shapes the chromatin environment for other transcription factors to bind and intensify oncogene expression. Unfortunately, the lack of a high-quality Tcf1 antibody for chromatin immunoprecipitation sequencing makes it difficult to separate direct vs indirect effects as downstream targets of Tcf1, like Gata3, have chromatin remodeling activity.[8] It also remains unclear whether transforming cells stimulated by weaker Notch signals, such as through Notch ligand-receptor interactions, access oncogenic regulatory regions through similar mechanisms. Given its importance as a primary regulator of chromatin accessibility in early T cells,[5] Tcf1 likely plays a role even in such cases. It is also unclear whether Tcf1 maintains chromatin architecture in established T-ALL or becomes superseded by other factors like Gata3.[8] Other groups showed that Notch signaling itself has limited activity in this regard in cancer.[9,10] Finally, it is unclear how Notch1/Tcf1 mechanisms at native cis-elements interact with a dysregulated sea of aberrancies in transcriptional regulators, DNA methylation, and mutations in promoters, enhancers, and insulators during human T-ALL initiation and maintenance. Future studies will be needed.

In an impressively ambitious and resource-heavy undertaking, Antoszewski et al rewards us with a genome-scale understanding of how Notch and Tcf1 transform chromatin into a nurturing environment for the development of T-cell leukemia. We have learned that Notch signaling directs Tcf1 to evict nucleosomes from repressed chromatin to recruit oncogenic transcription factors that fire up enhancers important for T-cell commitment and expansion (see figure). We have even seen that a previously unrecognized *MYC* enhancer (TMe) can separate oncogenic from physiological Notch functions, which might be key to targeting Notch without intolerable side effects. By dissecting the interactions at these oncogenic enhancers, we might find safe ways to quench their activities and reprogram malignant T cells back to their normal selves.

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

REFERENCES

1. Antoszewski M, Fournier N, Ruiz Buendía GA, et al. Tcf1 is essential for initiation of oncogenic Notch1-driven chromatin topology in T-ALL. *Blood.* 2022;139(16):2483-2498.
2. Radtke F, Wilson A, Stark G, et al. Deficient T cell fate specification in mice with an induced inactivation of Notch1. *Immunity.* 1999;10(5):547-558.
3. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science.* 2004;306(5694):269-271.
4. McCarter AC, Wang Q, Chiang M. Notch in leukemia. *Adv Exp Med Biol.* 2018;1066:355-394.
5. Johnson JL, Georgakilas G, Petrovic J, et al. Lineage-determining transcription factor TCF-1 initiates the epigenetic identity of T cells. *Immunity.* 2018;48(2):243-257.e10.
6. Weber BN, Chi AW, Chavez A, et al. A critical role for TCF-1 in T-lineage specification and differentiation. *Nature.* 2011;476(7358):63-68.
7. Germar K, Dose M, Konstantinou T, et al. T-cell factor 1 is a gatekeeper for T-cell specification in response to Notch signaling. *Proc Natl Acad Sci USA.* 2011;108(50):20060-20065.
8. Belver L, Yang AY, Albero R, et al. GATA3-controlled nucleosome eviction drives *MYC* enhancer activity in T-cell development and leukemia. *Cancer Discov.* 2019;9(12):1774-1791.
9. Kloetgen A, Thandapani P, Ntziachristos P, et al. Three-dimensional chromatin landscapes in T cell acute lymphoblastic leukemia. *Nat Genet.* 2020;52(4):388-400.
10. Petrovic J, Zhou Y, Fasolino M, et al. Oncogenic Notch promotes long-range regulatory interactions within hyperconnected 3d cliques. *Mol Cell.* 2019;73(6):1174-1190.e12.

DOI 10.1182/blood.2021015364

LYMPHOID NEOPLASIA

Comment on Wilson et al, page 2499

CNS prophylaxis in DLBCL: first do no harm

Craig A. Portell | University of Virginia

In this issue of *Blood*, Wilson et al[1] present a retrospective analysis of the timing of intravenous high-dose methotrexate (HD-MTX) during first-line treatment of diffuse large B-cell lymphoma (DLBCL). They found that intravenous HD-MTX given at the end of treatment (EOT) was equally effective for central nervous system (CNS) prophylaxis and less toxic than MTX given earlier (ie, between cycles of anthracycline-based immunochemotherapy).

Secondary progression of DLBCL into the CNS after initial treatment with standard anthracycline-based immunochemotherapy is a devastating event. Several groups have identified risk factors for secondary CNS progression and have developed the CNS International Prognostic Index (CNS-IPI)[2] that identifies high-, intermediate-, and low-risk groups. Because the high-risk group has an ~10% risk of progression in the CNS at 2 years, strategies to prevent CNS progression have been explored.[3] The blood-brain barrier prevents many treatments from entering the CNS space, and MTX has long been used as a method for treating and preventing CNS progression. It has been speculated that CNS progression occurs early in the course of treatment, and thus, should be intercalated into standard immunochemotherapy.[3] However, guidance has largely come from expert opinion developed from subgroup analyses of prospective and retrospective studies. Rigorous evaluations focused on the utility of CNS prophylaxis are lacking.

Thus, Wilson et al performed a wide-ranging retrospective analysis of the timing of CNS prophylaxis with intravenous HD-MTX occurring during or after immunochemotherapy. Their study includes 1384 patients with DLBCL or high-grade B-cell lymphoma not otherwise specified who were diagnosed between 2007 and 2020 at 47 centers in Europe, Australia, and North America. It should be noted that roughly half the patients (46.1%) also received intrathecal MTX, with more patients in the group that received MTX after immunochemotherapy than in the group that received MTX during immunochemotherapy. Ultimately, there was no significant difference in CNS progression rates when HD-MTX was given

during or after immunochemotherapy; however, patients had more chemotherapy delays and increased toxicity when MTX was given during immunochemotherapy. Ultimately, the authors thoughtfully conclude that CNS prophylaxis based on HD-MTX is best performed at the end of primary immunochemotherapy (EOT), a clinically meaningful finding which will limit toxicity to our patients.

Although this study was not meant to evaluate the utility of CNS prophylaxis in general, Wilson et al did note a rate of CNS relapse in their patients similar to that of historical controls who had received limited CNS prophylaxis.[2] Other recently reported retrospective studies that were focused on other aspects of MTX-based CNS prophylaxis in DLBCL (intrathecal vs intravenous HD-MTX,[4] outcomes in older patients,[5] and single-institution analyses[6,7]) have also failed to show improvements compared with historical CNS relapse rates. These retrospective studies will likely be the highest level of data we will have. A prospective randomized study that evaluates the utility of MTX-based prophylaxis for CNS progression will need a very large numbers of participants to detect a small difference in CNS progression rates, and it may detract from other potential studies of emerging biologics-based targets.

The utility of CNS prophylaxis in DLBCL is increasingly questioned. The Wilson et al study, in the context of other recently presented studies, suggests that MTX, given intrathecally or intravenously or during or after immunochemotherapy, is not adequately preventing CNS progression. However, there is evidence that suggests that in the groups with highest risk, such as those with extranodal disease involving testes, kidneys, or adrenal glands, MTX may provide a level of protection.[8] Thus, it is my current practice to limit MTX prophylaxis to these situations. On the basis of the study by Wilson et al, intravenous HD-MTX (if used) should be given at the EOT for at least 2 cycles and likely reserved for fit patients. In addition, the priority for treatment should be on anthracycline-based immunochemotherapy and not on CNS prophylaxis. If CNS prophylaxis delays or otherwise interferes with curative-intent immunochemotherapy, it should be abandoned.

What does the future hold? The lymphoma field is hopeful that therapies directed at the biology of DLBCL will replace MTX-based strategies for CNS prophylaxis. Small-molecule inhibitors have a chance of reaching the CNS through the blood-brain barrier. Some that are already known to do so (ibrutinib[9] and lenalidomide[10]) have already been tested with rituximab, plus cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP). It is possible that these and other agents may be effective in preventing secondary CNS progression, particularly if therapy is chosen on the basis of predicted activity identified with new classification systems that are focused on DLBCL biology.[9] It is also possible that better, more effective therapies against systemic DLBCL will prevent secondary CNS progression. Finally, improvements in the detection of CNS involvement through DNA testing of cerebrospinal fluid, or better risk assessment with circulating tumor DNA could potentially define a group of patients with extremely high risk who warrant additional treatment.

Given the uncertainty around CNS prophylaxis, upcoming studies of first-line treatment for DLBCL should not mandate CNS prophylaxis, particularly if it is paired with novel agents known to cross the blood-brain barrier. CNS-specific progressions should be reported so that researchers can identify new ways of preventing devastating CNS relapses. With the currently available data, intravenous HD-MTX should be limited to the EOT and to patients with the highest risk.

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

REFERENCES

1. Wilson MR, Eyre TA, Kirkwood AA, et al. Timing of high-dose methotrexate CNS prophylaxis in DLBCL: a multicenter international analysis of 1384 patients. *Blood*. 2022;139(16):2499-2511.
2. Schmitz N, Zeynalova S, Nickelsen M, et al. CNS international prognostic index: a risk model for CNS relapse in patients with diffuse large B-cell lymphoma treated with R-CHOP. *J Clin Oncol*. 2016;34(26):3150-3156.
3. Abramson JS, Hellmann M, Barnes JA, et al. Intravenous methotrexate as central nervous system (CNS) prophylaxis is associated with a low risk of CNS recurrence in high-risk patients with diffuse large B-cell lymphoma. *Cancer*. 2010;116(18):4283-4290.
4. Orellana-Noia VM, Reed DR, McCook AA, et al. Single-route CNS prophylaxis for aggressive non-Hodgkin lymphomas: real-world outcomes from 21 US academic institutions. *Blood*. 2022;139(3):413-423.
5. Eyre TA, Kirkwood AA, Wolf J, et al. Stand-alone intrathecal central nervous system (CNS) prophylaxis provide unclear benefit in reducing CNS relapse risk in elderly DLBCL patients treated with R-CHOP and is associated increased infection-related toxicity. *Br J Haematol*. 2019;187(2):185-194.
6. Bobillo S, Joffe E, Sermer D, et al. Prophylaxis with intrathecal or high-dose methotrexate in diffuse large B-cell lymphoma and high risk of CNS relapse. *Blood Cancer J*. 2021;11(6):113.
7. Jeong H, Cho H, Kim H, et al. Efficacy and safety of prophylactic high-dose MTX in high-risk DLBCL: a treatment intent-based analysis. *Blood Adv*. 2021;5(8):2142-2152.
8. Conconi A, Chiappella A, Orsucci L, et al. Intensified (intravenous and intrathecal) CNS prophylaxis in primary testicular diffuse large B-cell lymphoma: 5-year results of the IELSG30 trial [abstract]. *Hematol Oncol*. 2021;39(S2):89-90. Abstract 048.
9. Wilson WH, Wright GW, Huang DW, et al. Effect of ibrutinib with R-CHOP chemotherapy in genetic subtypes of DLBCL. *Cancer Cell*. 2021;39(12):1643-1653.e3.
10. Ayed AO, Chiappella A, Pederson L, et al. CNS relapse in patients with DLBCL treated with lenalidomide plus R-CHOP (R2CHOP): analysis from two phase 2 studies. *Blood Cancer J*. 2018;8(7):63.

DOI 10.1182/blood.2022015432