

Timing of high-dose methotrexate CNS prophylaxis in DLBCL

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

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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.

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74

75

76 **Key points:**

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

81

82 **Abstract:**

83 Prophylactic high-dose methotrexate (HD-MTX) is often used for diffuse large B-cell
84 lymphoma (DLBCL) patients at high risk of central nervous system (CNS) relapse, despite
85 limited evidence demonstrating efficacy or the optimal delivery method. We conducted a
86 retrospective, international analysis of 1,384 patients receiving HD-MTX CNS prophylaxis
87 either intercalated (i-HD-MTX) (n=749) or at the end (n=635) of R-CHOP/R-CHOP-like
88 therapy (EOT).

89 There were 78 CNS relapses (3-year rate 5.7%), with no difference between i-HD-MTX and
90 EOT; 5.7% vs 5.8%, p=0.98, 3-year difference: 0.04% (-2.0% to 3.1%). Conclusions were
91 unchanged on adjusting for baseline prognostic factors or on 6-month landmark analysis
92 (n=1,253). In patients with high CNS international prognostic index (n=600), 3-year CNS
93 relapse rate was 9.1% with no difference between i-HD-MTX and EOT. On multivariable
94 analysis, increasing age and renal/adrenal involvement were the only independent risk
95 factors for CNS relapse. Concurrent intrathecal prophylaxis was not associated with
96 reduction in CNS relapse. R-CHOP delays of ≥ 7 days were significantly increased with i-HD-
97 MTX versus EOT, with 308/1573 (19.6%) i-HD-MTX treatments resulting in delay to
98 subsequent R-CHOP (median 8 days). Increased risk of delay occurred in older patients
99 when delivery was later than day 10 in the R-CHOP cycle.

100 In summary, we found no evidence that EOT delivery increases CNS relapse risk versus i-HD-
101 MTX. Findings in high-risk subgroups were unchanged. Rates of CNS relapse in this HD-MTX-
102 treated cohort were similar to comparable cohorts receiving infrequent CNS prophylaxis. If

103 HD-MTX is still considered for certain high-risk patients, delivery could be deferred until R-
104 CHOP completion.

105

106 **Introduction**

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

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

122 Various attempts have been made to incorporate CNS-penetrating prophylaxis into front-
123 line therapy, aiming to minimise interruption of systemic treatment whilst reducing CNS
124 relapses in those most at risk. There remains a lack of robust evidence to guide
125 management, with national guidelines and position papers relying on mainly retrospective
126 data to make pragmatic recommendations about prophylactic strategies.⁹ High-dose
127 methotrexate (HD-MTX) is widely recommended as CNS prophylaxis in preference to
128 intrathecal (IT) therapy as the majority of relapses are parenchymal and the growing
129 evidence suggests IT therapy alone is ineffective.^{10,11} Historical retrospective studies suggest
130 that HD-MTX may be effective CNS prophylaxis¹²⁻¹⁴, but no randomised trials have been
131 performed to confirm this. Recent analyses cast doubt on HD-MTX efficacy, including a

132 retrospective study of approximately 2,300 patients demonstrating no apparent benefit in
133 high risk patients.¹⁵⁻¹⁹ Assuming HD-MTX may provide benefit to some high-risk patients,
134 there is uncertainty over how to safely integrate this into front-line therapy. Advocates of an
135 'intercalated' (i-HD-MTX) approach hypothesize that delivery between early cycles of R-
136 CHOP may prevent very early CNS relapses, whilst others prefer delivering HD-MTX at end of
137 treatment (EOT) to avoid interruptions/delays to potentially curative systemic therapy.

138 We previously analysed 334 patients treated with either i-HD-MTX or EOT HD-MTX.²⁰
139 Delays to R-CHOP were significantly increased by i-HD-MTX compared to EOT, and although
140 no differences in CNS relapse rate or survival between approaches were identified, the
141 event rate was too low to draw definitive conclusions. Given the critical importance of
142 maintaining dose intensity of systemic DLBCL therapy, and the increasing scrutiny over HD-
143 MTX efficacy as CNS prophylaxis, we conducted a large international study (n=1,384) with
144 the primary aim of determining whether EOT HD-MTX is as effective as i-HD-MTX in
145 preventing CNS relapse. Secondary endpoints included impact of HD-MTX timing on
146 survival, toxicity and delays to R-CHOP cycles and risk factors for CNS relapse including the
147 influence of concurrent IT prophylaxis.

148

149 **Methods**

150 We conducted a multicenter retrospective analysis of patients ≥ 16 years with DLBCL or high-
151 grade B-cell lymphoma NOS diagnosed between 2007-2020 from 47 centers in Europe,
152 Australia, and North America. The study received ethical approval from the West of
153 Scotland Research Ethics Committee (REC:20/WS/0114). Data were collected in compliance
154 with national and/or local regulations and data transfer agreements used where required.

155 Patients were included if they received frontline R-CHOP or R-CHOP-like therapy with
156 curative intent as well as HD-MTX CNS prophylaxis. HD-MTX was defined as any intravenous
157 MTX dose intended to cross the blood brain barrier and exert prophylactic effect, given for
158 ≥ 1 cycle. Diagnosis was established by local hematopathology review, with no central
159 pathological review performed. Patients with previously untreated transformed low-grade
160 NHL were included and concurrent IT prophylaxis was permitted. Patients with HIV-

161 associated DLBCL were included but those with immunosuppression-related
162 lymphoproliferative disorders and Burkitt lymphoma were excluded. Patients with known
163 CNS involvement at diagnosis and those treated with more intensive regimens, including
164 dose-adjusted etoposide, prednisolone, vincristine, cyclophosphamide, doxorubicin,
165 rituximab (DA-EPOCH-R), were excluded. Baseline CNS evaluation was performed according
166 to local clinician discretion.

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

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

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

179 We aimed to exclude a $\geq 5\%$ difference in CNS relapse rate between EOT HD-MTX and i-HD-
180 MTX, i.e. that EOT HD-MTX was not more than 5% inferior, using a pre-planned power
181 calculation (*supplementary materials*). Time-to-CNS relapse was calculated from diagnosis
182 date until CNS relapse with systemic only relapse and death in remission treated as
183 competing events. Patients alive without relapse were censored at date last seen. Analyses
184 used competing risks by the Fine and Gray method. Time to isolated CNS relapse was
185 analysed in the same manner, but with concurrent systemic relapse (defined as CNS and
186 systemic relapse occurring within 30 days of each other) also counted as a competing event.
187 Due to violations in the proportional hazards (PH) assumption for other prognostic factors of
188 interest, an analysis using pseudo-observation methods²² (difference in 3-year cumulative
189 incidence and lifetime lost over 10 years) was also performed. PFS and OS were analysed
190 using Kaplan Meier survival analysis and Cox regression with times measured from date of

191 diagnosis until the first event, and patients without an event were censored at the date last
192 seen. Treatment delays were analysed using logistic regression (endpoint: any delay ≥ 7
193 days during chemotherapy) and mixed effects logistic regression models (delays after each
194 cycle of i-HD-MTX). Analyses were performed with STATA v16.1 (STATAcorp, Texas).

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

199 **Results**

200 Baseline characteristics for all 1,384 patients (i-HD-MTX n=749, EOT n=635) are summarized
201 in **Table 1**. Median follow-up was 37.9 months. Characteristics of i-HD-MTX and EOT groups
202 were closely matched, with no statistically significant differences in risk factors included in
203 the CNS-IPI except for advanced stage (i-HD-MTX 86.4% vs EOT 80.2%, $p=0.002$). Overall,
204 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
205 risk estimates from the validation cohort in the CNS-IPI publication (0.8%, 3.9% and 12% for
206 CNS-IPI risk groups respectively), the estimated risk in our whole population was 7.0%.

207 There was a trend towards a higher CNS-IPI score for i-HD-MTX patients ($p=0.083$), however
208 there was no significant difference in the numbers with score 4-6 (45.1% vs 43.0%, $p=0.45$).

209 The group with low CNS-IPI ($n=203$) was enriched for patients considered to have a high-risk
210 EN site involvement (181/203 (89.2%)), the most common of which were testicular (37.6%),
211 craniofacial (22.1%) and breast (10.5%). Detailed reasons for CNS prophylaxis in
212 *Supplemental Table 1*.

213 80.5% of patients had baseline PET-CT and 50.8% had baseline CNS evaluation (9.3% CT or
214 MRI and CSF analysis, 8.1% CT or MRI only, 33.4% CSF analysis only).

215 Treatment details, including HD-MTX delivery, are outlined in *Supplemental Table 2*.

216 Frontline immunochemotherapy was R-CHOP-21 (87.4%), R-CHOP-14 (9.4%) or R-CHOP-like
217 therapy (3.2%). 91.8% received ≥ 6 cycles. Overall, 46.1% received IT prophylaxis in addition
218 to HD-MTX, with significantly more in the EOT group compared to i-HD-MTX (55.7% vs
219 38.0%, $p<0.0001$).

220 The median number of HD-MTX cycles delivered was 2 for both groups. Similar numbers
221 received ≥ 2 cycles (87.7% vs 85.6%, $p=0.25$), however, significantly more patients received
222 ≥ 3 in the i-HD-MTX group (36.8% vs 12%, $p<0.0001$) and the patient number receiving a
223 total cumulative dose of $>6 \text{ g/m}^2$ HD-MTX was greater in the i-HD-MTX group (46.4% vs
224 23.2%, $p<0.0001$).

225 There were 78 CNS relapses in the entire population (i-HD-MTX $n=41$, EOT $n=37$). CNS
226 relapse was parenchymal in 41 (53%), parenchymal and leptomeningeal in 16 (21%) and
227 leptomeningeal in 21 (27%) with similar distribution in both groups. The median time to
228 CNS relapse was 8.5 months (interquartile range, IQR:6.1-16.7) for the i-HD-MTX group and
229 10.3 months (IQR 6.4-27.0) for the EOT group.

230 There was no difference in the 3-year CNS relapse rates between i-HD-MTX and EOT groups:
231 5.7% vs 5.8%, hazard ratio (HR) 1.01 (95% confidence interval (CI) 0.65-1.57), $p=0.98$ (*Figure*
232 *1a*). This remained similar when adjusted for baseline prognostic factors: HR 1.06 (0.67-
233 1.66), $p=0.82$, and the 3-year difference (EOT – i-HD-MTX) excluded the non-inferiority limit
234 of +5% when calculated using the unadjusted or adjusted HR, difference: 0.04% (-2.0% to
235 3.1%) or 0.3% (-1.8% to 3.6%) (**Table 2**). On landmark analysis of patients alive and free
236 from progression at 6 months ($n=1253$), conclusions were unchanged: 3-year rates: 4.7% vs
237 4.7%, and 3-year differences of -0.03% (-1.0 to 3.0%) and -0.2% (-2.1 to 3.0%) using the
238 unadjusted and adjusted HRs (*Figure 1b*). Baseline characteristics and details of events in
239 excluded patients are described in *Supplemental Tables 3 and 4*. Analyses performed using
240 pseudo-observation methods also concurred.

241 Sub-analyses of CNS relapse in high-risk patients are summarised in **Table 3**. In patients
242 with CNS-IPI 4-6 ($n=600$) or CNS-IPI 5-6 ($n=210$), the overall 3-year CNS relapse rates were
243 9.1% and 10.5% respectively. Although this study was not powered for non-inferiority
244 comparisons within small high-risk subgroups, with the exception of breast involvement
245 ($n=56$ with only 5 events), all HRs were below or very close to 1, and 3-year differences
246 between i-HD-MTX and EOT were under +0.2%. In a composite high-risk group ($n=885$)
247 including CNS-IPI 4-6 and/or any of the following: ≥ 3 extranodal sites, renal, adrenal,
248 testicular or breast involvement, there was no difference in 3-year CNS relapse rates
249 between groups (i-HD-MTX 7.4% vs EOT 7.7%, HR 1.00 (95% CI 0.61-1.62)) and we could
250 again exclude the +5% non-inferiority margin; 3-year difference: 0.0% (-2.8 to 4.3). Applying

251 the same subgroup analyses to the landmark cohort did not change these conclusions and
252 the 3-year difference within the composite high-risk group just met the non-inferiority
253 margin: 0.6% (-2.1 to 5.0%). (*Supplemental Table 5*).

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

264 CNS relapses were isolated in 57/78 (73.1%) cases with the remainder occurring in
265 combination with systemic progression. Sites of isolated relapse were parenchymal in
266 35/57 (61%), leptomeningeal in 16/57 (28%) and both in 6/57 (11%). Median times to
267 isolated CNS relapse in the i-HD-MTX and EOT groups were 8.3 months (IQR 6.1-18.2) and
268 12.2 (7.4-29.2) months respectively. There was no difference in 3-year cumulative incidence
269 of isolated CNS relapse between groups (**Table 4**).

270 With a median follow-up of 37 months, PFS and OS were significantly inferior in the i-HD-
271 MTX group compared to EOT, with differences persisting in a model adjusted for sex, age,
272 ECOG performance status, presence of ≥ 2 EN sites, renal/adrenal involvement and stratified
273 by stage and LDH (PH violations): adjusted PFS HR 0.79 (95% CI 0.64-0.98), $p=0.024$ and OS
274 HR 0.67 (95% CI 0.52-0.88), $p=0.003$ (*Figure 2A-B*). However, on landmark analysis there
275 was no significant difference in PFS or OS between groups in univariable or adjusted analysis
276 (model including aforementioned baseline characteristics as well as treatment parameters
277 and chemotherapy delays): adjusted PFS HR 1.05 (95% CI 0.81-1.36), $p=0.72$ and OS HR 0.85
278 (95% CI 0.61-1.18), $p=0.32$ (*Figure 2C-D*).

279 Non-relapse mortality (NRM) was reported in 55/1384 (4.0%) patients. Although no NRM
280 events were reported as being directly attributable to HD-MTX, there was a trend towards

281 higher 3-year cumulative incidence of NRM in the i-HD-MTX group compared to EOT (3.9%
282 vs 2.4%, HR 0.60 (95% CI 0.34-1.04), p=0.06) (*Supplemental Figure 1*). This did not seem to
283 be driven by deaths during treatment as the landmark analysis remained similar: HR:0.56
284 (95% CI 0.31-1.02), p=0.055.

285 The median OS of the 78 patients experiencing any CNS relapse was 5.4 months (IQR 2.8-
286 6.9) with no survival difference between i-HD-MTX and EOT groups (*Supplemental Figure*
287 *2a*). When analysed according to presence of isolated CNS or synchronous systemic/CNS
288 relapse, there was a trend towards inferior survival in patients with synchronous relapse (HR
289 1.69 (95% CI 0.96-2.98), p=0.069) (*Supplemental Figure 2b*). There was no difference in
290 survival according to site of CNS relapse (parenchymal vs leptomeningeal vs both,
291 *Supplemental Figure 2c*).

292 Univariable and multivariable analyses of risk factors for any delay of ≥ 7 days during
293 frontline therapy are displayed in **Table 5**. The only significant risk factor for delays was i-
294 HD-MTX delivery (odds ratio, OR, 0.44 (95% CI 0.33-0.59), p<0.0001). Results were
295 unchanged using ordinal regression with number of delays throughout therapy categorized
296 as 0, 1-2 and ≥ 3 .

297 A total of 1573 cycles of HD-MTX were given intercalated between cycles of R-CHOP/R-
298 CHOP-like therapy, with most patients receiving first HD-MTX delivery after cycle 1 or 2
299 (28.5% and 44.4% respectively, see *Supplemental Figure 3a-b*). The median day post-R-
300 CHOP of i-HD-MTX delivery was 10 (IQR 1-14) and median number of intercalated cycles per
301 patient was 2 (IQR 1-2). 308/1573 (19.6%) of intercalated HD-MTX cycles resulted in
302 subsequent R-CHOP delay (median delay 8 days (IQR 6-19)).

303 Survival analyses in the landmark cohort demonstrated a significantly inferior PFS in patients
304 who had a delay of ≥ 7 days vs those who did not (adjusted HR 1.52 (95% CI 1.15-2.03),
305 p=0.004) and a trend towards inferior OS (adjusted HR 1.38 (95% CI 0.96-1.98), p=0.085).

306 Univariable and multivariable analyses of risk factors for delays following i-HD-MTX are
307 displayed in **Table 6**. Increasing age and baseline creatinine clearance were the only
308 significant factors associated with delays on UVA, with increasing age the only variable
309 approaching statistical significance on MVA (p=0.055). Clinicians reported infection (19.5%),
310 renal toxicity (11.7%), cytopenias (11.7%), administrative (8.1%) and mucositis (3.9%) as the

311 most frequent reasons for delays after i-HD-MTX. Mixed effects logistic regression models
312 were used to assess delays at each cycle of i-HD-MTX (*Supplementary* for full details). The
313 only baseline factor significant in this analysis was older age, though there were interactions
314 with dose and timing which suggested that the increase in risk was only present for patients
315 treated with higher doses ($\geq 3\text{g}/\text{m}^2$) and later in the R-CHOP cycle (>10 days). There was no
316 clear evidence that delays were associated with the R-CHOP cycle in which the dose was
317 given, or the i-HD-MTX dose number.

318 The most frequent toxicities observed post HD-MTX administration were febrile
319 neutropenia, renal toxicity and mucositis. No direct comparison between i-HD-MTX and EOT
320 groups are possible, as some events for i-HD-MTX may be related to concurrent systemic
321 chemotherapy. However, we observed numerically greater febrile neutropenia (15.2% vs
322 2.5%), mucositis (15.4% vs 4.6%) and renal toxicity (17.8% vs 13.9%) in patients in i-HD-MTX
323 vs EOT.

324

325 **Discussion**

326 Most DLBCL patients are cured with frontline chemoimmunotherapy, and there have been
327 significant advances in recent years for patients with relapsed/refractory systemic
328 disease.²³⁻²⁶ However, patients with CNS involvement at relapse (occurring in almost 1/3 of
329 relapses in high-risk DLBCL²⁷) are frequently excluded from trials of novel agents and cellular
330 therapies and their prognosis is extremely poor (median OS 5-6 months).⁵

331 There is no broad consensus worldwide regarding how best to reduce the risk of CNS
332 relapse.²⁸ HD-MTX has been widely adopted as CNS prophylaxis in DLBCL, with initial
333 supporting evidence derived from studies demonstrating efficacy in treatment of primary
334 CNS lymphoma.²⁹ Historical, retrospective non-randomised studies also suggested a benefit
335 of HD-MTX in DLBCL patients at high risk of CNS relapse, either intercalated with R-CHOP¹⁴
336 or delivered at EOT.¹³ Recently, large retrospective analyses have demonstrated no
337 apparent benefit of HD-MTX in reduction in CNS relapse risk.^{18,19} Patients at highest risk of
338 CNS relapse are also those at greatest risk of systemic treatment failure, and therefore there
339 has been a lack of agreement about how HD-MTX should be incorporated alongside R-

340 CHOP, with the risk of early CNS progression balanced against the risk of interrupting
341 systemic treatment. Our previous UK study demonstrated increased delays to R-CHOP with
342 i-HD-MTX compared to EOT, but the number of CNS relapse events were too small to
343 conclude that the approaches were equivalent in efficacy.²⁰

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

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

365 Much of the literature addressing CNS relapse in DLBCL does not distinguish between
366 isolated CNS relapse and CNS relapse occurring either with or after systemic progression.
367 Indeed, Schmitz *et al* does not give this detail.⁶ Arguably, any CNS relapse occurring
368 concurrent with or after systemic relapse represents a failure of systemic therapy, with the
369 aim of prophylactic HD-MTX being purely to prevent isolated CNS events. A recent
370 retrospective analysis (n=226) reported a significant reduction in isolated CNS relapses with

371 HD-MTX but no difference in overall survival or concomitant CNS-systemic relapses.³⁰ We
372 excluded any CNS relapse occurring after first systemic DLBCL relapse/progression, and
373 recorded data on whether the CNS relapse was isolated. Considering that isolated CNS
374 relapses are likely to occur because of occult clones taking sanctuary in the CNS either at
375 diagnosis or early in the disease course, there is theoretical rationale that early HD-MTX
376 delivery may be important. However, in the 73.1% of cases where CNS relapse was isolated,
377 we found no benefit for i-HD-MTX.

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

393 Direct comparison of HD-MTX toxicity between i-HD-MTX and EOT approaches is
394 problematic, as some of the toxicities with i-HD-MTX may be influenced by concurrent R-
395 CHOP. We were unable to record toxicities between R-CHOP cycles in the EOT group to
396 serve as the most accurate comparator. However, the observed rates of febrile
397 neutropenia, mucositis and renal toxicity (all 15-17%) associated with i-HD-MTX are of
398 concern, particularly when benefit is questionable.

399 Concurrent IT therapy was used in a significant proportion of patients, particularly in the
400 EOT group, likely due to clinician concern that some form of CNS-directed therapy should be
401 delivered early. However, there is cumulative data to suggest that IT therapy is ineffective

402 in reducing CNS relapses in DLBCL, including a large systematic review of over 7,000 DLBCL
403 patients which demonstrated no benefit of standalone IT therapy in preventing CNS
404 relapse.¹⁰ We demonstrate that use of concurrent IT prophylaxis was not associated with
405 reduction in CNS relapse on multivariable analysis, and there was no evidence of an
406 interaction with HD-MTX timing. However, all patients were given HD-MTX and therefore
407 we were unable to assess whether IT prophylaxis without HD-MTX shows benefit.

408 The overall rate of CNS relapse observed raises concern about any potential efficacy of HD-
409 MTX, irrespective of delivery timing. The observed overall 3-year rate of 5.7% was only
410 marginally less than the predicted risk of 7% when the CNS-IPI risk model was applied to our
411 cohort. Furthermore, our 3-year cumulative incidence of CNS relapse in high CNS-IPI
412 patients was 9.1%, which is almost identical to that observed in the original CNS-IPI study,
413 where no systemic HD-MTX was used in the design cohort and very few in the validation
414 cohort.⁶ Recent retrospective analyses demonstrate no apparent benefit of HD-MTX
415 prophylaxis¹⁵⁻¹⁷, including a multicenter analysis of approximately 2,300 high-risk patients
416 which found no difference in CNS relapse between patients who receiving HD-MTX vs not.¹⁹
417 Furthermore, the overall rate of CNS relapse of 9% in the latter study, which included 1,890
418 patients receiving no HD-MTX, was identical to the rate observed in patients with CNS-IPI 4-
419 6 in our analysis.

420 To answer the question of HD-MTX efficacy definitively, a randomised controlled trial of HD-
421 MTX versus no prophylaxis is required, but sample size would present significant logistical
422 challenges. Our data, in conjunction with other recent literature, suggest a limited benefit
423 for HD-MTX for the majority of DLBCL patients, irrespective of timing of delivery. However,
424 even the large *Lewis et al* analysis is limited in its ability to exclude benefit of HD-MTX in the
425 highest risk subgroups, such as those with CNS-IPI 6 or with high risk EN site involvement
426 (e.g. testicular, breast). There is also prospective data to suggest a benefit of HD-MTX for
427 patients with testicular DLBCL, with recently presented results from the IELSG30 trial
428 demonstrating no CNS relapses following IV and IT CNS prophylaxis.³¹

429 To date, no other agent has been shown to reduce risk of CNS relapse in DLBCL. Novel
430 agents, such as ibrutinib and lenalidomide, have been proposed as potential agents capable
431 of influencing CNS relapse risk due to their ability to cross the blood-brain barrier. Although
432 both agents have shown promising activity in primary and secondary CNS involvement with

433 B-cell malignancies, neither have shown overall benefit for patients with DLBCL when
434 incorporated into R-CHOP in large prospective trials.^{32,33} Whether these drugs could
435 specifically benefit the small subset of patients at most risk of CNS relapse remains an
436 unanswered question. Until a more effective prophylactic strategy is demonstrated, some
437 may still reasonably choose to use HD-MTX for the most high-risk patients, and we provide
438 valuable data to support decision-making around its delivery.

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

455 In conclusion, in an international cohort of 1,384 patients, we demonstrate that delivery of
456 EOT HD-MTX did not increase the risk of CNS relapse compared to early integration during
457 R-CHOP/R-CHOP-like therapy. CNS relapse rate observed in high-risk patients in our study
458 were relatively high despite the use of HD-MTX, raising further concern about the efficacy of
459 HD-MTX as CNS prophylaxis. We cannot conclude from our data that HD-MTX, intercalated
460 or not, does not benefit a small subset of very high-risk patients although we recognise that
461 usage is likely to decrease substantially in light of the recent presented and published data.
462 In the selected patients where HD-MTX may still be considered we provide data to support
463 EOT delivery for most patients. i-HD-MTX should be used with caution in older patients or

464 those at increased risk of toxicity, and if employed the HD-MTX should be delivered earlier
465 in the R-CHOP cycle (prior to day 10) to reduce R-CHOP delays. It may be that investigating
466 the incorporation of novel agents and using more sophisticated techniques (e.g. CSF ctDNA)
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488

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522

523 **References**

- 524 1. Sehn LH, Salles G. Diffuse Large B-Cell Lymphoma. *N Engl J Med*. 2021;384(9):842-858.
525 2. Klanova M, Sehn LH, Bence-Bruckler I, et al. Integration of cell of origin into the clinical CNS
526 International Prognostic Index improves CNS relapse prediction in DLBCL. *Blood*. 2019;133(9):919-
527 926.
528 3. Gleeson M, Counsell N, Cunningham D, et al. Central nervous system relapse of diffuse large
529 B-cell lymphoma in the rituximab era: results of the UK NCRI R-CHOP-14 versus 21 trial. *Annals of*
530 *Oncology*. 2017;28(10):2511-2516.

- 531 4. Villa D, Connors JM, Shenkier TN, Gascoyne RD, Sehn LH, Savage KJ. Incidence and risk
532 factors for central nervous system relapse in patients with diffuse large B-cell lymphoma: the impact
533 of the addition of rituximab to CHOP chemotherapy. *Annals of Oncology*. 2010;21(5):1046-1052.
- 534 5. El-Galaly TC, Cheah CY, Bendtsen MD, et al. Treatment strategies, outcomes and prognostic
535 factors in 291 patients with secondary CNS involvement by diffuse large B-cell lymphoma. *Eur J*
536 *Cancer*. 2018;93:57-68.
- 537 6. Schmitz N, Zeynalova S, Nickelsen M, et al. CNS International Prognostic Index: A Risk Model
538 for CNS Relapse in Patients With Diffuse Large B-Cell Lymphoma Treated With R-CHOP. *Journal of*
539 *Clinical Oncology*. 2016;34(26):3150-3156.
- 540 7. Ollila TA, Kurt H, Waroich J, et al. Genomic subtypes may predict the risk of central nervous
541 system recurrence in diffuse large B-cell lymphoma. *Blood*. Vol. 137; 2021:1120-1124.
- 542 8. Bobillo S, Crespo M, Escudero L, et al. Cell free circulating tumor DNA in cerebrospinal fluid
543 detects and monitors central nervous system involvement of B-cell lymphomas. *Haematologica*.
544 2021;106(2):513-521.
- 545 9. McKay P, Wilson MR, Chaganti S, Smith J, Fox CP, Cwynarski K. The prevention of central
546 nervous system relapse in diffuse large B-cell lymphoma: a British Society for Haematology Good
547 Practice Paper. *Br J Haematol*. 2020.
- 548 10. Eyre TA, Djebbari F, Kirkwood AA, Collins GP. A systematic review of the efficacy of CNS
549 prophylaxis with stand-alone intrathecal chemotherapy in diffuse large B cell lymphoma patients
550 treated with anthracycline-based chemotherapy in the rituximab era. *Haematologica*. 2019;epub
551 ahead of print: DOI 10.3324/haematol.2019.229948.
- 552 11. Eyre TA, Kirkwood AA, Wolf J, et al. Stand-alone intrathecal central nervous system (CNS)
553 prophylaxis provide unclear benefit in reducing CNS relapse risk in elderly DLBCL patients treated
554 with R-CHOP and is associated increased infection-related toxicity. *British Journal of Haematology*.
555 2019.
- 556 12. Cheah CY, Herbert KE, O'Rourke K, et al. A multicentre retrospective comparison of central
557 nervous system prophylaxis strategies among patients with high-risk diffuse large B-cell lymphoma.
558 *British Journal of Cancer*. 2014;111(6):1072-1079.
- 559 13. Ferreri AJ, Bruno-Ventre M, Donadoni G, et al. Risk-tailored CNS prophylaxis in a mono-
560 institutional series of 200 patients with diffuse large B-cell lymphoma treated in the rituximab era.
561 *British Journal of Haematology*. 2015;168(5):654-662.
- 562 14. Abramson JS, Hellmann M, Barnes JA, et al. Intravenous methotrexate as central nervous
563 system (CNS) prophylaxis is associated with a low risk of CNS recurrence in high-risk patients with
564 diffuse large B-cell lymphoma. *Cancer*. 2010;116(18):4283-4290.
- 565 15. Puckrin R, El Darsa H, Ghosh S, Peters A, Owen C, Stewart D. Ineffectiveness of high-dose
566 methotrexate for prevention of CNS relapse in diffuse large B-cell lymphoma. *Am J Hematol*.
567 2021;96(7):764-771.
- 568 16. Bobillo S, Joffe E, Sermer D, et al. Prophylaxis with intrathecal or high-dose methotrexate in
569 diffuse large B-cell lymphoma and high risk of CNS relapse. *Blood cancer journal*. 2021;11(6).
- 570 17. Jeong H, Cho H, Kim H, et al. Efficacy and safety of prophylactic high-dose MTX in high-risk
571 DLBCL: a treatment intent-based analysis. *Blood Adv*. 2021;5(8):2142-2152.
- 572 18. Orellana-Noia VM, Reed D, McCook AA, et al. Single-route CNS prophylaxis for aggressive
573 non-Hodgkin lymphomas: real-world outcomes from 21 US academic institutions. *Blood*. 2021.
- 574 19. Lewis KL, Jakobsen LH, Villa D, et al. High-Dose Methotrexate Is Not Associated with
575 Reduction in CNS Relapse in Patients with Aggressive B-Cell Lymphoma: An International
576 Retrospective Study of 2300 High-Risk Patients [abstract]. *Blood*. 2021;138:181. Abstract 181
- 577 20. Wilson MR, Eyre TA, Martinez-Calle N, et al. Timing of high-dose methotrexate CNS
578 prophylaxis in DLBCL: an analysis of toxicity and impact on R-CHOP delivery. *Blood Adv*.
579 2020;4(15):3586-3593.

- 580 21. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging,
581 and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin*
582 *Oncol.* 2014;32(27):3059-3068.
- 583 22. Overgaard M, Anderson PK, Parner ET. Regression analysis of censored data using pseudo-
584 observations: An update. *The Stata Journal.* 2015;15(3):809-821.
- 585 23. Schuster SJ, Tam CS, Borchmann P, et al. Long-term clinical outcomes of tisagenlecleucel in
586 patients with relapsed or refractory aggressive B-cell lymphomas (JULIET): a multicentre, open-label,
587 single-arm, phase 2 study. *Lancet Oncol.* 2021.
- 588 24. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in
589 Refractory Large B-Cell Lymphoma. *N Engl J Med.* 2017;377(26):2531-2544.
- 590 25. Salles G, Duell J, González Barca E, et al. Tafasitamab plus lenalidomide in relapsed or
591 refractory diffuse large B-cell lymphoma (L-MIND): a multicentre, prospective, single-arm, phase 2
592 study. *Lancet Oncol.* 2020;21(7):978-988.
- 593 26. Sehn LH, Herrera AF, Flowers CR, et al. Polatuzumab Vedotin in Relapsed or Refractory
594 Diffuse Large B-Cell Lymphoma. *J Clin Oncol.* 2020;38(2):155-165.
- 595 27. Frontzek F, Ziepert M, Nickelsen M, et al. Rituximab plus high-dose chemotherapy
596 (MegaCHOEP) or conventional chemotherapy (CHOEP-14) in young, high-risk patients with
597 aggressive B-cell lymphoma: 10-year follow-up of a randomised, open-label, phase 3 trial. *Lancet*
598 *Haematol.* 2021;8(4):e267-e277.
- 599 28. Martinez-Calle N, Wilson MR, Eyre TA, Cwynarski K, McKay P, Fox CP. Interpretation of
600 retrospective data evaluating high-dose methotrexate as central nervous system prophylaxis in
601 diffuse large B-cell lymphoma; caution required. *Am J Hematol.* 2021;96(9):E338-e339.
- 602 29. Ferreri AJ, Guerra E, Regazzi M, et al. Area under the curve of methotrexate and creatinine
603 clearance are outcome-determining factors in primary CNS lymphomas. *Br J Cancer.* 2004;90(2):353-
604 358.
- 605 30. Ong SY, de Mel S, Grigoropoulos NF, et al. High-dose methotrexate is effective for
606 prevention of isolated CNS relapse in diffuse large B cell lymphoma. *Blood Cancer J.* 2021;11(8):143.
- 607 31. Conconi A, Chiappella A, Orsucci L, et al. Intensified (Intravenous And Intrathecal) CNS
608 Prophylaxis In Primary Testicular Diffuse Large B-Cell Lymphoma: 5-Year Results Of The IELSG30 Trial
609 [abstract]. *Hematological Oncology.* 2021;39(S2).
- 610 32. Younes A, Sehn LH, Johnson P, et al. Randomized Phase III Trial of Ibrutinib and Rituximab
611 Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone in Non-Germinal Center B-Cell
612 Diffuse Large B-Cell Lymphoma. *J Clin Oncol.* 2019;37(15):1285-1295.
- 613 33. Nowakowski GS, Chiappella A, Gascoyne RD, et al. ROBUST: A Phase III Study of
614 Lenalidomide Plus R-CHOP Versus Placebo Plus R-CHOP in Previously Untreated Patients With ABC-
615 Type Diffuse Large B-Cell Lymphoma. *J Clin Oncol.* 2021;39(12):1317-1328.

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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 discrete 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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633 **Table 2 – Univariable and multivariable models for difference in 3-year CNS relapse rates**
 634 **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) |

635 ¹HR for EOT vs i-HD-MTX

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

638 ³Difference in cumulative incidence rates allowing for competing risks at 3 years using pseudo
 639 observations.

640 ⁴Full model adjusted for sex, age, advanced stage, extra nodal disease (≥2 sites), ECOG (≥2),
 641 renal/adrenal involvement, raised LDH (plus ITs, HDMTX≥2 doses, and cumulative dose >6g/m² for
 642 landmark cohort).

643 ⁵Adjusted for only variables significant with backwards selection (based on survival time lost): age
 644 and renal/adrenal involvement for CNS relapse and age alone for isolated CNS relapse.
 645 The 10-year cut off for lifetime lost was chosen as close to the end of follow-up (131 months, and
 646 after the last event).

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

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658 **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 | |
| End of treatment | 8.6% (5.6 – 13.1) | 21/263 | 0.92 (0.52 – 1.62) | -0.7% (-4.4 to 5.4) |
| CNS IPI 5-6 | 10.5% (5.9 – 16.0) | 21/210 | | |
| Intercalated | 11.8% (6.7 – 20.1) | 12/118 | 1.00 | |
| End of treatment | 9.1% (4.6 – 17.4) | 9/92 | 0.96 (0.41 – 2.29) | -0.4% (-6.8 to 13.1) |
| Testicular involvement | 7.5% (4.2 – 13.2) | 14/175 | | |
| Intercalated | 6.0% (2.3 – 15.3) | 8/80 | 1.00 | |
| End of treatment | 8.5% (4.1 – 17.2) | 6/95 | 0.92 (0.32 – 2.68) | -0.4% (-4.0 to 9.3) |
| Renal/adrenal involvement | 11.3% (7.6 – 16.7) | 25/240 | | |
| Intercalated | 14.4% (8.9 – 23.0) | 16/138 | 1.00 | |
| End of treatment | 7.6% (3.7 – 15.5) | 9/102 | 0.67 (0.30 – 1.52) | -4.5% (-9.9 to 6.6) |
| Breast involvement | 9.7% (3.6 – 24.6) | 5/56 | | |
| Intercalated | 5.3% (1.3 – 19.5) | 3/38 | 1.00 | |
| End of treatment | 20.5% (5.6 – 60.3) | 2/18 | 1.56 (0.26 – 9.39) | 2.8% (-3.9 to 34.5) |
| 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 | |
| End of treatment | 7.1% (4.0 – 12.3) | 13/162 | 1.01 (0.48 – 2.10) | 0.0% (-4.1 to 8.1) |
| Any high-risk factor above | 7.6% (5.9 – 9.7) | 65/885 | | |
| Intercalated | 7.4% (5.2 – 10.4) | 34/482 | 1.00 | |
| End of treatment | 7.7% (5.3 – 11.1) | 31/403 | 1.00 (0.61 – 1.62) | 0.0% (-2.8 to 4.3) |

659 *EOT vs intercalated. Events post 3 years: 8 events (5 EOT and 3 intercalated). Five-year rates: EOT:
 660 7.3% (5.2 – 10.1) and 6.5 (4.7 – 9.1) intercalated.

661 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%
 662 (6.6 – 13.5) EOT and 8.6% (5.9 – 12.4) intercalated

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

678 *Survival time is measured up to 10 years, for example, in univariable analysis, a patient given EOT*
679 *HD-MTX has a CNS-relapse free life expectancy over 10 years that is 0.43 months shorter than for a*
680 *patient given i-HD-MTX. The MVA shows variables remaining significant with backwards selection (p-*
681 *value for rejection 0.05). With a rare event, lifetime lost is not easily clinically interpretable, but at 3*
682 *years, this translates to a difference in cumulative incidence of 6.58% for patients with renal and*
683 *adrenal involvement when compared to those without, and an increase in incidence of 1.12% for*
684 *each decade of age.*

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

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689 **Table 5 – Univariable and multivariable analyses of risk factors for any delay of ≥ 7 days**
 690 **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 |

691 *A more conservative analysis which excluded any patient in the iHDMTX group given <6 cycles of*
 692 *treatment (i.e. a patient group who may not have been given EOT MTX even if it was the intention)*
 693 *found very similar results for treatment approach: HR: 0.44 (0.33 – 0.59), p <0.001 (UVA) and HR*
 694 *0.47 (0.35 – 0.64), p <0.001 (MVA).*

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

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709 **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 |

710 *MVA, with backwards selection (p=0.05 for inclusion), age is the only factor that remains: OR: 1.19 (1.05 –*
711 *1.35), p = 0.008 (N=735) [Note this is slightly different from the UVA quoted (despite being the only variable*
712 *left) as it included complete cases only]*

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

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725 **Figure Legends**

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

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

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Figure 1A

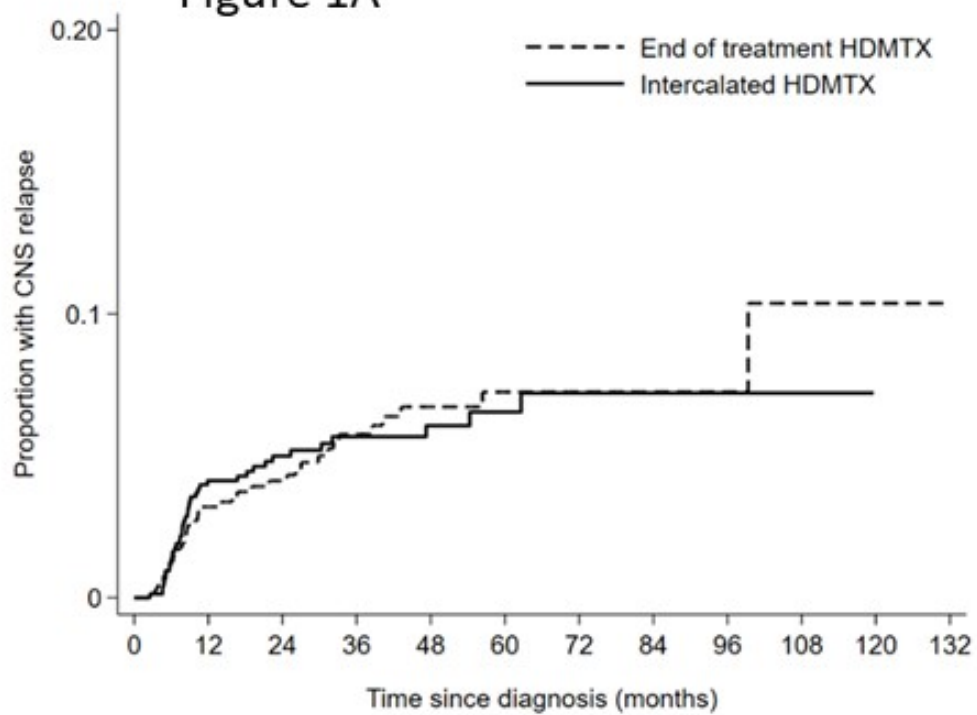


Figure 1B

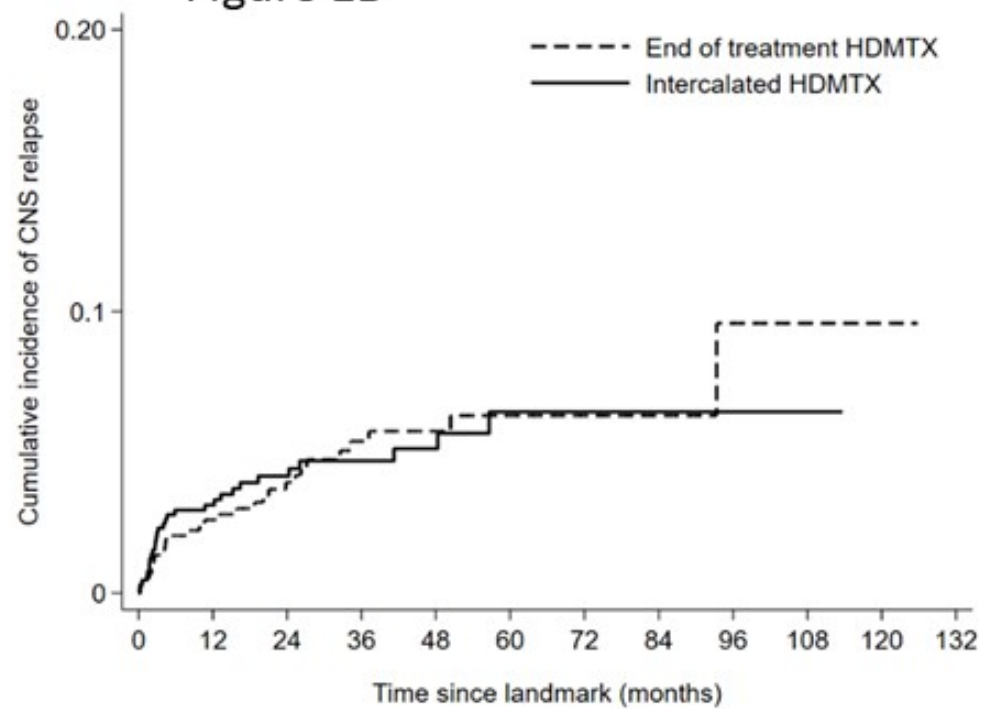
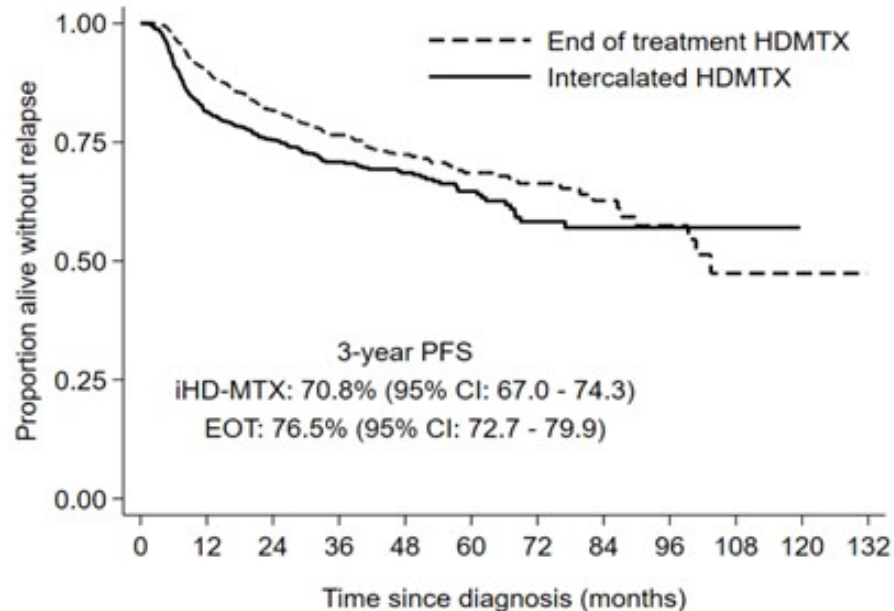
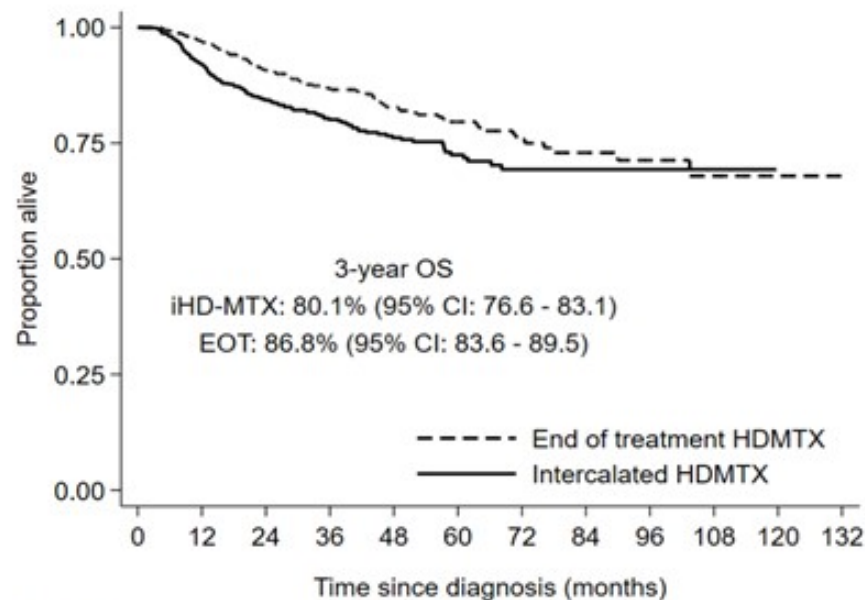


Figure 2A



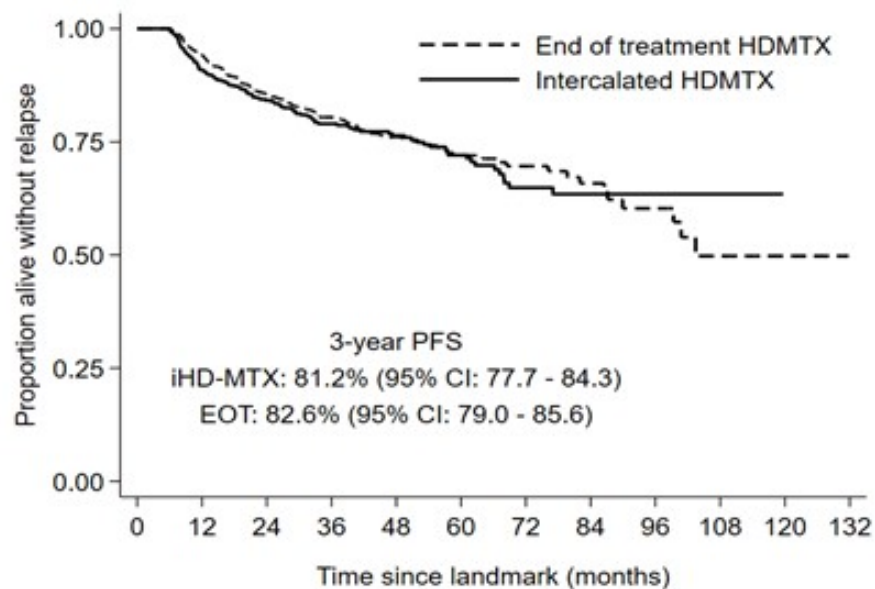
| Number at risk | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | 108 | 120 | 132 |
|------------------|-----|-----|-----|-----|-----|-----|----|----|----|-----|-----|-----|
| End of treatment | 635 | 535 | 398 | 270 | 185 | 118 | 72 | 40 | 24 | 10 | 3 | 0 |
| Intercalated | 749 | 562 | 374 | 257 | 175 | 106 | 57 | 33 | 17 | 7 | 0 | 0 |

Figure 2B



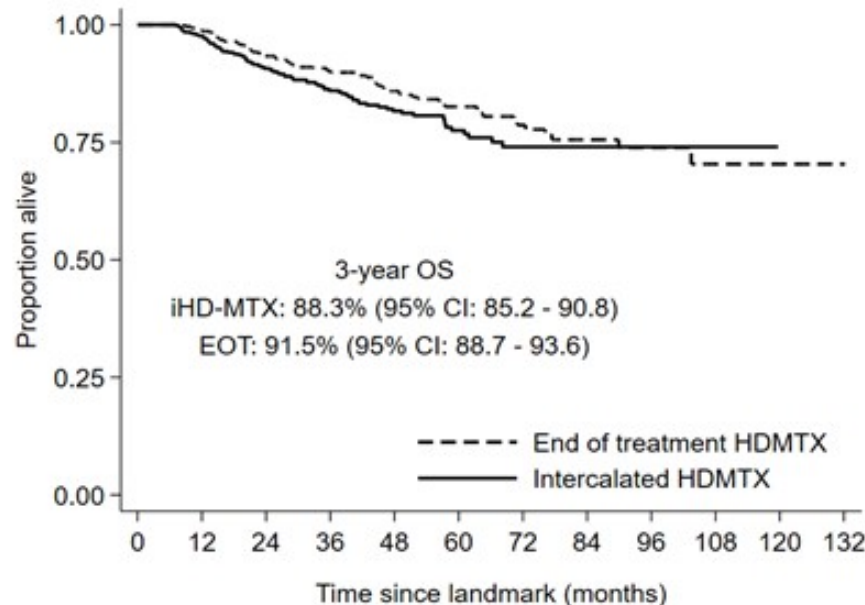
| Number at risk | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | 108 | 120 | 132 |
|------------------|-----|-----|-----|-----|-----|-----|----|----|----|-----|-----|-----|
| End of treatment | 635 | 574 | 440 | 310 | 215 | 144 | 85 | 52 | 33 | 17 | 6 | 1 |
| Intercalated | 749 | 631 | 417 | 293 | 193 | 114 | 67 | 39 | 21 | 8 | 0 | 0 |

Figure 2C



| Number at risk | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | 108 | 120 | 132 |
|------------------|-----|-----|-----|-----|-----|-----|----|----|----|-----|-----|-----|
| 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 | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | 108 | 120 | 132 |
|------------------|-----|-----|-----|-----|-----|-----|----|----|----|-----|-----|-----|
| 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 |