High-Dose Methotrexate for the Prevention of Central Nervous System Relapse in Diffuse Large B-cell Lymphoma

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Submitted in fulfilment of the requirements for the Degree of:

Doctor of Medicine (MD) - Published Work

College of Medical, Veterinary and Life Sciences

University of Glasgow

2024

SUMMARY

Central nervous system (CNS) relapse in diffuse large B-cell lymphoma (DLBCL) is a rare event, occurring in approximately 2-5% of patients overall, but is associated with a poor prognosis. Certain patient and disease characteristics significantly increase the risk of CNS relapse. In an attempt to prevent this serious complication, CNS-directed prophylactic therapy has often been added to first-line chemoimmunotherapy regimens (e.g. rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP)) in patients with DLBCL deemed to be at highest risk.

Previous UK guidance on this topic was published in 2013 and recommended intrathecal (IT) chemotherapy as standard CNS prophylaxis in DLBCL. In the years since this guidance was published, it became clear that IT prophylaxis has limited efficacy in DLBCL, based on biological rationale as well as a number of publications showing no benefit. Gradually, clinicians moved towards use of systemic intravenous high-dose methotrexate (HD-MTX) instead.

In 2019, a writing group was formed with the aim of producing an updated version of BSH guidance on this subject **(Paper 1)**. During this process, it became clear that the evidence supporting the now wide-spread use of HD-MTX was of relatively poor quality. Although there appeared to be sufficient cumulative evidence to support its use, there was significant uncertainty about how and when HD-MTX should be incorporated into first-line DLBCL therapy. There was significant variation in practice around the timing of delivery, with some centres delivering early in between cycles of R-CHOP therapy (intercalated, i-HD-MTX) while others waited and delivered at end of R-CHOP treatment (EOT).

An initial audit of practice at BWOSCC revealed significant toxicity with an i-HD-MTX approach and subsequent delays to vital systemic therapy. A larger, multicentre analysis with other UK centres was therefore proposed, aiming to compare the deliverability/toxicity of i-HD-MTX vs EOT delivery **(Paper 2).** This study demonstrated a significant increased risk of toxicity and R-CHOP delay with i-HD-MTX versus EOT delivery. As a secondary analysis, CNS relapse rates appeared to be comparable between the two approaches. Some clinicians felt that the results were sufficient to abandon i-HD-MTX altogether, citing the clear risks of toxicity and systemic therapy interruption. However, others had a more cautious interpretation of the data, highlighting that the statistical power

of the study was insufficient to definitively exclude a benefit of i-HD-MTX over EOT with regards to CNS relapse reduction.

A much larger, international study was therefore proposed, aimed at achieving a sample size with sufficient statistical power to determine non-inferiority of EOT HD-MTX delivery in preventing CNS relapse **(paper 3).** A database of 1,384 patients receiving HD-MTX as CNS prophylaxis from 37 centres worldwide was created, by far the largest dataset of this type in existence. It was again demonstrated that i-HD-MTX was associated with increased risk of R-CHOP delay, but importantly this study also definitively showed no benefit in terms of CNS relapse reduction compared to EOT delivery. This work was widely acknowledged by lymphoma clinicians across the world as practice-changing, resulting in cessation of i-HD-MTX and move towards EOT delivery only in the vast majority of centres.

Whilst these data clearly showed no benefit of i-HD-MTX vs EOT delivery, concerningly high rates of CNS relapse overall were observed, despite the use of HD-MTX. In 2023, a study lead by colleagues in Australia was published which aimed to address the important question of whether HD-MTX has any efficacy at all, irrespective of when it is delivered. Lewis *et al* reported on a retrospective analysis of 2,418 patients deemed at high risk of CNS relapse, of whom 425 received HD-MTX with the remainder receiving no HD-MTX. They found no clinically meaningful reduction in risk of CNS progression with HD-MTX. Although this study was the most robust to date addressing the HD-MTX efficacy question, it had important caveats, not least the relatively low number of HD-MTX treated patients in the highest-risk subgroups.

The publication of the HD-MTX timing study, and the data presented by Lewis *et al*, stimulated discussion amongst the lymphoma community about how to interpret these new data and what change, if any, there should be to recommended practice. A number of review articles on this difficult area were produced, discussing the emerging evidence in detail and the potential implications for DLBCL management **(papers 4-6)**.

Despite the data from Lewis *et al* and other smaller studies suggesting a lack of efficacy of HD-MTX, it was clear that some clinicians were not ready to abandon its use altogether, especially given the lack of alternative strategies available. During analysis of the HD-MTX timing study, it was clear that there was a lack of consensus on what the optimal dosage and number of cycles of HD-MTX is when used as prophylaxis. Given the potential significant toxicity of

HD-MTX, and the uncertainty around its efficacy, a further study was designed specifically analysing the impact of HD-MTX dosage **(paper 7)**. The key finding from this analysis was that increasing HD-MTX dose was associated with increased toxicity but with no significant impact on CNS relapses, progression-free or overall survival. It was concluded that, if HD-MTX is still to be used in this setting, no more than 2 cycles should be given at doses higher than 3-3.5g/m2.

In 2024, an updated BSH guideline on this topic was proposed, aimed at summarising the additional evidence available since the publication of the 2020 guideline (**paper 8**). A series of pragmatic recommendations were produced to guide clinicians in this controversial topic. The main changes were to use HD-MTX in a much more selected manner, with acknowledgment that omission entirely was reasonable based on current available evidence. Where HD-MTX is used, there was now much more definitive guidance on how and when to deliver it based on the aforementioned research.

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PREFACE

This work was initiated on a part-time basis during a 1 year fellowship in Lymphoma at the Beatson West of Scotland Cancer Centre in 2018. I then continued the work over the next 6 years in my own time while working as a haematology specialty trainee and then subsequently following appointment to a full-time NHS Consultant post.

ACKNOWLEDGEMENTS

I am hugely indebted to my mentor, and now consultant colleague, Dr Pam McKay. Dr McKay gave me the opportunity to contribute to a BSH guideline on this topic in 2018, at which point I don't think either of us foresaw how this would be the starting point for multiple presentations and publications which would be practice-changing across the world. Throughout she has been a vital source of wisdom and encouragement for me. Her work ethic and passion for patient care are a constant inspiration which I can only hope to emulate in my career going forward.

One of the joys of this work has been forging new relationships with colleagues across the country and beyond. In particular, I would like to thank Dr Kate Cwynarski and Dr Toby Eyre. Their input to this body of work has been crucial and their stature in the lymphoma community has no doubt helped provide a platform for its success and wider recognition. I am also very grateful to the many other clinicians across the world who submitted data for our projects, which was often time consuming but carried out with a shared desire to improve outcomes for our patients.

Amy Kirkwood has provided expert statistical input for the majority of this work. Her ability to provide meticulously thorough statistical output along with her insight and knowledge of the clinical relevance is unparalleled and I fully appreciate that this was carried out in addition to her vast clinical trial workload.

Professor Chris Halsey has been a huge support both in the proposal of this MD and in the writing of the thesis. I am very grateful to her for agreeing to supervise this submission and for all her expertise and wisdom.

I dedicate this thesis to my family. My parents have been an ever-present source of support and have always encouraged me to fulfil my academic aspirations. To my wonderful wife Katy – thank you for your understanding during the busy periods when evenings and weekends were consumed by this work. Finally, to our children Calum and Sophie who arrived while this work was undertaken – I have you both to thank for making me determined to work harder and more efficiently than ever to ensure I could spend as much time with you as possible.

LIST OF PUBLICATIONS AND PERMISSIONS

1. McKay P, **Wilson MR**, Chaganti S, Smith J, Fox CP, Cwynarski K. The prevention of central nervous system relapse in diffuse large B-cell lymphoma: a British Society for Haematology Good Practice Paper. Br J Haematol 2020 Sep;190(5):708-714 PMID: 32433789
   https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.16866
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2. **Wilson MR,** Eyre TA, Martinez-Calle N, et al. Timing of high dose methotrexate CNS prophylaxis in DLBCL: an analysis of toxicity and impact on R-CHOP delivery. Blood Advances 2020 Aug11;4(15):3586-3593. PMID: 32761231
   https://ashpublications.org/bloodadvances/article/4/15/3586/461703/Timing-of-high-dose-methotrexate-CNS-prophylaxis
   Permission: Permission from publisher (Elsevier) to include final published article.

3. **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 Apr 21;139(16):2499-2511. PMID: 34995350
   https://ashpublications.org/blood/article/139/16/2499/483371/Timing-of-high-dose-methotrexate-CNS-prophylaxis
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4. Eyre TA, Savage KJ, Cheah CJ, El-Galaly TC, Lewis KL, McKay P, **Wilson MR,** Evens AM, Bobillo S, Villa D, Maurer MJ, Cwynarski K, Ferreri AJ. CNS prophylaxis in diffuse large B-cell lymphoma. Lancet Oncology 2022 September 1;23(9):E416-426 PMID: 36055310
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5. **Wilson MR**, Bobillo S, Cwynarski K. CNS Prophylaxis in Aggressive B-Cell Lymphoma**.** Hematology Am Soc Hematol Educ Program. 2022 Dec 9; 2022(1): 138-145 PMID: 36485105
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6. Bobillo S, **Wilson MR,** Cwynarski K. Controversies in central nervous system prophylaxis of high-risk diffuse large B-cell lymphoma. Curr Opin Oncol 2023 Sep 1;35(5):382-388. PMID: 37551947
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7. **Wilson MR,** Kirkwood AA, Wong Doo N et al. Dosage of high-dose methotrexate as CNS prophylaxis in DLBCL: A detailed analysis of toxicity and impact on CNS relapse. Am J Hematol 2024 Feb;99(2):E46-E50. PMID: 38037530
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8. **Wilson MR,** Cwynarksi K, Eyre TA et al. Central nervous system prophylaxis in large B-cell lymphoma: A British Society for Haematology Good Practice Paper. In press, BJHaem July 2024
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PRESENTATIONS AND ABSTRACTS

- I presented an initial audit of outcomes from Beatson West of Scotland Cancer Centre (BWOSCC) in poster format at the International Conference on Malignant Lymphoma in June 2019:
    - *Tolerability Of High Dose Intravenous Methotrexate For CNS Prophylaxis Intercalated With R-CHOP - A Single Centre Retrospective Analysis*

- I presented Paper 2 in oral form at the European Haematology Association Annual Meeting in June 2020:
    - *High Dose Methotrexate CNS Prophylaxis In Diffuse Large B-Cell Lymphoma (DLBCL): A Multicentre Analysis Of Toxicity And Impact On R-CHOP Delivery*
    - I received an EHA Abstract Achievement Award for this work

- I presented Paper 3 in oral form at the 63rd American Society of Hematology Annual Meeting and Exposition, December 2021:
    - *Early Integration of High Dose Methotrexate to Frontline DLBCL Therapy Does Not Impact CNS Relapse Compared to End of Treatment Delivery: A Multicentre International Analysis of 1384 Patients*
    - I received the ASH-BSH Abstract Achievement Award for this work

LIST OF ABBREVIATIONS

ABC	Activated B-cell subtype
ALL	Acute lymphoblastic leukaemia
BSH	British Society of Haematology
BWOSCC	Beatson West of Scotland Cancer Centre
CNS	Central nervous system
CNS-IPI	Central nervous system international prognostic index
CSF	Cerebrospinal fluid
CT	Computed tomography
DLBCL	Diffuse large B-cell lymphoma
DSHNHL	German High-Grade Non-Hodgkin Lymphoma Study Group
EOT	End of treatment
FISH	Fluorescent in situ hybridisation
HD-MTX	High dose methotrexate
i-HD-MTX	Intercalated high-dose methotrexate
IT	Intrathecal
LDH	Lactate dehydrogenase
MRI	Magnetic resonance imaging
NHL	Non-Hodgkin lymphoma
R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone

PAPER 1

McKay P, **Wilson MR**, Chaganti S, Smith J, Fox CP, Cwynarski K. The prevention of central nervous system relapse in diffuse large B-cell lymphoma: a British Society for Haematology Good Practice Paper. Br J Haematol 2020 Sep;190(5):708-714 PMID: 32433789

https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.16866

Journal impact factor: 6.5 Number of citations: 40

Summary of contribution:

Conceptualisation	Yes – with all co-authors
Data Curation	Yes – I performed the literature search
Formal Analysis	Yes – all co-authors contributed to analysis of literature, writing of recommendations and formal grading
Investigation	N/A
Methodology	N/A
Project Administration	Yes – with PM
Visualisation	Yes – with PM
Writing – original draft	Yes – with all co-authors
Writing – review & editing	Yes – with PM

The prevention of central nervous system relapse in diffuse large B-cell lymphoma: a British Society for Haematology Good Practice Paper

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Methodology:

This Good Practice Paper was compiled according to the BSH process at http://www.b-s-h.org.uk/guidelines/proposing-and-writing-a-new-bsh-guideline/. The British Society for Haematology (BSH) produces Good Practice Papers to recommend good practice in areas where there is a limited evidence base but for which a degree of consensus or uniformity is likely to be beneficial to patient care. The Grading of Recommendations Assessment, Development and Evaluation (GRADE)

nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria can be found at http://www.gradeworkinggroup.org

Literature review details

Ovid MEDLINE, Embase and Cochrane databases were searched for English language articles up to February 2020 using the keywords: diffuse large B cell lymphoma, central nervous system prophylaxis, CNS prophylaxis, central nervous system recurrence, CNS recurrence. The references from relevant publications were searched and published guidelines by the European Society for Medical Oncology were noted.

Review of the manuscript

Review of the manuscript was performed by the British Society for Haematology (BSH) Guidelines Committee Haematology Oncology Task Force, the BSH Guidelines Committee and the Haematology Oncology sounding board of BSH. It was also posted on the members section of the BSH website for comment.

Introduction

Central nervous system (CNS) relapse in patients with diffuse large B cell lymphoma (DLBCL) is an uncommon event and often confers a poor prognosis. Estimates of incidence vary from 1.9-6.4% with discrepancy in the literature as to whether the introduction of rituximab has reduced this risk (Boehme*, et al* 2009, Gleeson*, et al* 2017, Mitrovic*, et al* 2012, Villa*, et al* 2010).

Retrospective analyses of large trial datasets have provided some insight into the pattern of CNS relapse in the rituximab era. The majority (70-80%) of relapses involve the brain parenchyma with isolated leptomeningeal relapses occurring in a minority of patients (Kansara*, et al* 2017, Klanova*, et al* 2019). Concurrent CNS and systemic relapses occur in a significant proportion of cases (46-48%, (Gleeson*, et al* 2017, Kansara*, et al* 2017).

There is a lack of robust evidence to clearly recommend which patients should receive CNS prophylaxis and how this should be delivered. The data are largely retrospective with a wide variation in selection criteria for which patients received prophylaxis, primary treatment regimen used and type of CNS prophylaxis given. Although there is no clear answer as to what level of risk warrants CNS prophylaxis, a pragmatic approach would be to consider any patient with an estimated CNS relapse rate of >10% as a candidate for prophylactic therapy, whilst taking individual patient considerations and risk of toxicity into account. Even with this approach, a significant proportion of patients will receive CNS prophylaxis ‘unnecessarily’, and the priority should be to ensure delivery of optimal systemic treatment.

Since the publication of BSH guidance on the prevention of CNS lymphoma relapse (McMillan, *et al* 2013), there is increasing evidence to support the use of high dose intravenous (IV) methotrexate and as such it was felt appropriate to update the guidance.

Baseline investigation

Baseline PET-CT should be performed in all patients who are being treated with curative intent as it has a higher sensitivity for detection of extranodal sites and thus influences the decision to give CNS prophylaxis.

Contrast-enhanced brain MRI and CSF including flow cytometry may detect occult CNS disease in a small proportion of patients (Wilson, *et al* 2014). This is recommended in ESMO guidelines (Hutchings, *et al* 2018) as positive results would require consideration of a CNS directed chemotherapy approach. This may be particularly relevant for patients who have disease sites in close proximity to the CNS.

Who should receive prophylaxis?

Clinical risk factors

Several large studies demonstrated that both elevated lactate dehydrogenase (LDH) and advanced stage at diagnosis are associated with increased risk of CNS relapse (Haioun, *et al* 2000, Hollender, *et al* 2002, Tomita, *et al* 2018, van Besien, *et al*

1998). Van Besien et al recommended raised LDH and ≥2 extra nodal sites to define patients at high CNS risk (van Besien, *et al* 1998) and this approach was recommended in the 2013 BSH guideline (McMillan et al 2013) for selecting patients to whom CNS prophylaxis should be offered.

More recently, the German High-Grade Lymphoma Study Group (DSHNHL) has developed the '**CNS-IPI score'** as a tool to estimate the risk of CNS relapse/progression in patients with DLBCL treated with R-CHOP (Schmitz, *et al* 2016). Univariable and multivariable analyses of potential risk factors for CNS relapse were performed on a training cohort of 2164 patients from prospective DSHNHL studies and the MabThera International Trial (MInT). The final model consists of the established IPI factors plus involvement of kidney and/or adrenal glands (table 1).

Table 1: CNS-IPI risk categories with corresponding 2 year rates of CNS relapse and proportion of patients in each category from the training (clinical trial patients) and validation (BCCA registry) cohorts (Schmitz, et al 2016). 1 point is scored for any of the following: age >60 years, LDH >normal, ECOG performance status >1, stage III/IV disease, extranodal involvement ≥2 sites, kidney and/or adrenal involvement.

CNS-IPI risk group	2-year rates of CNS relapse	Proportion of patients: training cohort	Proportion of patients: validation cohort
Low (0-1 points)	**0.6%**	**46%**	**31%**
Intermediate (2-3 points)	**3.4%**	**41%**	**46%**
High (4-6 points)	**10.2%**	**12%**	**23%**

The model was validated on a population based cohort of 1597 patients from the British Columbia Cancer Agency (BCCA) with similar results, suggesting it can be applied to routine clinical practice. However, this approach still means approximately 90% of patients in the high CNS-IPI group potentially receive prophylaxis unnecessarily. Moreover, the model has suboptimal sensitivity with a significant proportion of CNS events occurring in the intermediate risk group. In an attempt to improve on the sensitivity of this model, a further retrospective analysis evaluated the impact of the number of extranodal sites identified by PET/CT imaging on CNS relapse rates (El-Galaly*, et al* 2017). From a cohort of 1532 patients, a group of 144 patients (9%) who had ≥3 extranodal sites was identified which had a 3-year cumulative incidence of CNS relapse of 15.2%. A pragmatic approach would be to offer CNS prophylaxis to patients with a high (4-6 points) CNS-IPI score and to any patient with involvement of 3 or more extranodal sites, irrespective of the CNS-IPI.

Anatomical risk factors

Historically, several specific extra-nodal localisations have been associated with a high risk of CNS relapse, however, many reflect stage III/IV disease or the presence of ≥2 extranodal sites and, outside of the IPI parameters, few are independently predictive.

Testicular involvement by DLBCL has the strongest evidence for a high risk of CNS relapse. Retrospective studies from the pre-rituximab era suggested a CNS relapse rate of 15-21% with the majority occurring in the brain parenchyma (64-85%) (Fonseca*, et al* 2000, Zucca*, et al* 2003). In an attempt to reduce this risk, the IELSG-10 study protocol included 4 doses of intrathecal methotrexate, with a 5 year

cumulative incidence of CNS relapse of 6% (Vitolo*, et al* 2011). However, it should be noted that this was a small cohort (n=53) and many patients had favourable IPI features. A subsequent trial (IELSG-30) involving intrathecal cytarabine intercalated with R-CHOP followed by 2 doses of IV methotrexate (1.5g/m2) is primarily assessing the feasibility of intensified CNS prophylaxis, and the results with regards to CNS relapse rate are awaited. As a result of the IELSG-10 data, many centres include intrathecal chemotherapy during first line therapy for testicular DLBCL, independent of decisions regarding systemically administered CNS prophylaxis.

Renal parenchymal and/or adrenal involvement has been shown to be an independent risk factor for CNS relapse and is incorporated into the CNS-IPI model for this reason (Schmitz*, et al* 2016, Villa*, et al* 2011).

Breast involvement with DLBCL is rare. Retrospective data suggest it is often localised at presentation (Jia*, et al* 2018), largely based on CT imaging rather than PET. Such patients are likely to be underrepresented in clinical trials but retrospective studies have demonstrated high CNS relapse rates of 12-16% (Hosein*, et al* 2014, Jeanneret-Sozzi*, et al* 2008, Yhim*, et al* 2010). Similarly, although uterine involvement in DLBCL is rare it does appear to carry a high risk of CNS relapse (41% , n=17) (El-Galaly*, et al* 2017)).

Intravascular large B-cell lymphoma is rare but carries a very high risk of CNS involvement, both at initial presentation and at relapse (Shimada*, et al* 2010).

Epidural, orbital and craniofacial involvement have previously been considered as high risk of CNS disease but there is no robust confirmatory evidence in the

rituximab era (Murawski*, et al* 2014). In such cases, the key question is whether the dura has been breached, as there is no evidence to suggest that proximity to the CNS per se is an indication for CNS prophylaxis. There is insufficient evidence to suggest that bone or bone marrow involvement confers sufficiently increased risk in isolation to offer CNS prophylaxis.

Biological risk factors

DLBCL with a MYC translocation occurring with a BCL2 and/or BCL 6 translocation (so-called double-hit (DHL) and triple-hit lymphomas (THL)), have been associated with an aggressive clinical course and poor outcomes. Estimates of CNS involvement in such patients vary widely in the literature, with early data likely overestimating risk as FISH was only performed on high risk patients (Savage 2017). More recent evidence suggests that the risk may not be as high as perceived – a retrospective analysis of a large dataset from the BCCA identified 24 patients with DHL/THL with a CNS relapse rate of 4.5% (Savage*, et al* 2016). Data from the phase III GOYA study showed a 5% risk of CNS relapse in 20 patients with DHL. The R-CHOP-14 versus 21 trial included 16 patients with DHL and a further 36 with isolated MYC rearrangement – no CNS relapses were reported in these patients (Gleeson*, et al* 2017). Although numbers of patients with DHL are small these were large, prospective trials with less bias than previous retrospective studies. The majority of patients with DHL/THL will meet other criteria for CNS prophylaxis and/or have primary intensified regimens but, for the uncommon situation where this is not the case, there does not appear to be sufficient evidence to recommend CNS prophylaxis due to DHL/THL status in isolation.

Dual expression of MYC and BCL2 protein (DEL) is more common than DHL (~30% vs 5% of DLBCL) but is also associated with poorer outcomes. Retrospective analysis of a BCCA dataset demonstrated that DEL is associated with an increased risk of CNS relapse (2-year risk 9.7%) (Savage*, et al* 2016). Contrary to this, analysis of data from the GOYA study on CNS relapse confirmed CNS-IPI and ABC cell of origin (gene expression by NanoString) as independent risk factors but not DEL (Klanova*, et al* 2019). There is currently insufficient evidence to recommend CNS prophylaxis in patients with DEL and, until the data on cell of origin are validated on a separate cohort, CNS prophylaxis cannot currently be recommended for ABC subtype per se.

HIV-related DLBCL:

There are insufficient data to determine whether HIV infection is an independent risk factor for secondary CNS involvement in DLBCL. Therefore, we recommend that the criteria for non-HIV associated DLBCL are applied to such patients, in line with current British HIV association guidelines (Bower*, et al* 2014).

Recommendations:

CNS prophylaxis should be offered to patients with any of these factors:

1. **High (4-6) CNS-IPI (1B).**
2. **Involvement of 3 or more extranodal sites irrespective of CNS-IPI (1B).**
3. **Anatomical sites: testicular, renal/adrenal, intravascular (1B).**

Consider CNS prophylaxis in patients with any of the following risk factors:

1. **Anatomical sites: breast, uterus (2C).**

What is the optimum CNS prophylaxis in the Rituximab era?

CNS involvement in DLBCL tends to occur early, either during systemic chemotherapy or shortly after its completion. The median times from diagnosis to CNS relapse in the recent NCRI R-CHOP-14 vs 21 and GOYA trials were 8.1 and 8.5 months respectively, with a wide range reported (e.g. 0.9-43.5 months in the GOYA trial) (Gleeson*, et al* 2017, Klanova*, et al* 2019). Thus, it is logical to aim to deliver CNS directed prophylaxis as early as possible for those at risk. This approach is being investigated by international study groups (Leppa*, et al* 2018).

It is also important to recognise that patients with high IPI DLBCL have a significant risk of systemic relapse, and some may receive regimens with more intensive protocols incorporating CNS-directed therapy, e.g. R-CODOX-M/R-IVAC. The additional value of intrathecal chemotherapy included in this protocol is uncertain when used for patients with DLBCL.

Intrathecal chemoprophylaxis

Intrathecal (IT) chemotherapy has been widely used in high-risk patients with DLBCL for many years despite a lack of robust evidence demonstrating its efficacy. This has come under more scrutiny in the rituximab era given the predominance of parenchymal relapse.

In the RICOVER-60 trial, lack of adherence to the CNS prophylaxis protocol allowed a comparison between patients who received IT prophylaxis versus those who did not, with no statistically significant influence on any type of CNS event demonstrated in patients who had received IT prophylaxis (Boehme*, et al* 2009). Retrospective analyses of other large clinical trials have also demonstrated no reduction in CNS relapse rates with IT prophylaxis (Bernstein*, et al* 2009, Cheah*, et al* 2014). A recent systematic review of the efficacy of IT CNS prophylaxis included fourteen studies and a total of 7357 patients treated with rituximab or obinutuzumab-based immunochemotherapy. IT prophylaxis was not found to be a univariable or multivariable factor associated with a reduction of CNS relapse in any study (Eyre*, et al* 2019a).

In summary, the benefit of IT prophylaxis remains unclear with no strong evidence to support this as an effective means of reducing CNS relapse risk. Given that IT chemotherapy does not meaningfully penetrate the brain parenchyma (the commonest CNS compartment for relapse) (Blasberg*, et al* 1975) it is reasonable to conclude that IT prophylaxis has a limited role in the prevention of CNS relapse.

Systemic CNS Prophylaxis

Reflecting the uncertainty around the efficacy of IT prophylaxis, systemically administered CNS prophylaxis in the form of high dose intravenous methotrexate (HD-MTX) has been increasingly employed in recent years. However, there has been no randomised study demonstrating a benefit of HD-MTX CNS prophylaxis and

there remains a lack of consensus regarding delivery (timing, dose and number of cycles).

It has been demonstrated that higher area under the curve of methotrexate is associated with superior outcome in primary CNS lymphoma, with the optimum way to achieve this being a short infusion (2-4 hours) with doses of at least 3g/m2 MTX (Ferreri*, et al* 2004). Given the predominantly renal excretion of methotrexate, patients should have a creatinine clearance of ≥50ml/min. Furthermore, patients should be deemed to have sufficient cardiac function to cope with the intravascular fluid volume shifts of this regimen.

A retrospective study investigated delivering HD-MTX at a dose of 3.5g/m2 on day 15 of alternating cycles of R-CHOP (Abramson*, et al* 2010). They demonstrated a low incidence of CNS relapse using this approach (3%), but there were issues with nephrotoxicity causing delay of chemotherapy in 8/65 (12%) patients and avoidance of further MTX in seven. A more recent multicentre retrospective analysis of 334 patients identified that intercalated HD-MTX significantly increased R-CHOP delays, mucositis and neutropenic fever compared to delivery after R-CHOP completion. Intercalated HD-MTX resulted in a delay of the subsequent R-CHOP cycle in 20% of instances (median 7 days), however delays were significantly reduced when HD-MTX was delivered before day 10 of the R-CHOP cycle (16% vs 26%, p=0.01). There was no difference in CNS relapse observed between the 2 approaches, however the event rate was low (19/334, 5.7%) and concurrent IT therapy in 60% of patients in the end of treatment group was a potential confounding factor (Wilson et al, 2020). Given the increased incidence of febrile neutropenia, G-CSF may be

considered as per institutional guidelines when HD-MTX is intercalated with R-CHOP.

A Nordic Lymphoma Group study investigated an aggressive chemotherapy and systemic CNS prophylaxis regimen for younger (age 18-65) patients with high risk DLBCL or grade III follicular lymphoma (Holte*, et al* 2013). Six cycles of R-CHOEP-14 were given followed by a course of high-dose cytarabine and a course of high-dose methotrexate (3g/m2 as 24 hour infusion). The CNS relapse rate of 4.5% was felt to be encouraging given the high risk nature of the patient group (56% stage IV, 26.5% with ≥2 extranodal sites), but with all CNS relapses occurring within 6 months it was proposed that delivering CNS directed therapy earlier may have improved outcomes. The same group are investigating this further in the NLG-LBC-05 trial, with initial results suggesting an improvement in CNS relapse risk by incorporation of HD-MTX at the beginning of therapy (Leppa*, et al* 2018).

Ferreri et al reported a retrospective analysis of 107 patients with high risk features for CNS relapse (involvement of specific extra nodal sites or advanced stage with high LDH) (Ferreri*, et al* 2015). 40/107 patients received CNS prophylaxis, the majority receiving HD-MTX +/- IT therapy. The CNS relapse rate in patients who received prophylaxis was 2.5% compared to 12% in those who did not, although the number of patients with high CNS-IPI was lower in the prophylaxis group.

Although none of the above studies in isolation are definitive, taken together the data support consideration of HD-MTX as an effective strategy for CNS prophylaxis.

CNS prophylaxis in older patients

Age >60 years is a factor in the CNS-IPI score and therefore a significant proportion of older patients with DLBCL will fall into the high-risk category for CNS relapse using this selection method. However, delivering sufficient relative dose intensity (RDI) of systemic therapy can be challenging in older patients, and when making decisions about CNS prophylaxis in this patient group one should carefully consider the potential impact on RDI and therefore risk of systemic relapse. The risk of renal toxicity with HD-MTX is particularly relevant in older patients and may be a limitation in delivering HD-MTX intercalated with R-CHOP.

The need for CNS prophylaxis in this group of patients has recently been questioned. A retrospective analysis of 270 patients with DLBCL aged >80 years from 2 multicentre LYSA trials treated with mini CHOP + rituximab or ofatumumab found that despite no patients receiving prophylaxis, CNS relapse rates were low at 3% (Cabannes-Hamy*, et al* 2018). A retrospective analysis of 690 patients aged ≥70 treated with R-CHOP also found the CNS relapse rate to be low at 2.6%. 81.2% of patients received no CNS prophylaxis, with 14.3% receiving IT MTX alone (Eyre*, et al* 2019b).

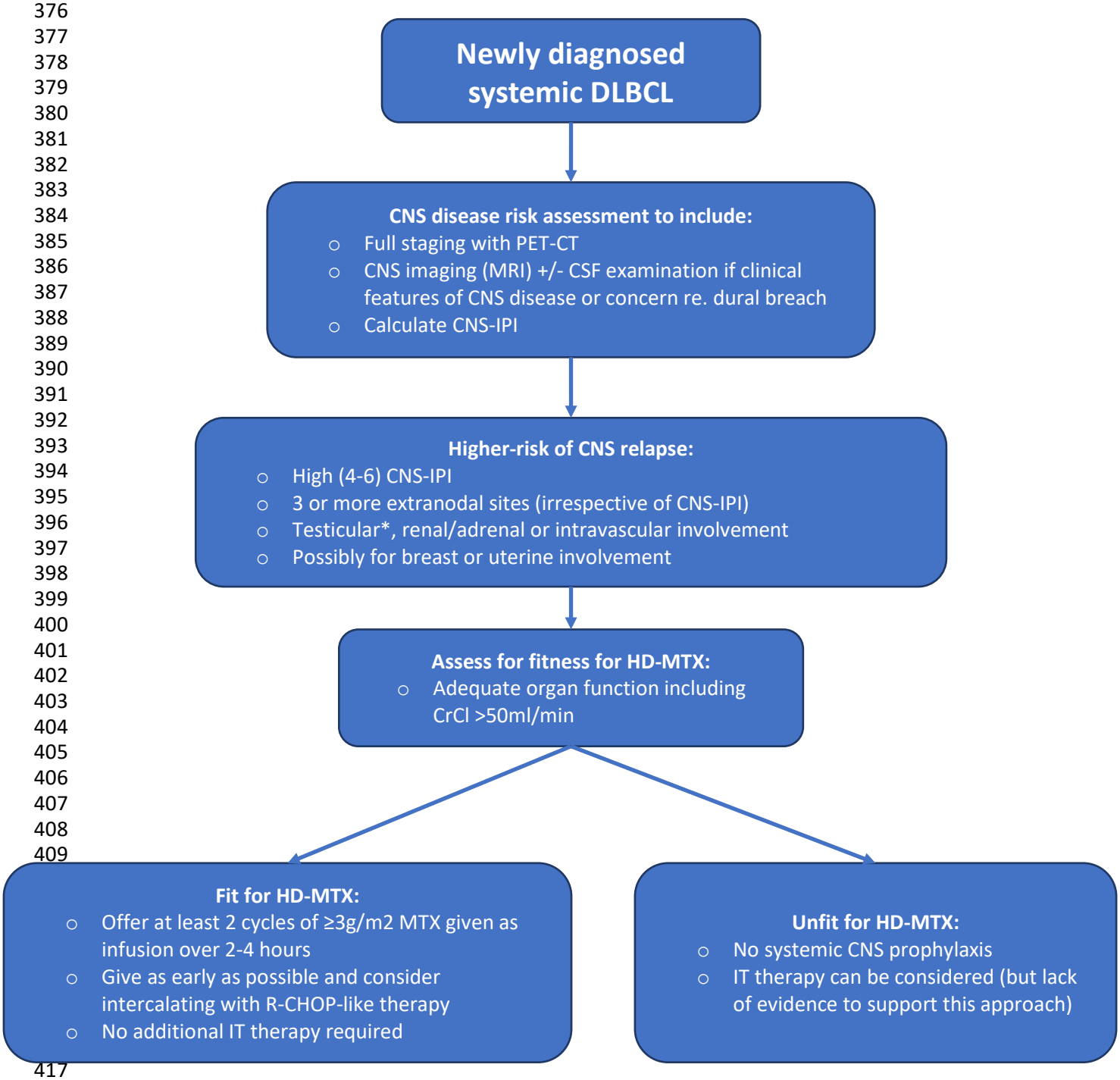
***Recommendations*:**

1. **Where CNS prophylaxis is indicated:**
    - **High dose intravenous methotrexate is preferred (2C).**
    - **Patients' physiological fitness for HD-MTX should be considered (including cardiac and renal function) (1B). Regarding renal function, we consider CrCl ≥50ml/min to be acceptable.**

- **2-3 cycles of at least 3 g/m^2 with an infusion time of 2–4 hours is recommended (2C).**
- **HD-MTX should be administered as early as possible as part of first line therapy without compromising dose and time intensity of R-CHOP-like treatment. Decisions on whether to intercalate or deliver at end of R-CHOP should be individualised, based on a careful analysis of competing risks (2C).**
- **If HD-MTX is intercalated with R-CHOP-21, the preferred scheduling appears to be before day 10 (2C).**

2. **If HD-MTX is successfully delivered then additional IT prophylaxis is not recommended (2C).**
3. **If unable to deliver HD-MTX, IT prophylaxis may be considered, however there is a paucity of data to support this approach (2C).**
4. **Patients with testicular lymphoma should be considered for IT as well as systemic prophylaxis (2B).**

Figure 1



**Patients with testicular involvement should be considered for IT as well as systemic prophylaxis*

Acknowledgements

All authors were involved in the formulation and writing of the manuscript, as well as approval of its final version. The BSH haematology oncology task force members at the time of writing this good practice paper is Pamela McKay. The authors would like to thank them, the BSH sounding board and the BSH guidelines committee for their support in preparing this Good Practice Paper.

Declaration of Interests

No expenses were incurred during the writing of this Good Practice Paper. All authors have made a declaration of interests to the BSH and Task Force Chairs which may be viewed on request.

Review Process

Members of the writing group will inform the writing group Chair if any new pertinent evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be archived and removed from the BSH current guidelines website if it becomes obsolete. If new recommendations are made an addendum will be published on the BSH guidelines website (www.b-s-h.org.uk/guidelines/).

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PAPER 2

Wilson MR, Eyre TA, Martinez-Calle N, et al. Timing of high dose methotrexate CNS prophylaxis in DLBCL: an analysis of toxicity and impact on R-CHOP delivery. Blood Advances 2020 Aug11;4(15):3586-3593. PMID: 32761231

https://ashpublications.org/bloodadvances/article/4/15/3586/461703/Timing-of-high-dose-methotrexate-CNS-prophylaxis

Journal impact factor: 7.5 Number of citations: 39

Summary of contribution:

Conceptualisation	Yes – with PM
Data Curation	Yes – I created data collection template and coordinated data collection from other UK centres
Formal Analysis	Yes – I amalgamated data, cleaned in preparation for analysis and performed all statistical analyses
Investigation	Yes – as above
Methodology	Yes – as above
Project Administration	Yes – with PM
Visualisation	Yes
Writing – original draft	Yes
Writing – review & editing	Yes

REGULAR ARTICLE

blood advances

Timing of high-dose methotrexate CNS prophylaxis in DLBCL: an analysis of toxicity and impact on R-CHOP delivery

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Key Points

- HD-MTX CNS prophylaxis intercalated with R-CHOP caused increased toxicity and R-CHOP delay compared with delivery at EOT.
- No differences in survival or CNS relapse were seen, and delays after i-HD-MTX were reduced by delivering R-CHOP before day 10.

High-dose methotrexate (HD-MTX) is increasingly used as prophylaxis for patients with diffuse large B-cell lymphoma (DLBCL) at high risk of central nervous system (CNS) relapse. However, there is limited evidence to guide whether to intercalate HD-MTX (i-HD-MTX) between R-CHOP-21 (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone given at 21-day intervals) or to give it at the end of treatment (EOT) with R-CHOP-21. We conducted a retrospective, multicenter analysis of 334 patients with DLBCL who received CNS prophylaxis with i-HD-MTX (n = 204) or EOT HD-MTX (n = 130). Primary end points were R-CHOP delay rates and HD-MTX toxicity. Secondary end points were CNS relapse rate, progression-free survival, and overall survival. The EOT group had more patients with a high CNS international prognostic index (58% vs 39%; $P < .001$) and more concurrent intrathecal prophylaxis (56% vs 34%; $P < .001$). Of the 409 cycles of i-HD-MTX given, 82 (20%) were associated with a delay of next R-CHOP (median, 7 days). Delays were significantly increased when i-HD-MTX was given after day 9 post–R-CHOP (26% vs 16%; $P = .01$). On multivariable analysis, i-HD-MTX was independently associated with increased R-CHOP delays. Increased mucositis, febrile neutropenia, and longer median inpatient stay were recorded with i-HD-MTX delivery. Three-year cumulative CNS relapse incidence was 5.9%, with no differences between groups. There was no difference in survival between groups. We report increased toxicity and R-CHOP delay with i-HD-MTX compared with EOT delivery but no difference in CNS relapse or survival. Decisions on HD-MTX timing should be individualized and, where i-HD-MTX is favored, we recommend scheduling before day 10 of R-CHOP cycles.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma,[1] comprising ~40% of all cases of lymphoma in large population-based registries. Despite being an aggressive malignancy, the majority of cases can be cured with R-CHOP chemoimmunotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) given at 21-day intervals.

Submitted 21 May 2020; accepted 29 June 2020; published online 6 August 2020.
DOI 10.1182/bloodadvances.2020002421.

Requests for data sharing may be submitted to the corresponding author (Matthew R. Wilson; e-mail: matthewwilson1@nhs.net).

The full-text version of this article contains a data supplement.

Systemic progression or relapse remains the most common cause of treatment failure in DLBCL, but central nervous system (CNS) relapse may also occur either in isolation or in combination with systemic disease recurrence. The prognosis from CNS relapse is dismal, with most studies reporting a median survival of <6 months.[2,3] Estimates of incidence of CNS relapse in DLBCL vary from 2% to 6%, with some discrepancy across published studies as to whether the introduction of rituximab has reduced this risk.[4-7]

Various patient and disease characteristics have been identified that confer a high risk of CNS relapse in DLBCL, including the total number of extranodal sites involved,[8] involvement of specific high-risk sites (eg, testicular, breast), advanced stage disease, and increased lactate dehydrogenase (LDH) levels.[9] Recently, the CNS international prognostic index (CNS-IPI) has increasingly been used to identify high-risk patients; this index was derived from a large population of patients in clinical trials for DLBCL and validated in a "real-world" registry.[10] It incorporates all standard IPI features as well as an additional point for renal and/or adrenal involvement.

Although the evidence for identifying patients at increased risk of CNS relapse is relatively robust, data on the most effective way to reduce this risk are lacking, with many studies being retrospective and incorporating significant selection bias. Intrathecal (IT) chemotherapy (eg, methotrexate [MTX]), incorporated into R-CHOP therapy, was used for many years as a prophylactic regimen. However, with an increased recognition that the pattern of CNS relapse in DLBCL is predominantly parenchymal,[11,12] an area inadequately penetrated by IT chemotherapy,[13] there has been increased focus on the use of systemic prophylaxis such as intravenous high-dose MTX (HD-MTX). Indeed, several recent publications have cast further doubt on any benefit of IT prophylaxis[14,15] as well as highlighting the potential for toxicity with this approach.[16]

Although several studies have suggested that HD-MTX is effective CNS prophylaxis in DLBCL,[15,17,18] no prospective randomized trial has been performed to show the benefit of this strategy, and there remains a lack of consensus regarding how it should be delivered (ie, timing, number of cycles, dose). CNS relapses tend to occur early, with the median time from DLBCL diagnosis to CNS relapse reported in most studies at between 6 and 8 months.[12,19] Therefore, there is rationale to deliver CNS prophylaxis as early as possible during treatment. "Intercalating" HD-MTX between cycles of R-CHOP has been adopted in many centers. However, the largest published study demonstrating this as a deliverable and effective strategy was retrospective in nature, single center, and included only 65 patients.[18] Given that failure of systemic therapy in DLBCL poses a much greater risk than CNS relapse, concern exists that the toxicity of intercalated HD-MTX (i-HD-MTX) may compromise delivery of R-CHOP therapy. An alternative approach is to wait until completion of systemic therapy before delivering HD-MTX with the aim to retain R-CHOP dose intensity, albeit with concern that such a delay in delivery may not abrogate early CNS relapse in some patients.

To address this clinically important and unanswered question, we conducted a retrospective, multicenter national analysis of patients with DLBCL who had received R-CHOP therapy as well as CNS prophylaxis with HD-MTX. Within this large data set, our primary aim was to analyze the toxicity of HD-MTX and its effect on R-CHOP relative dose intensity, comparing an i-HD-MTX approach to delivery at end of treatment (EOT). Secondary aims were to determine whether there were differences in survival and relapse outcome (including rates of CNS recurrence).

Methods

Data on 334 consecutive patients with DLBCL who received R-CHOP given at 21-day intervals in addition to HD-MTX CNS prophylaxis between 2011 and 2018 were collected from 11 centers in the United Kingdom who used either the i-HD-MTX or the EOT approach according to center preference. Patients with transformed indolent non-Hodgkin lymphoma were included, but patients with HIV-associated DLBCL, posttransplant or immunosuppression-related lymphoproliferative disorders, and any patients with known CNS involvement at diagnosis were excluded. Baseline CNS evaluation was not mandated but was performed according to local treating clinician discretion for patients with clinical suspicion of CNS disease at diagnosis. Patients receiving additional IT prophylaxis were not excluded.

Patients were selected for CNS prophylaxis per local policies on the basis of published risk models, including involvement of ≥2 extranodal sites plus increased LDH levels[9] or high CNS-IPI score,[10] or due to involvement of specific high-risk sites (testicular, renal/adrenal, breast, paranasal sinus, paraspinal, or ovarian involvement).

Baseline characteristics were collected, including several risk factors known to influence CNS relapse rates. Continuous variables are expressed as median and range; intergroup comparisons were performed by using the Mann-Whitney *U* test. Categorical variables are presented as proportions and were compared by using the χ^2 test.

R-CHOP was scheduled in 21-day cycles for all patients. R-CHOP delays were analyzed in 2 ways. First, all cycles of i-HD-MTX administered were reviewed and any delays to subsequent R-CHOP cycles recorded, with univariable and multivariable analyses (MVA) of risk factors for delay performed using logistic regression. Second, to determine if i-HD-MTX was an independent risk factor for delay, an analysis of all R-CHOP delays throughout therapy for both groups was performed, including MVA with timing of HD-MTX included as a risk factor.

Progression-free survival (PFS), overall survival (OS), and time to CNS relapse were determined by using Kaplan-Meier survival analysis[20] and Cox regression with comparison between treatment groups made using the log-rank test. Time-to-event analyses were measured from the date of initial DLBCL diagnosis. An "event" for PFS was defined by CNS or systemic relapse, or death from any cause. Patients were censored at the date last seen if alive and event free. Time-to-CNS relapse and the cumulative incidence of CNS relapse at 2 and 3 years were calculated. Landmark survival analyses of PFS, OS, and CNS relapse were performed for patients who were alive and event free at 6 months from diagnosis to address potential immortality bias. Statistical analyses were performed by using IBM SPSS Statistics for Windows, version 26 (IBM SPSS Statistics, IBM Corporation, Armonk, NY) with 95% confidence intervals presented and $P < .05$ considered significant.

Results

Baseline characteristics

Baseline characteristics of all 334 patients are summarized in Table 1, with further stratification by timing of HD-MTX (i-HD-MTX [n = 204] vs EOT [n = 130]). Across both cohorts, the median age

Table 1. Baseline characteristics

Characteristic	All patients (N = 334)	Intercalated (n = 204)	EOT (n = 130)	P
Age, median (range), y	61 (20-82)	60 (20-81)	62 (20-82)	.78
Male sex	197 (59)	116 (57)	81 (62)	.32
Creatinine clearance, median (range), mL/min	111 (44-299)	115 (45-299)	107 (44-236)	.03*
Advanced stage	266 (82)	168 (82)	107 (82)	.99
Elevated LDH	242 (72)	143 (70)	99 (76)	.33
ECOG PS ≥2	88 (27)	45 (22)	44 (34)	.02*
1 EN site	123 (37)	81 (40)	42 (32)	.38
2 EN sites	116 (35)	66 (32)	50 (38)	
≥3 EN sites	90 (27)	55 (27)	35 (27)	
Renal/adrenal involvement	55 (16)	26 (13)	29 (22)	.02*
"Double hit"†	10 (3)	5 (3)	5 (4)	.65
CNS-IPI				
Low (0-1)	51 (16)	32 (16)	19 (15)	
Intermediate (2-3)	123 (35)	88 (45)	35 (27)	
High (4-6)	151 (46)	77 (39)	74 (58)	<.001*
IT prophylaxis	142 (42)	69 (34)	73 (56)	<.001*
Received 6 cycles of R-CHOP	319 (96)	194 (95)	125 (96)	.65
No. HD-MTX received, median (range)	2 (1-4)	2 (1-4)	2 (1-3)	.62
Received ≥3 g/m^2 HD-MTX	309 (93)	191 (94)	118 (91)	.33

Data are n (%) unless otherwise noted. Missing data: LDH, n = 8; PS, n = 3; Renal/adrenal involvement, n = 2; Double hit, n = 38; CNS-IPI, n = 9. ECOG PS, Eastern Cooperative Oncology Group performance status; EN, extranodal; IT, intrathecal.

*Statistically significant.

†Presence of *MYC* with *BCL2* and/or *BCL6* translocations.

was 61 years (range, 20-82 years) with a male predominance (59%). Sixty-two percent had involvement of ≥2 extranodal sites, and 46% had a high CNS IPI (score, 4-6). Only 3% had "double-hit" lymphoma (presence of *MYC* with *BCL2* and/or *BCL6* translocations), reflecting the preference in most centers to treat such patients with more intensive regimens than R-CHOP. Ninety-six percent of patients received 6 cycles of R-CHOP, and the median number of cycles of HD-MTX delivered was 2 (range, 1-4 cycles).

Baseline characteristics were broadly similar between the 2 treatment groups. Of note, the EOT group had a higher proportion of patients with poor performance status and with renal/adrenal involvement; as a result, more patients were in the high CNS-IPI category in this group. A higher proportion of patients in the EOT group (73 of 130 [56%]) received IT prophylaxis in addition to HD-MTX compared with the intercalated group (69 of 204 [34%]). The most frequently used IT chemotherapy was MTX, with a median number of treatments of 2 (range, 1-6).

Delays with i-HD-MTX

A total of 409 cycles of HD-MTX were given intercalated between cycles of R-CHOP from 204 patients. Eighty-two (20%) of these were associated with a delay in the subsequent R-CHOP cycle, with a median delay of 7 days (range, 2-150 days). Clinicians were asked to determine whether they felt the R-CHOP delay was directly attributable to HD-MTX. Fifty-six (14%) of 409 cycles had an R-CHOP delay attributed to MTX, with reasons for delay as follows: infection (n = 19), mucositis (n = 11), cytopenias (n = 10), renal toxicity (n = 7), delayed MTX clearance (n = 2), hepatotoxicity (n = 2), and other/unknown (n = 4). Delays were significantly increased when i-HD-MTX was given after day 9 following R-CHOP (48 of 185 [26%] vs 32 of 207 [16%]; $P = .01$). Univariable and multivariable analysis of factors associated with R-CHOP delay after intercalated MTX identified that delivering MTX later in the R-CHOP cycle (on or after day 10) was the most significant factor contributing to R-CHOP delay (Table 2). Full details of timing of delivery of i-HD-MTX are displayed in supplemental Figure 1.

Table 2. Univariable and multivariable analysis of factors influencing delay of subsequent R-CHOP when i-HD-MTX given

	Univariable		Multivariable	
Parameter	Odds ratio (95% CI)	P	Odds ratio (95% CI)	P
i-HD-MTX given after day 9 post R-CHOP	1.92 (1.16-3.16)	.01*	1.74 (1.03-2.93)	.04*
Age	1.02 (1.00-1.04)	.07	1.02 (0.99-1.05)	.16
Male sex	1.41 (0.86-2.32)	.17	1.50 (0.88-2.56)	.13
Advanced stage	0.61 (0.34-1.11)	.11	0.56 (0.26-1.21)	.14
ECOG PS ≥2	0.84 (0.52-1.71)	.84	0.98 (0.52-1.84)	.94
No. extranodal sites	0.95 (0.74-1.20)	.65	1.00 (0.76-1.33)	.98
Elevated LDH	1.12 (0.65-1.93)	.70	1.72 (0.87-3.40)	.12
Baseline creatinine clearance	1.00 (0.99-1.00)	.16	1.00 (0.99-1.01)	.80

Missing data: day of i-HD-MTX, n = 17.

*Statistically significant.

Comparison of R-CHOP delays between treatment groups

Sixty-five (32%) of 203 patients in the i-HD-MTX group had at least one R-CHOP delay during therapy of ≥7 days compared with 18 of 119 (15%) in the EOT group ($P = .001$). Ninety (44%) of 203 had at least 1 delay of ≥3 days in the i-HD-MTX group compared with 27 of 119 (23%) in the EOT group ($P < .001$). Further breakdown of number of cycles delayed for each patient is outlined in supplemental Table 1. On multivariable analysis of the whole cohort, including several baseline and prognostic factors, intercalation of HD-MTX and male sex were the only parameters independently associated with increased R-CHOP delays (Table 3).

MTX toxicity

Toxicity data were collected for a total of 729 cycles of HD-MTX (Table 4). The overall rate of renal toxicity was 5% and was similar across groups. Focusing on the period post–HD-MTX administration, i-HD-MTX was associated with significantly increased mucositis (10% vs 4%; $P = .001$), neutropenic fever (10% vs 2%; $P < .001$), and longer median inpatient stay (5 vs 4 days; $P < .001$), likely reflecting the delivery of MTX during the neutrophil nadir after R-CHOP.

Survival outcomes and CNS relapse

There were 19 CNS relapses in the whole study cohort (5.7%), with a median time from diagnosis to relapse of 8.1 months (range, 5-46 months). Fourteen were parenchymal (74%), 2 (11%) involved both the parenchyma and leptomeninges, and 3 (16%) were isolated to the leptomeninges. Four of the five patients with leptomeningeal involvement at relapse had received concurrent IT prophylaxis. Two of the patients who experienced a CNS relapse had only received 1 cycle of HD-MTX (both in the i-HD-MTX group), with the remainder receiving ≥2 cycles.

The overall estimated 2- and 3-year cumulative incidence of CNS relapse was 5.1% (95% CI, 2.7-7.5) and 5.9% (95% CI, 3.0-8.8), respectively. According to HD-MTX timing, the 3-year cumulative incidence of CNS relapse was: i-HD-MTX, 6.8% (95% CI, 2.9-10.7); and EOT, 4.7% (95% CI, 1.0-8.4). There was no statistically significant difference between the groups (unadjusted hazard ratio, 1.21; 95% CI, 0.48-3.07; $P = .691$) (Figure 1A).

On univariable analysis, the only significant risk factor for CNS relapse identified was involvement of ≥2 extranodal sites ($P = .04$). Timing of HD-MTX and use of IT prophylaxis were not associated with CNS relapse risk on MVA (supplemental Table 2). There was no reduction in CNS relapse rate in the 72 patients in the EOT group who had IT prophylaxis compared with those who did not (5.8% vs 5.5%; $P = .96$).

An analysis focusing on patients who developed early CNS relapse (defined as earlier than 8 months from original DLBCL diagnosis) identified 9 patients in this category, with clinical and prognostic features described in supplemental Table 3. Of note, these patients were enriched for high-risk features such as advanced stage and raised LDH levels (all patients), number of extranodal sites (5 of 9 with ≥3 extranodal sites), and renal or adrenal involvement (4 of 9 patients). However, 4 of 9 patients did not fall into the high-risk CNS-IPI category (due to age ≤60 years and Eastern Cooperative Oncology Group performance status <2). The outcomes were poor, with all but 1 patient dying of lymphoma. Of note, 6 of 9 patients had concurrent systemic progression at the time of CNS relapse.

Table 3. Univariable and multivariable analysis of factors associated with R-CHOP delays in whole study population

	Univariable		Multivariable	
Parameter	**Odds ratio (95% CI)**	***P***	**Odds ratio (95% CI)**	***P***
i-HD-MTX	2.64 (1.48-4.72)	.001*	3.06 (1.62-5.77)	.001*
Age	1.01 (0.99-1.03)	.58	1.00 (0.97-1.03)	.88
Baseline creatinine clearance	1.00 (0.99-1.01)	.78	1.00 (0.99-1.01)	.39
Male sex	1.60 (0.95-2.70)	.08*	1.84 (1.04-3.26)	.04*
Advanced stage	0.82 (0.43-1.56)	.54	0.69 (0.29-1.63)	.40
ECOG PS ≥2	0.81 (0.45-1.43)	.46	0.86 (0.46-1.60)	.63
≥2 extranodal sites	1.23 (0.73-2.07)	.44	1.76 (0.89-3.45)	.10
Elevated LDH	0.91 (0.51-1.60)	.73	0.94 (0.50-1.91)	.94
IT therapy given	0.63 (0.38-1.06)	.08	0.74 (0.42-1.30)	.29
HD-MTX dose	0.76 (0.45-1.30)	.31	0.65 (0.37-1.16)	.14

*Statistically significant.

With a median follow up of 2.4 years (range, 0.3-8.7 years), the 3-year PFS and OS of the i-HD-MTX group were 71.2% (95% CI, 64.0-78.4) and 80.6% (95% CI, 73.8-87.4), respectively, and in the EOT group, 3-year PFS and OS were 76.3% (95% CI, 68.2-84.5) and 85.3% (95% CI, 78.1-92.6). There was no statistically significant difference in either PFS ($P = .26$) or OS ($P = .32$) between the groups (Figure 1B-C). On landmark analysis including only those who were alive and event-free at 6 months, there remained no difference in PFS, OS, or CNS relapse rate between the 2 groups (supplemental Figure 2). No significant difference in CNS relapse, PFS, or OS was seen when analysis was restricted to patients with high CNS-IPI, but an increased risk of treatment delays remained with i-HD-MTX (data not shown). There was no significant difference in 3-year PFS between patients who did or did not have ≥1 R-CHOP delay of ≥7 days (66.8% vs 75.1%; $P = .12$).

Table 4. Summary of HD-MTX toxicity

Parameter	**All (N = 729)**	**Intercalated (n = 409)**	**EOT (n = 320)**	***P***
No. of inpatient days, median (range)	5 (2-60)	5 (2-60)	4 (3-80)	<.001*
Toxicity				
Renal (any)	38 (5)	21 (5)	17 (5)	.92
Grade 1 (creatinine 1.5-1.9 × baseline)	22 (3)	12 (3)	10 (3)	
Grade 2 (creatinine 2-2.9 × baseline)	6 (1)	3 (1)	3 (1)	
Grade 3 (creatinine >3 × baseline)	10 (1)	6 (1)	4 (1)	
Liver (grade 2 or worse)	17 (2)	7 (2)	10 (3)	.21
Mucositis	54 (7)	42 (10)	12 (4)	.001*
Neutropenic fever	49 (7)	42 (10)	7 (2)	<.001*

Data are n (%) unless otherwise noted.
*Statistically significant.

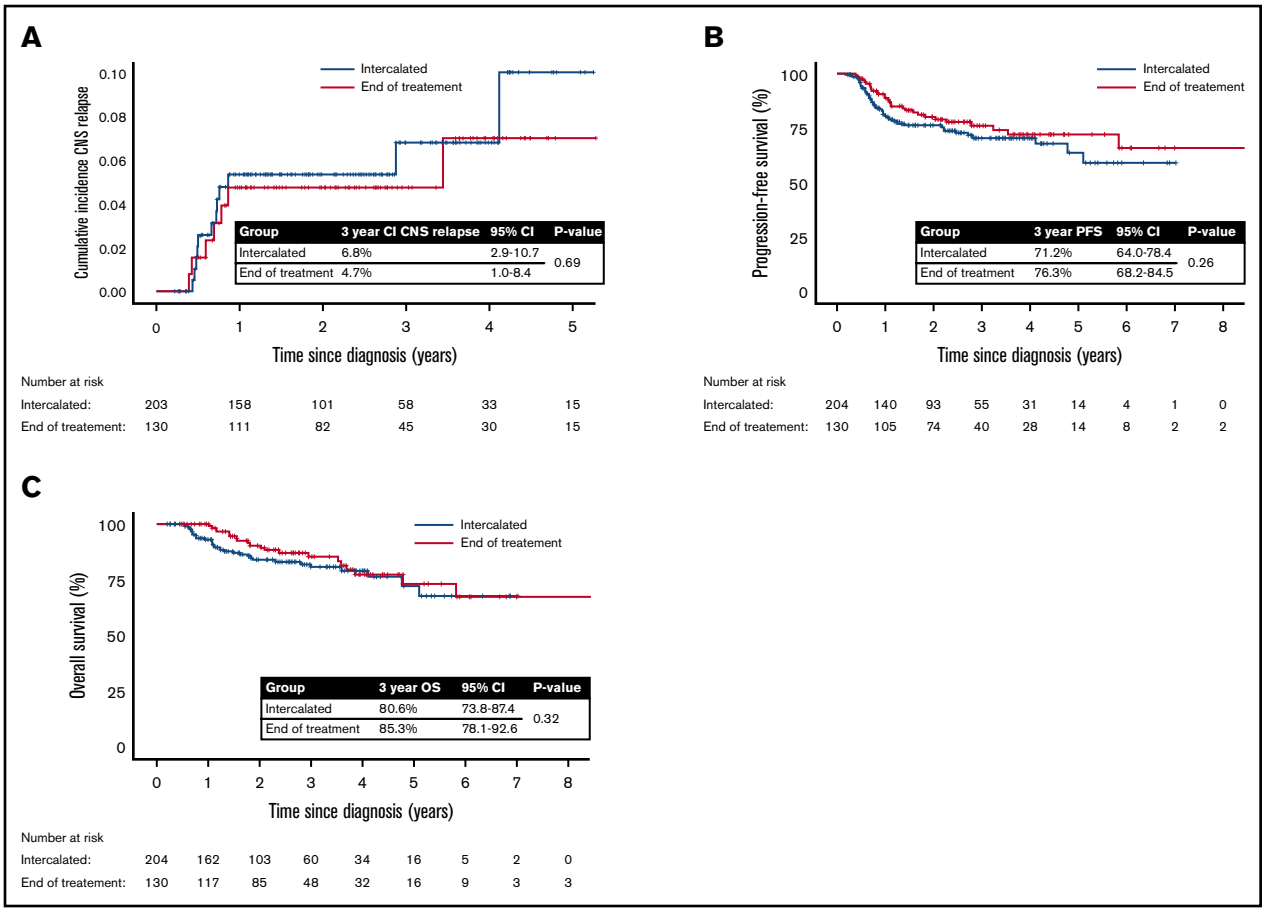

Figure 1. CNS relapse rates and survival outcomes according to timing of HD-MTX CNS prophylaxis. (A) Cumulative incidence of CNS relapse according to HD-MTX timing. (B) PFS according to HD-MTX timing. (C) OS according to HD-MTX timing.

Discussion

CNS prophylaxis in DLBCL is a contentious issue, with wide variation in practice throughout the United Kingdom and worldwide. This disparity is largely due to the paucity of robust, prospective evidence to guide how patients are selected for prophylaxis and the optimum method of delivery. Cumulatively, there appears to be sufficient data to suggest that intravenous HD-MTX is an effective method for delivering CNS prophylaxis. Although the median time from diagnosis to CNS relapse reported in most studies is 6 to 8 months,[12,19] early CNS relapses during primary R-CHOP therapy do occur. Therefore, although not supported by prospective data, there is theoretical rationale for administering HD-MTX as early as possible. However, HD-MTX can result in significant toxicity, and careful patient selection is crucial. Patients at highest risk of CNS relapse are also those at greater risk of systemic treatment failure, and there are concerns that delivering HD-MTX in an intercalated fashion with R-CHOP may compromise the timing and relative dose intensity of systemic therapy. Some clinicians fear that this risk outweighs the relatively low likelihood of early CNS relapse, and they choose to wait until after R-CHOP completion before administering HD-MTX.

To the authors' knowledge, this multicenter retrospective analysis of 334 patients is the largest of its type, specifically assessing the deliverability and toxicity of HD-MTX as CNS prophylaxis with R-CHOP chemoimmunotherapy, either intercalated or delivered at the end of systemic treatment. We have shown that intercalating a cycle of HD-MTX resulted in a delay of the subsequent R-CHOP cycle in 20% of instances, with a median delay of 7 days. Although clinicians reported that the HD-MTX itself caused a delay in 14% of cycles, delays due to the inherent toxicity of R-CHOP are inevitable for some patients, and it is difficult to ascertain the true contribution of HD-MTX in these delays.

We addressed this issue by comparing patients receiving i-HD-MTX vs those who received it after R-CHOP; the latter group acted as a "control" to show how many delays are seen with R-CHOP alone in this high-risk patient group. We acknowledge that those who received EOT HD-MTX were potentially more likely to have completed R-CHOP therapy without significant complication, and there may be a degree of selection bias in using this group as a control for delays. However, we found that 32% of patients in the intercalated group had at least one R-CHOP delay of ≥7 days compared with only 15% in the EOT group. Importantly, on multivariable analysis, timing of MTX (intercalated vs EOT) was the only independent risk factor influencing number of R-CHOP delays. Although a delay of ≥3 days may not be considered clinically relevant in isolation, it should be noted that 15% of patients in the intercalated group had ≥2 delays of ≥3 days during treatment compared with only 1% in the EOT group.

Although we have shown that i-HD-MTX increased the risk of R-CHOP delay, the clinical significance of this finding is a matter for debate. Given the need to maintain dose intensity in a high-grade, proliferative malignancy such as DLBCL, delays of ≥7 days might be considered as potentially clinically relevant. This is particularly concerning in this patient cohort who are inherently at high risk of systemic relapse, as shown by a median IPI of 3 and 123 (37%) of 334 with an IPI of 4 to 5. On analysis of all patients who experienced a delay of ≥7 days, there was a trend toward improved PFS in the no delay group, but this did not reach statistical significance. We should acknowledge there may be other confounding variables associated with delay that we have not identified in this retrospective analysis. Furthermore, it should be noted that for 56 of the 65 patients in the i-HD-MTX group who had a delay of ≥7 days, the rest of the cycles were delivered with no further delays of a similar length.

When considering patients for i-HD-MTX CNS prophylaxis, clinicians must assess the patients' fitness for such an approach. However, other than ensuring adequate renal function, this is done in a mainly subjective manner, and often it can be difficult to predict the tolerability of this approach in individual patients. From this data set, we attempted to identify factors that may help identify patients more likely to experience R-CHOP delays after i-HD-MTX. Timing of i-HD-MTX following R-CHOP was the most significant factor identified on both univariable and multivariable analyses, with a higher rate of delay seen when i-HD-MTX was given on day 10 or later. Therefore, based on these data, it may be more suitable to bring forward i-HD-MTX to earlier within the R-CHOP 21-day cycle to minimize the risk of delay to the next treatment. It is recognized that such an approach cannot be substantiated with high-quality evidence and may lead to as-yet unidentified toxicities.

The rate of CNS relapse in the entire cohort was low (5.7%). Although the study was not designed or powered to address the efficacy of HD-MTX CNS prophylaxis, given the high-risk nature of the patient group, this does seem to be a relatively low rate of CNS recurrence. For example, patients with a high CNS IPI (score, 4-6) have a predicted 2-year CNS relapse rate of 10.2%[10]; 151 patients in our study fell into this category but had a 2-year CNS relapse risk of 6.4%. We feel that we have provided some indirect evidence of efficacy of HD-MTX CNS prophylaxis but acknowledge that this is an area requiring further investigation, ideally within the setting of a prospective randomized trial. Furthermore, with such a low event rate, it is difficult to draw definitive conclusions on any potential difference in CNS relapse risk when considering the different approaches for delivering HD-MTX. However, there did not seem to be any signal toward a difference in CNS recurrence between the 2 strategies.

Toxicity is the main concern when selecting patients for HD-MTX, and it was therefore important to assess and quantify the frequency of various toxicities in this real-world cohort. Accepting that this is a patient group deemed by clinicians to be "fit" for HD-MTX, we showed that renal toxicity occurred in 5% of HD-MTX cycles, with the majority being relatively mild and only 2% of cycles causing grade 2 toxicity or worse. There was a significant increase in mucositis and infection after i-HD-MTX, which is likely to be the main explanation for the longer median inpatient stay with this approach.

The main limitations of the current study are those inherent to retrospective, nonrandomized observation analyses, with some imbalances in baseline characteristics between groups. We acknowledge that selection criteria for CNS prophylaxis varied between centers, reflecting the limited evidence to guide such decisions, particularly before the introduction of the CNS-IPI. Survival outcomes were a secondary end point of the study with no preplanned power calculation, and thus there is a risk that the study is underpowered to detect a difference in PFS or OS between the 2 groups. There is also potential for survivorship bias in retrospectively identifying patients who had HD-MTX after R-CHOP completion, as data from those who progressed early or died before R-CHOP completion may not have been captured. However, data from recent large prospective trials suggest that the number of patients with disease progression or treatment-related mortality during R-CHOP induction therapy is very small (approximately <5%).[21-23] Furthermore, on landmark analysis including only those who were alive and event free at 6 months, there remained no difference in PFS, OS, or CNS relapse rate between the groups.

Despite a higher proportion of patients with high CNS-IPI in the EOT group, there appeared to be no increased CNS relapse with this approach. However, the number of patients receiving IT therapy in this group (56%) may be considered a confounding factor. Accepting the caveat of low event rates in a retrospective analysis, in the EOT group there was no increase in CNS relapse rate in the 54 patients who had no IT therapy, and in the whole study population use of IT therapy was not found to be a significant predictor for CNS relapse on multivariable analysis. Furthermore, there is growing evidence to suggest that IT therapy is ineffective in reducing CNS relapses in DLBCL,[14-16] although no prospective trial has definitively answered this question.

The current study addressed 2 methods for HD-MTX delivery (intercalated or at end of R-CHOP therapy), but a potential third option is to attempt delivery at the beginning of treatment. This approach was investigated in a recent phase 2 trial in which HD-MTX was given with the first 2 cycles of 14-day R-CHOP therapy, followed by an additional 4 cycles of 14-day R-CHOP and etoposide with IT cytarabine given as further CNS prophylaxis.[24] Although the rates of systemic and CNS relapse were low, whether this intensive approach is deliverable in a routine clinical setting remains to be seen. Other potential methods for reducing CNS relapse in DLBCL under investigation mainly involve incorporation of novel agents capable of crossing the blood–brain barrier. For example, ibrutinib, a Bruton tyrosine kinase inhibitor, has shown activity in CNS involvement of mantle cell lymphoma,[25] lymphoplasmacytic lymphoma,[26] and DLBCL.[27] Similarly, the immunomodulatory agents lenalidomide and pomalidomide have shown activity in primary and secondary CNS involvement with B-cell malignancies.[28,29] Both ibrutinib and lenalidomide have failed to show overall benefit for patients with DLBCL when incorporated into R-CHOP therapy in large phase 3 trials[30,31]; whether these drugs could specifically benefit the small subset of patients at high risk of CNS relapse remains an unanswered question.

In conclusion, although our data suggest that HD-MTX may be deferred until EOT with less risk of causing R-CHOP delay, the clinical significance of such delays is unclear, and the additional value of IT therapy during R-CHOP in this setting remains uncertain. There continues to be theoretical rationale for intercalating HD-MTX with R-CHOP to reduce the risk of very early CNS relapse and, where this approach is favored, we recommend that HD-MTX is

scheduled before day 10 of the R-CHOP cycle to minimize risk of delay to the next treatment. Delivery at EOT seems to be a valid alternative strategy, particularly where there is concern about fitness and ability to maintain R-CHOP dose intensity, accepting a risk that early CNS relapse may not be prevented. In the absence of a prospective, randomized trial to inform decision-making in this area, our data may help make a careful analysis of competing risks on an individual patient basis.

Authorship

Contribution: M.R.W., P.M., C.P.F., F.M., and K.C. conceived the study; M.R.W. coordinated the collection of national data; M.R.W., T.A.E., N.M.-C., M.A., K.E.P., G.P., J.K., J. Schofield, J.E., K.L., A.M.K., N.S., C.-K.C., M.A.T., T.C., and J. Smith collected data; and M.R.W. performed statistical analysis and wrote the manuscript, which all authors critically reviewed.

Conflict-of-interest disclosure: G.P. received travel expenses from Takeda and AbbVie. J. Smith received travel expenses from AbbVie and Janssen. K.C. served a consulting/advisory role and received travel expenses from Roche and Janssen; and served a consulting/advisory role for Celgene. M.A. received honoraria from Roche; served a consultancy role for Takeda and Gilead; received travel expenses from AbbVie; and received research funding from Pfizer. N.M.-C. received travel support and honoraria from AbbVie. N.S. served consultancy roles for AbbVie and Roche. P.M. served a consultancy role and received travel expenses from Roche. T.A.E. received honoraria from Roche, Janssen, and Celgene. K.C. received consultancy/speaker fees from AbbVie, AZ, Celgene, Gilead, Janssen, Roche, and Takeda; and received research funding from Adienne, AbbVie, Roche, Gilead, and Takeda. The remaining authors declare no competing financial interests.

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Supplementary materials table 1. Comparison of R-CHOP delays by timing of HD-MTX CNS prophylaxis

	All (n=322)	Intercalated (n=203)	End of Treatment (n=119)	P value
Number of patients any R-CHOP delay ≥ 7 days	83 (26%)	65 (32%)	18 (15%)	0.001
1 cycle delayed ≥ 7 days	72 (22%)	56 (28%)	16 (13%)	
2 cycles delayed ≥ 7 days	7 (2%)	6 (3%)	1 (1%)	
3 cycles delayed ≥ 7 days	3 (1%)	3 (1%)	0 (0%)	
4 cycles delayed ≥ 7 days	1 (<1%)	0 (0%)	1 (1%)	
Number of patients with R-CHOP delay ≥ 3 days	117 (36%)	90 (44%)	27 (23%)	<0.001
1 cycle delayed ≥ 3 days	85 (26%)	60 (30%)	25 (21%)	
2 cycles delayed ≥ 3 days	21 (7%)	20 (10%)	1 (1%)	
3 cycles delayed ≥ 3 days	10 (3%)	10 (5%)	0 (0%)	
4 cycles delayed ≥ 3 days	1 (<1%)	0 (0%)	1 (1%)	

**Data missing: Intercalated n=1, End of treatment n=11*

Supplementary materials table 2. Univariable and multivariable analysis of risk factors for CNS relapse in the whole study population

	UNIVARIABLE		MULTIVARIABLE	
Parameter	**Hazard Ratio (95% CI)**	**P value**	**Hazard Ratio (95% CI)**	**P value**
Age	1.00 (0.96-1.03)	1.00	0.63 (0.18-2.18)	0.46
Intercalated vs EOT HD-MTX	0.84 (0.32-2.10)	0.69	1.32 (0.49-3.57)	0.59
Male sex	0.79 (0.32-1.94)	0.79	0.76 (0.30-1.91)	0.56
Advanced stage	1.92 (0.44-8.3)	0.39	0.46 (0.07-3.20)	0.43
ECOG≥2	0.51 (0.15-1.75)	0.28	0.41 (0.09-1.82)	0.24
2 or more EN sites	3.67 (1.07-12.6)	**0.04**	4.06 (0.76-21.61)	0.10
Renal/adrenal involvement	1.38 (0.46-4.16)	0.57	0.70 (0.18-2.72)	0.61
LDH > ULN	6.63 (0.89-49.69)	0.07	6.38 (0.75-54.25)	0.09
High CNS IPI	1.33 (0.54-3.28)	0.53	0.95 (0.20-4.58)	0.95
IT therapy given	1.50 (0.78-2.88)	0.22	1.46 (0.73-2.88)	0.28
2 or more HD-MTX given	1.07 (0.25-4.46)	0.93	0.89 (0.19-4.13)	0.88

EOT denotes end of treatment; HD-MTX, high dose methotrexate; LDH, lactate dehydrogenase; ULN, upper limit of normal; IT, intrathecal.

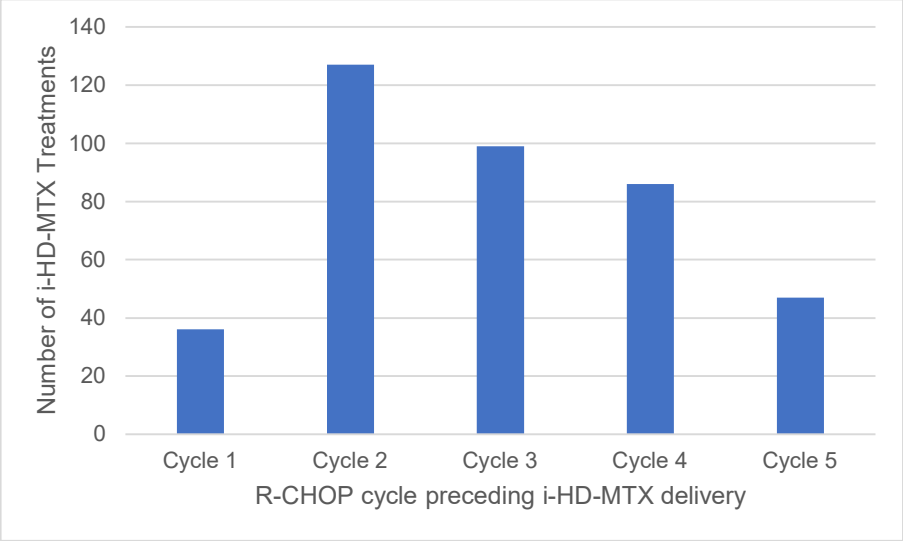
Supplementary materials table 3. Features of 9 patients with CNS progression within 8 months of initial DLBCL diagnosis.

Patient	1	2	3	4	5	6	7	8	9
Intercalated vs EOT	EOT	EOT	IC	IC	IC	IC	IC	EOT	IC
Age	53	53	59	56	77	77	47	35	73
Sex	F	M	F	M	M	F	F	F	M
Advanced stage	Y	Y	Y	Y	Y	Y	Y	Y	Y
ECOG PS≥2	N	Y	N	N	N	N	N	N	N
No. extranodal sites	2	3	3	2	2	4	4	4	1
Renal/adrenal involvement	N	N	N	N	Y	Y	Y	Y	N
LDH > ULN	Y	Y	Y	Y	Y	Y	Y	Y	Y
CNS-IPI	3	5	3	3	4	4	4	4	3
No. HD-MTX received	2	2	3	3	1	3	2	2	3
Concurrent IT prophylaxis	Y	Y	N	Y	N	Y	Y	N	N
Time to CNS relapse (months)	4.6	5.1	5.1	5.5	5.7	5.8	5.9	7.2	7.9
Concurrent systemic progression	Y	Y	Y	N	Y	N	Y	N	Y
Alive/dead	Dead	Dead	Dead	Alive	Dead	Dead	Dead	Dead	Dead

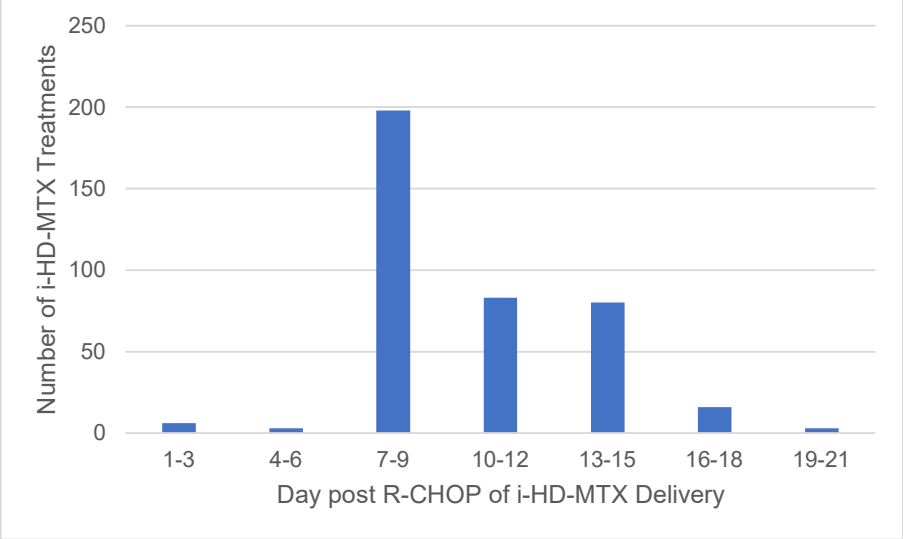
EOT denotes end of treatment; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; ULN, upper limit of normal; CNS-IPI, central nervous system international prognostic index; HD-MTX, high dose methotrexate; and IT, intrathecal.

Supplementary materials Figure 1: Timing of i-HD-MTX delivery. A) According to cycle of R-CHOP where i-HD-MTX was delivered (data available for 395 i-HD-MTX treatments and B) According to timing within the R-CHOP 21 day cycle (data available for 389 i-HD-MTX treatments)

A)

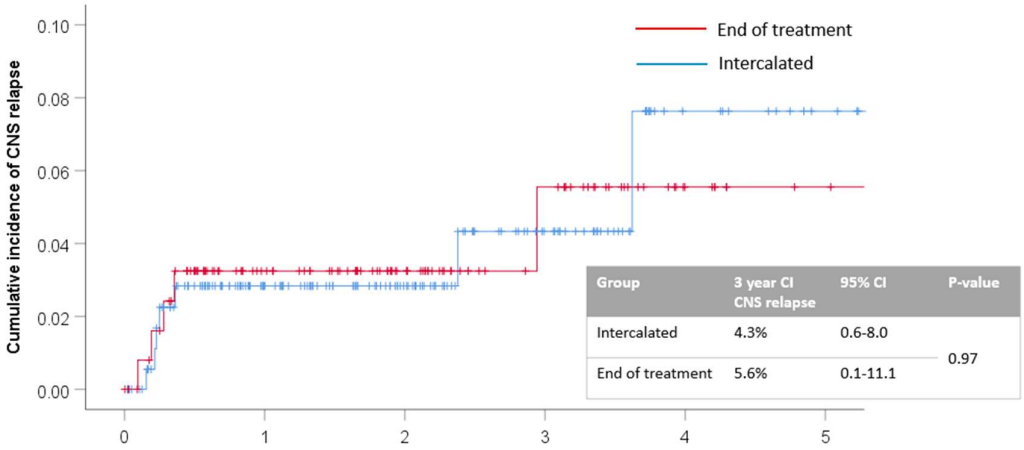


B)



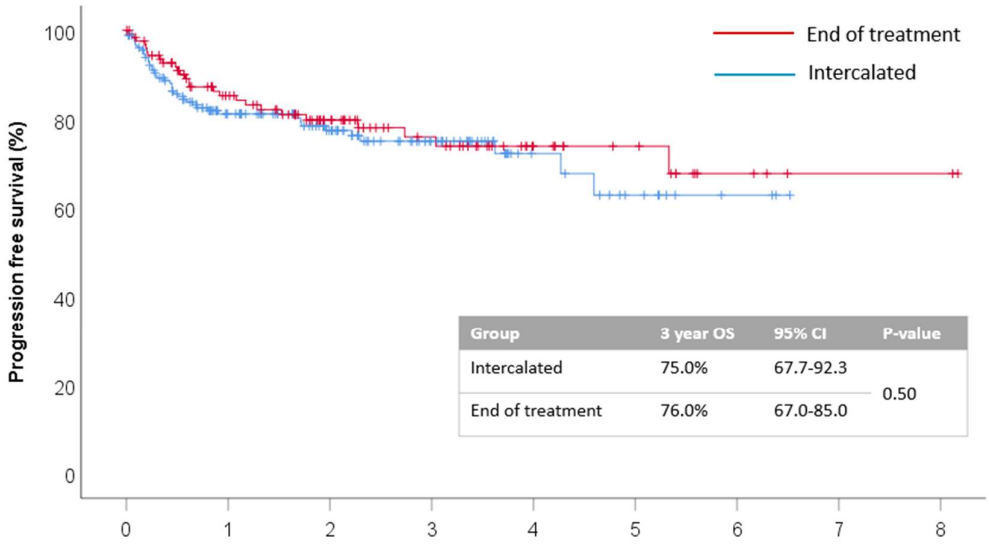
Supplementary materials Figure 2: Landmark analysis including only patients alive and event-free at 6 months. A) cumulative incidence of CNS relapse B) progression free survival and C) overall survival by MTX timing

A)



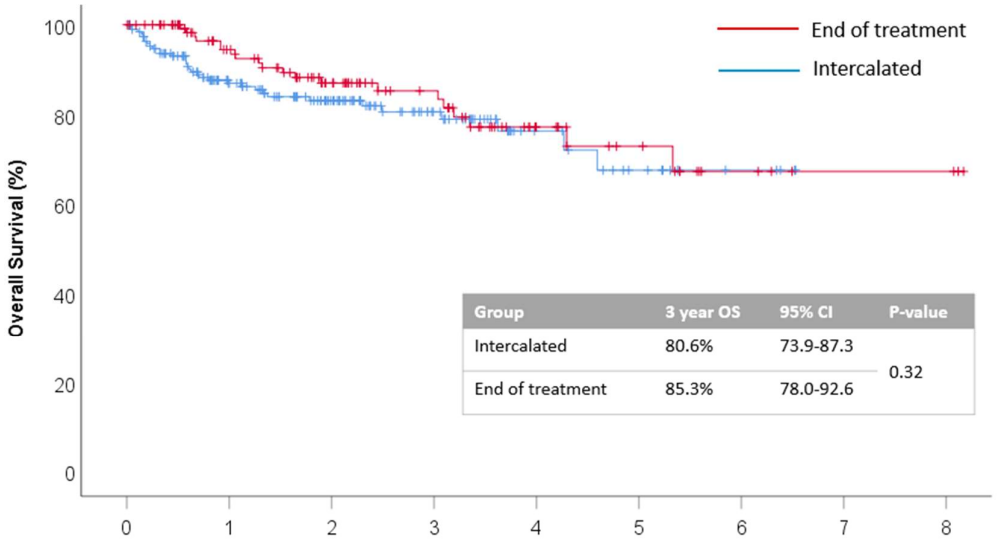
Number at risk						
Intercalated:	188	124	84	48	18	10
End of treatment:	128	91	63	41	20	14

B)



Number at risk									
Intercalated:	183	110	75	45	16	9	3	0	0
End of treatment:	127	84	56	36	19	13	5	2	1

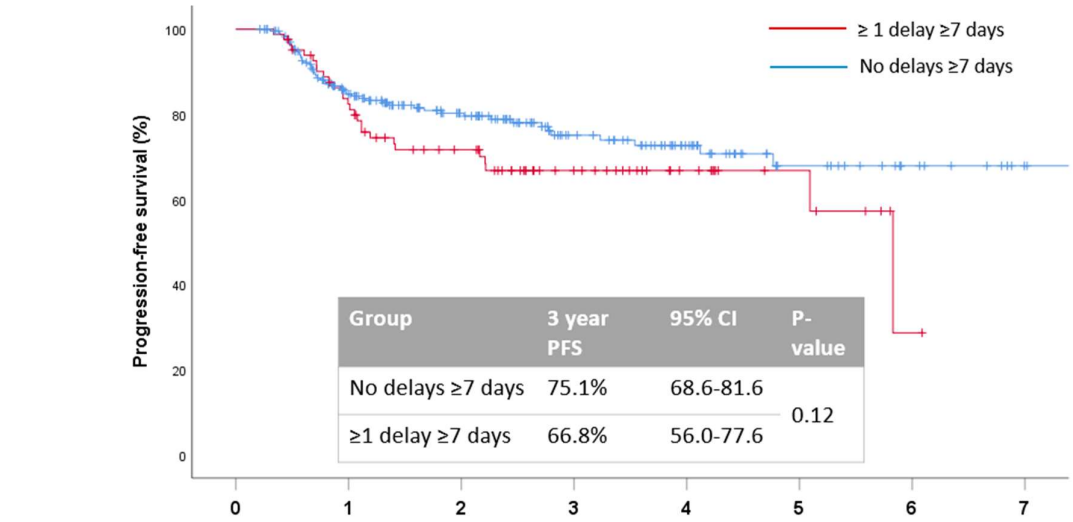
C)



Group	3 year OS	95% CI	P-value
Intercalated	80.6%	73.9-87.3	0.32
End of treatment	85.3%	78.0-92.6	

Number at risk									
Intercalated:	193	126	86	50	19	11	4	0	0
End of treatment:	130	96	66	45	22	14	6	2	2

Supplementary materials Figure 3: **Progression-free survival of whole study population according to R-CHOP delays ≥7 days**



Group	3 year PFS	95% CI	P-value
No delays ≥7 days	75.1%	68.6-81.6	0.12
≥1 delay ≥7 days	66.8%	56.0-77.6	

Number at risk								
No delays ≥ 7 days	239	172	118	67	43	21	11	3
≥ 1 delay ≥ 7 days	83	64	47	27	15	7	1	0

PAPER 3

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 Apr 21;139(16):2499-2511. PMID: 34995350

https://ashpublications.org/blood/article/139/16/2499/483371/Timing-of-high-dose-methotrexate-CNS-prophylaxis

Journal impact factor: 20.3
Number of citations: 45

Summary of contribution:

Conceptualisation	Yes – with TE, KC and PM
Data Curation	Yes – I obtained ethical approval, co-ordinated data sharing agreements, created a data collection template and coordinated data collection from 37 centres
Formal Analysis	Yes, with AK. I amalgamated data and cleaned in preparation for analysis. AK performed statistical analyses
Investigation	Yes – as above
Methodology	Yes – as above
Project Administration	Yes – with PM
Visualisation	Yes
Writing – original draft	Yes
Writing – review & editing	Yes

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Regular Article

LYMPHOID NEOPLASIA

Timing of high-dose methotrexate CNS prophylaxis in DLBCL: a multicenter international analysis of 1384 patients

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KEY POINTS

- **End of treatment HD-MTX did not increase risk of CNS relapse compared with intercalated delivery and caused fewer delays to R-CHOP therapy.**
- **CNS relapse rates in this large analysis of HD-MTX-treated patients were similar to published cohorts receiving minimal CNS prophylaxis.**

Prophylactic high-dose methotrexate (HD-MTX) is often used for diffuse large B-cell lymphoma (DLBCL) patients at high risk of central nervous system (CNS) relapse, despite limited evidence demonstrating efficacy or the optimal delivery method. We conducted a retrospective, international analysis of 1384 patients receiving HD-MTX CNS prophylaxis either intercalated (i-HD-MTX) (n = 749) or at the end (n = 635) of R-CHOP/R-CHOP-like therapy (EOT). There were 78 CNS relapses (3-year rate 5.7%), with no difference between i-HD-MTX and EOT: 5.7% vs 5.8%, *P* = .98; 3-year difference: 0.04% (−2.0% to 3.1%). Conclusions were unchanged on adjusting for baseline prognostic factors or on 6-month landmark analysis (n = 1253). In patients with a high CNS international prognostic index (n = 600), the 3-year CNS relapse rate was 9.1%, with no difference between i-HD-MTX and EOT. On multivariable analysis, increasing age and renal/adrenal involvement were the only independent risk factors for CNS relapse. Concurrent intrathecal prophylaxis was not associated with a reduction in CNS relapse. R-CHOP delays of ≥7 days were significantly increased with i-HD-MTX vs EOT, with 308 of 1573 (19.6%) i-HD-MTX treatments resulting in a delay to subsequent R-CHOP (median 8 days). Increased risk of delay occurred in older patients when delivery was later than day 10 in the R-CHOP cycle. In summary, we found no evidence that EOT delivery increases CNS relapse risk vs i-HD-MTX. Findings in high-risk subgroups were unchanged. Rates of CNS relapse in this HD-MTX-treated cohort were similar to comparable cohorts receiving infrequent CNS prophylaxis. If HD-MTX is still considered for certain high-risk patients, delivery could be deferred until R-CHOP completion.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL). Between 60% and 70% of cases are cured with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) immunochemotherapy.[1] Systemic disease progression is the primary cause of treatment failure; however, relapse within the central nervous system (CNS) occurs in ~2% to 5%[2-4] with poor outcomes.[5]

The CNS international prognostic index (CNS-IPI) is the most established model for predicting CNS relapse risk and incorporates IPI factors plus an additional point for renal and/or adrenal involvement.[6] Patients with CNS-IPI 4-6 have a risk of CNS relapse of ~10%, and CNS-IPI ≥5 patients incur a risk of 15% to 30%. Although the CNS-IPI has improved on earlier models for selecting high-risk patients, the specificity remains unsatisfactory, subjecting many patients to unnecessary prophylaxis. Advances have been made in using molecular subtyping to identify patients at highest risk of CNS relapse, as well as using baseline cerebrospinal spinal fluid (CSF) circulating tumor DNA (ctDNA) assessment; however, this is costly, invasive, and these findings require validation in larger cohorts before being incorporated into routine practice.[7,8]

Various attempts have been made to incorporate CNS-penetrating prophylaxis into frontline therapy, aiming to minimize interruption of systemic treatment while reducing CNS relapses in those most at risk. There remains a lack of robust evidence to guide management, with national guidelines and position papers relying on mainly retrospective data to make pragmatic recommendations about prophylactic strategies.[9] High-dose methotrexate (HD-MTX) is widely recommended as CNS prophylaxis in preference to intrathecal (IT) therapy as the majority of relapses are parenchymal, and the growing evidence suggests IT therapy alone is ineffective.[10,11] Historical retrospective studies suggest that HD-MTX may be effective CNS prophylaxis,[12-14] but no randomized trials have been performed to confirm this. Recent analyses cast doubt on HD-MTX efficacy, including a retrospective study of approximately 2300 patients demonstrating no apparent benefit in high-risk patients.[15-19] Assuming HD-MTX may provide benefit to some high-risk patients, there is uncertainty over how to safely integrate this into frontline therapy. Advocates of an 'intercalated' (i-HD-MTX) approach hypothesize that delivery between early cycles of R-CHOP may prevent very early CNS relapses, while others prefer delivering HD-MTX at the end of treatment (EOT) to avoid interruptions/delays to potentially curative systemic therapy.

We previously analyzed 334 patients treated with either i-HD-MTX or EOT HD-MTX.[20] Delays to R-CHOP were significantly increased by i-HD-MTX compared with EOT, and although no differences in CNS relapse rate or survival between approaches were identified, the event rate was too low to draw definitive conclusions. Given the critical importance of maintaining dose intensity of systemic DLBCL therapy and the increasing scrutiny over HD-MTX efficacy as CNS prophylaxis, we conducted a large international study (n = 1384) with the primary aim of determining whether EOT HD-MTX is as effective as i-HD-MTX in preventing CNS relapse. Secondary endpoints included the impact of HD-MTX timing on survival, toxicity, and delays to R-CHOP cycles and risk factors for CNS relapse, including the influence of concurrent IT prophylaxis.

Methods

We conducted a multicenter retrospective analysis of patients ≥16 years with DLBCL or high-grade B-cell lymphoma not otherwise specified diagnosed between 2007 and 2020 from 47 centers in Europe, Australia, and North America. The study received ethical approval from the West of Scotland Research Ethics Committee (REC:20/WS/0114). Data were collected in compliance with national and/or local regulations and data transfer agreements used where required.

Patients were included if they received frontline R-CHOP or R-CHOP-like therapy with curative intent as well as HD-MTX CNS prophylaxis. HD-MTX was defined as any IV MTX dose intended to cross the blood-brain barrier and exert a prophylactic effect, given for ≥1 cycle. Diagnosis was established by local hematopathology review, with no central pathological review performed. Patients with previously untreated transformed low-grade NHL were included, and concurrent IT prophylaxis was permitted. Patients with HIV-associated DLBCL were included, but those with immunosuppression-related lymphoproliferative disorders and Burkitt lymphoma were excluded. Patients with known CNS involvement at diagnosis and those treated with more intensive regimens, including dose-adjusted etoposide, prednisolone, vincristine, cyclophosphamide, doxorubicin, rituximab (DA-EPOCH-R), were excluded. Baseline CNS evaluation was performed according to local clinician discretion.

Patient records were reviewed by local investigators. Data were recorded in a standardized, study-specific collection sheet and returned to principal investigators for secure central database storage.

Patients were selected for CNS prophylaxis according to local policies based on published risk models or due to the involvement of specific high-risk sites. Delivery of HD-MTX (i-HD-MTX or EOT) was determined according to local center preference, with i-HD-MTX defined as any patient receiving HD-MTX before the final R-CHOP cycle.

Standard baseline characteristics and prognostic indicators were recorded for all patients. Response to frontline therapy was recorded according to the Lugano classification.[21] The number of delays to R-CHOP cycles of ≥7 days throughout therapy was recorded for all patients. All i-HD-MTX treatments were reviewed with the number of days delay to subsequent R-CHOP cycles reported.

We aimed to exclude a ≥5% difference in CNS relapse rate between EOT HD-MTX and i-HD-MTX (ie, that EOT HD-MTX was not more than 5% inferior), using a preplanned power calculation (supplementary Materials). Time-to-CNS relapse was calculated from diagnosis date until CNS relapse with systemic-only relapse and death in remission treated as competing events. Patients alive without relapse were censored at the date last seen. Analyses used competing risks by the Fine and Gray method. Time to isolated CNS relapse was analyzed in the same manner, but with concurrent systemic relapse (defined as CNS and systemic relapse occurring within 30 days of each other) also counting as a

competing event. Due to violations in the proportional hazards (PHs) assumption for other prognostic factors of interest, an analysis using pseudo-observation methods[22] (difference in 3-year cumulative incidence and lifetime lost over 10 years) was also performed. Progression-free survival (PFS) and overall survival (OS) were analyzed using Kaplan Meier survival analysis and Cox regression with times measured from the date of diagnosis until the first event, and patients without an event were censored at the date last seen. Treatment delays were analyzed using logistic regression (endpoint: any delay ≥7 days during chemotherapy) and mixed-effects logistic regression models (delays after each cycle of i-HD-MTX). Analyses were performed with STATA v16.1 (StataCorp, College Station, TX).

When identifying these patients in a retrospective manner, there is a risk that some patients planned for EOT HD-MTX are missed due to early progression. To address this potential survivorship bias in the EOT group, a secondary analysis for patients who had responded and were alive and progression-free at 6 months was also performed.

Results

Baseline characteristics for all 1384 patients (i-HD-MTX n = 749, EOT n = 635) are summarized in Table 1. Median follow-up was 37.9 months. Characteristics of i-HD-MTX and EOT groups were closely matched, with no statistically significant differences in risk factors included in the CNS-IPI except for advanced stage (i-HD-MTX 86.4% vs EOT 80.2%, $P = .002$). Overall, 44.2% had a CNS-IPI 4-6, 40.9% had a CNS-IPI 2-3, and 14.9% had a CNS-IPI 0-1. Applying the CNS relapse risk estimates from the validation cohort in the CNS-IPI publication (0.8%, 3.9%, and 12% for CNS-IPI risk groups, respectively), the estimated risk in our whole population was 7.0%. There was a trend toward a higher CNS-IPI score for i-HD-MTX patients ($P = .083$); however, there was no significant difference in the numbers with scores 4-6 (45.1% vs 43.0%, $P = .45$). The group with low CNS-IPI (n = 203) was enriched for patients considered to have a high-risk EN site involvement (181/203 [89.2%]), the most common of which were testicular (37.6%), craniofacial (22.1%), and breast (10.5%). Detailed reasons for CNS prophylaxis are in supplemental Table 1.

Patients with baseline positron emission tomography-computed tomography was 85%, and 50.8% had baseline CNS evaluation (9.3% CT or MRI and CSF analysis, 8.1% CT or MRI only, 33.4% CSF analysis only).

Treatment details, including HD-MTX delivery, are outlined in supplemental Table 2. Frontline immunochemotherapy was R-CHOP-21 (87.4%), R-CHOP-14 (9.4%), or R-CHOP-like therapy (3.2%); 91.8% received ≥6 cycles. Overall, 46.1% received IT prophylaxis in addition to HD-MTX, with significantly more in the EOT group compared with i-HD-MTX (55.7% vs 38.0%, $P < .0001$).

The median number of HD-MTX cycles delivered was 2 for both groups. Similar numbers received ≥2 cycles (87.7% vs 85.6%, $P = .25$); however, significantly more patients received ≥3 in the i-HD-MTX group (36.8% vs 12%, $P < .0001$) and the patient number receiving a total cumulative dose of >6 g/m^2 HD-MTX was greater in the i-HD-MTX group (46.4% vs 23.2%, $P < .0001$).

There were 78 CNS relapses in the entire population (i-HD-MTX n = 41, EOT n = 37). CNS relapse was parenchymal in 41 (53%), parenchymal and leptomeningeal in 16 (21%), and leptomeningeal in 21 (27%) with similar distribution in both groups. The median time to CNS relapse was 8.5 months (interquartile range [IQR], 6.1-16.7) for the i-HD-MTX group and 10.3 months (IQR, 6.4-27.0) for the EOT group.

There was no difference in the 3-year CNS relapse rates between i-HDMTX and EOT groups: 5.7% vs 5.8%; hazard ratio (HR), 1.01; 95% CI, 0.65-1.57; $P = .98$ (Figure 1A). This remained similar when adjusted for baseline prognostic factors: HR, 1.06 (0.67-1.66); $P = .82$, and the 3-year difference (EOT - i-HD-MTX) excluded the noninferiority limit of +5% when calculated using the unadjusted or adjusted HR, difference: 0.04% (−2.0% to 3.1%) or 0.3% (−1.8% to 3.6%) (Table 2). On landmark analysis of patients alive and free from progression at 6 months (n = 1253), conclusions were unchanged: 3-year rates: 4.7% vs 4.7%, and 3-year differences of −0.03% (−1.0% to 3.0%) and −0.2% (−2.1% to 3.0%) using the unadjusted and adjusted HRs (Figure 1B). Baseline characteristics and details of events in excluded patients are described in supplemental Tables 3 and 4. Analyses performed using pseudo-observation methods also concurred.

Subanalyses of CNS relapse in high-risk patients are summarized in Table 3. In patients with CNS-IPI 4-6 (n = 600) or CNS-IPI 5-6 (n = 210), the overall 3-year CNS relapse rates were 9.1% and 10.5%, respectively. Although this study was not powered for noninferiority comparisons within small high-risk subgroups, with the exception of breast involvement (n = 56 with only 5 events), all HRs were below or very close to 1, and 3-year differences between i-HD-MTX and EOT were under +0.2%. In a composite high-risk group (n = 885) including CNS-IPI 4-6 and/or any of the following: ≥3 extranodal sites, renal, adrenal, testicular, or breast involvement, there was no difference in 3-year CNS relapse rates between groups (i-HDMTX 7.4% vs EOT 7.7%; HR, 1.00; 95% CI, 0.61-1.62) and we could again exclude the +5% noninferiority margin; 3-year difference: 0.0% (−2.8 to 4.3). Applying the same subgroup analyses to the landmark cohort did not change these conclusions, and the 3-year difference within the composite high-risk group just met the noninferiority margin: 0.6% (−2.1% to 5.0%) (supplemental Table 5).

Univariable and multivariable analyses of risk factors for CNS relapse in the whole population and landmark cohort are described in Table 4. Multiple variables violated the PH assumption in both univariable and multivariable analysis, so an analysis was performed using a method comparing the expected CNS relapse-free "lifetime lost" over 10 years, allowing for systemic-only relapse and death in remission as competing events. Age and renal/adrenal involvement were the only independent risk factors in whole cohort and landmark analyses. Due to the potential for immortal time bias, other treatment parameters (use of concurrent IT prophylaxis, HD-MTX cycle number given, and cumulative HD-MTX dosage) were included only in landmark analyses. There was no evidence of associations with time to CNS relapse nor of interactions with HD-MTX timing.

Table 1. Baseline characteristics of the whole study population

	All n = 1384 (%)	End of treatment n = 635 (%)	Intercalated n = 749 (%)	*P*
Age (y), median (range)	62.5 (17-88)	63.0 (18-86)	62.0 (17-88)	.065
Follow-up (mo), median (IQR)	37.9 (21.8-59.6)	41.0 (25.0-63.2)	35.2 (19.6-56.5)	
Baseline creatinine clearance, median (range)	98.2 (33.3-345.2)	94.5 (33.3-345.2)	101.9 (35.5-332)	.0001
Male sex	840 (60.7)	393 (61.9)	447 (59.7)	.40
Advanced stage	1156 (83.5)	509 (80.2)	647 (86.4)	.0019
Raised LDH baseline	943 (70.0)	410 (68.0)	533 (71.5)	.16
Missing/unknown	36	32	4	
ECOG ≥2	358 (25.9)	158 (25.0)	200 (26.7)	.47
Missing/unknown	3	3	0	
Extranodal sites				
0-1	586 (42.3)	282 (44.4)	304 (40.6)	.11*
2	421 (30.4)	191 (30.1)	230 (30.7)	
≥3	377 (27.2)	162 (25.5)	215 (28.7)	
Renal or adrenal involvement	240 (17.3)	102 (16.1)	138 (18.4)	.25
Testicular involvement	175 (12.7)	95 (15.0)	80 (10.7)	.016
Breast involvement	56 (4.1)	18 (2.8)	38 (5.1)	.037
Double or triple hit	66 (6.1)	32 (6.7)	34 (5.7)	.47
Missing/unknown	308	159	149	
CNS IPI				
Low (0-1)	203 (14.9)	107 (17.5)	96 (12.9)	.083*
Intermediate (2-3)	555 (40.9)	241 (39.4)	314 (42.0)	
High (4-6)	600 (44.2)	263 (43.0)	337 (45.1)	
Missing/unknown	26	24	2	
Baseline CNS assessment	703 (50.8)	382 (60.2)	321 (42.9)	<.0001

P values are χ^2 for discrete variables (*for trend) and Wilcoxon Mann-Whitney for continuous.

CNS IPI, central nervous system international prognostic index; ECOG, Eastern Cooperative Oncology Group performance status; IQR, interquartile range; LDH, lactate dehydrogenase.

CNS relapses were isolated in 57 of 78 (73.1%) cases, with the remainder occurring in combination with systemic progression. Sites of isolated relapse were parenchymal in 35 of 57 (61%), leptomeningeal in 16 of 57 (28%), and both in 6 of 57 (11%). Median times to isolated CNS relapse in the i-HD-MTX and EOT groups were 8.3 months (IQR 6.1-18.2) and 12.2 (7.4-29.2) months, respectively. There was no difference in the 3-year cumulative incidence of isolated CNS relapse between groups (Table 4).

With a median follow-up of 37 months, PFS and OS were significantly inferior in the i-HD-MTX group compared with EOT, with differences persisting in a model adjusted for sex, age, ECOG performance status, presence of ≥2 EN sites, renal/adrenal involvement, and stratified by stage and lactate dehydrogenase (LDH) (PH violations): adjusted PFS HR, 0.79; 95% CI 0.64-0.98; $P = .024$; and OS HR, 0.67; 95% CI, 0.52-0.88; $P = .003$ (Figure 2A-B). However, on landmark analysis, there was no significant difference in PFS or OS between groups in univariable or adjusted analysis (model including aforementioned baseline characteristics as well as treatment parameters and chemotherapy delays): adjusted PFS HR, 1.05; 95% CI, 0.81-1.36; $P = .72$; and OS HR, 0.85; 95% CI, 0.61-1.18; $P = .32$ (Figure 2C-D).

Nonrelapse mortality (NRM) was reported in 55 of 1384 (4.0%) patients. Although no NRM events were reported as being

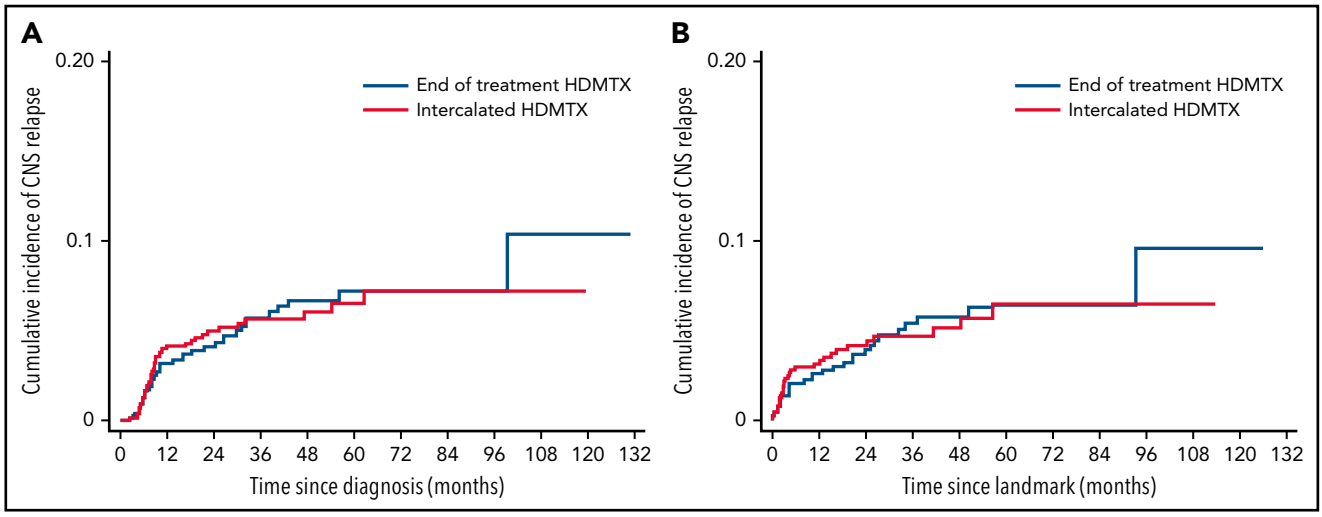

Figure 1. Cumulative incidence of CNS relapse. (A) CNS relapse in the whole population, (B) CNS relapse in landmark population.

directly attributable to HD-MTX, there was a trend toward a higher 3-year cumulative incidence of NRM in the i-HD-MTX group compared with EOT (3.9% vs 2.4%; HR, 0.60; 95% CI, 0.34-1.04; $P = .06$) (supplemental Figure 1). This did not seem to be driven by deaths during treatment as the landmark analysis remained similar: HR, 0.56; 95% CI, 0.31-1.02; $P = .055$.

The median OS of the 78 patients experiencing any CNS relapse was 5.4 months (IQR, 2.8-6.9), with no survival difference between i-HD-MTX and EOT groups (supplemental Figure 2A). When analyzed according to the presence of isolated CNS or synchronous systemic/CNS relapse, there was a trend toward inferior survival in patients with synchronous relapse (HR, 1.69; 95% CI, 0.96-2.98; $P = .069$) (supplemental Figure 2B). There was no difference in survival according to the site of CNS relapse (parenchymal vs leptomeningeal vs both) (supplemental Figure 2C).

Univariable and multivariable analyses of risk factors for any delay of ≥7 days during frontline therapy are displayed in Table 5. The only significant risk factor for delays was i-HD-MTX delivery (odds ratio, 0.44; 95% CI, 0.33-0.59; $P < .0001$). Results were unchanged using ordinal regression with the number of delays throughout therapy categorized as 0, 1 to 2, and ≥3.

A total of 1573 cycles of HD-MTX were given intercalated between cycles of R-CHOP/R-CHOP-like therapy, with most patients receiving first HD-MTX delivery after cycle 1 or 2 (28.5% and 44.4%, respectively) (supplemental Figure 3A-B). The median day post-R-CHOP of i-HD-MTX delivery was 10 (IQR, 1-14), and the median number of intercalated cycles per patient was 2 (IQR, 1-2). Of the 1573 intercalated HD-MTX cycles, 308 (19.6%) resulted in subsequent R-CHOP delay (median delay 8 days [IQR, 6-19]).

Survival analyses in the landmark cohort demonstrated a significantly inferior PFS in patients who had a delay of ≥7 days vs those who did not (adjusted HR, 1.52; 95% CI, 1.15-2.03; $P = .004$) and a trend toward inferior OS (adjusted HR, 1.38; 95% CI, 0.96-1.98; $P = .085$).

Univariable and multivariable analyses of risk factors for delays following i-HD-MTX are displayed in Table 6. Increasing age and baseline creatinine clearance were the only significant factors associated with delays on univariate analysis, with increasing age the only variable approaching statistical significance on multivariate analysis ($P = .055$). Clinicians reported infection (19.5%), renal toxicity (11.7%), cytopenias (11.7%), administrative (8.1%), and mucositis (3.9%) as the most frequent reasons for delays after i-HD-MTX. Mixed-effects logistic regression models were used to assess delays at each cycle of i-HD-MTX (see supplemental Materials for full details). The only baseline factor significant in this analysis was older age, though there were interactions with dose and timing, which suggested that the increase in risk was only present for patients treated with higher doses (≥3 g/m^2) and later in the R-CHOP cycle (>10 days). There was no clear evidence that delays were associated with the R-CHOP cycle in which the dose was given or the i-HD-MTX dose number.

The most frequent toxicities observed post-HD-MTX administration were febrile neutropenia, renal toxicity, and mucositis. No direct comparison between i-HD-MTX and EOT groups is possible, as some events for i-HD-MTX may be related to concurrent systemic chemotherapy. However, we observed numerically greater febrile neutropenia (15.2% vs 2.5%), mucositis (15.4% vs 4.6%), and renal toxicity (17.8% vs 13.9%) in patients in i-HD-MTX vs EOT.

Discussion

Most DLBCL patients are cured with frontline chemoimmunotherapy, and there have been significant advances in recent years for patients with relapsed/refractory systemic disease.[23-26] However, patients with CNS involvement at relapse (occurring in almost 1 of 3 of relapses in high-risk DLBCL[27]) are frequently excluded from trials of novel agents and cellular therapies, and their prognosis is extremely poor (median OS 5-6 months).[5]

There is no broad consensus worldwide regarding how best to reduce the risk of CNS relapse.[28] HD-MTX has been widely adopted as CNS prophylaxis in DLBCL, with initial supporting

Table 2. Univariable and multivariable models for the difference in 3-y CNS relapse rates between i-HD-MTX and EOT groups, for all CNS relapses and for isolated CNS relapse only

	HR* (95% CI)	3-y difference, % (HR)†	3-y difference, %‡
All patients			
EOT HD-MTX (UVA)	1.01 (0.65-1.57)	0.04 (−2.0 to 3.1)	0.06 (−2.63 to 2.76)
EOT HD-MTX (adjusted§)	1.06 (0.67-1.66)	0.3 (−1.8 to 3.6)	0.79 (−1.95 to 3.52)
EOT HD-MTX (adjusted‖)			0.07 (−2.59 to 2.73)
Landmark cohort only			
EOT HD-MTX (UVA)	0.99 (0.60-1.66)	−0.03 (−1.0 to 3.0%)	0.02 (−2.58 to 2.63)
EOT HD-MTX (adjusted§)	0.96 (0.55-1.67)	−0.2 (−2.1 to 3.0%)	0.47 (−2.18 to 3.12)
EOT HD-MTX (adjusted‖)			−0.11 (−2.70 to 2.48)
Isolated CNS relapse			
EOT HD-MTX (UVA)	1.07 (0.63-1.81)	0.3 (−1.4 to 3.0%)	0.47 (−1.84 to 2.78)
EOT HD-MTX (adjusted§)	1.10 (0.64-1.87)	0.4 (−1.4 to 3.2)	1.00 (−1.38 to 3.30)
EOT HD-MTX (adjusted‖)			0.33 (−2.00 to 2.63)
Isolated CNS relapse, landmark cohort			
EOT HD-MTX (UVA)	1.07 (0.60-1.93)	0.2 (−1.3 to 2.9%)	1.11 (−1.34 to 3.56)
EOT HD-MTX (adjusted§)	1.05 (0.57-1.95)	0.2 (−1.7 to 3.6)	1.02 (−1.33 to 3.37)
EOT HD-MTX (adjusted‖)			0.93 (−1.51 to 3.36)

The 10-y cut off for lifetime lost was chosen as close to the end of follow-up (131 mo, and after the last event).

CNS, central nervous system; ECOG, Eastern Cooperative Group performance status; EOT, end of treatment; HD-MTX, high-dose methotrexate; HR, hazard ratio; i-HD-MTX, intercalated high-dose methotrexate; IT, intrathecal; LDH, lactate dehydrogenase; UVA, univariate analysis.

*HR for EOT vs i-HD-MTX.

†Calculated by applying the hazard ratio to the 3-y rate in the i-HD-MTX group to get the corresponding rate in the EOT group, and then taking the difference.

‡Difference in cumulative incidence rates allowing for competing risks at 3 y using pseudo-observations.

§Full model adjusted for sex, age, advanced stage, extra nodal disease (≥2 sites), ECOG (≥2), renal/adrenal involvement, raised LDH (plus ITs, HDMTX ≥2 doses, and cumulative dose >6 g/m^2 for landmark cohort).

‖Adjusted for only variables significant with backward selection (based on survival time lost): age and renal/adrenal involvement for CNS relapse and age alone for isolated CNS relapse.

evidence derived from studies demonstrating efficacy in the treatment of primary CNS lymphoma.[29] Historical, retrospective, nonrandomized studies also suggested a benefit of HD-MTX in DLBCL patients at high risk of CNS relapse, either intercalated with R-CHOP[14] or delivered at EOT.[13] Recently, large retrospective analyses have demonstrated no apparent benefit of HD-MTX in the reduction in CNS relapse risk.[18,19] Patients at the highest risk of CNS relapse are also those at greatest risk of systemic treatment failure, and therefore there has been a lack of agreement about how HD-MTX should be incorporated alongside R-CHOP, with the risk of early CNS progression balanced against the risk of interrupting systemic treatment. Our previous UK study demonstrated increased delays to R-CHOP with i-HD-MTX compared with EOT, but the number of CNS relapse events was too small to conclude that the approaches were equivalent in efficacy.[20]

To our knowledge, this international, multicenter collaboration represents the largest dataset of patients with DLBCL receiving HD-MTX as CNS prophylaxis. The study achieved its primary endpoint of demonstrating noninferiority of EOT HD-MTX compared with i-HD-MTX with regards to CNS relapse risk. This finding was observed despite an increased cumulative HD-MTX dosage in i-HD-MTX compared with EOT patients. When identifying these patients retrospectively, there is a risk that some patients planned for EOT HD-MTX are missed due to early progression. Indeed, the inferior PFS and OS in the i-HD-MTX group suggest this. To address this, we performed a landmark analysis assessing only those patients alive and progression-free at 6 months. This included 90.5% of patients and again demonstrated noninferiority and, importantly, no PFS/OS difference.

The proportion of CNS-IPI 4 to 6 patients in our study was relatively low (44%). However, the CNS-IPI is an imperfect tool, with a high-risk score resulting in a positive predictive value of only 12%. Other established, independent risk factors include specific EN site involvement (eg, testicular, renal/adrenal, and breast) and the total number of EN sites involved. We performed analyses aimed at determining whether the timing of HD-MTX delivery had any influence on CNS relapse in the most high-risk patients. Again, differences were small, though we acknowledge restricting analyses to small subgroups may result in small differences between groups being missed. However, we could still exclude a 5% difference for the composite high-risk group (absolute difference +0.2%), and, although not quite excluded for the high CNS-IPI group, the absolute difference favored EOT (−0.7%) and the upper confidence interval only just crossed +5% (+5.4%).

Much of the literature addressing CNS relapse in DLBCL does not distinguish between isolated CNS relapse and CNS relapse

Table 3. Results within specific high-risk groups

	3-y CNS relapse rates, %	Events/n	HR* (95% CI)	3-y difference,% (EOT, intercalated)
CNS IPI 4-6	**9.1 (6.9-11.9)**	**49/600**		
Intercalated	9.4 (6.5-13.5)	28/337	1.00	−0.7 (−4.4-5.4)
End of treatment	8.6 (5.6-13.1)	21/263	0.92 (0.52-1.62)	
CNS IPI 5-6	**10.5 (5.9-16.0)**	**21/210**		
Intercalated	11.8 (6.7-20.1)	12/118	1.00	−0.4 (−6.8-13.1)
End of treatment	9.1 (4.6-17.4)	9/92	0.96 (0.41-2.29)	
Testicular involvement	**7.5 (4.2-13.2)**	**14/175**		
Intercalated	6.0 (2.3-15.3)	8/80	1.00	−0.4 (−4.0-9.3)
End of treatment	8.5 (4.1-17.2)	6/95	0.92 (0.32-2.68)	
Renal/adrenal involvement	**11.3 (7.6-16.7)**	**25/240**		
Intercalated	14.4 (8.9-23.0)	16/138	1.00	−4.5 (−9.9-6.6)
End of treatment	7.6 (3.7-15.5)	9/102	0.67 (0.30-1.52)	
Breast involvement	**9.7 (3.6-24.6)**	**5/56**		
Intercalated	5.3 (1.3-19.5)	3/38	1.00	2.8 (−3.9-34.5)
End of treatment	20.5 (5.6-60.3)	2/18	1.56 (0.26-9.39)	
≥3 extranodal sites	**7.6 (5.2-10.9)**	**29/377**		
Intercalated	8.0 (5.0-12.8)	16/215	1.00	0.0 (−4.1-8.1)
End of treatment	7.1 (4.0-12.3)	13/162	1.01 (0.48-2.10)	
Any high-risk factor above	**7.6 (5.9-9.7)**	**65/885**		
Intercalated	7.4 (5.2-10.4)	34/482	1.00	0.0 (−2.8-4.3)
End of treatment	7.7 (5.3-11.1)	31/403	1.00 (0.61-1.62)	

High risk CNS IPI: 9.5% (6.2-14.4) EOT and 9.4% (6.5-13.5) intercalated. High risk (all factors): 9.5% (6.6-13.5) EOT and 8.6% (5.9-12.4) intercalated.

CNS IPI, central nervous system international prognostic index; EOT, end of treatment; HR, hazard ratio.

*EOT vs intercalated. Events post 3 years: 8 events (5 EOT and 3 intercalated). Five-year rates: EOT: 7.3% (5.2-10.1) and 6.5 (4.7-9.1) intercalated.

occurring either with or after systemic progression. Indeed, Schmitz and colleagues do not give this detail.[6] Arguably, any CNS relapse occurring concurrently with or after systemic relapse represents a failure of systemic therapy, with the aim of prophylactic HD-MTX being purely to prevent isolated CNS events. A recent retrospective analysis (n = 226) reported a significant reduction in isolated CNS relapses with HD-MTX but no difference in OS or concomitant CNS-systemic relapses.[30] We excluded any CNS relapse occurring after the first systemic DLBCL relapse/progression and recorded data on whether the CNS relapse was isolated. Considering that isolated CNS relapses are likely to occur because of occult clones taking sanctuary in the CNS either at diagnosis or early in the disease course, there is a theoretical rationale that early HD-MTX delivery may be important. However, in the 73.1% of cases where CNS relapse was isolated, we found no benefit for i-HD-MTX.

We demonstrate that i-HD-MTX significantly increases the risk of R-CHOP delay, with 19% of i-HD-MTX treatments resulting in a delay to subsequent R-CHOP and 26% of patients in the i-HD-MTX group experiencing ≥1 delay of ≥7 days during therapy vs 13% in the EOT cohort, though we acknowledge that some patients planned for EOT HD-MTX who suffered complications and R-CHOP delays may have had HD-MTX omitted, and therefore are not captured in this study. Given the need to maintain relative dose intensity in DLBCL, these delays are clinically relevant, especially in patients inherently at high risk of systemic treatment failure. We found that increasing age was an independent risk factor for delays with i-HD-MTX, suggesting i-HD-MTX should be used with particular caution in older patients, though our repeated measures analysis suggested that earlier delivery (before day 10) may be associated with a lower risk of delay. Although we found no clear evidence of an increase in risk by dose, R-CHOP cycle number, or HD-MTX dose number, HD-MTX delivery was decided by site and may have been guided by the deliverability of previous cycles, possibly biasing our data. To understand these relationships, an analysis based on patients treated on 1 protocol is needed.

Direct comparison of HD-MTX toxicity between i-HD-MTX and EOT approaches is problematic, as some of the toxicities with i-HD-MTX may be influenced by concurrent R-CHOP. We were unable to record toxicities between R-CHOP cycles in the EOT group to serve as the most accurate comparator. However, the

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Table 4. Univariable and multivariable analyses of risk factors for all CNS relapse and isolated CNS relapse only

Risk factor	All patients		Landmark	
	Survival time lost (mo)	*P*	Survival time lost (mo)	*P*
All CNS relapses, UVA				
EOT HD-MTX	0.52 (−3.04-4.09)	.77	0.43 (−3.13-3.99)	.82
Sex	0.71 (−2.99-4.40)	.71	0.14 (−3.58-3.85)	.94
Age (for a 10-y increase)	1.61 (0.58-2.64)	.002	1.64 (0.61-2.66)	.002
Advanced stage	2.53 (−2.27-7.33)	.30	1.22 (−3.66-6.11)	.62
Extranodal sites ≥2	4.39 (1.00-7.79)	.011	1.99 (−1.48-5.47)	.26
ECOG ≥2	0.86 (−2.94-4.67)	.66	0.40 (−3.39-4.19)	.84
Renal/adrenal involvement	7.64 (2.28-13.00)	.005	6.06 (0.62-11.51)	.029
Raised LDH	3.02 (−0.29-6.34)	.074	1.63 (−1.67-4.94)	.33
ITs given			1.10 (−2.48-4.68)	.55
HD-MTX doses ≥2			−2.87 (−8.57-2.84)	.33
Cumulative dose >6 g/m^2			−2.19 (−5.47-1.09)	.19
All CNS relapses, MVA				
Age (for a 10-y increase)	1.60 (0.59-2.61)	.002	1.33 (0.39-2.27)	.006
Renal/adrenal involvement	7.65 (2.31-13.00)	.005	5.45 (0.23-10.66)	.041
Isolated CNS relapse, UVA				
EOT HD-MTX	0.71 (−2.51-3.94)	.66	0.79 (−2.93-4.51)	.68
Sex	0.46 (−2.89-3.81)	.79	0.59 (−3.39-4.56)	.77
Age (for a 10-y increase)	1.42 (0.51-2.34)	.002	1.47 (0.44-2.49)	.005
Advanced stage	0.24 (−4.48-4.95)	.92	−0.52 (−5.81-4.77)	.85
Extranodal sites ≥2	2.21 (−0.89-5.31)	.16	0.82 (−2.79-4.42)	.66
ECOG ≥ 2	−0.69 (−3.90-2.52)	.67	−1.63 (−5.11-1.85)	.36
Renal/adrenal involvement	3.89 (−0.54-8.32)	.086	2.29 (2.45-7.03)	.34
Raised LDH	1.17 (−1.86-4.19)	.45	0.03 (−3.27-3.32)	.99
ITs given			1.21 (−2.59-5.00)	.53
HD-MTX doses ≥2			−2.43 (−7.95-3.10)	.39
Cumulative dose >6 g/m^2			−3.59 (−6.84 to −0.35)	.030
Isolated CNS relapse, MVA				
Age (for a 10-y increase)	1.41 (0.52-2.31)	.002	1.47 (−0.44-2.49)	.005

Survival time is measured up to 10 y; for example, in univariable analysis, a patient given EOT HDMTX has a CNS-relapse-free life expectancy over 10 y that is 0.43 mo shorter than for a patient given i-HD-MTX. The MVA shows variables remaining significant with backward selection (*P* value for rejection =.05). With a rare event, lifetime lost is not easily clinically interpretable, but at 3 years, this translates to a difference in cumulative incidence of 6.58% for patients with renal and adrenal involvement when compared with those without, and an increase in incidence of 1.12% for each decade of age.

ECOG, Eastern Cooperative Group performance status; EOT, end of treatment; HD-MTX, high-dose methotrexate; IT, intrathecal; LDH, lactate dehydrogenase; MVA, multivariable analysis; UVA, univariable analysis.

observed rates of febrile neutropenia, mucositis, and renal toxicity (all 15% to 17%) associated with i-HD-MTX are of concern, particularly when the benefit is questionable.

Concurrent IT therapy was used in a significant proportion of patients, particularly in the EOT group, likely due to clinician concern that some form of CNS-directed therapy should be delivered early. However, there is cumulative data to suggest that IT therapy is ineffective in reducing CNS relapses in DLBCL, including a large systematic review of over 7000 DLBCL patients, which demonstrated no benefit of standalone IT therapy in preventing CNS relapse.[10] We demonstrate that the use of concurrent IT prophylaxis was not associated with a reduction in CNS relapse on multivariable analysis, and there was no evidence of an interaction with HD-MTX timing. However, all patients were given HD-MTX, and therefore we were unable to assess whether IT prophylaxis without HD-MTX shows benefit.

The overall rate of CNS relapse observed raises concern about any potential efficacy of HD-MTX, irrespective of delivery timing. The observed overall 3-year rate of 5.7% was only marginally less than the predicted risk of 7% when the CNS-IPI risk model was applied to our cohort. Furthermore, our 3-year cumulative incidence of CNS relapse in high CNS-IPI patients was 9.1%,

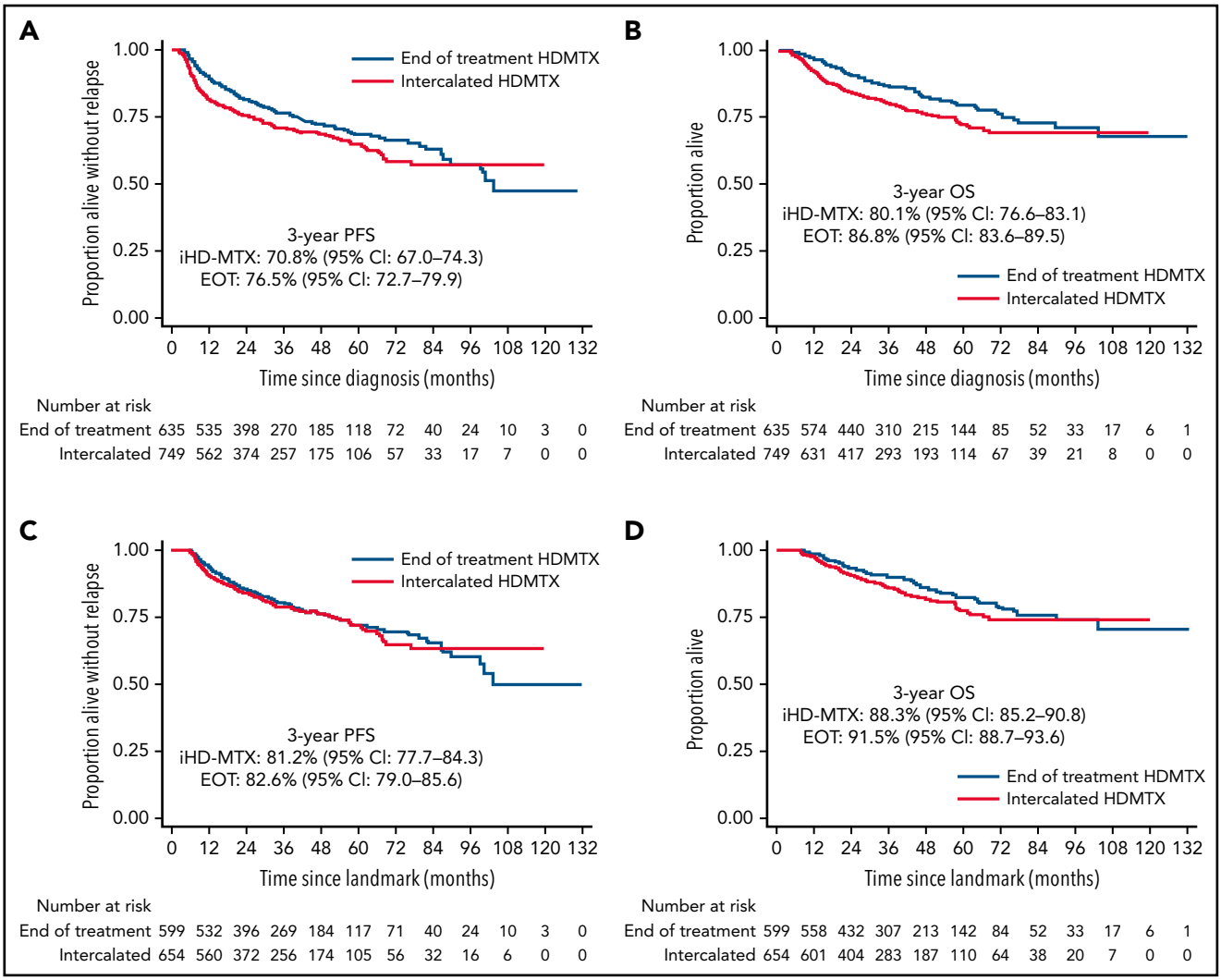

Figure 2. Progression-free and overall survival. Whole cohort (A-B) and landmark cohort (C-D).

which is almost identical to that observed in the original CNS-IPI study, where no systemic HD-MTX was used in the design cohort and very few in the validation cohort.[6] Recent retrospective analyses demonstrate no apparent benefit of HD-MTX prophylaxis,[15-17] including a multicenter analysis of approximately 2300 high-risk patients, which found no difference in CNS relapse between patients receiving HD-MTX vs not.[19] Furthermore, the overall rate of CNS relapse of 9% in the latter study, which included 1890 patients receiving no HD-MTX, was identical to the rate observed in patients with CNS-IPI 4-6 in our analysis.

To answer the question of HD-MTX efficacy definitively, a randomized controlled trial of HD-MTX vs no prophylaxis is required, but sample size would present significant logistical challenges. Our data, in conjunction with other recent literature, suggest a limited benefit for HD-MTX for the majority of DLBCL patients, irrespective of the timing of delivery. However, even the large Lewis and colleagues analysis is limited in its ability to exclude the benefit of HD-MTX in the highest risk subgroups, such as those with CNS-IPI 6 or with high-risk EN site involvement (eg, testicular and breast). There is also prospective data to suggest a benefit of HD-MTX for patients with testicular DLBCL, with recently presented results from the IELSG30 trial demonstrating no CNS relapses following IV and IT CNS prophylaxis.[31]

To date, no other agent has been shown to reduce the risk of CNS relapse in DLBCL. Novel agents, such as ibrutinib and lenalidomide, have been proposed as potential agents capable of influencing CNS relapse risk due to their ability to cross the blood-brain barrier. Although both agents have shown promising activity in primary and secondary CNS involvement with B-cell malignancies, neither have shown overall benefit for patients with DLBCL when incorporated into R-CHOP in large prospective trials.[32,33] Whether these drugs could specifically benefit the small subset of patients at most risk of CNS relapse remains an unanswered question. Until a more effective prophylactic strategy is demonstrated, some may still reasonably choose to use HD-MTX for the most high-risk patients, and we provide valuable data to support decision-making around its delivery.

Table 5. Univariable and multivariable analyses of risk factors for any delay of ≥7 d during frontline therapy

	Univariable			Multivariable	
Risk factor	**Events/n**	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
≥7-d delay (all patients)					
HD-MTX approach					
Intercalated	196/743	1.00	<.0001	1.00	<.0001
EOT	79/616	0.41 (0.31-0.55)		0.44 (0.33-0.59)	
Age (for an increase of 10 y)	275/1359	0.96 (0.87-1.06)	.37	0.92 (0.82-1.04)	.20
Sex					
Male	166/825	1.00	.90	1.00	.95
Female	109/534	1.02 (0.78-1.33)		0.99 (0.75-1.32)	
Advanced stage					
Stage 1-2	46/221	1.00	.82	1.00	.90
Stage 3-4	229/1138	0.96 (0.67-1.37)		0.97 (0.63-1.50)	
ECOG					
0-1	210/1004	1.00	.32	1.00	.43
2+	65/353	0.85 (0.63-1.16)		0.88 (0.63-1.22)	
2+ extranodal sites					
<2	115/576	1.00	.83	1.00	.62
2+	160/783	1.03 (0.79-1.35)		1.08 (0.79-1.48)	
LDH					
Normal	93/401	1.00	.12	1.00	.088
>ULN	180/925	0.80 (0.60-1.06)		0.76 (0.56-1.04)	
Baseline CrCl	272/1321	0.94 (0.68-1.30)	.71	0.73 (0.49-1.10)	.14

A more conservative analysis which excluded any patient in the iHDMTX group given <6 cycles of treatment (ie, a patient group who may not have been given EOT MTX even if it was the intention) found very similar results for treatment approach: HR: 0.44 (0.33-0.59), $P < .001$ (UVA); and HR 0.47 (0.35-0.64), $P < .001$ (MVA).

CI, confidence interval; CrCl, creatinine clearance; ECOG, Eastern Cooperative Group performance status; EOT, intercalated; HD-MTX, high-dose methotrexate; LDH, lactate dehydrogenase; OR, odds ratio; ULN, upper limit of normal.

The strengths of this study are the multicenter design, large sample size, preplanned power calculation, and the granularity of data, particularly with regards to HD-MTX delivery and CNS relapse. The main limitations are those inherent to retrospective, nonrandomized observational analyses, with potential for selection bias and imbalance between treatment groups, in particular, the immortal time bias for EOT patients due to the lack of recorded data on "intention-to-treat with EOT HD-MTX." The EOT cohort could not, by definition, have experienced an event during therapy and remained fit to receive HD-MTX at this point. This may have excluded frailer patients who experienced delays during immunochemotherapy. However, both groups were extremely well balanced for baseline characteristics, with all analyses of relapse and survival including adjusted models to account for potential imbalances, and importantly, our results held within the landmark cohort, who should not be prone to immortal time bias. The selection criteria for CNS prophylaxis varied between centers, reflecting the limited evidence to guide such decisions, particularly before the introduction of the CNS-IPI. Only 50% of patients had baseline CNS evaluation, which introduces a potential risk of selection bias and of including patients with occult CNS involvement at diagnosis.

In conclusion, in an international cohort of 1384 patients, we demonstrate that delivery of EOT HD-MTX did not increase the risk of CNS relapse compared with early integration during R-CHOP/R-CHOP-like therapy. The CNS relapse rate observed in high-risk patients in our study was relatively high despite the use of HD-MTX, raising further concern about the efficacy of HD-MTX as CNS prophylaxis. We cannot conclude from our data that HD-MTX, intercalated or not, does not benefit a small subset of very high-risk patients, although we recognize that usage is likely to decrease substantially in light of the recently presented and published data. In the selected patients where HD-MTX may still be considered, we provide data to support EOT delivery for most patients. i-HD-MTX should be used with caution in older patients or those at increased risk of toxicity, and if employed, the HD-MTX should be delivered earlier in the R-CHOP cycle (prior to day 10) to reduce R-CHOP delays. It may be that investigating the incorporation of novel agents and

Table 6. Risk factors for delays following intercalated HD-MTX

Risk factor	Univariable			Multivariable	
	Events/n	OR (95% CI)	*P*	OR (95% CI)	*P*
Age (for an increase of 10 y)	214/748	1.20 (1.05-1.36)	.006	1.16 (1.00-1.35)	.055
Sex					
Male	131/447	1.00	.61	1.00	.74
Female	83/301	0.92 (0.66-1.27)		0.95 (0.67-1.33)	
Advanced stage					
Stage 1-2	30/102	1.00	.85	1.00	.82
Stage 3-4	184/646	0.96 (0.60-1.51)		1.06 (0.63-1.81)	
ECOG					
0-1	163/548	1.00	.26	1.00	.37
2+	51/200	0.81 (0.56-1.17)		0.84 (0.57-1.23)	
2+ extranodal sites					
<2	87/303	1.00	.96	1.00	.98
2+	127/445	0.99 (0.72-1.37)		1.00 (0.70-1.45)	
LDH					
Normal	69/212	1.00	.15	1.00	.21
>ULN	145/532	0.78 (0.55-1.10)		0.79 (0.54-1.15)	
Baseline CrCl (for an increase of 100)	212/738	0.66 (0.44-0.99)	.043	0.84 (0.52-1.37)	.48

MVA, with backward selection (P = .05 for inclusion), age is the only factor that remains: OR: 1.19 (1.05-1.35), P = .008 (n = 735). Note, this is slightly different from the UVA quoted (despite being the only variable left) as it included complete cases only.

See Table 5 for definitions.

using more sophisticated techniques (eg, CSF ctDNA) to identify high-risk patients are areas where the field should focus attention.

Acknowledgments

The authors thank the following health care professionals for their expert dedication to data collection: Catherine Thieblemont (Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris), Sridhar Chaganti (University Hospitals Birmingham), George Follows (Cambridge University Hospitals NHS Foundation Trust), Anca Prica (Princess Margaret Cancer Centre), Adam Olszewski (Brown University and Lifespan Cancer Institute), Barbara Botto (AOU Città della Salute e della Scienza di Torino), Corinne Haioun (Hospital Henri Mondor), Caroline Besson (Centre Hospitalier de Versailles), Olivier Tournilhac (Service d'Hématologie et de Thérapie Cellulaire, CHU Estaing, Université Clermont Auvergne), Pietro Di Ciaccio (St Vincent's Hospital Sydney), Agnes Olivrie and Julie Abraham (Hématologie Clinique et Thérapie Cellulaire, CHU de Limoges), Dipti Talaulikar and Caitlin Coombes (Australian National University and Canberra Health Services), Raul Cordoba (Fundacion Jimenez Diaz University Hospital, Health Research Institute), Adolgo de la Fuente (MD Anderson, Madrid, Spain), Rebecca Oliver and Laura Percy (University Hospitals Bristol NHS Foundation Trust), Kamel Laribi and Catherine Truong (Centre Hospitalier Le Mans, Le Mans), Ruth Clifford (University Hospital Limerick), Jordan Carter and Andrew Evens (Rutgers Cancer Institute), Brian Henessy (University Hospital Waterford), Wendy Osborne and Thomas Creasey (Newcastle Hospitals NHS Foundation Trust), and Javier Penalver and Maria Garcia Roa (Hospital Universitario Fundacion Alcorcon).

Authorship

Contribution: M.R.W., T.A.E., A.A.K., K.C., and P.M. designed the study, analyzed data, and wrote the paper; A.A.K. performed all statistical analyses; and all authors participated in the collection of data and writing/reviewing the manuscript.

Conflict-of-interest disclosure: M.R.W. received conference fees from Takeda, Janssen, and Kite/Gilead; honoraria from Abbvie and Kite/Gilead. T.A.E. received honoraria from Roche, Kite/Gilead, Janssen, Abbvie, AstraZeneca, Loxo Oncology, Beigene, and Secura Bio; provided consultancy for Roche, Abbvie, Loxo Oncology, Incyte, Secura Bio. M.A. received honoraria from Takeda and Roche; research funding from Pfizer. E.S. received honoraria from Riemser Pharma GmbH; research funding from Roche and Abbvie. M.K. provided consultancy to Roche, Antegene, and Genor Biopharma. M.N. received research funding from TG Therapeutics, Genmab, Genentech/Roche, and Gilead. K.L.L. received honoraria from AstraZeneca, Janssen, and Roche; patents and royalties from Janssen and Novartis; provided consultancy to AstraZeneca. A.K.Ø. received travel expenses from Abbvie. A.S. received honoraria from Janssen. N.S. received honoraria and membership on an entity's board of directors or advisory committees for Abbvie, Janssen, and Roche. L. Roulin received travel expenses from Janssen. K.M. received travel and meeting expenses from Bristol-Myers Squibb. N.H. holds membership on an entity's board of directors or advisory committees and speakers bureau for Novartis. A.G. received speaker honoraria from Roche, Janssen, Abbvie, Celgene, Fresenius, and Novo Nordisk; travel and accommodation expenses from Roche, Janssen, and Abbvie. T.C.E. ended employment in the past 24 months with Roche; received speaker fee from Abbvie. C.Y.C. provided consultancy, received honoraria and other (advisory) for/from Roche, Janssen, MSD, Gilead, Ascentage Pharma,

Beigene, AstraZeneca, Loxo/Lilly, and TG Therapeutics; received research funding from Abbvie and Celgene. A.J.M.F. has membership on an entity's board of directors or advisory committees for Gilead, Novartis, Juno, PletixaPharm, Roche, and Incyte; received research funding from BMS, Beigene, Pharmacyclics, Hutchison Medipharma, Amgen, Genmab, ADC Therapeutics, Gilead, Novartis, and Pfizer. C.P.F. received honoraria and has membership on an entity's board of directors or advisory committees and received research funding for/from Roche; received speaker fees from Janssen. K.C. provided consultancy and received travel expenses to scientific conferences and speakers bureau for/from Roche, Janssen, Kite/Gilead, and Takeda; provided consultancy and speakers bureau for Gilead and Incyte; provided consultancy for Celgene and Atara; received travel expenses to scientific conferences for BMS/Celgene. P.M. received honoraria and has membership on an entity's board of directors or advisory committees from/for Roche, Kite, Takeda, and Beigene; received travel support from Gilead and Janssen. The remaining authors declare no competing financial interests.

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Footnotes

Submitted 28 October 2021; accepted 30 December 2021; prepublished online on *Blood* First Edition 7 January 2022. DOI 10.1182/blood.2021014506.

These data were presented at the 63rd Annual Meeting of the American Society of Hematology, 11-14 December 2021.

Qualified researchers may request data from the corresponding author.

The online version of this article contains a data supplement.

There is a *Blood* Commentary on this article in this issue.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

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Supplementary materials – methods:

Power calculation:

Based on previous studies, we assumed that the rate in the i-HD-MTX group would be approximately 5% at 3 years and that 60% would receive i-HD-MTX and 40% EOT. Using a 2.5% 1-sided alpha, recruiting 1200 or more patients would result in ~80% power to exclude this difference (60 events).

Supplementary Materials Table 1: Reasons for CNS prophylaxis:

	All N=1384	**End of treatment** N=635	**Intercalated** N=749
Indication for CNS Prophylaxis			
Double/Triple hit lymphoma	8 (0.6)	5 (0.8)	3 (0.4)
EN sites 2+ & high LDH	179 (12.9)	64 (10.1)	115 (15.4)
High CNS IPI	432 (31.2)	209 (32.9)	223 (29.5)
Number of EN sites	68 (4.9)	41 (6.5)	27 (3.6)
Other/unknown	56 (4.1)	29 (4.6)	27 (3.6)
Specific high-risk site	641 (46.3)	287 (45.2)	354 (47.3)
Bone	*78 (12.2)*	*24 (8.4)*	*54 (15.3)*
Breast	*43 (6.7)*	*11 (3.8)*	*32 (9.0)*
Craniofacial	*81 (12.6)*	*36 (12.5)*	*45 (12.7)*
Kidney/adrenal	*88 (13.7)*	*39 (13.6)*	*49 (13.8)*
Paraspinal	*87 (13.6)*	*39 (13.6)*	*48 (13.6)*
Testicular	*146 (22.8)*	*81 (28.2)*	*65 (18.4)*
*Other**	*75 (11.9)*	*48 (16.1)*	*27 (7.6)*
Unknown	*43 (6.7)*	*9 (3.1)*	*34 (9.6)*

**other sites were bone marrow, bowel, heart, liver, lung, ovary, pancreas, parotid/salivary glands, peritoneum, pleura, prostate, skin/soft tissue, stomach, tonsils, uterus.*

EN, extranodal; LDH, lactate dehydrogenase; CNS IPI, central nervous system international prognostic index ;

Supplementary Materials Table 2: Treatment details for whole study cohort

	All N=1384	End of treatment N=635	Intercalated N=749	P
Chemotherapy regimen, N (%)				
R-CHOP-14	130 (9.4)	62 (9.8)	68 (9.1)	
R-CHOP-21	1210 (87.4)	540 (85.0)	670 (89.5)	
Other*	44 (3.2)	33 (5.2)	11 (1.4)	
Six cycles of chemotherapy given, N (%)	1271 (91.8)	582 (91.7)	689 (92.0)	0.82
Number of cycles of chemotherapy, median (range)	6.0(1 - 8)	6.0(2 - 8)	6.0(1 - 8)	0.0005
Number of cycles of chemotherapy				
3 and under	25 (1.8)	3 (0.5)	22 (2.9)	0.003*
4-6	1220 (88.2)	595 (93.7)	625 (83.4)	
7-8	139 (10.0)	37 (5.8)	102 (13.6)	
Number of cycles of HD-MTX, median (range)	2.0(1 - 8)	2.0(1 - 6)	2.0(1 - 8)	<0.0001
IT prophylaxis given, N (%)	636 (46.1)	352 (55.7)	284 (38.0)	<0.0001
Missing/unknown	4	3	1	
No. of ITs given, median (range)**	2 (1-12)	3 (1-6)	1 (1-12)	<0.0001
Two cycles+ HD-MTX given?, N (%)	1198 (86.6)	557 (87.7)	641 (85.6)	0.25
Number cycles of HD-MTX (grouped)				
1	186 (13.4)	78 (12.3)	108 (14.4)	<0.0001*
2	846 (61.1)	481 (75.7)	365 (48.7)	
≥3	352 (25.4)	76 (12.0)	276 (36.8)	
Cumulative HD-MTX dose (g/m2), median (range)	6.0(1 - 24)	6.0(1 - 24)	6.0(1 - 24)	<0.0001
Cumulative dose HD-MTX (g/m2)				
≤6	883 (64.2)	484 (76.8)	399 (53.6)	<0.0001
>6	492 (35.8)	146 (23.2)	346 (46.4)	
Missing/unknown	9	5	4	

***Other regimens were R-miniCHOP (n=8), R-CHOEP (n=7), R-CEOP (n=4), R-COMP (n=15), R-GCVP (n=2), R-CHOP-21 x 4 and IVE x 2 n=8)*

***For those patients receiving IT therapy*

HD-MTX, high dose methotrexate; IT, intrathecal.

Supplementary Materials Table 3: Baseline characteristics and treatment details of landmark cohort (patients alive and free from progression at 6 months)

	All N=1253	End of treatment N=599	Intercalated N=654	p-value
Baseline				
Age (years) , median (range)	62.0 (17 - 88)	63.0 (18 - 86)	62.0 (17 - 88)	0.033
Baseline Creatinine Clearance, median (range)	97.8 (33.3 - 332)	94.4 (33.3 - 304.0)	101.6 (35.5 - 332)	<0.0001
Male Sex, N (%)	754 (60.2)	367 (61.3)	387 (59.2)	0.45
Advanced stage, N (%)	1032 (82.4)	476 (79.5)	556 (85.0)	0.010
Raised LDH baseline, N (%)	832 (68.1)	381 (66.8)	451 (69.3)	0.36
Missing/unknown	32	29	3	
ECOG ≥2, N (%)	309 (24.7)	144 (24.2)	165 (25.2)	0.6
Missing/unknown	3	3	0	
Extra-nodal sites, N (%)				
0-1	541 (43.2)	268 (44.7)	273 (41.7)	0.30*
2	382 (30.5)	179 (29.9)	203 (31.0)	
≥3	330 (26.3)	152 (25.4)	178 (27.2)	
Renal or adrenal involvement, N (%)	204 (16.3)	94 (15.7)	110 (16.8)	0.59
Testicular involvement, N (%)	164 (13.1)	92 (15.4)	72 (11.0)	0.022
Breast involvement, N (%)	164 (13.1)	92 (15.4)	72 (11.0)	0.022
Double or triple hit, N (%)	54 (5.6)	28 (6.3)	26 (5.0)	0.41
Missing/unknown	288	151	137	
CNS IPI, N (%)				
Low (0-1)	199 (16.2)	106 (18.3)	93 (14.2)	0.24*
Intermediate (2-3)	510 (41.4)	229 (39.6)	281 (43.0)	
High (4-6)	522 (42.4)	243 (42.0)	279 (42.7)	
Missing/unknown	22	21	1	
Baseline PET performed?	1013 (80.9)	515 (86.0)	498 (76.3)	<0.0001
Baseline CNS assessment	645 (51.5)	366 (61.1)	279 (42.7)	<0.0001
Treatment				
Chemotherapy regimen, N (%)				
R-CHOP-14	125 (10.0)	61 (10.2)	64 (9.8)	
R-CHOP-21	1087 (86.8)	506 (84.4)	581 (88.8)	
Other*	41 (3.2)	32 (5.4)	9 (1.4)	
Six cycles of chemotherapy given, N (%)	1176 (93.9)	550 (91.8)	626 (95.7)	0.0041
Number of cycles of chemotherapy	6.0(2 - 8)	6.0(2 - 8)	6.0(2 - 8)	<0.0001
Number of cycles of chemotherapy				
3 and under	11 (0.9)	3 (0.5)	8 (1.2)	<0.0001*
4-6	1113 (88.8)	561 (93.7)	552 (84.4)	
7-8	129 (10.3)	35 (5.8)	94 (14.4)	

	All N=1253	End of treatment N=599	Intercalated N=654	p-value
Number of cycles of HD-MTX	2.0(1 - 8)	2.0(1 - 6)	2.0(1 - 8)	<0.0001
IT prophylaxis, N (%)	574 (45.9)	331 (55.4)	243 (37.2)	<0.0001
Missing/unknown	3	2	1	
Two cycles+ HD-MTX given?, N (%)	1096 (87.5)	526 (87.8)	570 (87.2)	0.73
Number cycles of HD-MTX (grouped)				
1	157 (12.5)	73 (12.2)	84 (12.8)	<0.0001*
2	780 (62.3)	455 (76.0)	325 (49.7)	
≥3	316 (25.2)	71 (11.9)	245 (37.5)	
Cumulative HD-MTX dose, median (range)	6.0(1 - 24)	6.0(1 - 24)	6.0(1 - 24)	<0.0001
Cumulative HD-MTX dose				
≤6	802 (64.4)	457 (76.9)	345 (52.9)	<0.0001
>6	444 (35.6)	137 (23.1)	307 (47.1)	
Missing/unknown	7	5	2	

*p-values are Chi squared for discreate variables (*for trend) and Wilcoxon Mann Whitney for continuous.*

***Other regimens were R-miniCHOP (n=8), R-CHOEP (n=5), R-CEOP (n=4), R-COMP (n=15), R-GCVP (n=2), R-CHOP-21 x 4 and IVE x 2 n=7)*

IQR, inter-quartile range; LDH, lactate dehydrogenase ; ECOG, Eastern Cooperative Oncology Group performance status; CNS IPI, central nervous system international prognostic index; HD-MTX, high dose methotrexate; IT, intrathecal.

Supplementary Materials Table 4: Details of patients excluded in landmark cohort:

Events	EOT		Intercalated	
	N	Median time (range)	N	Median time
CNS relapse	8	4.6 (2.6 – 5.9)	11	5.1 (2.6 -6.8)
Parenchymal	2		3	
Leptomeningeal	3		5	
Both	3		3	
Systemic PD	19	6.3 (5.1 – 21.8)	59	6.3 (5.0 – 21.8)
Death without relapse	3	4.6, 5.0 and 30.5	7	4.1 (0.9 – 7.3)

EOT, end of treatment; PD, progressive disease.

Supplementary materials Table 5: Results within specific high-risk groups and treatment parameters for landmark cohort

The three-year difference is calculated at 3 years post diagnosis, i.e. 30 months from the landmark

		HR* (95% CI)	Events/N	3-year rates	3-year difference (EOT – intercalated)	3-year rate (overall)
CNS IPI High						
	Intercalated	1.00	18/279	7.7% (4.9 – 12.2)	-0.7% (-4.1 to 5.7)	7.3% (5.2 – 10.3)
	End of treatment	0.90 (0.45 – 1.79)	15/243	6.8% (4.1 – 11.4)		
CNS IPI 5-6						
	Intercalated	1.00	9/95	11.2% (5.9 – 20.8)	-5.3% (-9.3 to 6.5)	7.9% (4.5 – 13.6)
	End of treatment	0.50 (0.16 – 1.64)	4/82	4.1% (1.3 – 12.2)		
Testicular involvement						
	Intercalated	1.00	6/72	6.4% (2.4 – 16.4)	-1.6% (-4.8 to 7.2)	7.1% (3.9 – 13.0)
	End of treatment	0.74 (0.25 – 2.21)	7/92	7.6% (3.5 – 16.1)		
Renal/adrenal involvement						
	Intercalated	1.00	11/110	13.3% (7.4 – 23.4)	-5.8% (-10.5 to 5.6)	9.2% (5.6 – 15.0)
	End of treatment	0.54 (0.20 – 1.47)	6/94	5.1% (1.9 – 13.1)		
Breast involvement						
	Intercalated	1.00	1/35	0%	-	6.5% (1.7 – 23.5)
	End of treatment	4.95 (0.45 – 54.8)	2/18	20.1% (5.6 – 60.3)		
3 or more extra nodal sites						
	Intercalated	1.00	8/178	5.0% (2.5 – 9.8)	1.8% (-2.2 to 11.5)	5.2% (3.2 – 8.4)
	End of treatment	1.38 (0.54 – 5.52)	10/152	5.5% (2.8 – 10.8)		
Any high-risk factor above						
	Intercalated	1.00	23/409	5.9% (3.8 – 9.0)	0.3% (-2.3 to 4.8)	6.2% (4.6 – 8.4)
	End of treatment	1.05 (0.60 – 1.86)	25/379	6.6% (4.3 – 9.9)		
Treatment parameters						
Cycles of HDMTX (interaction p = 0.23)						
< 2 cycles						
	Intercalated	1.00	3/84	2.9% (0.7 – 11.2)	3.3% (-1.3 - 19.5)	4.3% (2.0 – 9.4)
	End of treatment	2.16 (0.54 – 8.66)	6/72	5.9% (2.3 – 15.0)		

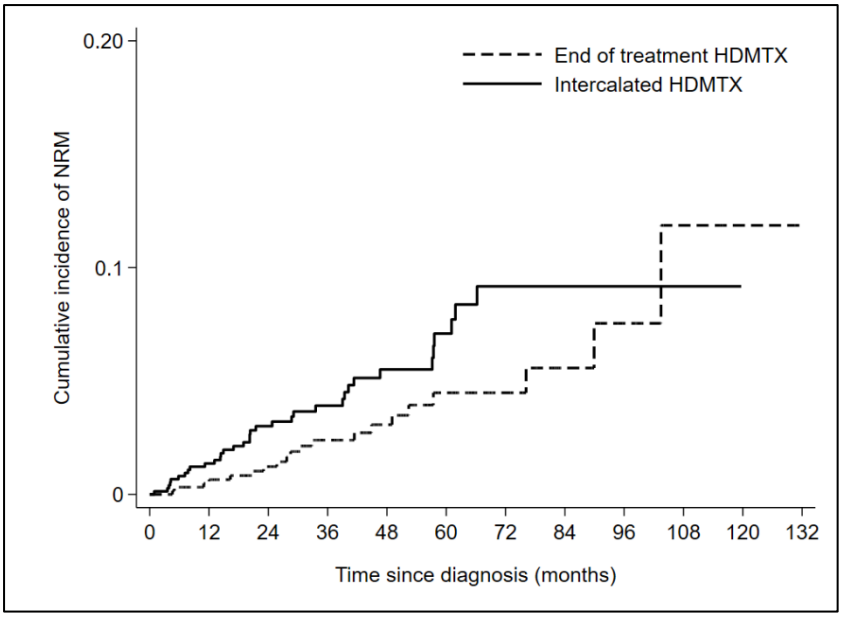
≥2 cycles						
	Intercalated	1.00	27/570	5.0% (3.6 – 7.3)	-0.6% (-2.5 to 2.5)	4.8% (3.6 – 6.4)
	End of treatment	0.87 (0.50 – 1.52)	23/527	4.6% (2.9 – 7.0)		
ITs (interaction p = 0.93)						
Not given						
	Intercalated	1.00	18/410	4.0% (2.4 – 6.5)	-0.3% (-2.2. to 3.6)	4.4% (3.0 – 6.4)
	End of treatment	0.92 (0.43 – 1.94)	11/266	4.9% (2.7 – 8.8)		
Given						
	Intercalated	1.00	12/243	4.6% (2.7 – 7.9)	-0.2% (-3.1. to 5.7)	5.2% (3.5 – 7.6)
	End of treatment	0.96 (0.46 – 2.02)	17/331	5.9% (3.4 – 10.3)		
Cumulative dose (interaction p = 0.14)						
≤6g/m2						
	Intercalated	1.00	15/345	3.9% (2.2 – 6.7)	0.8% (-1.4 to 4.7)	4.9% (3.5 – 6.8)
	End of treatment	1.20 (0.63 – 2.28)	25/457	5.5% (3.6 – 8.4)		
>6g/m2						
	Intercalated	1.00	15/307	5.7% (3.4 – 9.5)	-3.2% (-4.5 to 2.4)	4.6% (2.9 – 7.2)
	End of treatment	0.42 (0.12 – 1.45)	3/137	2.2% (0.7 – 6.8)		

**EOT vs intercalated. Events post 3 years: 8 events (5 EOT and 3 intercalated). Five-year rates: EOT: 6.3 (4.3 – 9.2) and 5.7% (3.8 – 9.7) intercalated.*

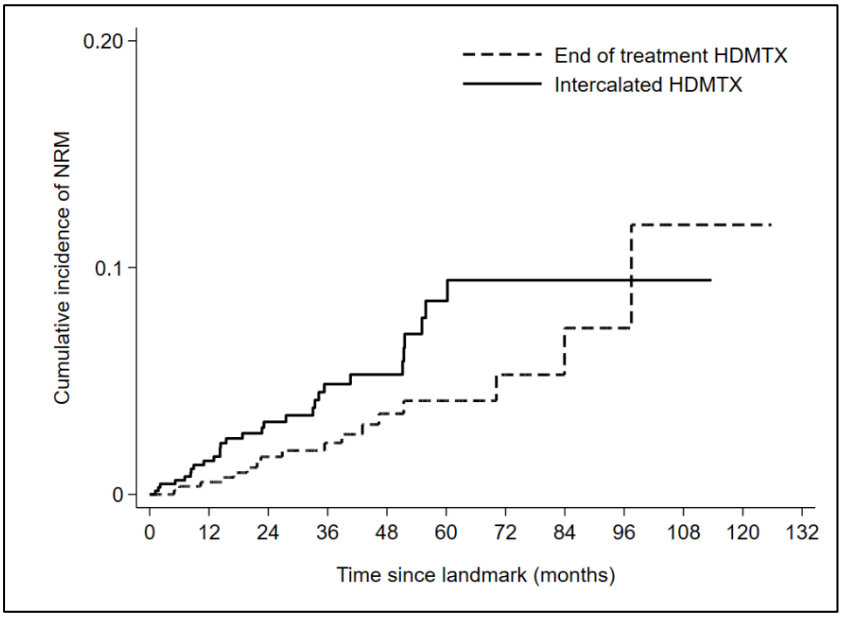
High risk CNS IPI: 7.8% (4.7 – 12.9) EOT and 7.7% (4.9 – 12.2) intercalated. High risk (all factors): 8.5% (5.6 – 12.6) EOT and 7.4% (4.7 – 11.6) intercalated

HR, hazard ratio; EOT, end of treatment; CNS IPI, central nervous system international prognostic index; IT, intrathecal; HD-MTX, high dose methotrexate

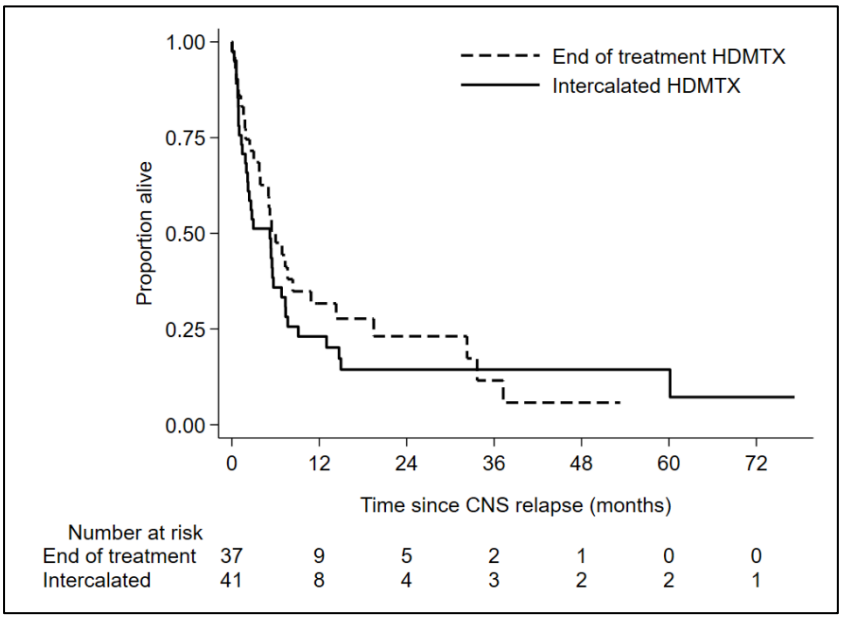
Supplementary materials Figure 1A – cumulative incidence of non-relapse mortality according to HD-MTX timing, whole study cohort (n=1384)



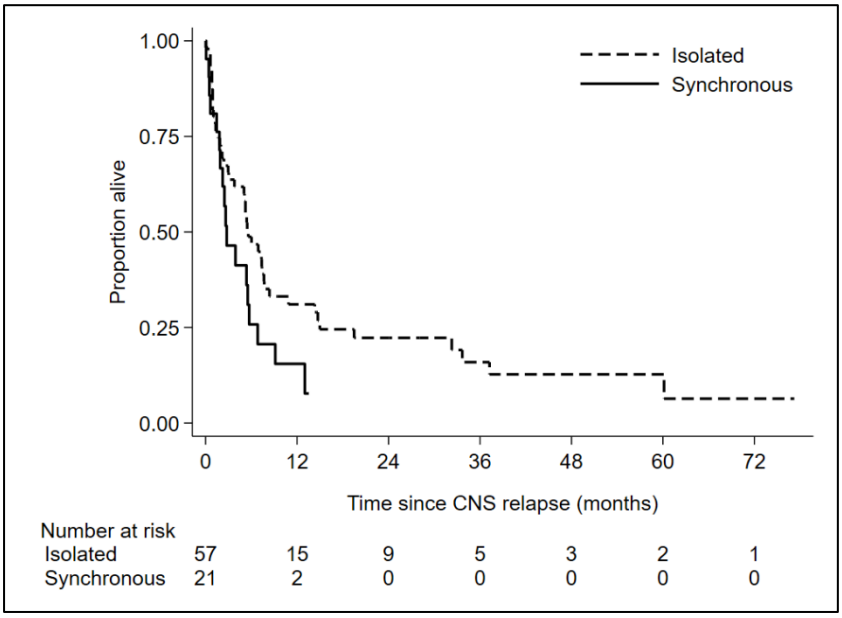
Supplementary materials Figure 1B – cumulative incidence of non-relapse mortality according to HD-MTX timing, landmark cohort (n=1253)



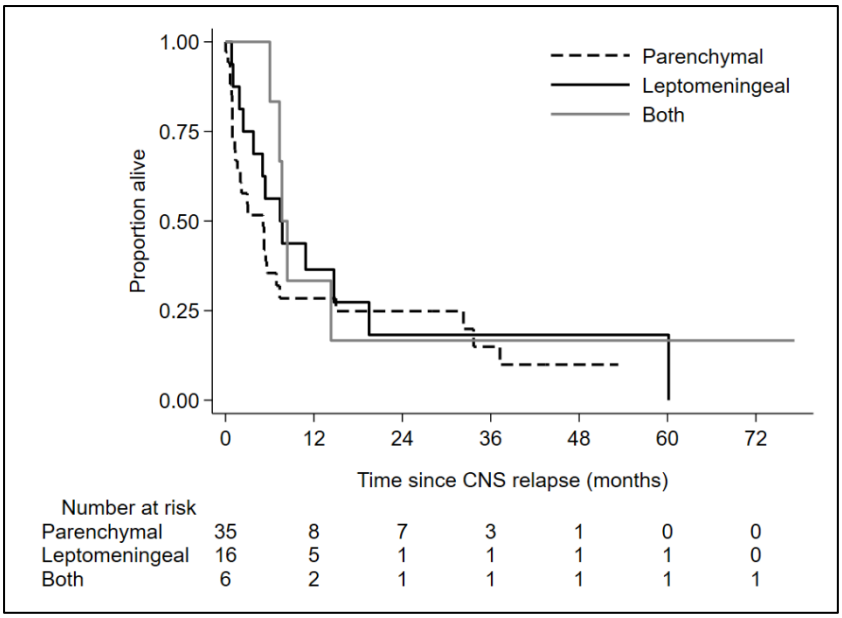
Supplementary Materials Figure 2A – overall survival in patients with any CNS relapse according to HD-MTX timing



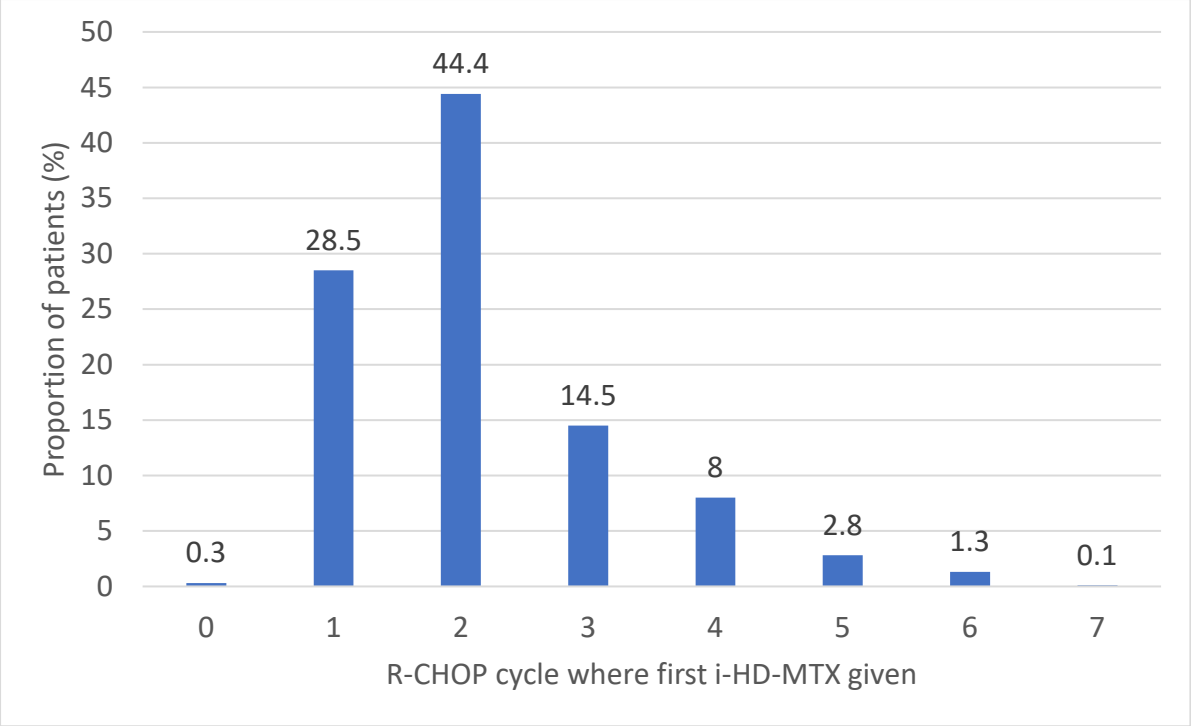
Supplementary Materials Figure 2B – overall survival according to isolated CNS relapse vs synchronous systemic/CNS relapse



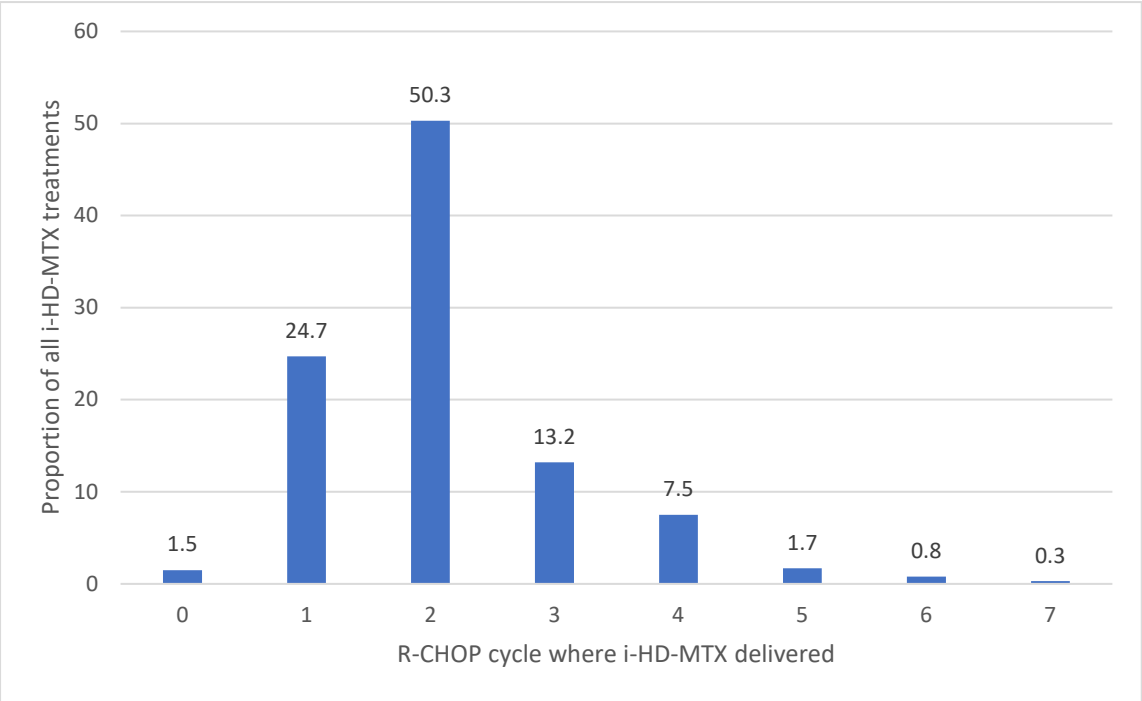
Supplementary Materials Figure 2C – overall survival according to site of CNS relapse



Supplementary Materials Figure 3a: timing of first delivery of intercalated HD-MTX:



Supplementary Materials Figure 3b: timing of delivery of all intercalated HD-MTX treatments



Supplementary results: Delays at each dose of HD-MTX

Mixed effects logistic regression models were used to assess delays at each dose of i-HD-MTX. This included risks factors as in ***Table 6***, as well as the timepoint of delivery in the R-CHOP cycle, HD-MTX dose number (1, 2 or 3+) and the dose (<3/≥3g/m^2). Again, age was the only baseline factor significantly associated with an increase in delays, though there were significant interactions with timing, dose and cycle number. A 10-year increase in age showed a reasonably large increase in risk of delay (ORs 2.44 cycles 1-2, 1.73 cycles 3-4 and 3.48 cycles 5+) when 3g/m^2 or more was given after 10 days, but no increase in risk for lower doses or when given earlier. Similarly, if we consider timing, we see differential results within the older and younger cohort; OR (<10 days vs ≥10 days): 0.86 (0.38 – 1.99) for patients ≤60 and 2.15 (1.91 – 4.56) for patients >60 years.

Dose

Firm conclusions about the associations between dose and delay are hard to draw in this population, as lower first doses were given to patients who were older (3.5% of patients aged ≤60 years started on <3g/m^2 vs 17.5% in >60 years), had lower creatinine clearance (median 84.4ml/min for those on <3g/m^2 vs 103.7 for those ≥3g/m^2, p <0.0001) or were ECOG 2+ (9.7% <3g vs 15.2%, p=0.036) , and delays post dose 1 were associated with dose reductions for dose 2 (doses decreased for 2.3% of patients without a delay compared to 9.0% for those with a delay, p = 0.001).

Cycle number

The effect of cycle number was not clear. Delays appeared to be less common in cycle 5+ (compared to 1-2) regardless of age, but the effects differed by age for cycles 3-4 which appeared to show an increase in risk in younger patients but no difference in older patients, a finding we cannot explain.

As HD-MTX delivery was decided by site, and may have been guided by the deliverability of previous cycles, we also looked at delays for dose 1 alone. The same patterns for age and timing and were seen: for doses ≥3g/m^2, the OR for an increase of 10 years was 1.08 (0.86 – 1.36) when delivered <10 days into the cycle and 1.45 (1.16 – 1.82) for ≥10 days, and the OR for ≥10 days vs <10 days was 0.78 (0.43 – 1.42) for age≤60 and 1.77 (1.09 – 2.89) for age >60. The interaction with cycle and age remained; driven by different effects for cycles 3-4, though cycle 5+ doses no longer showed a reduction in risk. This might suggest that the cycle 5 results could be biased by dose number. 46.7% of the doses given from cycle 5 onwards were the 3rd or later dose, (compared to 0% and 29.5% for cycles 1-2 and 3-4) i.e. patients planned for cycle 5+ doses may have stopped early if they did not tolerate those given earlier.

PAPER 4

Eyre TA, Savage KJ, Cheah CJ, El-Galaly TC, Lewis KL, McKay P, **Wilson MR,** Evens AM, Bobillo S, Villa D, Maurer MJ, Cwynarski K, Ferreri AJ. CNS prophylaxis in diffuse large B-cell lymphoma. Lancet Oncology 2022 September 1;23(9):E416-426 PMID: 36055310

https://www.thelancet.com/journals/lanonc/article/PIIS1470-2045(22)00371-0/fulltext
Journal impact factor: 51.1
Number of citations: 13

Summary of contribution:

Conceptualisation	Yes – with all co-authors
Data Curation	N/A
Formal Analysis	N/A
Investigation	N/A
Methodology	N/A
Project Administration	TE
Visualisation	TE
Writing – original draft	I co-wrote section of the review on 'Arguments against CNS prophylaxis' with TEG
Writing – review & editing	Led by TE, I assisted with reviewer responses and final proof checking

CNS prophylaxis for diffuse large B-cell lymphoma

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CNS relapse in the brain parenchyma, eyes, or leptomeninges is an uncommon but devastating complication of diffuse large B-cell lymphoma. CNS prophylaxis strategies, typically involving intrathecal or high-dose antimetabolites, have been developed in the front-line treatment setting with the aim to reduce this subsequent risk. Clinical and biological features associated with elevated risk are increasingly well defined and are discussed in this Review. This Review summarises both the historical and current developments in this challenging field, provides a nuanced discussion regarding current reasons for and against standard prophylactic measures, outlines evidence for the timing of prophylactic measures when delivered, and reflects on possible future developments.

Lancet Oncol 2022; 23: e416–26

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Introduction

CNS involvement is an uncommon and often fatal event, occurring in around 5% of patients with systemic diffuse large B-cell lymphoma (DLBCL) during primary treatment or shortly after completion. Tumour cells reach the CNS by the haematogenous route, direct infiltration from neighbouring organs, or dissemination through neurovascular axes, and affect the brain parenchyma, meninges, the cerebrospinal fluid, or the eyes. The current management strategy consists of identifying patients with an increased risk of CNS recurrence and incorporating CNS-penetrating treatments into front-line therapy as prophylaxis. In this Review, we critically analyse available evidence supporting the use of prognostic models for CNS relapse and the different CNS prophylaxis strategies used. We discuss data for and against the most used prophylactic options and consider open questions for future studies.

CNS relapse of DLBCL

Secondary CNS lymphoma: clinical risk factors

There has been considerable interest in defining patients at high risk of CNS relapse. The CNS International Prognostic Index (CNS-IPI) is the best-validated prognostic model developed in the rituximab era. It is comprised of the standard five IPI factors (age >60 years, elevated lactate dehydrogenase, performance status ≥2, extranodal sites >1, stage 3 or 4 disease), as well as kidney or adrenal involvement, for a total of six risk factors.[1] The risk model was developed in aggressive B-cell lymphoma (80% DLBCL) patients from the German High Grade Non-Hodgkin Lymphoma Study Group and validated in a population-based database of rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone or prednisolone (R-CHOP)-treated patients with DLBCL from the BC Cancer Agency. This model stratifies patients into low, intermediate, and high-risk groups, with the high-risk group representing 12% of patients in clinical trials and 23% of patients in real-world settings who have a risk of CNS relapse of 10% or higher (table 1).[1,3,4] In this model, the low-risk group (n=1002 [46·4%]) score 0–1 point, the intermediate-risk group score 2–3 points (n=896 [41·5%]), and the high-risk group score 4–6 points (n=263 [12·2%]). The respective 2-year rates for the occurrence of CNS relapse were 0·6% (95% CI 0%–1·2%) for the low-risk group, 3·4% (2·2%–4·4%) for the intermediate-risk group, and 10·2% (6·3%–14·1%) for the high-risk group.

Although the CNS-IPI is useful to compare studies and evaluate the independent relevance of biomarkers, it does not capture the full spectrum of patients at high risk and has low specificity. Moreover, the CNS-IPI does not delineate which patients benefit, and which do not, from prophylaxis. In some studies, other high-risk extranodal sites have included bone marrow,[5] uterine,[6] testis,[7] and breast involvement[7] (table 2). With the introduction of rituximab, the risk of CNS relapse associated with some extranodal sites (eg, sinus) appears to have diminished.[12] Further, a retrospective analysis[2] of 1532 patients evaluated the impact of the number of extranodal sites identified by PET or CT on CNS relapse risk and identified a group of 144 patients (9%) with three or more extranodal sites with a 3-year cumulative CNS relapse incidence of 15·2%.

Overall, any possible difference in the incidence of CNS relapse before and after the introduction of rituximab is somewhat unclear from the available literature,[13,14] with small underpowered series suggesting only a small possible reduction.

	Number of patients	All patients with DLBCL 2-year CNS rate of relapse	Patients with low risk (0–1 factors) 2-year CNS rate of relapse	Patients with intermediate risk (2–3 factors) 2-year CNS rate of relapse	Patients with high risk (4–6 factors) 2-year CNS rate of relapse
DSHNHL*[1]	2164†	4%	0·6%	3·4%	10·2%
BC Cancer*[1]	1597	4%	0·8%	3·9%	12%
Multi-centre[2]	1532	4% (3-year)	0·4% (3-year)	3% (3-year)	11% (3-year)
GOYA[3]	1418	2·8%	0·8%	1·9%	8·9%
UK NCRI[4]	1080	1·9% (All)	0%	2·2%	5·2%

DLBCL=diffuse large B-cell lymphoma. R-CHOP=rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone or prednisolone. CNS-IPI=International Prognostic Index. DSHNHL=German Aggressive non-Hodgkin lymphoma study group now referred to as German Lymphoma Alliance. BC=British Columbia. NCRI=National Cancer Research Institute. *Studies that formed the basis for the CNS-IPI. †DLBCL n=1735 (80%); sensitivity analysis with DLBCL alone produced similar results.

Table 1: **CNS relapse risk in large scale DLBCL patient studies receiving R-CHOP or R-CHOP-like therapy according to the CNS-IPI**

	Disease frequency	2-year risk of CNS relapse	Comment
Clinical risk factors			
High risk CNS-IPI ≥4[1,3,4]	12–23%	10–12%	Robust CNS risk model; low specificity
Extranodal sites ≥3[2]	9·5%	15·3%	Greater specificity, but lower sensitivity
Kidney[1,3,4]	2%	~40%	Very high CNS risk with concurrent testicular involvement
Testicular[7]	5%	10% (limited*), 24% (advanced†)	Predominantly ABC; rituximab is not protective of CNS relapse
Uterine[6]	2%	44% (4-year)	Independent risk factor; ovarian not risk factor, isolated involvement does not seem to confer same risk, but large-scale studies are lacking
Breast[7]	<2%	16% (overall risk)	Predominantly ABC
Biomarkers			
MYC⁺ *BCL2*⁺ double hit[8]	~5–10%	13–50%	Exclusively GCB; estimates highly variable depending on selection criteria
ABC DLBCL[3,9]	30–40%	7–9%	..
MYC⁺ *BCL2*⁺ dual expressers[9]	~30%	All 9·3% CNS-IPI high 22·7% CNS-IPI intermediate 11%	Two-thirds are ABC subtype
MCD DLBCL subtype[10]	~15%	38% (overall risk)	No large-scale studies
CD5⁺ DLBCL[11]	5–10%	12·7%	No large-scale studies

DLBCL=diffuse large B-cell lymphoma. R-CHOP=rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone or prednisolone. CNS-IPI=central nervous system International Prognostic Index. ABC=activated B-cell subtype. GCB=germinal centre B-cell subtype. MCD=MYD88 and CD79B gene mutations co-occurence. *Limited=stage 1 or 2 DLBCL. †Advanced=stage 3 or 4 DLBCL

Table 2: **Clinical markers and biomarkers of CNS relapse risk in patients with DLBCL treated with R-CHOP or R-CHOP-like therapy**

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CNS relapse: impact of biology, genetics, and biomarkers

MYC translocation coupled with a *BCL2* translocation, with or without a *BCL6* translocation (ie, double-hit or triple-hit), occurs in approximately 5–10% of patients with DLBCL. Formally termed high grade B-cell lymphoma with *c-MYC* and *BCL2* or *BCL6* rearrangements by the WHO classification, this entity has historically been associated with a high CNS risk; however, studies are subject to selection bias and often include other high-grade histologies.[8] The putative risk of CNS relapse in DLBCL could relate to high-risk clinical features associated with double-hit or triple-hit rather than disease biology, although this remains incompletely explored. The activated B-cell subtype of DLBCL is also associated with a higher risk than the germinal centre B-cell subtype.[3,9] Combining the CNS-IPI and cell-of-origin phenotype defined by gene expression profiling resulted in the distinction of a high-risk subgroup (8% of the GOYA cohort) associated with a 2-year CNS relapse rate of 15%.[3] Dual expression of MYC and BCL2 proteins occurs predominantly in activated B-cell or non-germinal centre B-cell subtypes of DLBCL and could further refine risk,[9] but differing methodologies might restrict application.[3] CD5 positivity is seen in activated B-cell or non-germinal centre B-cell subtypes of DLBCL and imparts an elevated CNS relapse risk[11] (table 2).

Two independent studies integrated multi-platform genetic analyses to propose a new taxonomy of DLBCL subclassification beyond cell-of-origin, with largely overlapping groups.[15–17] The MCD and C5 clusters described are activated B-cell subtypes typified by a high frequency of *MYD88*^{L265P} or *CD79B* aberrations, or both, which are also noted in primary extranodal lymphomas of immune privileged sites (eg, CNS, testis, breast).[15] Almost 75% of MCD tumours have aberrations in genes that might facilitate immune evasion, including in MHC class 1, *PDL1* or *PDL2*, and *CD58*.[15] A separate small study[10] supported the hypothesis that CNS relapse is associated with MCD subtype (38% *vs* 8%, p=0·003), and applying a simplified hierarchical clustering based on commonly-mutated genes captured 84% of the LymphGen MCD and almost half of CNS recurrences, with the remainder being germinal centre B-cell subtypes (either EZHB [and double-hit by FISH] or within hcTP53). Therefore, next generation sequencing could more precisely identify patients at risk.

Secondary CNS lymphoma outcomes

The outcome of patients developing secondary CNS lymphoma is poor, even for those fit enough to receive intensive therapies. Prospective studies of intensive regimens followed by high-dose chemotherapy and autologous stem-cell transplantation show complete response rates of 25–63% (appendix p 1).[18–21] However, responses are often non-durable and 2-year overall survival rarely exceeds 50%. Outcomes are most favourable in patients who are able to proceed to autologous stem-cell transplantation and those receiving thiotepa-based conditioning,[18,19,21] although many are carefully selected on the basis of age, fitness, and chemosensitivity. Retrospective real-world studies of patients with secondary CNS lymphoma receiving heterogeneous treatments have reported median overall survival of approximately 6–12 months (appendix p 1).[21–24]

Historical CNS prophylaxis data

Intrathecal chemotherapy

Reports from the 1970s and 1980s showed the risk of secondary CNS lymphoma.[25] A handful of subsequent non-controlled studies suggested a benefit for intrathecal or intravenous high-dose methotrexate, or both.[26–29] A CNS relapse rate of 2·2% combining intrathecal and intravenous methotrexate was reported in a pooled analysis[28] of 974 patients with aggressive non-Hodgkin lymphoma; however, patients received consolidative therapy with other CNS-penetrating agents (ifosfamide, cytarabine), limiting the analysis. Arkenau and colleagues[29] examined 259 patients who were newly diagnosed, from 1996 to 2005. 51 (20%) patients considered high-risk received intrathecal prophylaxis,

most with 12·5 mg of single-agent intrathecal methotrexate (median 3 [min 1, max 7] doses), and the reported CNS relapse rate was 1·1%. Intrathecal therapy has historically been most commonly delivery via lumbar puncture, although an Ommaya reservoir (an intraventricular catheter delivery system) can also be used when either many intrathecal doses are required or delivered over a longer timeframe, or when lumbar punctures are technically challenging.

Additional evidence of effectiveness of intrathecal chemotherapy is largely extrapolated from patients with aggressive B-cell lymphomas with a high risk of CNS disease (up to 40%), including Burkitt lymphoma and lymphocytic lymphoma.[27,30–32] Integrated intrathecal chemotherapy is standard in these cases. Intrathecal chemotherapy has also been evaluated in primary testicular lymphoma.[33] In a prospective study (IELSG10)[34] of R-CHOP and four early intrathecal methotrexate prophylaxis doses (12 mg/dose) in R-CHOP-21 cycles 1–2, the 5-year cumulative CNS relapse incidence was 6%, comparing favourably to historical rates of 10–30%. Another analysis has questioned the contribution of intrathecal chemotherapy in primary testicular lymphoma.[35]

Intravenous high-dose methotrexate

High-dose methotrexate has been increasingly used as CNS prophylaxis in patients with DLBCL, partly due to the observation that most (70–80%) CNS relapses are parenchymal in the rituximab era and therefore unlikely to be prevented by intrathecal chemotherapy alone.[3,24] The optimal dose or timing of high-dose methotrexate remains undefined, with data to inform dosage extrapolated from primary CNS lymphoma studies. Pharmacokinetic studies show considerable variation in methotrexate concentrations among individuals receiving the same dose, leading to different drug exposure.[36] Methotrexate area under the curve is important for primary CNS lymphoma outcomes, and doses of 3 g/m² or more in a short infusion (4–6 h) appears to result in the optimal area under the curve.[37] The role of simultaneous intrathecal treatment has progressively lessened in primary CNS lymphoma when the methotrexate dose used is 3 g/m² or more,[38] but this remains to be investigated in patients with secondary CNS lymphoma.

Intrathecal prophylaxis

Although some historical data suggest a potential benefit for intrathecal prophylaxis, most clinical studies of intrathecal prophylaxis are challenging to interpret because of their retrospective (or post hoc) nature, variability in target populations and dosing strategies, simultaneous delivery of high-dose antimetabolites, and the low CNS relapse event rates. Previous studies of intrathecal methotrexate penetration suggest that therapeutic levels might only occur in the subarachnoid space and in 2–3 mm of the superficial CNS parenchyma due to interstitial fluid pressure.[39] Specific analyses of the timing of intrathecal treatment are missing. Although there is a theoretical advantage of early intrathecal delivery alongside immunochemotherapy, the evidence for this specific strategy compared with delivery at the end of induction is weak. Few studies have actively analysed specific morbidity associated with intrathecal delivery.

A systematic analysis[40] done in the anti-CD20 monoclonal antibody era assessed the role of stand-alone intrathecal prophylaxis. Three post-hoc trial analyses (RICOVER-60, RCHOP-14/21, and GOYA),[3,4,41] one prospective database, and ten retrospective series were included and a total of 7357 patients were analysed. A median of 11·9% of patients received intrathecal prophylaxis across variable risk groups or by investigator discretion. The cumulative incidence of CNS relapse ranged from 1·9% at 6·5 years to 8·4% at 5 years. Most CNS relapses (73%) involved brain parenchyma. Although a specific meta-analysis was not performed, no individual study showed a reduction of CNS relapse rate with intrathecal prophylaxis by univariable or multivariable analysis. Although toxicity data are scarce, intrathecal delivery is known to cause discomfort and one large retrospective study described an independent association with infection-related hospitalisation and use of intrathecal methotrexate in older patients who received intrathecal alongside R-CHOP.[42]

Antimetabolite prophylaxis

Agents used and evidence base

Despite the biological rationale for using CNS-penetrating agents as a prophylaxis, no randomised studies have been completed specifically in patients with aggressive B-cell lymphoma with a high risk of CNS relapse. Systemic high-dose methotrexate is widely used, but evidence supporting its efficacy to prevent CNS recurrence is conflicting. Retrospective studies investigating antimetabolites alongside or after front-line anthracycline-based chemoimmunotherapy describe a reduction of CNS relapse events in high-risk patients to 0–3%, compared with the expected rate of approximately 10%,[1] in patients receiving 1 g/m² or more of high-dose methotrexate.[43–46] The definition of high-risk varies between studies (table 3).

Intensified front-line regimens incorporating CNS-penetrating agents have been studied in phase 2 trials as an alternative prophylactic approach and also to improve systemic disease control in intermediate-risk and high-risk aggressive B-cell lymphoma. Regimens include rituximab, cyclophosphamide, vincristine sulfate, doxorubicin, methotrexate, ifosfamide, etoposide, and cytarabine (R-CODOX-M-IVAC; n=111, CNS-IPI=3–5, 2-year CNS relapse=3·6%),[52] R-CHOP plus etoposide (R-CHOEP-14) followed by cytarabine and high-dose methotrexate (n=145, age-adjusted CNS-IPI=2–3, 3-year CNS relapse=4·5%),[53] and doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone (ACVBP)

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See Online for appendix

followed by intrathecal methotrexate and 2 cycles of 3 g/m² high-dose methotrexate and consolidation including 4 cycles of ifosfamide–etoposide and cytarabine in some patients[28] (pre-rituximab era, n=974 patients with aggressive B-cell lymphoma, CNS relapse=2·2% overall; 4·1% for high-risk patients). These studies did not include a comparative group of patients with similar characteristics treated without CNS-penetrating drugs. Notably, ACVBP has been compared with CHOP in a randomised trial of patients with low CNS-IPI risk and aggressive B-cell lymphomas. ACVBP was associated with a reduction of CNS relapse rate from 8·3% (CHOP)

	Number of patients	Study design and patient characteristics	Definition of high risk	Intervention	CNS relapse
Studies reporting benefit of HD-MTX in CNS prophylaxis					
Cheah et al[43]	217 (intervention 1 n=49, intervention 2 n=125, intervention 3 n=43)	Multicentre retrospective; DLBCL; high risk of CNS relapse	≥2 of the following: multiple extranodal sites, elevated lactate dehydrogenase, B symptoms; or extranodal involvement of any of the following: bone marrow, breast, testis, kidney, adrenal, paranasal sinus, nasopharynx, liver, paravertebral	(1) R-CHOP + intrathecal methotrexate (2) R-CHOP + intrathecal methotrexate + HD-MTX × 2 cycles (3) R-HCVAD or R-CODOX-M-IVAC (containing intrathecal methotrexate or HD-MTX)	3-year CNS relapse for intervention 1=18·4%, intervention 2=6·9%, intervention 3=2·3%
Ferreri et al[44]	200 (high risk patients=107)	Single centre retrospective; HIV negative; age ≥18 years;[18] FDG-PET staged at diagnosis DLBCL; rituximab + anthracycline-based chemotherapy	Extranodal involvement of any of the following: testis, spine, skull, paranasal sinuses, orbit, nasopharynx, kidney, adrenal, breast; or elevated lactate dehydrogenase plus stage III or IV	High risk patients 2008-HD-MTX 3 g/m² × 3–4 cycles with or without intrathecal cytarabine (liposomal); HD-MTX: following R-CHOP completion intrathecal cytarabine: D4 or 5 of R-CHOP cycle	Median follow-up 5 years; CNS relapse in high risk patients: 0/33 (0%; HD-MTX with or without intrathecal cytarabine), 9/74 (12%; no CNS prophylaxis or intrathecal cytarabine alone)
Ferreri et al[45]	242 (patients with CNS-IPI score of 4–6 n=75)	Single centre retrospective; HIV negative; age 18–89 years; patients with DLBCL	CNS-IPI score of 4–6; or testicular involvement	HD-MTX 3 g/m² × 3–4 cycles with or without intrathecal cytarabine (liposomal); HD-MTX: following R-CHOP completion intrathecal cytarabine: D4 or 5 of R-CHOP cycle	Median follow-up 65 months, CNS relapse in high risk patients: 0/24 (0%; HD-MTX); 10/51 (20%; no HD-MTX)
Abramson et al[46]	65	Single centre retrospective; age 25–79 years; patients with DLBCL; CHOP with or without rituximab depending on known CNS risk factors	Hollender score of 4–5; or ≥2 extranodal sites plus elevated lactate dehydrogenase; or extranodal involvement of any of: bone marrow, paranasal sinuses, testis, epidural, liver, adrenal, renal, orbit	(R)-CHOP × 6–8 cycles; HD-MTX × 1–8 cycles (3·5 g/m²) intercalated following cycle 2, 4, 6 of chemotherapy or following chemotherapy completion	Median follow-up 33 months, CNS relapse: 2/65 (3%)
Studies reporting no benefit of HD-MTX in CNS prophylaxis					
Bobillo et al[47]	585 (intervention 1 n=253, intervention 2 n=42, intervention 3 n=290)	Single centre retrospective; patients with DLBCL; R-CHOP or R-CHOP-like therapy	CNS-IPI score of 4–6; or extranodal involvement of any of: testis, breast, kidney, adrenal, bone marrow; or *MYC* and *BCL2* rearrangement	(1) intrathecal methotrexate or intrathecal cytarabine, or both (2) HD-MTX with or without intrathecal methotrexate or intrathecal cytarabine (3) No CNS prophylaxis	5-year CNS relapse risk overall=6·5%; intervention 1=5·5%; intervention 2=5%; intervention 3=7·5%
Puckrin et al[48]	906 (high risk patients n=326)	Multicentre retrospective; age 18–70 years; patients with DLBCL	CNS-IPI score of 4–6; or *MYC* and *BCL2* rearrangement; or extranodal involvement of testis	HD-MTX	Median follow-up 35·3 months; CNS relapse in high risk patients: HD-MTX=12·2% *vs* no HD-MTX=11·2%
El Galaly et al[2]	1532	Multicentre retrospective; patients with DLBCL;[18] FDG-PET staged; R-CHOP or R-CHOP-like therapy	Not required for inclusion, outcomes described for CNS-IPI 4–6 cohort	HD-MTX or intrathecal methotrexate (or both)	3-year cumulative incidence CNS relapse for patients with CNS-IPI score of 4–6: HD-MTX=11·2% *vs* no HD-MTX=10·2%
Orellana-Noia et al[49]	1162	Multicentre retrospective; age ≥18 years; patients with DLBCL or other aggressive B-NHL; received single route CNS prophylaxis	No specific CNS risk criteria required for inclusion—all patients must have received CNS prophylaxis	Intrathecal methotrexate (n=894), HD-MTX (n=236)	Overall=5·7%; intrathecal methotrexate =5·4%; HD-MTX=6·8%
Lewis et al[50]	2300 (all high risk)	Multicentre retrospective; age 18–80 years; patients with DLBCL; R-CHOP-like or DA-EPOCH-R-like therapy	CNS-IPI score of 4–6; or *MYC* and *BCL2* rearrangement; or primary testicular or breast lymphoma	HD-MTX with or without intrathecal methotrexate (n=410)	5-year cumulative incidence of CNS relapse: HD-MTX=9·1% *vs* no HD-MTX=8·4% (patients in CR at end of systemic treatment: HD-MTX=4·5% *vs* no HD-MTX=6·0%)
Wilson et al[23]	1384	Multicentre retrospective; patients with DLBCL; R-CHOP-like therapy	No specific CNS risk criteria required for inclusion—all received HD-MTX	iHD-MTX (n=749) or EOT HD-MTX (n=635)	3-year cumulative incidence of CNS relapse: overall=5.7%, CNS-IPI score of 4–6=9·1%; no difference between i-HD-MTX *vs* EOT HD-MTX

(Table 3 continues on next page)

	Number of patients	Study design and patient characteristics	Definition of high risk	Intervention	CNS relapse
(Continued from previous page)					
Prospective trials of intensive frontline combination regimens					
Tilly et al[51]	635 (ACVBP therapy n=323; CHOP therapy n=312)	Prospective randomised; pre-rituximab era; mixed histology: patients with DLBCL, lymphocytic lymphoma, or Burkitt lymphoma; age 61–69 years	aaIPI≥1	ACVBP treatment arm: ACVBP + intrathecal methotrexate × 4 cycles; HD-MTX (3g/m^2) × 2 cycles; etoposide + ifosfamide × 4 cycles; subcutaneous cytarabine × 2 cycles	ACVBP=2·8%, CHOP=8·3%
McMillan et al[52]	111	Prospective phase 2 single arm trial; age 18–65 years; patients with DLBCL; CNS staging at enrolment	CNS-IPI score of 3–5	R-CODOX-M-IVAC	2-year CNS relapse rate=3·6%
Holte et al[53]	156	Prospective phase 2 single arm trial; age 18–65 years; ECOG 0–3; patients with DLBCL or grade 3 (A or B) follicular lymphoma; CNS staging at enrolment	aaIPI 2–3	R-CHOEP-14 × 6; intravenous cytarabine × 1; HD-MTX × 1	7/156 (4·5%) all within 6 months of diagnosis
Leppä et al[54]	139	Prospective Phase 2 single arm trial; age 18–64 years; patients with DLBCL or grade 3B follicular lymphoma	aaIPI 2–3; or ≥2 extranodal sites; or extranodal involvement of any of; testis paranasal sinus, orbit, bone marrow	HD-MTX + R-CHOP-14 × 2 cyles; R-CHOEP-14 × 4 cyles; rituximab-cytarabine × 1 cyle; intrathecal cytarabine (liposomal) intercalated	Median follow-up 5 years; CNS relapse=2·3%

HD-MTX=high-dose (ie, intravenous) methotrexate. DLBCL=diffuse large B-cell lymphoma. R-CHOP=rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone. R-HCVAD=rituximab, dexamethasone, cyclophosphamide, doxorubicin, vincristine, methotrexate, and cytarabine. R-CODOX-M-IVAC=rituximab, doxorubicin, vincristine, cyclophosphamide, cytarabine, methotrexate, ifosfamide, and etoposide. FDG-PET=fluorodeoxyglucose-PET. CNS-IPI=central nervous system international prognostic index. CHOP=cyclophosphamide, doxorubicin, vincristine, and prednisolone. B-NHL=B cell non-Hodgkin lymphoma. DA-EPOCH-R=dose-adjusted rituximab, etoposide, cyclophosphamide, prednisolone, doxorubicin, and vincristine. iHD-MTX=intercalated HD-MTX. EOT=end of treatment. ACVBP=doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone. aaIPI=age-adjusted international prognostic index. ECOG=Eastern Cooperative Oncology Group. R-CHOEP=rituximab, cyclophosphamide, doxorubicin, vincristine, etoposide, and prednisolone.

Table 3: **Summary of prospective and retrospective studies of aggressive B-cell lymphoma assessing the value of HD-MTX**

to 2·7% (ACVBP).[51] Whether this difference was due to improved systemic disease control or specific CNS-penetrating agents (and to which agents) remains undefined. This observation was not supported in a similar randomised trial performed in the rituximab era (CNS relapse=0% [R-ACVBP] *vs* 1% [R-CHOP]; p=0·52).[55] In these studies, baseline screening for occult CNS disease (cerebral spinal fluid cytology or flow cytometry and neuroimaging) varied, but requirements for such procedures restrict comparability to observational studies without systematic CNS screening (table 3). Historical observations suggest high CNS relapse rates (10–30%) in primary testicular lymphoma,[33] with the observed event rate substantially diminished with high-dose methotrexate prophylaxis at 1·5 g/m² and intrathecal prophylaxis in the IELSG30 trial (54 patients with primary testicular lymphoma).[34,56]

In contrast, several large retrospective series have not shown that high-dose methotrexate reduces CNS relapse, with rates of 6–12% in patients with high-risk aggressive B-cell lymphoma (most studies report cases as DLBCL), regardless of high-dose methotrexate prophylaxis[2,47–49] (table 3). This includes a retrospective cohort of 2300 patients treated in the rituximab era at high risk of CNS relapse (CNS-IPI=4–6 [89·2%], double-hit, triple-hit, primary testicular lymphoma, or breast DLBCL). High-dose methotrexate at various doses during or following chemoimmunotherapy was not associated with a significant reduction in CNS relapse (8·4% with high-dose methotrexate *vs* 9·1% without high-dose methotrexate, p=0·1).[50] Furthermore, in a retrospective cohort of 1384 patients who all received high-dose methotrexate, 3-year CNS relapse risk was 9·1% in 600 patients with DLBCL and CNS-IPI scores were 4–6,[23] remarkably similar to the cohorts examined in the original CNS-IPI development and validation cohorts (10·2%) where minimal CNS prophylaxis was used.

Timing of high-dose methotrexate delivery

With CNS relapse typically occurring at a median of 6–9 months from initial DLBCL diagnosis,[3,4] there is theoretical rationale to deliver high-dose methotrexate as early as possible. The first study to report intercalated high-dose methotrexate between R-CHOP cycles was a small retrospective series (n=65).[46] The CNS relapse rate was low (3%), but delays to systemic therapy were noted in 12% of patients.

Two single-arm, phase 2 trials observed a low CNS relapse rate following high-dose methotrexate-based prophylaxis in young high-risk patients[53,54] (table 3). In the first study, high-dose methotrexate and cytarabine delivered after R-CHOEP-14 was associated with a 4·5% CNS relapse rate at a median follow-up of 52 months.[53] In the second study, early high-dose methotrexate in combination with R-CHOP or R-CHOEP-14 was associated with a CNS relapse rate of 2·5% at a

median follow-up of 60 months.[54] These data also suggested that early prophylaxis delivery might reduce CNS relapse risk, although confidence intervals were wide and high-dose methotrexate infusion times varied. High-dose methotrexate was delivered as a 24 h infusion in the initial trial and as 3 h infusion (delivery associated with improved CNS bioavailability) in the subsequent study.

A recent large retrospective study addressed the question of high-dose methotrexate timing, comparing patients receiving high-dose methotrexate delivered either intercalated (n=749) or at end of R-CHOP (n=635).[23] Delays to R-CHOP of 7 days or more were significantly increased with intercalated high-dose methotrexate versus delivery at the end of R-CHOP, with 20% of intercalated high-dose methotrexate treatments associated with a delay to subsequent R-CHOP. There was no difference in CNS relapse rate between the groups, including on multivariable analyses and when restricting analyses to the highest-risk patients. Overall, intercalated high-dose methotrexate is associated with R-CHOP interruption and delay,[23,57] compromising delivery and possibly the effectiveness of R-CHOP. Accepting the limitations of retrospective data, from which these results on high-dose methotrexate originate, results from this study support an end-of-R-CHOP delivery.

Appraisal of the evidence for CNS prophylaxis

The evidence for the utility of CNS prophylaxis in high-risk DLBCL is scarce, with no randomised trials evaluating this question. Evaluation of the utility of CNS prophylaxis is further complicated by several factors, including the heterogeneity of DLBCL and risk assessment for CNS involvement, multiple methods of CNS prophylaxis, and the relative rarity of CNS relapse, leading to numerous small and underpowered observational studies. As discussed, data to support CNS prophylaxis were predominantly driven by extrapolation from other lymphoma subtypes, small single-institution series, and comparison of observed rates of CNS relapse while using CNS prophylaxis with expected rates of relapse without CNS prophylaxis. Comparison of results from single-arm clinical trials or observational studies with historical rates must be done with caution, as outcomes in DLBCL can be highly sensitive to selection bias, even while controlling for factors such as the CNS-IPI.[58]

Despite the absence of strong data to support its use, CNS prophylaxis has been widely used, although large retrospective studies suggest limited utility (table 3). These studies are complicated by heterogeneity in the use of CNS prophylaxis by treating physicians or institutions. Furthermore, there is likely to be treatment selection bias, as patients receiving CNS prophylaxis tend to be younger and fitter than patients who receive no prophylaxis. Careful analytical approaches to adjust for clinical factors can partly adjust for this selection bias. Broadly, the clinical approach and evidence base suggests a loss-aversion scenario,[59] in which there is little evidence to support a strong effect for CNS prophylaxis, but the perceived risk of not treating for CNS relapse has often outweighed the lack of evidence regarding its use.

Arguments in favour of high-dose methotrexate-based prophylaxis

The poor outcome of secondary CNS lymphoma is attributed to several factors: poor CNS penetrance of chemotherapeutics, impaired neurocognitive function and patient performance status, contributing to increased treatment-related toxicity,[18] and recurrent genetic aberrations conferring treatment resistance.[60] Thus, there is broad agreement that CNS relapse risk should be minimised. A crucial issue is whether any patients truly benefit from prophylaxis and, if so, what the most effective therapeutic strategy is.

Unfortunately, data to inform practice are largely retrospective with a wide variation in selection criteria for prophylaxis, type of prophylaxis, and primary treatment regimen. Studies comparing patients treated with or without CNS prophylaxis are often imperfectly matched for high-risk features, and there might be other biases guiding treatment decisions. Caution should be adopted when interpreting retrospective series reporting no impact of CNS prophylaxis[47–49] and their limitations should be acknowledged.

In some cases, current practice has resulted in a large proportion of DLBCL patients receiving CNS prophylaxis unnecessarily, including approximately 90% of patients with CNS-IPI scores of 4–6 who might never have developed CNS relapse.[1] Moreover, the CNS-IPI score model has shown suboptimal sensitivity with a significant proportion of CNS events occurring in the intermediate-risk group.[1] Thus, other risk factors including those described herein (ie, testicular, renal, adrenal, ≥3 extranodal sites) should be considered. Focusing strategies on these high-risk groups is recommended. Conversely, evidence is less clear for uncommon DLBCL subtypes, such as double-hit, where CNS relapse risk could be due to concomitant high-risk features[8] rather than to biological reasons; however, large scale studies of the CNS risk in double-hit with DLBCL histology alone are also scarce.

The comparison between two prospective IELSG trials on isolated primary testicular lymphoma argues in favour of high-dose methotrexate prophylaxis.[34,56] Primary testicular lymphoma is an important model, as patients in one study treated without prophylaxis exhibit a 5-year CNS relapse rate of 19%.[33] In the IELSG10 trial,[34] 53 patients with stage I–II primary testicular lymphoma received R-CHOP-21, contralateral testicular radiotherapy, and four doses of intrathecal methotrexate. Three patients had CNS relapse (two leptomeningeal), resulting in a long-term CNS relapse rate of 6% (median follow-up 65 months). In the following IELSG30 trial,[56] 54 patients with primary testicular lymphoma were treated with intrathecal liposomal cytarabine and an

additional two doses of methotrexate at 1·5 g/m²; no CNS relapses were observed at a median follow-up of 73 months. With all the limitations of cross-comparing single-arm trials, these results suggest a possible benefit of high-dose methotrexate prophylaxis in primary testicular lymphoma. Testicular involvement with advanced stage DLBCL represents a group with an even greater CNS risk and could also be reasonably considered for prophylaxis despite the evidence limitations.

A study[61] of 103 patients with aggressive B-cell lymphomas showed that, with careful patient selection and strict protocol guidance, two cycles of high-dose methotrexate at 3 g/m² can be delivered in an outpatient setting, without methotrexate serum-level monitoring, but using fixed-dose leucovorin rescue and oral hyperhydration. Only eight patients did not receive the second methotrexate dose due to toxicity, suggesting the feasibility of this approach.

Arguments against high-dose methotrexate-based prophylaxis

The frequency of asymptomatic CNS involvement at initial DLBCL diagnosis has not been well studied, with no large studies consecutively screening all high-risk patients with cerebral spinal fluid flow cytometric assessment and comprehensive CNS imaging (including MRI). A single-centre study[62] examined 154 patients with newly diagnosed DLBCL, of whom 93 (60%) had baseline cerebral spinal fluid flow cytometry. MRI imaging was only performed in symptomatic patients. 12 of 101 samples obtained in patients without neurological symptoms had positive cerebral spinal fluid flow cytometry, with a substantial proportion not otherwise considered at high-risk of CNS involvement. Although only a minority of patients with positive flow-cytometry had CNS relapse, the use of CNS prophylaxis and the short follow-up period limit the interpretation of the clinical relevance of infiltration detected by flow cytometry. Another study[63] reported that 11 out of 51 patients with high-risk DLBCL had occult CNS involvement at diagnosis, defined by positive flow-cytometry results, and five of these 11 patients later relapsed in the CNS. Use of high-dose methotrexate was not described. These data suggest that a substantial number of patients without overt neurological symptoms might have occult CNS involvement at baseline, which is also in line with the observed early presentation of CNS relapse. These patients could also represent a significant proportion of those later diagnosed with CNS relapse. Therefore, a first important step in the prevention of secondary CNS involvement is to consider systematic screening of very high-risk patients (imaging and cerebral spinal fluid analysis with cytology and flow) and to develop more sensitive techniques to capture minimal CNS involvement. By medical consensus, these patients are likely to be better managed by intensive treatment regimens with CNS-penetrating agents rather than R-CHOP.

The consideration of CNS prophylaxis in its true sense is only relevant for patients without baseline CNS involvement. Delivering high-dose methotrexate to all CNS-IPI high-risk patients is unlikely to be cost-effective. The CNS-IPI high-risk group constitutes 12–23% of all patients with DLBCL and the 2-year rate of CNS relapse in this group is approximately 12%.[1,2] Although outpatient administration is feasible, most patients receiving high-dose methotrexate are still managed as inpatients due to complex hydration and rescue regimens. High-dose methotrexate takes approximately 6 days to complete, providing a substantial administrative and financial burden to hospitals and patients. High-dose and intrathecal methotrexate are also rarely associated with potentially serious leukoencephalopathy and myelopathy.[64]

Two recent large studies show similar secondary CNS lymphoma rates in CNS-IPI high-risk patients treated with or without high-dose methotrexate[50] and with different high-dose methotrexate schedules.[23] In one of the studies,[50] 2300 patients with high risk of CNS relapse (CNS-IPI 4–6: 89·2%)—mostly treated with R-CHOP-like therapy (93·8%)—were analysed according to use of high-dose methotrexate or not. A total of 410 patients (17·8%) received high-dose methotrexate and 32 of 410 (7·8%) had CNS relapse as compared with 169 of 1890 (8·9%) among patients treated without high-dose methotrexate. The adjusted 5-year CNS relapse rates were 8·4% in the high-dose methotrexate group versus 9·1% in the no high-dose methotrexate group. Since high-dose methotrexate is associated with guaranteed time bias against development of CNS-relapse until high-dose methotrexate is delivered, patients in complete response were analysed. Among 1455 patients who had a complete response, 284 (19·5%) received high-dose methotrexate with 16 of 284 (5·6%) experiencing CNS relapse as compared with 68 of 1171 (5·8%) treated without high-dose methotrexate. Again, no difference in the 5-year risk of CNS relapse risk between the groups was observed (5·0% *vs* 6·0%). If the true difference is a 1% decrease in CNS relapse in favour of high-dose methotrexate, this means that 100 patients would need to be treated with high-dose methotrexate to avoid one CNS relapse. These results should be interpreted with some caution as the cohort treated with high-dose methotrexate included a higher proportion of patients with high-risk features, including more than two extranodal sites (44% *vs* 30%), and high-risk extranodal sites (47% *vs* 24%). Overall, these data suggest that, although high-risk patients can be identified, the current prophylaxis measures at our disposal might be insufficient.

The ongoing application of CNS prophylaxis, despite an absence of robust evidence showing its efficacy, has been driven in part by dismal secondary CNS lymphoma outcomes. Although these poor outcomes are driven mainly by patients with concurrent systemic and CNS

relapse, as seen from the results of the MARIETTA/IELSG42 trial,[18] subgroups including patients with isolated CNS relapse—arguably the only secondary CNS lymphoma category potentially prevented by CNS prophylaxis—also show unsatisfactory outcomes with intensive immunochemotherapy.[18] There remains a need to continue to improve on these outcomes in all patients, but patients with concurrent systemic or CNS relapse require the most attention, for whom systemic treatment and CNS prophylaxis failure are both concerns, potentially requiring different strategies.

It is also important to recognise the impact of increasing the availability of chimeric antigen receptor (CAR) T-cell therapy and novel oral therapeutics for patients with secondary CNS lymphoma. The immunomodulatory agent lenalidomide and the Bruton's tyrosine kinase inhibitor ibrutinib have clear clinical activity in CNS lymphoma.[65,66] Results from multiple case series have shown CAR T-cell therapy activity in primary CNS lymphoma and secondary CNS lymphoma, suggesting the potential to greatly improve outcomes for these patients.[67,68] Although further CAR T-cell studies are needed, this therapeutic strategy could influence the risk-to-benefit balance when making decisions around CNS prophylaxis, but it is recognised that performance status might preclude eligibility and, in many countries, it is not readily available due to cost and resource impact.

Advances in our understanding of the molecular DLBCL biology have identified genetic subgroups with predilection for CNS relapse. This has implications both for diagnostics and therapeutics, with the potential to identify higher-risk patients with greater specificity and to investigate the use of novel targeted agents with augmented systemic disease control and CNS penetration. A more personalised approach using such targeted agents in patients with known high-risk molecular sub-types could improve on the specificity and effectiveness of traditional CNS prophylaxis.

Future directions

Novel therapy approaches in preventing CNS relapse

Data from studies of lenalidomide and ibrutinib have shown CNS penetration in primary CNS lymphoma and activity in systemic activated B-cell or non-germinal B-cell DLBCL subtypes,[65,66] leading to front-line trials integrating these agents. A recent post-hoc analysis[69] of the randomised, phase 3 REMARC trial suggested that lenalidomide maintenance post-R-CHOP in patients with DLBCL aged 60–80 years (CNS-IPI ≥1) was not associated with lower rates of CNS relapse (2-year CNS relapse rate=3·3% lenalidomide *vs* 0·9% placebo). CNS prophylaxis was given per local practice or investigator discretion and did not alter outcomes. The phase 3 ROBUST trial[70] and phase 2 ECOG-ACRIN E1412 trial[71] evaluating R-CHOP-lenalidomide versus R-CHOP have not yet reported CNS rates. The phase 3 PHOENIX trial,[72] comparing R-CHOP-ibrutinib to R-CHOP in ABC DLBCL, showed overall low and similar CNS relapse rates (2·4% *vs* 3·8%). An immune escape phenotype is evident in some DLBCL subtypes, especially MCD or C5 DLBCL,[15–17] highlighting a potential role for PD1 inhibitors. Overall, although these studies did not show a benefit of biological drugs in preventing CNS relapse, they are hypothesis-generating examples that open new options for future research into CNS prophylaxis. In this context, CAR T-cell therapy could play a relevant role as it is efficacious in refractory DLBCL, with responses observed in half of patients with CNS lymphoma,[73,64] and as CAR T-cells can expand in the periphery and traffic to the CNS without active disease at infusion.[74,75] More widespread use of CAR T-cells as part of first-line and second-line treatment for patients with DLBCL might theoretically help prevent CNS relapse. In the recent phase 2 ZUMA-12 study,[76] none of the 40 patients with DLBCL with CNS-IPI score of 3 or more and PET$^+$ disease after two courses of anthracycline-based chemotherapy treated with axicabtagene ciloleucel had CNS relapses at a median follow-up of 15·9 months. This overall hypothesis deserves further investigation.

Circulating tumour DNA analysis in the cerebrospinal fluid

In addition to imaging, high-risk patients with DLBCL often have cerebral spinal fluid cytology and flow cytometry analysis; however, sensitivity remains poor.[63] Cell-free circulating tumour DNA (ctDNA) has recently emerged as a non-invasive prognostic biomarker in patients with lymphoid malignancies[77] and could have a role in cerebral spinal fluid analysis. A recent study[78] of 67 patients with CNS lymphoma (including 12 with isolated secondary CNS lymphoma) identified ctDNA in all cerebral spinal fluid pretreatment samples (100%) and showed a significant correlation of plasma ctDNA concentration with tumour volume and outcomes.

Two studies have also examined the potential utility of cerebral spinal fluid ctDNA to predict CNS relapse in high-risk B-cell lymphoma. Bobillo and colleagues analysed specific tumour-derived mutations in the cerebral spinal fluid from 12 patients with newly diagnosed B-cell lymphoma. One of two patients with CNS relapse had detectable amounts of ctDNA in a cerebral spinal fluid sample collected 3 months before the relapse.[79] A separate study[80] used an NGS-minimal residual disease assay to analyse 22 patients with high-risk B-cell lymphoma. Clonotypic DNA was detected at diagnosis in the cerebral spinal fluid in eight (36%) patients, of whom two relapsed in the CNS, with a 12-month cumulative risk of CNS recurrence of 29% versus 0% risk for patients with negative cerebral spinal fluid.[80] Further, in primary testicular lymphoma, where *MYD88* mutations occur in approximately 70% of cases, this information might play a role in cerebral

Search strategy and selection criteria

References for this Review were identified through PubMed searches with the terms "CNS prophylaxis", "central nervous system prophylaxis", "secondary CNS lymphoma", "aggressive B-cell lymphoma", "diffuse large B-cell lymphoma", "intrathecal", "high dose methotrexate", and "methotrexate" for articles published from 1975 until March 31, 2022. Articles were also identified through searches of the authors' own files. Only papers published in English were reviewed. The final reference list was generated on the basis of originality and relevance to the scope of this Review.

spinal fluid CNS detection in paucicellular cases.

Conclusion

Decision making with regards to the use of CNS prophylaxis must be pragmatic, considering (1) the estimated risk of secondary CNS lymphoma, (2) the effectiveness and toxicity of currently available prophylactic strategies, (3) the treatment options available for secondary CNS lymphoma should it arise, (4) preferences of the patient, and (5) health-care resource use. To date, there is an absence of robust prospective data informing risk estimation and the definitive benefit of prophylactic strategies. Future developments should focus on integrating molecular (ie, activated B-cell, genetic DLBCL subtyping) and clinical risk factors (ie, CNS-IPI, number of extranodal sites, and high-risk sites as described) to identify very high-risk patients and expanding on ultrasensitive technology to detect occult CNS involvement at presentation including ctDNA or *MYD88* mutation testing of the cerebral spinal fluid (or both) to direct patients for CNS treatment strategies. Importantly, biological agents active against DLBCL with good CNS bioavailability could improve front-line treatment effectiveness and reduce CNS dissemination. Finally, should secondary CNS lymphoma arise despite these strategies, there are several novel approaches in development, with CAR T-cell treatment showing potential promise in a poor-risk patient group. There remains an urgent need for adequately-powered, prospective, international, collaborative studies of uniformly treated patients at high risk of CNS relapse to address this important clinical question.

Contributors

All authors contributed equally to writing and editing the paper, the analysis, and conception. All authors reviewed the manuscript and approved submission.

Declaration of interests

TCE-G is partly funded from a grant from the Danish Cancer Society. AJMF declares research funding from ADC Therapeutics, Bayer HealthCare Pharmaceuticals, Beigene, Bristol Myers Squibb (BMS), Genmab, Gilead, Hutchison Medipharma, Incyte, Janssen Research & Development, MEI Pharma, Novartis, PletixaPharm, Pharmacyclics, Protherics, Roche, and Takeda; consulting fees from Gilead, Juno, Novartis, PletixaPharm, and Roche; speaker fees from Gilead and Roche; and advisory work for Gilead, Juno, Novartis, PletixaPharm, and Roche. PM declares speaker fees from Janssen. TAE declares advisory work from Janssen, Kite Pharma, Roche, AbbVie, AstraZeneca, Loxo Oncology, Beigene, Incyte, and Secura Bio; speaker bureau fees from Janssen, Kite Pharma, Roche, AbbVie, AstraZeneca, Loxo Oncology, Beigene, and Incyte; and travel support from Roche and AbbVie. MJM recognises the National Cancer Institute: University of Iowa Mayo Clinic Lymphoma SPORE (P50-CA097274) grant; declares advisory work from Adaptive Biotechnologies, Pfizer, Kite Pharma, and Genmab; and research support from BMS, Genentech, Genmab, and Morphosys to his institution. KLL declares speaker bureau from Janssen, AstraZeneca, and Roche; travel support from Novartis and Janssen; and advisory work from IQVIA and Loxo at Lilly. KC declares consulting fees from Roche, BMS, KITE Pharma, and Janssen; speaker bureau from Roche, KITE Pharma, and BMS; and travel support from Roche, KITE Pharma, and BMS. CYC declares speaker bureau from Beigene, Janssen, and Gilead; and advisory work from TG Therapeutics, Roche, Novartis, Janssen, Beigene, AstraZeneca, Eli Lilly, BMS, Gilead, and BMS. KJS declares advisory work from BMS, Seattle Genetics, Merck, Janssen, Novartis, and Kyowa; and DSMC Regeneron, Steering Committee Beigene, research funding from BMS. TCE-G declares previous employment with Roche (March, 2019 until February, 2021) and an honorarium from AbbVie. All other authors declare no competing interests.

Acknowledgments

There was no funding from external agencies or pharmaceutical companies to support this study. TE recognises the Oxford NIHR Biomedical Research Centre. Views expressed are those of the authors and not necessarily those of the NHS or the NIHR or the United Kingdom's Department of Health.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Eyre TA, Savage KJ, Cheah CY, et al. CNS prophylaxis for diffuse large B-cell lymphoma. *Lancet Oncol* 2022; **23:** e416–26.

Supplementary Table 1. Prospective and retrospective studies of aggressive B-cell lymphomas with SCNS relapse in the rituximab era. Retrospective studies with n>75 included.

Reference	n	Cohort Details	Intervention	Outcomes
Prospective Studies				
Ferreri *et al* [18]	75	MARIETTA multicenter phase 2 trial Isolated SCNSL 20% CNS parenchyma 45%	MATRIx x3 + R-ICE x3, followed by high-dose carmustine/thiotepa and ASCT (ASCT rate 49%)	ORR 61% CR 55% 2-year PFS and OS both 46% 2-year PFS and OS both 83% in patients who received ASCT
Ferreri *et al* [19]	38	Italian multicenter phase 2 trial Isolated SCNSL 39% CNS parenchyma 76%	Multiphase regimen with HD-MTX and cytarabine, plus other cytotoxics, followed by high-dose carmustine/thiotepa and ASCT (ASCT rate 53%)	ORR CR 63% 5-year EFS 40% 5-year OS 41% (68% ASCT)
Doorduijn *et al* [20]	36	HOVON-80 multicentre phase 2 trial Isolated SCNS 44% CNS parenchyma 67%	R-DHAP + HD-MTX + IT rituximab, followed by high-dose busulfan/cyclophosphamide and ASCT (ASCT rate 42%)	ORR 53% CR 22% 1-year PFS 19% 1-year OS 25%
Korfel *et al* [79]	30	Multicentre phase 2 trial Isolated SCNSL 70% CNS parenchyma 77%	HDMTX + thiotepa + ifosfamide + cytarabine, followed by high-dose carmustine/thiotepa/etoposide and ASCT (ASCT rate 80%)	CR 26% after induction CR 63% after ASCT 2-year TTF 49% (58% ASCT) 2-year OS 63% (68% ASCT)
Retrospective Studies				
El-Galaly *et al* [22]	291	International multicentre cohort with SCNSL during/after frontline R-CHOP (or similar). Isolated SCNSL 61% CNS parenchyma 68%	Various regimens; 60% received intensive therapies including HMDTX (52%) and ASCT (14%)	mOS 4 months 2-year OS 20% Young fit patients treated with HDMTX-based therapy for isolated SCNS had 2-year OS 62%
Akin *et al* [80]	102	Single center cohort consecutively and exclusively treated with ASCT – MD Anderson Cancer Centre Isolated SCNSL 24% CNS parenchyma 41%	Largely HD-MTX and/or cytarabine-based regimens (85%) prior to ASCT (100%). 24% conditioning regimens contained thiotepa.	4-year PFS 48% 4-year OS 57% Improved outcomes in patients with CR at time of ASCT and those with ≤2 prior lines of therapy.
Bromberg *et al* [21]	92	International multicenter cohort – International PCNSL Study Group Isolated SCNSL 5% (incomplete data) CNS parenchyma 51%	Various regimens, mostly HD-MTX (>50%) and/or cytarabine-containing, WBRT.	mOS 7 months Improved OS in patients who received ASCT (2-year OS 54%)
Kansara *et al* [24]	84	Single institution cohort – BC Cancer, Canada Isolated SCNSL 56% CNS parenchyma 73%	Various treatments including HD-MTX, WBRT, steroids.	mOS 2.5 months Isolated SCNSL and parenchymal involvement had better 2-year OS (~20%)
Wilson *et al* [23]	78	International multicentre with SCNSL after frontline R-CHOP with HD-MTX prophylaxis Isolated SCNSL 74% CNS parenchyma 53%	Not described	mOS 5 months Trend towards improved OS in isolated SCNSL.

Abbreviations: SCNSL: secondary central nervous system lymphoma, CNS: central nervous system, ORR: overall response rate, CR: complete response, ASCT: autologous stem cell transplantation, WBRT: whole brain radiotherapy, mOS: median overall survival, PS: performance status, HD-MTX: high dose methotrexate, R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone, EFS: event free survival, PCNSL: primary central nervous system lymphoma, R-DHAP: rituximab, cisplatin, cytarabine, dexamethasone, R-ICE: rituximab, ifosphamide, carboplatin, etoposide, IT: intrathecal, MATRIx: methotrexate, cytarabine, thiotepa, rituximab. Studies n>75 included.

PAPER 5

Wilson MR, Bobillo S, Cwynarski K. CNS Prophylaxis in Aggressive B-Cell Lymphoma**.** Hematology Am Soc Hematol Educ Program. 2022 Dec 9; 2022(1): 138-145 PMID: 36485105 -

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9820554/

Journal impact factor: 3
Number of citations: 7

Summary of contribution:

Conceptualisation	Yes with KC and SB
Data Curation	Yes – performed literature review along with SB
Formal Analysis	N/A
Investigation	N/A
Methodology	N/A
Project Administration	Yes
Visualisation	Yes
Writing – original draft	Yes with SB and KC
Writing – review & editing	Yes

CNS prophylaxis in aggressive B-cell lymphoma

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The prevention of central nervous system (CNS) relapse in diffuse large B-cell lymphoma (DLBCL) continues to be one of the most contentious areas of lymphoma management. Outcomes for patients with secondary CNS lymphoma (SCNSL) have historically been very poor. However, in recent years improved responses have been reported with intensive immunochemotherapy approaches, and there is a growing interest in potential novel/cellular therapies. Traditional methods for selecting patients for CNS prophylaxis, including the CNS International Prognostic Index, are hampered by a lack of specificity, and there is accumulating evidence to question the efficacy of widely employed prophylactic interventions, including intrathecal and high-dose methotrexate (HD-MTX). Given the potential toxicity of HD-MTX in particular and the ongoing need to prioritize systemic disease control in high-risk patients, there is an urgent need to develop more robust methods for identifying patients at highest risk of CNS relapse, as well as investigating prophylactic interventions with greater efficacy. Here we review new evidence in this field from the last 5 years, focusing on the potential use of molecular diagnostics to improve the identification of high-risk patients, recent large data sets questioning the efficacy of HD-MTX, and the current approach to management of patients with SCNSL. We provide a suggested algorithm for approaching this very challenging clinical scenario.

LEARNING OBJECTIVES

- Understand the currently available methods for identifying patients at high risk of CNS relapse and the potential for novel molecular diagnostics to improve patient selection in the future
- Review recent evidence to question the efficacy of traditional methods for delivering CNS prophylaxis and to evaluate the increasing focus on alternative interventions for this important clinical problem

CLINICAL CASE

A 62-year-old man with no previous medical history presented in January 2020 with a short history of weight loss, night sweats, hip pain, and bilateral groin lymphadenopathy. His lactate dehydrogenase (LDH) was elevated (>3 times the upper limit of normal). Fluorodeoxyglucose-positron emission tomographic (FDG-PET) imaging revealed widespread hypermetabolic lymphadenopathy (largest lesion, 4cm diameter) as well as pathological FDG uptake in multiple areas of bone (scapula, L3 vertebra, left hemipelvis) and in the left kidney. A core biopsy from a left inguinal lymph node demonstrated a diagnosis of diffuse large B-cell lymphoma (DLBCL), non-germinal center subtype (Hans algorithm), with overexpression of *MYC* (>90%) and *BCL2* (>60%) by immunohistochemistry—that is, the double-expressor subtype. Fluorescence in situ hybridization (FISH) studies showed no evidence of *MYC*, *BCL2*, or *BCL6* rearrangements. His Eastern Cooperative Oncology Group (ECOG) performance status (PS) was 1, resulting in an international prognostic index (IPI) of 4 (age >60, stage IVB, raised LDH, ≥2 extranodal (EN) sites) and a central nervous system (CNS) IPI of 5 (aforementioned IPI factors plus renal involvement). Six cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) therapy were planned at 21-day intervals. Consideration was given as to whether CNS prophylaxis should be incorporated to reduce risk of CNS relapse.

Introduction

CNS relapse (otherwise referred to as secondary CNS lymphoma [SCNSL]) is a relatively rare but often devastating complication for patients with DLBCL. Estimates of CNS relapse incidence vary, occurring overall in approximately 5% of DLBCL patients but with subgroups in which the risk is significantly higher.[1,2] Most CNS relapse events occur

Table 1. Summary of consensus guideline recommendations for CNS prophylaxis in DLBCL

Guideline	Patient selection	Method for CNS prophylaxis suggested
British Society for Haematology (2021)[8]	*Offer to:* • High (4–6) CNS-IPI • ≥3 EN sites • High-risk EN site involvement—testicular, renal/adrenal, intravascular *Consider in:* • Breast involvement • Uterine involvement	• HD-MTX (≥3g/m^2 for 2-3 cycles) as early as possible as part of first-line therapy without compromising dose and time intensity of R-CHOP-like treatment • IT prophylaxis not recommended if HD-MTX successfully delivered • Consider IT as well as systemic prophylaxis in testicular DLBCL
NCCN (2022)[48]	*Consider in:* • High (4–6) CNS-IPI • Double/triple-hit HGBL • High-risk EN site involvement—testicular, breast, primary cutaneous, renal/adrenal	• HD-MTX (3-3.5g/m^2 for 2-4 cycles) during or after the course of treatment and/or • IT methotrexate and/or cytarabine (4-8 doses) during or after the course of treatment
ESMO (2018)[49]	*Consider in:* • High IPI • High-risk EN site involvement—testicular, renal/adrenal, breast, bone marrow, bone	• HD-MTX is "an option . . . even though the level of supporting evidence is low" • "Little or no role" for IT therapy

ESMO, European Society for Medical Oncology; HGBL, high-grade B-cell lymphoma; NCCN, National Comprehensive Cancer Network.

either during or closely following frontline immunochemotherapy, with a median time in recent prospective clinical trials of 6 to 8 months.[1,3] Management of SCNSL is often challenging, with historically poor outcomes. As a result, much attention has focused on both the identification of patients at highest risk for this complication, as well as prophylactic treatments aimed at abrogating risk as much as possible. Although our understanding of which patients are at highest risk of SCNSL has improved, particularly with the introduction of the CNS-IPI and increased understanding of the molecular biology of DLBCL,[4] decision-making around prophylactic interventions continues to be based either on retrospective analyses or data extrapolated from other disease subtypes, with no prospective randomized trials performed aimed at addressing CNS prophylaxis efficacy directly.

Clinicians often are faced with the dilemma of trying to prevent such a feared complication whilst ensuring that the patient is not exposed to additional therapy with associated risk of toxicity and a limited evidence-base to demonstrate its efficacy. The limitations of the evidence to inform decision-making are reflected in the variation between national guidelines on the topic (Table 1), as well as the significant disparity in practice between centers within the same health care system.

A 2017 American Society of Hematology Educational Program review gave a comprehensive overview of the risk factors for CNS relapse and evidence to guide prophylactic interventions at that time.[5] In this article we focus on updates in the field in the last 5 years, with particular attention to the advances in molecular diagnostics and implications for SCNSL, as well as new evidence to question the efficacy of high-dose methotrexate (HD-MTX).

How do we identify patients at high risk of CNS relapse?

Clinical risk factors

Numerous studies have investigated potential risk factors for CNS relapse in DLBCL.[5] In 2016 the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL) developed a prognostic model (CNS-IPI) incorporating the 5 standard IPI factors as well as involvement of the kidneys or adrenal glands, stratifying patients into 3 risk categories (Table 2).[4] Notably, patients with 5 or 6 risk factors had a much higher risk of CNS relapse of 15% and 32.5%, respectively. Although the CNS-IPI is a robust model and has been validated in subsequent studies, it lacks specificity, and half of events occur among patients with low to intermediate scores. It should also be noted that although a small number of patients in the DSHNHL trials used to formulate the CNS-IPI had Burkitt lymphoma, the final model is validated for patients with DLBCL only, and CNS prophylaxis strategies for Burkitt lymphoma should be considered separately.

Certain EN sites have been associated with a higher risk of CNS recurrence, with kidney/adrenal involvement included in the CNS-IPI model and intravascular lymphoma a distinct entity with a well-established risk of CNS involvement at baseline or at relapse. Testicular involvement has long been recognized as a risk factor, in the context of both limited and advanced stage, with a 10-year CNS relapse risk of 10% to 25% (see section Testicular DLBCL).[6] Breast involvement has been associated with a higher risk of CNS relapse (~15%) in retrospective series,[7] whereas other EN sites such as the uterus, blood, bone marrow, or epidural area showed more inconsistent results and are unlikely to be independently predictive of CNS relapse.[8] Finally, a large retrospective study reported that the involvement of 3 or more EN sites as determined by PET-computed tomography conferred a 3-year cumulative risk of CNS relapse of 15%.[9]

Biological risk factors

The dual overexpression of *MYC* and *BCL2*, determined by immunohistochemistry (double-expressor DLBCL), has not been consistently associated with a high risk of CNS relapse.[3,10] However, most double-expressor cases are classified as the activated B-cell (ABC) subtype, which, when determined by gene expression profiling, has been associated with a CNS relapse risk of 7% to 9% and 15% when combined with a high CNS-IPI.[3,10]

Recently, multiplatform analysis defined new molecular subgroups, or clusters.[11,12] The MCD and C5 clusters, characterized by a high frequency of *MYD88*^{L265P} and *CD79* mutations, occur almost exclusively in the ABC subtype. Genetic alterations defining these subtypes are also recurrently mutated in primary

Table 2. CNS-IPI risk categories with corresponding 2 year rates of CNS relapse and proportion of patients in each category from training (DSHNHL) and validation (BCCA) cohorts

Risk group	Risk factors	DSHNHL cohort		BCCA cohort	
		N (%)	2-year risk of CNS relapse	N (%)	2-year risk of CNS relapse
Low	0-1	1002 (46)	0.6%	463 (31)	0.8%
Intermediate	2-3	896 (41)	3.4%	694 (46)	3.9%
High*	4	188 (9)	7.4%	344 (23)	12%
High	5	62 (3)	15%		
High	6	13 (1)	32.5%		

One point is scored for any of the following: age >60 years, LDH > normal, ECOG performances status >1, stage III/IV disease, extranodal involvement ≥2 sites, kidney and/or adrenal involvement.
BCCA, British Colombia Cancer Agency.
*High risk group (4–6 factors) overall 2-year risk of CNS relapse of 10.2% in DSHNHL cohort

EN lymphomas originating in the CNS, testes, breasts, skin, and intravascular spaces. Interestingly, a recent series of SCNSL (n=13) confirmed a higher prevalence of the MCD subtype than a reference cohort of relapsed DLBCL with no CNS involvement (38% vs 8%; *P*=.003).[13] Furthermore, the hcMCD subtype defined by *MYD88*^{L265P} mutation or more than 3 mutations in *CD79*, *PIM1*, *ETV6*, *BTG1*, *PRDM1*, or *PBL1XR1* constituted almost half of the patients with CNS recurrence (46%). The remaining cases were either double-hit lymphoma (DHL) or associated with *TP53* mutations. Although these data need to be validated, there is clear potential for next generation sequencing analysis to help identify patients at risk of CNS relapse.

High-grade B-cell lymphomas harboring *MYC* translocation along with *BCL2* and/or *BCL6* translocation (DHL or triple-hit lymphomas] have historically been associated with a high risk of CNS involvement). However, there is accumulating evidence to suggest that early data overestimated this risk, as FISH was not performed consistently,[10] and such patients often meet other clinical criteria.

Baseline screening

Baseline screening with brain imaging and lumbar puncture/cerebrospinal fluid (CSF) analysis is increasingly used to identify high-risk patients with CNS involvement who may benefit from CNS-directed therapies. Several studies have shown that CSF analysis with flow cytometry is more sensitive than cytology for the detection of occult CNS involvement.[14] However, a proportion of patients with a negative flow cytometry result relapse in the CNS shortly after treatment, suggesting the need for more sensitive techniques. Cell-free circulating tumor DNA (ctDNA) has recently appeared as a prognostic biomarker in patients with CNS lymphoma, with good correlation between ctDNA levels (*MYD88*^{L265P} mutation) and treatment response and outcomes.[15-17] Two studies have assessed the role of CSF ctDNA analysis in patients with high-risk B-cell lymphoma.[17,18] The first analyzed specific tumor-derived mutations in sequential CSF samples from 12 patients receiving frontline treatment, and CSF ctDNA was detected 3 months before CNS relapse in 1 of 2 patients in whom this occurred.[17] More recently, Olszewski et al analyzed CSF from 22 patients with aggressive B-cell lymphoma using a next generation sequencing-minimal residual assay.[18] At diagnosis, CSF ctDNA was identified in 8 patients, 2 of whom relapsed in the CNS, with a 12-month cumulative risk of CNS recurrence of 29% in patients with a positive analysis vs a 0% risk for patients with negative CSF.[18] Taken together, acknowledging the limitation of the small number of patients, these results suggest the potential utility of CSF ctDNA to identify patients with a higher risk of CNS relapse. Further studies are ongoing to validate these findings before the technology can be incorporated into routine clinical practice.

How do we manage patients with CNS relapse/SCNSL?

Historically, SCNSL has been associated with a dismal prognosis and median overall survival (OS) of approximately 6 months.[19] Identifying effective therapeutic approaches remains challenging, and the majority of patients still progress or relapse shortly after treatment.

In recent years, combinations of intensive chemotherapies including HD-MTX followed by autologous stem cell transplantation (ASCT) have been adopted, with 2-year OS of 25% to 68% reported.[20,21] Recently, the MARIETTA phase 2 study examined the efficacy of 3 courses of MATRix (rituximab, MTX, cytarabine, thiotepa) plus 3 courses of RICE (rituximab, ifosfamide, etoposide, carboplatin) followed by carmustine and thiotepa-conditioned ASCT in 75 patients with CNS involvement at diagnosis or relapse. Two-year OS for the intention-to-treat population was 46%,[22] while those undergoing ASCT (37/75 patients) had 2-year OS of 83%. Two-year progression-free survival (PFS) of 71% was reported in patients with SCNSL at initial diagnosis, but PFS was only 28% in patients previously treated with R-CHOP. Although this study was restricted to patients under the age of 70, an ECOG PS of 3 or lower, and adequate organ function, thiotepa-based ASCT is increasingly utilized in older patients.[23] However, induction chemotherapy regimens are intense and less well tolerated in older patients in the real-world setting.[24]

Chimeric antigen receptor (CAR) T-cell therapy has shown promising results in relapsed/refractory DLBCL, including in older and unfit patients. There is accumulating data demonstrating the efficacy and safety of CAR T cells in CNS lymphoma.[25] In SCNSL, the TRANSCEND study demonstrated complete

remission (CR) in 3 of 6 patients, with grade 3 neurological events in 2 cases (27).[26] Similarly, small (≤8 patients) series of patients with highly refractory SCNSL treated with commercial agents have been reported as having CR rates of approximately 50% with no significant toxicity.[27,28] These findings suggest that CAR T cells may be a viable salvage treatment for this challenging population, especially for patients not fit enough to receive intensive immunochemotherapy. Furthermore, a number of phase 1/2 studies evaluating CAR T cells in CNS lymphoma are currently ongoing (NCT04608487, NCT04464200, NCT03484702).

Methods for delivery of CNS prophylaxis

Intrathecal chemotherapy

For many years, intrathecal (IT) cytotoxic chemotherapy was used as CNS prophylaxis in DLBCL, with supporting evidence derived mainly from nonrandomized, retrospective analyses as well as data extrapolated from other B-cell malignancies. More recently, a systematic review of stand-alone IT prophylaxis analyzed a total of 7357 patients treated with anti-CD20 monoclonal antibody–based immunochemotherapy, incorporating 3 post hoc trial analyses and 10 retrospective studies.[29] Overall, IT prophylaxis was not found to be associated with a reduction in CNS relapse rate on univariable or multivariable analyses. The delivery of IT therapy can be challenging and uncomfortable for the patient, with some evidence to suggest an association with infection-related hospitalization in older patients.[30] With an increasing recognition that the majority of CNS relapses in DLBCL involve the brain parenchyma, an area not penetrated by IT therapy alone, IT use has diminished in this setting, with a move toward systemic antimetabolite therapy instead. An exception to this is in testicular DLBCL, where IT therapy may continue to have a role based on data from prospective IELSG trials (see below).

HD-MTX

Approximately 70% to 80% of CNS relapses in DLBCL involve the brain parenchyma,[31] and therefore there is a rationale for prophylactic therapies that cross the blood-brain barrier and penetrate all CNS compartments. Intravenous HD-MTX has increasingly been used over the last 10 years as CNS prophylaxis in DLBCL, with initial supporting evidence mainly derived from its efficacy in primary CNS lymphoma. Over the years several studies that have the common theme of being nonrandomized, retrospective analyses have addressed this area, but they have been of variable size and have produced discrepant results (Table 3).[32-40] While there has been widespread incorporation of HD-MTX as

Table 3. Summary of recent studies evaluating use of HD-MTX in DLBCL

Study (year)	n	Design	Risk factors	Systemic treatment	CNS Prophylaxis	CNS relapse	Comments
Lewis et al[32] (2022)	2300	Multicenter, retrospective	CNS-IPI ≥4 Testicular, breast involvement DHL	R-CHOP (94%) R-EPOCH (6%)	1. HD-MTX (18%) 2. No HD-MTX (82%)	1. 9.2% (5y) 2. 8.1% (5y)	No benefit HD-MTX
Wilson et al[33] (2022)	1384	Multicenter, retrospective	High-risk EN sites CNS-IPI ≥4 ≥2 EN and LDH ↑	R-CHOP	1. HD-MTX (all, intercalated, or EOT)	1. 5.7% (3y) 2. 5.8% (3y)	No difference between EOT and intercalated HD-MTX
Orellana-Noia et al[34] (2022)	1030	Multicenter, retrospective	Not described	R-CHOP (48%) R-EPOCH (45%) Other (7%)	1. HD-MTX (20%) 2. IT (77%)	1. 6.8% 2. 5.4%	No benefit HD-MTX vs IT
Puckrin et al[35] (2021)	326	Multicenter, retrospective	CNS-IPI ≥4 Testicular DHL LDH ↑ + ECOG >1 + >1 EN	R-CHOP (85%) Intensive chemotherapy (15%)	1. HD-MTX (35%) 2. No HD-MTX (65%)	1. 12.2% 2. 11.2%	No benefit HD-MTX
Bobillo et al[36] (2021)	585	Single-center, retrospective	CNS-IPI ≥4 High-risk EN sites DHL	R-CHOP (68%) R-EPOCH (15%) Other (17%)	1. HD-MTX (7%) 2. IT MTX (43%) 3. None (50%)	1. 7.5% (5y) 2. 5.5% (3y) 3. 5%	No benefit (IT or HD-MTX)
Ong et al[37] (2021)	226	Multicenter, retrospective	High-risk EN sites CNS-IPI ≥4	R-CHOP	1. HD-MTX (29%) 2. No HD-MTX (71%)	1. 3.1% (3y, isolated) 2. 14.6% (3y, isolated)	HD-MTX significantly reduced risk of isolated CNS relapse
Wilson et al[38] (2020)	334	Multicenter, retrospective	CNS-IPI ≥4 High-risk EN sites ≥2 EN sites and LDH ↑	R-CHOP	1. HD-MTX (all, intercalated, or EOT)	1. 6.8% (3y) 2. 4.7% (3y)	No difference between EOT and intercalated HD-MTX
Lee et al[39] (2019)	130	Single-center, retrospective	CNS-IPI ≥4 High-risk EN sites ≥2 EN and LDH ↑	R-CHOP	1. HD-MTX (49%) 2. None (51%)	1. 6.9% (2y) 2. 8.1% (2y)	No benefit HD-MTX
Goldschmidt et al[40] (2019)	480	Multicenter, retrospective	High-risk EN sites Stage IV, LDH ↑, ≥1 EN	CHOP +/−R (80%)	1. HD-MTX (27%) 2. None (73%)	1. 6.9% 2. 6.3%	No benefit HD-MTX

prophylaxis for high-risk patients, disagreement has arisen about the safest and most effective way to incorporate it into front-line therapy. The practice of "intercalated" HD-MTX was first described in a single-center retrospective study of 65 patients in which HD-MTX was delivered at days 10 to 15 in between cycles of R-CHOP, resulting in a CNS relapse rate of 3%.[41] While this approach delivers early CNS-directed therapy, potentially advantageous given the often early onset of CNS relapse, it introduces potential toxicity and delays to systemic R-CHOP therapy, with many choosing to wait and deliver HD-MTX after R-CHOP completion instead.

In the last year, a number of studies in this area have been published to date, arguably providing the most robust data to inform our practice in the absence of prospective clinical trials. These studies have addressed 2 separate questions: (1) Is HD-MTX effective?, and (2) How should it be incorporated into front-line (R-CHOP/R-CHOP-like) therapy? Lewis et al carried out an international multicenter analysis of 2300 patients deemed at high risk for SCNSL on the basis of high CNS-IPI, the presence of double-hit FISH abnormalities, or the involvement of high risk sites (breast or testicular).[32] Patients received either HD-MTX (n=410) with or without concurrent IT therapy, IT therapy alone, or no CNS prophylaxis. There was no significant difference in the 5-year cumulative incidence of CNS relapse between the HD-MTX and no–HD-MTX arms (9.1% vs 8.4%, respectively), with results unchanged when analyses were restricted to patients achieving CR at the end of systemic treatment (5.0% vs 6.0%) and in subanalyses of patients with "ultra"–high-risk characteristics. These findings are consistent with those of another large retrospective study by Orellana-Noia et al, in which no reduction in CNS relapse was seen in patients receiving HD-MTX (n = 236) compared to those receiving IT prophylaxis alone (n = 894).

Wilson et al reported an international multicenter analysis of 1384 patients, all of whom received HD-MTX CNS prophylaxis delivered either intercalated (n = 749) or at the end of R-CHOP therapy (n = 635).[33] There was no difference in CNS relapse between the 2 delivery approaches (3-year rate of 5.7% vs 5.8%, respectively), with intercalated delivery causing significantly higher rates of R-CHOP delay. Notably, in analyses restricted to patients with high CNS-IPI (n = 600), the 3-year rate of CNS relapse was 9.1%, very similar to the rates reported in the original CNS-IPI study in which minimal CNS prophylaxis was used.[4]

Both these studies carry inherent caveats associated with retrospective data collection. Notably, the Lewis et al study had a relatively low number of patients in the HD-MTX arm, with potential for a signal toward benefit in very high-risk patients being missed as a result. The Wilson et al study had wide variation in the criteria used for selection for CNS prophylaxis, and both data sets contained significant numbers of patients receiving concurrent IT prophylaxis. However, both studies add compelling data to the argument that HD-MTX may not significantly reduce rates of CNS relapse for the majority of patients deemed to be "high risk" by traditional criteria. If the absolute risk reduction of 1% with HD-MTX from the Lewis et al study is accurate, 100 high-risk patients would need to be treated to avoid 1 CNS relapse. HD-MTX carries a significant risk of toxicity, including acute kidney injury, mucositis, and hepatotoxicity.[38] Considering that systemic treatment failure is a greater risk than CNS relapse, it appears likely that the balance of risks for the vast majority of patients favors prioritizing systemic therapy and forgoing HD-MTX altogether or, at the very least, delivering at end of treatment (EOT). An alternative approach for some very high-risk patients may be to use intensified systemic regimens that incorporate HD-MTX—for example, R-CODOX-M/IVAC, which has promising data in a phase 2 trial but has not been demonstrated to be superior to R-CHOP in a randomized trial and is associated with significant toxicity.[42]

Testicular DLBCL

Testicular lymphoma has been associated with a high risk of long-term CNS relapse, with a 5-year risk of 10% and 25% for limited (primary testicular lymphoma [PTL]) and advanced disease in the rituximab era, respectively.[6] Biologically, over 75% of testicular lymphomas resemble the ABC subtype and are enriched for the somatic mutations commonly seen in CNS lymphoma, such as *MYD88*^{L265P} and *CD79*, which are present in up to 70% of cases.

Two prospective studies explored the role of CNS prophylaxis in the rituximab era. The IELSG-10 phase 2 study demonstrated that patients with PTL treated with R-CHOP and contralateral radiation therapy plus 4 doses of IT MTX had a lower risk of CNS relapse compared to historical series (5-year cumulative risk of 6% vs 20%).[43] More recently, the IELSG-30 trial included a total of 54 patients with PTL receiving R-CHOP, contralateral radiation therapy, and 2 courses of HD-MTX (dose, 1.5g/m^2), along with 4 doses of IT liposomal cytarabine. Preliminary results showed no CNS relapses after a median follow-up of 5 years.[44] According to these studies, patients with testicular lymphoma may benefit from CNS prophylaxis incorporating HD-MTX and/or IT chemotherapy.

CLINICAL CASE (Continued)

Due to the presence of high-risk features for SCNSL, the patient had a baseline MRI head/spine and lumbar puncture with CSF analysis (flow cytometry) with no CNS disease evident. He went on to receive 6 cycles of R-CHOP-21, with intercalated HD-MTX (3g/m^2) planned on day 8 of R-CHOP cycle 2 and cycle 4. No IT prophylaxis was administered. Following the first HD-MTX treatment after R-CHOP cycle 2, the patient experienced a 7-day hospital admission with grade 2 renal toxicity and line infection. As a result, cycle 3 R-CHOP was delayed by 10 days. No further HD-MTX was given, and he received the remaining cycles of R-CHOP on schedule, with end-of-treatment PET-computed tomography demonstrating a complete metabolic response. At 12 months' follow-up, he remains well with no evidence of systemic or CNS disease relapse.

How do we approach CNS prophylaxis in 2022?

The above case, treated prior to the publication of the most recent large HD-MTX analyses,[32,33] demonstrates the difficulties in decision-making in this area. The patient had a CNS-IPI score of 5, corresponding to a 2-year risk of CNS relapse of 15% according to the trial data sets used in the formulation of the score. Had his ECOG performance status been 2 instead of 1, the CNS-IPI score would have been 6, conferring an estimated risk of 33%, although it should be emphasized that only 13 patients were in this category in the CNS-IPI trial data set. However, the patient had an inherently greater risk of systemic

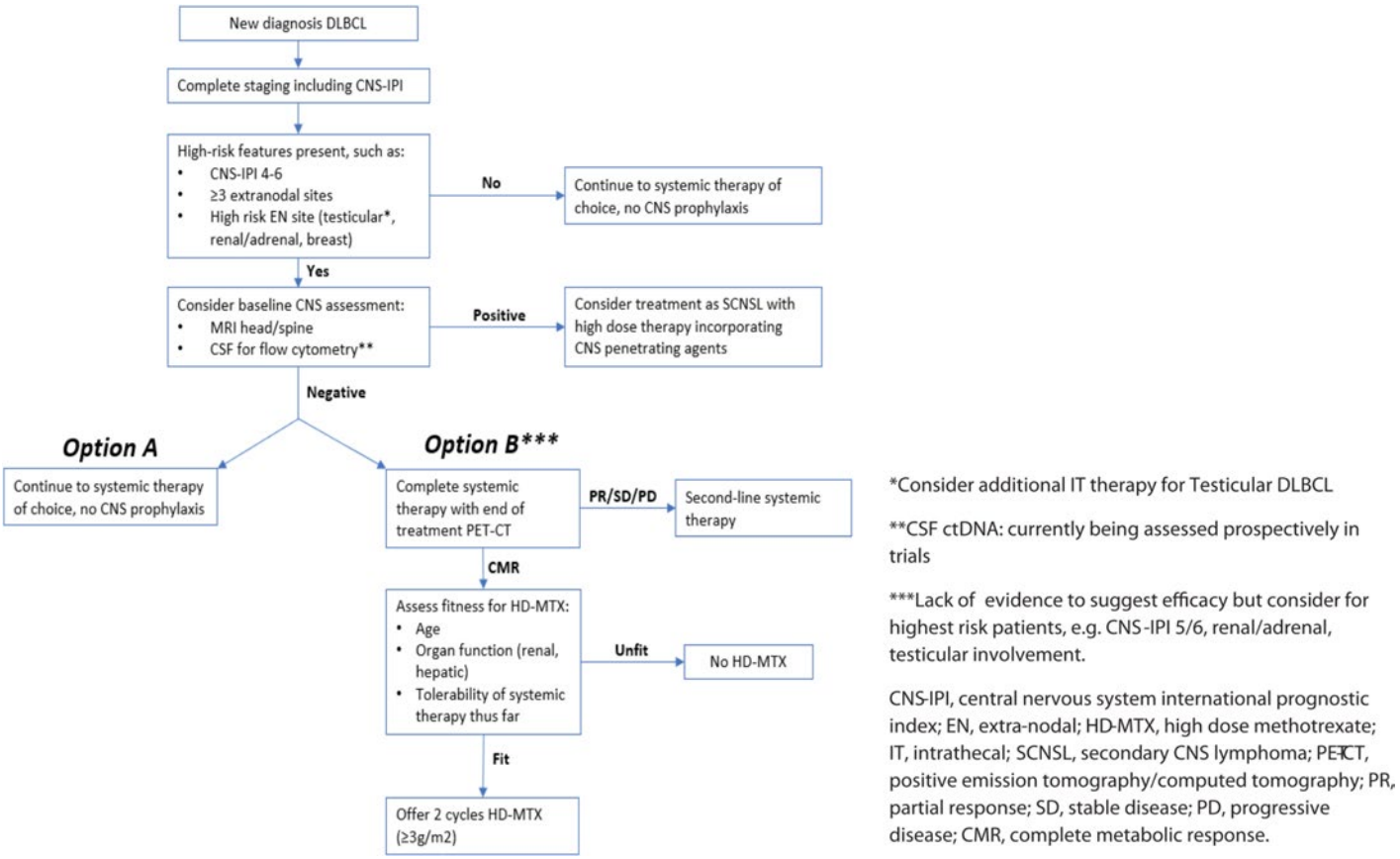

Figure 1. Proposed algorithm for CNS prophylaxis in DLBCL in 2022. CMR, complete metabolic response; CT, computed tomography; PD, progressive disease; PR, partial response; SD, stable disease.

treatment failure. He experienced significant toxicity following the first intercalated HD-MTX, resulting in delayed R-CHOP therapy that could potentially have had detrimental effect on systemic disease control.

If this patient were to present now for treatment, suggested approaches based on recent data are outlined in Figure 1. The decision-making involved essentially places greater emphasis on baseline screening for occult CNS involvement in high-risk patients, as well as more judicious use of HD-MTX.

Future directions and conclusions

Although we have seen advances in this extremely contentious area of DLBCL management in the last 5 years, it is clear that we need to continue to develop more specific methods of identifying patients at highest risk of CNS relapse and to investigate more effective prophylactic interventions for those at highest risk. As outlined above, the use of ctDNA as a baseline screening tool carries much potential for improving patient selection for prophylaxis. The incorporation of novel agents able to cross the blood-brain barrier is likely to be an area of ongoing research. Although trials thus far have not demonstrated an overall benefit with the addition of ibrutinib or lenalidomide to R-CHOP,[45,46] studies such as REMoDL-A (NCT04546620) investigating the addition of acalabrutinib are ongoing, and results with regard to CNS relapse rates will be of interest. Improving systemic disease control may be an effective way to reduce CNS relapses, particularly those that occur concurrent with systemic relapse. The recent POLARIX trial demonstrated an additional agent (polatuzumab vedotin) that can improve PFS over R-CHOP alone for the first time, raising the question of whether broad adoption of such a frontline regimen could have an impact on CNS events over time.[47] Until then, we must use currently available risk-stratification models to carefully select patients for more stringent baseline screening for CNS disease and exercise greater caution in the use of prophylactic HD-MTX in light of recently published data.

Conflict-of-interest disclosure

Matthew R. Wilson: speakers' bureau: Kite/Gilead, Janssen; consultancy/advisor: Veriton; conference/travel support: Takeda, Janssen, Kite/Gilead; research funding: Abbvie.
Sabela Bobillo: speakers' bureau: Janssen, Roche, Gilead; travel support: Gilead.
Kate Cwynarski: consultancy/advisor: Roche, Takeda, Celgene, Atara, Gilead, KITE, Janssen, Incyte; speakers' bureau: Roche, Takeda, KITE, Gilead, Incyte; research funding: Roche, Takeda, KITE, Janssen, Bristol Myers Squibb.

Off-label drug use

Matthew R. Wilson: nothing to disclose.
Sabela Bobillo: nothing to disclose.
Kate Cwynarski: nothing to disclose.

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DOI 10.1182/hematology.2022000331

PAPER 6

Bobillo S, **Wilson MR,** Cwynarski K. Controversies in central nervous system prophylaxis of high-risk diffuse large B-cell lymphoma. Curr Opin Oncol 2023 Sep 1;35(5):382-388. PMID: 37551947

https://journals.lww.com/co-oncology/fulltext/2023/09000/controversies_in_central_nervous_system.6.aspx

Journal impact factor: 3.4
Number of citations: 1

Summary of contribution:

Conceptualisation	Yes with KC and SB
Data Curation	Yes – performed literature review along with SB
Formal Analysis	N/A
Investigation	N/A
Methodology	N/A
Project Administration	SB
Visualisation	SB
Writing – original draft	Led by SB. I wrote sections on clinical/biological risk factors for CNS relapse and future directions.
Writing – review & editing	SB

Title: Controversies in the CNS prophylaxis of high-risk diffuse large B cell lymphoma

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Abstract

Purpose of review: Central nervous system (CNS) relapse in patients with diffuse large B cell lymphoma (DLBCL) is an uncommon but devastating complication with an overall survival of less than 6 months. This article will review the recent updates on CNS prophylaxis including new potential advances in the identification of high-risk patients.

Recent findings: The identification of patients at high risk of CNS relapse based on clinical and biological features has improved over recent years, however, the effectiveness of different CNS prophylaxis strategies including intrathecal chemotherapy and high-dose methotrexate have been recently questioned in several large retrospective studies. The analysis of cell free circulating tumor DNA (ctDNA) in the cerebrospinal fluid has been shown to identify patients with a high risk of CNS involvement and work is ongoing to identify how this can be used as a prognostic biomarker.

Summary: Recent clinical retrospective data have questioned the effectiveness of intrathecal and high-dose methotrexate in the prevention of CNS relapse in high-risk DLBCL patients. The role of more sensitive methods to detect CNS involvement and the benefit of novel therapies in CNS relapse prevention are currently under evaluation.

Keywords: CNS prophylaxis, high-dose methotrexate, cell free circulating tumor DNA, diffuse large B cell lymphoma.

Introduction:

Central nervous system (CNS) relapse is an uncommon yet often fatal complication that usually occurs within the first year after initial diagnosis of diffuse large B-cell lymphoma (DLBCL).[1] The incidence of CNS relapse in patients with DLBCL is approximately 5%; however, the presence of certain risk factors may increase the risk up to 15%-20%. [1, 2] Management of patients with CNS relapse, either isolated or concurrent with systemic lymphoma, remains challenging, especially for patients not fit enough to receive intensive therapy and autologous stem cell transplantation.[3*,4*,5]

Given the dismal outcomes associated with CNS relapse, in recent years there has been growing interest in defining patients at highest risk of this complication as well as exploring the role of CNS prophylaxis in this group. The central nervous system international prognostic index (CNS-IPI)(6) and the recent new molecular classification of DLBCL [7, 8] have contributed to a better classification of high-risk patients, however the effectiveness of current CNS prophylaxis strategies including intrathecal methotrexate (IT MTX) and high-dose intravenous methotrexate (HD-MTX) remains controversial.[9-15] This article will focus on the recent updates on CNS prophylaxis, including new advances in defining patients at high risk of CNS relapse and the potential role of more sensitive methods in early detection of CNS involvement.

Clinical risk factors:

The CNS-IPI is the most robust risk model for predicting CNS relapse in the rituximab era.(6) The model was developed from a population of patients with aggressive B-cell lymphoma from the German High Grade Non-Hodgkin Lymphoma Study Group (including 80% with DLBCL), and subsequently validated in a patient cohort from the BC Cancer Agency treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). One point is allocated for each of the standard five IPI risk factors (age >60 years, elevated lactate dehydrogenase,

performance status ≥2, extranodal sites ≥2, advanced stage disease) as well as kidney or adrenal involvement. As outlined in **Table 1**, this results in three risk groups with the high-risk group traditionally targeted for CNS prophylaxis. However, the model lacks positive predictive value as approximately half of CNS relapse events occur in the low/intermediate risk groups.[6]

In addition to the factors included in the CNS-IPI, other clinical risk factors have been suggested as predictors of CNS relapse, especially the involvement of certain extranodal sites. However, increasingly there is a recognition that a number of extranodal sites (e.g. bone marrow, uterus and epidural involvement) are strongly associated with advanced stage disease or other high-risk features and lack independent prognostic power.[16] The most robust evidence for individual sites portending higher risk relates to renal/adrenal (independent risk factor in the CNS-IPI) and testicular involvement, which has been associated with CNS relapse rates in the rituximab era of 10 and 25% for limited and advanced stage disease respectively.[17] Breast involvement is more controversial, but has been associated with a higher risk of CNS relapse (~15%) in retrospective series.[18]

Biological risk factors:

High grade B-cell lymphomas with *MYC* translocation as well as *BCL2* and/or *BCL6* translocation (so called double (DHL) or (previously described) triple hit lymphomas) have previously been associated with increased CNS relapse risk. However, early studies addressing this question were confounded by selection bias with non-uniform application of fluorescence in situ hybridization (FISH) studies.[19] Recently, a retrospective series of 191 patients with DHL from British Colombia was presented, with cases only included from a time period when FISH was routinely incorporated for all new aggressive B-cell lymphoma cases.[20] The 2 year risk of CNS relapse in the series was lower than historical estimates at 6%, and the CNS-IPI remained predictive of CNS relapse suggesting that the risk is driven by other established clinical factors rather than the DHL status itself.

Activated B-cell (ABC) subtype DLBCL is associated with higher CNS relapse risk than germinal centre subtype, with the increased risk more clearly defined when cell-of-origin (COO) is assessed using gene expression profiling. A sub-analysis of the GOYA study found a cohort with CNS relapse risk of 15% defined by combining ABC-subtype (by gene expression profiling) with high CNS-IPI.[21] DLBCL with overexpression of MYC and BCL2 by immunohistochemistry, so-called 'double expressor' subtype, has also been associated with increased risk, possibly related to the high proportion of such cases which are ABC-subtype COO.[19, 21]

Recently, the biological heterogeneity of DLBCL has been more thoroughly explored, with application of next generation sequencing (NGS) to identify distinct molecular subtypes.[7, 8] Two independent scientific groups have published new molecular classifications of DLBCL. The 'MCD' and 'C5' clusters are characterised by ABC subtype COO and a high frequency of *MYD88*^{L265P} and *CD79* mutations. These mutations are also frequently observed in extranodal lymphomas of immune-privileged sites, e.g. CNS, testicular and breast DLBCL. A recent analysis of 26 patients who experienced CNS relapse after rituximab-based immunochemotherapy demonstrated a higher prevalence of MCD subtype than in a reference cohort of relapsed DLBCL with no CNS involvement (38% vs 8%, p.003).[22**] Using a simplified hierarchical clustering based on commonly-mutated genes included in most clinically available NGS panels, 84% of MCD tumors could be identified and this group comprised 46% of CNS relapses. Although larger studies are required to validate these findings, there is clear potential for NGS to more precisely identify patients at highest risk of CNS relapse.

Baseline screening

Baseline screening usually comprises brain imaging and cerebral spinal fluid (CSF) analysis including cytology and flow cytometry. Although flow cytometry is a more sensitive technique than cytology, a proportion of patients with negative flow cytometry will still experience CNS relapse either during or

soon after first-line therapy. Given the controversy regarding the efficacy of CNS prophylaxis in high-risk patients, recently there has been considerable interest in developing more sensitive technologies for early detection of CNS involvement. The analysis of cell free circulating tumor DNA (ctDNA) in the CSF has emerged as a new prognostic marker in primary CNS lymphoma and several studies have recently demonstrated the presence of *MYD88*^{L265P} mutation in the CSF from patients with primary or secondary CNS lymphoma.[23, 24] Furthermore, a recent large study including 92 patients with CNS lymphoma demonstrated that CSF ctDNA levels at diagnosis and during treatment were predictive of clinical outcomes.[25**] Two studies have explored the role of ctDNA in predicting CNS relapse in patients with high-risk B cell lymphoma so far.[26*, 27*] Bobillo et al. analysed specific tumor-derived mutations in the CSF from 12 patients with newly diagnosed high-risk B-cell lymphoma.[26*] Interestingly, CSF ctDNA was detected 3 months before CNS recurrence in one of two patients with CNS relapse. Olszewski et al. analysed 22 patients with high-grade B-cell lymphoma and detected clonotypic DNA at diagnosis in the CSF from 8 patients, of whom 2 relapsed in the CNS, with a 12-month cumulative risk of CNS relapse of 29% vs. 0% for patients with a positive vs. negative result, respectively.[27*] According to these studies, CSF ctDNA could be a more sensitive technique to identify patients with higher risk of CNS who may benefit from directed CNS-therapies. Further studies including a larger number of patients are needed to validate these findings.

CNS prophylaxis strategies:

Intrathecal chemotherapy has been historically used as CNS prophylaxis in DLBCL based on data extrapolated from other aggressive lymphomas such as Burkitt lymphoma and evidence from retrospective series conducted before the introduction of rituximab.[1, 2, 28, 29] A systematic review of stand-alone IT prophylaxis including 7,357 patients from 3 post hoc analysis and 10 retrospective studies treated in the rituximab era demonstrated no reduction in CNS relapse rate.[9] As a result, the use of IT prophylaxis in patients with high-risk DLBCL has diminished significantly in

the last 5 years. An exception to this is testicular DLBCL, where IT therapy may continue to have a role based on data from prospective trials.[30, 31*] The IELSG-10 phase 2 study demonstrated that patients with primary testicular lymphoma treated with R-CHOP, contralateral radiation therapy (RT) and 4 doses of IT MTX had a 5-year cumulative risk of CNS relapse of 5% which is lower than historical series (~20%).[30] More recently, the same group reported no CNS relapses in 54 patients with testicular lymphoma treated in the IELSG-30 trial with R-CHOP, contralateral RT along with 2 cycles of HD-MTX (dose 1.5g/m^2) and 4 doses of IT chemotherapy, after a median follow-up of 5 years.[31*] Although longer follow-up is needed to identify late CNS relapses, these results support a possible role for IT MTX along with HD-MTX and contralateral RT in this particular setting.

High-dose intravenous methotrexate has been increasingly used over the last 10 years given the concern over lack of efficacy of IT prophylaxis and in the rituximab era the observation that CNS relapses frequently involve the brain parenchyma, an area not penetrated by IT therapy.[32] Although initial retrospective studies described lower CNS relapse events in patients receiving HD-MTX,(33-35) several retrospective series in the last 3 years have controversially found no apparent benefit of HD-MTX prophylaxis in high-risk patients (**Table 2**).[10**-13, 36] The largest study conducted to date was presented at the ASH conference in 2021, and analysed 2,300 high-risk patients (high CNS IPI, double-hit, or testicular or breast involvement) of whom 410 received CNS prophylaxis with HD-MTX +/- IT MTX. [10**] The 5-year cumulative incidence of CNS relapse was not significantly different between patients who received HD-MTX and those who did not (8.1% vs. 9.2%, respectively). Although this and previous retrospective studies showed lack of benefit of HD-MTX, it is important to note that none of these studies were powered to detect a difference in the very high-risk subgroups (e.g. CNS-IPI 5-6, renal/adrenal or testicular involvement). Other limitations are the retrospective nature of the studies, the different criteria used to define high-risk groups and presumed treatment selection bias since patients who received CNS prophylaxis tended to be younger, have better performance status and more had CNS staging (scan+/- CSF) than patients who did not.

The optimal timing of HD-MTX administration has also been recently investigated. Wilson et al. conducted an international retrospective study of 1,384 patients receiving HD-MTX either intercalated between cycles (n=749) or following completion of systemic therapy (n=635).[36*] Intercalating HD-MTX did not reduce CNS relapse compared with end of treatment HD-MTX (3-year rate of 5.7% vs 5.8% respectively) and resulted in significant delays to delivery of systemic therapy.[36*] Furthermore, the overall rate of CNS relapse seen in the group with high CNS-IPI (n=600) was remarkably similar to the rates seen in the Lewis et al study and in the original CNS-IPI publication at 9.1%, despite the use of HD-MTX in all patients which challenges whether such an approach is efficacious.

Finally, for patients with very high-risk features, the use of intensified systemic regimens incorporating HD-MTX and other drugs crossing the blood brain barrier (BBB) may be an option, although such regimens have been associated with significant toxicity, especially in older and unfit patients.[37, 38] The French Lymphoma Study Alliance (LYSA) and the German Lymphoma Alliance (GLA) recently analysed the risk of CNS relapse in 2203 younger patients (age < 60 years) treated in several prospective phase II and phase III trials with R-CHOP, R-CHOEP (R-CHOP plus etoposide), dose-escalated R-CHOEP followed by stem cell transplantation (R-MegaCHOEP) or rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycine, prednisone (R-ACVBP) plus consolidation including drugs crossing the BBB. The cumulative incidence of CNS relapse was generally low in the high-risk patients (age adjusted IPI of 2 -3, n=627), especially in those receiving R-ACVBP plus consolidation vs. (Mega)-R-CHO(E)P with a 3-year CI of 1.6% vs. 4%, (HR 2.4 (95% CI: 0.8-7.4), respectively (p=0.118).[39] Although this data is retrospective it is of interest and supports intensive approaches incorporating agents that cross the blood brain barrier in fit patients.

Future directions:

CNS prophylaxis continues to be one of the most contentious areas of DLBCL management due to the lack of prospective trials addressing this rare, but clinically important, problem. There are a number of potential hurdles when considering the design of a potential randomised control trial in this area. Firstly, the sample size required to power a trial with CNS relapse as a primary endpoint would be exceptionally large. Secondly, there has been a lack of agreement about what form CNS prophylaxis should take in an experimental arm. Although HD-MTX has been the most widely adopted agent in recent years, in the last two years large (albeit retrospective) studies have cast significant doubt over whether it has clinically meaningful efficacy in this setting. Therefore, it is challenging to design a trial in 2023 which randomises patients to HD-MTX vs no prophylaxis. Finally, in an era where we have an increasing knowledge of the biological heterogeneity of DLBCL, as well as an expanding array of novel agents capable of targeting recurring genetic aberrations in high-risk subgroups, the focus has understandably shifted towards prospective evaluation of incorporation of these agents instead of interventions such as HD-MTX.

One area where there is growing consensus is the timing of HD-MTX, with general acceptance now that if HD-MTX is used, it can be delivered at end of R-CHOP therapy rather than intercalated between cycles. Indeed, some ongoing prospective DLBCL trials investigating incorporation of novel agents, e.g. REMoDL-A (NCT04546620), permit the use of HD-MTX but only after completion of systemic therapy. REMoDL-A is investigating the addition of the BTK-inhibitor acalabrutinib to R-CHOP, and results with regards to CNS relapse rates in this trial will be of interest. An earlier trial investigating the addition of ibrutinib to R-CHOP showed no survival benefit or reduction in CNS relapses[40] , however it is hoped that the reduced toxicity of new BTK-inhibitors may result in greater benefit. Given the lack of benefit demonstrated in several trials investigating the addition of novel agents to all DLBCL patients[40, 41] , it seems clear that future studies need to more specifically target the patients most likely to benefit from these therapies.

The use of modern technologies such as NGS, gene expression profiling and ctDNA has the potential to allow a sophisticated risk adapted approach to prospective DLBCL trials, and may improve outcomes for patients at greatest risk of systemic treatment failure. Given that this patient group is also at highest risk of CNS relapse, this could potentially reduce the incidence of SCNSL, particularly if novel agents capable of crossing the blood-brain barrier are used. Furthermore, ctDNA analysis of CSF has clear potential to be a highly sensitive and specific tool for identifying patients at greatest risk of CNS relapse, although further studies are required to determine the optimum treatment for this group.

Conclusion

CNS prophylaxis remains a challenge due to the lack of prospective data addressing this important problem. Integration of molecular biomarkers with classical clinical risk factors might improve the selection of patients for CNS prophylaxis. International collaboration to address these important questions will be crucial, while for now it appears reasonable to exercise greater caution in the use of prophylactic HD-MTX in light of recently published data.

Key bullet points:

- Patients with high-risk diffuse large B-cell lymphoma should be carefully considered for CNS prophylaxis in light of recently published data
- Integration of clinical risk factors with new molecular biomarkers might improve the selection of patients for CNS prophylaxis.
- Baseline analysis of CSF circulating tumor DNA may have a role in detecting occult CNS involvement in patients with aggressive diffuse large B-cell lymphoma.

Conflict of interest:

Conflict of interest: SB: Speakers's Bureau: AstraZeneca, Abbvie, Janssen. Conferences/Travel support: Roche, Janssen. MW: Honoraria/consultancy – Kite/Gilead, Janssen, Veriton Conference/travel support – Takeda, Janssen, Kite/Gilead, Abbvie. KC: Consulting/Advisory Role: Roche, Takeda, Celgene, Atara, Gilead, KITE, Janssen, Incyte, Abbvie. Speakers' Bureau: Roche, Takeda, KITE, Gilead, Incyte. Conferences/Travel support: Roche, Takeda, KITE, Janssen, BMS

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Table 1: Summary of clinical and biological risk factors for CNS relapse in DLBCL

CNS, central nervous system; CNS-IPI, central nervous system international prognostic index; MVA, multivariable analysis; ABC, activated B-cell; DHL, diffuse large B-cell lymphoma with *MYC* and *BCL2* rearrangements; NGS, next generation sequencing.

Table 2. Summary of the most recent studies analyzing HD-MTX

DLBCL, diffuse large B-cell lymphoma; HD-MTX: high-dose methotrexate; CNS: central nervous system; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; IT MTX: intrathecal methotrexate; LDH: lactate dehydrogenase; R-HCVAD: rituximab, dexamethasone, cyclophosphamide, doxorubicin, vincristine, methotrexate, cytarabine; R-CODOX-M-IVAC: rituximab, doxorubicin, vincristine, cyclophosphamide, cytarabine, methotrexate, ifosfamide, etoposide; DLBCL: diffuse large B-cell lymphoma; CNS-IPI: central nervous system international prognostic index; DA-EPOCH-R: dose-adjusted rituximab, etoposide, cyclophosphamide, prednisolone, doxorubicin, vincristine; EOT: end of treatment; EN: extranodal; y, years; n, number; isol, isolated.

[*] breast, testis, kidney/adrenal
[†] testis, breast, kidney, adrenal, bone marrow

Table 1

	Frequency	Estimate of CNS relapse risk	Comments
Clinical risk factors			
CNS-IPI[6] :			
Low (0-1)	31-46%	0.6-0.8%	Robust risk-model but lacking positive predictive value High risk includes 'ultra high-risk group' with score 5/6 (CNS relapse rate 15-33%)
Intermediate (2-3)	41-46%	3.4-3.9%	
High (4-6)	12-23%	10.2-12.0%	
Renal/adrenal[6, 42]	2%	25-40%	Independent risk factor on MVA in CNS-IPI study
Testicular[43, 44]	~5%	10% (limited) – 25% (advanced)	Predominantly ABC subtype, enriched for somatic mutations seen in CNS lymphoma e.g. *MYD88*^{L265P} and *CD79*
Breast[45]	<2%	12-16%	Often localised, potentially underrepresented in clinical trials
≥3 extranodal sites[43]	10%	15%	
Biological risk factors			
'Double hit' DLBCL[19, 46]	5-10%	5-15%	Risk overestimated in early studies, DHL status itself may not be independently predictive
'Double expressor' DLBCL[19, 47]	20-30%	~10%	Majority are ABC-DLBCL
ABC cell-of-origin[21]	~25%	7%	CNS relapse ~15% when ABC subtype and high CNS-IPI
MCD molecular subtype[22, 48]	14%	38%	Larger studies required, NGS not currently widely available

Table 2

Study (year)	n	Risk factors	Chemotherapy	CNS Prophylaxis	CNS relapse	Comments
Ong *et al* (2021)[14]	226	High risk EN sites* CNS-IPI ≥ 4	R-CHOP	1. HD-MTX (29%) 2. No HD-MTX (71%)	1. 3.1% (3y, isolated) 2. 14.6% (3y, isolated)	HD-MTX reduced the risk of isolated CNS relapse (p=0.046)
Bobillo *et al* (2021)[13]	585	High risk EN sites† CNS-IPI ≥ 4 double-hit (*MYC/BCL2*)	1. R-CHOP (68%) 2. R-EPOCH (15%) 3. Other (17%)	1. None 2. IT MTX (43%) 3. HD-MTX (7%)	1. 7.5% (5y) 2. 5.5% (5y) 3. 3. 5% (5y)	No benefit MTX IT vs. HD-MTX vs. none
Puckrin *et al* (2021) [12]	906 (326 high-risk)	CNS-IPI ≥ 4, testicular, double-hit, LDH ↑ +, ECOG >1 + > 1 EN	1. R-CHOP 2. Intensive chemotherapy	1. None 2. HD-MTX (35%)	1. 12.2% 2. 11.2%	No benefit
Orellana-Noia *et al* (2022)[11]	1162	All patients received CNS prophylaxis	R-CHOP DA-EPOCH-R	1. HD-MTX (20%) 2. IT (77%)	1. 6.8% 2. 5.4%	No benefit MTX iv vs. IT MTX. No benefit in the subgroup analysis
Wilson et *al* (2022)[36]	1384	All patients received prophylaxis with HD-MTX	R-CHOP-like	1. MTX intercalated (n=749) 2. HD-MTX EOT (n=635)	1. 5.7% (3y) 2. 5.8% (3y)	No difference between EOT and intercalated
Lewis *et a*l (2022)[10]	2300	CNS-IPI ≥ 4, double-hit (*MYC/BCL2*), primary testicular or primary breast	R-CHOP-like or DA-EPOCH-R-like	1. None (n=1890) 2. HD-MTX +/- IT (n=410)	1. 9.1% (5y) 2. 8.4% (5y)	No benefit

PAPER 7

Wilson MR, Kirkwood AA, Wong Doo N et al. Dosage of high-dose methotrexate as CNS prophylaxis in DLBCL: A detailed analysis of toxicity and impact on CNS relapse. Am J Hematol 2024 Feb;99(2):E46-E50. PMID: 38037530

https://onlinelibrary.wiley.com/doi/full/10.1002/ajh.27167

Journal impact factor: 12.8
Number of citations: 0

Summary of contribution:

Conceptualisation	Yes with TE
Data Curation	Yes – database created from paper 3 used for this analysis – see details above
Formal Analysis	AK performed statistical analyses
Investigation	Yes – with AK
Methodology	Yes – with AK
Project Administration	Yes
Visualisation	Yes
Writing – original draft	Yes
Writing – review & editing	Yes

Received: 18 August 2023 | Revised: 27 October 2023 | Accepted: 6 November 2023

DOI: 10.1002/ajh.27167

CORRESPONDENCE

Dosage of high-dose methotrexate as CNS prophylaxis in DLBCL: A detailed analysis of toxicity and impact on CNS relapse

To the Editor:

Central nervous system (CNS) relapse in diffuse large B-cell lymphoma (DLBCL) is a rare event, occurring in 2%–5% and is associated with a poor prognosis.[1] Certain patient and disease characteristics significantly increase this risk.[2] CNS-directed prophylaxis has often been incorporated into first-line therapy in patients at highest risk. In light of cumulative evidence suggesting that intrathecal (IT) therapy is ineffective,[3] high-dose intravenous methotrexate (HD-MTX) has become widely used as prophylaxis, based largely on retrospective, underpowered analyses suggesting a potential benefit.[4]

We published an analysis of 1384 patients receiving HD-MTX prophylaxis either intercalated between R-CHOP (i-HD-MTX) or at "end-of-treatment" (EOT), demonstrating increased R-CHOP delays with i-HD-MTX and, crucially, similar rates of CNS relapse between the approaches.[5] EOT HD-MTX is now considered the optimal approach. The overall rate of CNS relapse seen in patients with a high CNS-IPI (9.1%), despite the use of HD-MTX, raised the question as to whether it has any benefit, irrespective of delivery time.

Several additional studies have addressed this question,[6–9] with the largest being a recent retrospective analysis of 2418 patients.[10] There was no clinically significant reduction in CNS relapse in patients in first complete remission who received HD-MTX ($n = 356$), nor any clear benefit in ultra-high risk subgroups. Accepting the limitations of retrospective analyses, there is now significant uncertainty about the role of HD-MTX as CNS prophylaxis in DLBCL. However, given the lack of alternative strategies, and concern that the aforementioned studies were underpowered to demonstrate benefit in ultra high-risk subgroups, it is likely that HD-MTX will still be used for selected patients. One such group is testicular DLBCL, where prospective IELSG trial data suggest a potential benefit of HD-MTX, albeit at doses of 1.5 g/m^2 and in combination with IT therapy.[11]

There remains a lack of consensus regarding the optimal dosage and HD-MTX cycle number when used as prophylaxis, with international guidelines lacking consensus on this matter.[4,12,13] In our prior international study, we found huge variation in practice, with 25% of patients having ≥3 cycles and some having up to 6.[5] Given the potential significant toxicity of HD-MTX and the uncertainty around its efficacy, we performed an analysis of the impact of HD-MTX dosage on both toxicity and patient outcome (survival and specifically CNS relapse).

The details of the HD-MTX database including inclusion/exclusion criteria, patient baseline characteristics, and treatments are previously described.[5] A total of 1384 patients were included, $n = 635$ receiving EOT HD-MTX and $n = 749$ i-HD-MTX; a total of 3111 HD-MTX cycles were analyzed. A landmark cohort of patients alive and in CR 8 months from diagnosis was used for all outcome analyses (CNS relapse, PFS and OS) to control for immortality bias and included $n = 1217$ (EOT, $n = 587$; i-HD-MTX, $n = 630$). Statistical methodology is described in Appendix S1.

Baseline characteristics are described previously[5] (Table S1). The median follow-up from 8-month landmark was 31.3 months (IQR, 15.6–52.6). Details of number and dose of HD-MTX cycles (cumulative and peak [maximum individual dose]) are displayed in Table S2. Although the median number of HD-MTX cycles and median cumulative dose were equal in the two groups (2 cycles, 6 g/m^2 respectively), significantly more patients received ≥3 cycles (37% vs. 12%, $p < .0001$) or had a cumulative dose >9 g/m^2 in the i-HD-MTX group. More patients had a peak HD-MTX dose of <3 g/m^2 in the EOT group (23% vs. 9%, $p < .001$): these patients were older, had lower baseline creatinine clearance, higher ECOG performance status, higher CNS-IPI, and were more likely to receive fewer HD-MTX cycles (Table S2). Analyses of factors influencing first HD-MTX dose are described in Appendix S3.

Numerically higher rates of Cycles 1 and 2 toxicities were recorded with i-HD-MTX (Figure 1A). However, due to the potential confounding effect of recent R-CHOP, only toxicities following EOT HD-MTX were analyzed in further detail (Tables S3 and S4); 252/635 (40%) experienced toxicity thought related to HD-MTX, with 44/635 (7%) grade ≥3. The most common were mucositis, hepatic, infection and renal with 14% experiencing renal toxicity (Grade ≥2, 6; Grade ≥3, 2%).

Higher doses in Cycle 1 were associated with an increased risk of mucositis, but no other toxicities. In Cycle 2, higher dose was associated with an increased risk of hepatic toxicities, in all patients, and those given at least 90% of the first cycle dose. No significant difference was seen for grade ≥3 events; however, numbers were small for Cycle 2 ($N = 16$) and not analyzable by type. Patients were less likely to be given a second cycle if they experienced toxicity in Cycle 1; 26% versus 5%, $p < .001$. This difference was greatest for renal toxicity; 51% versus 7%, $p < .001$ with no patients experiencing grade ≥3 continuing; 100% versus 10.3%, $p < .001$. Similar findings were

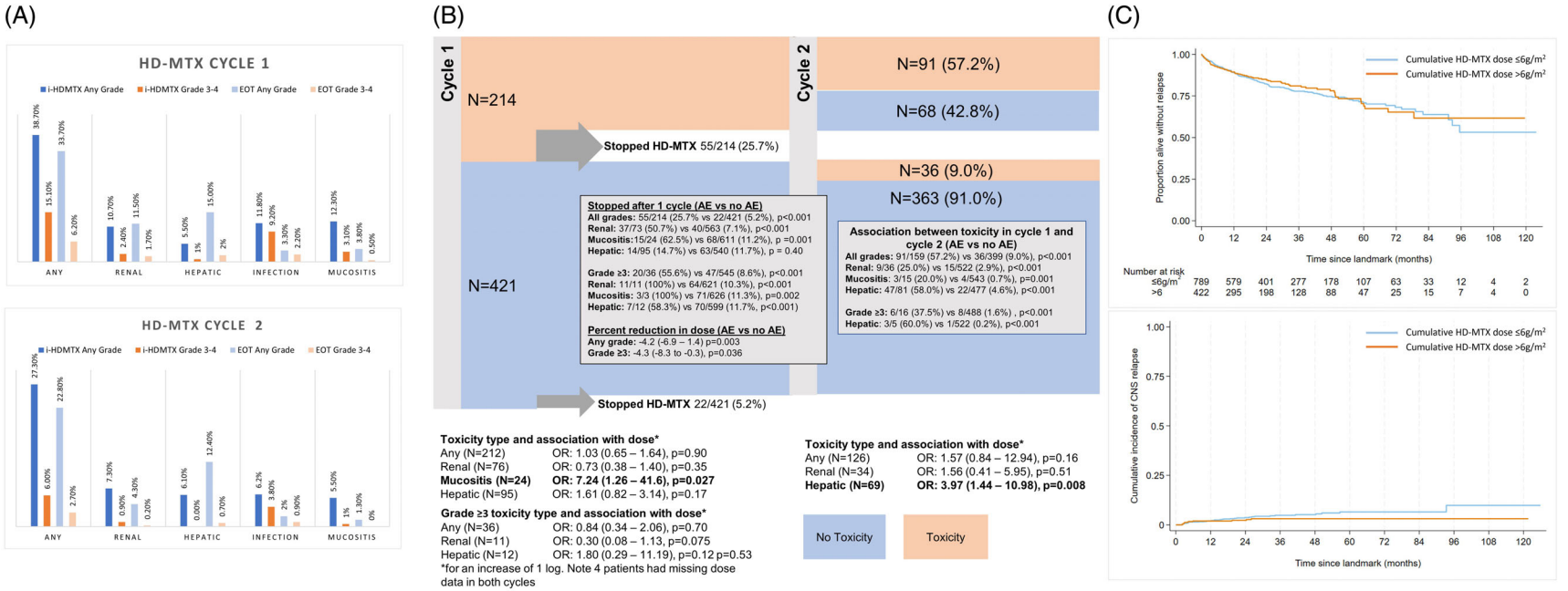

FIGURE 1 (A) Details of toxicities experienced with Cycles 1 and 2 HD-MTX for both intercalated (i-HD- MTX) and EOT (end of treatment) delivery. (B) Details of toxicity with EOT HD-MTX delivery, including proportion of patients continuing to Cycle 2 and reasons for stopping, association between toxicity in Cycles 1 and 2, and association of toxicity with HD-MTX dose. (C) Progression-free survival and cumulative incidence of CNS relapse according to cumulative dose of HD-MTX.

observed for mucositis ($p < .001$, any and grade ≥3) and hepatic toxicity (grade ≥3 only, $p < 0.001$).

Patients who experienced toxicity in Cycle 1 were at higher risk of another event in Cycle 2; this was significant for all events analyzed and included a 58% risk of a hepatic event compared to 5% risk in those who had not experienced one in Cycle 1 ($p < .0001$). Patients without grade ≥3 events in Cycle 1 were at very low risk of having a grade ≥3 event in Cycle 2 even when treated with ≥90% of the dose (1.7%).

In the landmark cohort, 47 CNS relapse events occurred ($n = 45$ with complete covariate data), 36 were isolated and 11 synchronous with systemic relapse. Twelve CNS relapse events occurred before the 8-month landmark (8 isolated, 4 synchronous). Full details of analyses on CNS relapse, PFS and OS are in Table S5. There was no significant reduction in CNS relapse with increasing HD-MTX dose, considering dose either cumulatively (HR, 0.69 [95% CI, 0.39–1.22], $p = .20$) (total dose: ≤6 g/m^2 vs. >6 g/m^2, Figure 1C) or as peak dose (HR, 0.99 [95% CI, 0.38–2.55], $p = .98$). Similarly, there was no significant difference in PFS for either cumulative HD-MTX dose (HR, 1.04 [95% CI, 0.77–1.41], $p = .80$) (Figure 1C) or peak dose (HR, 1.06 [95% CI, 0.63–1.77], $p = .83$). Non-relapse mortality (NRM) was reported in 55/1384 (4.0%) of patients overall, and 44 in the landmark cohort. There was no association between NRM and cumulative HD-MTX dose (Appendix S2).

We present the largest study of its kind, analyzing 1384 patients receiving a total of 3111 HD-MTX cycles, specifically assessing the impact of HD-MTX dose on toxicity, CNS relapse, and survival. We demonstrated no reduction in CNS relapse with higher cumulative or peak doses of HD-MTX. We used a multivariable landmark analysis to mitigate for immortality bias and to account for potential early events, preventing HD-MTX completion.

We limited our detailed analysis of HD-MTX toxicity to the EOT group, given the potential impact of concurrent R-CHOP with i-HD-MTX. However, it is noteworthy that the i-HD-MTX group had significantly more patients with ≥3 cycles and higher cumulative dosage, and we did observe numerically greater toxicity in the i-HD-MTX group. Although we did not record toxicities occurring with R-CHOP alone in the EOT arm to serve as a comparator, the rates of infection (16.4%) and mucositis (15%) recorded with i-HD-MTX are higher than that described with R-CHOP alone in previous Phase 3 trials.[14]

In the EOT group, toxicity was still relatively frequent (40%, 7% grade ≥3). We demonstrated a low (2%) rate of grade ≥3 renal toxicity in the EOT group, which provides some reassurance; however, there were clearly age based adjustments made, and it is also possible that physicians made judgments on risk of renal toxicity and implemented additional precautions, which are not recorded. Although increasing cumulative or peak HD-MTX dose did not significantly increase the overall risk of HD-MTX toxicity, we found an increased risk of mucositis with higher dose in Cycle 1 and increased liver toxicity with higher doses in Cycle 2.

Our dataset provides valuable insight into prescribing patterns with HD-MTX. Patients experiencing any toxicity were more likely to stop after 1 HD-MTX cycle, with renal toxicity showing the strongest association. If patients continued to Cycle 2, those who had experienced toxicity in Cycle 1 were much more likely to do so again. Although we did not see any evidence that the grade was likely to increase, this needs to be caveated by the fact clinicians may have already stopped for patients they felt were at higher risk of worsening toxicity.

We observed that most patients received doses of HD-MTX of either 3 or 3.5 g/m^2. The evidence for this practice is derived from PCNSL studies, where pharmacokinetic analyses determined that HD-MTX doses of ≥3 g/m^2 are required to reach CNS tumoricidal concentrations.[15] Our sub-analyses showed some evidence of increased toxicity with 3.5 g/m^2 versus 3 g/m^2 (renal, mucositis), in keeping with our overall observation that toxicity increases with higher doses (Table S6). However, the event number was small and dose choices are potentially subject to clinician bias.

Our data do not allow determination of a clear cut-off for HD-MTX dose which significantly minimizes toxicity, especially considering that clinicians made dose decisions based on patient characteristics. It was reassuring to observe that patients who did not experience toxicity with cycle 1 HD-MTX were highly unlikely to have a toxicity event with Cycle 2. However, considering the clear association between increased dosage and toxicity observed, and the intention to deliver an effective HD-MTX dose, it appears reasonable to deliver doses of no more than 3–3.5 g/m^2 for a maximum of 2 cycles.

The strengths of this study are the multicenter design, large sample size, and granularity of the HD-MTX data. The main limitations pertain to its retrospective, non-randomized design that leaves potential for selection bias, particularly when considering patients who were retrospectively identified as having received EOT HD-MTX. We had no data on patients who were intended to receive EOT HD-MTX but ultimately did not receive it due to toxicity with R-CHOP or disease progression. We acknowledge that some toxicities may have occurred but were not recorded in case-notes. We also recognize that, although the sample is large, the number of CNS relapses remained relatively small and despite multivariable adjustments, there may have been other factors affecting dose which may confound the treatment effects.

In summary, we found no evidence for increased efficacy with higher doses of HD-MTX when used as CNS prophylaxis in DLBCL, and demonstrated greater risk of toxicity with increased dose. Patients who experienced toxicity with Cycle 1 HD-MTX were much more likely to do so again if they continued to Cycle 2. Therefore, in the increasingly uncommon scenario where HD-MTX is used as CNS prophylaxis, our recommendation would be that a maximum of two cycles should be given at doses no higher than 3–3.5 g/m^2 following R-CHOP. Where toxicity is encountered with first HD-MTX delivery, there does not appear to be rationale in continuing with subsequent cycles.

AUTHOR CONTRIBUTIONS

Matthew R. Wilson, Toby A. Eyre, Amy A Kirkwood, Kate Cwynarski, and Pam McKay designed the original HD-MTX timing study. Amy A Kirkwood performed all statistical analyses. Matthew R. Wilson, Amy

A Kirkwood, and Toby A. Eyre analyzed data and wrote the paper. All other authors participated in collection of data and in writing/reviewing the manuscript.

ACKNOWLEDGMENTS

The authors would like to thank the following health care professionals for their expert dedication to data collection: Catherine Thieblemont (Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris), Sridhar Chaganti and Graham Mcilroy (University of Birmingham), George Follows and Anna Santarsieri (Cambridge University Hospitals NHS Foundation Trust), Anca Prica and Qin Liu (Princess Margaret Cancer Centre, Toronto), Adam Olszewski (Brown University and Lifespan Cancer Institute), Barbara Botto and Francesco Vassallo (AOU Città della Salute e della Scienza di Torino), Corinne Haioun and Louise Roulin (Hospital Henri Mondor), Caroline Besson and Naelle Lombion (Centre Hospitalier de Versailles), Olivier Tournilhac (Service d'Hématologie et de Thérapie Cellulaire, CHU Estaing, Université Clermont Auvergne), Pietro Di Ciaccio and Nada Hamad (St Vincent's Hospital Sydney), Agnes Olivrie and Julie Abraham (Hématologie Clinique et Thérapie Cellulaire, CHU de Limoges), Dipti Talaulikar and Caitlin Coombes (Australian National University and Canberra Health Services), Raul Cordoba and Alberto Lopez-Garcia (Fundacion Jimenez Diaz University Hospital, Health Research Institute IIS-FJD, Madrid), Adolgo de la Fuente (MD Anderson, Madrid), Rebecca Oliver and Laura Percy (University Hospitals Bristol NHS Foundation Trust), Kamel Laribi and Catherine Truong (Centre Hospitalier Le Mans, Le Mans), Ruth Clifford (University Hospital Limerick), Jordan Carter and Andrew Evens (Rutgers Cancer Institute), Brian Henessy (University Hospital Waterford), Wendy Osborne and Thomas Creasey (Newcastle Hospitals NHS Foundation Trust), Javier Penalver and Maria Garcia Roa (Hospital Universitario Fundacion Alcorcon, Madrid), Marie-Pierre Moles-Moreau and Aline Clavert (Service des Maladies du Sang, CHU Angers, Angers, France), Teresa Calimeri (IRCCS San Raffaele Scientific Institute, Milano, Italy), Andreas Kiesbye Øvlisen (Aalborg University Hospital, Aalborg, Denmark), Timothy J. Ebsworth (University Hospital Southampton NHS Foundation Trust, Southampton, UK), Johnathan Elliot (The Christie NHS Foundation Trust, Manchester, UK), Laure Ricard (Hospital Saint-Antoine Ap-Hp, Paris, France), Nimish Shah (Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, UK), Adam S. Zayac (Brown University and Lifespan Cancer Institute, Providence, RI, USA), Laure Lebras (Centre Léon Bérard, Lyon, France), Manos (Austin Hospital, Melbourne, Australia), Ruben Fernandez (Hospital de Cabueñes, Gijon, Spain), Deirdre O'Mahony (Bon Secours Cork Cancer Centre, Cork, Ireland), Praveen Gounder (Concord Clinical School, Concord Hospital University of Sydney, Sydney, Australia), Nathalie Forgeard (La Pitie Salpetriere Hospital, APHP-Sorbonne Universite, Paris, France), Kossi Agbetiafa (Institut Curie Hôpital René Huguenin, Saint-Cloud, France), Elisabeth Schorb (Department of Medicine, University Medical Center Freiburg, Freiburg, Germany), Thura Win Htut (Aberdeen Royal Infirmary, Aberdeen, UK), Hamish Scott (St Vincent's Private Hospital Melbourne, Melbourne), Anna Guidetti (Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy), Brett R Barlow (University of Alabama, Birmingham, USA), Emmanuelle Tchernonog (CHU de Montpellier, Montpellier, France), Fiona Miall (University Hospitals of Leicester NHS Trust, Leicester, UK), Chan Y. Cheah (Linear Clinical Research and Sir Charles Gairdner Hospital, WA, Australia), Nicolás Martinez-Calle (Nottingham University Hospitals NHS Trust, Nottingham, UK).

CONFLICT OF INTEREST STATEMENT

M. R. W.: Conference fees—Takeda, Janssen and Kite/Gilead, Honoraria—Abbvie, Kite/Gilead, Veriton Pharma, Takeda. TAE: Honoraria—Roche, Kite/Gilead, Janssen, Abbvie, AstraZeneca, Loxo Oncology, Beigene, Secura Bio. Consultancy—Roche, Abbvie, Loxo Oncology, Incyte, Secura Bio, Autolus. MA: Conference fees—Takeda, Honoraria—Takeda and Roche, Research funding—Pfizer. J. S.: Conference fees/honoraria—AbbVie, MSD and Astra-Zeneca. M. K.: Consultancy—Roche, Antegene, Genor Biopharma. M. N.: Research funding—TG Thereapeutics, Genmab, Genentech/Roche, Gilead. K. L.: Honoraria—AstraZeneca, Janssen, Roche. Patents and royalties—Janssen and Novartis, Consultancy—AstraZeneca. A. F.: Membership on an entity's Board of Directors or advisory committees—Gilead, Novartis, Juno, PletixaPharm, Roche, Incyte, Research Funding—BMS, Beigene, Pharmacyclics, Hutchison Medipharma, Amgen, Genmab, ADC Therapeutics, Gilead, Novartis, Pfizer. C. F.: Honoraria, Membership on an entity's Board of Directors or advisory committees and Research Funding—Roche. Other: speaker fees—Janssen. K. C.: Consultancy, Other: travel to scientific conferences and Speakers Bureau—Roche, Janssen, Kite/Gilead, Takeda. Consultancy and Speakers Bureau—Gilead, Incyte. Consultancy—Celgene, Atara. Travel to scientific conferences—BMS/Celgene. P. M.: Honoraria and Membership on an entity's Board of Directors or advisory committees—Roche, Kite, Takeda, Beigene. Travel support: Gilead, Janssen. All other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1

The registry received ethical approval from the West of Scotland Research Ethics Committee (REC:20/WS/0114). Data were collected in compliance with national and/or local regulations and data transfer agreements used where required. Data were de-identified or anonymized, as required.

Baseline characteristics and dosing in i-HD-MTX vs EOT groups were compared using Chi-squared tests and Wilcoxon Mann-Whitney tests. Linear regression was used to assess the associations between baseline characteristics and first HD-MTX dose (not affected by toxicity led reductions) and the association between toxicity in cycle 1 and dose in cycle 2. Associations between dose and toxicity were assessed using logistic regression. Time to event endpoints were all calculated from 8 months from diagnosis (to allow a minimum of 2 cycles to be delivered at EOT) until the first event to control for immortality time bias, with Cox regression and the log rank test used for progression free (PFS) and overall survival (OS) (events; progression and death and death respectively). Time to CNS relapse and non-relapse mortality (NRM) were analysed using competing risks survival analysis by the method of Fine and Grey with NRM and relapse treated as competing risks respectively. All time to event analyses are adjusted for other known risk factors (see table footnotes for factors included). All analyses were performed in STATA version 16.1 (STATACORP, Texas).

Appendix S2

Non-relapse mortality:

NRM was reported in 55/1384 (4.0%) of patients overall, and 44 in the landmark cohort. Two deaths were reported as being directly attributable to HD-MTX. One patient died from febrile neutropenia after i-HD-MTX cycle 1, and one patient died from infection after EOT HD-MTX cycle 2. However, we acknowledge that direct causality to HD-MTX cannot be definitively proven in retrospect, particularly for the former patient where concurrent R-CHOP toxicity may also have been a factor. There was a trend towards higher 3-year cumulative incidence of NRM in the i-HD-MTX group compared to EOT (3.9% vs 2.4%, HR 0.60 (95% CI 0.34-1.04), p=0.06). However, further analyses of causes and timing of deaths (6-month landmark showed similar results: HR: 0.56 (95% CI 0.31-1.02), p=0.055) did not show a pattern consistent with methotrexate causality. Therefore, it may be that the observed difference in NRM between groups relates to differences in baseline characteristics. Analysis of dosage did not demonstrate an association between risk of NRM and cumulative HD-MTX dose (HR 1.28 (95%CI 0.68-3.40), p=0.44), peak dose (HR 1.39 (95%CI 0.49-3.97), p=0.54) or grouped peak dose <1.5 g/m2/1.5-3 g/m2/≥3 g/m2 (p=0.22).

Appendix S3

Factors influencing first HD-MTX dose and reductions for subsequent cycles:

Older age was associated with lower first HD-MTX dose for both groups, but with more marked dose reductions seen for those receiving i-HD-MTX (on average 0.19 g/m^2 lower for each 10-year increment of age compared to 0.11 g/m^2, p=0.002). Creatinine clearance at baseline/diagnosis was recorded but not specifically prior to HD-MTX. Lower creatinine clearance was associated with lower HD-MTX first dose but this effect was not seen when adjusted for age. ECOG performance status was also only recorded at baseline – patients receiving i-HD-MTX with baseline ECOG ≥2 had lower first HD-MTX doses (p=0.001). For patients receiving EOT HD-MTX, delays during R-CHOP did not appear to influence HD-MTX dose reductions.

In patients continuing to cycle 2, the majority were not dose reduced (as % of dose 1: median 100% (IQR 100-100), range 33-100%), though reductions were more likely if cycle 1 toxicity had occurred (p=0.003) and reduced more if this was renal (p<0.001). Patients with mucositis or hepatic toxicity (any grade) did not show increased dose reductions, but patients with grade ≥3 mucositis were more likely to be reduced (p=0.045).

Table S1: Baseline characteristics of whole study population with subgroups by maximum individual (peak) HD-MTX dose

	All N=1384	Peak dose ≥3 g/m2 N=1166	Peak dose <3g/m2 N=212	p-value
Age (years), median (IQR)	62.5 (53-69)	61 (52-67)	70 (65-75)	<0.0001
range	17-88	17-8	19 – 88	
Baseline Creatinine Clearance, median (IQR)	98.2 (76.9 – 125.8)	102 (80.1 – 130.5)	82.9 (64.5 – 101.3)	<0.0001
Range	33.3 – 345.2	33.3 – 345.2	35.5 – 291.2	
Male sex, N (%)	840 (60.7)	695 (59.6)	141 (66.5)	0.058
Advanced stage, N (%)	1156 (83.5)	982 (84.2)	169 (79.7)	0.10
Raised LDH baseline, N (%)	943 (70.0)	800 (70.7)	138 (65.7)	0.15
Missing/unknown	36	34	2	
ECOG ≥2, N (%)	358 (25.9)	289 (24.9)	68 (32.1)	0.027
Missing/unknown	3	3	0	
Extra-nodal sites, N (%)				
0-1	586 (42.3)	485 (41.6)	98 (46.2)	0.42*
2	421 (30.4)	364 (31.2)	56 (26.4)	
≥3	377 (27.2)	3317 (27.2)	58 (27.4)	
Renal or adrenal involvement, N (%)	240 (17.3)	199 (17.1)	339 (18.4)	0.64
Testicular involvement, N (%)	175 (12.7)	127 (10.9)	47 (22.2)	<0.001
Breast involvement, N (%)	56 (4.1)	50 (4.3)	6 (2.8)	0.32
Double or triple hit, N (%)	66 (6.1)	51 (5.6)	15 (9.2)	0.080
Missing/unknown	3	259	49	
CNS IPI, N (%)				
Low (0-1)	203 (15.0)	170 (14.9)	32 (15.1)	0.005*
Intermediate (2-3)	555 (40.9)	491 (43.1)	63 (29.7)	
High (4-6)	600 (44.2)	479 (42.0)	117 (55.2)	
Missing/unknown	26	26	0	
Baseline CNS assessment, N(%)	703 (50.8)	560 (48.0)	138 (65.1)	<0.001
HD-MTX treatment				
HD-MTX approach				
EOT	635 (45.9)	489 (41.9)	142 (67.0)	<0.001
i-HD-MTX	749 (54.1)	677 (58.1)	70 (33.0)	
Total cycles, median (IQR)	2 (2-3)	2 (2-3)	2 (2-2)	0.0025
range	1-8	1-8	1-6	
HD-MTX cycles ≥2	1199 (86.6)	1019 (87.4)	177 (83.5)	0.12

p-values are Chi squared for discreate variables (*for trend) and Wilcoxon Mann Whitney for continuous.

IQR, inter-quartile range; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group performance status; CNS IPI, central nervous system international prognostic index; HD-MTX, high dose methotrexate; i-HD-MTX, intercalated high-dose methotrexate.

Table S2: HD-MTX dosage analysed as total number of cycles, cumulative dose and peak dose for both EOT and i-HD-MTX delivery, for whole study population and landmark cohort of patients alive and event-free at 8 months

	All patients (n=1384)			Landmark cohort (n=1217)		
	EOT (n=635)	**iHDMTX (n=749)**	**p-value**	**EOT (n=587)**	**iHDMTX (n=630)**	**p-value**
Total cycles given, median (IQR)	2 (2-2)	2 (2-3)	<0.0001	2 (2-2)	2 (2-3)	<0.0001
Range	1-6	1-8		1-6	1-8	
1	77 (12.1)	108 (14.4)	<0.0001*	70 (11.9)	84 (13.3)	<0.0001*
2	482 (75.9)	365 (48.7)		448 (76.3)	316 (50.2)	
≥3	76 (12.0)	276 (36.9)		69 (11.8)	230 (36.5)	
Cumulative dose, median (IQR)#	6 (3.5 – 6)	6 (6 – 10)	<0.0001	6 (3.5 – 6)	6 (6 – 10)	<0.0001
range	1- 24	1-24		1- 24	1-24	
<3	45 (7.2)	16 (2.2)	<0.0001*	43 (7.4)	13 (2.1)	<0.0001*
3-6	165 (26.2)	133 (17.9)		156 (26.8)	105 (16.7)	
6-9	348 (55.3)	345 (47.5)		319 (54.8)	309 (49.1)	
>9	71 (11.3)	242 (32.5)		64 (11.0)	202 (32.1)	
Peak dose, median (IQR)**	3 (3 – 3)	3 (3-3.5)	<0.0001	3 (3 – 3)	3 (3-3.5)	<0.0001
range	1-8	1-6.8		0.52-8	1-6.8	
Peak dose (grouped)**						
<1.5	27 (4.3)	7 (0.9)	<0.001*	25 (4.3)	5 (0.8)	<0.001*
1.5-3	115 (18.2)	63 (8.4)		112 (19.2)	52 (8.3)	
≥3	489 (77.6)	677 (90.6)		447 (76.5)	572 (90.9)	

*trend

Cumulative MTX dose is expressed in g/m2.

**Peak refers to maximum individual HD-MTX dose

Table S3 – impact of HD-MTX dose on risk of toxicity (EOT HD-MTX only)

Toxicity	Any Grade			Grade 3-5		
	Event/ N	OR* (95% CI)	p-value	Event/N	OR* (95% CI)	p-value
Cycle 1						
Any toxicity	212/631	1.03 (0.65 – 1.64)	0.90	35/577	0.81 (0.33 – 2.00)	0.65
Renal toxicity	76/631	0.73 (0.38 – 1.40)	0.35	11/628	0.30 (0.08 – 1.13)	0.075
Mucositis	24/631	7.24 (1.26 – 41.6)	0.027	-	-	-
Hepatic	95/631	1.61 (0.82 – 3.14)	0.17	12/607	1.80 (0.29 – 11.19)	0.53
Cycle 2						
Any toxicity	126/554	1.57 (0.84 – 2.94)	0.16	-	-	-
Renal toxicity	34/554	1.56 (0.41 – 5.95)	0.51	-	-	-
Hepatic**	69/554	3.97 (1.44 – 10.98)	0.008	-	-	-

**For an increase in 1 log of dose. **Although significant, this was driven by a lack of events in the patients given very low doses (<1.5mg/m^2), restricting the analysis to those given ≥1.5mg/m2 showed increase in risk.*

Grade 3-5 events and Mucositis were not analysed at cycle 2 as numbers were small (N=14 any, N=1 renal, N=4 hepatic and N=7 Mucositis (any grade))

Table S4 - Relationships between toxicity in cycle 1, and delivery of and toxicity in cycle 2

		No toxicity cycle 1	Toxicity cycle 1	p-value
Any Toxicity				
Continued to cycle 2?				
	No	22 (5.2%)	55 (25.7%)	<0.001
	Yes	399 (94.8%)	159 (74.3%)	
Mean % dose reduction C1 to C2 toxicity vs no toxicity		-4.15 (95% CI: -6.91 to -1.40)		0.003
Any toxicity in cycle 2?				
	No	363 (91.0%)	68 (42.8%)	<0.001
	Yes	36 (9.0%)	91 (57.2%)	
Hepatic toxicity				
Continued to cycle 2?				
	No	63 (11.7%)	14 (14.7%)	0.40
	Yes	477 (88.3%)	81 (85.3%)	
Mean % dose reduction C1 to C2 toxicity vs no toxicity		-1.94 (95% CI: -5.4 to 1.51)		0.27
Hepatic toxicity in cycle 2?				
	No	455 (95.4%)	34 (42.0%)	<0.001
	Yes	22 (4.6%)	47 (58.0%)	
Renal toxicity				
Continued to cycle 2?				
	No	40 (7.1%)	37 (50.7%)	<0.001
	Yes	522 (92.9%)	36 (49.3%)	
Mean % dose reduction C1 to C2 toxicity vs no toxicity		-9.70 (95% CI: -14.90 to -4.50)		<0.001
Renal toxicity in cycle 2?				
	No	507 (97.1%)	27 (75.0%)	<0.001
	Yes	15 (2.9%)	9 (25.0%)	
Mucositis toxicity				
Continued to cycle 2?				
	No	68 (11.2%)	9 (37.5%)	0.001
	Yes	543 (88.9%)	15 (62.5%)	
Mean % dose reduction C1 to C2 toxicity vs no toxicity		-3.3 (95% CI: -7.3 to 0.66)		0.10
Mucositis toxicity in cycle 2?				
	No	539 (99.3%)	12 (80.0%)	0.001
	Yes	4 (0.7%)	3 (20.0%)	
Infection/febrile neutropenia toxicity				
Continued to cycle 2?				
	No	72 (11.8%)	5 (23.8%)	0.16
	Yes	542 (88.3%)	16 (76.2%)	
Mean % dose reduction C1 to C2 toxicity vs no toxicity		-1.34 (95% CI: -2.94 to 0.27)		0.10
Infection/febrile neutropenia toxicity in cycle 2?				
	No	536 (98.9%)	11 (68.8%)	<0.001
	Yes	6 (1.1%)	5 (31.3%)	
		No grade 3-4 toxicity cycle 1*	**Grade 3-4 toxicity cycle 1***	

Any toxicity				
Continued to cycle 2?				
	No	47 (8.6%)	20 (55.6%)	<0.001
	Yes	498 (91.4%)	16 (44.4%)	
Mean % dose reduction C1 to C2 toxicity vs no toxicity		-4.31 (95% CI: -8.34 to -0.28)		0.036
Any toxicity in cycle 2?				
	No	480 (98.4%)	10 (62.5%)	<0.001
	Yes	8 (1.6%)	6 (37.5%)	
Hepatic toxicity				
Continued to cycle 2?				
	No	70 (11.7%)	7 (58.3%)	<0.001
	Yes	529 (88.3%)	5 (41/7%)	
Mean % dose reduction C1 to C2 toxicity vs no toxicity		-10.12 (95% CI: -20.01 to -0.24)		0.045
Hepatic toxicity in cycle 2?				
	No	521 (99.8%)	2 (40.0%)	<0.001
	Yes	1 (0.2%)	3 (60.0%)	
Renal toxicity				
Continued to cycle 2?				
	No	64 (10.3%)	11 (100%)	<0.001
	Yes	557 (89.7%)	0	
Mucositis Toxicity				
Continued to cycle 2?				
	No	71 (11.4%)	3 (100)	0.002
	Yes	555 (88.7%)	0	
Infection/febrile neutropenia toxicity				
Continued to cycle 2?				
	No	73 (11.8%)	4 (28.6%)	0.079
	Yes	546 (88.2%)	10 (71.4%)	
Mean % dose reduction C1 to C2 toxicity vs no toxicity		-1.33 (95% CI: -2.9 to 0.26)		0.10
Infection/febrile neutropenia toxicity in cycle 2?				
	No	43 (9d9.5%)	8 (80.0%)	0.003
	Yes	33 (0.6%)	2 (20.0%)	

Table S5 – impact of HD-MTX dose on PFS, OS, CNS relapse and non-relapse mortality

	Events/N	HR (95% CI)	p-value
PFS[1]			
Cumulative dose (1 log increase)	202/1039	1.04 (0.77 – 1.42)	0.78
Cumulative dose (grouped)			
<3	13/49	1.32 (0.73 – 2.41)	0.33
3-6	41/229	0.83 (0.58 – 1.19)	
6+	148/761	1.00	
Peak dose (1 log increase)	202/1040	1.07 (0.64 – 1.79)	0.79
Peak dose (grouped)			
<1.5	8/27	1.00	0.34
1.5-3	26/140	0.56 (0.25 – 1.25)	
3+	168/873	0.68 (0.32 – 1.43)	
OS[1]			
Cumulative dose (1 log increase)	126/1039	0.84 (0.58 – 1.22)	0.37
Cumulative dose (grouped)			
<3	11/49	2.01 (1.02 – 3.96)	0.13
3-6	30/229	1.10 (0.71 – 1.70)	
6+	85/761	1.00	
Peak dose (1 log increase)	126/1040	0.76 (0.42 – 1.39)	0.37
Peak dose (grouped)			
<1.5	7/27	1.00	0.19
1.5-3	18/140	0.46 (0.19 – 1.12)	
3+	101/873	0.48 (0.21 – 1.09)	
CNS relapse[2]			
Cumulative dose (1 log increase)	45/1181	0.72 (0.41 – 1.26)	0.25
Cumulative dose (grouped)			
<3	2/53	0.98 (0.23 – 4.13)	0.99
3-6	11/258	1.00 (0.49 – 2.03)	
6+	32/870	1.00	
Peak dose (1 log increase)	45/1182	0.96 (0.37 - 2.49)	0.93
Peak dose (grouped)			
<1.5	1/29	1.00	0.97
1.5-3	7/161	0.78 (0.09 – 6.38)	

3+	37/992	0.99 (0.44 – 2.25)	
NRM[3]			
Cumulative dose (1 log increase)	44/1211	1.28 (0.68 – 3.40)	0.44
Cumulative dose (grouped)			
<3	1/56	0.44 (0.06 – 3.28)	0.49
3-6	13/261	1.30 (0.67 – 2.54)	
6+	30/894	1.00	
Peak dose (1 log increase)	44/1213	1.39 (0.49 – 3.97)	0.54
Peak dose (grouped)			
<1.5	0/30	-	0.22
1.5-3	8/164	1.00	
3+	36/1019	0.94 (0.42 – 2.07)	

Adjusted for: [1]HD-MTX type, age, sex, number of extra nodal sites, ECOG, renal/adrenal involvement, baseline creatinine, raised LDH, ITs (yes/no). [2]Adjusted for CNS IPI, number of extra nodal sites and testicular involvement. [3]Adjusted for age. Interactions were considered between dose and HD-MTX type, and between ITs and HD-MTX type (PFS/CNS relapse). Neither were significant.

Table S6 – Sub-analysis comparing toxicity with peak doses of 3g/m2 vs 3.5g/m2

Toxicity	Any Grade			Grade 3-5*		
	Event/N	OR (95% CI)	p-value	Event/N	OR* (95% CI)	p-value
Any toxicity						
3g/m^2	119/346	1.00	0.71	17/311	1.00	0.77
3.5g/m^2	40/123	0.92 (0.59 – 1.42)		7/113	1.14 (0.46 – 2.83)	
Renal						
3g/m^2	32/346	1.00	0.036	4/346	1.00	0.69
3.5g/m^2	20/123	1.91 (1.04 – 3.48)		2/122	1.43 (0.26 – 7.88)	
Hepatic						
3g/m^2	72/346	1.00	<0.001	8/324	1.00	0.29
3.5g/m^2	6/123	0.20 (0.08 – 0.46)		1/123	0.32 (0.04 – 2.62)	
Mucositis						
3g/m^2	9/346	1.00	0.011	0/344	1.00	0.26
3.5g/m^2	10/123	3.31 (1.31 – 8.36)		1/119	-	
Infections/FN						
3g/m^2	10/246	1.00	0.79	7/344	1.00	0.78
3.5g/m^2	3/123	0.84 (0.23 – 3.10)		2/123	0.80 (0.16 – 3.88)	

OR, odds ratio; FN, febrile neutropenia

PAPER 8

Wilson MR, Cwynarksi K, Eyre TA et al. Central nervous system prophylaxis in large B-cell lymphoma: A British Society for Haematology Good Practice Paper. In Press, BJHaem June 2024

Journal impact factor: 6.5
Number of citations: N/A

Summary of contribution:

Conceptualisation	Yes – with all co-authors
Data Curation	I designed literature review search terms, the literature search was performed by an external agency (Niche Technologies)
Formal Analysis	Yes – all co-authors contributed to analysis of literature, writing of recommendations and formal grading
Investigation	N/A
Methodology	N/A
Project Administration	Yes
Visualisation	Yes
Writing – original draft	Yes
Writing – review & editing	Yes

Central nervous system prophylaxis in large B-cell lymphoma: A British Society for Haematology Good Practice Paper

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Summary

This Good Practice Paper provides recommendations for the baseline investigation, risk stratification and use of prophylactic interventions for patients with large B-cell lymphoma at risk of central nervous system relapse. Recent evidence which has questioned the role of high-dose methotrexate in this clinical scenario is discussed in detail.

Methodology (this is a main heading following style Heading 1)

This guideline was compiled according to the BSH process at [https://b-s-h.org.uk/media/16732/bsh-guidance-development-process-dec-5-18.pdf]. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria can be found at http://www.gradeworkinggroup.org and is summarised in appendix 3 of the guidance document linked above.

Literature review details (this is a sub-heading following style Heading 2)

A literature review was performed using the PubMed database using the following search terms: high grade B-cell lymphoma; high grade lymphoma; diffuse large B-cell lymphoma; central nervous system relapse; central nervous system prophylaxis; central nervous system recurrence; high-dose methotrexate. The search was limited to publications written in English, publications with abstracts, studies carried out in humans, Clinical Studies, Clinical Trials, Comparative Studies, Evaluation Studies, Guidelines, Meta-Analyses, Observational Studies, Systematic Reviews, Validation Studies, and published between 01/01/2013 – 18/12/2023.

Review of the manuscript

Review of the manuscript was performed by the British Society for Haematology (BSH) Haematology Oncology Task Force and the BSH Guidelines Committee. It was also on the members section of the BSH website for comment and has been reviewed by Lymphoma Action. These organisations do not necessarily approve or endorse the contents.

Introduction

Relapse within the central nervous system (CNS) is a relatively rare but potentially devastating complication for patients with large B-cell lymphoma (LBCL). Often referred to as secondary CNS lymphoma (SCNSL), it is important to distinguish this scenario from patients with SCNSL where both CNS and systemic disease is evident at first presentation. The incidence of CNS relapse in LBCL is approximately 5% for all patients, but greater within subgroups where the risk is 15-30%.[1-3] Management of patients with SCNSL, where CNS relapse is either isolated or concurrent with systemic relapse, is challenging with median overall survival typically <6 months.[4-6]

A previous British Society for Haematology (BSH) good practice paper (GPP) in 2020 summarised the relatively weak evidence base to guide strategies aimed at reducing risk of CNS relapse in LBCL.[7] At that time, there was consensus that sufficient cumulative evidence existed to support recommendations on the use of high-dose methotrexate (HD-MTX) for patients with certain high-risk characteristics. Since the publication of this GPP, several important additional studies have been published which have introduced significant uncertainty about HD-MTX efficacy in this setting. This revised GPP summarises evidence published since 2020 and provides pragmatic guidance for clinicians around decision-making on CNS prophylaxis in adults with the various subtypes of diffuse large B-cell lymphoma (DLBCL) and high grade B-cell lymphoma (HGBCL) included under the umbrella term LBCL in recent classification systems.[8-10]

Identification of high-risk patients

Given the rarity of CNS relapse overall in LBCL, it is clear that treating all patients with additional CNS prophylaxis would result in over-treatment and exposure to unnecessary toxicity for the vast majority. Therefore, there is an ongoing need to identify patients at highest risk of SCNSL and to investigate interventions which may mitigate this risk.

The CNS International Prognostic Index (CNS-IPI)

Since its introduction in 2016, the CNS-IPI has been widely used as a tool for CNS relapse risk estimation in LBCL.[3] This model was developed from analyses of large prospective LBCL trials and validated on a population-based cohort, resulting in a 6-point scoring system incorporating the standard IPI factors together with renal/adrenal involvement. Those with a high-risk score (4-6) constitute 12-23% of all patients with LBCL, but have an overall estimated CNS relapse risk of ~10-12%. Consequently, the CNS-IPI lacks sufficient positive predictive value in that offering CNS prophylaxis to this group results in the vast majority being exposed to potentially toxic additional treatment when they would not have gone on to develop CNS relapse. It also has insufficient negative predictive value, as approximately half of CNS relapse events occur in patients with a low-intermediate score.[3] Furthermore, the CNS-IPI does not predict whether an intervention, including HD-MTX prophylaxis, can meaningfully reduce this risk.

Anatomical risk factors

A number of anatomical sites have previously been associated with risk of CNS relapse, but most are not independently predictive in multivariable analyses.[7,11] The strongest evidence is for renal/adrenal involvement and testicular LBCL, where historical estimates of CNS relapse risk are as high as 30%.[12,13]

Testicular LBCL is one of the only areas where prospective trial evidence exists suggesting a possible benefit of CNS prophylaxis. The IELSG30 trial investigated rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone (R-CHOP) with concurrent intrathecal (IT) liposomal cytarabine, contralateral testicular radiotherapy and two cycles of intermediate dose (1.5 g/m^2) intravenous (IV) methotrexate after R-CHOP completion. No CNS relapses were reported from 54 patients at a 5-year analysis.[14]

Although these results are encouraging, this was a small, non-randomised study and it remains unclear which therapeutic components have most impact on CNS relapse risk. The dose of 1.5 g/m^2 methotrexate was selected in IELSG30 to provide a balance between toxicity (in a typically older patient population with testicular LBCL) and efficacy. MTX doses between 1-3g/m2 can penetrate the CNS parenchyma, whereas doses of ≥3g/m2 are required to achieve tumoricidal levels in the CSF.[15] It was postulated that the addition of IT chemotherapy to this intermediate MTX dose would ensure both parenchymal and leptomeningeal coverage. Until there is more evidence to support this dosing strategy, for now we suggest that HD-MTX is delivered at the more widely established dose of 3-3.5g/m2, thus ensuring adequate CSF penetrance, with or without additional IT therapy as per the IELSG30 trial. Where HD-MTX is contraindicated, standalone IT prophylaxis can be considered in this particular entity, acknowledging there is a lack of robust evidence to support this.

Epidural, orbital and craniofacial involvement have previously been considered as high risks of CNS disease but there is no robust confirmatory evidence in the rituximab era.[16] In such cases, the key question is whether the dura has been breached, as there is no evidence to suggest that proximity to the CNS per se is an indication for CNS prophylaxis. A number of retrospective studies have suggested primary breast LBCL confers a risk of CNS relapse of 5-15%.[17-19] Intravascular lymphoma is a distinct entity from other LBCL subtypes with a well-established high risk of CNS disease at baseline or relapse, where a small single arm prospective study suggested promising results with incorporation of HD-MTX and IT therapy with R-CHOP.[20] Finally, in a large retrospective study the number of extranodal (EN) sites involved using positron emission tomography–computed tomography (PET-CT) predicted a 3-year cumulative CNS relapse risk of 15% in patients with ≥3 EN sites.[21]

Biological risk factors

Recently revised classification systems[8,9] retain high-grade B-cell lymphoma (HGBCL) with *MYC* and *BCL2* rearrangements (with or without *BCL6* rearrangement) as a distinct entity associated with relatively adverse prognosis. HGBCL with *MYC* and *BCL6* rearrangements only is described separately with prognosis more akin to other LBCL subtypes. These so-called 'double hit lymphomas' (DHL) have previously been associated with high CNS relapse risk. However, there is accumulating evidence to suggest that early data overestimated this risk, as FISH was performed selectively in high risk patients[22]. A recent retrospective series of 191 patients with DHL, identified during a time period where FISH was routinely incorporated for all new HGBCL cases, showed a relatively low 2-year risk of CNS relapse at 6%.[23] Furthermore, the CNS-IPI remained predictive of CNS relapse suggesting that the risk is driven by other factors rather than the DHL status itself. Activated B-cell subtype (ABC) LBCL appears to confer increased risk when determined using gene expression profiling. However, this technology is not incorporated into routine clinical practice. Similarly, certain molecular subtypes, or 'clusters', have been described using multi-platform genetic analyses, with the 'MCD' and 'C5' clusters (both characterised by a high frequency of *MYD88*^{L265P} and *CD79* mutations) in particular showing an association both with primary CNS involvement and risk of CNS relapse.[24] However, until this classification is validated and applied uniformly in LBCL diagnostics, it cannot be routinely applied to inform clinical decision making on CNS prophylaxis.

The role of baseline screening

Whilst it is well established that patients with symptoms suggestive of CNS disease should be investigated with CNS imaging and cerebrospinal fluid (CSF) analysis, there is less evidence to support routine baseline screening for clinically occult LBCL in the CNS. The frequency of asymptomatic CNS involvement at baseline has not been well studied, with

no large screening studies of consecutive patients undergoing sensitive analyses of CSF and CNS imaging using magnetic resonance imaging (MRI). Several small studies have suggested that occult CNS involvement may be detectable using these modalities in a minority of high-risk, asymptomatic patients.[25,26] However, it remains unclear whether all such patients will experience clinical CNS progression.

A recent retrospective analysis of 510 high-risk LBCL patients who had uniform screening of CSF with flow cytometry (+/- imaging) detected baseline CNS disease in 54/510 (11%).[27] These patients had inferior survival compared to patients with no CNS disease at baseline, but had better outcomes than those with no CNS disease at baseline who went on to have CNS relapse. These data are in line with findings from the MARIETTA trial[5], which demonstrated superior survival for patients with SCNSL who had CNS disease at baseline compared to those with later CNS progression. Therefore, if CNS disease is detected at baseline (using conventional methodology), an intensified chemoimmunotherapy approach with incorporation of CNS penetrating agents should be considered.

Recently, cell-free circulating tumour DNA (ctDNA) has emerged as a non-invasive prognostic biomarker in lymphoid malignancies, and there is interest in its application to CSF analysis in CNS lymphoma.[28,29] Whilst early data suggests that ctDNA in the CSF offers greater sensitivity for detecting occult CNS involvement and may predict CNS relapse in some patients, this approach requires validation in larger prospective studies before it can be applied in practice.

Routine screening of all asymptomatic patients would have substantial resource implications, would potentially delay start of systemic therapy and would introduce risk of complications to patients from lumbar puncture. Therefore, it appears reasonable to consider CNS screening (MRI brain/spine with contrast and/or CSF analysis including flow cytometry) for those at highest risk of CNS relapse (i.e. CNS-IPI 5-6, renal/adrenal or

testicular involvement, involvement of ≥3 EN sites) if achievable without delays to systemic therapy.

High-dose methotrexate as CNS prophylaxis

As described, HD-MTX has been widely used in recent years as CNS prophylaxis in LBCL in place of the historical approach of IT chemotherapy.[7] There is now general acceptance that IT therapy has a limited role as CNS prophylaxis in LBCL[30,31], with the potential exception being in testicular LBCL (see above). The justification for HD-MTX use is based on a combination of scientific rationale, extrapolation from its efficacy in CNS lymphoma treatment, and a number of small retrospective analyses suggesting potential benefit as prophylaxis.[32,33] However, recent evidence has questioned its efficacy in this setting.

Timing of delivery

Most CNS relapses occur either during or shortly after first-line chemoimmunotherapy, with a median time to CNS relapse of 6-8 months.[1,34] Therefore, there is rationale to deliver CNS prophylaxis as early as possible. However, there has been uncertainty over the safest and most effective way to incorporate HD-MTX with systemic therapy, with some centres 'intercalating' HD-MTX between cycles of R-CHOP (i-HD-MTX) and others delivering at end-of-treatment (EOT) to avoid interruptions to systemic therapy.[32,35] A recent large, multicentre, retrospective analysis addressed this question, collecting data on 1,384 patients receiving HD-MTX as CNS prophylaxis either as i-HD-MTX (n=749) or delivered at EOT (n=635).[36] There was no difference in CNS relapse between the approaches (3-year rate 5.7% vs 5.8% respectively), and i-HD-MTX delivery caused significantly increased delays to R-CHOP delivery. As a result of these data, there is now

broad consensus that if HD-MTX is to be used, it should be delivered after R-CHOP (or similar), ideally having confirmed systemic complete response (CR).

Toxicity and dosage of HD-MTX

Guidelines worldwide lack consensus on this issue.[37] Doses of 3-3.5 g/m^2 are generally recommended, based on evidence primarily from primary CNS lymphoma (PCNSL) studies where pharmacokinetic analyses determined that doses of ≥3 g/m^2 are required to reach CNS tumoricidal concentrations in both CNS parenchyma and CSF.[15] However, the number of cycles given varies widely, with 25% of patients having ≥3 cycles and some having up to 6 in the aforementioned HD-MTX timing study. Recently, a sub-analysis of the HD-MTX timing study was published, aimed at addressing the uncertainty around optimal dosage and number of cycles of HD-MTX when used as CNS prophylaxis.[38] Wilson *et al* found no evidence for superior efficacy with increasing cumulative dose of HD-MTX and demonstrated greater risk of toxicity with increased dose. Those who experienced toxicity with cycle 1 HD-MTX were much more likely to do so again if they received further cycles. Although the study cannot definitively define an 'optimal' dose of HD-MTX beyond which toxicity increases significantly, where HD-MTX is used it seems reasonable to deliver doses of no more than 3-3.5 g/m^2 for a maximum of 2 cycles. It should be noted that data on infusion times, known to be an important determinant of HD-MTX bioavailability, were lacking in this analysis and in other studies which question the efficacy of HD-MTX as CNS prophylaxis. We recommend that short infusion times of 2-4 hours are used, in line with published evidence demonstrating higher peak MTX concentration and superior outcome in primary CNS lymphoma.[15]

While a specific chronological age threshold cannot be recommended for HD-MTX 'fitness', patients should be carefully assessed for adequate performance status and organ function (in particular creatinine clearance >50ml/min and satisfactory left ventricular

ejection fraction) prior to HD-MTX administration. Pragmatically, patients who are not deemed fit for full dose anthracycline would not normally be considered for HD-MTX as the co-morbidities driving sub-optimal first-line therapy also increase the risk of HD-MTX toxicity, with uncertain benefit.

Efficacy of HD-MTX

In the 600 patients with high CNS-IPI in the HD-MTX timing study[36], the 3-year CNS relapse rate was 9.1% despite the use of HD-MTX, raising the important question of whether it has any efficacy at all. In recent years, numerous retrospective analyses have addressed this question (**Table 1**), the largest being a multicentre retrospective analysis of 2,418 patients.[39] Lewis *et al* included patients treated with curative intent who were deemed at high risk of CNS relapse defined as either CNS-IPI 4-6, patients with high grade B-cell lymphoma with rearrangements of *MYC* plus *BCL2* and/or *BCL6*, primary breast/testicular LBCL or renal/adrenal involvement irrespective of CNS-IPI. The number of patients included in the HD-MTX treated group (n=425) fell short of the pre-planned power calculation target of 581, however the non-HD-MTX treated cohort exceeded target (n=1993). To mitigate for immortality bias from retrospective identification of patients who were deemed fit enough to receive HD-MTX and responding sufficiently to systemic therapy, the authors performed separate landmark analyses of patients in CR at end of systemic therapy (CR group). Although a statistically significant reduction in CNS relapse was seen in the HD-MTX group (5-year risk 6.9% vs 8.5%, 95% CI -1.1 to 4.4%) when all patients were included, significance was not retained when analyses were restricted to the CR group.

Subgroup analyses of patients with the highest risk characteristics were underpowered but did not appear to show benefit of HD-MTX in patients with CNS IPI 5-6, testicular, renal or breast involvement. However, it should be noted that there was an imbalance in those

with very high-risk features between the HD-MTX and no HD-MTX groups. For example, the proportion of patients with ≥2 EN sites or with involvement of renal/adrenal/testes was 85% vs 69% and 50% vs 25% respectively. In theory, one could argue that the baseline risk of the HD-MTX group was higher and therefore the fact that there was essentially equivalent rates between groups is suggestive of some benefit from HD-MTX use. Finally, among patients with CNS progression, isolated CNS relapse was more frequent in patients not receiving HD-MTX (75.0% vs 61.1%) with remaining patients experiencing synchronous CNS/systemic relapse.

Only one prospective, randomised trial in this area has been performed.[40] This phase III trial from 14 centres in Korea randomised 142 patients to either IT methotrexate (n=73) or intercalated HD-MTX (3 g/m^2 in ≤70 years, 2 g/m^2 in >70 years) (n=69). Although there was no significant difference in 2-year CNS relapse rates between the arms (5.5% vs 4.9% respectively), the trial lacked sufficient statistical power to answer this question definitively.

Current recommendations and rationale

The aforementioned studies represent the highest quality evidence currently available to address this difficult clinical question. It is unlikely that an adequately powered prospective trial will be performed, given the rarity of CNS relapse and the extremely large sample size required. Given the weak evidence base which led to the use of HD-MTX as CNS prophylaxis and the recent accumulation of evidence suggesting minimal (if any) benefit, many clinicians have already significantly restricted their use of prophylactic HD-MTX.

The Lewis *et al* study showed a statistically significant reduction in CNS relapse with HD-MTX in the whole study population, however the clinical significance of such a marginal reduction is debatable and it appears likely that HD-MTX will not benefit most patients. HD-MTX also confers toxicity risks for patients and has significant impact on hospital

resources. Counter to these arguments is the lack of definitive evidence to exclude benefit of HD-MTX in the highest risk subgroups, the devastating impact of SCNSL and the ongoing need to consider any feasible method to negate this risk.

Recently, the POLARIX trial demonstrated a progression-free survival (PFS) benefit with the substitution of the antibody-drug conjugate polatuzumab vedotin for vincristine in R-CHOP (so-called Pola-R-CHP).[41] This is now licensed and approved in the UK for patients with LBCL and IPI score of ≥2. The POLARIX trial reported CNS relapses of 3% in both arms, with no detail on whether relapses were isolated vs synchronous with systemic progression. Although specific data is lacking on this issue, polatuzumab vedotin has a large molecular weight (~150 kDa) and is unlikely to cross the blood-brain-barrier. It appears reasonable to conclude that more widespread use of this regimen will not have a meaningful impact on isolated CNS relapses and therefore does not influence decision-making around CNS prophylaxis at present. Trials investigating the addition of novel agents capable of crossing the blood-brain-barrier to first-line chemoimmunotherapy are ongoing, and results with regard to CNS relapse risk reduction will be of interest. We must continue to investigate more specific methods for identifying patients at highest risk, with technology such as ctDNA showing much promise. Until then, the following serve as pragmatic recommendations based on currently available evidence. The underlying principle is that consideration of CNS prophylaxis should be made carefully on a case-by-case basis and discussed at a dedicated lymphoma Multidisciplinary Team (MDT) meeting, whilst acknowledging that omission of HD-MTX is now considered a reasonable approach even for patients at highest risk of CNS relapse. The patient should be involved in the final decision, after discussion of the potential risks and benefits in their individual situation.

Recommendations:

- **If feasible, without causing clinically significant delay to systemic therapy, consider baseline CNS screening (MRI brain/spine with contrast and/or CSF analysis including flow cytometry) for patients with disease in close proximity to the CNS and in those at highest risk of CNS relapse (2C):**
    - **CNS-IPI 5/6**
    - **≥3 EN sites**
    - **Renal/adrenal, testicular or breast involvement**
- **If SCNSL is confirmed on baseline investigation, offer intensified chemoimmunotherapy incorporating CNS-penetrating agents for appropriately selected patients as per SCNSL guidelines (1B)**
- **The decision-making process around CNS prophylaxis should involve a lymphoma MDT and the patient (1A)**
- **Offer CNS prophylaxis to patients with testicular LBCL with IT chemotherapy and/or HD-MTX (1B)**
- **Routine stand-alone IT prophylaxis is not recommended other than in selected patients with testicular LBCL in whom HD-MTX is contraindicated (1C)**
- **Consider HD-MTX CNS prophylaxis for other patients at highest risk of CNS relapse (CNS-IPI 5/6, ≥3 EN sites, renal/adrenal or breast involvement) weighing risk vs benefit on an individual patient basis (2C)**
- **Where HD-MTX is used:**
    - **Ensure adequate performance status and organ function (renal and cardiac) prior to HD-MTX administration (1C)**
    - **Deliver at end of treatment after confirmation of systemic complete metabolic response (1C)**
    - **Deliver a maximum of 2 cycles at doses of 3-3.5 g/m^2 (1C)**

Acknowledgements

The authors wish to thank Kitty Johnson (Niche Science & Technology) for her help in undertaking the initial literature review and to Lymphoma Action for providing input to the manuscript.

The BSH haemato-oncolgy task force members at the time of writing this guideline were Dr Nilima Parry-Jones (chair), Dr Toby Eyre (deputy chair), Dr Dima El-Sharkawi, Dr Simon Stern, Dr Tracey Chan, Dr Matthew Wilson, Dr Robert Sellar, Dr Austin Kulasekararaj, Dr Andrew Clark and Dr Jahanzaib Khwaja. The authors would like to thank them, the BSH sounding board, and the BSH guidelines committee for their support in preparing this guideline.

Declaration of Interests

The BSH paid the expenses incurred during the writing of this guidance.

All authors have made a declaration of interests to the BSH which may be viewed on request.

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Members of the writing group will inform the writing group chair if any new evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be reviewed regularly by the relevant task force and the literature search will be re-run every five years to search systematically for any new evidence that may have been missed. The document will be archived and removed from the BSH current guidelines website if it becomes obsolete. Please check the BSH guidelines website (www.b-s-h.org.uk/guidelines) for any addenda that may be produced after the initial publication.

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Table 1: Summary of recent studies evaluating use of HD-MTX in DLBCL

Study (year)	n	Design	Risk factors	Systemic treatment	CNS Prophylaxis	CNS relapse	Summary
Lewis KL (2023)[39]	2418	Retrospective	CNS-IPI ≥4 Testicular, breast involvement DHL	R-CHOP (91%) DA-EPOCH-R (9%)	1. HD-MTX (18%) 2. No HD-MTX (82%)	1. 6.9% (5y) 2. 8.5% (5y)	**1.6% risk reduction with HD-MTX but not maintained when restricted to patients in CR at EOT**
Yahng S (2023)[40]	142	Randomised phase III trial	IPI ≥ 4 Age-adjusted IPI ≥2 + LDH ↑ + ≥1 EN site High risk EN sites	R-CHOP	1. HD-MTX (51%) 2. IT MTX (49%)	1. 4.9% (2y) 2. 5.5% (2y)	**No benefit HD-MTX vs. IT**
Bennett R (2023)[27]	387	Retrospective	CNS-IPI ≥4 DHL ≥2 EN sites High risk EN sites	R-CHOP	1. HD-MTX (44%) 2. No HD-MTX (56%)	1. 6.2% (5y) 2. 5.6% (5y)	**No benefit HD-MTX vs no HD-MTX (all patients had baseline CSF screening)**

Wilson MR (2022)[36]	1384	Retrospective	High risk EN sites CNS-IPI ≥ 4 ≥2 EN sites and LDH ↑	R-CHOP	1. HD-MTX (EOT) 2. HD-MTX (intercalated)	1. 5.8% (3y) 2. 5.7% (3y)	**No difference between EOT and intercalated HD-MTX**
Orellana-Noia V (2022)[42]	1030	Retrospective	Not described	R-CHOP (48%) R-EPOCH (45%) Other (7%)	1. HD-MTX (20%) 2. IT (77%)	1. 6.8% 2. 5.4%	**No benefit HD-MTX vs. IT**
Puckrin R (2021)[43]	326	Retrospective	CNS-IPI ≥ 4 Testicular DHL LDH ↑ + ECOG >1 + >1 EN	R-CHOP (85%) Intensive chemotherapy (15%)	1. HD-MTX (35%) 2. No HD-MTX (65%)	1. 12.2% 2. 11.2%	**No benefit HD-MTX**
Bobillo S (2021)[44]	585	Retrospective	CNS-IPI ≥ 4 High risk EN sites DHL	R-CHOP (68%) R-EPOCH (15%)	1. HD-MTX (57%) 2. IT MTX (43%)	1. 7.5% (5y) 2. 5.5% (3y) 3. 5%	**No benefit (IT or HD-MTX)**

				Other (17%)	3. None (50%)		
Ong SY (2021)[45]	226	Retrospective	High risk EN sites CNS-IPI ≥ 4	R-CHOP	1. HD-MTX (29%) 2. No HD-MTX (71%)	1. 3.1% (3y, isolated) 2. 14.6% (3y, isolated)	**HD-MTX significantly reduced risk of isolated CNS relapse**
Lee K (2019)[46]	130	Retrospective	CNS-IPI ≥ 4 High risk EN sites ≥2 EN and LDH ↑	R-CHOP	1. HD-MTX (49%) 2. None (51%)	1. 6.9% (2y) 2. 8.1% (2y)	**No benefit HD-MTX**
Goldschmidt N (2019)[47]	480	Retrospective	High risk EN sites Stage IV, LDH ↑, ≥1 EN	CHOP +/- R (80%)	1. HD-MTX (27%) 2. None (73%)	1. 6.9% 2. 6.3%	**No benefit HD-MTX**

CNS-IPI, central nervous system international prognostic index; EN, extra-nodal; HD-MTX, high dose methotrexate; IT, intrathecal; DHL, double-hit high grade B-cell lymphoma with MYC + BCL2 and/or BCL6 translocations; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group performance status; EOT, end of treatmen

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DISSERTATION

Introduction to CNS Relapse in DLBCL

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL), comprising approximately 40% of all NHL cases.[1] It is a high-grade, aggressive malignancy but can be cured in 60-70% of patients using a combination of chemoimmunotherapy called 'R-CHOP' (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone).[2]

Whilst progression/relapse with systemic lymphoma is by far the most common cause of treatment failure in DLBCL, relapse within the central nervous system (CNS) may also occur, either in isolation or in combination with systemic disease. The standard chemoimmunotherapy regimens used in DLBCL (e.g. R-CHOP) do not incorporate agents which can cross the blood-brain barrier.[3] Tumour cells may reach the CNS by a haematogenous route, direct infiltration from neighbouring organs, or dissemination through neurovascular axes, and can affect the brain/spinal cord parenchyma, meninges, cerebrospinal fluid (CSF) or the eyes.[4] CNS relapse typically occurs early, at a median of 6-9 months from initial DLBCL diagnosis.[5,6]

Investigation for CNS involvement by DLBCL (either at presentation or during/post-treatment) is typically triggered by neurological signs or symptoms. The diagnosis can be confirmed by demonstrating the presence of typical radiological features on computed tomography (CT) or magnetic resonance imaging (MRI) (with or without confirmatory tissue biopsy), or by the presence of DLBCL cells in the CSF using flow cytometry. The frequency of asymptomatic CNS involvement at initial DLBCL diagnosis has not been well studied, with no large studies consecutively screening all patients with CSF analysis and CNS imaging. Two small studies attempting to answer this question found asymptomatic CNS involvement in 12% and 20% respectively, with the important caveat that only high-risk patients underwent screening investigations.[7,8] Interestingly, not all of these patients went on to have CNS progression. For this reason, as well as considering the time and resource implications of these investigations in patients who often need to commence systemic therapy rapidly, universal screening of asymptomatic high-risk patients has not been widely adopted.

While outcomes for patients with systemic DLBCL relapse have improved in recent years[9], the prognosis from CNS relapse remains dismal with median survival of <6 months typically reported.[10,11] Therefore, although CNS relapse is rare (estimates of the incidence vary from 2% to 6%)[6,12-14], this is a clinically important problem with devastating consequences for patients. As a result, there has been great interest from lymphoma clinicians over the years in developing methods for more accurately identifying patients at greatest risk of this complication as well as additional 'prophylactic' interventions which may help reduce CNS relapse events.

Clinical risk factors:

It is well established that the risk of CNS relapse can be linked for many patients to baseline clinical characteristics, in particular advanced stage disease and the involvement of particular extranodal organs at diagnosis.[14-17] Historically, a number of specific extranodal sites have been associated with a high risk of CNS relapse, although in recent years it has become clear that many of these are not independently predictive and are simply a reflection of lymphoma with advanced stage disease and a predilection for extranodal involvement.[18] The strongest evidence for anatomical sites which are independently predictive of CNS relapse exists for testicular[19-21], renal/adrenal[22,23], breast[24-27] and uterine[28] involvement.

In 2016 the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL) developed a prognostic model (the CNS international prognostic index, CNS-IPI) which incorporated the existing 5 risk factors from the long established DLBCL IPI as well as involvement of the kidney or adrenal glands, stratifying patients into 3 risk categories (**Table 1**)[22]. This scoring system is useful in the clinical setting but has a number of limitations. The high-risk category (score 4-6) has typically been used to identify patients where additional CNS prophylaxis should be considered. However, within this group there is significant variation in risk (e.g. CNS-IPI 4; 10% 2-year risk, CNS-IPI 6; 33% 2-year risk). It also lacks sensitivity, with approximately half of CNS relapse events occurring in patients with low to intermediate scores.

Table 1: CNS-IPI risk categories with corresponding 2 year rates of CNS relapse and proportion of patients in each category from training (DSHNHL) and validation (BCCA) cohorts.[22] 1 point is scored for any of the following: age >60 years, lactate dehydrogenase (LDH) >normal, ECOG performances status >1, stage III/IV disease, extranodal involvement ≥2 sites, kidney and/or adrenal involvement.

		DSHNHL cohort		BCCA cohort	
Risk group	Risk factors	N (%)	2-year risk of CNS relapse	N (%)	2-year risk of CNS relapse
Low	0-1	1002 (46)	0.6%	463 (31)	0.8%
Intermediate	2-3	896 (41)	3.4%	694 (46)	3.9%
High*	4	188 (9)	7.4%	344 (23)	12%
High	5	62 (3)	15%		
High	6	13(1)	32.5%		

DSHNHL, German High-Grade Non-Hodgkin Lymphoma Study Group; BCCA, British Colombia Cancer Agency

**High risk group (4-6 factors) overall 2-year risk of CNS relapse of 10.2% in DSHNHL cohort*

Biological risk factors:

With increasing knowledge of the biological heterogeneity in DLBCL[29,30], there is now greater recognition that certain genetic or molecular aberrations may be linked

to risk of CNS involvement. The presence of translocations involving the *MYC* and *BCL2* genes, so-called 'double-hit' lymphoma, is well established as an adverse prognostic marker overall in DLBCL but also potentially confers a greater risk of CNS relapse.[31] However, early studies have probably overestimated this risk as often fluorescence in-situ hybridisation (FISH) analysis was only performed in patients with high-risk clinical features, and there is now uncertainty over whether the increased risk is driven by biological or clinical characteristics.[32,33] The 'cell of origin' of DLBCL (germinal centre vs activated B-cell (ABC) subtype) can also help prognosticate, with the ABC subtype linked to higher risk of CNS relapse.[5] However, the gene expression profiling technology required to analyse this accurately has not been incorporated into routine clinical practice due to uncertainty over its implications for treatment choice.

More recently, two independent studies have used multi-platform genetic analyses to propose a new molecular taxonomy for DLBCL subclassification, with particular 'clusters' demonstrating a high frequency of extranodal involvement including CNS localisation.[29,30] The so-called 'MCD' or 'C5' clusters, characterised by a high frequency of *MYD88*^{L265P} and *CD79* mutations, occur almost exclusively in the ABC cell-of-origin subtype. Genetic alterations defining these subtypes are also recurrently mutated in primary extranodal lymphomas originating in the CNS, testes, breast, skin and intravascular space. A series of patients with SCNSL (n=13) confirmed a higher prevalence of the MCD subtype than in a reference cohort of relapsed DLBCL with no CNS involvement.[34] Furthermore, the 'hcMCD' subtype, defined by *MYD88*^{L265P} mutation or more than 3 mutations in *CD79*, *PIM1*, *ETV6*, *BTG1*, *PRDM1*, or *PBL1XR1* constituted almost half of the patients with CNS recurrence. Although these data clearly show great potential, larger studies are required to validate the findings and the technology required to sub-categorise DLBCL in this way is not used in routine diagnostics, due to resource limitations and a lack of robust evidence to demonstrate how it could impact treatment decisions.

The history of CNS prophylaxis in DLBCL:

The risk of CNS relapse in DLBCL as a particular entity was first described in the 1970s.[35,36] For many years, prophylactic intrathecal (IT) chemotherapy was incorporated into first-line therapy (with e.g. R-CHOP or similar systemic regimens) for patients deemed at high risk of secondary CNS involvement.[37,38] A number of cytotoxic drugs can be delivered in this way, most commonly methotrexate, cytarabine and hydrocortisone. The initial rationale for use of IT therapy as CNS prophylaxis was based largely on evidence extrapolated from other haematological malignancies with high rates of CNS disease, including Burkitt lymphoma and acute lymphoblastic leukaemia (ALL).[39] In the latter diseases, IT therapy has well-established efficacy, likely due to the high rates of leptomeningeal invasion in these entities.[40,41] Indeed, early studies demonstrating potential benefit of IT prophylaxis in DLBCL are hampered by the fact that patients with Burkitt lymphoma were often included.[42]

More recently, there has been recognition that CNS relapses in DLBCL involve the CNS parenchyma in approximately 70-80% of cases, an area which is inadequately

penetrated by intrathecal chemotherapy alone.[43,44] A number of studies have now failed to demonstrate any benefit of IT prophylaxis in DLBCL[6,12,14,45-49], the most robust being a systematic review of 7,357 DLBCL patients which demonstrated no reduction in CNS relapse rates with the use of stand-alone IT prophylaxis.[50] The delivery of IT therapy can be logistically challenging and uncomfortable for the patient, with some evidence to suggest increased toxicity particularly in older patients.[49] As a result of this evidence, IT use has vastly diminished in recent years, with a move towards systemically delivered antimetabolite therapy instead.

Intravenous high-dose methotrexate (HD-MTX) has been widely used as CNS prophylaxis over the last 10 years. The initial rationale for its use was derived from its efficacy in the treatment of primary CNS lymphoma, where robust clinical and pharmacokinetic studies have demonstrated efficacy of HD-MTX at doses of 3g/m2 given in a short (4 hour) infusion.[51] However, the evidence for its efficacy as prophylaxis in DLBCL is conflicting, with no consensus on timing of delivery, dosage or number of cycles in the prophylactic setting. Furthermore, HD-MTX has potential significant toxicity, in particular mucositis, renal dysfunction and hepatic injury.

A review of the literature and BSH guidance in 2020

British Society of Haematology (BSH) guidance on CNS prophylaxis in DLBCL was first published in 2013.[38] This guideline summarised the conflicting evidence base on the topic, with the authors at the time concluding that sufficient evidence existed to recommend IT prophylaxis as standard prophylaxis for patients with DLBCL at high risk of CNS relapse (defined as any patient with a raised LDH as well as more than 2 or more extranodal sites, or those patients with either testicular, breast or epidural involvement). Systemic HD-MTX was mentioned as an option in addition to IT therapy, notably stating *'there are no data to confirm that HD-MTX alone can replace IT therapy, and if this strategy is followed it is essential that the practice is carefully audited.'*

In the intervening years, the growing body of evidence questioning the efficacy of IT prophylaxis drove increased interest in use of systemic HD-MTX instead. In 2019, a writing group was formed to update BSH guidance on this topic, recognising a need to update the 2013 recommendations and ensure they were more reflective of current UK practice. Whilst undertaking my lymphoma fellowship at the Beatson West of Scotland Cancer Centre (BWOSCC), I was invited to participate in the writing group by my mentor Dr Pam McKay. The final 'good practice paper (GPP)' was published in 2020 (**Paper 1**). As per standard BSH guideline processes, all recommendations in the GPP were graded according to the GRADE criteria (**Table 2**).[52] GRADE is a framework for developing and presenting summaries of evidence and provides a systematic approach for making clinical practice recommendations. It is the most widely adopted tool for grading quality of evidence and is used by over 100 organisations worldwide.

Table 2: The GRADE nomenclature for evaluating levels of evidence and assessing the strength of recommendations in guidelines. Adapted from[52]

Quality of Evidence	**General guidance**
High (A)	E.g. Randomised Controlled Trial – further research is very unlikely to change our confidence in the estimate of effect
Moderate (B)	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate
Low (C)	E.g. Observational study – further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the outcome
Very low (D)	Any estimate of effect is very uncertain
Decrease grade if:	Serious/very serious limitation to study quality Important inconsistency Some or major uncertainty about directness Imprecise or sparse
Increase grade if:	Strong evidence of association – significant relative risk of >2 (<0.5) based on consistent evidence from two or more observational studies, with no plausible confounders (+1) Very strong evidence of association – significant relative risk of >5 (<0.2) based on direct evidence with no major threats to validity (+2) Evidence of a dose response gradient (+1) All plausible confounders would have reduced the effect
Strength of recommendations:	
Strong (Grade 1)	Clinicians are certain that benefits do, or do not, outweigh risks and burdens. Grade 1 recommendations can be applied uniformly to most patients and words such as 'recommend', 'offer' and 'should' are appropriate.
Weak (Grade 2)	Clinicians believe that benefits and risks and burdens are finely balanced, or appreciable uncertainty exists about the magnitude of benefits and risks. In addition, clinicians are becoming increasingly aware of the importance of patient values and preferences in clinical decision making. Grade 2 recommendations require judicious application to individual patients and words such as 'suggest' and 'consider' are appropriate

The main questions addressed in the 2020 BSH GPP were as follows:

- Who should receive CNS prophylaxis?
- What form should CNS prophylaxis take?
- When should CNS prophylaxis be given?

A comprehensive literature review on the topic was undertaken and summarised in the paper. Since the previous 2013 guideline, the CNS-IPI had been developed and increasing data around high-risk extranodal sites were available, resulting in a change to recommendations around which patients should be considered for CNS prophylaxis:

- CNS prophylaxis should be offered to patients with any of these factors
    - High (4–6) CNS-IPI (GRADE 1B).
    - Involvement of three or more extranodal sites irrespective of CNS-IPI (GRADE 1B).
    - Anatomical sites: testicular, renal/adrenal, intravascular (GRADE 1B).
- Consider CNS prophylaxis in patients with any of the following risk factors:
    - Anatomical sites: breast, uterus (GRADE 2C).

The evidence around route of CNS prophylaxis was more challenging to interpret. Since the previous 2013 guideline, a number of studies were reviewed which appeared to show a benefit of HD-MTX as CNS prophylaxis.[53-56] It was acknowledged, however, that no prospective randomised evidence existed to support HD-MTX use in this setting. Ultimately, we concluded that '*although none of the above studies in isolation are definitive, taken together the data support consideration of HD-MTX as an effective strategy for CNS prophylaxis.'*

There was even less definitive evidence available to inform guidance on the delivery of HD-MTX as CNS prophylaxis: when should it be given, at what dose, and how many cycles? It is important to highlight that administration of HD-MTX is cumbersome for patients, involving an inpatient stay of 3-5 days, during which large volumes of intravenous fluid are required to ensure renal clearance of the drug. It is associated with particularly toxicities, in particular acute kidney injury, mucositis and hepatic injury. The aforementioned studies supporting HD-MTX use employed varying strategies for delivery. The most controversial area was around timing of delivery. Considering the early onset of many CNS relapses, with most occurring either during or shortly after first-line systemic therapy, there is rationale to deliver CNS prophylaxis as early as possible. The practice of 'intercalated HD-MTX (i-HD-MTX)' was first described in a single-centre retrospective analysis of 65 patients.[53] This involved delivery of HD-MTX at days 10-15 in between planned 3-weekly doses of R-CHOP chemoimmunotherapy. Although rates of CNS relapse were low (3%) in this small study, notably there were concerningly high rates of renal toxicity and delays to systemic therapy observed. Considering these potential issues and the need to maintain dose intensity of systemic therapy in DLBCL, many clinicians instead chose to deliver HD-MTX after R-CHOP completion (end of treatment, EOT). The potential disadvantage to this approach is the risk of early CNS progression before prophylactic therapy can be delivered.

Ultimately, as a writing group we made pragmatic recommendations in the 2020 GPP based on the relatively poor quality evidence. The main messaging was that HD-MTX was now the recommended modality for CNS prophylaxis, delivered 'as early as possible' as part of first-line therapy. The many discussions we had around the lack of evidence to support decision-making in this area proved to be the stimulus for our subsequent research studies.

Timing of HD-MTX delivery: toxicity and impact on R-CHOP delivery

It became clear during discussions around the BSH GPP that there was significant variation across the UK with regards to timing of HD-MTX delivery. At the BWOSCC, our practice for several years had been to deliver i-HD-MTX, driven by our concern that waiting until EOT may fail to prevent early CNS progression. After an initial audit of our local practice revealed significant toxicity and delays to systemic therapy for some patients, we carried out a brief survey across UK lymphoma leads and found that there was approximately a 50:50 split of centres delivering i-HD-MTX vs EOT. Therefore, we saw an opportunity to carry out a multicentre retrospective analysis across the UK, the aim being to determine whether i-HD-MTX delivery in a 'real-world' setting caused increased toxicity and delays to systemic therapy (**Paper 2**). This work was designed as an audit/service evaluation exercise with full anonymisation of data and therefore ethical approval was not sought.

The controversy around this topic and uncertainty amongst UK lymphoma clinicians was evident, and we were able to recruit 11 centres for data collection. I created a data collection tool (*Appendix 3*), coordinated data collection across the 11 centres, performed statistical analyses and wrote the first draft of the resultant paper. The data collection tool ensured that details on baseline clinical characteristics, treatment delivery as well as data on HD-MTX delivery were collected for every patient.

Data on 334 patients were collected (n=204 i-HD-MTX, n=130 EOT). The primary endpoints of the study were R-CHOP delay rates and HD-MTX toxicity. We demonstrated a statistically significant increase in R-CHOP delays with i-HD-MTX delivery, with 20% of i-HD-MTX treatments being associated with subsequent delay to the next R-CHOP cycle. We observed concerningly higher rates of mucositis, febrile neutropenia and longer inpatient admission with i-HD-MTX versus EOT, the caveat being that concurrent R-CHOP therapy likely impacted on these toxicities in the i-HD-MTX group.

A secondary endpoint of the study was CNS relapse rates according to HD-MTX timing. Given the overall rarity of CNS relapse, we were conscious that the sample size in this study would not allow statistically robust conclusions to be drawn on comparisons of CNS relapse rates. Acknowledging this limitation, we observed no apparent difference in 3-year CNS relapse rates between the 2 groups.

I presented these data as an oral presentation at the European Haematology Association annual meeting in 2020, with the full manuscript published in 2020. The presentation was selected in the 'highlights in lymphoma' session and results stimulated much discussion both in the UK and across the world. The observation of increased R-CHOP delays with i-HD-MTX could not be disputed, and reflected what many clinicians observed in clinical practice. However, the sample size was felt by many to be inadequate to definitively exclude a benefit of i-HD-MTX with regards to CNS relapse prevention. For centres who already delivered EOT HD-MTX, the results were seen as practice-affirming. Some centres who traditionally delivered i-HD-MTX changed practice to EOT on the basis of the study findings (including at BWOSCC), whereas others continued i-HD-MTX due to the aforementioned concerns around efficacy.

Timing of HD-MTX and impact on CNS relapse

Due to the conflicting interpretation of our initial UK study, Dr McKay and I formed a study working group to discuss carrying out a much larger, international analysis aimed at answering the question around HD-MTX timing and the impact specifically on CNS relapse rates **(Paper 3).** The working group included Dr Toby Eyre and Dr Kate Cwynarski, lymphoma clinicians from Oxford and University College Hospital London respectively, as well as Amy Kirkwood, a medical statistician who has specific expertise in haemato-oncology (in particular lymphoma) research.

The key initial step was to perform a power calculation which would estimate the sample sizes required in each group to answer this question. Based on previous studies, we assumed that the CNS relapse rate in the i-HD-MTX group would be approximately 5% at 3 years and that 60% of patients would receive i-HD-MTX vs. 40% receiving EOT. We aimed to exclude a ≥5% difference in CNS relapse rate between EOT and i-HD-MTX, i.e. that EOT was not more than 5% inferior. Using a 2.5% 1-sided alpha, we estimated that recruiting 1200 patients would result in 80% power to exclude this difference.

Thanks to the international connections of clinicians on the project working group, and by utilising the significant global interest in this question from the lymphoma community, we were able to recruit 37 centres from Europe, Australia and North America to contribute data to the study. I obtained ethical approval for the study from the West of Scotland Research Ethics Service (*Appendix 1*). Many of the international sites had regulations in place about data transfer, and we therefore created a Data Transfer Agreement in collaboration with NHS Greater Glasgow and Clyde Information Governance team (*Appendix 2)*. I designed a detailed data collection tool – this was similar to that used for Paper 2 but with modifications based on feedback from clinicians who contributed to Paper 2 and to include new datapoints which we felt would enable more robust analysis (*Appendix 4)*.

We collected data on 1,384 patients in total (749 i-HD-MTX, 635 EOT) and therefore met our pre-planned sample requirement for statistical power. We showed no difference in 3-year CNS relapse between i-HD-MTX and EOT delivery, including when using multivariable analysis to adjust for baseline prognostic factors and when restricting to a landmark analysis of patients alive and free from lymphoma progression at 6 months. The latter analysis was important as the retrospective nature of patient identification introduced a risk of omitting patients planned for EOT HD-MTX who had progression or death before this could be delivered (so-called ‘immortal time bias’). We also provided further confirmation of our findings from paper 2 around R-CHOP delays, with significantly increased delays of ≥7 days in the i-HD-MTX group. We showed that these delays are clinically significant, with significantly inferior survival observed in patients who had any delay of ≥7 days. Therefore, we were now able to conclude in a much more robust manner that delivery of HD-MTX at EOT is safer, does not introduce any risk of delay to vital systemic therapy, and most importantly does not increase the risk of CNS relapse compared to intercalated delivery.

This work was accepted for oral presentation at the American Society of Hematology annual congress in December 2021 with the final manuscript published in 2022. It was widely recognised as being practice-changing, in that clinicians who previously had delivered i-HD-MTX now moved to EOT delivery.

Increasing scrutiny over the efficacy of HD-MTX

The overall rate of CNS relapse observed in our HD-MTX timing study raised a larger question: does HD-MTX have any efficacy in reducing CNS relapse at all, regardless of timing of delivery? In the patients with CNS-IPI score of 4-6 in our study, the 3-year CNS relapse rate observed was 9.1%, despite all patients receiving HD-MTX. This is almost identical to that observed in the original CNS-IPI study, where no HD-MTX was used in the cohort used to design the score and in very few patients from the validation cohort.

In addition to the concerns from our study around overall HD-MTX efficacy, a number of additional retrospective analyses have emerged over the last few years which have attempted to answer this question.[57-62] These studies were described in detail in a number of review articles which I co-authored **(papers 4-6)**, including an invited review for Lancet Oncology (**paper 4**) and an educational review for the American Society of Hematology annual meeting in 2022 (**paper 6**).

The largest and most robust single study addressing the question of HD-MTX efficacy was first presented by Dr Katharine Lewis in December 2021, with the final results published in October 2023.[63] Lewis *et al* analysed 2,418 patients deemed at high risk of CNS relapse and compared those treated with HD-MTX CNS prophylaxis (n=425) vs those who did not receive HD-MTX (n=1993). It is important to note that the number of patients in the HD-MTX treated group fell short of their pre-planned power calculation target of 581. Nevertheless, this enormous international effort is likely to be the most robust data we have addressing this specific question. The authors found a small, but statistically significant, reduction in CNS relapse in the HD-MTX group when all patients were included, but the significance was not retained when analyses were restricted to patients in CR at end of systemic therapy (the latter analyses importantly addressing potential immortal time bias). Lewis *et al* concluded that the use of HD-MTX was not associated with a clinically meaningful reduction in risk of CNS progression.

The findings from this study stimulated much debate amongst the lymphoma community. Many took these results as sufficient to omit HD-MTX as CNS prophylaxis for the vast majority of patients, citing the significant risk of toxicity of the treatment with now cumulative evidence to suggest a lack of efficacy. However, there are some important caveats to the Lewis *et al* study. Sub-group analyses of those patients at 'ultra-high' risk of CNS relapse were performed, and appeared to show no benefit of HD-MTX, but these were significantly underpowered. Given the devastating consequences of CNS relapse, many clinicians felt that the data was insufficient to completely exclude a benefit for patients at highest risk.

Dosage of HD-MTX and impact on toxicity/CNS relapse

Despite the data from Lewis *et al* and other smaller studies suggesting a lack of efficacy of HD-MTX, it was clear that some clinicians were not ready to abandon its use altogether, especially given the lack of alternative strategies available to reduce CNS relapse in those patients at highest risk. During analysis of our HD-MTX timing study, it was clear that there was a complete lack of consensus on what the optimal dosage and number of cycles of HD-MTX is when used as prophylaxis. National guidelines vary significantly in their recommendations on this subject.[18,64,65] Although there is tentative agreement that doses of ≥3g/m2 are required to have adequate tumoricidal effect throughout the CNS, this is based on data extrapolated from patients with primary CNS lymphoma and it is unclear whether the same applies in the prophylactic setting.[51] We found a huge variation in the number of cycles delivered in our HD-MTX timing study, with 25% of patients having ≥3 cycles and some having up to 6. Given the potential significant toxicity of HD-MTX, and the uncertainty around its efficacy, we saw an opportunity to use our database to analyse the impact of HD-MTX dosage on both toxicity and CNS relapse rates.

The ethical approval from the original HD-MTX timing study was valid for any further analyses conducted on the registry. We ensured that all centres who contributed to the original study were happy for their data to be used for this additional analysis.

We had data on 3,111 HD-MTX treatments given to 1,384 patients for analysis. We found that increasing HD-MTX dose was associated with greater risk of certain toxicities – in particular mucositis and liver dysfunction. There was no significant impact on CNS relapse rate or survival with increasing HD-MTX dose, considering either cumulative (i.e. total given to each patient) or peak (maximum individual dose given) doses.

Whilst our findings were of interest, how to pragmatically apply them to clinical practice was more challenging. We acknowledged that use of HD-MTX as CNS prophylaxis was declining overall, but felt that our data could be extremely useful for situations where clinicians still wish to deliver it. We could not determine a clear cut-off for HD-MTX dose which significantly minimises toxicity. However, given the clear association between increased dosage and toxicity observed, and the intention to deliver an effective HD-MTX dose (widely acknowledged as ≥3-3.5g/m2), we concluded that it was reasonable to deliver doses of no more than 3-3.5g/m2 for a maximum of 2 cycles. We found that patients experiencing toxicity with cycle 1 HD-MTX were much more likely to do so again with cycle 2. Where toxicity is encountered with first HD-MTX delivery, we recommended not continuing to subsequent cycles. Again, these data could potentially be practice-changing, especially when considering the significant number of clinicians who have historically given 3 or more doses of HD-MTX in this setting.

Updated UK guidance in 2024

In the BSH GPP published in 2020, we recommended HD-MTX CNS prophylaxis for all high-risk patients, with no specific guidance on how/when to deliver. By 2024, it

was clear that this guidance was already outdated due to the flurry of additional publications on the topic in the intervening years. There was significant uncertainty throughout the UK lymphoma community around how to interpret this recent data, and whether their practice should change accordingly. Therefore, we were asked by BSH to produce an updated GPP to help guide clinicians in this difficult area (**paper 8**). I was privileged to be asked to lead the writing group which included a number of expert lymphoma clinicians.

We had multiple meetings as a writing group to discuss the content of this GPP. We addressed a number of specific questions in the paper:

- Should we consider baseline investigation for CNS disease in asymptomatic patients (something which had not previously been recommended)?
- How should patients with occult CNS disease at baseline be managed?
- Who should receive HD-MTX CNS prophylaxis?
- Where HD-MTX CNS prophylaxis is used, how many cycles should be given and when?

As outlined in **paper 8**, we produced a series of recommendations based on the available evidence since 2020 and the collective expertise of the writing group. We outlined in detail the thought processes behind the updated recommendations, and summarised the most recent evidence in the field. The most notable points from the updated guidance are:

- Acknowledgement that omission of HD-MTX is reasonable, even for patients at highest risk of CNS relapse.
- Patients at highest risk of CNS relapse should be screened for CNS disease at baseline if feasible, with consideration of an intensified chemoimmunotherapy approach for those with positive CNS screening.
- Patients with testicular DLBCL should continue to be offered CNS prophylaxis with IT chemotherapy and/or HD-MTX
- HD-MTX can be ‘considered’ in other patients at highest risk of CNS relapse, with careful consideration of risks and benefits on an individual patient basis:
    - CNS-IPI score 5-6
    - ≥3 extranodal sites
    - Renal/adrenal or breast involvement
- Where HD-MTX is used, it should be delivered at end of treatment, with maximum of 2 cycles given at doses of 3-3.5g/m2

Conclusions and future directions

The topic of CNS prophylaxis in DLBCL continues to be contentious and is frequently debated at lymphoma multi-disciplinary team meetings. As discussed in this dissertation, the last 4 years has seen a number of important publications in this area which have resulted in significant changes in clinical practice. Whilst it has been extremely gratifying to be involved in this work, and we have a greater understanding

of the role of HD-MTX in particular, arguably we still have a huge amount to do in addressing this important clinical problem.

If HD-MTX has any efficacy in this setting, it is likely to be minimal and only for a very small number of patients. It is associated with clear risks of toxicity and morbidity for patients, and therefore its use must be restricted to those deemed most likely to benefit. Unfortunately, we still do not have definitive data to inform who are the patients where the risk-benefit balance is in favour of delivering HD-MTX.

We have considered whether an adequately powered, randomised trial specifically addressing this problem is feasible. Unfortunately, this has a number of potential hurdles which are likely to be insurmountable. The sample size required to power a trial with CNS relapse as a primary endpoint would be exceptionally large. This would require international, multicentre collaboration, and there would likely be significant disagreement about what the experimental arm would entail. With the available data from the last 4-5 years, many clinicians would now feel uncomfortable randomising patients to a treatment with such questionable efficacy and risk of toxicity. Finally, in an era wherein we have an increasing knowledge of the biological heterogeneity of DLBCL, as well as an expanding array of novel agents capable of targeting recurring genetic aberrations, the focus has understandably shifted towards prospective evaluation of these agents instead of interventions like HD-MTX.

There are a huge number of ongoing prospective trials incorporating novel agents in first-line DLBCL therapy capable of crossing the blood-brain-barrier, and results with regards to CNS relapse rates will be of interest. For example, the ongoing REMoDL-A clinical trial (NCT04546620) is investigating the addition of the bruton's tyrosine kinase (BTK) inhibitor acalabrutinib to R-CHOP. BTK inhibitors are able to cross the blood-brain-barrier in clinically meaningful concentrations, are highly effective in a number of B-cell malignancies and have promising data showing efficacy in the treatment of CNS lymphoma.[66,67] There is also hope that earlier use of cellular and T-cell engaging therapies in DLBCL will potentially reduce CNS progressions. For example, chimeric antigen receptor (CAR) T-cell therapy has been transformative for management of relapsed/refractory systemic DLBCL[68,69], and there is increasing data demonstrating that these treatments are effective for CNS lymphoma (both primary and secondary).[70] With clinical trials investigating earlier use of CAR T-cells, including in the first-line setting[71], it may be that CNS relapses are seen less frequently in the future.

One area of great potential when considering superior methods for identifying patients at highest risk of CNS relapse is the use of circulating tumour DNA (ctDNA) in CSF at baseline. Emerging data suggests that this technology may be able to predict future CNS progression events in a much more robust manner than currently available clinical risk models.[72,73] These data are very promising but require validation in larger datasets before considering implementation in clinical practice, and the potential resource implications for routine healthcare diagnostics would be considerable.

For now, HD-MTX will continue to be used by some clinicians as CNS prophylaxis, albeit in a much more selected manner. This is driven by a) the dismal prognosis for

patients with CNS relapse and b) the current lack of viable alternative strategies. While we await results of the aforementioned trials investigating more effective methods for reducing CNS relapse, there is now clearer guidance on when and how HD-MTX should be delivered, potentially avoiding unnecessary toxicity for many patients.

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Appendix 1 – Ethics approval letter

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

Dr Matthew Wilson
NHS Greater Glasgow and Clyde (Beatson West of Scotland Cancer Centre)
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Date 02 October 2020
Direct line 0141 314 0213
E-mail WoSREC4@ggc.scot.nhs.uk

Dear Dr Wilson

Title of the Research Database: **Timing of HD-MTX CNS Prophylaxis in Diffuse Large B-cell Lymphoma**
REC reference: **20/WS/0114**
IRAS project ID: **287691**

Thank you for your letter of 23 September 2020, responding to the Committee's request for further information on the above research database and submitting revised documentation.

The further information was considered at the meeting of the Sub-Committee of the REC. A list of the members who were present at the meeting is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion of the above research database on the basis described in the application form and supporting documentation as revised.

Publication of Your Research Summary

We will publish your research summary for the above study on the research summaries section of our website, together with your contact details, no earlier than three months from the date of this favourable opinion letter.

Should you wish to provide a substitute contact point, make a request to defer, or require further information, please visit: https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/

N.B. If your study is related to COVID-19 we will aim to publish your research summary within 3 days rather than three months.

During this public health emergency, it is vital that everyone can promptly identify all relevant research related to COVID-19 that is taking place globally. If you haven't already done so,

please register your study on a public registry as soon as possible and provide the HRA with the registration detail, which will be posted alongside other information relating to your project. We are also asking sponsors not to request deferral of publication of research summary for any projects relating to COVID-19. In addition, to facilitate finding and extracting studies related to COVID-19 from public databases, please enter the WHO official acronym for the coronavirus disease (COVID-19) in the full title of your study. Approved COVID-19 studies can be found at: https://www.hra.nhs.uk/covid-19-research/approved-covid-19-research/

Duration of ethical opinion

The favourable opinion is given for a period of five years from the date of this letter provided that you comply with the standard conditions of ethical approval for Research Databases set out in the attached document. You are advised to study the conditions carefully. The opinion may be renewed for a further period of up to five years on receipt of a fresh application. It is suggested that the fresh application is made 3-6 months before the 5 years expires, to ensure continuous approval for the research database.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering letter on headed paper [Cover letter for resubmission]		23 September 2020
Other [CV]		
Other [Data collection]		
Protocol for management of the database	3.0	24 September 2020
REC Application Form [RD_Form_17082020]		17 August 2020

Research governance

Under the UK Policy Framework for Health and Social Care Research, there is no requirement for NHS research permission for the establishment of research databases in the NHS. Applications to NHS R&D offices through IRAS are not required as all NHS organisations are expected to have included management review in the process of establishing the database.

Research permission is also not required by collaborators at data collection centres (DCCs) who provide data under the terms of a supply agreement between the organisation and the database. DCCs are not research sites for the purposes of the RGF.

Database managers are advised to provide R&D offices at all DCCs with a copy of the REC application for information, together with a copy of the favourable opinion letter when available. All DCCs should be listed in Part C of the REC application.

NHS researchers undertaking specific research projects using data supplied by a database must apply for permission to R&D offices at all organisations where the research is conducted, whether or not the database has ethical approval.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK

After ethical review

Reporting requirements

The attached standard conditions give detailed guidance on reporting requirements for research databases with a favourable opinion, including:

- Notifying substantial amendments
- Submitting Annual Progress reports

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Learning

We are pleased to welcome researchers and research staff to our HRA Learning Events and online learning opportunities– see details at: https://www.hra.nhs.uk/planning-and-improving-research/learning/

IRAS project ID: 287691	Please quote this number on all correspondence

Yours sincerely

On behalf of
Dr Ken James
Chair

Appendix 2 - Data transfer agreement

DATA TRANSFER AGREEMENT

This Data Transfer Agreement ("**Agreement**") is entered into between:

Greater Glasgow Health Board, constituted pursuant to The National Health Service (Scotland) Act 1978, and having its headquarters at JB Russell House, Gartnavel Royal Hospital Campus, 1055 Great Western Road, GLASGOW G12 0XH, (the "**Recipient**")

and
[ ] (the "**Sender**")

BACKGROUND

A. The Recipient wishes to carry out the Study and create the Database (each as defined below).

B. The Recipient wishes to receive the Data (as defined below) for use in the Study and Database and the Sender has agreed to provide same on the terms set out in this Agreement.

The Parties agree as follows:

1. STUDY AND DATA DETAILS

1.1. The Recipient has received ethical approval ("**Ethical Approval**") to:

i. carry out the clinical research study known as "Timing of HD-MTX CNS Prophylaxis in Diffuse Large B-cell Lymphoma" ("**Study**") to assess outcomes of patients receiving HD-MTX CNS prophylaxis in DLBCL, comparing an intercalated approach to delivery at end of R-CHOP therapy; and

ii. create a database for the Study which may, subject to certain conditions and permission being granted by the Access Committee (as defined below), also be used by the Recipient, the Sender and/or other organisations contributing to the Database (each of the foregoing being a "**Contributing Organisation**") for other retrospective, observational studies in the future, as described further below ("**Database**").

1.2. The Parties have agreed that the Sender will, on or as soon as practicable after its signature of this Agreement, transfer to the Recipient, such data for participants in the Study identified by the Sender as is set out in Part 1 of the appendix ("**Appendix**") attached to and forming part of this Agreement ("**Data**"), in the manner described in the Appendix.

1.3. The Sender will ensure that the Data is fully de-identified (anonymous) prior to sending to the Recipient. The Sender will allocate each of the participants with a unique Study number and will retain a separate record (a "**Participant Log**") of names and identifiers linked to participant Study numbers which will be stored securely by the Sender. The Sender will not provide the Recipient with any access to the Participant Log. The Recipient agrees not to re-identify or attempt to re-identify the Data.

1.4. Given the anonymous status of the Data when received by the Recipient, the conditions of transfer, obligations of confidentiality and prohibition on re-identification, the Parties agree that when received by the Recipient, the Data does not contain Personal Data as defined in the UK Data Protection Act 2018.

2. PERMITTED USE

2.1. The Recipient shall be entitled to use the Data for the purposes of:

i. the Study; and
ii. creation of the Database for use in connection with the Study and for the purposes of certain other studies, as described in the Ethical Approval,

all subject to the terms of the Ethical Approval, (together the "**Permitted Use**") and for no other purpose.

2.2. Any Contributing Organisation may submit a request to access to the Database for the purposes of certain other retrospective, observational studies. The requirements for such studies are set out in the Ethical Approval and approval of any such study must be granted by the dedicated "**Access Committee**" set up by the Recipient (as required by the Ethical Approval). A study which has been approved by the Access Committee is an "**Approved Further Study**".

3. RECIPIENT RESPONSIBILITY

3.1. The Recipient shall only disclose the Data to such of its employees, officers, contractors, subcontractors, students or volunteers (together its "**Personnel**") who require it for the purposes of the Study or Approved Further Study as applicable and shall not otherwise disclose it except as required by law.

3.2. The Recipient shall ensure that all of its Personnel who have access to any Data, handle such Data as required by this Agreement.

3.3. The Recipient shall ensure that the Data is stored securely with appropriate technical and organisational safeguards in place.

3.4. The Recipient shall promptly report to the Sender, any use or disclosure of the Data not authorised by this Agreement, together with any remedial or mitigating action taken or proposed to be taken by the Recipient with respect thereto.

4. INTELLECTUAL PROPERTY

Nothing in this Agreement is intended to grant any rights to the Recipient under any patent, copyright or other intellectual property rights of the Sender, nor shall this Agreement grant the Recipient any rights in or to the Data except as expressly set forth in this Agreement.

5. PUBLICATION

5.1. Other than as set out in Clause 5.2 below, neither Party shall use the name or logo of the other Party in any public announcement or advertising without the other Party's prior written approval.

5.2. The Recipient anticipates that the results of the Study will be submitted for presentation at an appropriate international forum in 2021 and submitted for publication in a peer reviewed scientific journal. In addition, the contribution of data to the Study by Contributing Organisations will be appropriately acknowledged by the Recipient.

6. TERMINATION FOR BREACH AND DELETION OF DATA

The Sender may terminate this Agreement with immediate effect upon written notice to the Recipient if the Recipient is in material breach of any of its obligations under this Agreement and fails to remedy such breach, where it is capable of remedy, within 14 days of a written notice from the Sender specifying the breach and requiring its remedy. In the event of such termination, the Recipient will securely delete the Data it holds as soon as reasonably practicable.

7. NO WARRANTY

All Data is provided "as is" and the Sender makes no warranty regarding the accuracy, completeness, suitability or performance of the Data disclosed under this Agreement.

8. RELATIONSHIP BETWEEN THE PARTIES

The Parties to this Agreement are independent parties and nothing in this Agreement creates a relationship of employer and employee, principal and agent, joint venture or partnership between the Parties.

9. GENERAL

9.1. Neither Party may assign or transfer any of its rights or obligations under this Agreement without the prior written consent of the other.

9.2. This Agreement contains everything the Parties have agreed in relation to the subject matter it deals with. No Party can rely on an earlier written document or anything said or done by or on behalf of the other Party before this Agreement was executed.

9.3. No variation of this Agreement will be of any force or effect unless it is in writing and signed by an authorised signatory of each Party.

9.4. This Agreement may be executed in one or more counterparts, each of which shall be deemed an original, but all of which together shall constitute one and the same instrument.

9.5. This Agreement is governed by the law of Scotland. The Parties submit to the exclusive jurisdiction of the courts of Scotland.

SIGNED

Signed for and on behalf of Greater Glasgow Health Board

.....Authorised Signatory Date:.....

..... Name (Please Print)

Signature of Witness

.....

..... Name (Please Print)

Signed for and on behalf of [ ]

..... Authorised Signatory Date:.....

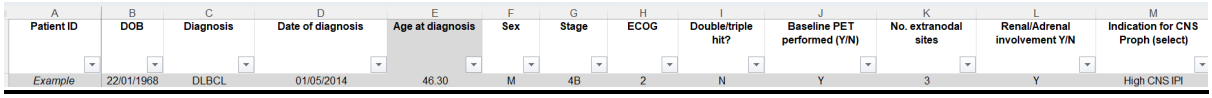
..... Name (Please Print)

Signature of Witness

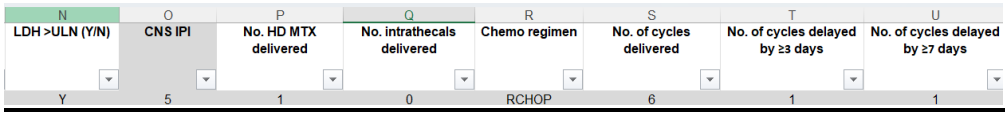
.....

..... Name (Please Print)

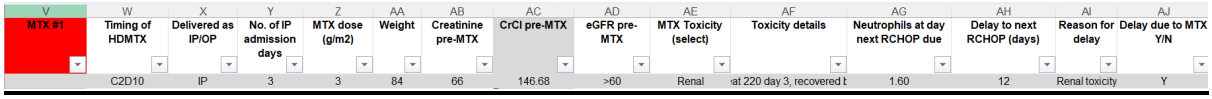
Appendix 3 – Data collection template for Paper 2



A	B	C	D	E	F	G	H	I	J	K	L	M
Patient ID	DOB	Diagnosis	Date of diagnosis	Age at diagnosis	Sex	Stage	ECOG	Double/triple hit?	Baseline PET performed (Y/N)	No. extranodal sites	Renal/Adrenal involvement Y/N	Indication for CNS Proph (select)
Example	22/01/1968	DLBCL	01/05/2014	46.30	M	4B	2	N	Y	3	Y	High CNS IPI



N	O	P	Q	R	S	T	U
LDH >ULN (Y/N)	CNS IPI	No. HD MTX delivered	No. intrathecals delivered	Chemo regimen	No. of cycles delivered	No. of cycles delayed by ≥3 days	No. of cycles delayed by ≥7 days
Y	5	1	0	RCHOP	6	1	1



V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
MTX #1	Timing of HDMTX	Delivered as IP/OP	No. of IP admission days	MTX dose (g/m2)	Weight	Creatinine pre-MTX	CrCl pre-MTX	eGFR pre-MTX	MTX Toxicity (select)	Toxicity details	Neutrophils at day next RCHOP due	Delay to next RCHOP (days)	Reason for delay	Delay due to MTX Y/N
	C2D10	IP	3	3	84	66	146.68	>60	Renal	at 220 day 3, recovered t	1.60	12	Renal toxicity	Y

Appendix 4 – Data collection template for paper 3

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Patient ID	DOB	Diagnosis	Transformed low grade lymphoma?	Date of diagnosis	Age at diagnosis	Sex	Stage	B symptoms	Performance status (ECOG)	Double /triple hit?	Baseline CNS assessment performed?	Baseline PET performed	No. extranodal sites	Extranodal site 1	Extranodal site 2
Example	*22/01/1945*	*DLBCL*	*No*	*01/05/2014*	*69.32*	*M*	*4*	*N*	*2*	*N*	*No*	*Y*	*4*	*Bone*	*Soft tissue*

Q	R	S	T	U	V	W	X	Y	Z	AA	AB
Extranodal site 3	Extranodal site 4	Renal/Adrenal involvement	Indication for CNS Proph	LDH	HD-MTX approach planned	No. HD MTX delivered	No. intrathecals delivered	Chemo regimen	No. of cycles R-CHOP delivered	No. of cycles delayed by ≥3 days	No. of cycles delayed by ≥7 days
Pleura	*Kidney/adrenal*	*Y*	*High CNS-IPI*	*x upper limit norm*	*Intercalated*	*1*	*0*	*R-CHOP-21*	*6*	*1*	*1*

AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL
If <2 HD-MTX delivered reason?	Response to R-CHOP (see criteria in tab)	Date of systemic relapse or progression	CNS Relapse?	Date of CNS relapse	Date of last FU or death	Follow up length (days)	Alive Y/N	Cause of death	Comments
Renal toxicity	*CR*	*NA*	*No*	*NA*	*07/05/2019*	*1832*	*Y*	*NA*	

A	B	C	D	E	F	G	H	I	J	K	L	M	N
Patient ID	HD-MTX course number	HD-MTX given post R-CHOP cycle:	Day post last R-CHOP cycle of HD-MTX Delivery	Delivered as IP/OP	No. of IP admission days	MTX dose (g/m2)	Weight	Creatinine pre-HD-MTX	MTX Toxicity (1)	Grade Toxicity (1) (see grading guide)	MTX Toxicity (2)	Grade Toxicity (2) (see grading guide)	Toxicity details (free text)
Example	2	6	21	IP	5	3	105.7	70	None	NA	None	NA	

O	P	Q	R	S	T
Neutrophils at day next RCHOP due	G-CSF support used?	Delay to next RCHOP (days)	Reason for delay	Delay due to MTX (clinician discretion)	Comments
NA	Y	NA			