

Protocol

Study Evaluating Relapses in Central Nervous System in Patients with Diffuse Large B-Cell Lymphoma Treated With Chemotherapy With or Without CNS Prophylaxis

Multicentric, prospective randomized phase III study

Brief Title: CNS Prophylaxis in Patients with Diffuse Large B-Cell Lymphoma

Sponsor: Czech Lymphoma Study Group

Study coordinating center: Czech Lymphoma Study Group (CLSG)

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INVESTIGATOR'S AGREEMENT

I have read the attached protocol and agree to abide by all provisions set forth therein. I agree to comply with all applicable international and local regulations related to the realization of the study. I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or realization of the clinical study without the prior written approval of the sponsor.

Investigator's name.....

Investigator's signature.....

Date.....

Protocol synopsis

Study ID: CLSG -CNS -01

Title: Study evaluating relapses in central nervous system in patients with diffuse large B-cell lymphoma (DLBCL) treated with chemotherapy with or without CNS prophylaxis.

Indication: All patients with newly diagnosed DLBCL at the age between ~~18-70~~ 18-72 years, that are treated with systemic chemotherapy 6x R CHOP+2xR or 6x DA EPOCH R+2x R.

Design: Multicentric, prospective, randomized phase III study

Primary objective: Comparison of cumulative incidence of CNS relapses in patients treated with 2 doses of intravenous methotrexate 3g/m² (arm A) or 6 doses of intrathecal methotrexate 12mg (arm B).

Secondary objectives:

- Evaluation and comparison of **overall, survival (OS)** in patients treated with 2 doses of intravenous methotrexate, 6 doses of intrathecal methotrexate and in patients without CNS prophylaxis.
- Evaluation of **overall response rate (ORR), complete remission rate (CRR)** in all three arms of the study (A, B, C), analysis of **treatment toxicity**.
- Evaluation of **progression-free survival (PFS)** in CNS and outside of CNS in all risk groups (low, intermediate and high risk).
- **Evaluation of definition of occult meningeal involvement:** cumulative incidence of CNS relapses in subgroups with occult meningeal involvement.

Number of patients: ~~500~~ 200

Recruitment period: ~~05/2015—12/2017~~ 07/2015-12/2023

Follow-up period: 1 year (~~2018, 2024~~)

Duration of the study: ~~05/2015—12/2018-~~ 07/2015-12/2024

Study protocol: All patients with DLBCL will undergo standard examination before the initiation of chemotherapy and risk factors for CNS relapse will be evaluated including lumbar puncture with collection and evaluation of cerebrospinal fluid (CSF): cytology, flow cytometry, miRNA and by **voluntary decision of the center also** IgVH rearrangement **and assessment of somatic mutations**

L265P MYD88 a Y196 CD79B. No intrathecal chemotherapy will be used during the first lumbar puncture.

All patients with systemic DLBCL without CNS involvement will receive chemotherapy 6 cycles R CHOP+2xR or 6 cycles DA EPOCH R+2xR (it means, that all patients will receive 8 doses of rituximab). Patients with risk factors for CNS relapse ≥ 2 or with occult meningeal involvement will be randomized in 1:1 ratio into arm A with methotrexate 3g/m² i.v. after the 3rd and 6th cycle of chemotherapy (R CHOP or DA EPOCH R), or into arm B with intrathecal(i.t.) methotrexate 12mg in all cycles of chemotherapy (it means 6x). Patients will be observed after the above mentioned treatment. Other patients with 0-1 risk factor will be allocated into arm C without CNS prophylaxis.

Risk factors for CNS relapse: age > 60 years, LDH > norm, clinical stage III/IV, performance status ECOG >1, kidney and/or adrenal involvement, extranodal involvement of more than one organ.

Low risk of CNS relapse: presence of 0-1 risk factor. Intermediate risk of CNS relapse: presence of 2-3 risk factors. High risk of CNS relapse: presence of 4-6 risk factors.

Patients with unclear meningeal infiltration will be randomized regardless of the number of risk factors within the intermediate risk group.

Patients with dry cerebrospinal fluid puncture will be monitored or randomized depending on the number of risk factors listed above.

In patients randomized to the arm with intrathecal methotrexate administration for whom intrathecal administration is not possible for technical reasons, the attending physician will decide whether to remain without prophylaxis or receive prophylactically intravenous methotrexate.

Primary endpoint

- **Comparison of cumulative incidence of CNS relapse in patients treated either with methotrexate i.v. or methotrexate i.t.**

Secondary endpoints

- **Complete remission rate (CRR)** – will be evaluated according to PET/CT-Cheson criteria (2014).
- **Overall response rate (ORR)** - rate of patients with complete or partial remission according to the Cheson criteria (2014) (1).
- **Overall survival (OS)** – date from diagnosis until date of death due to any reason.

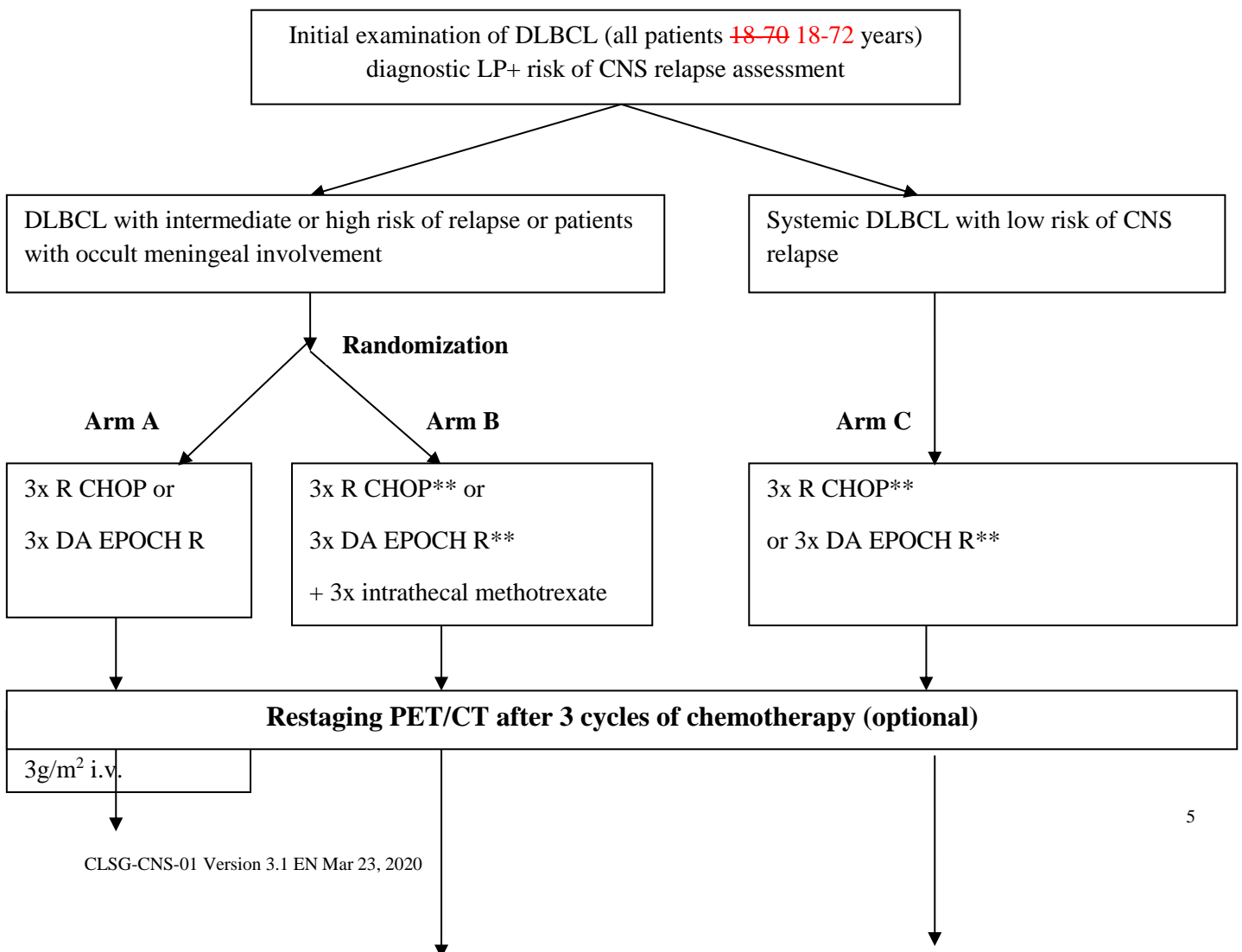
- **Progression-free survival (PFS) in risk subgroups** (low, intermediate and high risk) – date from enrollment into the study until disease relapse/progression (in or outside CNS) or until a death of patient after study treatment.

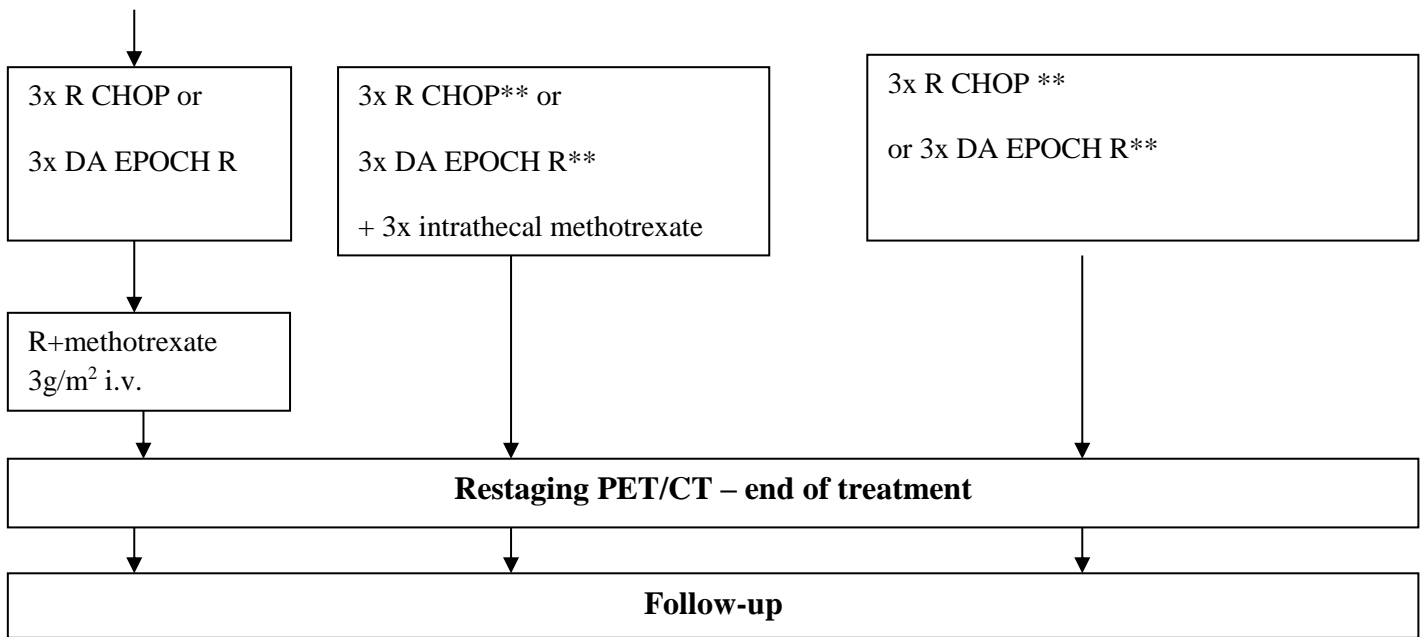
Statistical analysis:

Primary endpoint: Comparison of cumulative incidence of CNS relapses in patients treated either with methotrexate i.v. or methotrexate i.t. will be analysed by Fisher test.

Secondary endpoints: Comparison of CRR and ORR in all subgroups will be analysed by Fisher test or M-L Chi-square test. OS and PFS will be analysed by Kaplan-Meier method and log-rank test for comparison of survival curves.

Flowchart of CNS prophylaxis in newly diagnosed DLBCL





***Risk factors for CNS relapse:** age > 60years, LDH > reference range, clinical stage III/IV, performance status ECOG >1, kidney and/or adrenal gland involvement, involvement of > 1 extranodal organ.

Low risk of CNS relapse: presence of 0-1 risk factor. Intermediate risk of CNS relapse: presence of 2-3 risk factors. High risk of CNS relapse: presence of 4-6 risk factors.

****Patients in arms B and C without R + methotrexate** will be treated with additional 2 doses of rituximab alone either in cycle 1 of chemotherapy (i.e. D8 and D15), or after 6 cycles of chemotherapy – according to the practices at the treatment centers.

~~Patients with occult meningeal involvement regardless of risk factors will be randomized into the group with intermediate risk.~~

Patients with unclear meningeal infiltration will be randomized according to the number of risk factors. If the number of risk factors is 0-1 they will be randomized to the intermediate risk group due to unclear meningeal infiltration.

Patients with dry lumbar puncture of CSF will be followed or randomized according to above mentioned risk factors.

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1. Responsibilities for management of the study and study plan

1.1. Title: Study evaluating relapses in central nervous system in patients with diffuse large B-cell lymphoma (DLBCL) treated with chemotherapy with or without CNS prophylaxis

1.2. Sponsor: Czech Lymphoma Study Group (CLSG)

1.3. Coordinating center of the study:

Czech Lymphoma study Group is responsible for: randomization of patients, preparation of clinical study forms (CRF), validation of all data, comments and queries from centers, study monitoring, distribution of newsletters, statistical analysis and its reporting, processing of proposals from investigators.

Each CLSG study center is responsible for: data entry to the CRF from the center, SAE reporting, evaluation of treatment response.

1.4. Investigators:

To become an active center, the process of authorization must take place. The minimum documentation required for authorization includes:

- principal investigator's CV
- local ethics committee approval
- multicentric ethics committee (or any equivalent) approval
- approval of the hospital, where the study is planned
- approval of the State Institute for Drug Control (SÚKL)

1.5. Monitoring:

CLSG will assign persons to monitor the study and will distribute its own standard operating procedures (SOP) to the centers.

2. Background of the study

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma and it represents 45% of all non-Hodgkin lymphomas in the Czech Republic. The standard treatment of DLBCL patients is immunochemotherapy: 6 - 8 cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristin, prednisone) or its modifications (2,3). Currently, a new regimen was proposed for DLBCL patients and it includes 6 - 8 cycles of dose adjusted EPOCH R, its preliminary results show higher efficacy when compared with R-CHOP (4), but definitive results of studies comparing both regimens are still awaited. Relapse of DLBCL in the central nervous system (CNS) occurs in 4.6% of patients (range 2 - 10.4%), (5-25) and its prognosis is even worse when compared to primary CNS lymphomas. Median survival of these patients ranges between 4 - 5 months and only 10% - 25% of patients survive more than one year after diagnosis of CNS relapse (26).

Risk factors for CNS relapse in DLBCL

Most of the above mentioned studies include following risk factors for CNS relapse in DLBCL: advanced clinical stage, higher international prognostic index (IPI) or aaIPI (age adjusted IPI), elevated lactate dehydrogenase (LDH) > reference range, the ability of lymphoma to involve extranodal sites. It is currently unclear, whether involvement of certain sites (e.g. bone marrow) is more predictable for spreading of lymphoma into CNS rather than into another extranodal sites. Scrotal involvement is obligatory indicated for CNS prophylaxis due to the high risk of CNS relapse. The significance of prognostic indexes using various risk factors, that potentially could identify patients for CNS prophylaxis is still controversial (8,19,20). Hollender et al. (8) defined five risk factors (elevated LDH > reference range, involvement of more than one extranodal organ, retroperitoneal involvement, albumin <35 g/l and age >60 years): patients with 4-5 risk factors had a 30% risk of CNS progression, however, this was not confirmed by other studies. Chihara et al. (19) defined three independent risk factors (massive involvement, absolute lymphocyte count < $1 \times 10^9/l$ and extranodal involvement), but majority of progressions occurred in low risk cohorts.

A new prognostic model for risk of CNS relapse in DLBCL patients was proposed by Schmitz during the 12 ICML conference in Lugano and it included following risk factors: age > 60 years, lactate dehydrogenase > reference range, clinical stage III/IV, performance status ECOG >1, kidney and/or adrenal gland involvement. This model was originally constructed from the data of 1104 patients that participated in DSHNHL studies and subsequently extended to 2164 patients (including 1735 patients with DLBCL) and it was validated on an independent group of 1597 patients from BCCA registry (28). Patients from the DSHNHL studies with 0-1 risk factor had a 2-year rate of CNS relapse 0.6% (95% CI 0-1.2%), patients with 2-3 risk factors had a 2-year rate of CNS relapse 3.4% (95% CI 2.2-4.6%) and patients with 4-6 risk factors had a 2-year rate of CNS relapse 10.2% (95% CI 6.3-14.1%). The risk of CNS relapse in patients from BCCA registry was similar: 0-1 risk factor 0.8% (0-1.6%), 2-3 risk factors 3.9% (2.3-5.5%), 4-6 risk factors 12% (7.9-16.1%). Based on this risk model Schmitz recommended following treatment guidelines, that should be further validated in clinical trials: the low risk group (0-1 risk factor, risk of CNS relapse $\leq 1\%$) does not need any diagnostic or prophylactic procedures. Intermediate risk group for CNS

relapse (2-3 risk factors) should have modern diagnostic work up including MRI of the brain, flow cytometric analysis of CSF and a small high risk group for CNS relapse (4-6 risk factors, risk of CNS relapse 17% at 2 years) should have all available diagnostic work up and possibly CNS-directed therapy even without definitive proof of CNS disease.

CNS prophylaxis in DLBCL: significance of intrathecal prophylaxis, high dose of methotrexate and rituximab

The benefit of intrathecal prophylaxis to prevent the CNS relapse was not proved in 5 of 7 studies with overall 6653 patients. The largest retrospective study analyzed 3258 patients treated with CHOP (72%) or R-CHOP (27%), (20). About 30% of patients received some type of CNS prophylaxis, but criteria of prophylaxis changed over the time as they were not fixed and were based on collective consensus on every patient (very problematic study from this point of view). All types of prophylaxis were substantially more intensive than the current standard: some patients underwent cranial irradiation up to 50Gy, the second group received intrathecal prophylaxis with methotrexate and hydrocortisone (some of them with additional cytarabine), the number of intrathecal doses varied up to 10 doses administered during the first year after initial diagnosis, the third group received high doses of methotrexate 4x4 g/m² in 2 week intervals. None of these approaches with CNS prophylaxis was successful in reducing the CNS relapse (5,9 %), this rate was similar to the group without prophylaxis. Intrathecal methotrexate, hydrocortisone cytarabine and their combinations were most frequently used in DLBCL based on the reduction of CNS relapse in Burkitt lymphoma and lymphoblastic lymphoma. Sixty eight patients received regimen ACOMP-B (modification of MACOP-B with nine systemic chemotherapy drugs in consolidation) in a small non randomized study and about a half of patients received intrathecal methotrexate and hydrocortisone (14). No CNS relapse occurred in the group with intrathecal treatment compared to six CNS progressions in the group without prophylaxis (p = 0.03). These results were not confirmed by subsequent other studies with more patients.

Currently, the intrathecal prophylaxis is declining and the interest is redirected to intravenous high dose methotrexate because most of CNS relapses (30% - 70%) occur in the brain and the intrathecal treatment does not affect the intraparenchymal involvement. The Groupe d'Etudes des lymphomes de l'Adulte (GELA) used ACVBP regimen that included intravenous high dose methotrexate as a consolidation therapy and reported a low number of CNS relapses with this approach (11). GELA performed a randomized study: patients were treated either with ACVBP regimen (4 induction cycles of CHOP-bleomycine in 2 week intervals and with intrathecal methotrexate followed by consolidation with 2 doses of methotrexate 3g/m² and 6 cycles of etoposide, ifosfamide, cytarabine) or with CHOP regimen without intrathecal or intravenous methotrexate (21). Patients treated with ACVBP experienced lower rate of CNS and systemic relapses and a better overall survival (2.8% vs 8.3%, p = 0.004) when compared with CHOP. ACVBP regimen is a complex treatment system

and the benefit of each component to improve the control of CNS disease is not easily assessable.

Current studies analyzed, whether the addition of anti-CD20 antibody rituximab to combined systemic chemotherapy could improve the disease control and could reduce the incidence of CNS relapses. Four out of eight studies enrolled 4334 patients (9,19,20,23) and did not confirm a reduced incidence of CNS relapses. Another 3 studies included 2035 patients (7,24,25) and reported a reduced rate of CNS relapses. The study by Schmitz et al. (12) confirmed a significant impact of rituximab on reduction of CNS progressions in a low risk patients with age adjusted international prognostic index (aaIPI) 0-1 only where the overall risk of CNS relapse was low. Taken it all together, it is possible, that a better systemic control of the disease could eradicate minimal residual disease in CNS.

The incidence of isolated CNS relapse in patients with primary mediastinal B lymphoma (PMBL) is low- up to 2% only (29) and patients with PMBL will not be enrolled into this study with CNS prophylaxis.

Possibilities of early detection of CNS relapse/progression

1/Examination of CSF

Despite the fact that CNS relapses in aggressive lymphomas could occur several years after primary diagnosis and treatment, most studies reported the median time to occurrence of CNS relapses 6-12 months after initial diagnosis (25).

This arises the possibility, that some of these patients could have occult CNS involvement at the initial diagnosis of DLBCL. Most physicians refuse to perform lumbar puncture in patients with DLBCL and low (aa)IPI during initial staging due to low sensitivity (low numbers of cells in CSF, missing of intraparenchymal involvement) and low specificity (reactive lymphocytes and its differentiation from tumor cells) of CSF cytology (30).

Magnetic resonance imaging (MRI) or computed tomography (CT) is not routinely indicated in asymptomatic patients due to the high costs, unclear sensitivity and low specificity in low risk patients (31,32).

Flow cytometry (FCM) of CSF can detect a small number of tumour cells based on the detection of specific surface antigens that indicates a clonal population. FCM has a higher sensitivity and specificity when compared to CSF cytology (33-37), however, the clinical significance of a small clonal population detected by FCM is unknown. Most studies included aggressive histological subtypes of lymphomas including Burkitt and lymphoblastic lymphomas, some of them included HIV positive patients, other studies analyzed newly diagnosed and relapsed patients. Initial CNS involvement detected by FCM of CSF can be found in approximately 20-25% of risk patients (> 1 extranodal involvement, elevated LDH> reference range, HIV positivity, highly aggressive histology). Some studies tailored treatment according to these results.

Sancho et al.(37) divided 108 patients according to FCM and cytology in CSF at diagnosis: patients without CNS involvement (FCM negative and cytology negative, 83 patients), patients with occult CNS involvement (FCM positive, cytology negative, 15 patients) and clear CNS involvement (FCM and cytology positive, 7 patients). CNS progressions were reported in all 3 groups: 2.4% of patients

in the group with negative initial CSF, 13% of patients in the group with occult CSF involvement and 28.5% in the group with clear meningeal involvement. Based on the above mentioned studies the risk of CNS progression is increased in patients with positive FCM and especially when it is combined with positive cytology of CSF, but it does not automatically mean, that the progression is confirmed. CNS prophylaxis/treatment in these patients with DLBCL remains unclear.

Eighty percent of patients with CNS lymphomas has changes in CSF. Number of cells in CSF is in reference range (normal) in 33% - 60% of patients with CNS lymphoma involvement (38). Sensitivity of CSF cytology ranges between 2% - 32%, the differentiation between tumour and reactive postinflammatory lymphocytes results in difficulties to confirm the diagnosis of meningeal involvement (38). The evaluation of CSF cytology is affected by technical factors. Sensitivity of CSF cytology increases with the larger volume of CSF obtained for analysis ($\geq 10,5\text{ml}$) and with series of CSF samples from the same patient. Sensitivity is affected by a period between the sample collection and analytical phase, steroid application that induces cytotoxicity (38). Beta-2-microglobulin (B2M) is a marker of increased cell turnover. Elevated levels of B2M in CSF were detected in 68% patients with CNS involvement, but the specificity of elevated B2M is low as it occurs in bacterial meningitis and other neurological diseases (38). Elevated LDH - isoenzyme 5 in CSF is also detected in CNS lymphomas, however, the specificity is low as it occurs in gliomas and bacterial meningitis (38). Low level of glucose in CSF was detected in 19% - 54% of patients with CNS lymphomas (37).

Testing of IgVH rearrangement by PCR analysis of CSF can help to detect meningeal involvement (37).

MicroRNAs (miRNAs) are small noncoding RNAs, that regulate gene expression on posttranscription level. Elevated levels of these molecules were detected in the tumour tissue, however, they occur in peripheral blood, serum and CSF and they can serve as biomarkers of various tumours. Elevated miR-21 is detected in the serum of DLBCL and correlates with good prognosis (40). Elevated levels of miR-19b, miR-21 and miR-92 were detected in CSF of primary CNS lymphomas. Levels of miRNAs decrease to undetectable levels in remission and their rising levels correlates with upcoming relapse in primary CNS lymphomas (41). Studies correlating levels of miRNA in CSF with the risk of CNS relapse in systemic lymphomas have not been published so far.

2/Evaluation of intraparenchymal CNS involvement

MRI is standard imaging method to detect suspected brain involvement. Only few publications analyzed occult intraparenchymal CNS involvement in asymptomatic DLBCL patients with imaging methods. Akkas et al. (42) used positron emission tomography (PET) of the brain for evaluation of 123 immunocompetent patients (68 patients at initial diagnosis and 55 patients at the time of relapse). Initial PET helped to diagnose an asymptomatic CNS involvement in 3 patients (4.4%) and another 3 (5.4%) at the time of relapse. Three of them had intraparenchymal lesions only and it could not be detected by CSF examination.

Treatment of CNS relapse in DLBCL

Prognosis of secondary CNS relapse in DLBCL is worse when compared to primary CNS lymphomas. Treatment possibilities of CNS relapse range from pure palliative treatment with intrathecal methotrexate or liposomal cytarabine (43), through therapy similar to primary CNS lymphomas (44), to intensive salvage therapy with autologous stem cell transplantation (45 - 49). Choice of treatment depends on the age of patient, performance status and on the extent of the disease: isolated CNS relapse or systemic relapse with CNS involvement.

Regardless of poor prognosis in most patients the data have shown, that a subgroup of them could achieve a longlasting survival after intensive systemic treatment. Combination of methotrexate with ifosfamide achieved 90% response rate in a population of patients with mean age of 65 years. Median relapse-free survival was only 9 months, however, at a median follow-up of 15 months median survival was not reached (45). Results of transplanted patients dramatically differ between the analysis of all patients intended to treat with ASCT and analysis of really transplanted patients. Studies by Doorduyn et al. and Bromberg et al. (46, 47) included all patients indicated for high dose therapy and ASCT. Median survival in both studies was 7 months. Out of really transplanted patients, Bromberg et al. reported 62% of patients surviving 1 year, 54% 2 years and 42% 3 years, respectively (corresponding data in non-transplanted patients: 25%, 17% and 14%, respectively). Similar survival as Bromberg were reported by studies, that analyzed really transplanted patients: Street et al. reported a 2year survival in 68% patients (48) and Korfel reported 63% patients (49). Most studies used methotrexate in salvage therapy and conditioning regimens before ASCT included busulphan and thiotepa. Thiotepa is more effective with higher toxicity when compared to BEAM in CNS lymphomas (50).

3. Definition of study endpoints

The aim of the study is to confirm the benefit of CNS prophylaxis using 2 doses of methotrexate 3g/m² i.v. (arm A) that is probably more effective than 6 doses of intrathecal methotrexate 12mg (arm B) to prevent CNS relapse in intermediate and high risk DLBCL patients and in patients with occult meningeal involvement treated with the first-line systemic immunochemotherapy R CHOP or DA EPOCH R. Another aim is to evaluate the incidence of CNS relapses in the the low risk group without CNS prophylaxis (arm C).

3.1.Primary endpoint: comparison of cumulative incidence of CNS relapses in patients treated either with methotrexate 3g/m² i.v. or 6 doses of intrathecal methotrexate 12mg.

3.2.Secondary endpoints

Complete remission rate and partial remission rate

Overall survival (OS) of patients is defined as period between the date from diagnosis until date of death due to any reason.

Progression-free survival (PFS) outside of CNS

Treatment toxicity

Evaluation of definition of occult meningeal involvement: cumulative incidence of CNS relapses in subgroups with occult meningeal involvement:

a/occult FCM positivity and concomitant CSF cytology negative;

b/FCM negative and concomitant occult positivity of CSF cytology;

Validation of clinical predictive risk model

4. Design of the study

4.1. Design of the study: Multicentric, prospective, randomized phase III study

Patients with histologically confirmed DLBCL that signed informed consent can be enrolled into the study.

Risk factors for CNS relapse will be evaluated in all patients enrolled: age > 60 years, LDH > norm, clinical stage III/IV, performance status ECOG >1, kidney and/or adrenal involvement, extranodal involvement of more than one organ.

Low risk of CNS relapse: presence of 0-1 risk factor. Intermediate risk of CNS relapse: presence of 2-3 risk factors. High risk of CNS relapse: presence of 4-6 risk factors.

Patients with unclear meningeal infiltration will be randomized regardless of the number of risk factors within the intermediate risk group.

Patients with dry cerebrospinal fluid puncture will be monitored or randomized depending on the number of risk factors listed above.

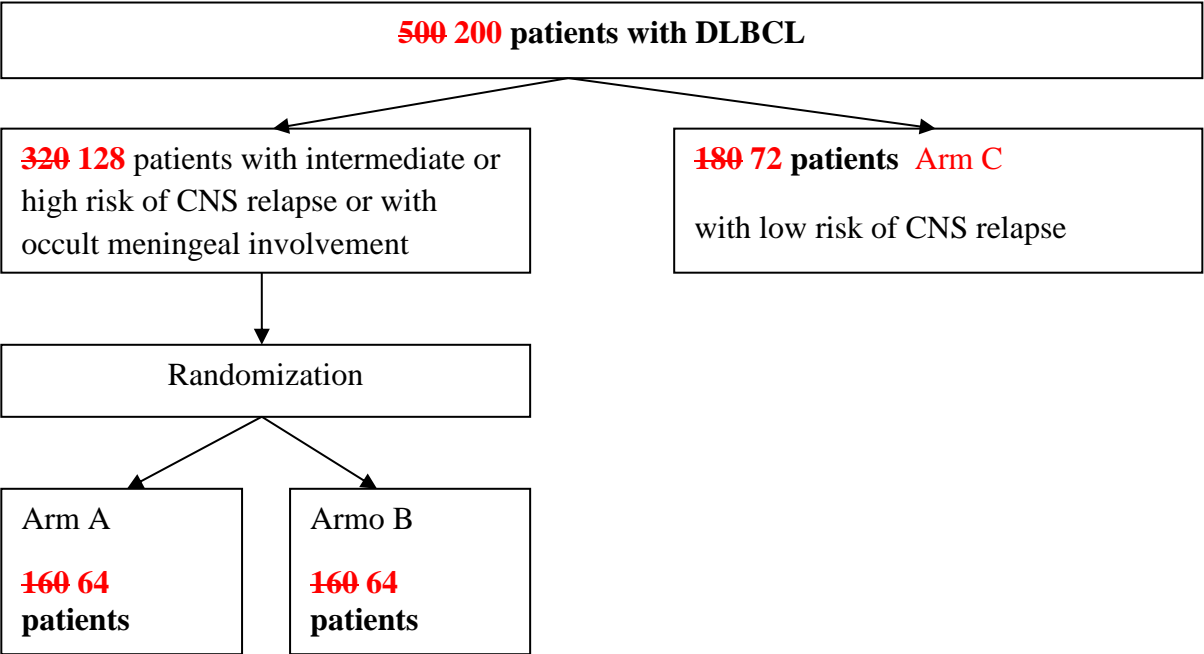
In patients randomized to the arm with intrathecal methotrexate administration for whom intrathecal administration is not possible for technical reasons, the attending physician will decide whether to remain without prophylaxis or receive prophylactically intravenous methotrexate.

All patients will be treated with systemic immunochemotherapy: either 6 cycles of R CHOP+2x R or 6 cycles of DA EPOCH R+2x R.

Patients with **intermediate and high risk** of CNS relapse and patients with occult meningeal involvement will be randomized in 1:1 manner either into arm **A** (application of 2 doses of methotrexate 3g/m² i.v.) or into arm **B** (application of 6 doses of intrathecal methotrexate 12mg).

Patients with **low risk of CNS relapse** and without meningeal involvement will be allocated into arm C without CNS prophylaxis.

4.2. Study population: ~~500 200~~ patients are planned for recruitment including ~~320 128~~ patients with intermediate or high risk of CNS relapse or with occult meningeal involvement and these patients will be randomized either into arm A or arm B. Another 180 patients with low risk of CNS relapse are planned to be allocated into arm C without CNS prophylaxis.



4.3. Recruitment period: ~~05/2015—12/2017~~ 07/2015-12/2023

4.4. Follow-up period: 1 year (~~2019~~ 2024)

4.5. Duration of the study: ~~05/2015—12/2018~~ -07/2015-12/2024

4.6. Duration of the study per patient: the overall treatment period is 22-24 weeks per patient and subsequent follow-up period lasts ~~2-years~~ 1 year.

5. Study population

5.1. Target study population

Patients with diffuse large B-cell lymphoma (DLBCL) at the age of ~~18-70~~ 18-72 years

5.2. Inclusion criteria

- 1/Histologically confirmed untreated DLBCL
- 2/Age ~~18-70~~ 18-72 years
- 3/Signed informed consent with the study
- 4/First-line treatment: either 6 cycles of R CHOP +2x R or 6 cycles of DA EPOCH R+ 2xR

5.3. Exclusion criteria

- 1/ Patients with DLBCL and concomitant initial CNS involvement
- 2/Patients with primary mediastinal B-cell lymphoma
- 3/ Patients with DLBCL treated with another chemotherapy than R CHOP or DA EPOCH R
- 4/ HIV positive, or active hepatitis B or C
- 5/ Other serious disease (based on the decision of the physician-investigator)
- 6/ Non-compliance of a patient
- 7/Any contraindication for application of anthracycline-based chemotherapy or high dose methotrexate
- 8/Pregnancy or breast-feeding

6. Schedule of procedures and assessments

6.1. Informed consent

Written informed consent (Appendix A) must be obtained from each subject prior to entering the trial and prior to performing any study - related procedure (PET/CT or CT and CSF assessment performed within 28 days before the screening initiation are permitted). However, emergency investigational and treatment procedures which can be considered as a standard of care do not need to be repeated. The patient and the investigator must personally date and sign two originals of informed consent forms. The patient shall receive one original of a fully completed informed consent form and the second completed original will be archived as a part of the medical documentation.

6.2. Allocation and randomization of patients

Based on screening examinations only patients with intermediate and high risk of CNS relapse or with occult meningeal involvement will be randomized either into arm A with 2 doses of methotrexate 3g/m² i.v. or into arm B with 6 doses of intrathecal methotrexate. Randomization will be performed centrally (provided by CLSG) and centers will receive written confirmation about a stratified randomization - allocation of the patient. Patients with low risk of CNS relapse will be allocated into arm C without CNS prophylaxis.

6.3. Schedule of procedures

The subject will be evaluated for eligibility during the screening period prior to administration of the first cycle of immunochemotherapy. **For technical reasons, screening lumbar puncture (without i.t. application) can be performed on day 1 of 1st immunochemotherapy cycle (prior to randomization). If the patient is randomized to an i.t. application of methotrexate, the lumbar puncture will be completed during the first cycle of treatment with an interval 1-2 weeks.** Laboratory assessments shall be realized within 2 weeks prior to administration of the first cycle of immunochemotherapy. Other assessments shall be performed within 28 days prior to administration of the first cycle of immunochemotherapy.

6.3.1. Screening procedures:

- Histologically confirmed diagnosis of DLBCL from the second review of histology;
- Complete history of a patient;
- Complete blood count with differential count of white blood cells and with the number of reticulocytes;
- Biochemistry (Na, Cl, K, Ca, Mg, P, urea, creatinine, uric acid, AST, ALT, total bilirubin, ALP, LDH, glucose, total protein, albumin);

- TSH, fT4;
- Serology (HBsAg, anti-HBC, anti-HIV1,2);
- Beta2microglobulin;
- Evaluation of aaIPI and classic IPI and assessment of risk factors for CNS relapse;
- Bone marrow examination: cytology, flow cytometry, histology;
- 12-lead electrocardiogram (ECG)
- Transthoracic echocardiography;
- PET/CT or CT of neck, chest, abdomen and pelvis (if PET is not available for screening evaluation) with contrast;
- MRI of the brain or MRS of the brain (could be performed during the first 2 cycles of chemotherapy- not strictly before therapy), examination of spinal cord and neurological examination in patients with intermediate or high risk of CNS relapse, in patients with occult meningeal involvement, and in suspected or apparent CNS involvement;
- Pregnancy test in female patients with childbearing potential.

6.3.2. Study screening examination

1. **Lumbar puncture: collect 5-10ml of CSF** into an empty vial (10ml) with a blue cap. Note: hemorrhagic CSF is not suitable for standard and study examinations, a new lumbar puncture with a new CSF collection is necessary (after a week delay).

CSF will be subsequently distributed into following vials:

1-3ml of CSF will be poured into a new empty vial (10ml) with a blue cap- CSF is indicated for FCM examination: complete the request FCM form and label the vial (CNS prophylaxis study) and deliver the vial and request form to FCM laboratory.

The sample should be processed within 1 hour after the CSF collection. If it is not possible to meet this requirement, the sample should be stabilized immediately after collection to avoid decline in viability in vitro ($\geq 50\%$ after 1 hour of collection). Options to ensure stability of CSF:

1. Transfix(Cytomark)- commercially available vials designated for collection and storage of CSF at 4⁰ C, declared stability from manufacturer up to 10 days, recommended time to examination: 72 hours.
2. RPMI + 5% FBS 2ml of CSF into 2 ml of medium, storage at 4⁰ C for 18 hrs.
3. RPMI without additives could be sufficient.

2-3ml of CSF will be poured into another empty vial (10ml) with a blue cap – CSF is indicated for cytology and biochemical examinations, complete the request cytology/biochemistry form and label the vial (CNS prophylaxis study). Note: CSF should be delivered into the cytology/biochemistry laboratory within 2 hours.

Laboratory manual of CSF examination including the request form for CSF examination is available at <https://www.likvor.cz/>. Following tests should be marked on the CSF request form: tick qualitative and quantitative cytology, biochemistry (total protein, glucose, lactate) and beta2microglobulin. The patient must always sign the CSF request form.

At the discretion of each center and at the expense of the dispatching center 1-3ml of CSF will be poured into a sterile vial – CSF is indicated for IgVH examination (by PCR) and assessment of somatic mutations L265P MYD88 a Y196 CD79B. transport by ambient temperature, not frozen and without other preservatives- complete the request form and label the vial (CNS prophylaxis study). This study vial should be delivered as soon as possible (within 48 hours) into the Center of

Molecular Medicine, CEITEC, Masaryk University, Brno (Prof.RNDr. Šárka Pospíšilová, PhD.).

Note: In case of discrepancies between negative FCM and positive cytology of CSF in the first sample of CSF, another lumbura puncture should be performed and sample of CSF should be collected with repeated FCM, cytology and new IgVH examination will be performed.

Comment on IgVH examination by PCR testing.

Sample of CSF is stable for 48 hrs after collection. Delivery of CSF samples for IgVH examination is recommended at ambient temperature during working hours (Monday – Thursday).

Results will be provided on written form and electronically to the physician that requested the examination.

1 ml of CSF in the initial vial with a blue cap is indicated for miRNA examination, complete the request form (miRNA) and label the vial (CNS prophylaxis study). This vial should be delivered to the Department of Laboratory Medicine- Hematology within 60-120 minutes after CSF collection! miRNA examination- see Appendix A.

Concomitant collection of peripheral blood:

10ml of serum, complete the request cytology/biochemistry form and label the vial (CNS prophylaxis study). Serum should be delivered concomitantly with CSF. Examination of CSF cytology and biochemistry- see request forms at <https://www.likvor.cz/>.

10 ml of peripheral blood: 5 ml pour into the first vial containing gel, fulfill the request miRNA serum form and label the vial (CNS prophylaxis study). Another 5ml pour into the second vial with EDTA, vortex it, complete the request miRNA plasma form and label the vial (CNS prophylaxis study). Both vials and request forms should be delivered to the Department of Laboratory Medicine- Hematology – see Appendix A.

6.3.3. Assessment during each cycle

Clinical and laboratory examinations will be performed according to standard local practices at each participating center. Adverse events and toxicity grade III/IV will be evaluated according to

Common Toxicity Criteria (CTCAE) version 4.0.

6.3.4. Assessment after 3 cycles of chemotherapy and at the end of treatment

Clinical evaluation and standard laboratory examination (complete blood count with differential count of white blood cells and reticulocyte count, biochemistry- see screening examinations)

Study examinations: 10ml of peripheral blood (for miRNA examination) will be collected during restaging after 3 cycles of treatment and at the end of the first-line treatment.

Requirements for blood collection- see screening procedures.

CT will be performed after 3 cycles of R-CHOP or DA EPOCH R chemotherapy (PET/CT after 3 cycles of chemotherapy is optional, but strongly recommended) and **PET/CT at the end of the first-line treatment (mandatory)**. Metabolic activity of PET will be evaluated by 5-point scale (51).

Patients with a PET negative complete remission after the first-line treatment will be observed only.

Patients randomized into arm A or B will undergo CSF examination: before the second dose of methotrexate in arm A and during the last i.t. application of methotrexate in arm B.

6.3.5. Follow-up during the 1st ~~and 2nd~~ year after treatment

Restaging every 6 months: it means 6, 12, ~~18 and 24~~ months after treatment

Clinical evaluation

Laboratory examinations: blood count with differential count of the white blood cells and reticulocyte count;

Adverse events

6.3.6. Diagnosis of CNS relapse

Suspicion of CNS relapse will be verified by a new CSF examination and MRI (or MRS) of the brain. New neurological symptoms or signs of neurological involvement or a new positive finding in CSF (FCM and/or cytology) or positive finding on MRI (or MRS) of the brain or spinal cord will establish the diagnosis of CNS relapse. (Stereotactic) biopsy of the brain in suspected CNS relapse is recommended but not mandatory to confirm the diagnosis of CNS relapse.

6.3.7. Study examinations in CNS relapse

Collection of 5-10ml of CSF, that will be distributed into following vials:

1-3ml of CSF into vial for FCM examination, see screening examination;

2-3ml of CSF into vial for cytology and biochemistry, see screening examination;

At the discretion of each center and at the expense of the sending center can be sent 1-3ml of CSF into vial for IgVH - PCR examination **and assessment of somatic mutations L265P MYD88 a Y196 CD79B**, see screening examination;

1ml of CSF into vial for miRNA examination, see screening examination;

Concomitant mandatory collection of peripheral blood:

-10ml of serum obtained concomitantly with cytology and biochemistry of CSF examination, requirements for blood collection-see screening examination

-10ml of peripheral blood- concomitant examination of miRNA in CSF and peripheral blood: requirements for blood collection- see screening examination.

7. Treatment and prophylaxis

All patients will receive treatment 6 cycles of R CHOP+2xR or 6 cycles of DA EPOCH R+ 2xR. The group of patients without methotrexate will receive two additional doses of rituximab, either during the first cycle of chemotherapy day 8 and day 15, or separately after the end of 6 cycles of chemotherapy- according to local practices. Finally, all patients will receive 8 doses of rituximab.

R-CHOP is a standard regimen of the first-line treatment (reduction of chemotherapy is possible since the first cycle of treatment based on performance status and comorbidities of patients).

Rituximab (R)- 375mg/m² i.v. day 1 (rituximab 1400 mg s.c. can be used from 2nd cycle instead of i.v. rituximab)

Cyclophosphamide (C)- 750mg/m² i.v. day 1

Doxorubicine (H)- 50mg/m² i.v. day 1

Vincristine (O)- 1,4mg/m² (max. 2mg) i.v. day 1

Prednisone (P)- 100mg p.o. days 1-5

Cycles repeated every 21 days

Supportive treatment: according to local practices of participating centers. Growth factors (G-CSF, pegylated G-CSF) can be used according to EORTC recommendations.

Delay of chemotherapy due to cytopenia – according to local practices in participating centers.

DA EPOCH-R – a new regimen for patients with DLBCL (mandatory application via central venous access and hospitalization):

Rituximab (R)- 375mg/m² i.v. day 1 (rituximab 1400 mg s.c. can be used from 2nd cycle instead of i.v. rituximab)

Etoposide (E) 50 mg/m²/d i.v. in continual infusion days 1,2,3,4 (96hrs.)

Doxorubicine (D) 10 mg/m²/d i.v. in continual infusion days 1,2,3,4 (96 hrs.)

Vincristine (O) 0,4 mg/m²/d i.v. in continual infusion days 1,2,3,4 (96hrs.)

Note: Etoposide, Doxorubicine and Vincristine are delivered together in one infusion

Bolus:

Cyclophosphamide (C) 750 mg/m²/d i.v. day 5

Prednisone (P) 60 mg/m²/d twice daily p.o. days 1,2,3,4,5

G CSF 5ug/kg/d s.c. since day 6 until neu recovery >5x10⁹/l after nadir

Blood count examination: 2x – 3x weekly

Cycles repeated every 21 days (neu ≥ 1x10⁹/l, platelet count ≥ 100x10⁹/l)

Dose modification in subsequent cycles:

Nadir neu ≥ 0,5x10⁹/l during cycle: 20% dose increment of etoposide, doxorubicine and cyclophosphamide in the following cycle.

Nadir neu < 0,5x10⁹/l during cycle in 1 or 2 measurements: unchanged dose of etoposide, doxorubicine and cyclophosphamide in the following cycle.

Nadir neu < 0,5x10⁹/l during cycle in 3 or more measurements: 20 % dose reduction of etoposide, doxorubicine and cyclophosphamide in the following cycle.

Nadir of platelet count $< 25 \times 10^9/l$ during cycle: 20% dose reduction of etoposide, doxorubicine and cyclophosphamide in the following cycle.

Cave: Vincristine is not initially capped to 2mg, but the estimated doses are applied in continual infusions. Reduction of vincristine is indicated if neuropathy occurs.

7.1. CNS prophylaxis

Besides the use of clinical risk model patients will be also allocated into treatment arms according to CSF examination:

a/ CSF without involvement (cytology of CSF negative, absolute number of clonal cells < 10 according to FCM);

b/ occult meningeal (CSF) involvement: unclear FCM positivity of CSF (presence of 10- 20 clonal cells) and concomitant negativity of CSF cytology; or negative FCM of CSF and concomitant positivity of CSF cytology (low number of cells). In case of discrepancies between a negative FCM finding and a positive cytology of CSF, **at the discretion of each center and at the expense of the sending center can be sent 1-3ml of CSF into vial for IgVH - PCR examination and assessment of somatic mutations L265P MYD88 a Y196 CD79B.** In case of positive results, the CSF will be considered positive for meningeal involvement. Negative IgVH in CSF will be considered as negative for meningeal involvement and the patient will be evaluated according to clinical risk model. In case of non-evaluable CSF or the sample is damaged it is recommended to repeat the CSF examination after one week or before the second cycle of chemotherapy. In case of negative CSF, the patient will be managed according to clinical risk model. In case of occult finding in CSF the patient will be randomized either into arm A with 2 doses of systemic methotrexate $3g/m^2$ i.v., or into arm B with intrathecal methotrexate 12mg prophylaxis in each cycle of systemic chemotherapy (overall 6x intrathecal methotrexate). Patients with clearly positive CSF for DLBCL involvement will be treated according to the regimens for systemic and secondary CNS involvement. If patient refuses a repeated lumbar puncture, patient will receive therapy for occult meningeal involvement. **For technical reasons, screening lumbar puncture (without i.t. application) can be performed on day 1 of 1st immunochemotherapy cycle (prior to randomization). If the patient is randomized to an i.t. application of methotrexate, the lumbar puncture will be completed during the first cycle of treatment with an interval 1-2 weeks.**

In patients with i.v. methotrexate $3g/m^2$ is it possible to start next cycle (R CHOP or DA EPOCH R) up to excretion of methotrexate and, in the case of toxicity (renal / hepatic) after the toxicity has been adjusted to grade ≤ 1 . The minimal interval between i.v. methotrexate and other chemotherapy are 14 days.

Definition of positive CSF according to FCM: presence of total number ≥ 25 clonal B-lymphocytes, or (in case of not evaluable kappa/lambda) presence of population ≥ 25 B-lymphocytes, that indicates DLBCL according to FSC and SSC characteristics and immunophenotype correlating with other patients' samples (bone marrow, lymph node);

Definition of positive CSF according to cytology: any finding of lymphocytes, that fulfill morphologic criteria for malignancy;

c/ CSF clearly positive for lymphoma involvement (FCM and cytology positive for lymphoma involvement). Patients with initial neurological symptoms will be treated according to the regimens for systemic and secondary CNS involvement.

Collection of CSF for miRNA examination will be performed during initial screening in all patients, miRNA will be examined centrally after enrollment of all planned patients at the Institute of Pathophysiology, First Faculty of Medicine, Charles University in Prague to ensure uniform conditions of examination.

MRI or MRS of the brain or spinal cord and neurological examination will be performed in patients with intermediate and high risk of CNS relapse or with occult meningeal involvement, suspected or clear CNS involvement. All effort should be made to perform a biopsy in case of positive MRI finding. However, if the biopsy would lead to a significant delay of treatment, the biopsy can be omitted.

The first Stratification step according to CSF: negative-unclear-positive

CSF negative: **IF** risk low- without prophylaxis

IF risk intermediate- high :stratification and randomization according this criterion

IF CSF intermediate: stratification into the group with with intermediate risk (including low risk CNS-IPI)

IF CSF positive: treatment according to CNS protocol, **it means out of CLSG-CNS-01 study**

CNS relapses will be assessed in all subgroups of patients without initial CNS involvement or with occult CNS involvement. CNS relapse is defined by a new onset of symptoms or signs of neurological involvement, or new clearly positive finding in CSF (FCM and/or cytology), or new positive finding assessed by MRI or MRS of the brain or spinal cord. In case of suspected CNS relapse, biopsy (stereotactic) of the brain is strongly recommended but not mandatory for the diagnosis of CNS relapse in suspected CNS relapse.

7.2. Study treatment and drug supply

The treatment is standard. All drugs will be applied in accordance with their Summary of Product Characteristics (SPC) and guidelines. Drugs will be reimbursed by insurance companies as other standard treatment and delivered through the hospital pharmacy. Drugs will be properly labeled and registered by a pharmacist responsible for the evaluated study drug and its dispensation acting

in compliance with Section 1, paragraph 19, letter d of a Decree No. 226/2008 Coll. and according to the SUKL guidelines LEK 12.

7.3. Other concomitant medication

Concomitant administration of any other chemotherapy agents or regimen during the study treatment is not allowed.

Standard supportive treatment (hydration, antiemetics, antimicrobial prophylaxis, G CSF, pegylated G CSF, blood component therapy) will be administered according to local standards used in each center.

8. Adverse events

Definition: an adverse and unintended response to the product administration which occurs at doses normally used for the prophylaxis, therapy or diagnosis of a disease or for the restoration, correction or other modification of physiological functions;

8.1. Reporting of adverse events

Only toxicities grade 3. and 4. are subjects of reporting according to Common Toxicity Criteria (CTCAE) version 4.0 or selected toxicities grade 2 (pneumonitis, headache, vertigo, thrombembolic complications). Reporting of adverse events means recording this event into an electronic CRF. In case the event fulfills criteria for serious adverse event (see below), it is necessary to report it in a special regimen for SAE reporting (see below).

Adverse events not listed on CTCAE will be graded according to the five-point scale:

Mild (grade 1)	Asymptomatic condition or mild symptoms, Requires clinical or diagnostic observation only, without intervention
Moderate (grade 2)	Requires minimal, local or non-invasive intervention.
Severe (grade 3)	Serious or clinically important, but not life-threatening condition; requiring hospitalization or prolonged hospitalization; care for his/her personal needs is reduced

Life threatening (grade 4)	Life threatening, urgent intervention is required
Death (grade 5)	Adverse event resulting in death

8.2. Electronic reporting of serious adverse events

Serious adverse event (SAE). adverse reaction that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity or is demonstrated as a congenital anomaly or birth defect in offsprings.

All serious adverse events (SAE) must be reported to the center of pharmacovigilance within 24hrs. since the diagnosis of SAE. Phnarmacovigilance reporting is regulated by Direction KLH 21 Version 5. The reponsible physician will fulfill all medical conditions into SAE reporting paper form and he/she will submit this form by fax to the center of pharmacovigilance. Concomitantly the SAE will be recorded into the electronic CRF with the date and type of event. Center of pharmacovigilance will send a confirmation about the acceptance of of SAE.

Minimal extent of the first SAE report must contain:

Identification of a patient;

Name of the person reporting SAE;

Description of SAE;

Relation to the treatment;

Follow-up extended SAE report - must be completed and submitted within 10 days.

Pharmacovigilance- fax: +420224963118

All further information after initial SAE report must be submitted to the principal investigator on a new SAE form. A new information will be labeled on the SAE form as follow-up report and sent. Patients withdrawn from the study due to adverse event (AE) will be followed at least until the resolution of the AE, even in case, when AE exceeds the duration of study. In case of SAE the patient will be followed until the recovery of the clinical status and until the recovery of laboratory finding(s), or until stabilization of worsened clinical health status. Follow-up of the patient continues even in case, when SAE exceeds the duration of study.

9. Criteria for premature termination of the study

9.1. Termination of the study: - Completion of a \geq 1-year follow-up

- Relapse/systemic progression
- CNS relapse/progression
- Initiation of another antitumor therapy

9.2. Premature withdrawal of a subject

Circumstances that lead to a premature withdrawal of a subject from the study must be clearly recorded by the investigator on the appropriate CRF. Criteria for subject withdrawal include (but not limited to) death, toxicity, infection, non-compliance (including loss of subject for follow-up), voluntary withdrawal of patient or responsible physician, failure to meet the eligibility criteria, etc. Patients are free to withdraw from the study at any time without affecting their standard treatment. When a patient decides to withdraw from the study, he/she should always be contacted in order to obtain information about the reason for withdrawal and to record adverse events. Every effort should be made to contact a patient failing to come for scheduled visits (by phone or in a written form and record it into the source documentation). When the patient interrupts any contact with the study center, the patient is considered lost for follow-up.

9.3. Premature termination of the study

The sponsor reserves the right to stop the trial anytime. Investigators will be informed about this decision in a written form. The same applies to any investigator wanting to discontinue his/her participation to the trial. The investigator must immediately inform the sponsor in a written form about this decision.

10. Analysis of the study data

10.1. Description of statistical methods

Softwares STATISTICA[®] (version 9.1 or higher) and R: A language and environment for statistical computing will be used for statistical analyses. All statistical tests will be two-sided and performed at a significance level of 5%. There will be no corrections of significance level during multiple testing. No interim analysis is planned for the study.

10.2. Planned number of subjects

Suggested number of subjects is based on the primary objective, that compares cumulative incidence of CNS relapses in the group of patients treated with methotrexate i.v. and i.t. It is assumed, that the rate of CNS relapses will be 6% after one year follow-up in arm B (methotrexate i.t.). Prophylactic treatment in arm A (methotrexate i.v.) is expected to reduce CNS relapses to 0%. Significance level α was adjusted to 5% and power of the test $1-\beta = 80\%$. Fisher test anticipates 158 subjects into each arm of the study for evaluation of cumulative incidence of CNS relapses 6% vs. 0%. The planned number of recruited subjects was increased to 160 into arms A and B. Overall ~~320~~ 128 patients with intermediate and high risk of CNS relapse will be enrolled into arm A or B for clinical evaluation. Moreover, it is suggested, that ~~180~~ 72 patients with low risk (without optimization of the sample size) will be concomitantly automatically enrolled into arm C. A total number of subjects enrolled into the clinical evaluation will be ~~500~~ 200.

10.3. Significance level

Significance level of $\alpha = 5\%$ will be used for final evaluation.

10.4. Procedures for accounting and reporting of missing, unused, and spurious data

No other techniques for addition of missing data will be used.

10.5. Procedures for reporting of deviations from original statistical plan

Any deviations from original statistical plan will be described and justified in the final report of clinical evaluation.

10.6. Selection of patients not included into the statistical analysis

Statistical analysis will be performed in the subgroups defined and stated below

Analysis of the whole group	All patients recruited into the protocol
Analysis of the group	All patients recruited into the protocol without documented major

according to the protocol	deviations per protocol
Safety analysis	All patients, that received at least one cycle or part of the study medication

Major deviations, that can lead to exclusion of the patient from the analysis per protocol:

- Failure to undergo randomization procedure in patients with intermediate or high risk of CNS relapse or with occult meningeal involvement;
- Initial reduction of chemotherapy;
- Application of any non-protocol treatment during the first line therapy.

10.7. Working hypothesis of the project

The aim of the project is to compare the efficacy of CNS prophylaxis using 2 cycles of methotrexate 3g/m² i.v. vs. 6 doses of intrathecal methotrexate 12mg in patients with DLBCL and intermediate or high risk of CNS relapse or with occult meningeal involvement and comparison of these subgroups with a low risk group of CNS relapse without CNS prophylaxis. Hypothesis assumes 6% occurrence of CNS relapses during one year, lowered (0%) incidence of CNS relapses in arm A with i.v. methotrexate and no influence on CNS relapses in the arm with i.t. methotrexate.

10.8. Data collection and analysis

After the informed consent form is signed by the patient and he/she will be registered in the databasis of CLSG in the study center. All these data will be centrally analyzed at the end of the study.

Planned number of recruited patients is ~~500~~ 200, and ~~320~~ 128 of them are anticipated to receive CNS prophylaxis: they will be randomized in 1:1 manner either into arm A with 2 doses of methotrexate 3g/m² i.v. or into arm B with 6 doses of intrathecal methotrexate 12mg during ~~3~~ 9 years (2015-~~2017~~-2023). In patients randomized to the arm with intrathecal methotrexate

administration for whom intrathecal administration is not possible for technical reasons, the attending physician will decide whether to remain without prophylaxis or receive prophylactically intravenous methotrexate.

Number of complete and partial remissions, stable diseases and progressions will be analyzed at the end of the first-line treatment. Patients will be observed during the follow-up in ~~2018~~ 2024 to detect CNS and/or a systemic relapse.

Primary endpoint: Comparison of cumulative incidence of CNS relapses in patients treated with CNS prophylaxis will be evaluated by Fisher test.

Secondary endpoints: Comparison of CR rates will be analyzed by Fisher test. Rate of CNS relapses will be evaluated in the group of patients without CNS prophylaxis as well. Comparison of 3 and more subgroups will be calculated by M-L Chi-square test.

OS and PFS will be calculated for all enrolled patients and for each risk subgroup. Final analyses of PFS and OS of the whole group and risk subgroups and the significance of occult meningeal involvement on the risk of CNS relapse is anticipated in December ~~2018~~ 2024. PFS and OS of the whole group will be calculated by Kaplan-Meier method and the subgroups will be analyzed by log-rank test. Cox model will be used for evaluation of prognostic significance of occult meningeal involvement and other risk factors for CNS relapse.

11. Responsibilities of investigators

The investigators are responsible for realization of the study in accordance with principles of good clinical practice, as stipulated by regulations of the Czech Republic. Investigators are responsible for correct and continuous completion of patients' CRFs, that were created for recording of all observations and data used for clinical research. Electronic CRFs were created for this study. All centers will receive instructions for correct completion of CRF data.

12. Ethical and regulatory principles

12.1 Ethical principles

This protocol is created in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and its amendments laid down by the 29th (Tokyo, 1975), the 35th (Venice, 1983) and the 41st (Hong Kong, 1989) World Medical Assemblies.

12.2 Laws and regulations

This protocol is performed in accordance with laws and regulations of the Czech Republic as well as with other standards of good clinical practice.

13 Administrative procedures

13.1 Secrecy agreement

All materials, information (oral or written) and unpublished documentation provided to the investigators including this protocol, the patient case report forms are the exclusive property of the sponsor of the study. They may not be given or disclosed by the investigator or by any person without the prior written formal approval of the study coordinator. The investigator shall consider the study confidential and shall take all necessary measures to ensure that there is no breach of confidentiality in respect of all information accumulated, acquired or deduced in the course of the study, other than that information to be disclosed by law.

13.2. Record retention in investigating centers

Laws and regulations of the Czech Republic determine the maximal period of data retention. Trials performed across the European Union should retain patient's study data at least for 15 years after the completion or discontinuation of the trial. Each center will notify the sponsor before destroying any data or records.

13.3. Ownership of data and use of the study results

Sponsor has the ownership of all data and results collected during this study. Sponsor has the right to use the data for presentation of the study, either in the form of case report forms or in the form of a report, with or without comments and with or without analysis, in order to submit them to the health authorities.

13.4. Publication policy

All study data and publications are the property of the sponsor. Study results will be published after data collection and evaluation. Interim results can be published before the final analysis. Any presentation of data - oral, poster or publication must be approved by the sponsor. The final report will include at least coordinator of the study, statistician, investigators according to the number of enrolled patients. Names of co-authors should change if several publications are performed.

13.5. Insurance

The insurance has been procured for patients according to the current Czech laws and regulations. The insurance company for this study is HDI Hannover Versicherung A.G. – organizational subdivision, that resides at the address Jugoslávská 29, 120 00 Prague 2, number of insurance policy 2.003.716.

13.6. Audit

The State Institute for Drug Control (SÚKL) may conduct a site audit or an inspection in any study center to ensure the compliance with good clinical practice and regulatory agency guidelines. By signing this protocol, the investigator agrees to allow the study coordinator and its representatives and SÚKL to enable a direct access to the study records for review. These personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information. The aim of audit is to review source documentation supporting adequacy and accuracy of data gathered in CRF, review of documentation required to be maintained, and review drug accountability.

13.8. Final report of the clinical study

Study coordinator is responsible for preparation and processing the final report.

13.9 Protocol amendments

Protocol amendments are integral parts of the protocol and are attached at the end of protocol. Changes or amendments of this protocol may be made only after discussion and agreement of all investigators, study coordinator and the sponsor. Any changes agreed upon will be recorded in written form, the written amendment will be signed by the investigators and by the sponsor and the signed amendment will be added to the protocol. Approval of amendments by multicentric ethics committee is required prior to their implementation to this protocol.

In some cases the change of protocol requires change of the informed consent of the patient. Before implementation of informed consent amendment a new signed approval of the multicentric ethics committee is required. If there is a change in the CRFs, these changes are required to add as a part protocol amendment. Protocol amendment must be submitted to the State Institute for Drug Control prior its implementation into practice.

14. Literature

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Appendix A. miRNA examination

Operating procedure in the hematological laboratory

All samples will be labeled by name of the study (CLS-CNS-01), by a code of participating center (VFN, FNKV, HK, Brno, Ostrava, Pilsen), unique code of a patient. date of sample collection and type of a sample (pellet, cell-free, serum, plasma).

Processing of CSF indicated for research

1. CSF indicated for research (labeled with miRNA) will be centrifuged (500x g/10min. at +4°C):
2. CSF supernatant – it means cell-free CSF approximately 1ml will be removed by a pipette and transferred into a cryovial 2ml (Eppendorf vials are permitted). The vial should be properly labeled and put into a freezer at -80°C – into the CSF-cell-free box.
3. CSF pellet (it means a layer of cells on the bottom of the vial after centrifugation) shall be removed by a pipette into a cryovial 2ml (Eppendorf vials are permitted). The vial should be properly labeled and put into a freezer at -80°C – into a CSF- pellet box.

Processing of serum and plasma indicated for research

4. Vial containing gel with peripheral blood and another vial with EDTA and peripheral blood should be centrifuged at 1500 x g /15min. at room temperature.
5. Serum (2ml) will be removed from the vial containing gel and transferred into a cryovial 2ml (Eppendorf vials are permitted). The vial should be properly labeled and put into a freezer at -80°C – into a Serum box.

6. Plasma (2ml) will be removed from the vial containing EDTA and transferred into a cryovial 2ml (Eppendorf vials are permitted). The vial should be properly labeled and put into a freezer at -80°C – into a Plasma box.

7. Cryopreserved samples indicated for miRNA examination will be registered **in a table** in each study center **according to workplace habits**. The samples indicated for miRNA examination will be shipped and **tables(in Exeell) lists with sampling dates and labels** from each center will be submitted before miRNA examination to the Institute of Pathophysiology, 1. LF UK in Prague.

Contact address for sample shipment:

Vitek Pospisil, Msc, PhD.

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Appendix B. Schedule procedures table

Examination	Screening d-28 - d-1	R CHOP/DA EPOCH R DI cycles 1-6	Methotrexate 3g/m ² i.v. (7)	Methotrexate 12mg i.t. (8)	Restaging after 3cycles R CHOP/DA EPOCH R	Restaging at the end of therapy	CNS relapse	Systemic relapse	Follow-up during the 1. 2 year after treatment (6,12, 18,24 m.)	Follow-up during the 2- 4 year after treatment (24,36,24 m.)
Informed consent	x									
History	x									
Physical examination	x	x	x	x	x	x	x	x	x	*
Blood count+diff.+retic.	x	x	x	x	x	x	x	x	x	*
Biochemistry (1)	x	x(2)	x(2)	x(2)	x(2)	x(2)	x(2)	x(2)		
Pregnancy test	X									
aaIPI, classical IPI	x									
Echokg, ECG	x									
Bone marrow examin. (3)	x							x		
PET/CT or CT	x				x	x	x	x		
MRI or MRS of brain (4)	x						x	x		
CSF (5)	x		x(9)	x(9)			x	x		
miRNA in CSF	x		x(9)	x(9)			x	x		
mi RNA in plasma & serum	x		x(9)	x(9)	x	x	x	x		
Randomization (6)	x									
Survival status									x	*
Toxicity		x	x	x	x	x	x	x	x	*
Adverse events		x	x	x	x	x	x	x	x	*

Legend:

- (1) Screening biochemistry examination- serum (Na, Cl, K, Ca, Mg, P, urea, creatinine, uric acid, AST, ALT, total bilirubin, ALP, LDH, glucose, total protein, albumin), serology (HBsAg, anti-HBC, anti-HIV1,2, beta2microglobulin);
- (2) Before each cycle of chemotherapy: Na, Cl, K, Ca, Mg, P, urea, creatinine, uric acid, AST, ALT, total bilirubin, ALP, glucose;
- (3) Bone marrow examination: cytology-aspiration, flow cytometric examination of bone marrow, histology;
- (4) MRI or MR spectroscopy of the brain, eventually of the spine and neurological examination in patients with intermediate or high risk of CNS relapse, in patients with occult meningeal involvement, suspected or clear CNS involvement.
- (5) Examination of CSF: cytology, biochemistry, flow cytometry, and in case of inconclusive IgVH examination. **For technical reasons, screening lumbal puncture (without i.t. application) can be performed on day 1 of 1st immunochemotherapy cycle (prior to randomization). If the patient is randomized to an i.t. application of methotrexate, the lumbal puncture will be completed during the first cycle of treatment with an interval 1-2 weeks.**
- (6) Randomization of patients with intermediate and high risk of CNS relapse, in patients with occult meningeal involvement.
- (7) In arm A only: **after 3- & 6.cycles of chemotherapy R-CHOP or DA-EPOCH-R before 1st dose of R+MTX i.v. and before 2nd dose of R+MTX i.v.**
- (8) In arm B Day1 of each chemotherapy cycle R-CHOP or DA-EPOCH-R.
- (9) In arm A concomitantly with the second dose of i.v. methotrexate and in arm B concomitantly with the last i.t. application of methotrexate.

Appendix C: Information for a patient

Study evaluating relapses in central nervous system in patients with diffuse large B-cell lymphoma treated with chemotherapy with or without CNS prophylaxis

Dear Madam, Dear Sir,

Diffuse large B-cell lymphoma (DLBCL) has been diagnosed in your organism. Your physician has informed you about necessary examinations before and during the treatment and discussed with you the suggested therapy and prognosis of the disease. As it was explained to you, lymphoma occurs preferentially in lymph nodes but it could occur in any organ. One of these organs is central nervous system (CNS), in other words: brain, spine, meninges. The probability of lymphoma incidence in CNS is low, however, some factors exist, that predispose to its increased risk, but even in such cases the incidence is around 6%. Currently, there is no consensus worldwide regarding most important factors defining the risk of CNS relapse, likewise, there is no consensus regarding the use and type of prophylaxis to prevent the relapse. The latest study has shown, that factors associated with higher risk of CNS relapse include: age > 60years, elevated lactate dehydrogenase in the peripheral blood > reference range, clinical stage III/IV, poor performance status evaluated according to ECOG scale > 1, involvement > 1 extranodal organ, kidney or adrenal gland involvement. There is a consensus worldwide, that patients with low risk of CNS relapse do not need any special measures, as the risk of CNS relapse is substantially lower than in other organs. Several possibilities of prophylaxis are investigated in patients with higher risk of CNS relapse. Administration of methotrexate is one of these possibilities, as this drug is efficacious in CNS involvement. This study compares two various ways of methotrexate application - either intrathecal (via lumbar puncture) or systemic (intravenous). If you have more than 1 of the above mentioned risk factors, we will give you cytostatics-methotrexate either intravenously or it will be administered directly to the cerebrospinal fluid by lumbar puncture (intrathecal application) to prevent CNS relapse. Decision, which form of CNS prophylaxis you will receive, will be realized in a randomized manner- it means you will be allocated by chance into one of two groups (randomization ratio 1:1). The probability of allocation into one of two arms is equal for all patients. The organization of the trial enables to compare two possibilities of CNS prophylaxis. Comparison of the treatment groups is provided on the random principle. If you don't have any of above mentioned risk factors or only one risk factor, you will not receive any form of CNS prophylaxis.

All patients regardless whether they are enrolled into the study or not are treated with standard immunochemotherapeutic protocol, that consists of targeted antibody rituximab treatment and systemic chemotherapy combining several cytostatics.

The primary goal of the trial is to determine which of two options is more effective in CNS prophylaxis in the group of patients with higher risk: either two doses of intravenous methotrexate 3g/m² or 6 doses of methotrexate delivered by lumbar puncture (intrathecal application). The secondary goal is to analyze the rate of CNS relapses in patients with low risk of CNS relapse and without CNS prophylaxis.

Study procedures

Initial examination program has to define the extent of lymphoma disease- DLBCL and to collect all necessary information for planning of directed treatment correctly. Initial collection of cerebrospinal fluid (CSF) is a part of this initial examination, standard examination of CSF includes: biochemistry, cytology, flow cytometry, and by voluntary decision of the center also IgVH rearrangement and assessment of somatic mutations L265P MYD88 a Y196 CD79B and we ask you to approve the molecular biology examination of a part of your CSF - especially for presence of micro RNA (miRNA), that could be more sensitive for CNS involvement, and your peripheral blood, that was collected during the standard diagnostic program, will be examined similarly. Maximal period of sample storage (CSF, plasma, serum) for miRNA examination is until the termination of the study, it means until December 31, ~~2018~~ 2024. By all means you will be treated with a standard immunochemotherapeutic regimen as it is used in the health care facility and as your treating physician informed you. After evaluation of all risk factors for CNS relapse, that include: age over 60 years, elevated lactate dehydrogenase in the blood over the reference range, clinical stage III/IV, poor initial performance status, involvement more than 1 extranodal organ, initial kidney or adrenal gland involvement, your treating physician has to inform you whether you belong to the group with higher or lower risk of CNS relapse. In case of higher risk you will be randomly (by chance) allocated either into arm A, where you will receive one dose of methotrexate 3g/m² intravenously after the 3rd and after the 6th cycle of standard systemic immunochemotherapy (it means you will receive 2 doses during the study), or into arm B where you will receive intrathecal methotrexate during each cycle of systemic immunochemotherapy (it means 6x). Low risk patients will receive systemic immunochemotherapy without methotrexate prophylaxis. If clinical and laboratory findings indicate low risk of CNS relapse, lumbar puncture with CSF

collection is not a standard, however, we cannot absolutely exclude, that meningeal involvement can occur even at diagnosis.

A possibility exists, that initial diagnostic examination of CSF will produce inconclusive results, it means, that it does not indicate a clinical involvement of CNS, but occult involvement cannot be excluded. In this case of occult meningeal involvement application of methotrexate (randomly assigned either into intravenous or intrathecal arm) will be offered to you as in cases with higher clinical risk of CNS relapse.

In case of proven lymphoma cells in CSF indicating CNS involvement a specific standard therapy will be offered to you. Final evaluation of results will be performed at the end of treatment and you will be observed during the follow-up. Collection of CSF will be no longer performed unless it is indicated due to your health status.

Except of standard collection of peripheral blood another 10ml of peripheral blood will be collected after 3 cycles of chemotherapy and at the end of treatment to evaluate the presence of miRNA.

Expected benefits for patients participating in the study

The study will permit to:

- validate, which strategy of CNS prophylaxis is more effective in reducing the incidence of CNS relapse in patients with its higher risk: either 2 cycles of high dose methototrexate or 6 intrathecal applications of methotrexate;
- evaluate the incidence of CNS relapse in low risk patients without CNS prophylaxis;
- evaluate, whether some CSF parameters could be more sensitive and more specific in predicting CNS relapse than standard clinical and laboratory tests.

Experimental examination procedures used in this study (molecular-biology and clearly defined FCM examination) could become a standard initial diagnostic tool in the future.

You should take into consideration, that the experimental diagnostic procedures performed within this study have no direct influence on you, as evaluation of results will ve performed after the end of your treatment.

There is absolutely no guarantee, that CNS relapse will not occur in your case despite the fact, that you will receive prophylactic high dose methotrexate i.v. or intrathecal methotrexate.

Information resulting from the study can help patients, that will come after you – similarly to the situation, that you can profit from older studies, that helped to find the best current standard chemotherapy.

The administered treatment can cause damage of fertility and menstruation (period). Damage of male fertility is usually permanent, female damage of fertility could recover after certain period since the end of therapy. Due to the possible risks mentioned above a double- barrier contraception is recommended for all male and female patients since the beginning of therapy until one year after the end of therapy.

Voluntary participation and the right to withdraw from the trial

Your participation in the trial is absolutely voluntary and you will have time enough for decision to participate in this study or not. Moreover, you can withdraw from the study anytime without statement of the reason. Withdrawing from the study would have absolutely neither influence on your further therapy nor on behaviour of the healthcare personal to you.

Compensation for the subject of evaluation

Your participation in the trial is voluntary and no financial compensation is planned.

Insurance

Patients will be insured during treatment and follow-up (for 4 years) – according to the current Czech legal laws and regulations, the insurance company for this study is HDI Hannover Versicherung A.G.- organizational subdivision, that resides at the address Jugoslávská 29, 120 00 Praha 2 (detailed information about the conditions of insurance can be obtained from your physician before the start of treatment), number of insurance policy 2.003.716. In case of health injury related to your participation in the study you are entitled to ask for compensation of injury according to the valid legal regulations in the Czech Republic.

Risks associated with the participation in the study

The most common complication associated with lumbar puncture is the overall feeling of discomfort and headache. High doses of methotrexate can lead to higher risk of hepatic and renal impairment.

The examination methods (collection of CSF, etc.) and treatment methods (immunochemotherapy, high dose of methotrexate, intrathecal application of methotrexate) are not specific for this study and you will undergo them during the standard treatment, you will be informed about their risks in a standard way concomitantly with obtaining the approval of informed consent valid for each healthcare facility. The study does not produce any new (or other) risks, when compared to situation if treated outside the study.

Personal data protection

The study anticipates to collect information about you and your disease that will be recorded in your medical files. If you decide to participate in the study, you will be asked to sign a written approval. We want to stress, that all data recorded with your name are confidential and they will be handled in conformance with the valid legal regulations in the Czech Republic. Your medical data will be encoded, it means that they will be labeled only by a numerical code and not by your name in the study. It is very important to collect the required data in the full extent, it means an occasional inspection of your medical records. Authorized persons only conducting this study (investigators), members of relevant ethics committee of your hospital, monitors and auditors and representatives of relevant regulatory institution (e.g. the State Institute for Drug Control) may have access to your medical data. All data are strictly confidential and your identity will never be disclosed.

Appendix D. Informed consent

Study evaluating relapses in central nervous system in patients with diffuse large B-cell lymphoma treated with chemotherapy with or without CNS prophylaxis

I have been properly and clearly informed by my treating physician about the goals of this study, its duration, possible problems and the risks of this clinical study. I have read *Information for a patient* carefully and I understand it. All my questions have been answered clearly and satisfactorily in details.

Data collected about my person are strictly confidential and will be processed in conformance with the valid legal regulations in the Czech Republic. Data can be used for research purpose only.

I will keep the instructions of the physician, that are necessary for the study execution, however, I reserve the right to withdraw from the study anytime without giving any explanation and without any sanction arising from study withdrawal.

My signature approves the authorized persons conducting this study and representatives of relevant regulatory institutions, that are responsible for conducting this study, to access and inspect my medical data in order to validate data obtained during this clinical trial.

I have received an original of *Information for a patient and Informed consent of a patient with the study recruitment*.

Place and date of informed consent:

Date of birth, name, surname and signature of a patient:

Treating center (stamp):

Name, surname and signature of a physician providing information:

Appendix E Study table- attached separately