Synopsis

SPONSOR	International Extranodal Lymphoma Study Group (IELSG)	
NAME PRODUCT / INTERVENTION	Methotrexate, cytarabine, rituximab, thiotepa, carmustine	
NAME OF ACTIVE PRINCIPLE *(if the product is a medicinal product)*	Methotrexate, cytarabine, rituximab, thiotepa, carmustine	
PROTOCOL CODE	IELSG32	
PROTOCOL TITLE	Randomized phase ii trial on primary chemotherapy with high-dose methotrexate and high-dose cytarabine with or without thiotepa, and with or without rituximab, followed by brain irradiation vs. high-dose chemotherapy supported by autologous stem cells transplantation for immunocompetent patients with newly diagnosed primary CNS lymphoma	
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STUDY SITES	Five countries were involved (Switzerland, Italy, Germany, Denmark, and the United Kingdom) and a total of 53 enrolling sites.	
STUDY PERIOD	First Patient Enrolled - 19.02.2010 Last Patient Enrolled - 07.10.2014	
DEVELOPMENT PHASE	II	
OBJECTIVES	<u>Primary Objective</u> The primary objective of the study at first randomization is to establish in a prospective, randomized trial the activity of three different chemotherapy combinations with high-dose methotrexate (HD-MTX) + high-dose cytarabine (HD-araC), HD-MTX + HD-araC + rituximab, and HD-MTX + HD-araC + rituximab + thiotepa in patients with newly diagnosed PCNSL. The primary objective of the study at the second randomization is to establish the efficacy of two consolidation strategies: conventional whole-brain radiotherapy (WBRT) versus high-dose chemotherapy supported by autologous stem cell transplantation (HDC + ASCT) in patients with newly diagnosed PCNSL.	
STUDY DESIGN AND METHODOLOGY	Enrolled PCNSL patients were stratified according to the IELSG score and randomized to receive MTX + Ara-C (Arm A), MTX + Ara-C + rituximab (Arm B), or MTX + Ara-C + rituximab + thiotepa (Arm C) as primary chemotherapy. Chemotherapy was administered every three weeks, with a maximum of four induction cycles. Patients achieving stable disease (SD) or better response after two cycles received two additional cycles of the same primary chemotherapy regimen. Stem cell harvest was performed in all three arms after the second cycle. After four cycles of chemotherapy, a response assessment was conducted. Patients who did not achieve SD or better response after the fourth cycle, those who experienced progressive disease (PD) at any time, or those with insufficient stem cell harvest received whole-brain radiotherapy (WBRT) at 36–40 Gy, with an optional tumor bed boost of 9 Gy. Patients achieving SD or better response after the fourth cycle were further stratified based on their objective response to primary chemotherapy (complete response [CR] vs. partial response [PR] + SD) and the chemotherapy regimen received (A vs. B vs. C). They were then randomly assigned to receive either WBRT at 36 Gy with or without a 9 Gy boost (Arm D) or BCNU + thiotepa followed by	

	autologous peripheral blood stem cell transplantation (APBSCT) as consolidation therapy (Arm E). Patients in CR after WBRT or APBSCT remained in follow-up. Those who did not achieve CR after WBRT were managed at the physician's discretion, while those who did not achieve CR after APBSCT were referred for WBRT.
SUBJECT POPULATION	Number of Subjects Planned: 227 Number of Subjects Enrolled: 227 Number of Subjects Randomized: 227 first randomization; 118 second randomization Number of Subjects for Each Analysis Population: Intention To Treat (ITT) n=219; For Protocol n=219 (first random); n=213 (second random); For Efficay n=219; For Safety n=219 <u>Brief description of demographic and baseline characteristics</u> *First randomization* Key features, such as age, sex distribution, ECOG performance status, LDH levels, presence of deep lesions, IELSG risk score, and disease involvement (e.g., meningeal, intraocular), were well-balanced across arms. All patients had diffuse large B-cell lymphoma. Notably, the median age was similar (around 57–58 years), the proportion of patients with poor performance status (ECOG >1) ranged from 32% to 36%, the deep brain lesions were common in all arms, especially in Arm C (85%) and around 60% of patients in each group had multiple lesions. Overall, baseline characteristics were comparable, indicating good randomization and allowing fair comparisons of treatment outcomes across the three groups. *Second randomization* The characteristics were generally balanced across the two arms, and no statistically significant differences were observed, as reflected by the p values, all above 0.05. The median age was identical in both groups (58 years), with similar interquartile ranges. The deep brain lesions, increased CSF protein, and multiple lesions were similarly prevalent in both groups. IELSG risk scores were comparable across arms, with most patients falling into the intermediate risk category. The two consolidation arms (WBRT and ASCT) had similar baseline characteristics, supporting the validity of outcome comparisons between these treatment strategies. <u>Brief description of subjects excluded from the primary analysis population</u> From the total of 227 patients initially randomized in the IELSG32 trial. 75 to Arm A (methotrexate–cytarabine), 74 to Arm B (methotrexate–cytarabine plus rituximab), and 78 to Arm C (methotrexate–cytarabine plus rituximab and thiotepa), eight patients were excluded from the primary analysis population. These exclusions occurred in Arm B (5 patients) and Arm C (3 patients), while no exclusions were reported in Arm A.

	The reasons for exclusion reflected significant deviations from the eligibility criteria: • Two patients started a valid treatment a few days before trial registration due to a miscommunication between physicians. (n=1 ARM B; n=1 ARM C) • Misdiagnosis: Some patients were found, upon further review, not to have primary CNS lymphoma but another condition • Systemic lymphoma: These cases involved patients whose disease was not confined to the central nervous system, violating the definition of primary CNS lymphoma and thus falling outside the trial's intended population. • Concomitant cancer: The presence of an additional malignancy had introduced confounding factors in treatment response and survival outcomes, warranting exclusion from the efficacy analysis. These exclusions underscored the importance of strict diagnostic criteria and centralized pathology review in multicenter trials, particularly for rare and diagnostically complex diseases such as primary CNS lymphoma. By removing these patients, the study ensured a more homogeneous and clinically appropriate population for evaluating treatment efficacy and safety, thus preserving the internal validity of the primary analysis.
ELIGIBILITY CRITERIA	<u>INCLUSION CRITERIA</u> To be eligible for inclusion in this trial, patients had to fulfill all the following criteria: • Histological or cytological assessed diagnosis of non-Hodgkin's lymphoma. • Diagnostic sample obtained by stereotactic or surgical biopsy, CSF cytology examination, or vitrectomy. • Disease exclusively localized in the central nervous system, CSF, cranial nerves, or eyes. • At least one measurable lesion. • Previously untreated patients (previous or ongoing steroid therapy admitted). • Age 18-65 years (with ECOG Performance Status 0-3) or 66-70 (with ECOG Performance Status 0-2). • Adequate bone marrow (PLT ≥ 100000 mm3, Hb ≥ 9 g/dl, ANC ≥ 2.000 mm3), renal (creatinine clearance ≥ 60 ml/min), cardiac (VEF ≥ 50%), and hepatic function (total serum bilirubin ≤ 3 mg/dL, AST/ALT or γGT ≤ 2 per upper normal limit value). • Sexually active patients of childbearing potential agree in implementing adequate contraceptive measures during study participation. • Absence of any familial, sociological, or geographical condition potentially hampering compliance with the study protocol and follow-up schedule. • Patient-signed informed consent obtained before registration.

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EXCLUSION CRITERIA

Patients with lymphomatous lesions outside the CNS.
Patients with a previous non-Hodgkin lymphoma at any time.
Previous or concurrent malignancies except surgically cured carcinoma in situ of the cervix, carcinoma of the skin, or other cancers without evidence of disease at least 5 years.
HBsAg and HCV positivity.
HIV infection, previous organ transplantation, or other clinically evident form of immunodeficiency.
Concurrent treatment with other experimental drugs.
Concurrent Pregnancy or lactation.
Patients not agreeing to take adequate contraceptive measures during the study.

Symptomatic coronary artery disease, cardiac arrhythmias uncontrolled with medication, or myocardial infarction within the last 6 months (New York Heart Association Class III or IV heart disease).
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<tr><td>STUDY PRODUCTS / DOSE AND MODE OF ADMINISTRATION/ INTERVENTIONS</td><td>
CHEMOTHERAPY REGIMENS (First randomization)
Patients registered according to the inclusion criteria were randomized for primary chemotherapy between:
<table>
<tr><td>Arm A</td><td></td><td></td><td></td></tr>
<tr><td>Methotrexate</td><td>3.5 g/m2</td><td>0.5 g/m2 in 15 min. +
3 g/m2 in 3-hr infusion</td><td>d 1</td></tr>
<tr><td>Cytarabine</td><td>2 g/m2</td><td>1 hr infusion, twice a day (every 12 h.)</td><td>d 2-3</td></tr>
<tr><td>Arm B</td><td></td><td></td><td></td></tr>
<tr><td>Rituximab</td><td>375 mg/m2</td><td>conventional infusion</td><td>d -5 & 0</td></tr>
<tr><td>Methotrexate</td><td>3.5 g/m2</td><td>0.5 g/m2 in 15 min. +
3 g/m2 in 3-hr infusion</td><td>d 1</td></tr>
<tr><td>Cytarabine</td><td>2 g/m2</td><td>1 hr infusion, twice a day (every 12 h.)</td><td>d 2-3</td></tr>
<tr><td>Arm C</td><td></td><td></td><td></td></tr>
<tr><td>Rituximab</td><td>375 mg/m2</td><td>conventional infusion</td><td>d -5 & 0</td></tr>
<tr><td>Methotrexate</td><td>3.5 g/m2</td><td>0.5 g/m2 in 15 min. +
3 g/m2 in 3-hr infusion</td><td>d 1</td></tr>
<tr><td>Cytarabine</td><td>2 g/m2</td><td>1 hr infusion, twice a day (every 12 h.)</td><td>d 2 - 3</td></tr>
<tr><td>Thiotepa</td><td>30 mg/m2</td><td>30 min. Infusion</td><td>d 4</td></tr>
</table>
Corticosteroids during treatment and their definitive interruption were dependent on clinical requirements. Routine use of G-CSF was recommended.
LEUKAPHERESIS AND CRYOPRESERVATION
From day 10 of the second chemotherapy cycle in all three arms, absolute CD34+ cell count per µL of blood was determined every day. The objective was to harvest a minimum of 5 x 106 CD34+ cells/kg of body weight with as few leukapheresis sessions as possible during consecutive days. CD34+ cells were collected, processed, and stored according to conventional guidelines.
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	Patients with insufficient stem cell harvest Patients with an insufficient stem cell harvest were excluded from the second randomization. They were treated with RT and considered evaluable for the first randomization endpoints.
	CONSOLIDATION THERAPY (Second randomization) Patients responsive (CR or PR) or with SD after primary chemotherapy (patient treated either with arm A, B, or C according to the protocol) were randomly assigned to receive: Arm D WBRT with 36 Gy in the case of CR to primary chemotherapy or the same WBRT dose followed by a tumor-bed boost of 9 Gy with 1-2 cm of margin surrounding enhanced residual lesion (total tumor-bed dose 45 Gy) in patients who achieved a PR or SD after primary chemotherapy. Photons of 4-10 MeV, 180 cGy per day, 5 weekly fractions. Whole-brain was irradiated by two opposite lateral fields, including the first two cervical vertebras and the posterior two-thirds of the orbits, which had to be shielded after 30 Gy (after 36 Gy in the case of evident intraocular disease at diagnosis). The tumor bed (boost or partial-brain RT) was irradiated by 2 to 4 isocentric treatment fields based on tumor location, with all portals treated per RT session. Arm E BCNU 400 mg/m2 in 500 ml saline sol, 1-hr inf day -6 Thiotepa 5 mg/kg in 250 ml saline sol, 2-hr inf., every 12 hrs days -5 -4 Reinfusion of PBSC ≥5 x 106 CD34+ cells/kg day 0
DURATION OF TREATMENT	CHEMOTHERAPY REGIMENS (First randomization) 4 cycles q21 for Arm A – Arm B – Arm C CONSOLIDATION THERAPY (Second randomization) Arm D (after 30 days from second randomization): 5 weekly fractions Arm E (after 6 days of high-dose chemiotherapy): conditioning regimen 3 days (from day -6 to day -4) and PBSC reinfusion 1 day (day 0).
STUDY ENDPOINTS	Primary endpoint at first randomization • Complete remission (CR) rate after primary chemotherapy. Primary endpoint at second randomization • 2-year failure-free survival (2-yr FFS). Secondary endpoints • Toxicity • Overall survival • Relapse rates and patterns • Early and late neurotoxicity • Quality of life • Neuropsychological evaluation with Mini-Mental Status Examination and IPCG neuropsychological tests battery, with the addition of two tests assessing the language function and pre-morbid intelligence.
STATISTICAL METHODS	The A'Hern-Fleming Single Stage Phase II design was used. For the first part of the trial (first randomization), the maximum CR rate considered of low interest was 45% (P0), and the minimum CR rate

	considered of interest was 65% (P1). To detect such a difference, a total number of 42 patients per arm was required (one-sided test, type I error 5% and power 80%). If at least 25 CR were observed among the 42 patients, the combination was considered active. For the second part of the trial (second randomization), the maximum 2-yr FFS rate considered of low interest was 65% (P0), and the minimum 2-yr FFS rate considered of interest was 85% (P1). In order to detect such a difference, a total number of 52 patients per arm was required (one-sided test, type I error 5% and power 80%). If at least 40 out of 52 patients were progression-free at 2 years, the combination was considered active. All primary analyses were based on intention-to-treat (IIT), where all randomized patients were included with the exception of patients who post-hoc objectively did not meet the eligibility criteria at the time of the randomization.
SUMMARY OF RESULTS	<u>Efficacy Results</u> Modern treatment for primary CNS lymphoma (PCNSL) involves a two-phase approach: induction chemotherapy followed by consolidation therapy. Historically, methotrexate-based chemotherapy, especially in combination with cytarabine, followed by whole-brain radiotherapy (WBRT), has been the standard regimen. However, despite initial effectiveness, this strategy has been associated with high relapse rates and significant long-term neurotoxicity, particularly in older patients. This has driven efforts to refine both the induction and consolidation phases. The IELSG32 trial is the first international randomized study designed to assess both optimal induction and consolidation strategies in newly diagnosed PCNSL patients. The MATRix regimen (Arm C) resulted in significantly higher complete remission rates (49%) than arms A (23%) and B (31%). It also led to superior 2-year progression-free survival (PFS) and overall survival (OS). The 7-year PFS and OS for MATRix were 52% and 56%, respectively. The MATRix arm showed a 34% gain in overall response rate and a 25% increase in PFS and OS compared to other regimens. These results establish MATRix as the new standard for induction therapy in PCNSL. In the second phase, patients who responded to induction therapy were randomized to receive either WBRT (36 Gy or 23.4 Gy in complete responders) and high-dose chemotherapy followed by autologous stem-cell transplantation (ASCT) with a thiotepa-based regimen. Both WBRT and ASCT were feasible and effective. No significant difference in long-term PFS or OS was observed between the two consolidation strategies.

	Safety Results The IELSG32 trial, a pivotal international randomized study, not only evaluated the efficacy of induction and consolidation therapies for primary CNS lymphoma (PCNSL) but also closely examined their toxicity profiles, tolerability, and cognitive outcomes, key considerations in the treatment of this aggressive but curable malignancy. *Induction Phase (MATRix vs. Methotrexate–Cytarabine Regimens)* The MATRix regimen (Arm C) resulted in higher rates of grade 4 neutropenia and thrombocytopenia, reflecting its increased hematologic intensity. Despite this, severe complications were comparable across all three groups, with only 16% of delivered cycles complicated by grade 3–4 febrile neutropenia or infections. Grade 4 neurotoxicity was extremely rare (<1%) in the three arms, and only two deaths (<1%) in arms A and B were attributed to late neurotoxicity. Treatment interruptions due to toxicity were uncommon: • Only 13 patients (6%) died from treatment-related toxicity. • 8 patients (4%) discontinued chemotherapy due to non-lethal toxicities. • In total, 28 patients (13%) discontinued therapy for non-disease-related reasons. *Consolidation Phase (WBRT vs. ASCT)* Toxicity was acceptable in both arms; Grade 4 non-hematologic toxicity was rare. ASCT-related mortality was 3%, lower than prior reports (9–14%) using different conditioning regimens. Late non-relapse mortality after ASCT was 5%, aligning with other modern studies *Cognitive Outcomes and Quality of Life* ASCT was associated with better cognitive outcomes and improved quality of life: patients receiving ASCT showed improvement in most cognitive domains, particularly executive function, attention, and visuospatial ability. In contrast, WBRT was linked to a mild but progressive cognitive decline, especially in attention and executive function, though the severity was less than previously reported, likely due to reduced radiation doses. The difference between WBRT and ASCT outcomes supports the hypothesis that radiation exposure, even at lower doses, can lead to lasting cognitive deficits, while ASCT may allow for better neurocognitive recovery.
CONCLUSIONS	The IELSG32 trial is the first international, randomized study evaluating two consolidation strategies, WBRT and ASCT, in patients with newly diagnosed PCNSL following high-dose methotrexate-based chemoimmunotherapy, particularly the MATRix regimen (methotrexate, cytarabine, thiotepa, and rituximab). Conducted across 53 centers in five European countries, the study offers robust data with broad geographic representation. The trial design reflects existing debates on the best consolidation strategy in PCNSL. Although WBRT has historically been the standard, its neurotoxicity has prompted growing interest in ASCT. Notably, the thiotepa-based ASCT regimen used in IELSG32 has demonstrated better CNS penetration and tolerability than older regimens like BEAM. The encouraging cognitive outcomes with ASCT

	reinforce findings from earlier single-arm studies, suggesting its role as a cognitively safer alternative to WBRT. The trial design did not aim to prove the superiority of one consolidation approach over the other, but to validate their feasibility and efficacy. Multivariate analysis confirmed that factors such as number of lesions, IELSG risk score, and induction regimen (MATRix vs. others) were independent predictors of survival, while the type of consolidation was not. Cognitive testing was limited to relapse-free survivors, potentially introducing selection bias, but similar proportions of patients were tested in both groups, minimizing systematic bias. Neurocognitive tests revealed that radiation dose is strongly associated with neurotoxicity, supporting the idea of dose-reduced WBRT when radiotherapy is used. The IELSG32 trial establishes MATRix as a highly effective induction regimen and confirms both WBRT and ASCT as viable consolidation strategies in PCNSL. Given the cognitive and quality-of-life advantages, ASCT may be preferred when feasible. These findings underscore the importance of individualized treatment decisions that consider patient age, performance status, treatment tolerance, and cognitive impact. This study sets a new benchmark for future randomized trials in PCNSL and supports wider adoption of MATRix and ASCT in clinical practice.
VERSION AND DATE OF THE REPORT	Version 1.0 dated 29.08.2025