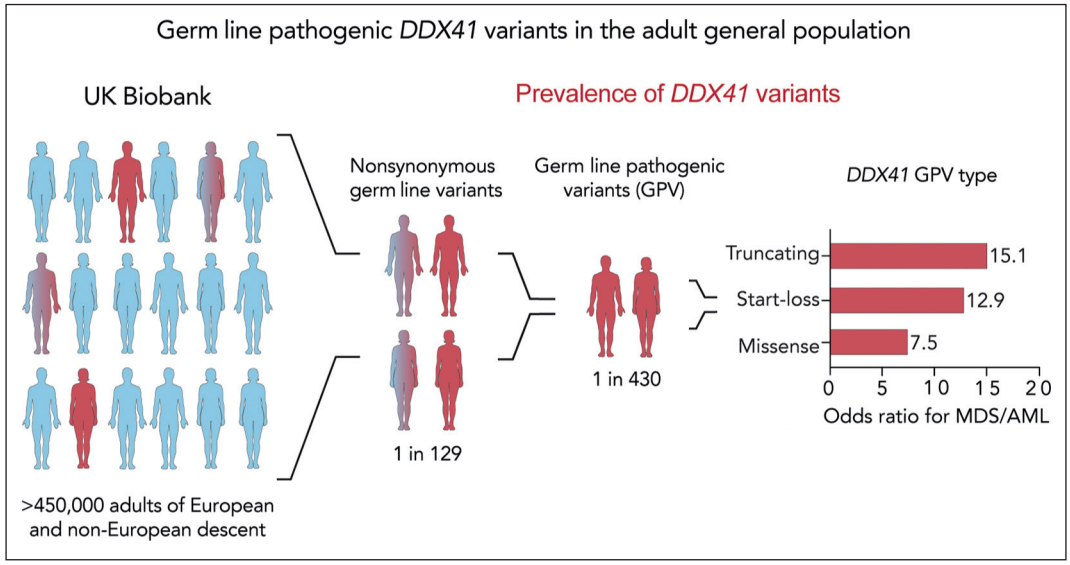

In a population-level analysis, Cheloor Kovilakam et al found that nonsynonymous germ line *DDX41* variants were present in 1 in 129 individuals and *DDX41*-GPV were present in 1 out of 430 adults in the UK Biobank. In addition, the *DDX41*-GPV variant type influenced the risk of MDS/AML, with truncating and start-loss alterations conferring the highest risk. Professional illustration by Somersault18:24.

et al suggests that hematologic indices and somatic sequencing may identify individuals at higher risk of MDS/AML. Prospective longitudinal studies incorporating error-corrected DNA sequencing are needed to develop improved prognostic models and evidence-based surveillance recommendations for *DDX41*-GPV carriers. Lastly, the prospect of donor-to-recipient transmission of *DDX41*-GPV is concerning given the known risk of donor cell leukemia.[10] Urgent registry-level studies are required to determine the impact of *DDX41*-GPV transmission on transplant outcomes. Indeed, this also raises additional questions regarding the utility and ethical considerations of routine *DDX41* genotyping in the evaluation of transplant donors, which also warrant investigation.

In summary, the study by Cheloor Kovilakam et al significantly advances our understanding of the population prevalence and myeloid malignancy risk associated with *DDX41*-GPV. It also demonstrates the utility of leveraging existing population-scale genotype/phenotype data to address clinically relevant questions related to germ line predisposition to hematologic neoplasms.

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

REFERENCES

1. Cheloor Kovilakam S, Gu M, Dunn WG, et al. Prevalence and significance of *DDX41* gene variants in the general population. *Blood*. 2023;142(14):1185-1192.
2. Makishima H, Bowman TV, Godley LA. DDX41-associated susceptibility to myeloid neoplasms. *Blood*. 2023;141(13):1544-1552.
3. Li P, Brown S, Williams M, et al. The genetic landscape of germline DDX41 variants predisposing to myeloid neoplasms. *Blood*. 2022;140(7):716-755.
4. Makishima H, Saiki R, Nannya Y, et al. Germ line DDX41 mutations define a unique subtype of myeloid neoplasms. *Blood*. 2023;141(5):534-549.
5. Sébert M, Passet M, Raimbault A, et al. Germline DDX41 mutations define a significant entity within adult MDS/AML patients. *Blood*. 2019;134(17):1441-1444.
6. Duployez N, Largeaud L, Duchmann M, et al. Prognostic impact of DDX41 germline mutations in intensively treated acute myeloid leukemia patients: an ALFA-FILO study. *Blood*. 2022;140(7):756-768.
7. Murdock HM, Kim HT, Denlinger N, et al. Impact of diagnostic genetics on remission MRD and transplantation outcomes in older patients with AML. *Blood*. 2022;139(24):3546-3557.
8. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
9. Luo X, Feurstein S, Mohan S, et al. ClinGen Myeloid Malignancy Variant Curation Expert Panel recommendations for germline RUNX1 variants. *Blood Adv*. 2019;3(20):2962-2979.
10. Gibson CJ, Kim HT, Zhao L, et al. Donor clonal hematopoiesis and recipient outcomes after transplantation. *J Clin Oncol*. 2022;40(2):189-201.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Gertz et al, page 1208

Birtamimab: a new amyloidosis treatment?

Antoine Huart | University Hospital of Toulouse

In this issue of *Blood*, Gertz et al[1] report a post hoc analysis of patients with severe cardiac amyloid light chain (AL) amyloidosis treated in the VITAL trial, a phase 3 randomized, double-blind, placebo-controlled clinical trial assessing the efficacy and safety of standard of care (SOC) chemotherapy plus

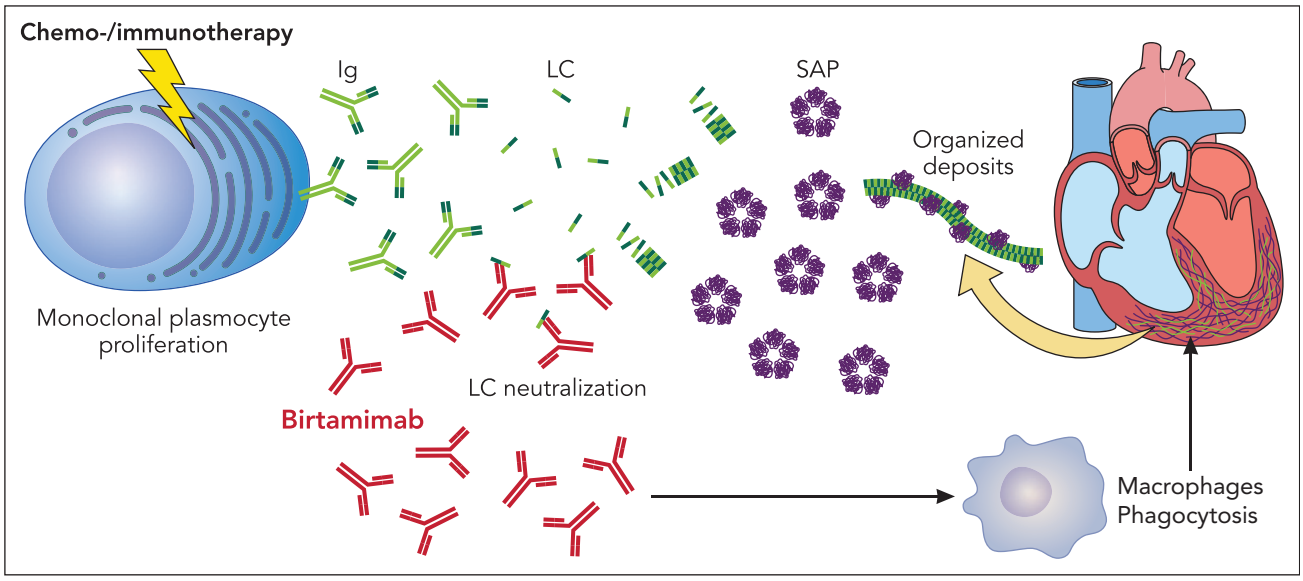
birtamimab, a humanized antibody designed to eliminate the toxic light chain (LC) oligomers and deposits (see figure). They found reduced all-cause mortality at 9 months for the subset of patients with Mayo stage IV cardiac AL amyloidosis receiving birtamimab compared with the placebo group.[1,2]

AL amyloidosis remains a very serious disease. Staging, especially of cardiac involvement through simple markers (troponin, proBNP), now identifies patients with a high risk of death. Mayo stage IIIB and IV patients have a median overall survival of only a few months.

There are 2 active areas of novel therapeutics for the management of the treatment of patients with amyloidosis. First, the efficacy of chemotherapy has improved. The randomized ANDROMEDA trial demonstrated that the addition of daratumumab, an anti-CD38 immunotherapeutic agent, to chemotherapy (bortezomib, cyclophosphamide, and dexamethasone) in patients with Mayo stage I to IIIA disease increased the hematological response rate (53.3% vs 18.1%) and improved the cardiac and renal response (decrease in proBNP and proteinuria) but without statistical significance on overall survival.[3] Kastritis et al tested daratumumab in monotherapy to stage IIIB patients and showed a doubling or more of overall survival compared with a historical control.[4] Thus, the mortality rate of stage IIIB/IV patients remains high, and there may be a concern that chemotherapy toxicity could actually contribute to the problem by destabilizing organ function. Second is the addition of therapeutics specifically to reduce the formation of deposits or to increase deposit clearance: The first trials date from 1995, when Gianni et al attempted to break down amyloid deposits by cyclin derivatives, without clear success.[5] Epigallocatechin-3-gallate, a green tea polyphenol, showed some efficacy in reducing the volume of the left ventricle by interfering with amyloid fibrillogenesis in patients with cardiopathy[6]; however, no additional studies were conducted. Recently, Wechalekar et al used a strategy against the serum amyloid P component (SAP). After depletion of serum SAP by miridesap, patients received dezamizumab, a monoclonal antibody against SAP. No improvement was found at 8 weeks of administration of the product, and adverse reactions like vasculitis were found.[7] NEOD001, or birtamimab, is a humanized monoclonal antibody directed against LC, designed to neutralize toxic soluble LC aggregates[8] and deplete insoluble organ-deposited amyloid via macrophage-induced phagocytosis.[9] It was tested in a phase I/II trial of 27 patients who had already received 1 or more lines of chemotherapy. The tolerance and safety of immunotherapy were excellent, and clinical improvements were noted.[10] The VITAL trial tested the addition of birtamimab to SOC bortezomib-based chemotherapy in a phase 3 randomized double-blind trial in 260 patients who were newly diagnosed. The unpublished trial was prematurely stopped at 9 months of treatment initiation after a futility analysis.

In this study, Gertz et al extracted data from the VITAL trial in a post hoc analysis of 77 stage IV patients (38 birtamimab/39 placebo) and found a difference in overall survival in favor of birtamimab. Admittedly, the statistical analysis must be viewed with great caution, as the VITAL trial was not designed for this analysis. The response to chemotherapy was identical, but disappointing, in both the birtamimab and placebo groups, with only 12 of 38 and 11 of 39 patients, respectively, achieving a hematological response better than or equal to a very good partial response at 3 months. This raises the question of the impact of earlier, more effective chemotherapy on the study outcome.

In the end, this post hoc analysis does suggest for the first time that there may be efficiency to an antideposit molecule. It therefore raises hope for the management of the most serious cases of AL amyloidosis. The small number of patients (77 patients) and the short duration of the study requires further validation for this indication. Indeed, the AFFIRM-AL study, a randomized phase 3 trial (2/1), will test birtamimab against placebo + SOC, including daratumumab, in 150 patients with stage IV amyloidosis and will hopefully prove informative. Finally, it should be noted that another trial (CAEL) is under development to test another immunotherapy antibody,



New therapeutic approaches in the treatment of amyloidosis. Ig, immunoglobulin. Professional illustration by Patrick Lane, ScEYEnce Studios.

11-1F4, directed against the amyloidogenic serum light chains in patients with stage IIIA and IIIB AL amyloidosis.

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

REFERENCES

1. Gertz MA, Cohen AD, Comenzo RL, et al. Birtamimab plus standard of care in light-chain amyloidosis: the phase 3 randomized placebo-controlled VITAL trial. *Blood*. 2023;142(14):1208-1218.
2. Kumar S, Dispenzieri A, Lacy MQ, et al. Revised prognostic staging system for light chain amyloidosis incorporating cardiac biomarkers and serum free light chain measurements. *J Clin Oncol*. 2012;30(9):989-995.
3. Kastritis E, Palladini G, Minnema MC, et al. Daratumumab-based treatment for immunoglobulin light-chain amyloidosis. *N Engl J Med*. 2021;385(1):46-58.
4. Kastritis E, Minnema MC, Dimopoulos MA, et al. P915: Efficacy and safety of daratumumab monotherapy in newly diagnosed patients with stage 3b light chain amyloidosis: a phase 2 study by the European Myeloma Network. *HemaSphere*. 2022;6(suppl):805-806.
5. Gianni L, Bellotti V, Gianni AM, Merlini G. New drug therapy of amyloidoses: resorption of AL type deposits with 4-iodo-4-deoxydoxorubicin. *Blood*. 1995;86(3):855-861.
6. Mereles D, Buss SJ, Hardt SE, Hunstein W, Katus HA. Effects of the main green tea polyphenol epigallocatechin-3-gallate on cardiac involvement in patients with AL amyloidosis. *Clin Res Cardiol*. 2010;99(8):483-490.
7. Wechalekar A, Antoni G, Al Azzam W, et al. Pharmacodynamic evaluation and safety assessment of treatment with antibodies to serum amyloid P component in patients with cardiac amyloidosis: an open-label Phase 2 study and an adjunctive immuno-PET imaging study. *BMC Cardiovasc Disord*. 2022;22(1):49.
8. Renz M, Torres R, Dolan PJ, et al. 2A4 binds soluble and insoluble light chain aggregates from AL amyloidosis patients and promotes clearance of amyloid deposits by phagocytosis (dagger). *Amyloid*. 2016;23(3):168-177.
9. Wall JS, Kennel SJ, Williams A, et al. AL amyloid imaging and therapy with a monoclonal antibody to a cryptic epitope on amyloid fibrils. *PLoS One*. 2012;7(12):e52686.
10. Gertz MA, Landau H, Comenzo RL, et al. First-in-human phase I/II study of NEOD001 in patients with light chain amyloidosis and persistent organ dysfunction. *J Clin Oncol*. 2016;34(10):1097-1103.

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PLATELETS AND THROMBOPOIESIS

Comment on Mobbs et al, page 1233

Pass the 12-LOX!

Enrico Di Cera | Saint Louis University School of Medicine

In this issue of *Blood*, Mobbs et al[1] report high-resolution structures of the enzyme 12S-lipoxygenase (12-LOX) obtained by cryogenic electron microscopy. The study elucidates the oligomeric states, conformational plasticity, and binding interactions of an intriguing enzyme involved in platelet activity and thrombosis. The new knowledge may boost the development of structure-based inhibitors.

The enzyme 12-LOX is expressed in platelets, where it promotes activation of αIIbβ3, glycoprotein VI, and protease activated receptor (PAR4), as well as in human islets, epidermal keratinocytes, and several tumor cell lines.[2] For this reason, 12-LOX constitutes an important molecular target for the treatment of thrombosis, heparin-induced thrombocytopenia (HIT), and platelet-mediated cancer progression. Many aspects of the function of 12-LOX in platelet biology and signaling remain incompletely understood. There is insufficient structural information and a lack of selective inhibitors to be used in animal models, where results from knockout mice have been conflicting.[3,4] As for other proteins relevant to hemostasis and thrombosis, advances in the structural biology of 12-LOX would afford a better understanding of function and eventually guide the development of selective inhibitors for therapeutic applications.

In this issue of *Blood*, Mobbs et al take advantage of the unique features of cryogenic electron microscopy (cryo-EM) in solving the structure of macromolecules under nativelike conditions. The study captures the molecular plasticity of 12-LOX for the first time from structures solved at very high resolution (1.7-2.8 Å) and from particles imaged from the same cryogenic grid. A snapshot of 12-LOX emerges with the protein distributed among different oligomeric forms and with the active site assuming "open" or "closed" conformations. The results are highly significant and will impact future functional and translational studies of this intriguing enzyme.

The structural organization of 12-LOX recapitulates the fold of typical LOX enzymes, with the active site housing a catalytic nonheme Fe^{2+} atom bound to 3 conserved His residues.[5] The entrance to the active site is lined by an arched helix and an α2 helix in an extended conformation. Protein from a "dimer peak" obtained from size exclusion chromatography produces multiple high-resolution cryo-EM structures of monomers, dimers, tetramers, and hexamers of 12-LOX from the same cryogenic grid. The dimer provides the functional unit of 12-LOX, with individual monomers assembled "head-to-toe" and stabilized by Van der Waals interactions and numerous H-bonds. The arrangement confirms the results of previous small-angle X-ray scattering (SAXS) analysis.[6] New for the cryo-EM study is the elucidation of higher oligomers of 12-LOX that show how the arrangement of the dimer is retained when tetramers and hexamers are assembled as dimers and trimers of dimers, respectively. The 2-ring arrangement of the hexamer creates a pore with a diameter of ~30 Å, which may have a physiological role and should stimulate further investigation. The hexamer is stabilized by a disulfide bond, unlike the dimer and tetramer, which suggests that formation of higher oligomers of 12-LOX may be sensitive to the oxidative environment of the cell. The different oligomeric states of 12-LOX likely regulate enzyme activity and interaction with membranes. The active site entrance and membrane-binding residues are positioned on the same surface in the 12-LOX dimer but are covered by dimer-dimer contacts in the tetramer and hexamer. Therefore, the dimer may be the only active oligomer of 12-LOX. Notably, the individual subunits