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DOI:
10.1182/blood.2021014506

American Society of Hematology
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Washington, DC 20036
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Timing of high dose methotrexate CNS prophylaxis in DLBCL: a multicenter international analysis of 1,384 patients

Tracking no: BLD-2021-014506R1

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Abstract:
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 1,384 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=0.98, 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=1,253). In patients with high CNS international prognostic index (n=600), 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 reduction in CNS relapse. R-CHOP delays of {greater than or equal to}7 days were significantly increased with i-HD-MTX versus EOT, with 308/1573 (19.6%) i-HD-MTX treatments resulting in 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 versus 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.

Conflict of interest: COI declared - see note

COI notes: MRW: Conference fees - Takeda, Janssen and Kite/Gilead, Honoraria - Abbvie and Kite/Gilead. TAE: Honoraria - Roche, Kite/Gilead, Janssen, Abbvie, AstraZeneca, Loxo Oncology, Beigene, Secura Bio. Consultancy - Roche, Abbvie, Loxo Oncology, Incyte, Secura Bio. MA: Honoraria - Takeda and Roche, Research funding - Pfizer. ES: Honoraria - Riemser Pharma GmbH, research funding - Roche, Abbvie. MK: Consultancy - Roche, Antegene, Genor Biopharma. MN: Research funding - TG Thereapeutics, Genmab, Genentech/Roche, Gilead. KLL: Honoraria - AstraZeneca, Janssen, Roche. Patents and royalties - Janssen and Novartis, Consultancy - AstraZeneca. AKO: Travel expenses - Abbvie. AS: Honoraria - Janssen. NS: Honoraria and Membership on an entity's Board of Directors or advisory committees - Abbvie, Janssen and Roche. LR: Travel - Janssen. KM: Travel and meetings - Bristol-Myers Squibb. NH: Membership on an entity's Board of Directors or advisory committees and Speakers Bureau - Novartis. AG: Speaker Honoraria - Roche, Janssen, Abbvie, Celgene, Fresenius, Novonordisk, Travel and accommodation - Roche, Janssen, Abbvie. TE: ended employment in past 24 months - Roche, Speakers fee - Abbvie. CYC: Consultancy, Honoraria and other (advisory) - Roche, Janssen, MSD, Gilead, Ascentage pharma, Beigene, AstraZeneca, Loxo/Lilly, TG Therapeutics, Research funding - Abbvie, Celgene. AF: 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. CF: Honoraria, Membership on an entity's Board of Directors or advisory committees and Research Funding - Roche. Other: speaker fees - Janssen. KC: 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. PM: Honoraria and Membership on an entity's Board of Directors or advisory committees - Roche, Kite, Takeda, Beigene. Travel support: Gilead, Janssen. All other authors: no competing financial interests to declare.

Preprint server: No;

Author contributions and disclosures: MRW, TAE, AAK, KC and PM designed the study, analysed data and wrote the paper. AAK performed all statistical analyses. All other authors participated in collection of data and in writing/reviewing the manuscript.

Non-author contributions and disclosures: Yes; The following healthcare professionals contributed to data collection at individual study sites. No funding source was used for their assisstance. Catherine Thieblemont (Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris), Sridhar Chaganti (University of Birmingham), George Follows (Cambridge University Hospitals NHS Foundation Trust), Anca Prica (Princess Margaret Cancer Centre, Toronto), 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 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).

Agreement to Share Publication-Related Data and Data Sharing Statement: Qualified researchers may request data from the corresponding author.

Clinical trial registration information (if any):

Timing of high dose methotrexate CNS prophylaxis in DLBCL: a multicenter international analysis of 1,384 patients

Short title for running head:

Timing of HD-MTX CNS prophylaxis in DLBCL

Scientific category:

Lymphoid neoplasia

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

Word count:

Abstract: 244

Main article: 4,459

Figures: 2

Tables: 6

References: 33

Key points:

- End of treatment HD-MTX did not increase risk of CNS relapse compared to 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.

Abstract:

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 1,384 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=0.98, 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=1,253). In patients with high CNS international prognostic index (n=600), 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 reduction in CNS relapse. R-CHOP delays of ≥7 days were significantly increased with i-HD-MTX versus EOT, with 308/1573 (19.6%) i-HD-MTX treatments resulting in 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 versus 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 commonest subtype of non-Hodgkin lymphoma (NHL). 60-70% 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 ~2-5%[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 15-30%. 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 tumour 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 front-line therapy, aiming to minimise interruption of systemic treatment whilst 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 randomised trials have been performed to confirm this. Recent analyses cast doubt on HD-MTX efficacy, including a

retrospective study of approximately 2,300 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 front-line therapy. Advocates of an 'intercalated' (i-HD-MTX) approach hypothesize that delivery between early cycles of R-CHOP may prevent very early CNS relapses, whilst others prefer delivering HD-MTX at end of treatment (EOT) to avoid interruptions/delays to potentially curative systemic therapy.

We previously analysed 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 to 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=1,384) with the primary aim of determining whether EOT HD-MTX is as effective as i-HD-MTX in preventing CNS relapse. Secondary endpoints included 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 NOS diagnosed between 2007-2020 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 intravenous MTX dose intended to cross the blood brain barrier and exert 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 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 were recorded for all patients. All i-HD-MTX treatments were reviewed with 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, i.e. that EOT HD-MTX was not more than 5% inferior, using a pre-planned 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 date last seen. Analyses used competing risks by the Fine and Gray method. Time to isolated CNS relapse was analysed in the same manner, but with concurrent systemic relapse (defined as CNS and systemic relapse occurring within 30 days of each other) also counted as a competing event. Due to violations in the proportional hazards (PH) 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. PFS and OS were analysed using Kaplan Meier survival analysis and Cox regression with times measured from date of

diagnosis until the first event, and patients without an event were censored at the date last seen. Treatment delays were analysed 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, Texas).

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 1,384 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=0.002). Overall, 44.2% had a CNS-IPI 4-6, 40.9% CNS-IPI 2-3 and 14.9% 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 towards a higher CNS-IPI score for i-HD-MTX patients (p=0.083), however there was no significant difference in the numbers with score 4-6 (45.1% vs 43.0%, p=0.45). 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 in *Supplemental Table 1*.

80.5% of patients had baseline PET-CT 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 to i-HD-MTX (55.7% vs 38.0%, p<0.0001).

The median number of HD-MTX cycles delivered was 2 for both groups. Similar numbers received ≥2 cycles (87.7% vs 85.6%, p=0.25), however, significantly more patients received ≥3 in the i-HD-MTX group (36.8% vs 12%, p<0.0001) 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<0.0001).

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% confidence interval (CI) 0.65-1.57), p=0.98 (*Figure 1a*). This remained similar when adjusted for baseline prognostic factors: HR 1.06 (0.67-1.66), p=0.82, and the 3-year difference (EOT – i-HD-MTX) excluded the non-inferiority 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.

Sub-analyses of CNS relapse in high-risk patients are summarised 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 non-inferiority 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% non-inferiority 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 non-inferiority margin: 0.6% (-2.1 to 5.0%). (*Supplemental Table 5*).

Univariable and multivariable analyses (MVA) 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 both 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.

CNS relapses were isolated in 57/78 (73.1%) cases with the remainder occurring in combination with systemic progression. Sites of isolated relapse were parenchymal in 35/57 (61%), leptomeningeal in 16/57 (28%) and both in 6/57 (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 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 to 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 LDH (PH violations): adjusted PFS HR 0.79 (95% CI 0.64-0.98), p=0.024 and OS HR 0.67 (95% CI 0.52-0.88), p=0.003 (*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=0.72 and OS HR 0.85 (95% CI 0.61-1.18), p=0.32 (*Figure 2C-D*).

Non-relapse mortality (NRM) was reported in 55/1384 (4.0%) patients. Although no NRM events were reported as being directly attributable to HD-MTX, 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) (*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=0.055.

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 analysed according to presence of isolated CNS or synchronous systemic/CNS relapse, there was a trend towards inferior survival in patients with synchronous relapse (HR 1.69 (95% CI 0.96-2.98), p=0.069) (*Supplemental Figure 2b*). There was no difference in survival according to 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, OR, 0.44 (95% CI 0.33-0.59), p<0.0001). Results were unchanged using ordinal regression with number of delays throughout therapy categorized as 0, 1-2 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, *see Supplemental Figure 3a-b*). The median day post-R-CHOP of i-HD-MTX delivery was 10 (IQR 1-14) and median number of intercalated cycles per patient was 2 (IQR 1-2). 308/1573 (19.6%) of intercalated HD-MTX cycles 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=0.004) and a trend towards inferior OS (adjusted HR 1.38 (95% CI 0.96-1.98), p=0.085).

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 UVA, with increasing age the only variable approaching statistical significance on MVA (p=0.055). 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 (*Supplementary* 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 (≥3g/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 are 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 1/3 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 evidence derived from studies demonstrating efficacy in treatment of primary CNS lymphoma.[29] Historical, retrospective non-randomised 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 reduction in CNS relapse risk.[18,19] Patients at 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 to EOT, but the number of CNS relapse events were too small to conclude that the approaches were equivalent in efficacy.[20]

To our knowledge, this international, multicentre collaboration represents the largest dataset of patients with DLBCL receiving HD-MTX as CNS prophylaxis. The study achieved its primary endpoint of demonstrating non-inferiority of EOT HD-MTX compared to 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 to 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 suggests 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 non-inferiority and importantly no PFS/OS difference.

The proportion of CNS-IPI 4-6 patients in our study was relatively low (44%). However, the CNS-IPI is an imperfect tool, with high-risk score resulting in a positive predictive value of only 12%. Other established, independent risk factors include specific EN site involvement (e.g. testicular, renal/adrenal and breast) and total number of EN sites involved. We performed analyses aimed at determining whether 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 favoured 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 occurring either with or after systemic progression. Indeed, Schmitz *et al* does not give this detail.[6] Arguably, any CNS relapse occurring concurrent 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 overall survival or concomitant CNS-systemic relapses.[30] We excluded any CNS relapse occurring after 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 theoretical rational 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 versus 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 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 one 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 observed rates of febrile neutropenia, mucositis and renal toxicity (all 15-17%) associated with i-HD-MTX are of concern, particularly when 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 7,000 DLBCL patients which demonstrated no benefit of standalone IT therapy in preventing CNS relapse.[10] We demonstrate that use of concurrent IT prophylaxis was not associated with 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%, 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 2,300 high-risk patients which found no difference in CNS relapse between patients who receiving HD-MTX vs not.[19] Furthermore, the overall rate of CNS relapse of 9% in the latter study, which included 1,890 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 randomised controlled trial of HD-MTX versus 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 timing of delivery. However, even the large *Lewis et al* analysis is limited in its ability to exclude benefit of HD-MTX in the highest risk subgroups, such as those with CNS-IPI 6 or with high risk EN site involvement (e.g. testicular, 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 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.

The strengths of this study are the multicentre design, large sample size, pre-planned 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, nonrandomised 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 immuno-chemotherapy. 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 1,384 patients, we demonstrate that delivery of EOT HD-MTX did not increase the risk of CNS relapse compared to early integration during R-CHOP/R-CHOP-like therapy. CNS relapse rate observed in high-risk patients in our study were 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 recognise that usage is likely to decrease substantially in light of the recent 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 using more sophisticated techniques (e.g. CSF ctDNA) to identify high-risk patients are areas where the field should focus attention.

Acknowledgements:

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 (University of Birmingham), George Follows (Cambridge University Hospitals NHS Foundation Trust), Anca Prica (Princess Margaret Cancer Centre, Toronto), 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 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).

Authorship Contributions:

MRW, TAE, AAK, KC and PM designed the study, analysed data and wrote the paper. AAK performed all statistical analyses. All other authors participated in collection of data and in writing/reviewing the manuscript.

Data Sharing Statement:

Qualified researchers may request data from the corresponding author.

Conflict of Interest Disclosures:

MRW: Conference fees – Takeda, Janssen and Kite/Gilead, Honoraria – Abbvie and Kite/Gilead. TAE: Honoraria – Roche, Kite/Gilead, Janssen, Abbvie, AstraZeneca, Loxo

Oncology, Beigene, Secura Bio. Consultancy - Roche, Abbvie, Loxo Oncology, Incyte, Secura Bio. MA: Honoraria – Takeda and Roche, Research funding – Pfizer. ES: Honoraria - Riemser Pharma GmbH, research funding – Roche, Abbvie. MK: Consultancy – Roche, Antegene, Genor Biopharma. MN: Research funding – TG Thereapeutics, Genmab, Genentech/Roche, Gilead. KLL: Honoraria – AstraZeneca, Janssen, Roche. Patents and royalties – Janssen and Novartis, Consultancy – AstraZeneca. AKO: Travel expenses – Abbvie. AS: Honoraria – Janssen. NS: Honoraria and Membership on an entity's Board of Directors or advisory committees - Abbvie, Janssen and Roche. LR: Travel – Janssen. KM: Travel and meetings - Bristol-Myers Squibb. NH: Membership on an entity's Board of Directors or advisory committees and Speakers Bureau – Novartis. AG: Speaker Honoraria – Roche, Janssen, Abbvie, Celgene, Fresenius, Novonordisk, Travel and accommodation – Roche, Janssen, Abbvie. TE: ended employment in past 24 months – Roche, Speakers fee – Abbvie. CYC: Consultancy, Honoraria and other (advisory) – Roche, Janssen, MSD, Gilead, Ascentage pharma, Beigene, AstraZeneca, Loxo/Lilly, TG Therapeutics, Research funding – Abbvie, Celgene. AF: 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. CF: Honoraria, Membership on an entity's Board of Directors or advisory committees and Research Funding – Roche. Other: speaker fees – Janssen. KC: 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. PM: Honoraria and Membership on an entity's Board of Directors or advisory committees – Roche, Kite, Takeda, Beigene. Travel support: Gilead, Janssen. All other authors: no competing financial interests to declare.

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623 **Table 1: Baseline characteristics of whole study population**

	All N=1384	End of treatment N=635	Intercalated N=749	p-value
Age (years), median (range)	62.5 (17 - 88)	63.0 (18 - 86)	62.0 (17 - 88)	0.065
Follow-up (months), 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)	0.0001
Male sex, N (%)	840 (60.7)	393 (61.9)	447 (59.7)	0.40
Advanced stage, N (%)	1156 (83.5)	509 (80.2)	647 (86.4)	0.0019
Raised LDH baseline, N (%)	943 (70.0)	410 (68.0)	533 (71.5)	0.16
Missing/unknown	36	32	4	
ECOG ≥2, N (%)	358 (25.9)	158 (25.0)	200 (26.7)	0.47
Missing/unknown	3	3	0	
Extra-nodal sites, N (%)				
0-1	586 (42.3)	282 (44.4)	304 (40.6)	0.11*
2	421 (30.4)	191 (30.1)	230 (30.7)	
≥3	377 (27.2)	162 (25.5)	215 (28.7)	
Renal or adrenal involvement, N (%)	240 (17.3)	102 (16.1)	138 (18.4)	0.25
Testicular involvement, N (%)	175 (12.7)	95 (15.0)	80 (10.7)	0.016
Breast involvement, N (%)	56 (4.1)	18 (2.8)	38 (5.1)	0.037
Double or triple hit, N (%)	66 (6.1)	32 (6.7)	34 (5.7)	0.47
Missing/unknown	308	159	149	
CNS IPI, N (%)				
Low (0-1)	203 (14.9)	107 (17.5)	96 (12.9)	0.083*
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, N(%)	703 (50.8)	382 (60.2)	321 (42.9)	<0.0001

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

625 IQR, inter-quartile range; LDH, lactate dehydrogenase ; ECOG, Eastern Cooperative Oncology Group
626 performance status; CNS IPI, central nervous system international prognostic index.

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

	HR[1] (95% CI)	3-year difference (HR)[2]	3-year difference[3]
All patients:			
EOT HD-MTX (UVA)	1.01 (0.65 – 1.57)	0.04% (-2.0 to 3.1)	0.06% (-2.63 – 2.76)
EOT HD-MTX (adjusted[4])	1.06 (0.67 – 1.66)	0.3% (-1.8 to 3.6)	0.79% (-1.95 to 3.52)
EOT HD-MTX (adjusted[5])			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[4])	0.96 (0.55 – 1.67)	-0.2% (-2.1 to 3.0%)	0.47% (-2.18 to 3.12)
EOT HD-MTX (adjusted[5])			-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[4])	1.10 (0.64 – 1.87)	0.4% (-1.4 to 3.2)	1.00% (-1.38 to 3.30)
EOT HD-MTX (adjusted[5])			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[4])	1.05 (0.57 – 1.95)	0.2% (-1.7 to 3.6)	1.02% (-1.33 to 3.37)
EOT HD-MTX (adjusted[5])			0.93% (-1.51 to 3.36)

[1] *HR for EOT vs i-HD-MTX*

[2] *Calculated by applying the hazard ratio to the 3-year rate in the i-HD-MTX group to get the corresponding rate in the EOT group, and then taking the difference.*

[3] *Difference in cumulative incidence rates allowing for competing risks at 3 years using pseudo observations.*

[4] *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 >6g/m2 for landmark cohort).*

[5] *Adjusted for only variables significant with backwards selection (based on survival time lost): age and renal/adrenal involvement for CNS relapse and age alone for isolated CNS relapse.*

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

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

Table 3: Results within specific high-risk groups

	3-year CNS relapse rates	Events/N	HR* (95% CI)	3-year 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 to 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 to 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 to 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 to 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 to 34.5)
End of treatment	20.5% (5.6 – 60.3)	2/18	1.56 (0.26 – 9.39)	
3 or more extra nodal sites	**7.6% (5.2 – 10.9)**	**29/377**		
Intercalated	8.0% (5.0 – 12.8)	16/215	1.00	0.0% (-4.1 to 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 to 4.3)
End of treatment	7.7% (5.3 – 11.1)	31/403	1.00 (0.61 – 1.62)	

**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.*

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

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

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

Risk factor	All patients		Landmark	
	Survival time lost (months)	p-value	Survival time lost (months)	p-value
All CNS relapses – UVA:				
EOT HD-MTX	0.52 (-3.04 to 4.09)	0.77	0.43 (-3.13 to 3.99)	0.82
Sex	0.71 (-2.99 to 4.40)	0.71	0.14 (-3.58 to 3.85)	0.94
Age (for a 10-year increase)	1.61 (0.58 to 2.64)	0.002	1.64 (0.61 to 2.66)	0.002
Advanced stage	2.53 (-2.27 to 7.33)	0.30	1.22 (-3.66 to 6.11)	0.62
Extra nodal sites ≥2	4.39 (1.00 to 7.79)	0.011	1.99 (-1.48 to 5.47)	0.26
ECOG ≥2	0.86 (-2.94 to 4.67)	0.66	0.40 (-3.39 to 4.19)	0.84
Renal/adrenal involvement	7.64 (2.28 to 13.00)	0.005	6.06 (0.62 to 11.51)	0.029
Raised LDH	3.02 (-0.29 to 6.34)	0.074	1.63 (-1.67 to 4.94)	0.33
ITs given			1.10 (-2.48 to 4.68)	0.55
HD=MTX doses ≥2			-2.87 (-8.57 to 2.84)	0.33
Cumulative dose >6g/m2			-2.19 (-5.47 to 1.09)	0.19
All CNS relapses – MVA:				
Age (for a 10-year increase)	1.60 (0.59 – 2.61)	0.002	1.33 (0.39 to 2.27)	0.006
Renal/adrenal involvement	7.65 (2.31 – 13.00)	0.005	5.45 (0.23 to 10.66)	0.041
Isolated CNS relapse – UVA:				
EOT HD-MTX	0.71 (-2.51 to 3.94)	0.66	0.79 (-2.93 to 4.51)	0.68
Sex	0.46 (-2.89 to 3.81)	0.79	0.59 (-3.39 to 4.56)	0.77
Age (for a 10-year increase)	1.42 (0.51 to 2.34)	0.002	1.47 (0.44 to 2.49)	0.005
Advanced stage	0.24 (-4.48 to 4.95)	0.92	-0.52 (-5.81 to 4.77)	0.85
Extra nodal sites ≥2	2,21 (-0.89 to 5.31)	0.16	0.82 (-2.79 to 4.42)	0.66
ECOG ≥2	-0.69 (-3.90 to 2.52)	0.67	-1.63 (-5.11 to 1.85)	0.36
Renal/adrenal involvement	3.89 (-0.54 to 8.32)	0.086	2.29 (2.45 to 7.03)	0.34
Raised LDH	1.17 (-1.86 to 4.19)	0.45	0.03 (-3.27 to 3.32)	0.99
ITs given			1.21 (-2.59 to 5.00)	0.53
HD-MTX doses ≥2			-2.43 (-7.95 to 3.10)	0.39
Cumulative dose >6g/m2			-3.59 (-6.84 to -0.35)	0.030
Isolated CNS relapse - MVA				
Age (for a 10-year increase)	1.41 (0.52 to 2.31)	0.002	1.47 (-0.44 to 2.49)	0.005

Survival time is measured up to 10 years, for example, in univariable analysis, a patient given EOT HDMTX has a CNS-relapse free life expectancy over 10 years that is 0.43 months shorter than for a patient given i-HD-MTX. The MVA shows variables remaining significant with backwards selection (p-value for rejection 0.05). 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 to those without, and an increase in incidence of 1.12% for each decade of age.

UVA, univariable analysis; EOT, end of treatment; HD-MTX, high dose methotrexate; ECOG, eastern cooperative group performance status; LDH, lactate dehydrogenase; IT, intrathecal; MVA, multivariable analysis

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

Risk factor	Univariable			Multivariable	
	Events/N	OR (95% CI)	p-value	OR (95% CI)	p-value
7+ day delay (all patients)					
HD-MTX approach					
Intercalated	196/743	1.00	<0.0001	1.00	<0.0001
EOT	79/616	0.41 (0.31 – 0.55)		0.44 (0.33 – 0.59)	
Age (for an increase of 10 years)	275/1359	0.96 (0.87 – 1.06)	0.37	0.92 (0.82 – 1.04)	0.20
Sex					
Male	166/825	1.00	0.90	1.00	0.95
Female	109/534	1.02 (0.78 – 1.33)		0.99 (0.75 – 1.32)	
Advanced stage					
Stage I-II	46/221	1.00	0.82	1.00	0.90
Stage III-IV	229/1138	0.96 (0.67 – 1.37)		0.97 (0.63 – 1.50)	
ECOG					
0-1	210/1004	1.00	0.32	1.00	0.43
2+	65/353	0.85 (0.63 - 1.16)		0.88 (0.63 – 1.22)	
2+ extra nodal sites					
<2	115/576	1.00	0.83	1.00	0.62
2+	160/783	1.03 (0.79 – 1.35)		1.08 (0.79 – 1.48)	
LDH					
Normal	93/401	1.00	0.12	1.00	0.088
>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)	0.71	0.73 (0.49 – 1.10)	0.14

A more conservative analysis which excluded any patient in the iHDMTX group given <6 cycles of treatment (i.e. 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 <0.001 (UVA) and HR 0.47 (0.35 – 0.64), p <0.001 (MVA).

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

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

Risk factor	Univariable			Multivariable	
	Events/N	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (for an increase of 10 years)	214/748	1.20 (1.05 – 1.36)	0.006	1.16 (1.00 – 1.35)	0.055
Sex					
Male	131/447	1.00	0.61	1.00	0.74
Female	83/301	0.92 (0.66 – 1.27)		0.95 (0.67 – 1.33)	
Advanced stage					
Stage I-II	30/102	1.00	0.85	1.00	0.82
Stage III-IV	184/646	0.96 (0.60 – 1.51)		1.06 (0.63 – 1.81)	
ECOG					
0-1	163/548	1.00	0.26	1.00	0.37
2+	51/200	0.81 (0.56 – 1.17)		0.84 (0.57 – 1.23)	
2+ extra nodal sites					
<2	87/303	1.00	0.96	1.00	0.98
2+	127/445	0.99 (0.72 - 1.37)		1.00 (0.70 – 1.45)	
LDH					
Normal	69/212	1.00	0.15	1.00	0.21
>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)	0.043	0.84 (0.52 – 1.37)	0.48

MVA, with backwards selection (p=0.05 for inclusion), age is the only factor that remains: OR: 1.19 (1.05 – 1.35), p = 0.008 (N=735) [Note this is slightly different from the UVA quoted (despite being the only variable left) as it included complete cases only]

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

Figure Legends

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

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

Figure 1A

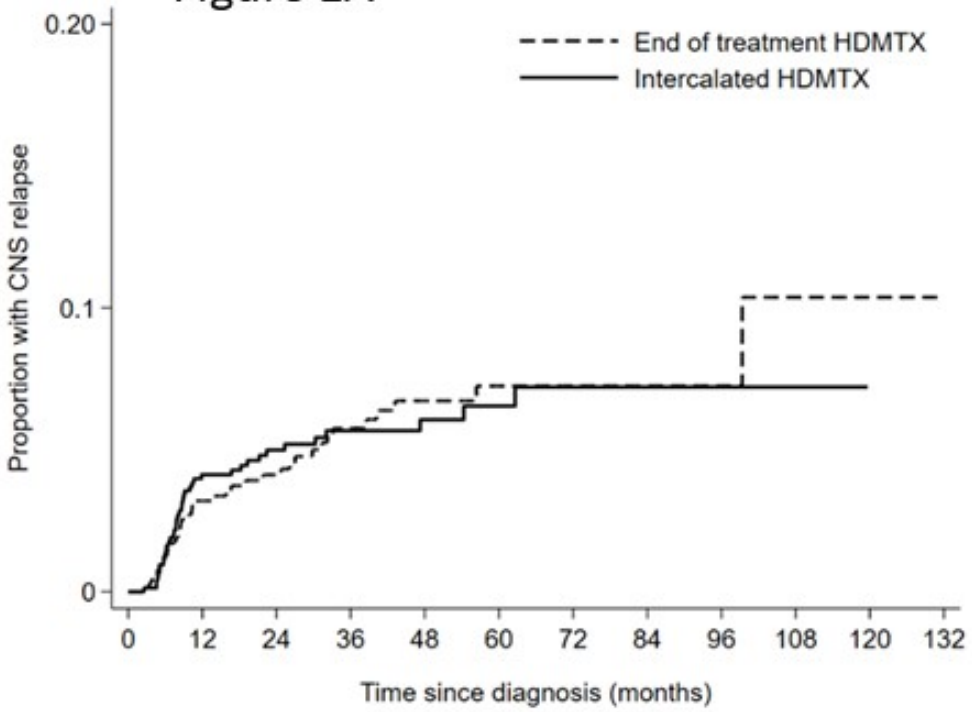


Figure 1B

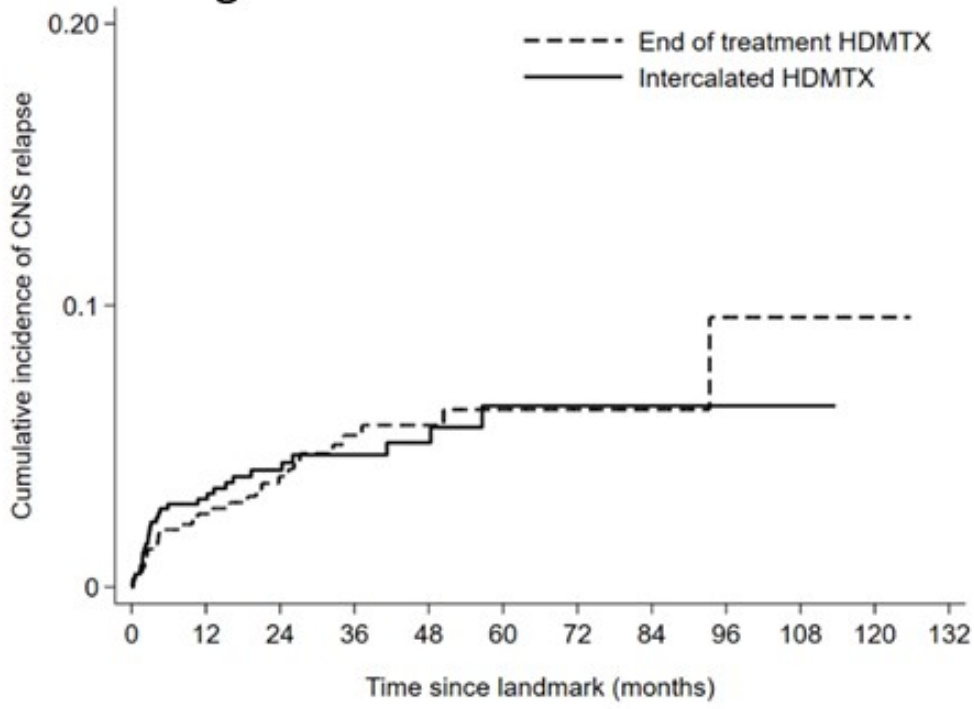
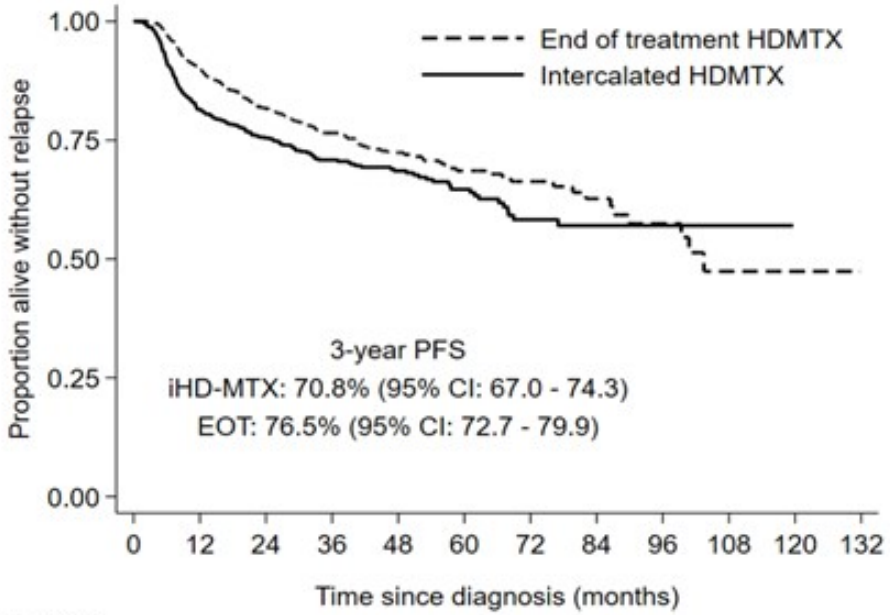


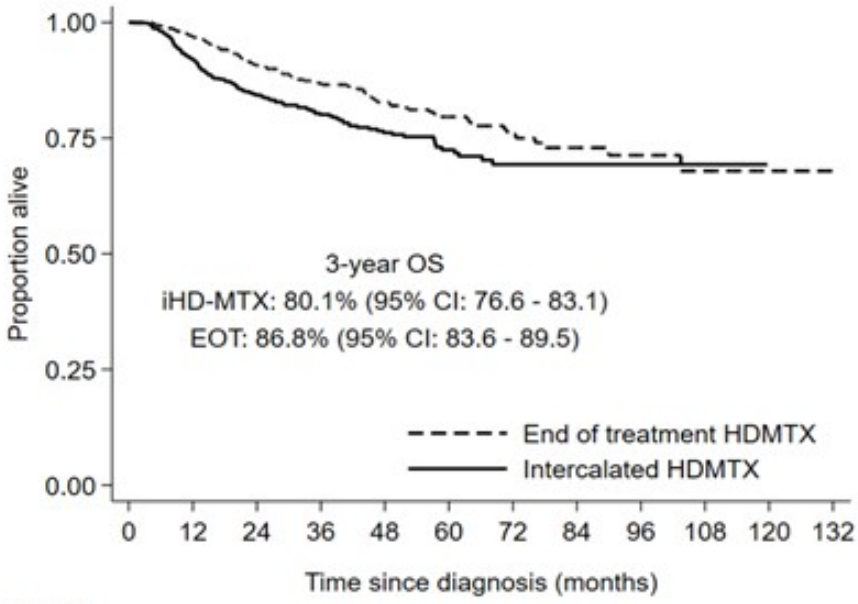
Figure 2A



Number at risk

End of treatment	635	535	398	270	185	118	72	40	24	10	3	0
Intercalated	749	562	374	257	175	106	57	33	17	7	0	0

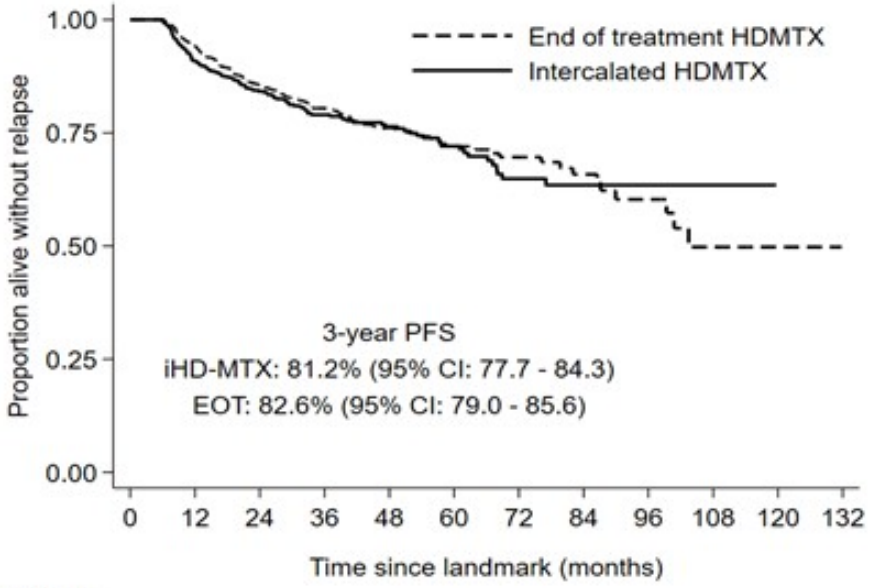
Figure 2B



Number at risk

End of treatment	635	574	440	310	215	144	85	52	33	17	6	1
Intercalated	749	631	417	293	193	114	67	39	21	8	0	0

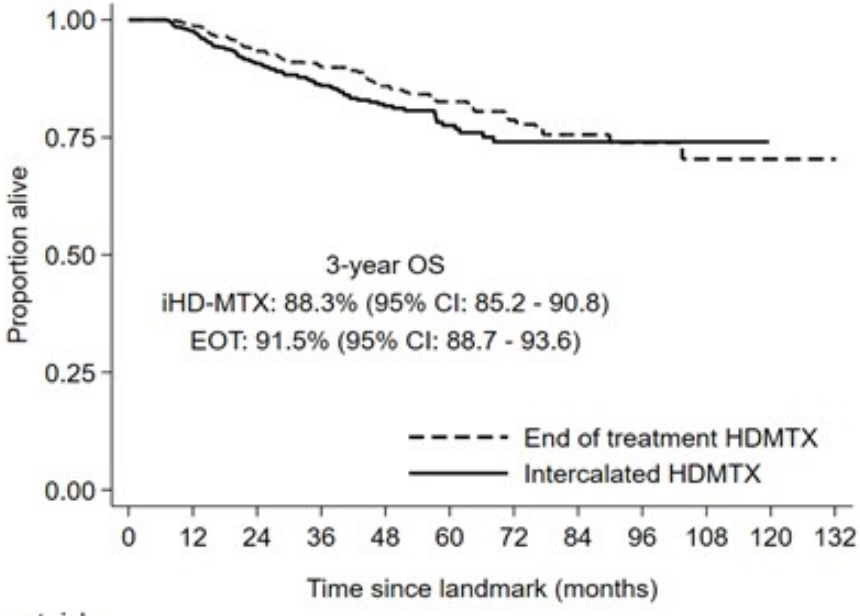
Figure 2C



Number at risk

End of treatment	599	532	396	269	184	117	71	40	24	10	3	0
Intercalated	654	560	372	256	174	105	56	32	16	6	0	0

Figure 2D



Number at risk

End of treatment	599	558	432	307	213	142	84	52	33	17	6	1
Intercalated	654	601	404	283	187	110	64	38	20	7	0	0