1

Yu Wei Zhang and Nina Cabezas-Wallscheid | Max Planck Institute of Immunobiology and Epigenetics

**In this issue of *Blood*, Keyvani Chahi et al<sup>1</sup> have described the transcription factor PLAG1 as a positive regulator of human cord blood (CB) hematopoietic stem cell (HSC) dormancy and self-renewal by repressing the expression of translational machinery that is customarily activated in response to ex vivo cultivation stress.**

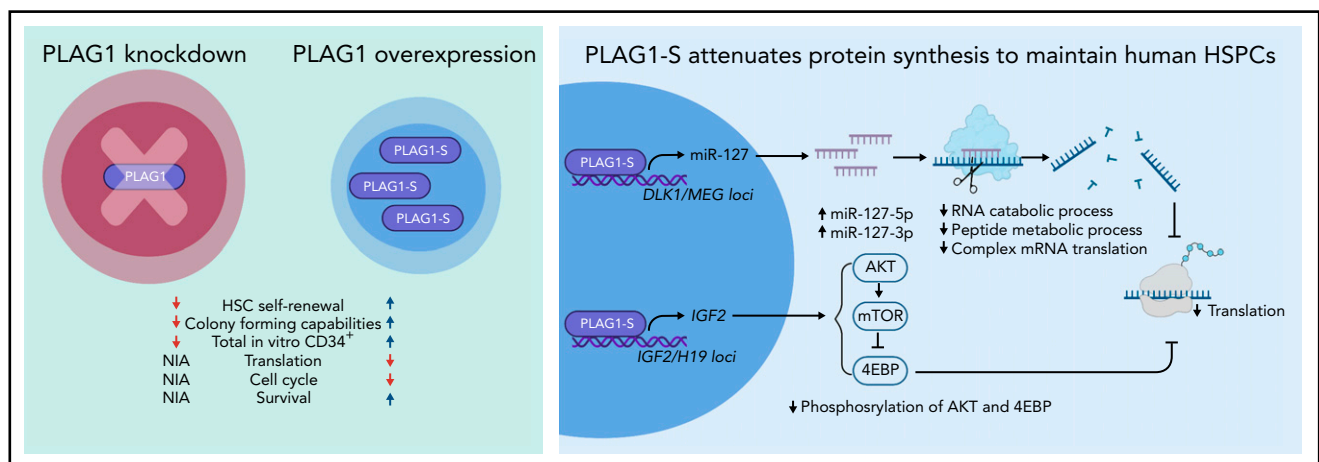
HSCs are capable of generating all blood cell lineages throughout a person's lifetime.<sup>2</sup> To preserve life-long stem cell

functionality, HSCs primarily reside in a state of dormancy and quiescence characterized by low levels of protein

synthesis.<sup>3,4</sup> Under stress conditions, such as ex vivo cultivation or transplantation, dormant HSCs are forced into a state of activation.<sup>5</sup> However, little is known about the preventative machinery that suppresses activation and promotes quiescence in human HSCs.

Previously, PLAG1 was shown to bind to the promoter of MSI2 to enhance in vitro expansion of hematopoietic stem and progenitor cells (HSPCs).<sup>6</sup> In their study, Keyvani Chahi et al showed that PLAG1 expression is restricted to non-cycling HSCs, which suggests a role for PLAG1 in modulating human HSPC function that is independent of the PLAG1/USF2-MSI2 regulatory axis. There are 3 human PLAG1 transcript variants encoding 2 protein isoforms. The shorter isoforms of PLAG1 (B and S) are enriched in CB HSCs, while the longer isoform (A) is expressed at much lower levels.<sup>6</sup> Knockdown of PLAG1 reduced the output of human HSPCs and was accompanied by impaired long-term bone marrow reconstitution, whereas the ectopic overexpression (OE) of the PLAG1-S isoform enhanced in vivo self-renewal of human HSCs.

From genomic binding and transcriptomic profiling of PLAG1-S<sup>OE</sup> HSPCs, the authors found that PLAG1-S can act as both a positive and a negative regulator of gene expression. For instance, ribosomal protein expression was downregulated in PLAG1-S<sup>OE</sup>, which pointed toward the ability of PLAG1-S to attenuate protein synthesis machinery and thus regulate stem cell



Loss and gain of PLAG1 function in human CB CD34<sup>+</sup> HSPCs (left). Mechanisms by which PLAG1-S represses protein synthesis to maintain human CB HSC dormancy and self-renewal (right). mRNA, messenger RNA; NIA, no information available. Figure created by BioRender.com.