or refractory primary mediastinal large B-cell lymphoma compared with diffuse large B-cell lymphoma. *Leuk Lymphoma.* 2008;49(7):1329-1336.

8. Rieger M, Osterborg A, Pettengell R, et al; MabThera International Trial (MInT) Group. Primary mediastinal B-cell lymphoma treated with CHOP-like chemotherapy with or without rituximab: results of the Mabthera International Trial Group study. *Ann Oncol.* 2011;22(3):664-670.
9. Dunleavy K, Pittaluga S, Maeda LS, et al. Dose-adjusted EPOCH-rituximab therapy in primary mediastinal B-cell lymphoma. *N Engl J Med.* 2013;368(15):1408-1416.
10. Aoki T, Izutsu K, Suzuki R, et al. Novel prognostic model of primary mediastinal large B-cell lymphoma (PMBL): a multicenter cooperative retrospective study in Japan [abstract]. *Blood.* 2013;122(12):638. Abstract 638.
11. Rodríguez J, Conde E, Gutiérrez A, et al. Primary mediastinal large cell lymphoma (PMBL): frontline treatment with autologous stem cell transplantation (ASCT). The GEL-TAMO experience. *Hematol Oncol.* 2008;26(3):171-178.
12. Sehn LH, Antin JH, Shulman LN, et al. Primary diffuse large B-cell lymphoma of the mediastinum: outcome following high-dose chemotherapy and autologous hematopoietic cell transplantation. *Blood.* 1998;91(2):717-723.
13. Hamlin PA, Portlock CS, Straus DJ, et al. Primary mediastinal large B-cell lymphoma: optimal therapy and prognostic factor analysis in 141 consecutive patients treated at Memorial Sloan Kettering from 1980 to 1999. *Br J Haematol.* 2005;130(5):691-699.
14. Aoki T, Shimada K, Suzuki R, et al. High-dose chemotherapy followed by autologous stem cell transplantation for relapsed/refractory primary mediastinal large B-cell lymphoma. *Blood Cancer J.* 2015;5:e372.
15. Jacobsen ED, Sharman JP, Oki Y, et al. Brentuximab vedotin demonstrates objective responses in a phase 2 study of relapsed/refractory DLBCL with variable CD30 expression. *Blood.* 2015;125(9):1394-1402.
16. Cheson BD, Pfistner B, Juweid ME, et al; International Harmonization Project on Lymphoma. Revised response criteria for malignant lymphoma. *J Clin Oncol.* 2007;25(5):579-586.

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To the editor:

Sinusoidal obstruction syndrome following CD33-targeted therapy in acute myeloid leukemia

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In late December 2016, Seattle Genetics, Inc., announced that the US Food and Drug Administration (FDA) had placed a hold or partial hold on several early-stage clinical trials of vadastuximab talirine (SGN-CD33A) in acute myeloid leukemia (AML) to further evaluate the potential risk of hepatotoxicity with this CD33 antibody–drug conjugate (ADC) when administered before or after allogeneic hematopoietic cell transplantation (HCT).[1] These holds arose from the identification of 6 patients experiencing hepatotoxicity, including several cases of sinusoidal obstruction syndrome (SOS; formerly known as veno-occlusive disease), with 4 resulting deaths. Medical details of these cases have not been provided, and it was not indicated whether SOS was confirmed through liver biopsy and to what degree other causes of liver injury were excluded if the diagnosis was made based on a typical clinical presentation. SOS is a well-recognized and often life-threatening complication following HCT, particularly in individuals undergoing myeloablative conditioning, which damages sinusoidal endothelial cells.[2] Thus, the occurrence of posttransplant SOS after SGN-CD33A exposure does not establish a causal link with this ADC. Still, these recent adverse events involving the liver are reminiscent of the experience with the first-generation CD33-directed ADC, gemtuzumab ozogamicin (GO), when given at the initial dosing of 9 mg/m^2 per dose, and call into question the safety of antibody-based therapeutics targeting CD33, at least in some patients.

In GO, a humanized CD33 antibody is conjugated to a disulfide derivative of calicheamicin-γ_1 via a hydrolyzable linker.[3,4] Free and conjugated calicheamicin caused nonspecific liver toxicity in preclinical testing, and GO was found to be preferentially distributed to the liver.[4] An association between GO and SOS was noted early in the clinic, and a subsequent FDA-requested prospective observational registry showed an SOS rate of around 10%, with a substantial subset of the cases being fatal.[5,6] Histologically, GO-associated liver damage is characterized by sinusoidal injury with extensive sinusoidal fibrosis, stellate cell activation, centrilobular hemorrhage into the space of Disse, and zone 3 hepatocyte necrosis.[7] The exact repair mechanisms after GO-mediated sinusoidal injury are unknown, but a protracted process is indicated by the fact that the association with SOS was particularly strong when GO was administered within 3 to 4 months from allogeneic HCT.[5,8] In animal models of sinusoidal injury marked by loss of sinusoidal endothelial cells, restoration of these cells is dependent on the presence of marrow-derived progenitor cells.[9]

Proposed mechanisms through which GO could damage hepatic sinusoids include exposure to unconjugated calicheamicin circulating in the bloodstream, nonspecific uptake of the ADC by Kupffer cells and liver sinusoidal endothelial cells, or CD33-mediated uptake of GO by 1 or more of the cell populations in the liver that express CD33 (Kupffer cells, sinusoidal endothelial cells, stellate cells).[7,10] It is tempting to consider a CD33-specific mechanism as the primary cause; however, antigen-mediated ADC uptake cannot explain the reported association between SOS (seen primarily after HCT) and prior exposure to inotuzumab ozogamicin (IO), an ADC in which the calicheamicin derivative is linked to a humanized CD22 antibody.[11] The latter suggests the importance of nonspecific (target-independent) hepatic toxicity, with ADCs containing moieties toxic to sinusoidal endothelial cells.

Recent studies with a nonbinding antibody-calicheamicin conjugate containing the same linker payload as GO and IO in cynomolgus monkeys showed loss of sinusoidal endothelial cells early after drug exposure and development of changes consistent with early SOS at later points.[12] Clearance of antibody-bound tumor cells in the liver through antibody-dependent phagocytosis by Kupffer cells is well documented for several antibody-based therapeutics.[13,14] Thus, ADCs, bound or unbound to their target antigen, may be taken up by Kupffer cells via Fc receptors, resulting in liver-dominant off-target delivery of the antineoplastic molecule. In a similar fashion, sinusoidal endothelial cells avidly take up immunoglobulins through endocytic pathways.[15-17] The proposed mechanisms underlying the development of SOS are

not mutually exclusive, and understanding the contributions of the upstream pathophysiologic processes, which may vary between individual CD33-targeted therapeutics and/or the antibody dose or dosing schedule used (eg, because CD33 target saturation is reached or not), will be of great clinical importance. Notably, if liver damage results predominantly from a target-independent mechanism, antibody-based therapeutics lacking an Fc domain such as fragment bispecific antibodies and chimeric antigen receptor (CAR)-modified T cells would have relatively little risk of SOS. On the other hand, if CD33-specific mechanisms were dominantly involved, the potential for SOS may increase with the potency of the individual therapeutics. Whatever the mechanism of damage to sinusoidal endothelial cells, the result is loss of signaling from sinusoidal endothelial cells to stellate cells, leading to stellate cell activation and filling of sinusoids with collagens.[18]

In SGN-CD33A, a humanized CD33 antibody is conjugated to a synthetic pyrrolobenzodiazepine (PBD) dimer via a protease-cleavable linker.[19] Sinusoidal liver toxicity of pyrrolizidine alkaloids has been known for decades,[20] but how this might relate to the pyrrole moiety in the PBD dimer and the possibility of SOS with PBD dimer-containing ADCs is unknown. Still, the apparent association between SGN-CD33A and SOS, be it through CD33- or Fc-mediated ADC uptake, suggests that the potential for this type of liver injury may extend beyond CD33-targeting with GO and other calicheamicin-based ADCs. Of particular interest may be IMGN779, a novel CD33 ADC in early clinical development that is using an indolino-benzodiazepine dimer as toxic payload.[21] To what degree other classes of CD33 antibody-based therapeutics are associated with sinusoidal injury is unclear. In the setting of allogeneic transplantation, SOS has been noted when radiolabeled CD33 antibodies were given to intensify conditioning regimens.[22]

On the other hand, clinically apparent SOS was not observed in randomized trials with lintuzumab (HuM195, SGN-CD33), an unconjugated CD33 antibody,[23,24] indicating that CD33 engagement with an antibody alone is not sufficient to cause signs and symptoms of SOS. However, because of the limited cytotoxic activity of lintuzumab against $CD33^+$ cells, the clinical experience with this monoclonal antibody does not convincingly argue against a direct role of CD33 in the pathogenesis of SOS. With new, highly potent CD33-targeted drugs, including second-generation ADCs, bispecific antibodies, and CAR-modified T cells, entering clinical testing, classes of therapeutics are now becoming available with which there is potential for significant damage to CD33-expressing cells in the liver. This damage could be exacerbated by myeloablative conditioning regimens given to patients in whom sinusoidal endothelial cells are already injured, with this concern being particularly relevant because some of these agents (eg, CAR T cells) may require allogeneic hematopoietic cell rescue to abrogate the duration of drug-induced cytopenias.

The experience with GO and SGN-CD33A should be a reminder to carefully monitor patients for signs and symptoms indicative of SOS. Because many factors can increase the risk of fatal SOS caused by conditioning regimens in the HCT setting, well-controlled, ideally randomized, studies will be necessary to fully understand the role of exposure to CD33-targeted therapeutics as an additional risk factor. Animal models have documented that sinusoidal endothelial cells can be protected from toxic injury by increasing their content of reduced glutathione or by matrix metalloproteinase inhibitors,[25] but these strategies have not yet been tested in man. For GO, some clinical data have indicated that defibrotide has a limited role for either prophylaxis or treatment of SOS.[3] However, the value of this drug in general is uncertain, especially since a recent randomized trial in children undergoing allogeneic HCT showed that defibrotide had no impact on survival.[26]

If the association between SOS and CD33-targeted drugs other than GO is substantiated, development of methods to prevent sinusoidal injury from CD33-targeted therapeutics, particularly in a primate model that expresses human CD33 in $CD33^+$ cells of the liver,[12] may become essential as an important strategy to improve the safety profile of the new generation of CD33 antibody-based drugs. Animal models and clinical epidemiology of cofactors associated with SOS in human trials may point the way to prevention strategies. Data from clinical trials with the new CD33-targeted agents—whether given alone, in combination, or in proximity to allogeneic HCT—will be hypothesis-generating but may not be definitive in view of uncontrolled variables (eg, baseline liver disease, concomitant liver-toxic medications, variable pharmacokinetics of drugs used in conditioning regimens, sepsis, endotoxemia). Utilization of a primate model of sinusoidal injury should yield carefully controlled studies that provide greater insight than clinical experience can. There is much to be learned regarding the biology of SOS and CD33, and those with interest in targeted therapy of AML will watch closely to see to what degree SOS will be dose-limiting for the use of SGN-CD33A, other potent CD33 antibody-based drugs, and possibly other antibody-toxin conjugates.

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References

1. Seattle Genetics announces clinical hold on several phase 1 trials of vadastuximab talirine (SGN-CD33A). http://www.businesswire.com/news/home/20161227005087/en/. Accessed 12 January 2017.
2. Mohty M, Malard F, Abecassis M, et al. Sinusoidal obstruction syndrome/veno-occlusive disease: current situation and perspectives-a position statement from the European Society for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant*. 2015;50(6):781-789.
3. Cowan AJ, Laszlo GS, Estey EH, Walter RB. Antibody-based therapy of acute myeloid leukemia with gemtuzumab ozogamicin. *Front Biosci (Landmark Ed)*. 2013;18:1311-1334.
4. Bross PF, Beitz J, Chen G, et al. Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. *Clin Cancer Res*. 2001;7(6):1490-1496.
5. McKoy JM, Angelotta C, Bennett CL, et al. Gemtuzumab ozogamicin-associated sinusoidal obstructive syndrome (SOS): an overview from the research on adverse drug events and reports (RADAR) project. *Leuk Res*. 2007;31(5):599-604.
6. Tallman MS, McDonald GB, DeLeve LD, et al. Incidence of sinusoidal obstruction syndrome following Mylotarg (gemtuzumab ozogamicin): a prospective observational study of 482 patients in routine clinical practice. *Int J Hematol*. 2013;97(4):456-464.
7. Rajvanshi P, Shulman HM, Sievers EL, McDonald GB. Hepatic sinusoidal obstruction after gemtuzumab ozogamicin (Mylotarg) therapy. *Blood*. 2002;99(7):2310-2314.
8. Wadleigh M, Richardson PG, Zahrieh D, et al. Prior gemtuzumab ozogamicin exposure significantly increases the risk of veno-occlusive disease in patients who undergo myeloablative allogeneic stem cell transplantation. *Blood*. 2003;102(5):1578-1582.

9. Wang L, Wang X, Xie G, Wang L, Hill CK, DeLeve LD. Liver sinusoidal endothelial cell progenitor cells promote liver regeneration in rats. *J Clin Invest*. 2012;122(4):1567-1573.

10. Harb R, Xie G, Lutzko C, et al. Bone marrow progenitor cells repair rat hepatic sinusoidal endothelial cells after liver injury. *Gastroenterology*. 2009;137(2): 704-712.

11. Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. *N Engl J Med*. 2016;375(8): 740-753.

12. Guffroy M, Falahatpisheh H, Biddle K, et al. Liver microvascular injury and thrombocytopenia of antibody-calicheamicin conjugates in cynomolgus monkeys—mechanism and monitoring [published online ahead of print 28 September 2016]. *Clin Cancer Res*.

13. van der Bij GJ, Bögels M, Otten MA, et al. Experimentally induced liver metastases from colorectal cancer can be prevented by mononuclear phagocyte-mediated monoclonal antibody therapy. *J Hepatol*. 2010;53(4): 677-685.

14. Montalvao F, Garcia Z, Celli S, et al. The mechanism of anti-CD20-mediated B cell depletion revealed by intravital imaging. *J Clin Invest*. 2013;123(12): 5098-5103.

15. Mousavi SA, Sporstøl M, Fladeby C, Kjeken R, Barois N, Berg T. Receptor-mediated endocytosis of immune complexes in rat liver sinusoidal endothelial cells is mediated by FcgammaRIIb2. *Hepatology*. 2007;46(3):871-884.

16. Ganesan LP, Kim J, Wu Y, et al. FcγRIIb on liver sinusoidal endothelium clears small immune complexes. *J Immunol*. 2012;189(10):4981-4988.

17. Sørensen KK, McCourt P, Berg T, et al. The scavenger endothelial cell: a new player in homeostasis and immunity. *Am J Physiol Regul Integr Comp Physiol*. 2012;303(12):R1217-R1230.

18. DeLeve LD. Liver sinusoidal endothelial cells in hepatic fibrosis. *Hepatology*. 2015;61(5):1740-1746.

19. Kung Sutherland MS, Walter RB, Jeffrey SC, et al. SGN-CD33A: a novel CD33-targeting antibody-drug conjugate using a pyrrolobenzodiazepine dimer is active in models of drug-resistant AML. *Blood*. 2013;122(8):1455-1463.

20. Neuman MG, Cohen L, Opris M, Nanau RM, Hyunjin J. Hepatotoxicity of pyrrolizidine alkaloids. *J Pharm Pharm Sci*. 2015;18(4):825-843.

21. Krystal WM, Walker R, Fishkin N., et al. IMGN779, a CD33-targeted antibody-drug conjugate (ADC) with a novel DNA-alkylating effector molecule, induces DNA damage, cell cycle arrest, and apoptosis in AML cells [abstract]. *Blood*. 2015;126(23). Abstract 1366.

22. Burke JM, Caron PC, Papadopoulos EB, et al. Cytoreduction with iodine-131-anti-CD33 antibodies before bone marrow transplantation for advanced myeloid leukemias. *Bone Marrow Transplant*. 2003;32(6):549-556.

23. Feldman EJ, Brandwein J, Stone R, et al. Phase III randomized multicenter study of a humanized anti-CD33 monoclonal antibody, lintuzumab, in combination with chemotherapy, versus chemotherapy alone in patients with refractory or first-relapsed acute myeloid leukemia. *J Clin Oncol*. 2005;23(18):4110-4116.

24. Sekeres MA, Lancet JE, Wood BL, et al. Randomized phase IIb study of low-dose cytarabine and lintuzumab versus low-dose cytarabine and placebo in older adults with untreated acute myeloid leukemia. *Haematologica*. 2013; 98(1):119-128.

25. Fan CQ, Crawford JM. Sinusoidal obstruction syndrome (hepatic veno-occlusive disease). *J Clin Exp Hepatol*. 2014;4(4):332-346.

26. Corbacioglu S, Cesaro S, Faraci M, et al. Defibrotide for prophylaxis of hepatic veno-occlusive disease in paediatric haemopoietic stem-cell transplantation: an open-label, phase 3, randomised controlled trial. *Lancet*. 2012;379(9823): 1301-1309.

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