regimens may open avenues for overcoming resistance to mogamulizumab.

*Conflict-of-interest disclosure: C.Q. served as a member of an advisory board or steering committee for Kyowa Kirin, Helsinn, and Mallinckrodt.* ■

## REFERENCES


1. Beygi S, Duran GE, Fernandez-Pol S, Rook AH, Kim YH, Khodadoust MS. Resistance to mogamulizumab is associated with loss of CCR4 in cutaneous T-cell lymphoma. *Blood*. 2022;139(26):3732-3736.
2. Yoshie O, Matsushima K. CCR4 and its ligands: from bench to bedside. *Int Immunol*. 2015;27(1):11-20.
3. Agar NS, Wedgeworth E, Crichton S, et al. Survival outcomes and prognostic factors in mycosis fungoides/Sézary syndrome: validation of the revised International Society for Cutaneous Lymphomas/European Organisation for Research and Treatment of Cancer staging proposal. *J Clin Oncol*. 2010;28(31):4730-4739.
4. Dobos G, Pohrt A, Ram-Wolff C, et al. Epidemiology of cutaneous T-cell lymphomas: a systematic review and meta-analysis of 16,953 patients. *Cancers (Basel)*. 2020;12(10):2921.
5. Ferenczi K, Fuhlbrigge RC, Pinkus J, Pinkus GS, Kupper TS. Increased CCR4 expression in cutaneous T cell lymphoma. *J Invest Dermatol*. 2002;119(6):1405-1410.
6. Kim YH, Bagot M, Pinter-Brown L, et al; MAVORIC Investigators. Mogamulizumab versus vorinostat in previously treated cutaneous T-cell lymphoma (MAVORIC): an international, open-label, randomised, controlled phase 3 trial. *Lancet Oncol*. 2018; 19(9):1192-1204.
7. Wang L, Ni X, Covington KR, et al. Genomic profiling of Sézary syndrome identifies alterations of key T cell signaling and differentiation genes. *Nat Genet*. 2015; 47(12):1426-1434.
8. Sugiyama D, Nishikawa H, Maeda Y, et al. Anti-CCR4 mAb selectively depletes effector-type FoxP3$^+$CD4$^+$ regulatory T cells, evoking antitumor immune responses in humans. *Proc Natl Acad Sci USA*. 2013; 110(44):17945-17950.
9. Ni X, Langridge T, Duvic M. Depletion of regulatory T cells by targeting CC chemokine receptor type 4 with mogamulizumab. *OncoImmunology*. 2015;4(7):e1011524.
10. de Masson A, Darbord D, Dobos G, et al. Macrophage-derived CXCL9 and CXCL11, T-cell skin homing, and disease control in mogamulizumab-treated CTCL patients. *Blood*. 2022;139(12):1820-1832.


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**MYELOID NEOPLASIA**

*Comment on Lewis et al, page 3737*

# Ceramide: improving Bcl-2 inhibitor therapy

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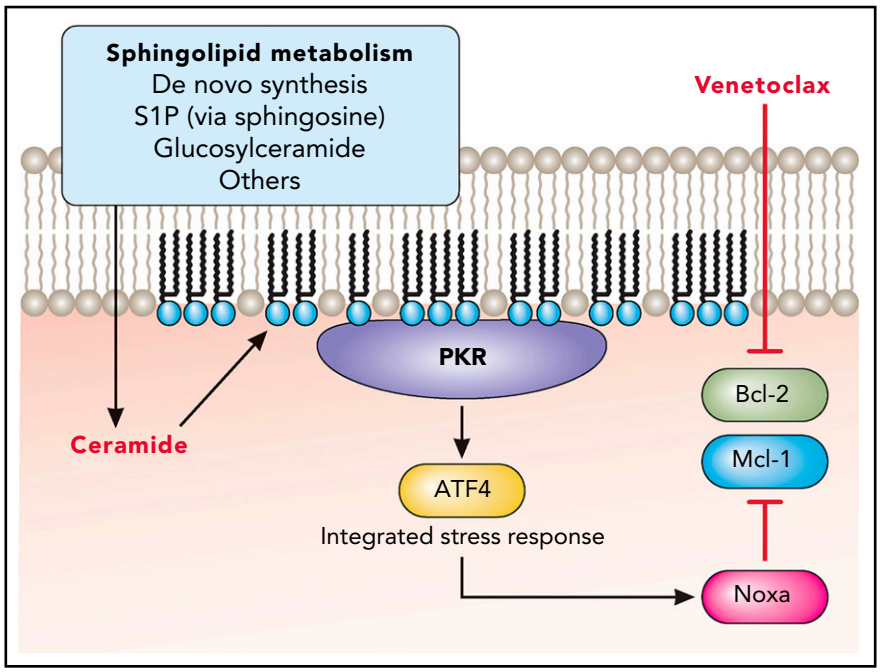
**In this issue of *Blood*, Lewis et al[1] highlight the importance of sphingolipid-regulating therapeutics by reporting a ceramide-mediated mechanism down-regulating myeloid cell leukemia 1 (Mcl-1) to restore venetoclax sensitivity of acute myeloid leukemia (AML).**

Here, Lewis et al build upon their earlier work demonstrating anti-AML efficacy for the sphingosine kinase inhibitor MP-A08.[2] The prior study showed that MP-A08 could regulate Mcl-1. However, the mechanism remained elusive as well as whether anti-AML efficacy was due to the lack of sphingosine-1-phosphate or alternatively to the accumulation of ceramide. In the present study, Lewis et al find that ceramide specifically generated in response to sphingosine kinase inhibition binds and activates protein kinase R (see figure). The integrated stress response is subsequently stimulated through activation of the transcription factor 4 (ATF4), and this mediates the production of Noxa, an endogenous inhibitor of Mcl-1. Importantly, Lewis et al validate this ceramide-dependent effect using MP-A08 as well as an acid ceramidase inhibitor. Therefore, a major implication of this study is that the accumulation of ceramide is essential to initiate an anti-AML mechanism downregulating Mcl-1. Moreover, this highlights the utility of ceramide-elevating therapeutics as a potentially major advance in the treatment of AML and related hematologic disorders.

Sphingolipids are a major class of lipid that plays important roles as regulators of cell fate and function in addition to being key membrane components.[3] The bioactive properties of sphingolipids are arguably some of their more interesting features, especially when considering the clinical relevance of sphingolipid-regulating therapeutics. Ceramide represents a major subtype of sphingolipid that is classically associated as a regulator of cell death and stress.[3] It can be generated through a de novo synthetic pathway or through the catabolism of many other sphingolipids. Ceramide also serves as a precursor to many of these other sphingolipids. Therefore, sphingolipid metabolism can be understood through a ceramide-centric perspective. Moreover, therapeutics that augment cellular ceramide levels may do so either by promoting ceramide generation, blocking its metabolism, or by delivery of exogenous ceramide.

Sphingolipid-regulating therapeutics, or more specifically ceramide-elevating therapeutics, are poised as potentially important anticancer agents. Fundamentally, this is because elevations in ceramide are associated with cancer cell death.[3] Several ceramide-elevating strategies have recently been reported to exert anti-AML efficacy. These include inhibitors of sphingosine kinases,[1,2] acid ceramidase,[4] as well as glucosylceramide synthase.[5] In addition, delivery of ceramide has been evaluated using a water-soluble ceramide analog,[6] as well as a nanoliposomal ceramide formulation.[7] Intriguingly, nanoliposomal ceramide exerted potent efficacy toward AML arising out of myelodysplastic syndrome. It is important to note that although dysfunctional sphingolipid metabolism may be a unifying theme across many subtypes of AML, it is not the case that all ceramide metabolic outlets are upregulated in each AML case.[7] For that reason, eventual clinical use of ceramide-elevating therapeutics may be situation-dependent and should be informed and adjusted by diagnostics that can follow these sphingolipid pathways.

Overcoming therapeutic resistance is another very important theme of Lewis et al. Venetoclax is a targeted therapeutic in the BH3 mimetic class, which targets the antiapoptotic B-cell lymphoma 2 (Bcl-2) protein.[8] It is used in the treatment of various hematologic malignancies, including AML, in combination with




Ceramide-elevating therapy promotes protein kinase R (PKR) activation through direct binding to ceramide. This promotes the integrated stress response through phosphorylation of eukaryotic initiation factor 2 alpha, which activates the transcription factor ATF4. Subsequent expression of Noxa blocks antiapoptotic Mcl-1 to overcome resistance to the Bcl-2 inhibitor venetoclax. Professional illustration by Patrick Lane, ScEYEnce Studios.

low-dose cytarabine. Resistance to venetoclax, or Bcl-2 inhibition, is often due to upregulation of other antiapoptotic Bcl-2 family members.[8] This includes Mcl-1, which has been the subject of additional drug discovery efforts. However, there may be limitations to the use of specific Mcl-1 inhibitors. The theoretical use of these inhibitors may be limited to short durations to avoid toxicity associated with the disruption of normal physiological roles for Mcl-1. There are also other pathways of resistance to Bcl-2 inhibition aside from upregulation of Mcl-1. This includes upregulation of mitogenic signaling pathways,[8] many which are known to be regulated by ceramide, including the Akt and MEK/Erk pathways.[3] Overall, Lewis et al's characterization of Mcl-1 downregulation by ceramide contributes profoundly to our understanding of the oncogenic pathways effected by ceramide. Therefore, ceramide-elevating therapies may hold more promise, as they can broadly target and limit multiple pathways of Bcl-2 inhibitor resistance.

As intriguing as their other findings are, the recent findings of Lewis et al aided in identifying the integrated stress response as a key pathway linking ceramide to regulation of Mcl-1. This is noteworthy because it may represent a unique vulnerability in cells of hematopoietic origin. Specifically, the ATF4-mediated integrated stress response was previously described as an attribute of hematopoietic stem cells, including AML stem cells.[9] This means that the ability of ceramide-elevating therapeutics to downregulate Mcl-1 may be possible owing to the prevalence of this integrated stress response in AML stem cells. Furthermore, the ability to impact the AML stem cell population means that ceramide-elevating therapeutics may be advantageously positioned to eradicate this key cellular population. Lewis et al showed that this leukemia-initiating population was disrupted by treatment with MP-A08, which was an effect also observed with other ceramide-elevating therapeutics.[7]

There are potential limitations to the utility of ceramide-elevating therapeutics. The first potential limitation is adaptive resistance owing to changes in ceramide metabolism, or more specifically, owing to upregulation of alternative ceramide metabolic outlets.[3,7] To manage this, it is important to understand how the molecular evolution in AML can manifest in changes to ceramide metabolism. This may help identify links between AML molecular subtypes and ceramide metabolic pathways, which could aid the clinical selection of specific ceramide-elevating therapeutics. Moreover, therapeutics simultaneously targeting multiple ceramide metabolic outlets may be useful to avoid adaptive resistance.[7] Last, it is imperative to define potentially harmful effects of ceramide-elevating therapeutics. A recent study linking disruption of sphingomyelin synthesis to the development of thrombocytopenia is noteworthy.[10] Specifically, it was observed that the lack of sphingomyelin, and not an elevation in ceramide, promoted thrombocytopenia. That effect was uncovered using transgenic knockout mice but could extrapolate to potential sphingomyelin synthase inhibitors, which also are ceramide-elevating therapeutics.

Overall, the study by Lewis et al has advanced our understanding of ceramide-elevating AML therapy by defining a mechanism downregulating Mcl-1 to overcome Bcl-2 inhibitor resistance. Ultimately, this rationalizes improvement to standard care AML therapy with agents such as venetoclax through combination with ceramide-elevating therapeutics.

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

## REFERENCES


1. Lewis AC, Pope VS, Tea MN, et al. Ceramide-induced integrated stress response overcomes Bcl-2 inhibitor resistance in acute myeloid leukemia. *Blood.* 2022;139(26):3737-3751.
2. Powell JA, Lewis AC, Zhu W, et al. Targeting sphingosine kinase 1 induces MCL1-dependent cell death in acute myeloid leukemia. *Blood.* 2017;129(6):771-782.
3. Morad SA, Cabot MC. Ceramide-orchestrated signalling in cancer cells. *Nat Rev Cancer.* 2013;13(1):51-65.
4. Tan SF, Liu X, Fox TE, et al. Acid ceramidase is upregulated in AML and represents a novel therapeutic target. *Oncotarget.* 2016;7(50):83208-83222.
5. Morad SA, Ryan TE, Neufer PD, et al. Ceramide-tamoxifen regimen targets bioenergetic elements in acute myelogenous leukemia. *J Lipid Res.* 2016; 57(7):1231-1242.
6. Dany M, Gencer S, Nganga R, et al. Targeting FLT3-ITD signaling mediates ceramide-dependent mitophagy and attenuates drug resistance in AML. *Blood.* 2016; 128(15):1944-1958.
7. Barth BM, Wang W, Toran PT, et al. Sphingolipid metabolism determines the therapeutic efficacy of nanoliposomal ceramide in acute myeloid leukemia. *Blood Adv.* 2019;3(17):2598-2603.

8. Roberts AW, Wei AH, Huang DCS. BCL2 and MCL1 inhibitors for hematologic malignancies. *Blood*. 2021;138(13): 1120-1136.

9. van Galen P, Mbong N, Kreso A, et al. Integrated stress response activity marks stem cells in normal hematopoiesis and leukemia. *Cell Rep*. 2018;25(5): 1109-1117.e5.

10. Fujii Y, Taniguchi M, Nagaya S, et al. A novel mechanism of thrombocytopenia by PS exposure through TMEM16F in sphingomyelin synthase 1 deficiency. *Blood Adv*. 2021;5(20):4265-4277.

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RED CELLS, IRON, AND ERYTHROPOIESIS

*Comment on Walter et al, page 3771*

# Pain without gain: steroids and sickle crisis

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**In this issue of *Blood*, Walter et al[1] report on the association of corticosteroid exposure and subsequent hospitalization for vaso-occlusive episodes (VOEs) in people with sickle cell disease (SCD).**

In theory, corticosteroids are an appealing treatment for severe SCD complications because they are inexpensive, are readily available, and have strong anti-inflammatory effects. However, evidence suggests that, in practice, the benefits may not outweigh the risks. Limited evidence highlights the concern: a randomized controlled trial of dexamethasone for acute chest syndrome in children with SCD reduced the need for blood transfusions, the duration of fever, and the need for oxygen and analgesic therapy.[2] However, rehospitalization occurred within 72 hours in 27% of children treated with dexamethasone compared with only 4.7% of children treated with placebo. A study of methylprednisolone to treat VOEs in children reported similar outcomes.[3] Subsequent retrospective studies and case series report an association of corticosteroid exposure with VOEs and even stroke.[4,5] A recent meta-analysis by Lopinto et al[6] that estimated the effect of corticosteroids on the clinical course of VOEs or acute chest syndrome included 6 studies (3 randomized controlled studies and 3 retrospective studies) and concluded that compared with standard treatment, corticosteroids were associated with an increased risk of hospital readmission.

Corticosteroid exposure for individuals with SCD is not limited to treating SCD complications. Corticosteroids are prescribed for myriad acute conditions ranging from bee stings and asthma to optic neuritis. Periprocedural exposure in which dexamethasone may be used as an antiemetic also occurs.[7] Prescribing physicians may be aware of the many established adverse effects of steroids such as immunosuppression, leukocytosis, hypertension, fluid retention, osteonecrosis, sleep disruption, and psychosis, but they often lack knowledge specific to SCD about the potential risks with steroid treatment for this patient population. In our experience, well-meaning clinicians prescribe corticosteroids for seemingly reasonable indications. This comes to our attention when those treatments precipitate what are often very severe VOEs. Neither the clinician nor the patient are aware of the risks associated with corticosteroid exposure in this population, likely because of the limited literature describing this phenomenon.

In this setting, Walter et al applied a rigorous, somewhat novel method to analyze real-world data about corticosteroid exposure and association with hospitalization for VOEs. The authors use a case-case time control design in a nationwide population-based cohort from the French national health insurance database. To adjust for temporal variations in the exposure, the authors used an adjusted control cohort in which the probability of exposure was similar to that for patients. They matched this cohort of future patients by using demographic characteristics, which resulted in 5151 patients for analysis. They excluded exposure to topical or inhaled corticosteroids and examined prescriptions of systemic corticosteroids as a proxy for patient exposure. During the study period, a remarkable 45% of patients were exposed at least once to systemic corticosteroids. The median time between dispensing of corticosteroids and hospital admission was 5 days. Corticosteroid exposure was associated with an increased risk of hospitalization for VOEs (adjusted odds ratio, 2.6; 95% confidence interval, 1.1-6.4), this risk of admission was lower in those prescribed hydroxyurea, in men, and in children.

The mechanistic explanation for corticosteroid association with VOEs is not well defined, but likely involves the interaction of established corticosteroid mechanisms with SCD inflammatory, hemolytic, and vasculopathic mechanisms.

Walter et al add important data about the association of corticosteroid use with subsequent VOEs. In so doing, they have contributed meaningfully to a growing body of evidence that suggests extreme caution is warranted when prescribing systemic corticosteroids to individuals with SCD. For circumstances in which corticosteroids are absolutely indicated, involving a provider with SCD expertise is essential. This study highlights several directions for future research. More data are needed to define whether causal and potentially targetable mechanisms explain the association described, to understand the sex difference identified here, and to define the use of prophylactic transfusion to mitigate corticosteroid-associated VOEs.[8] Transfusion may be valuable when there are no reasonable alternatives to corticosteroids.

Attending to corticosteroid use in individuals with SCD is tied to larger trends in corticosteroid prescribing. Dexamethasone is emerging as a treatment standard for patients with hypoxia associated with COVID infection. This reality highlights the pressing need for more information about corticosteroid treatment in individuals with SCD during acute illness. In addition, there is growing recognition that corticosteroids are overprescribed. A striking percentage of study patients were exposed to systemic corticosteroids in this study. In the United States, a study of 1.5 million adults enrolled in a health insurance plan for 2 years found that 21% of patients were prescribed a short course