Synopsis

SPONSOR	International Extranodal Lymphoma Study Group (IELSG)	
NAME PRODUCTS / INTERVENTION	Methotrexate, cytarabine, rituximab, thiotepa, etoposide, ifosfamide, carboplatin, carmustine, liposomial cytarabine	
NAME OF ACTIVE PRINCIPLE	Methotrexate, cytarabine, rituximab, thiotepa, etoposide, ifosfamide, carboplatin, carmustine, liposomial cytarabine	
PROTOCOL CODE	IELSG42	
PROTOCOL TITLE	An international phase II trial assessing tolerability and efficacy of sequential Methotrexate-Aracytin-based combination and R-ICE combination, followed by high-dose chemotherapy supported by autologous stem cell transplant, in patients with systemic B-cell lymphoma with central nervous system involvement at diagnosis or relapse (MARIETTA regimen)	
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STUDY SITES	Five countries were involved (Italy, Switzerland, United Kingdom, Czech Republic, The Netherlands), and a total of 24 enrolling sites.	
STUDY PERIOD	First Patient Enrolled – 30 March 2015 Last Patient Enrolled – 03 August 2018	
DEVELOPMENT PHASE	II	
OBJECTIVE	To evaluate the efficacy and feasibility of a new sequential combination of HD-MTX-AraC-based chemoimmunotherapy, followed by R-ICE regimen, and by high-dose chemotherapy supported by Autologous Stem Cell Transplant (ASCT).	
STUDY DESIGN AND METHODOLOGY	Treatment consisted of a sequential combination of two standardized chemotherapy regimens, MATRIX (methotrexate, cytarabine, thiotepa, and rituximab) for the first 3 cycles, followed by 3 cycles of R-ICE (rituximab, etoposide, ifosfamide, and carboplatin), followed by carmustine–thiotepa and autologous hematopoietic stem-cell transplantation (HSCT) as consolidation therapy. Treatment also included intrathecal chemotherapy, and whole-brain radiotherapy (WBRT) was administered to a subset of patients with refractory CNS disease before or after autologous HSCT. Patients with concurrent systemic and central nervous system (CNS) disease at the time of initial diagnosis received one to two cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) before MATRIX, and most patients enrolled at the time of CNS relapse had received R-CHOP previously.	
SUBJECT POPULATION	Number of Subjects Planned	76
	Number of Subjects Enrolled	79
	Number of Subjects Randomized	NA
	Number of Subjects for Each Analysis Population	Overall, 75 subjects
	<u>Brief description of demographic and baseline characteristics</u> Seventy-nine patients were enrolled in the study. Seventy-five patients with a median age of 58 years were assessable and received the first MATRIX cycle, 38 (51%) of whom were male and 37 (49%) were female. The disease at enrolment involved CNS (32 patients), isolated CNS relapse (15 patients) and concomitant CNS-systemic localization (28 patients). A total of 43 (57%) patients had a previous treatment. R-CHOP was the first-line treatment in most of the 43 patients registered at relapse, with a median time to CNS involvement of 5 months.	

	Brief description of subjects excluded from primary analysis population Four patients were excluded after enrolment before the start of the study treatment because of unrelated laboratory abnormalities (2 patients), disease only at flow cytometry examination of the cerebrospinal fluid (1 patient), and death at the same time as registration (1 patient).
ELIGIBILITY CRITERIA	**Inclusion Criteria** 1. Histologically confirmed diagnosis of diffuse large B-cell lymphoma. 2. CNS involvement (brain, meninges, cranial nerves, eyes and/or spinal cord) at diagnosis (concomitant to extra-CNS disease) or relapse after conventional chemo(-immune) therapy. 3. Diagnosis of CNS involvement either by brain biopsy or CSF cytology examination. Neuroimaging alone is acceptable when stereotactic biopsy is formally contraindicated or when the disease has been previously histologically documented in other areas and the CNS localization is concomitant with a diffuse progression of systemic disease. 4. No previous treatment with high-dose methotrexate-based chemotherapy and/or brain irradiation. 5. Age 18-70 years. 6. ECOG performance status 0-3. 7. Adequate bone marrow (platelets count ≥100x10^3/mm^3, haemoglobin ≥9 g/dL, neutrophils count ≥1.5x10^3/mm^3), renal (creatinine clearance ≥60 mL/min), cardiac (LVEF ≥50%), and hepatic function (total serum bilirubin ≤3 mg/dL, AST, and ALT and GGT ≤2.5 per upper normal limit value), unless the abnormality is due to lymphoma infiltration. 8. Absence of HIV infection and of detectable HCV-RNA and/or HBsAg and/or HBV-DNA. 9. No concurrent malignancies. Previous malignancies are accepted if surgically cured or if there was no evidence of disease in the last 3 years at a regular follow-up. 10. Absence of any familial, sociological, or geographical condition potentially hampering compliance with the study protocol and follow-up schedule. 11. Female patients must be non-pregnant and non-lactating. Sexually active patients of childbearing potential must implement adequate contraceptive measures during study participation. Highly effective contraceptive precautions are required until 12 months after the completion of trial treatment. Pregnancy test must be performed within 7 days of commencing study treatment. 12. No treatment with other experimental drugs within the 6 weeks before enrolment. 13. Given written informed consent prior to any study specific procedures, with the understanding that the patient has the right to withdraw from the study at any time, without any prejudice. Informed consent signed by a patient's guardian is acceptable if the patient is not able to decide inclusion in the study due to cognitive impairment. **Exclusion Criteria** 1. Other lymphoma categories other than diffuse large B-cell lymphoma. Patients with primary mediastinal lymphoma,

	intravascular large B-cell lymphoma or leg-type large B-cell lymphoma are excluded. 2. Patients with positive flow cytometry examination of the CSF, but negative results in CSF conventional cytology, and without any other evidence of CNS disease. 3. Patients with exclusive CNS disease at presentation (primary CNS lymphoma) are excluded. 4. Previous treatment with support of autologous or allogeneic stem cells/bone marrow transplantation. 5. Symptomatic coronary artery disease, cardiac arrhythmias not well controlled with medication or myocardial infarction within the last 6 months (New York Heart Association Class III or IV heart disease) 6. Any other serious medical condition which could impair the ability of the patient to participate in the trial. 7. Any contraindications as identified in relation to the relevant SmPCs.
STUDY PRODUCTS / DOSE AND MODE OF ADMINISTRATION/ INTERVENTIONS	Experimental treatment commenced within three weeks of the baseline assessment of target lesions. The treatment protocol included six cycles of chemoimmunotherapy: the first three cycles consisted of a high-dose methotrexate (HDMTX)-based combination, followed by three cycles of the R-ICE regimen. This was followed by a BCNU-thiotepa-containing conditioning regimen and subsequent ASCT. The interval between the first day of two consecutive cycles was three weeks, while the interval between the final chemoimmunotherapy cycle and conditioning ranged from 4 to 6 weeks. Intrathecal chemotherapy was administered to all enrolled patients, regardless of cerebrospinal fluid (CSF) cytology status, due to the high frequency of false-negative results with conventional cytology, particularly when flow cytometry was not routinely employed. Accordingly, a dose of intrathecal liposomal cytarabine or conventional triple-drug chemotherapy was given on day 5 of each MATRIX cycle and on day 4 of each R-ICE cycle. The protocol stipulated that patients with stable or progressive disease during MATRIX treatment would immediately switch to the R-ICE regimen. Those experiencing subsequent CNS progression events were to undergo WBRT before proceeding with autologous HSCT consolidation. For patients presenting with concomitant, extensive, and life-threatening extra-CNS disease, the treatment program could begin with one or two cycles of the conventional R-CHOP regimen, administered as per local practice, following the completion of baseline assessments. **R-CHOP (cycles 1 or 2)** Rituximab 375 mg/m² d1 Cyclophosphamide 750 mg/m² d1 Doxorubicin 50 mg/m² d1 Vincristine 1·4 mg/m² d1 (max 2 mg) Prednisone 75 mg d1-5 **MATRIX (cycles 1,2,3)** Rituximab 375 mg/m² d0 Methotrexate 3.5 g/m² d1 the first 0.5 g/m2 in 15 min + 3 g/m2 in 3-hour IV infusion Cytarabine 2 g/m² d2 & d3 every 12-hour in 1-hour IV infusion Folinic rescue (levo-folinic) 15 mg/m²

		IV every 6 hours for 12 doses, adjested on MTX level**	
	Thiotepa	30 mg/m^2	d4 in 30 minutes IV infusion
	Liposomial Cytarabine***	50 mg*	d5
	G-CSF	d6 to d12 both filgastrim or lenogastrim can be used as per local practice	

**LEUCOVORIN RESCUE AND POST-MTX HYDRATION
Folinic (THF) rescue should commence 24 hours after the start of MTX infusion. Levo-folinic acid was administered at a dose of 15 mg/m2 (folinic 30 mg/m2) intravenous push/PO every six hours for 12 doses. The post MTX hydration (including the solutions used to administrate Ara-C, antiemetics and other drugs) should reach a total volume of 2000 ml (or as per local practice).

MTX SERUM LEVEL DETERMINATION
MTX serum level determinations to be taken at 48 hours post methotrexate., and it was repeated every 24 hours until a concentration < 5 x 10-8M/L was reached. In the case of high MTX serum levels persisting after 48 hours (or more) after the end of infusion, leucovorin rescue was modified according to MTX levels as follows:

if MTX < 5 x 10-7 M/L	*levofolinic acid 15 mg/m2/6 hours (folinic acid – racemic 30 mg/m2/6hours)*
if MTX < 1 x 10-6 M/L	*levofolinic acid 50 mg/m2/6 hours (folinic acid – racemic 100 mg/m2/6hours)*
if MTX > 1 x 10-6 M/L	*levofolinic acid100 mg/m2/6 hours. (folinic acid – racemic 200 mg/m2/6 hours)*

The rescue at 72 and 96 hours follows the dosing guidance for 48 hours.

****If liposomal cytarabine was not available, standard intrathecal chemotherapy with "methotrexate 10-12 mg + cytarabine 40-50 mg + hydrocortisone 50 mg" could be administered (doses to be consistent with local institutional guidelines). Oral steroids (as per local practice) were suggested for 2-5 days after intrathecal liposomal cytarabine delivery to prevent chemical or aseptic meningitis/arachnoiditis.*

R-ICE (**cycles 4,5,6)**

Rituximab	375 mg/m2	d1
Etoposide	100 mg/m2	d1, d2, d3 in 500-1000ml over 30-60 minutes IV infusion
Ifosfamide	5 g/m2	d2 in 1000ml in 24-hour IV infusion with MESNA support, as per local practice
Carboplatin	5 AUC	d2, capped dose of carboplatin is 800mg, total dose in 500ml in 1-hour IV infusion
Liposomial Cytarabine*	50 mg	d4

***If liposomal cytarabine (depocyte) was not available, standard intrathecal chemotherapy with "methotrexate 10 mg + cytarabine 40 mg + hydrocortisone 50 mg" could be administered. Oral steroids were suggested for 2-3 days after intrathecal liposomial cytarabine delivery to prevent chemical or aseptic meningitis/arachnoiditis.*

Patients with a complete or partial response after MATRIX–R-ICE and with adequate ASCT harvest received autologous HSCT. Myeloablative chemotherapy consisted of carmustine on day –6,

	thiotepa every 12 hours on day –5 and –4, supported by autologous HSCT. **Conditioning and ASCT** BCNU (carmustine)* 400 mg/m^2 d-6 in 500 ml in 1-2 hours infusion Thiotepa 5 mg/kg d-5 & d-4 in saline solution in 2-hour infusion every 12 hours Auto-transplantation (ASCT) 5 x 10^6 CD34+cells/kg d0 *In case of BCNU unavailability, the recommended conditioning regimen (Phase IV) was: Thiotepa 5 mg/kg d-6 & d-5 in saline solution by 2-hour infusion Busulfan 3.2 mg/kg d-4, d -3, d-2, administered in 4 doses per day corresponding to 0.8 mg/kg each dose, by 2-hour infusion or 3.2 mg/kg as a once daily infusion given over 3 hours. Clonazepam prophylactically from the day before busulfan therapy to the day after completion of busulfan therapy. ASCT 5 x 10^6 CD34+cells/kg d0 Patients with residual disease in the brain parenchyma after autologous HSCT received WBRT. The patients with residual disease in the cerebrospinal fluid after autologous HSCT received additional, intensified intrathecal chemotherapy (methotrexate 12 mg; cytarabine 50 mg; hydrocortisone 50 mg on days 1 and 8 each month for 3 months, or thiotepa 10 mg plus rituximab 25 mg on days 4 and 11, each month for 3 months). **Post ASCT** WBRT within 4 weeks from ASCT, with 36 Gy + tumor-bed boost 10 Gy in patients with residual disease in the parenchymal brain/cerebellum.
DURATION OF TREATMENT	MATRIX 3 cycles of 21 days – RICE 3 cycles of 21 days followed by ASCT.
ENDPOINTS	Primary Endpoint 1-year progression-free survival (PFS) Secondary Endpoints 1. Complete remission rate before autologous stem cell transplantation 2. Response duration 3. Overall survival 4. Safety
STATISTICAL METHODS	The Fleming design was used. The maximum 1-year PFS rate considered of low interest was 50% (P0) and the minimum 1-year PFS rate considered of interest was 65% (P1). To detect such a difference a total number of 69 patients was required (one-sided test, type I error 5% and power 80%), with a drop-out of 10%, 76 patients were needed. If at least 41 patients were progression-free survivors at 1 year, the strategy would be considered effective.

	All primary analyses were based on intention to treat, where all registered patients were included except for patients who post-hoc objectively did not meet the eligibility criteria at the time of registration. Differences in response rate among subgroups of interest were tested using the test chi-square. Survival curves were estimated using the Kaplan-Meier method and the Log rank test was used to compare outcome of the different subgroups of interest. Independent association between studied variables and survival were tested using the Cox proportional hazard model.
SUMMARY OF RESULTS	<u>Efficacy Results</u> Between March 30, 2015, and August 3, 2018, a total of 79 patients were enrolled in the study, 75 of whom were assessable. Out of the 450 planned cycles, 319 (71%) were delivered. At one year from enrollment, the primary endpoint was achieved, with 42 patients remaining progression-free, resulting in a PFS rate of 58%. After completing the MATRIX–R-ICE regimen, 49 patients (65%) achieved an objective response, including 29 patients (39%) with a complete response. Of the responders, 37 proceeded to autologous HSCT. By the end of the treatment program, 46 patients (61%) had an objective response, with a median duration of response of 26 months. At a median follow-up of 29 months, 35 patients remained progression-free, and 33 were alive, corresponding to a 2-year overall survival (OS) rate of 46%. <u>Safety Results</u> The MARIETTA programme included two standardised regimens (i.e., MATRIX and R-ICE) used in routine practice in several countries and showed a good safety profile. The safety profile of this study could be considered to be favourable with only 13% of grade 3–4 infections or febrile neutropenia and 5% treatment-related mortality. Importantly, MATRIX and R-ICE are two standardised regimens that are used in routine practice in several countries. A study from June 2020 demonstrates that MATRIX is widely used in routine practice across many cancer centers, with outcomes in terms of activity and tolerability comparable to those observed in prospective clinical trials.
CONCLUSIONS	The MARIETTA study is the largest prospective trial focused on patients with secondary CNS lymphoma; it was done in 24 centres in four countries, representing the most geographically extensive trial to date, which supports the generalisability of the results. This trial showed that the sequential combination of MATRIX and R-ICE followed by autologous HSCT was active in this population of patients with a very poor prognosis. Patients aged up to 70 years and with an ECOG Performance Status of 3 or less were included, representing a real-world cohort of patients. The MARIETTA programme is active in every subgroup of secondary CNS lymphoma, with 1-year progression-free survival of 58% and encouraging 2-year progression-free survival of 71% in patients with CNS disease at initial lymphoma diagnosis. Moreover, the MARIETTA programme included two standardised regimens (i.e., MATRIX and R-ICE) used in routine practice in several countries and showed a good safety profile. Combined with existing evidence, the results of the MARIETTA trial show that a growing proportion of patients with secondary CNS lymphoma can have durable remissions with intensified chemoimmunotherapy. Patients with CNS disease at initial lymphoma diagnosis and patients who had CNS dissemination during or after upfront R-CHOP therapy have different outcomes, suggesting that

	these two populations of patients with secondary CNS lymphoma might benefit from different treatments. Patients with CNS involvement at initial diagnosis seemed to benefit from treatment with debulking R-CHOP followed by MATRIX–R-ICE and autologous HSCT. Further efforts are required to improve remission rates before autologous HSCT, especially in patients with CNS involvement at first relapse.
VERSION AND DATE OF THE REPORT	Version 1.0 – 17.03.2025