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**LYMPHOID NEOPLASIA**

*Comment on Orellana-Noia et al, page 413*

# CNS prophylaxis in DLBCL: time to say goodbye?

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**In this issue of *Blood*, Orellana-Noia et al report that central nervous system (CNS) relapse rates following either intrathecal (IT) or systemic (IV) administration of methotrexate (MTX) are not significantly different, shedding doubt on the overall efficacy of both approaches.[1]**

For patients with diffuse large B-cell lymphoma (DLBCL), the overall incidence of relapse or progression in the CNS has been estimated at 5% with percentages varying from 1% to >15% in different risk groups. Current guidelines recommend CNS prophylaxis with IT or IV MTX for patients with high CNS–international prognostic index (IPI).[2] In a large retrospective study, Orellana-Noia et al now compare CNS relapses occurring after single-route IT or IV MTX. Do their findings suggest that both routes of administration are comparably effective, or ineffective? What are the clinical implications?

Because the overall incidence of CNS disease in DLBCL is ~5%, not all patients are deemed high-risk candidates requiring prophylaxis. The CNS-IPI identifies a high-risk group carrying a 10% rate of CNS disease.[3] Because this rate was not considered high enough to justify CNS prophylaxis in every high-risk patient, the search for alternative predictors continued. Indeed, more recently, combinations of CNS-IPI and molecular characteristics (activated B-cell subtype, double-hit lymphoma, distinct genetic signatures) were reported to increase the risk of CNS relapse.[4,5] Disappointingly, however, a very high-risk group unequivocally warranting aggressive CNS prophylaxis in any patient carrying these characteristics has not been identified so far.

As early as 2009, we and others started to report that IT MTX was not effective in preventing CNS relapse in the rituximab era.[6] Similarly, studies investigating the systemic administration of MTX also gave equivocal results. Thus, although studies on prophylaxis with IT or IV MTX became increasingly controversial, guidelines and most clinicians continue to recommend MTX for prophylaxis to prevent secondary CNS involvement (see table).

The study by Orellana-Noia et al adds to our doubts on the current practice of prophylactically administering IV or IT MTX to patients with DLBCL. The authors report that CNS relapse rates do not significantly differ between patients receiving IT or IV MTX (5.4% vs 6.8%, $P = .4$) and, importantly, differences in CNS relapse rates by route of administration failed to show significant differences also when patient groups were stratified by CNS-IPI, National Comprehensive Cancer Network–IPI, and double-hit status. Interpretation of the data and arriving at appropriate recommendations are not easy. One extreme would be to completely abandon CNS prophylaxis because increasing numbers of studies failed to demonstrate a benefit of MTX administration regardless of the route (see table). A more cautious approach would possibly return to IT MTX because, if not more effective, it is undoubtedly less toxic than IV MTX. Both conclusions seem premature because all studies have limitations (eg, patients in the current study receiving IT MTX were mostly treated with dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (DA-EPOCH-R) while patients given IV MTX mostly received rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). Although the key message remained unchanged after this imbalance had been taken care of by statistical modeling, the retrospective nature of this and other studies does not definitely exclude that the results were influenced by known and unknown confounding factors.

The comparison of CNS relapses occurring after CHOP vs cyclophosphamide, doxorubicin, vincristine, etoposide, and prednisone (CHOEP) or R-CHOP vs CHOP was an early demonstration that not only the prophylactic regimen but also first-line therapy may influence the incidence of CNS relapses.[7,8] We recently reported that rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone (R-ACVBP) including 4 IT injections of MTX followed by consolidative CNS prophylaxis with IV MTX, rituximab, ifosfamide, etoposide, and cytosine arabinoside resulted in very low CNS relapses. Patients with age-adjusted International Prognostic Index (aaIPI) 2 or 3 experienced a 3-year

**Major studies investigating the role of CNS prophylaxis in DLBCL**

| Study | Centers | Patients | First-line treatment and CNS prophylaxis | CNS relapse |
|---|---|---|---|---|
| Orellana-Noia et al, *Blood* 09/2021 | 21 US centers | n = 1130<br>Median age, 62 y<br>30% high CNS-IPI | R-CHOP/R-CHOP-like with IT MTX: n = 894<br>with HD MTX: n = 236 | 5.4% (IT MTX) vs 6.8% (HD MTX) ($P$ = .4) |
| Ong et al, *Blood Cancer Journal* 08/2021 | Oligocentric Singapore | n = 226<br>Median age, 65 y<br>85% high CNS-IPI | R-CHOP<br>with HD MTX: n = 66<br>w/o: n = 160 | 3-y risk (isolated CNS relapse): 3.1% (HD MTX) vs 14.6% (w/o) ($P$ = .032) |
| Bobillo et al, *Blood Cancer Journal* 06/2021 | Monocentric New York | n = 585<br>Median age, 68 y<br>68% high CNS-IPI | R-CHOP/R-CHOP-like with IT MTX: n = 253<br>with HD MTX: n = 42<br>w/o: n = 290 | 5-y risk: 5.5% (IT MTX), 5% (HD MTX), and 7.5% (w/o) ($P$ = .34) |
| Jeong et al, *Blood Advances* 04/2021 | Monocentric Seoul | n = 258<br>Median age, 62 y<br>49% high CNS-IPI | R-CHOP with HD MTX: n = 128<br>w/o: n = 130 | 2-y cumulative incidence: 12.4% (HD MTX) vs 13.9 (w/o) ($P$ = .96) |
| Puckrin et al, *American Journal of Hematology* 04/2021 | Alberta Cancer Registry Canada | n = 326<br>Median age, 63 y<br>88% high CNS-IPI | R-CHOP-like or more intense with HD MTX: n = 115 w/o: n = 211 | 11.2% (HD MTX) vs 12.2% (w/o) ($P$ = .82) |
| Eyre et al, *British Journal of Hematology* 10/2019 | 8 UK centers | n = 690<br>Median age, 77 y<br>40% high CNS-IPI | R-CHOP with IT MTX: n = 97<br>with HD MTX: n = 14<br>both: n = 17<br>w/o: n = 552 | Adjusted for CNS IPI: HR 1.34 (95% CI, 0.46-3.86) ($P$ = .59) (IT MTX/HD MTX/both vs w/o) |
| Klanova et al, *Blood* 02/2019 | Multicentric GOYA (phase 3 trial) | n = 1418<br>Median age, 62 y<br>17% high CNS-IPI | R/G-CHOP with IT MTX and/or AraC: n = 140<br>w/o: n = 1278 | 2-y risk: 2.8% (IT MTX) vs 2.6% (w/o) |
| Boehme et al, *Blood* 04/2009 | Multicentric RICOVER-60 (phase 3 trial) | n = 1222<br>Median age, 68 y | (R)-CHOP with IT MTX: n = 273<br>w/o: n = 949 | CNS events: 2.5% (IT MTX) vs 4.4% (w/o) |

AraC, cytosine arabinoside; CI, confidence interval; G, obinutuzumab, HD, high dose; HR, hazard ratio; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; w/o, without prophylaxis.

cumulative incidence of CNS relapse of 1.6% treated with R-ACVBP vs 4% following R-CHOP, R-CHOEP, or dose-escalated R-CHOEP (R-MegaCHOEP).[9] Although the difference was not significant, the low rate of CNS relapse after R-ACVBP may suggest that the combination of aggressive first-line therapy, IT MTX, and systemic administration of multiple drugs crossing the blood-brain barrier may reduce the incidence of CNS relapse.

Although patients with low or intermediate CNS-IPI can be spared CNS prophylaxis because of the low relapse rates reported, CNS imaging and fluorescence-activated cell sorter analysis of the cerebrospinal fluid will identify CNS involvement at diagnosis in some patients with high CNS-IPI. Patients with high CNS-IPI but no evidence of CNS involvement by modern screening technologies remain prime candidates for CNS prophylaxis. In light of the devastating prognosis of CNS relapse, and before results of studies involving alternative molecules (lenalidomide, Bruton tyrosine kinase, or BCL2 inhibitors) become available, this small group of patients (<<10% of DLBCL patients) may benefit from the combination of aggressive first-line therapy, IT MTX, and systemic administration of multiple drugs crossing the blood-brain barrier.

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

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**MYELOID NEOPLASIA**

*Comment on Davis et al, page 424*

# Driving differentiation: targeting APA in AML

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**In this issue of *Blood*, Davis et al show that the posttranscriptional alternative polyadenylation (APA) process contributes to acute myeloid leukemia (AML) pathogenesis and that targeting the APA regulator FIP1L1 in AML cell lines promotes differentiation.[1] The authors quantify changes in APA patterns by performing 3′ untranslated region (UTR) extraction and deep sequencing (3′ READS) (see figure) on samples from patients with primary AML and on hematopoietic stem and progenitor cells (HSPCs) from healthy donors. Mechanistic analysis suggests that targeting APA promotes differentiation of AML cells.**

More than 70% of all genes harbor more than 1 polyadenylation site, and differential usage of these sites is termed APA.[2] Depending on the exact localizations of the different polyadenylation sites, this process can affect the 3′ UTR as well as the coding DNA sequence (CDS). APA can influence RNA stability, RNA output, protein localization, and protein isoform expression. Occurring within the 3′ UTR, APA determines the length of the UTR, which directly affects the presence or absence of binding motifs and regulatory sequences. In contrast, differential usage of polyadenylation sites within exons, introns, and alternative 3′ UTRs leads to changes in the CDS of the transcript.

Large-scale sequencing of transcriptomes in different cancer entities and the application of novel 3′ sequencing methods have revealed that changes in APA are a common feature of malignant transformation.[3] Although multiple studies have dealt with the prevalence of APA in solid tumors, few reports have focused on hematologic diseases and leukemia. Recently, APA was studied in healthy murine HSPCs and samples from patients with primary acute lymphoblastic leukemia, chronic lymphocytic leukemia, and AML.[4-7] These studies suggest that APA plays a key role in the development of hematologic diseases. To date, however, mechanistic insights into how APA is involved in leukemia pathogenesis have remained elusive, which has also limited the potential for therapeutic exploitation. The study by Davis et al serves as a resource for APA patterns in samples from patients with AML and from HSPCs from healthy individuals, provides a comprehensive mechanistic analysis of the signaling pathways deregulated by APA in AML, and introduces APA as a putative target for AML therapy.

Davis et al performed 3′ READS on primary CD34$^+$ AML blasts from patients