

Sponsor Protocol N°: IGR2009/1593

EudraCT N°: 2010-019224-31

**INTERGROUP TRIAL FOR CHILDREN OR ADOLESCENTS
WITH B-CELL NHL OR B-AL: EVALUATION
OF RITUXIMAB EFFICACY AND SAFETY IN HIGH RISK
PATIENTS**

European Intergroup for Children Non Hodgkin's Lymphoma (EICNHL)
and
Children Oncology Group (COG)

Abbreviated Title of Protocol: Inter-B-NHL ritux 2010
Version n°4.0 – Date: December 14th, 2015

COORDINATING INVESTIGATORS	Véronique Minard-Colin (SFCE) & Thomas Gross (COG)
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Version 4: The randomisation is halted in phase III after the first interim analysis on November 20th 2015. The study continues as a single arm to answer to the secondary objective questions

SIGNATURE PAGE

**« Intergroup trial for children or adolescents with B-Cell NHL or B-AL:
evaluation of Rituximab efficacy and safety in high risk patients»**

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Version n°4.0 December 14th, 2015

Investigator center:

Department:

Name and address of center:

I, Dr/Pr_____, certify that I have read the entire protocol entitled “Intergroup trial for children or adolescents with B-Cell NHL or B-AL: evaluation of Rituximab efficacy and safety in high risk patients” and I agree to conduct the study according to this protocol and to comply with requirements subject to ethical and safety considerations.

Date:

Signature:

ABBREVIATIONS

Abbreviation	Term
ANC	Absolute Neutrophil Count
Ara-C	Aracytine = Cytarabine
ASH	American Society of Hematology
B-AL	Mature B-cell Acute Leukemia = Burkitt AL= L3 AL = B-ALL
BFM	“Berlin-Frankfurt-Münster”
BM	Bone Marrow
CHOP	Cyclophosphamide, Hydroxydaunorubicin (doxorubicin), Oncovin (vincristine), Prednisone
CNS	Central Nervous System
CPM	Cyclophosphamide
CSF	CerebroSpinal Fluid
COG	Children Oncology Group
COP	Cyclophosphamide, Oncovin (vincristine), Prednisone
COPADM	Cyclophosphamide, Oncovin (vincristine), Prednisolone, Adriamycin (doxorubicin), Methotrexate
CR	Complete Remission
CTC	Common Terminology Criteria
CYM	CYtarabine (Aracytine, Ara-C), Methotrexate
CYVE	CYtarabine (Aracytine, Ara-C), VEposide (VP16)
DA-EPOCH-R	DA-EPOCH with Rituximab
DA-EPOCH	Dose-Ajusted EPOCH
DLBCL	Diffuse Large B-Cell Lymphoma
EFS	Event Free Survival
EICNHL	European Intergroup for Childhood Non Hodgkin’s lymphoma
EPOCH	Etoposide, Prednisone, Oncovin (vincristine), Cyclophosphamide Hydroxyldaunorubicin (doxorubicin)
GFR	Glomerular Filtration Rate
HBV	Hepatitis B Virus
HC	Hydrocortisone
HD	High Dose
HIV	Human Immunodeficiency Virus
Ig	Immunoglobulin
IT	IntraThecal (injection)
IV	Intra Venous
LDH	Lactate DeHydrogenase
LMB	“Lymphome Malin B”
m	Maintenance
MALT	Mucosa-Associated Lymphoid Tissue

MDD	Minimal Disseminated Disease
MRD	Minimal Residual Disease
MRI	Magnetic Resonance Imaging
MTX	Methotrexate
NCI	National Cancer Institute
NHL	Non-Hodgkin's Lymphoma
OS	Overall Survival
PA	Posterior Anterior
PET(-CT)	Positron Emission Tomography (- Computed Tomography)
PFS	Progression Free Survival
PLM	Progressive Multifocal Leukoencephalopathy
PMLBL	Primary Mediastinal Large B-Cell Lymphoma
RR	Response Rate
SFCE	Société Française de lutte contre les Cancers et leucémies de l'Enfant et de l'adolescent
SFOP	Société Française d'Oncologie Pédiatrique (=French Society of Pediatric Oncology)
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
TIT	Triple Intrathecal Therapy (3 drugs are injected intra thecally)
VCR	Vincristine
VP16	Etoposide

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The COG centers are localized in USA, Canada and Australia.

The study was activated in COG on June 11th, 2012.

Additional annexes (from J to Q) are dedicated to the COG participating sites.

1.1.3. Japanese group

Japanese group cannot participate in the study due to drug supply issue. However, the Japanese group will conduct a parallel study and a meta analysis will be done with the Inter-B NHL Ritux 2010 data.

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2. SYNOPSIS

Identification	Sponsor Protocol N°: IGR2009/1593 EudraCT N°: 2010-019224-31 Version n°4.0 December 14 th , 2015
Title	Intergroup trial for children or adolescents with B-cell NHL or B-AL: evaluation of Rituximab efficacy and safety in high risk patients
Abbreviated Name	Inter-B-NHL ritux 2010
Study patients	Phase III trial: Untreated children or adolescents with stage III and LDH > Nx2 or stage IV B-cell NHL or B-AL (Burkitt AL or L3 AL) Phase II trial: Untreated children or adolescents with PMLBL
Coordinating sponsor	Gustave Roussy 114 rue Edouard Vaillant 94805-Villejuif cedex France
Coordinating Investigators	Véronique Minard-Colin and Thomas Gross
Participating Group/National coordinating Investigator	Italy : AIEOP (Associazione Italiana di Ematologia ed Oncologia Pediatrica) / M Pillon Belgium : BSPHO (Belgian Society of Paediatric Haematology and Oncology) / A Uyttebroeck UK : UK NCRI CCL CSG (UK National Cancer Research Institute Children's Cancer and Leukaemia Clinical Studies Group) / A Burke Netherlands : DCOG (Dutch Childhood Oncology Group) / J Zsiros Hungary : Hungarian Society of Pediatric Oncologist and Pediatric Hematologist / C Csoka Poland : PPLLSG (Polish Paediatric Leukemia/Lymphoma Study Group) / B.Kazanowska Spain : SEHOP (Sociedad Española de Hematología y Oncología Pediátricas) / R Delgado France : SFCE (Société Française des Cancers de l'Enfant) / V Minard-Colin Hong Kong : HKPHOSG (Hong Kong Paediatric Haematology and Oncology Study Group) / A Chiang North America and Oceania : COG (Children's Oncology Group) / T Gross. COG will coordinate centers localized in USA, Canada and Australia.
Rationale	Rituximab (antiCD20) in association with chemotherapy has extended the survival of adult patients with diffuse large B-cell lymphoma (DLBCL) and become the standard treatment of B-cell lymphoma in adults. However, there has never been a definitive clinical trial evaluating efficacy and safety of rituximab added to chemotherapy for childhood B-cell lymphoma. Results from adult B-lymphoma cannot be assumed to apply to children because of differences in the biology of childhood DLBCL and because >75% of childhood B lymphoma are Burkitt type, where efficacy of rituximab has never undergone definitive evaluation (either in adults or children). Because Event Free Survival (EFS) is already high in children with the current intensive chemotherapy regimen, and because rituximab is an expensive medication which could produce potential severe side effects (e.g. prolonged lymphoid B-cell depletion), a large randomized trial is necessary to evaluate whether rituximab can add benefit to the current chemotherapy regimen. Two pilot studies in children provide preliminary evidence of safety and activity of rituximab in this disease setting that support such a study. A Berlin-Frankfurt-Münster group (BFM) study, which tested a single dose of rituximab administered prior to chemotherapy, has shown tumor responses. A COG pilot study tested the safety and tolerability of the combination of rituximab with LMB chemotherapy and showed no increased short term toxicity. While EFS is 90% with either with the LMB or the BFM regimen, EFS of stages III with LDH level \geq Nx2 and of stages IV & B-AL is around 84%, indicating a need for therapeutic improvement and identifying a higher risk

	<p>population in which to evaluate the potential benefit of rituximab. Primary mediastinal large B-cell lymphoma (PMLBL) constitutes another high risk group, shown to have a worse outcome in the previous paediatric studies. In adults, there is accumulating evidence of a benefit of Rituximab in this disease including a National Cancer Institute (NCI) study reported 100% Progression-Free Survival (PFS) with a rituximab plus DA-EPOCH regimen. Due to these compelling results in adults, and because PMLBL is understood to be the same disease in children and adults, rituximab must be considered in its treatment. Nonetheless, while it is not deemed appropriate to randomize patients to a study arm without rituximab, it is important to evaluate a rituximab-based regimen in the setting of a phase II clinical study in order to establish the safety profile and EFS in children. Although PMLBL will be treated on a separate phase II study, this is included in the same protocol with the phase III study for feasibility because of the small population and because all the patients have forms of B-cell lymphoma and will be treated and followed in a similar manner.</p> <p><u>November 2015:</u> Based on the first interim analysis indicating a superior event free survival in the arm with rituximab, the randomization was halted on November 20th 2015. This amendment is to continue the study with a single arm combining rituximab and chemotherapy in order to be able to answer to secondary objectives.</p>
Objectives	<p><u>PRIMARY OBJECTIVES</u></p> <ul style="list-style-type: none"> ▪ Phase III study: For the patients with advanced stage B-cell NHL/B-AL (stage III and LDH > Nx2, any stage IV or B-AL) to test whether adding 6 injections of rituximab to standard LMB chemotherapy regimen improves the EFS compared with LMB chemotherapy alone. <p><u>November 2015:</u> the first interim analysis allowed to answer positively.</p> <ul style="list-style-type: none"> ▪ Phase II study: To determine the efficacy of DA-EPOCH-R in children and adolescent PMLB in terms of EFS. <p><u>SECONDARY OBJECTIVES</u></p> <ul style="list-style-type: none"> ▪ In Phase III and phase II studies: <ul style="list-style-type: none"> - To study the complete remission (CR) rate and the overall survival (OS). - To evaluate safety on all study arms: including toxic deaths, adverse events recorded using the NCI-CTC V4 (non haematological toxicity grade ≥3, infections grade 3 to 5), cardiac toxicity (CTC grade 2-5 and evolution of left ventricular ejection fraction and left ventricular shortening fraction), number of days with platelets transfusion, number of days with red cells transfusion, rituximab infusion reactions and intensive care unit admission. - To study the rate of patients with Ig (IgM, IgA, IgG) level abnormally low and lymphocyte count abnormally low at 1 year and until 5-year follow-up, and to study the need for immunoglobuline infusions and levels of post (previous and re-)vaccination antibodies at 1 year. ▪ In Phase III study: <ul style="list-style-type: none"> - To study long term (at least 5 years) risks of the use of rituximab plus chemotherapy compared with LMB chemotherapy alone in children and adolescents with advanced stage B-NHL/B-AL (all events related (certain and probable) to therapy). <p><u>November 2015:</u> a longer follow up and a greater number of patients are necessary to evaluate the secondary objectives.</p> <p>This will be studied in patients prospectively registered in the single arm treatment combining chemotherapy and rituximab study until a total of 300 patients treated with rituximab as initially planned.</p> <ul style="list-style-type: none"> ▪ In Phase II study: <ul style="list-style-type: none"> - To study the long term risk of DA-EPOCH-R regimen, especially the cardiac risk related to doxorubicin given at higher dose than usual in children, but infused over 96h (i.e. evaluation of CTC grade 2-5 and

	<p>evolution of left ventricular ejection fraction and left ventricular shortening fraction).</p> <p>EXPLORATORY SECONDARY OBJECTIVES (not done in all countries)</p> <ul style="list-style-type: none"> ▪ In phase III and phase II studies: <ul style="list-style-type: none"> - To obtain data on PET(-CT) in childhood pediatric B-cell NHL. ▪ In phase III study: <ul style="list-style-type: none"> - To evaluate the potential prognostic value of Minimal Dissiminated Disease (MDD) and Minimal Residual disease (MRD) in correlation with outcome. - To perform an economic study comparing the cost-effectiveness ratio between 2 therapeutic strategies: LMB chemotherapy with versus LMB chemotherapy without Rituximab. <p><u>November 2015:</u> this economic study is halted paralely to the halt of the randomisation</p> <ul style="list-style-type: none"> - To characterize the pharmacokinetics of rituximab in combination with LMB chemotherapy in a subset of patients.
Study Design	<p>Prospective Phase-III, multicentre (intergroup) open-labelled, randomised trial for advanced stage B-cell NHL/B-AL, except PMLBL, treated according to a LMB scheme</p> <p><u>November 2015:</u> the randomization is halted. All patients are treated according to the arm with rituximab.</p> <p>Phase II single arm study for PMLBL with the DA-EPOCH-Rituximab regimen</p>
Number of patients	<p>Phase III study- non PMLBL patients: 600 eligible patients are required, randomized 1:1 into two treatment arms (i.e. 300 patients in each arm). Expected accrual period = 4-5 years.</p> <p><u>November 2015:</u> the randomization is halted after the randomization of 362 patients, 181 in each arm. 120 other patients will be prospectively registered in the single arm treatment with rituximab.</p> <p>Phase II study - PMLBL patients: 47 eligible patients will be included in the single DA-EPOCH-R treatment arm. Expected accrual period = around 4 years.</p>
Diagnosis and criteria for inclusion:	<p>Children and adolescents aged less than 18 years with untreated advanced stage B-cell NHL or B-AL.</p> <p><u>INCLUSION CRITERIA:</u></p> <p><u>HISTOLOGY AND STAGING DISEASE</u></p> <p>Phase III study:</p> <ul style="list-style-type: none"> - Histologically or cytologically proven B-cell malignancies, either Burkitt lymphoma or B-AL (=Burkitt leukaemia = L3-AL) or diffuse large B-cell NHL or aggressive mature B-cell NHL non other specified or specifiable. - Stage III with elevated LDH level ("B-high"), [LDH > twice the institutional upper limit of the adult normal values (> Nx2)] or any stage IV or B-AL. <p>Phase II study:</p> <ul style="list-style-type: none"> - Histolo-cytologically proven PMLBL. - PMLBL without CNS involvement. <p><u>GENERAL CONDITIONS</u></p> <ul style="list-style-type: none"> - 6 months to less than 18 years of age at the time of consent. - Males and females of reproductive potential must agree to use an effective contraceptive method during the treatment, and after the end of treatment: during twelve months for women, taking into account the characteristics of rituximab and during five months for men, taking into account the characteristics of methotrexate. <p><u>INITIAL WORK-UP</u></p> <ul style="list-style-type: none"> - Complete initial work-up within 8 days prior to treatment. <p><u>OTHERS</u></p> <ul style="list-style-type: none"> - Able to comply with scheduled follow-up and with management of toxicity. - Signed informed consent from patients and/or their parents or legal

	<p>guardians.</p> <p><u>EXCLUSION CRITERIA</u></p> <p><u>HISTOLOGY AND STAGING DISEASE</u></p> <ul style="list-style-type: none"> - Follicular lymphoma, MALT and nodular marginal zone are not included into this therapeutic study. - In phase II study (PMLBL) patients with CNS involvement are not eligible. <p><u>GENERAL CONDITIONS</u></p> <ul style="list-style-type: none"> - Patients with congenital immunodeficiency, chromosomal breakage syndrome, prior organ transplantation, previous malignancy of any type, or known positive HIV serology. - Evidence of pregnancy or lactation period. - There will be no exclusion criteria based on organ function. <p><u>PRIOR THERAPY</u></p> <p>Past or current anti-cancer treatment except corticosteroids of less than 7 days duration in total</p> <p><u>EXCLUSION CRITERIA RELATED TO RITUXIMAB:</u></p> <ul style="list-style-type: none"> - Tumor cell negative for CD20 (absence of result due to technical problems in the presence of other characteristics suggestive of BL/DLBCL, including genetic and phenotypic features, is not an exclusion criteria) - Prior exposure to rituximab. - Severe active viral infection, especially hepatitis B. Severe infection (such as sepsis, pneumonia, etc..) should be clinically controlled at the time of randomisation. Contact the national co-investigator for further advice if necessary. - Hepatitis B carrier status history of HBV or positive serology.
Chemotherapy treatment	<p>Phase III - non PMLBL patients:</p> <p>Prephase (COP) for all groups followed by:</p> <ul style="list-style-type: none"> • group B: 4 courses: 2 COPADM + 2 CYM, with MTX 3g/m² • group C: 6 courses: 2 COPADM + 2 CYVE + 2 maintenance courses, with MTX 8g/m², in 4h in C1, in 24h in C3 (except the 1st course) <p>CNS + patients receive additional IT before each CYVE courses and HDMTX between CYVE courses.</p> <p>Folinic rescue after HDMTX is started at H24, except in C3 at H36 after 24h infusion HDMTX.</p> <p>Group B and C1 patients who have no tumor response at D7 are switched respectively to C1 and C3 treatment, without or with rituximab as initially assigned. Those in group B with histologically confirmed viable residual tumor after the course n°3 (1st CYM) are switched to C1 arm starting at CYVE n°1 without or with rituximab (only with the first CYVE) as initially assigned.</p> <p>November 2015: the randomization is halted. All patients receive rituximab.</p> <p>Phase II – PMLBL patients:</p> <p>6 courses of DA-EPOCH-R</p>
Rituximab	<p>Rituximab is given at a dosage of 375 mg/m² i.v.</p> <p>Randomized phase III-non PMLBL high risk advanced stage patients (LMB groups B and C): in the arm with Rituximab, 6 injections of Rituximab are given: two doses at 48h interval are given at D-2 and D1 of the 2 first courses (COPADM) and one dose at the beginning of the 2 following courses (CYM or CYVE). The “slow responders” randomised to receive rituximab and who are switched to group C after CYM1, will receive rituximab only with the first CYVE in order to receive a total of 6 courses.</p> <p>November 2015: the randomization is halted. All patients receive rituximab</p> <p>Phase II in PMLBL patients: 6 injections of Rituximab are given to all patients: one injection at each of the 6 courses of EPOCH.</p> <p>Roche will provide Rituximab.</p>
Statistical considerations	<p>Phase III - non PMLBL patients:</p> <p>Assuming one-sided 5% level of statistical significance, occurrence of 72 events will provide 90% power to detect an increase in the EFS long-term</p>

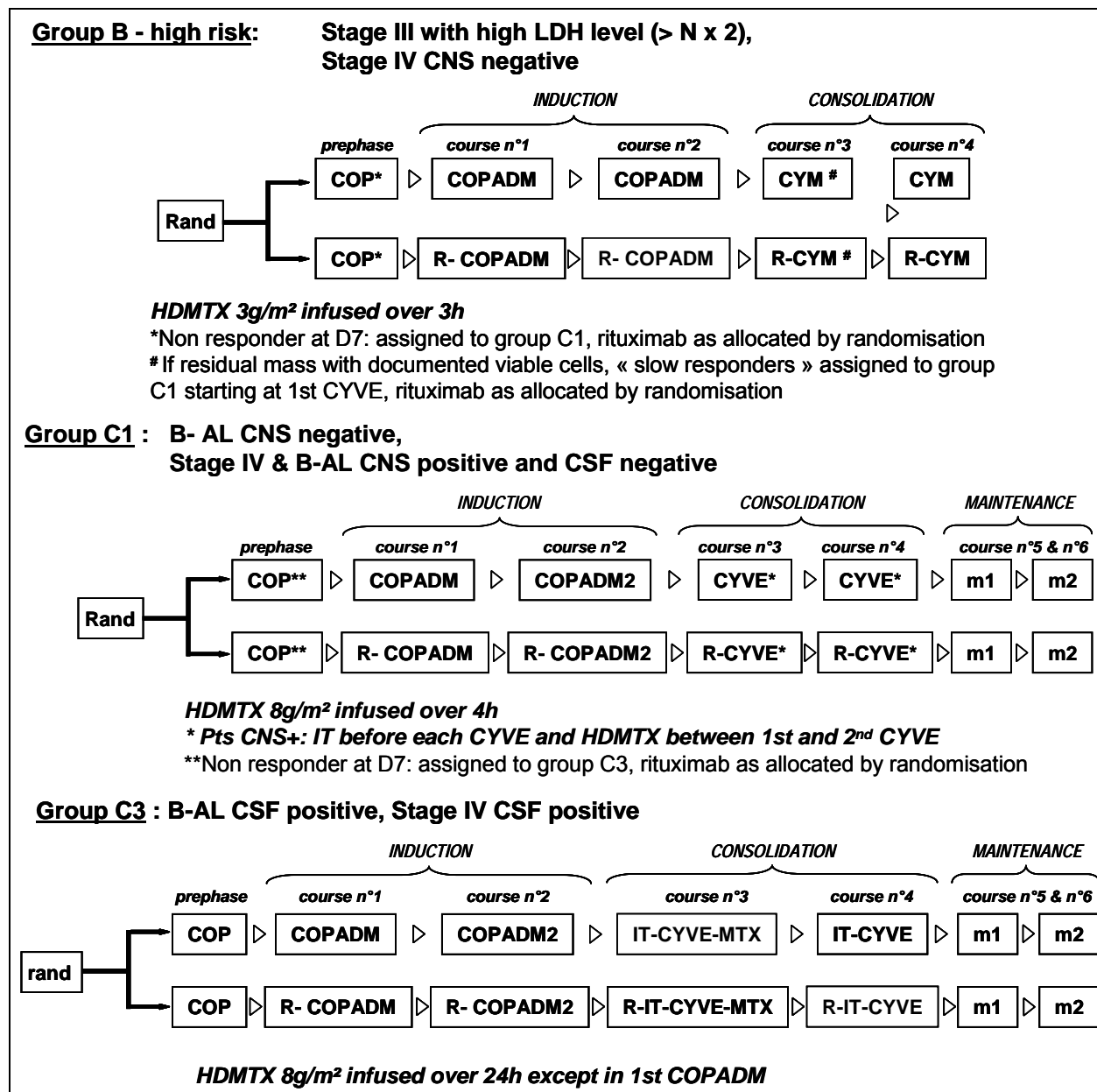
	<p>from 84% to 92% (corresponding to a 50% reduction in the risk of failure). A total of 72 events are expected out of 600 eligible randomized patients (300 per treatment arm). The enrolment rate for these patients is expected to be 130-150 patients per year, suggesting a total enrolment period of 4-5 years. Interim analyses for efficacy and futility will be performed approximately after 1/3 of EFS events and yearly thereafter.</p> <p>November 2015: The randomization is halted after the first interim analysis based on 27 events.</p> <p>Phase II – PMLBL patients:</p> <p>Historically, long-term EFS in pediatric PMLBL is of 67% with most events occurring in the first two years. We will compare the EFS with DA-EPOCH-R to a fixed outcome, reflecting the historical pediatric PMLBL experience: null hypothesis $EFS(t) = 0.67 + 0.33\{\exp(-1.5t)\}$ versus alternative $EFS^*(t) = [0.67 + 0.33\{\exp(-1.5t)\}]^R$, where R is less than 1.0, using a one-sample log-rank test. Testing will be done at the 10% level of statistical significance (1-sided). The sample of 40 patients will provide 90%, 80% power to detect a true long-term EFS of respectively 84.6% and 82.4%.</p> <p>The analysis will be done when all patients will be followed for at least 18 months. A single futility analysis, testing the hypothesis that the true event-free survival is $S^*(t) = [0.67 + 0.33\{\exp(-1.5t)\}]^{0.406}$ (corresponding to a long-term EFS of 85% will be performed once 6 events (half of the expected events under the null hypothesis) have been observed.</p> <p>The accrual duration will be of around 4 years.</p>
Duration of study	<p>Inclusion period: 4-5 years</p> <p>Follow-up period: 5 years</p> <p>Expected overall duration of study: 10 years</p>

3. STUDY DESIGN

3.1. HIGH RISK B-NHL AND B-AL: PHASE III

- For children/adolescents with B-cell lymphoma (except PMLBL) and stage III + LDH > Nx2, Stage IV or B-AL (= Burkitt leukaemia= L3-AL).
- Treatment according to LMB scheme.
- Randomisation assignment to not receive rituximab or to receive rituximab, 2 doses during the 2 first induction courses (COPADM) and one dose during the 2 consolidation courses (CYM or CYVE).

November 2015: the randomization is halted: all patients receive the arm with rituximab



3.2. PMLBL: PHASE II

All patients receive rituximab

6 courses of EPOCH with rituximab, with dose adaptation (DA) at each course based on previous course ANC nadir



Doxorubicin, VCR, VP16 infused over 96h, no IT, no HDMTX

3.3. DURATION OF PATIENT PARTICIPATION

Patients will participate between 5.3 and 5.5 years according to the treatment group (B, C or PMLBL). This includes a treatment period of 3.5 – 5.5 months and a 5 year follow up period.

3.4. DURATION OF THE STUDY

This study will be completed in approximately 10 years (accrual and treatment: 5 years, follow-up after treatment end: 5 years). The end of the study is the last follow up visit of the last treated subject, i.e. 5 years after the end of treatment of the last subject and approximately 10 years after the trial start.

3.5. NUMBER OF PATIENTS

This study will be conducted in approximately 350 centers.

Phase III: 600 patients will be randomized.

November 2015: 181 patients were randomized in each arm. After the stop of the randomization on November 20th, 120 patients will be included in the prospective single arm treatment. This number of patients corresponds to approximately one year of enrollment.

Phase II: 47 patients will be enrolled.

4. BACKGROUND AND RATIONALE

4.1. BACKGROUND IN CHILDHOOD AND ADOLESCENCE B-CELL LYMPHOMA (B-NHL) AND LEUKEMIA (B-AL)

4.1.1. LMB studies

Results

Since 1981, the French Society of Paediatric Oncology (SFOP) has conducted several consecutive multicentre “LMB” studies in France, and also 2 centres in Belgium and 1 in the Netherlands (Patte, 1986, 1990, 1991, 2001). The general scheme of the LMB protocols was the same along the studies: it begins with a prephase, called COP, with small doses of vincristine, cyclophosphamide, and prednisone, which induces a good tumour reduction and permits the management of metabolic or other acute problems without myelosuppression. The intensive induction phase starts one week later with two consecutive courses of COPADM based on fractionated high dose (HD) cyclophosphamide and HD methotrexate (MTX) in association with vincristine, adriamycin (doxorubicin), prednisone. The two consolidation courses are based on 5 days of continuous infusion of Cytarabine (Ara-C). CNS prophylaxis is given by HD MTX and intrathecal injections (IT) of MTX ± Ara-C. Maintenance therapy, made of 5 days monthly courses with the previously used drugs, varied along the studies and was progressively shortened.

The major conclusions of the LMB studies were as follows:

- While Event Free Survival (EFS) of Central Nervous system (CNS) negative advanced stages increased to 75-80% for patients during the 2 first **LMB 81 and 84** studies (Patte 1986, 1991), duration of treatment was progressively reduced from 12 to 5 courses, and toxicity, especially the toxic death rate, decreased parallel to the investigators' experience. CNS prophylaxis by HD MTX (3 g/m² in 3h infusion) and IT MTX was efficient with a CNS relapse rate <2%. It was shown that partial remission (with documented viable cells in the residual mass) after 3 courses had a poor outcome, but could be salvaged by treatment intensification, and that the absence of tumour reduction after the COP was indicative of a poor prognosis (EFS = 29%).
- With the introduction of higher dose of MTX (8 g/m² in 4 h), HD Ara-C [CYVE courses = combination of Ara-C (in continuous infusion and HD) with VP16], and repeated triple IT in the **LMB86** study, the EFS of patients with initial CNS involvement increased from 19% to 75% (Patte, 1990). This intensified strategy was also beneficial to patients with “mature” B-cell Acute Leukemia [(B-AL), also called Burkitt leukaemia and previously L3-ALL]: those without CNS involvement could achieve an EFS rate around 90%. To note, in this LMB86 protocol, the patients received a cranial irradiation.

Based on the results of these studies, the following **LMB 89** study was designed (Patte, 2001). Three risk groups were defined which received treatment of progressive intensity: Group A patients (resected stage I and resected abdominal stage II) received 2 courses of COPAD without IT, nor HDMTX. Group B patients (not eligible for groups A or C) received a five course treatment identical to the short arm of the LMB84 protocol: after the prephase, 2 courses of COPADM followed by 2 courses of CYM (HD MTX with Ara-C in 5 days continuous infusion) and a maintenance (m1) similar to a COPADM course with lower dose of cyclophosphamide. Group C patients (with CNS involvement and B-AL with ≥70% of blasts in bone marrow) received the most intensive 8 course treatment similar to that of the LMB86 protocol: after the prephase, 2 courses of COPADM with MTXHD at the dose of 8g/m², 2 courses of CYVE and 4 maintenance courses (m1 to m4). Cranial irradiation was only for patients with CNS involvement. Treatment was further intensified for group B patients who did not respond to COP and any patient with residual viable cells after the consolidation phase, by “switching” to group C regimen. EFS of the 420 patients with Burkitt lymphoma was 92%, similar to EFS of patients with diffuse large B-cell lymphoma (DLBCL) or non-subclassified B-cell lymphoma also included in the study. EFS was 93%, 99%, 91%, 87% and 87% for stages 1, 2, 3, 4 and B-AL respectively, and 100%, 92% and 84% for group A, B and C respectively. Prognostic factors remained LDH (serum lacticodeshydrogenase) level, non-response to COP (although EFS increased from 29% to 70%), and CNS disease (EFS 79% compared to 90% for the patients in group C CNS neg). Concerning LDH level, the cut off was twice the upper limit of the normal value defining those with low LDH level (LDH<Nx2) and those with high LDH level (LDH > Nx2): EFS was 95% for patients with low LDH level compared to 87% for those with high LDH level and in stage III, it was respectively 94% vs 89% (p=0.06). To note, no further decline was observed in the EFS when the LDH level increased beyond Nx2.

The next study, the **FAB LMB96** (May 1996-June 2001), was a randomised international trial with the participation of the SFOP, the United Kingdom Children's Cancer Study Group (UKCCSG) from Great Britain and the Children's Cancer Group (CCG) from the USA (Gerrard 2008, Patte 2007, Cairo 2007).

It was an attempt to reduce total drug dosage further especially that of cyclophosphamide to avoid sterility in boys, to reduce treatment duration, and to suppress cranial irradiation in patients with initial CNS involvement. The study confirmed the excellent outcome of group A on a larger number of patients [n=132 patients, 4y EFS of 98.3%, 4y OS of 99.2%] (Gerrard, 2008). For the patients of group B being good responder to COP and in complete remission after the third course of chemotherapy, treatment can be decreased to 4 courses delivering only 3.3 g/m² of cyclophosphamide and 120 mg/m² of doxorubicin (Patte, 2007). In group C, the diminution by 1/3 of HD Ara-C dose and by half of VP16 dose lead to a decrease of 10% of the EFS, so the trial was closed after the third interim analysis (Cairo, 2007). An interesting observation in group B is the prognostic value of the delay between the two induction courses. Patients who started COPADM2 more than 21 days after COPADM1 have a significantly 8% lower survival rate than those who started within D21 (Patte, American Society of Haematology [ASH] 2003).

Based on the results of the BFM95 study (see below), in the observational on-going study SFOP/SFCE LMB 2001/2003, the possibility to improve the outcome of the patients with CNS positive or with bad response to COP is being tested, by increasing the duration of the HD MTX infusion from 4 to 24h. The number of patients is small, especially of those with blasts in the CSF but the preliminary results indicate improved outcome of these patients. However toxicity, especially mucositis rate and intensity, appears higher than with the shorter infusion time.

Treatment-related toxicity and morbidity in LMB regimens

Myelosuppression is the main treatment complication, especially during the COPADM and CYVE courses. More than 80% of the patients experience febrile neutropenia requiring intravenous antibiotics, and more than 50% require transfusions. Morbidity is higher in group C and is correlated with the higher dose of MTX and HD Ara-C but also with initial BM involvement for most of the patients in this group. 1st COPADM is generally less well tolerated although dose of cyclophosphamide is double in COPADM2 (in regimen "B4" which is now used in this protocol, dose of cyclophosphamide is not double in the 2nd course of COPADM).

The SFOP conducted a randomised trial for patients included in LMB89, LMT89 and HM91 protocols comparing COPAD(M) courses with or without G-CSF (**trial GL93**) (Patte, 2002). The trial on 148 patients showed that G-CSF did not decrease treatment-related morbidity, nor increase the dose-intensity after COPAD(M) induction courses. In details: although duration of neutropenia <500 was 3 days shorter in G-CSF+ patients ($p = 10^{-4}$), incidence of febrile neutropenia (89% v 93% in the 1st course, 88% v 88% in the 2nd course), durations of hospitalization and antimicrobial therapy, percentages of infections, mucositis, and transfusions were not significantly different. Although the percentage of G-CSF+ patients commencing the following course on day 21 was significantly higher (84% v 68% after the 1st and 57% v 38% after the 2nd course; $p < .05$), the median delay between the 2 courses was only 1 day less in G-CSF+ patients (median delay after 1st COPAD(M), 19 v 20 days, $p=0.01$; after 2nd, 21 v 22 days, NS). Remission and survival rates were similar in both arms.

Mucositis, the second main complication in the COPADM courses, is due to the combination of HDMTX and doxorubicin, because it does not occur in COPAD or CYM courses where only one of these drugs is administered. A parallel increased incidence of mucositis was observed when the doxorubicin infusion was prolonged. Thus in group C of FAB LMB 96, the rates of mucositis grade III-IV in COPADM1 and COPADM2 were 68% and 52%, respectively, with 6-hour infusion (n =202), 74, and 63% with 48-hour infusion (n=31). In the pilot study of the COG in which doxorubicin was given in 1h, the rate of mucositis decreased to 30% and 23% respectively. In group C3 of LMB 2001/03 in which HDMTX given in 24h and doxo in 1h, the rate of grade 3/4 mucositis is high (28/50% and 29/41% in first and second COPADM respectively), reason why in this study MTX will be given in 4h in the first course of COPADM.

The tables below report the percentage of complications occurring after the different courses in the different protocols. The dose of cyclophosphamide and MTX, and duration of infusion of doxorubicin are indicated.

Protocol N° and Type of course MTX g/m ² /h Doxo infusion CPM TD g/m ²	LMB81 1 st COPADM 3/3 bolus 1.5	LMB84 1 st COPADM 3/3 bolus 1.5	LMB81 2 nd COPADM 3/3 bolus 3	LMB84 2 nd COPADM 3/3 bolus 3	LMB89 1 st + 2 nd COPADM 3/3 bolus 1.5/3	LMB 96** 1 st COPADM 3/3 6 h 1.5	LMB 96** 2 nd COPADM 3/3 6h 1.5	LMB 96** 2 nd COPADM 3/3 6 h 3
No. of Patients	114	212	105	205	750	717	325	378
PN < 500 (%)	88	78	96	87				
Fever (%)	89	80	96	80	82	61	53	65

Major infection* (%)	24	29	14	21	12	9	6	9
RBC transfusion(%)	66	47	61	46	58	44	45	67
Platelet transf. (%)	20	9	11	9	17	10	8	26
Mucositis (%)	44	28	34	21	38	43	31	42
Gr3 (%)					23	21	17	23
Gr4 (%)					15	22	14	19
Toxic deaths					2	1	0	0
Due to infection	4	7	1	0		1		
not due to infection	2	0	0	0		0		

*in the 3 tables, major infection is defined by sepsis, cutaneous and/or pulmonary infections, meningitis, or collapse.

** In FAB LMB96 toxicity is reported only for patients receiving doxo in 6h infusion (after amendment in march 1997 reducing doxo infusion from 48h to 6h)

	GL93 1 st COPADM no G-CSF all groups	GL93 2 nd COPADM no G-CSF all groups		LMB84 CYM 1	LMB84 CYM2	LMB 89 CYM 1 +2
No. of patients	73	72		200	79	716
PN < 500 (%)	99	99		29	26	
Fever (%)	93	88		25	19	25
Major infection* (%)	14 (gr3-4)	10 (gr3-4)		7	7	5
RBC transfusion (%)	63	67		28	14	31
Platelet transf. (%)	14	24		9	6	15
Mucositis gr3-4(%)	53	56		2	1	1
Toxic deaths						
Due to infection				1	0	1
Not due to infection				1	0	

Protocol n° and Name of the course MTX g/m ² /h Doxo infusion CPM TDg/m ²	LMB89 C 1 st + 2 nd COPADM 8/4h bolus 1.5/3	LMB96 C** 1 st COPADM 8/4h 6h 1.5	LMB96 C** 2 nd COPADM 8/4h 6h 1.5	LMB89 1 st + 2 nd CYVE	LMB96 1 st CYVE CNS -/+	LMB96 2 nd CYVE CNS -/+
No of patients	261	195	191	271	70 / 65	69 / 55
PN < 500 (%)						
Fever (%)	85	78	76	73	73 / 82	77 / 78
Major infection* (%)	20	18	12	21	17 / 22	26 / 25
RBC transfusion (%)	71	79	82	84	87 / 91	91 / 93
Platelet transf. (%)	53	65	62	82	84 / 89	93 / 94
Mucositis (%)	53	68	51		9 / 20	13 / 28
Gr 3 (%)	33	29	24	6	9 / 6	10 / 19
Gr 4 (%)	21	39	27	0	0 / 14	3 / 9
Toxic deaths	2	1	2	1	0 / 1	1 / 0
Due to infection		0	1		0 / 1	1 / 0
Not due to infection		1	1		0 / 0	0 / 0

** In FAB LMB96 toxicity is reported only for patients receiving doxo in 6h infusion (after amendment in march 1997 reducing doxo infusion from 48h to 6h)

4.1.2. BFM studies

Since 1981 the Berlin-Frankfurt-Münster (BFM) group developed a specific treatment childhood and adolescent B-cell non-Hodgkin lymphomas (B-NHL) and conducted six multicenter studies (BFM 81, 83, 86, 90, 95 and ongoing BFM04 study) with the participating countries Austria, Germany, Switzerland and recently the Czech Republik.

EFS rates of 89 % were achieved for the B-NHL patients in studies NHL-BFM 90 and 95 (Reiter 1999, Woessmann 2005). Since BFM95 study, patients are stratified in four risk groups taking into account stage (St. Jude staging system), resection of localized tumors, pre-treatment LDH and CNS involvement. Treatment intensity is adapted to these risk groups:

R1: localised resected tumor: 2 courses A, B;

R2: stage I and II non resected, stage III with LDH level < 500: 4 courses: A, B, A, B.

R3: Stage III, stage IV CNS neg, B-AL CNS neg with LDH between 500 and 999: 5 courses: AA,BB,CC, AA, BB.

R4: LDH > 1000 and CNS pos: 6 courses: AA,BB, CC, AA, BB, CC.

In BFM 95, EFS rates were 94%, 94%, 85%, and 81 % in respectively risk groups R1, R2, R3, and R4. In the ongoing study BFM NHL04, dose of HDMTX is 1g/m² in 4h infusion in R1 and R2, 5 g/m² in 24h infusion in R3 and R4.

Main conclusions from BFM studies are:

- for stage IV and Burkitt leukaemia (BFM 86) and for stage III and high LDH ≥ 500 U/L (BFM 90), results increased significantly from around 50% to > 80% when the dose of MTX was increased from 0.5 g/m² to 5 g/m² (Reiter 1992, 1995, 1999).
- In the randomised BFM 95 the 4h infusion time of MTX proved less efficacious in R3 and R4 compared to the standard 24 hour infusion, while for lower risk patients (stage I, II, III-LDH<500 U/L: R1 and R2) receiving 1g/m², there was no difference in efficacy between both infusion schedules (Woessmann 2005).
- In this BFM95 study, HD Ara-C was introduced with VP16 (course CC in R3 and R4). The EFS of R3+R4 treated with the "BFM95 standard arm" (HDMTX in 24h infusion) is much higher than in corresponding BFM90 R3 arm (HDMTX in 24h infusion but without course CC): 93%vs 78%, and in fact EFS of the "reduced arm" (HDMTX in 4h infusion) : 77% is similar to that of BFM90 R3 arm.
- Except for patients with primary mediastinal large B-cell lymphoma (PMLBL) (Seidemann K 2002), there was no difference in EFS between other subtypes, especially between Burkitt lymphoma and DLBCL.
- Initial CNS involvement remains the main poor prognostic parameter, especially in patients with Burkitt lymphoma/leukemia, it was the strongest predictor of relapse in multivariate analysis (Salzburg 2007).

However, the increased risk of relapse is only true for those patients with cerebrospinal fluid (CSF) blasts while the relapse rate is not significantly increased in patients with isolated cranial nerve pulsly or an intracerebral mass without CSF blasts, see below. Of note, pEFS of 12 patients with epidural locations but without CSF blasts and treated as CNS negative was 92 %.

4.1.3. Comparative analysis of LMB and BFM studies

Thus, the French SFCE LMB trials and the Austrian-German-Swiss BFM trials have succeeded to obtain an overall survival (OS) of 90% in childhood and adolescent B-cell NHL and B-AL. So, these two treatment strategies became the most frequently applied for these patients in the last two decades. However, although the overall results as well as outcome of patients according to stage of disease were almost identical the treatment strategies differed in terms of stratification of patients into risk groups and treatment intensity and the choice, dose and schedule of drugs. Therefore, a comparative analysis patient characteristics, treatment strategies, and treatment outcome was performed based upon pooled data from both study groups. The aim was to find out useful conclusions from differences between the treatment strategies in relation to patient outcome which might be useful for the design of future common treatment protocols.

Data of 935 patients enrolled from 04/96-12/05 into the study NHL-BFM 95 (Woessmann 2005) and the ongoing study B-NHL-BFM 04, and 692 patients enrolled from 07/96-12/05 in the SFCE part of the FAB LMB 96 (Cairo 2007, Patte 2007, Gerrard 2008) and the ongoing study LMB 2001/03 were merged and analysed by Anne Aupérin and M Zimmermann, the 2 statisticians of each group.

Histology: The distribution of histological subtypes in LMB and BFM series, were respectively: Burkitt/B-AL: 77% and 68%, DLBCL 13% and 16% , PMLBL 3% and 2%, "other" 7% and 13%. The risk for relapse did not differ between histological subtypes in both BFM and LMB studies, except for patients with PMLBL. In the subsequent comparative analysis of LMB and BFM studies patients with PMLBL in the LMB (n=21) and the BFM (n=21) series were excluded.

Patient characteristics

The stages and CNS disease differed slightly between the LMB and BFM series (see the table below).

Comparative analysis of LMB and BFM studies patients without PMBCL

The 4y pEFS was 90% for 671 LMB and was 89% (SE=0.01) for 914 BFM patients, respectively (ns). Treatment outcome was also comparable between both series regarding stages, LDH and CNS involvement (table below), except for stage III with low LDH level who had a better outcome in the LMB series.

Combined analysis of BFM and LMB studies: Treatment outcome

Inclusion of Patients: BFM from 3/96 to 12/2005, LMB from 5/96 to 12/2005, 21 patients each with PMLBL excluded from BFM and LMB series.

	LMB (N=671)		BFM (N=914)		P(LR)
	% patients	4y pEFS	% patients	pEFS (4y)	
Total group	100	0.90	100	0.89	.74
Stages					
I	9	0.98	10	0.97	.86
II	17	0.96	25	0.98	.30
III	43	0.92	41	0.88	.13
IV	13	0.84	8	0.76	.27
B-AL	19	0.81	16	0.81	.85
CNS involved	13	0.79	9	0.72	.39
LDH					
<2UNL	50	0.95			
≥2UNL	50	0.85			
<500U/L			58	0.93	
>500U/L			42	0.84	
Stage III, <2UNL	45	0.96			
Stage III, ≥2UNL	55	0.87			
Stage III <500U/L			51	0.86	
Stage III, ≥500U/L			49	0.89	
Stage III-LDH≥2UNL+st IV+B-AL	50	0.84			
Stage III-LDH≥500U/L+st IV+B-AL			43	0.84	.86

4.1.4. Who are the high risk patients in children NHL/B-AL?

Looking at the table above, we can consider as **high risk** patients those whose EFS is below 90%, i.e. those with stage III and “high” LDH level, with stage IV and B-AL. And as indicated, the EFS of these patients taken together are 84% for both series.

Patients with **CNS involvement** have the worst outcome. CNS involvement is defined by the presence of: blasts in CSF, cranial nerve palsies, intra cerebral mass. The improvement of radiological explorations tended to enlarge the definition to masses with intra cranial or intra spinal extension beyond meninx and to intraspinal masses with neurological deficits.

The review of the combined BFM and LMB data showed that among the patients with CNS involvement, there was a clear difference between those with **presence of blasts in CSF** vs those without. In the BFM series, the EFS was 70.2% (n=54) vs 84.3% (n=26) respectively. In the FAB LMB96 series of patients treated with arm C1, it is 75% (n=24) vs 94.7% (n=19). In LMB 2001 group C3 (pts until 2006), it was 84.6 (n=13) vs 97.1% (n=39). There is a trend to a higher outcome with the C3 regimen, but the numbers are very small. Surprisingly, the proportion of patients with blasts in CSF is very low in this last series.

On the basis of these results only patients with CSF positivity will be considered as “very high risk” patients who will receive the more toxic regimen C3 with HDMTX in 24h infusion.

Patients CNS positive, but CSF negative, will receive the standard C1 regimen with a course of HDMTX between 1st and 2nd CYVE and 2 double IT before each CYVE as in FAB LMB96 and LMB2001. This strategy tended to improve by 4-5% EFS of these patients compared to the results in LMB89.

4.1.5. Rationale for choice of chemotherapy regimen

The similarity in results between the LMB and BFM strategies for advanced stage disease allow either to be taken forward as a common platform for this current study. The LMB strategy has been chosen in the absence of BFM participation in studying the question of the efficacy of Rituximab in increasing the EFS when added to chemotherapy in high risk B-NHL.

4.1.6. Modifications of the LMB regimen in this trial compared to the previous ones

Patients with stage III high LDH level and stage IV without CNS involvement will be treated in group B according to B4 arm of the FAB LMB96 study. Patients with B-AL CNS negative will be treated in group C according to group C1 arm of the FAB LMB96 study. Stage IV and B-AL with CNS involvement but CSF negative will be treated according to C1 CNS positive arm of the FAB LMB96 study. Stage IV and B-AL with CSF positive will be treated according to C3 regimen of the SFCE LMB2001-03 protocol.

- **Duration of infusion of doxorubicin:**

As previously indicated, the duration of the infusion changed over the time, being IVD or 15mn in the first LMB 81, 84, 86, 89 studies. It was changed to 48h just before start of FABLMB96, but due to a very high rate of mucositis, we returned to 6h during FAB LMB96 trial. In LMB2001/03, it was 6h except in C3 where it is 1h because of the 24h infusion of MTX. As there is no evidence that the different durations of infusion has a different antitumor effect, it was decided to administer doxorubicin in 1 hour to reduce the mucositis rate in COPADM and M1 courses.

- **Group C**

Duration of maintenance: Until now, there are 4 maintenance courses in group C. The attempt to reduce maintenance to 1 course was part of the FABLMB96 trial in addition to the reduction of doses of Ara-C and VP16 in the CYVE courses. Both reductions were in the “reduced arm”. The reduced arm was a failure with a 10% reduction of the EFS rate. It was not possible to differentiate the role of the reduction of the dose in CYVE and of the treatment duration. However, knowing the importance of the early dose intensity, the possibility to reduce the duration of treatment in group B without jeopardizing outcome, the early occurrence of failures or relapse in group C and the fact that the BFM gives a total of 6 courses, it was decided to eliminate the 2 last maintenance courses (m3, m4) without performing a trial but with monitoring of the efficacy of this treatment (see point 12.2.3.2. Monitoring of efficacy of Group C treatment standard arm). So in group C, there will be a total of 6 courses (2 COPADM, 2 CYVE and m1 and m2).

C3 regimen will be reserved to patients with presence of blasts in the CSF. This regimen piloted in the LMB2001/03 gives a high rate of prolonged oral mucositis and gastrointestinal mucositis. Knowing that the first COPADM is generally less well tolerated by the patients, the decision was taken to start the 24H MTX infusion only with the second COPADM.

4.2. BACKGROUND AND RATIONALE FOR RITUXIMAB ADDED TO CHEMOTHERAPY

4.2.1. Background (Cartron, 2004)

CD20 is almost exclusively expressed on B cells. Its expression begins at early pre B-cell stage and is lost during plasma cell differentiation, so it is expressed during all stages of B-cell differentiation, but it is not expressed on stem cells and plasmocytes. CD20 is essential for the regulation of cell cycle and cell differentiation. CD 20 is expressed in many B-cell lymphoma cells, especially in follicular lymphoma, DLBCL, Burkitt lymphoma and chronic lymphocytic leukemia. It has a stable expression, no modulation or internalization, which makes it an ideal target.

The chimeric murine/human anti-CD20 monoclonal antibody rituximab is a human IgG1-antibody with variable regions isolated from a murine anti-CD20 monoclonal antibody. This chimeric antibody is produced by the transfected Chinese hamster ovary (CHO) cell line clone 8-8F12-5E5-50C9 and binds to CD20-positive cells with high affinity. The antibody is effective in in-vitro models and has shown to deplete B cells in vivo.

The mode of action of rituximab comprises complement-mediated lysis of B cells and involves antibody-dependent cellular cytotoxicity. Other potential mechanisms of rituximab against B cells include induction of apoptosis, blockade of G/S-transmission, blockade of differentiation and increase in the phosphorylation of cellular proteins.

4.2.2. Background in DLBCL in adults

A **phase II** in relapsed non follicular B-cell lymphoma demonstrated a 37% response rate (RR) in DLBCL (Coiffier, 1998).

Three **phase III** studies showed the benefit of rituximab added to CHOP or CHOP-like chemotherapy: The GELA trial (Groupe d'Etude des Lymphomes de l'Adulte LNH-98-5 trial) for elderly patients (> 60 years) (Coiffier, 2002, Feugier 2005)), the US Intergroup phase 3 trial (ECOG /CALGB/ SOG) (Haberman 2006) also in elderly, and the MabThera International Trial (MInT) for “young” adults (18-59 years) having no or one risk factor according to age-adjusted International Prognostic Index (IPI) (Pfreundschuh, 2006). These studies have demonstrated that rituximab added to chemotherapy not only improves progression free survival but also overall survival in DLBCL.

Benefit of rituximab in subgroups:

In the GELA LNH-98-5 trial, to establish whether or not rituximab reduces bcl-2-associated treatment failure, **bcl-2 protein** expression and clinical outcome were studied (N Mounier, Blood, 2003). Results are summarized in the following table.

	bcl2 + 193 pts			bcl2 – 99 pts		
	R-CHOP	CHOP	p	R-CHOP	CHOP	p
RR (response rate)	78%	60%	.004	76%	73%	NS
OS	67%	48%	.004	72%	67%	NS
EFS	58%	32%	<.01	60%	40%	NS

Multivariate analysis confirmed the significant benefit for survival and EFS of R-CHOP in bcl-2+ patients. These results suggested that rituximab was able to prevent chemotherapy failure in patients with bcl-2 protein overexpression.

Similarly, **Bcl-6 protein** expression, known to be associated with a favorable prognosis in DLBCL, was studied in the US Intergroup phase 3 trial (Winter, Blood 2006). It was observed a reduction in treatment failures and death with the addition of R to CHOP in Bcl-6⁺ DLBCL cases only.

4.2.3. Data in adult Burkitt lymphoma

Data on Burkitt lymphoma are anecdotal. The more relevant data are the following:

- D Thomas (Thomas, 2006) compared a series of 31 Burkitt or B-ALL treated with hyper-CVAD regimen with rituximab to an historical series of 48 patients treated with the same chemotherapy regimen, but without rituximab. Results were significantly lower in this historical series, but patients had significantly more unfavourable prognostic factors (CNS disease, percentage of bone marrow involvement, presence of blasts in blood) making direct comparisons difficult.

- At the Lugano meeting of June 2008 Hoelzer compared the results of the prospective B-NHL 2002 study for Burkitt, L3-AL and DLBCL to the previous study B-NHL 90 (n=270) in which OS was 56%. Treatment was based on childhood BFM90 regimen. In the new series rituximab was introduced for all and HD Ara-C for patients with advanced stage aged less than 55 years. The EFS was 85% for Burkitt, 77% for L3-AL and 79% for DLBCL. Results were similar in Burkitt whatever the age, but worse in L3-AL older than 55 years with a PFS of 37%. EFS increased in the recent study, except for the patients who did not receive HD Ara-C, making difficult to determine the respective role of rituximab and Ara-C, the latest being known for its high efficacy in advanced stage Burkitt lymphoma or in B-AL.

- The only phase III in adult Burkitt lymphoma and L3-AL is currently performed in France: patients over 18 years of age are treated according to the LMB scheme with adaptation of the dose of MTX in older patients (Divine, 2005) and are randomized to receive or not rituximab at the beginning of each chemotherapy course. This study is on going.

4.2.4. Safety and tolerability of rituximab in adults

A review (Held, 2006) indicated that ~ 600 000 adult patients has been treated with rituximab. In general, the antibody is well tolerated. The more frequent reactions include infusion reaction (10%, generally at the 1st infusion), muco-cutaneous reaction, non IgE-mediated hypersensitivity reactions,

cardiac arrhythmia and renal failure. The percentage of neutropenia, anemia and thrombocytopenia is slightly increased in combined treatment compared to chemotherapy alone. Late onset of neutropenia is described in < 0.02% pts. B depletion associated with serum Ig deficiency reaches significance in a minority of patients. Serious infection was reported in only 2% of the patients. After combined chemotherapy and rituximab, there are rather viral infections than bacterial or fungal (Aksoy, 2007) (Koo, 2008). Recently, cases of interstitial lung diseases (Wagner, 2007) and of leukoencephalopathy (Goldberg, 2002) (Boren, 2008), some being fatal, have been reported after treatment with rituximab.

4.2.5. Rituximab in children

In children, there are fewer reports due to the small number of patients treated with the antibody. Severe and unusual viral infections have been reported (Seifert, 2006) (Quartier, 2003).

Concerning rituximab in childhood lymphoma, there are very few data (anecdotal reports).

- An international phase II was planned for relapsed patients with rituximab in front line before the start of the salvage chemotherapy. The study was planned in several European countries, but because of the difficulties to implement the European Directive on Clinical Trials in the different countries, it was opened only in France and had to close early because of insufficient accrual. In the 3 patients enrolled, no response was observed.

- COG conducted a study for relapsed patients with B-cell lymphoma, combining rituximab to the regimen ICE already used in relapsed lymphoma (Griffin, 2009). The study had to close prematurely because of insufficient accrual. 20 patients were accrued. 3 of the 6 DLBCL achieved complete remission and 4 of the 14 Burkitt. Six patients were able to proceed to HD consolidation and stem cell transplantation. The conclusion was that R-ICE was associated with an encouraging objective response and an acceptable toxicity profile.

- COG conducted a pilot study ANHL01P1 to evaluate the toxicity of addition of rituximab to LMB regimen in group B (stage III/IV, n=51) and group C (n=46) (Cairo, Goldman, Lugano, 2008; ASH 2008 and 2010). Two doses of rituximab were administered at 48h interval at the beginning of each COPADM courses and one dose at the beginning of each consolidation courses (CYM in group B, CYVE in group C). Pharmacokinetics of rituximab was also performed. This study show there were no more toxicities observed than would be expected with the regimen without rituximab. Pharmacokinetics was similar to that of adults. This study is the basis for the modalities of administration of rituximab in the Inter B NHL ritux 2010.

As of the final report (Fall 2010) on ANHL01P1, the estimated 3-year event-free survival was: Group B pilot (N=40): 92% (95% confidence interval [CI]: 78%, 98%); Group C pilot (N=37): 86% (95% confidence interval [CI]: 70%, 94%). The estimated 3-year overall survival was: Group B pilot (N=40): 95% (95% confidence interval [CI]: 80%, 99%); Group C pilot (N=37): 89% (95% confidence interval [CI]: 73%, 96%). These results demonstrated addition of rituximab to standard FAB therapy did not result in greater than expected acute toxicity. The outcome data suggests addition of rituximab may provide benefit and justifies a large randomized trial.

- The BFM group conducted a phase 2 upfront window of rituximab 5 days before the start of the regular chemotherapy (BFM 04 trial). Despite difficulties to include all the eligible patients, the study showed that rituximab has activity as single agent in pediatric B-NHL. Of 87 evaluable patients, 36 were responders (RR 41.4% [95%-CI 31-52%]), Response was defined as $\geq 25\%$ decrease of at least one lesion or BM or PB blasts. There was no difference between the RR of children with Burkitt lymphoma (27 responders of 67 patients) and those with DLBCL (7 responders of 15 patients). A response was more frequently observed in Bone Marrow (BM) (12 of 18 sites) compared with solid tumor lesions (36 of 108 evaluated sites; $P = .007$) (Meinhardt 2010).

4.2.6. Rationale for the rituximab trial

- There are no data from randomized studies on whether Rituximab can improve treatment outcome of patients with Burkitt lymphoma in general (adults and children/adolescents) or whether Rituximab can improve treatment outcome of children with other aggressive B-cell lymphoma. Childhood DLBCL, excluding primary mediastinal large B-cell lymphoma (PMLBL) (see paragraph 4.3.5), has a different biology from that of adults and a better outcome. Furthermore, they are bcl-2 negative and bcl-6 positive, the categories of DLBCL which in adults do not seem to benefit from rituximab. It is

the reason why rituximab is not given systematically in childhood DLBCL and why a randomisation is necessary to assess the efficacy of rituximab in childhood DLBCL.

So a prospective randomized trial is necessary to assess the potential benefit of rituximab in childhood/adolescent B-Cell lymphoma.

- There is a category of patients with a lower outcome who can benefit from an improvement of outcome: those with stage III with high LDH level or stage IV or B-ALL.
- The modalities of administration of rituximab will be those used in the COG pilot study that is 2 administrations of rituximab at 48h interval at the beginning of the 2 first courses of chemotherapy and one administration of rituximab at the beginning of the 2 following courses of chemotherapy.

4.2.7. Rituximab and immune function

There is extensive experience with using rituximab alone and in combination with chemotherapy regimens used to treat B-cell malignancies in adult patients. The use of rituximab has been associated with serious viral reactivations, notably hepatitis B virus (HBV), but serious infections with cytomegalovirus (CMV), varicella-zoster virus (VZV) have also occurred (MabThera SmPC). Fatal progressive multifocal leukoencephalopathy (PML) associated with JC virus reactivation has been associated with rituximab usage (Carson KR et al, 2009). The median time of PML from last dose of rituximab was 5.5 months (0.3 – 66 months). A study of 166 patients with indolent lymphoma who received rituximab only, demonstrated abnormally low levels of circulating B-cell after 3 doses of rituximab and 83% of patients had sustained low levels of circulating B-cells for 6-9 months after completion of rituximab. Despite this reduction in B-cells only 14% of patients developed low IgG or IgM levels (Laughlin P. and al 1998). However, other studies have reported with more than four doses of rituximab given as single agent reported 73% of patients had abnormally low IgM levels at 1 year following rituximab (Ghielmini et al, 2005).

When B-cells do recover, it appears there is a relative deficiency of memory B-cells, i.e. CD27 expression (Sidner RA, et al, 2004). These data raise concern not only about patient's ability to fight infections, but to retain immunity from past exposures and vaccinations.

Indeed, one small series of patients had impaired responses to recall antigens (polio and tetanus) (van der Kolk LE, 2002).

There has been some suggestion of more infections when rituximab has been added to chemotherapy, but there is little data about the effect on B-cell recovery and immunoglobulin (Ig) levels. Ig levels were assessed in a cohort of pediatric patients treated for B-cell NHL, who either received standard chemotherapy or only 1 dose of rituximab just prior to chemotherapy. The results showed that 50% of patients in both cohorts had \geq Ig subtype level abnormally low at 1 year after diagnosis. The frequency of patients with decreased Ig levels at 1 year after diagnosis increased with the intensity of the chemotherapy performed. 1/3 of the B-NHL/AL pts had already revealed Ig levels below LNL at diagnosis (personal communication – Alfred Reiter).

This randomized study provides an opportunity to study immune reconstitution and potential additive effect of rituximab in comparison with what is seen with standard chemotherapy for children and adolescents with aggressive, mature B-cell NHL. Parameters to be studied are: a) lymphocyte and lymphocyte B recovery (other subtypes of lymphocytes if possible), b) Ig levels (IgG, IgA, Ig M), c) titers to previous vaccinations (tetanus, polio and pneumococcus) and d) if titers are non-protective, ability to respond (4-fold increase in titer) to re-vaccination. Especially, titers against haemophilus influenza and pneumococcus will be studied. Patients will be evaluated during the 5 year follow up of the trial. Additionally, patients with infections that require interventions (hospitalizations, antibiotics prescribed, IVIG supplementation) will be collected.

4.2.8. November 2015: Rationale for a prospective single arm study with rituximab

The first interim analysis was based on 27 events that occurred among 310 patients, randomized between December 2011 and June 2015: 155 allocated to the control arm and 155 allocated to the Rituximab arm (174 from European centers and 136 from COG centers). 27 events represent 37.5% of the total expected number of events (72 events) among the planned 600 patients. The 27 events were reviewed and validated by the steering committee. 20 events were observed in the control arm versus 7 in the Rituximab arm. The 1-year EFS rate was 81.5% (95%CI=73.0%-87.8%) in the control arm and 94.2% (95%CI=88.5%-97.2%) in the Rituximab arm .

The adjusted EFS hazard ratio was 0.33 (95% confidence interval = 0.14-0.79), one-sided p-value=0.006. This p-value is higher than the nominal alpha (0.0014) of this first interim analysis performed on 37.5% of the expected information.

Thus, the upper boundary of the first interim analysis was not crossed. However, the conditional power analysis indicated that, if the true efficacy advantage of the Rituximab arm was on the order of what is currently observed (i.e, a 67% reduction in the risk of event), the trial result would support with certainty the conclusion of Rituximab superiority. Even if there was more subtle advantage of Rituximab (i.e., a 25% reduction in risk of event as compared to a 67% reduction), the trial would with high likelihood support the conclusion of Rituximab superiority either at a future interim analysis or at the end of the study.

Based on these analyses and the results of the French LMBA randomized study in adults, the IDMC recommended on November 9th:

1. that the randomization in the Rituximab randomized trial in children/adolescents with high risk B cell lymphoma has to be halted.
2. Patients already enrolled who are currently receiving treatment without rituxumab should have rituxumab added during subsequent courses at points in therapy where they would otherwise receive it
3. If enrollment to the trial is to continue, future patients enrolled on the trial should receive the rituxumab-containing regimen.

The members of the steering committee agreed with these conclusions. The sponsors halted the randomization on November 20th 2015 and suspended the study until decision is taken on the future of the study.

The members of the steering committee discussed on the benefit of continuing to register patients in a prospective single arm with rituximab. They felt that more patients were needed to answer to the secondary objectives: in priority those concerning safety (more patients are needed to better know about rare toxicities and long term toxicities) and immunology status. More patients were also needed in the PET and the MDD/MRD studies.

The suggestion to the sponsors was to continue the study until a total of 300 patients treated with rituximab as initially planned. This corresponds to approximately one year of registration.

The sponsors agreed to reopen the study. This is the object of the amendment to continue to treat patients in a prospective single arm study with rituximab.

4.3. BACKGROUND FOR THE PMLBL

4.3.1. Generalities on PMLBL

PMLBL is a pathologic and clinical entity of non-Hodgkin's lymphoma (NHL) derived from mature thymic B cells. It was first described in 1981 and is now well recognized as a separate entity of aggressive B-cell NHL (B-NHL). Histologically, it is characterized by diffuse proliferation of large cells with clear cytoplasm, invasive growth pattern, and various degrees of sclerosis leading to a typical compartmentalization pattern. Tumor cells are of B-cell origin, expressing CD79a, CD19, CD20, and CD22, but not CD5 or CD10. Characteristically, PMLBL cells do not show immunoglobulin G expression and are negative for CD21, which indicates that PMLBL is derived from medullary thymic B cells.

Clinically, they present as large, bulky mediastinal tumors, frequently involving adjacent mediastinal structures and lung tissue and often presenting as superior vena cava obstruction or with pleural and/or pericardic effusions. Extrathoracic manifestations are frequent and sub diaphragmatic, often involving the kidneys, but also pancreas or liver. Bone marrow and CNS involvements are infrequent. In adults, PMLBL is predominantly diagnosed in young female adults (median age at diagnosis, 28 to 35 years). Intensive multidrug chemotherapy proved to be efficacious in the treatment of adult patients with PMLBL. The place of radiotherapy is controversial.

Whereas in adults, PMLBL are described as of better prognostic compared to the other DLBCL, in children they appeared as of worse prognostic than the other DLBCL. However in both cases, EFS is around 65-75%. Series in children are rare. The larger series published so far is that of the German-Austrian group on 28 patients treated according to the BFM protocols (Seidemann, 2003). The 5-year EFS was 70% and the only prognostic factors was LDH level: more or less than 500 U/L. In the

FAB/LMB96 (M Gerard, SIOP 2004), PMLBL has a less favourable outcome than the other B-cell lymphoma in children. Size and LDH were prognostic on a series of 43 pts.

In order to define prognostic factors in a larger series of children and adolescents, with the aim of designing a multicenter international study, data of several paediatric study groups were merged and analysed.

4.3.2. Retrospective international paediatric study

Data on PMLBL of the 2 AIEOP studies 92 and 97 of the Italian group (1992-2002: 24 patients) (Pillon, 2004), the 3 BFM studies 86, 90 and 95 of the BFM/GPOH group (1986-2003: 40 patients) (Reiter 1995, 1999, Woessmann, 2005), the SFOP LMB 89 study of the French group (1989-1996: 8 patients) (Patte, 2001), and the International FAB LMB96 of the collaborative British, American and French groups (1996-2001: 42 patients) (Gerrard, 2005)(Patte, 2007), were merged in a single database and analysed by Anne Aupérin of the biostatistics department of Institut Gustave Roussy (Patte, 2005).

Treatment strategy was based on the BFM-B strategy in the BFM and AEIOP studies and on the LMB strategy in the LMB studies. Radiotherapy was not part of the treatment, except in Italian patients where “final” mediastinal irradiation was done in many cases, as recommended by the adult haematologists in this country.

•118 pts registered patients

114 patients were eligible for analysis: 47 males and 67 females (59%). The median age was 14.5 years (1.4 – 19.7). 52 patients (46%) were ≥ 15 years and 33 (29%) ≥ 16 years. According to St Jude's staging classification, there were 112 stages 3 and 2 stages 4 (bone marrow involvement). LDH level was more than twice the normal value (or > 500 U/L in BFM studies) in 43/110 patients (39%). In addition to the mediastinal mass, there was pleural involvement in 31 patients and pericardium +/- lung involvement in 38 patients. Extra thoracic involvement was: cervical node: 13, axillary nodes: 6, lumbo-aortic node: 8, kidney: 17 and other intra abdominal viscera: 28.

There were some differences in the characteristics of the patients as shown in the following table, but due to the small numbers, only size > 10 cm was significantly more frequent in the LMB series.

	AIEOP	BFM	LMBs	p
Sex (M %)	42	45	38	0.8
Age ≥ 15 y (%)	34	37	58	0.06
≥ 16 y (%)	25	20	38	0.15
LDH $> Nx2$ (%)	33	26	50	0.1
Size > 10 cm (%)	33	NA	60	0.05
Lumbo aortic n (%)	8	12.5	2	

In the LMB protocols, patients who have a tumor reduction $< 20\%$ at D7 (bad response to COP) received a more intensive treatment with HD Ara-C + VP16 in the consolidation phase (CYVE course). 8/50 patients were in such a case.

Complete remission was difficult to assess because of the high frequency of a residual mass which was managed differently by the national groups and the individual investigators. Eight patients in AEIOP, 17 (including 2 progressions) in BFM and 36 in LMB, were declared as not in CR at time of assessment of CR. Among the 36 LMB patients, 28 had biopsies or removal of the residual mass and viable cells were found in 4 cases; seven patients received chemotherapy intensification.

•In progressing patients

Radiotherapy was performed in 3 identified failing patients (BFM and AEIOP) as rescue treatment. It was done as “final” treatment (medical decision) in 13 AEIOP patients (7 no residual mass, 4 “residual mass”) and in 4 BFM patients having “partial remission”.

There were 34 events: 1 toxic death (FAB LMB96), 10 “progressions” (3 AIEOP, 1 BFM, 6 LMBs), 22 “relapses” (2 AIEOP, 10 BFM, 10 LMBs), and one 2nd malignancy (Hodgkin's lymphoma). The difference between progression and relapse was not easy to differentiate depending on how was considered a residual mass, but in total, 32 patients had a failure. Twenty-three patients died indicating that some failures could be rescued.

Survival of all patients was 78.6% and EFS 67.4%. According to the national groups, EFS was 72.7% for AIEOP, 65.4% for LMB and 66.7% for BFM (non significant). Considering radiotherapy, survival was 81.9% for the patients who received radiotherapy and 78% for those who did not (non significant).

The factors that were found prognostic were:

- LDH level: \leq Nx2 or 500 U/L (67 pts) vs $>$ Nx2 or 500 U/L (43 pts): EFS 75.8% vs 50.4% ($p=0.009$)
- lumbo aortic nodes: absence (106) vs presence (8 pts) EFS: 69.9% vs 37.5% ($p=0.02$)
- size of the mediastinal mass (not known for many BFM pts) \leq 10cm (37pts) vs $>$ 10 cm (43pts) EFS: 81% vs 57% ($p=0.025$)

For patients having no adverse prognostic factor (24 pts), EFS was 83%, whereas it was 57% for those having one or more bad prognostic factors (64 pts) ($p=0.024$).

4.3.3. Adult literature before rituximab

Several institutions/groups retrospectively reviewed their series of PMLBL.

- **The Memorial Sloan Kettering** (Hamlin, 2005) reviewed a series of 141 patients treated between 1980 and 1999. The conclusions were: (1) dose intense chemotherapy may be superior to CHOP, (2) the impact of radiotherapy requires randomised controlled trial, (3) the age adjusted IPI did not predict survival (4) HD chemotherapy should be reserved for patients refractory to anthracycline-based therapy or in a clinical trial for high-risk patients.

- **Todeschini et al** (Todeschini, 2005) retrospectively reviewed a series of 138 patients treated between 1982 and 1999 in 13 **Italian institutions**. In a multivariate analysis, achievement of CR ($P<0.0001$) and type of CT (MACOP-B/VACOP-B) retained the significance for OS ($P=0.008$) and EFS ($P=0.03$). The conclusions were that the more intensive treatment positively influenced OS and EFS and that consolidation with IF-RT (involved field radiotherapy) to the mediastinum further improved the outcome of CR patients.

- **Zinzani et al** (Zinzani, 2002) reviewed a series of 426 patients treated between August 1981 and December 1999 in 20 institutions of the **IELSG** (international extranodal Lymphoma study group, with Italian, UK, Swiss, Greek institutions) with either CHOP/CHOP-like regimens, third generation (MACOPB, VACOP-B, ProMACE CytaBOM) regimens or high-dose chemotherapy (HDS/ABMT). The conclusions were: MACOP-B plus radiation therapy may be a better strategy than other treatments; these retrospective data need to be confirmed by prospective studies; the encouraging survival results after HD chemotherapy require confirmation in selected high-risk patients.

- The series of 105 adult patients treated at **Institut Gustave Roussy** between July 1989 and November 2003 was reviewed (Massoud, 2008). Conclusions were that: 1) EFS is superior with ACVBP regimen (80% vs 65%), 2) RTH did not add benefit for patients treated with the dose dense regimen, 3) In the multivariate analysis for OS, a poor performance status and CHOP chemotherapy remained associated with a poor outcome ($p=0.02$ and 0.02 , respectively).

4.3.4. Rituximab in PMLBL

Two relevant publications report on their series of patients treated with rituximab in addition to chemotherapy

- The **National Cancer Institute** (NCI) (Dunleavy, 2006) reported at ASH 2006 a small series of 44 patients with mediastinal large B-cell lymphoma treated with DA-EPOCH \pm rituximab without routine radiation: 18 patients received DA-EPOCH alone and the subsequent 26 received DA-EPOCH+rituximab. DA-EPOCH was administered for 6–8 cycles. At a median potential follow-up of 9.5 and 4.2 years, EFS and OS was 67% and 78% for DA-EPOCH and 91% and 100% for DA-EPOCH-R, respectively. Rituximab was associated with a significantly improved EFS ($p=.038$) and OS ($p=0.023$) by 2-tailed exact log-rank test with caveats associated with any non-randomized comparison. Three patients on DA-EPOCH-R had positive Positron Emission Tomography (PET) and biopsy after treatment. One received radiation (event), one received salvage chemotherapy and radiation (event), and one no further treatment after biopsy. The conclusion was that rituximab may significantly improve EFS and OS with DA-EPOCH-based treatment and that accrual continues.

The series with an extended number of patients was presented at Frankfurt in June 2009 at the international meeting on NHL in children and adolescents, then at the ASH meeting in December 2009. Mediastinal grey zone lymphoma ($n=11$) had clearly a worse prognostic and were considered

separately. For PMLBL, PFS and OS at 4 years median F/U were 100% for 35 patients with PMLBL without RT. Comparison to historical PMLBL (n=17) treated with DA-EPOCH (PFS 65% and OS 77% at 10 years) indicated a significant benefit of rituximab (PFS: P=0.0012) and (OS: P=0.013).

There are 2 originalities in this regimen:

- 3 drugs (doxorubicin, VCR and VP16) are given as continuous infusion over 4 days (=96h).
- Doses in the following course are adapted to the previous course absolute neutrophil count (ANC) nadir, with an approximately 20% increase of the infusional drugs. This may lead to administer almost 400 mg/m² of doxorubicin, which is a higher dose than usually used nowadays in children and adolescents. The infusion over 96h might decrease the anthracycline cardiac toxicity in comparison with the bolus administration.

- Recently, the 87 PMLBL registered in the **MinT study** were analysed (10% of the cases) and compared to the other DLBCL of the trial. Rituximab increased the rates of CR (unconfirmed) (54% vs 80%, P = 0.015), virtually eliminated progressive disease (2.5% vs 24%, P < 0.001) and improved 3y EFS and OS (78% vs 52%, P = 0.012) (89% versus 78%, P = 0.158) respectively. The conclusion was that in young patients with PMBCL (age-adjusted International Prognostic Index 0-1), rituximab added to six cycles of CHOP-like chemotherapy increases response rate and EFS to the same extent as other DLBCL.

4.3.5. Rationale and principles for a phase II study in PMLBL

PMLBL is a distinct entity of lymphoma among large cell lymphoma, but which appeared to be similar in children, adolescents and young adults. This was confirmed in a recent publication: the review of a pediatric series (mostly BFM cases and some Italian cases) showed that the 44 cases with primary mediastinal large B-cell lymphoma had similar a pattern with respect of histology, immunophenotype or gains at 9p (59%) and 2p (41%) compared to published data from the adult counterpart. Only 4 cases of mediastinal gray zone lymphomas were identified.

This similarity with the adult counterpart was an indication for the use of rituximab in childhood PMLBL.

We first designed a LMB based therapeutic scheme taking into account the data presented above. From the adult literature, we took in the benefit of “dose dense” chemotherapy with anthracyclines and of rituximab in DLBCL. We considered PET(-CT) as potential tool to assess response. Concerning radiotherapy, we considered that there was no clear demonstration of its benefit on survival and that its potential late effects do not encourage using it systematically.

The main principles of the therapeutic scheme were:

- the protocol scheme is a modified LMB scheme,
- rituximab is given to all patients, at least 6 infusions,
- consolidation is with ICE which demonstrated its great effectiveness in relapse of adult DLBCL,
- response will be assessed by CT scan and if available PET(-CT),
- High dose chemotherapy with autologous stem cell rescue will be performed in “bad responders”,
- no radiotherapy will be recommended given the lack of data to support its use in children and adolescents with this disease and in those adults who have received dose intense chemotherapy and Rituximab.

Because of the results of the **DA-EPOCH-R** regimen presented by NCI at ASH 2009, after the review of the series of pediatric PMLBL indicating that mediastinal marginal grey zone were exceptional, it was finally decided to start with a phase II evaluating this regimen.

- If the excellent results were reproduced, this regimen will be chosen as standard treatment although doxorubicin dose might be as high as almost 400 mg/m². The next stage would be to try to limit the upper dose of doxorubicin. An effort will be made to monitor cardiac function after the end of treatment.

- If the expected results were not achieved with DA-EPOCH-R regimen, in a multicenter prospective study then a second phase II will be proposed. One proposal will be to test the efficacy of the modified LMB regimen associated with rituximab. The decision will be taken according to the results of the ongoing modified LMB2001 study for PMLBL and according to the more recent data in the literature.

4.4. PLACE OF PET(-CT) IN EVALUATION OF TUMOR RESPONSE IN NHL

In adult NHL, several studies have shown the superiority of PET-CT compared to classic radiological investigations, for the initial work up and for the assessment of the remission. Other studies also tend to show the prognostic value of the persistence of PET(-CT) positivity after 2 courses, which could be an indication for an early intensification of the therapy. Interim results of the PETAL trial, designed to investigate whether a change in treatment protocol may improve the outcome for patients with aggressive NHL who have a positive scan after two cycles of (R) –CHOP, indicate that, in patients with a positive scan, relapses were six times more frequent than in patients with a negative scan (Duhrsen 2009). In another recent study of PMLBL and DLBCL patients, midtreatment PET was shown to identify a significantly higher event free and overall survival in the PET negative group and correspondingly lower survival in the PET positive group (Zinzani 2010).

Data about specificity and sensitivity in childhood NHL (except in PMLBL which is similar in children and in adults) are very limited, however many teams tend to perform this investigation, either for initial work up, or for evaluation of the complete remission. In PMLBL, data are more consistent; however the definition of local remission is still a major issue.

This protocol gives the opportunity to collect data on PET(-CT) scan in a large series of children/adolescents with high risk B NHL. As PET(-CT) scan is not easily available in all countries participating in this protocol, **the ability to perform PET(-CT) scan is not mandatory for participation in the protocol and it is reminded, as stated in paragraph 11.2.4, that no decision must be taken on PET(-CT) scan only.** However, when PET scan or PET(-CT) scan is performed, data will be collected. At the end of the study, if the quality of collected data allows, some preliminary analysis will be performed to guide future studies in this area (see section 133 on Ancillary studies).

4.5. MRD/MDD

Please refer to the annexe G.

5. OBJECTIVES

5.1. PRIMARY OBJECTIVES

Phase III study:

For the patients with advanced stage B-cell NHL/B-AL (stage III and LDH > Nx2, any stage IV or B-AL) to test whether adding 6 injections of rituximab to standard LMB chemotherapy regimen, improves the EFS compared with LMB chemotherapy alone.

November 2015: The first interim analysis showed a benefit of rituximab in addition to the chemotherapy. Therefore the randomization was halted on November 20th 2015.

Phase II study:

For patients with PMLB, to evaluate the EFS following treatment with the regimen DA-EPOCH-rituximab.

5.2. SECONDARY OBJECTIVES

In Phase III and Phase II studies:

- To study the complete remission (CR) rate and the overall survival (OS).
- To evaluate safety on all study arms: including toxic deaths, adverse events recorded using the NCI-CTC V4 (non haematological toxicity grade ≥3, infections grade 3 to 5), cardiac toxicity (CTC grade 2-5 and evolution of left ventricular ejection fraction and left ventricular shortening fraction), number of days with platelets transfusion, intensive care unit admission and number of days with red cells transfusion, rituximab infusion reactions.
- To study the rate of patients with Ig (G, A and M) level abnormally low and lymphocyte count abnormally low at 1 year and until 5-year follow-up, and to study the need for immunoglobuline infusions and levels of post (previous and re-)vaccination antibodies at 1 year.

In Phase III study:

- To study long term (at least 5 years) risks of the use of rituximab plus chemotherapy compared with LMB chemotherapy alone in children and adolescents with advanced stage B-NHL/B-AL (all events related (certain and probable) to therapy).

November 2015: the randomization was halted since the answer to the primary objective was positive. However a greater number of patients are necessary to answer to the secondary objectives, in priority those concerning safety (including rare toxicities and long term toxicities) and immunology status. This is the reason why the study continues with a single arm in order to register 120 other patients receiving rituximab combined to chemotherapy until a total of 300 patients treated with rituximab as initially planned.

In Phase II study:

- To study the long term risk of DA-EPOCH-R regimen, especially the cardiac risk related to doxorubicin given at higher dose than usual in children, but infused over 96h (i.e. evaluation of CTC grade 2-5 and evolution of left ventricular ejection fraction and left ventricular shortening fraction).

5.3. EXPLORATORY SECONDARY OBJECTIVES

(Parallel studies not done in all countries)

- In phase III and phase II studies:

- To obtain data on PET(-CT) scan in childhood pediatric B-cell NHL.

- In phase III study:

- To evaluate the potential prognostic value of Minimal Dissiminated Disease (MDD) and Minimal Residual disease (MRD) in correlation with outcome.
- To perform an economic study comparing the cost-effectiveness ratio between 2 therapeutic strategies: LMB chemotherapy with versus LMB chemotherapy without Rituximab.
- To characterize the pharmacokinetics of rituximab in combination with LMB chemotherapy in a subset of patients.

November 2015: As said above for the secondary objectives, the continuation with a single arm study will be useful to answer to the secondary exploratory objectives (except the economic study).

6. SELECTION OF STUDY PATIENTS

Children and adolescents aged less than 18 years with untreated advanced stage B-cell NHL or B-AL.

6.1. INCLUSION CRITERIA:

Histology and staging disease

Phase III study:

- Histologically or cytologically proven* B-cell malignancies, either Burkitt lymphoma or B-AL (=Burkitt leukaemia = L3-AL) or diffuse large B-cell NHL or aggressive mature B-cell NHL non other specified or specifiable.
- Stage III with elevated LDH level ("B-high"), [LDH > twice the institutional upper limit of the adult normal values (> Nx2)] or any stage IV or B-AL.

Phase II study:

- Histolo-cytologically proven* PMLBL.
- PMLBL without CNS involvement.

** Slides will be reviewed by the national pathology panel, but review is not mandatory before registration.*

General conditions

- 6 months to less than 18 years of age at the time of consent.
- Males and females of reproductive potential must agree to use an effective contraceptive method during the treatment, and after the end of treatment: during twelve months for women, taking into account the characteristics of rituximab and during five months for men, taking into account the characteristics of methotrexate.

Initial work-up

- Complete initial work-up within 8 days prior to treatment that allows definite staging.

Others

- Able to comply with scheduled follow-up and with management of toxicity.
- Signed informed consent from patients and/or their parents or legal guardians.

6.2. EXCLUSION CRITERIA:

Histology and staging disease

- Follicular lymphoma, MALT and nodular marginal zone are not included into this therapeutic study
- In phase II study (PMLBL) patients with CNS involvement are not eligible.

General conditions

- Patients with congenital immunodeficiency, chromosomal breakage syndrome, prior organ transplantation, previous malignancy of any type, or known positive HIV serology.
- Evidence of pregnancy or lactation period.
- There will be no exclusion criteria based on organ function. Dosing guidelines for organ dysfunction are provided in annexe D1.

Prior therapy

Past or current anti-cancer treatment except corticosteroids of less than 7 days duration in total.

Exclusion criteria related to rituximab:

- Tumor cell negative for **CD20** (absence of result due to technical problems in the presence of other characteristics suggestive of BL/DLBCL, including genetic and phenotypic features, is not an exclusion criteria).
- Prior exposure to rituximab.
- Severe active viral **infection**, especially hepatitis B. Severe infection (such as sepsis, pneumonia, etc.) should be clinically controlled at the time of randomisation. Contact the national co-investigator for further advice if necessary.
- **Hepatitis B** carrier status history of HBV or positive serology. A patient is considered as HBV carrier or to have (had) HBV infection in case of:
 - Unimmunized and HBsAg and/or anti-HBs antibody and/or anti- HBc antibody positive,
 - Immunized and HBsAG and/or anti-HBc antibody positive.

Important note: For the Phase III trial, a patient without a known history of hepatitis B could be randomized in the study if the serology results are not available at the time of the randomization. However, if the serology results are positive or not available at day 6 (the first day would be due to receive rituximab, if so randomized), the patient must be withdrawn from the study whatever the allocated treatment arm. The data center must be informed immediately. For the phase II trial, the hepatitis B serology results must be available before registration.

In each case indicating a carrier status or history for hepatitis B infection, the patients must not receive rituximab, and therefore must not be included in the rituximab trials on any treatment arm. In case of high-risk patients, the recommendation is to treat these patients with the standard LMB regimen corresponding to the patient prognostic group. In the case of PMLBL the physician is left to choose the most appropriate therapy.

Others

- Participation in another investigational drug clinical trial.
- Patients who, for any reason, are not able to comply with the national legislation.

6.3. WITHDRAWAL CRITERIA:

The only criteria for patient withdrawal from the trial are:

- HBV positive serology known after randomization
- Parent or patient consent withdrawal

7. INITIAL WORK UP

Important note: All eligibility studies must be performed prior the randomization/registration. All clinical and laboratory data required for initial work up of any included patient must be available in the patient's medical/research record which will serve as the source document for verification at the time of audit.

7.1. HISTOLOGICAL DIAGNOSIS

Refer to annexe F

.Histo-cytological diagnosis is necessary. Tumour sample or tumour cells should be sent fresh to the local cyto-pathology laboratory for:

- Histology and cytology (touch imprint, fluid, bone marrow),
- Immuno-phenotyping,
- Freezing of a piece of tumour or tumour cells,
- Cytogenetics (optional according to cell availability),
- Molecular biology (optional according to cell availability).

It is important to also collect normal cells (see annexe F.2.3) (according to national regulation)

7.2. PRE-TREATMENT INVESTIGATIONS

- Complete history and physical examination, performance status (evaluated according to WHO, Karnowski or Lansky scoring), B symptoms
- Biology:
 - Full blood count, differential, and platelet count
 - Electrolytes, urea, creatinine, uric acid, calcium, phosphorous, albumin
 - SGOT, SGPT, gamma GT, bilirubin
 - LDH level and determine if below or above twice the adult upper normal value of the institution
 - Haemostasis/coagulation
- Bilateral BM aspirate
- Bilateral BM biopsy (mandatory for DLBCL and PMLBL)
- Lumbar puncture, cell count, differential, cytology, glucose, and protein (although not recommended, it is accepted that first intrathecal chemotherapy (IT) may be performed at the same time)
- Radiological investigations:

- Chest x-ray (PA and lateral) and abdominal ultrasound are minimal investigations
- Chest and Abdominal CT are recommended according to the sites of the tumors
- CT scan and/or MRI in head and neck tumors
- Bone scan if clinically applicable (if no PET(-CT) available) and x-ray or MRI of the suspect areas
- Cranial and/or spinal MRI if clinically applicable
- PET(-CT) scan if available locally, and if the clinical condition of the patient allows it, and if it does not delay start of treatment
- Assessment of Cardiac Function by echocardiogram and measurement of shortening fraction and ejection fraction (can be done after start of treatment but must be done before the first dose of doxorubicin)
- Immunity status:
 - The total peripheral blood lymphocyte count and CD19⁺CD20⁺ B cells evaluated using flow cytometry.
 - The serum levels of IgG, IgA and IgM.
 - Serum antibodies to polioviruses and tetanus and diphtheria toxoids will be measured using enzyme-linked immunosorbent assays, as well as serum antibodies to pneumococcus and haemophilus influenzae.
 - Optional evaluation; peripheral blood counts of various lymphocytes subsets (CD3⁺, CD3⁺CD4⁺, CD3⁺CD8⁺, as well as CD3⁻CD16⁺ (or CD3⁻CD56⁺, or CD3⁻CD16⁺CD56⁺) NK cells) using flow cytometry.
 - 5 ml of serum for banking in order to be able to make comparison later if necessary (i.e. in case of severe infection or significant other abnormalities) (according to national regulation).
- HIV antibody test (according to local regulation)
- Hepatitis B screening:
 - Patient's Hepatitis B immunization status (Vaccination Yes or No)
 - HBs AG, anti-HBs antibody, antiHBc antibody IgG, IgM.

Important note: a patient without a known history of hepatitis B could be randomized in the study if the serology results are not available at the time of the randomization. However, if the serology results are positive or not available at day 6, the patient must be withdrawn from the study whatever the allocated treatment arm. The data center must be informed immediately.
- In pubertal boys, sperm banking should be discussed and if performed, sperm should be cryopreserved before starting chemotherapy
- In pubertal girls, pregnancy test (urinary or blood test according to local regulation)

8. STAGING AND THERAPY STRATIFICATION

• Phase III study - Non PMLBL Patients:

Staging is done according to the **St Jude's** (Murphy's) stratification (see annexe E): stage III and stage IV [bone marrow involvement (< 25%) and/or CNS involvement] and B-AL (bone marrow involvement ≥25%). Among stage III, only the patients having LDH level above twice the adult upper limit of normal for institution range are eligible for the randomisation (they are called "B-high" risk patients).

The **therapy stratification** is similar to the previous stratifications of the protocols LMB. Patients are either treated according to group B or C (based on the previous FAB LMB 96 trial) therapy depending on the stage and CNS involvement.

• Phase II study - PMLBL Patients:

Any patient without CNS involvement is eligible and there is no therapy stratification. In the improbable case of a patient with CNS involvement at diagnosis, the patient is not eligible for the study.

9. REGISTRATION/RANDOMISATION PROCEDURES

9.1. CONSENT

Registration/randomization procedures should not be performed until the informed consent for the study is signed. Registration before the start of COP is only for COG centers. In Europe, registration and randomization are done at the same time. Randomization may be delayed until day 3 at the latest to allow further discussion.

9.2. PHASE III STUDY

Randomization of patients will occur following initial work-up and must be performed at the latest 3 days after the start of COP. The randomization will be stratified on the following characteristics:

- National Group (COG, SFCE, UK NCRI CCL CSG, AIEOP, BSPHO, DCOG, SEHOP, PPLLSG, and Hungarian Society of Pediatric Oncologist and Pediatric Hematologist)
- Histology: Large Cell, non Large Cell (Burkitt, atypical Burkitt, B-AL or L3-AL)
- Chemotherapy group: B, C1, C3

For the European countries, randomization will be done using TenAlea software (NKI, Amsterdam) via internet and the two arms will be assigned by minimization, but with an alea parameter of 0.80. The definition of the randomization parameters will be implemented by the Biostatistic Unit of the Gustave Roussy Institut, France.

Modalities of the randomization may depend on the administrative organisation of each national group. The investigators and delegated site staff can perform randomisation directly by internet (username and password will be given to the investigators) or by faxing the randomization form to the Biostatistic Unit of the Gustave Roussy Institut, France or to their national data center. The modality of randomization chosen by each national group must be defined before the start of the trial in the country. In case of problem with web connection, the investigator must fax the randomization form to the Biostatistic Unit of the Gustave Roussy Institut and can contact Gisèle GOMA (phone: 33 (0)1 42 11 54 72 or Beep 33 (0)1 42 11 49 00) or Anne Aupérin (phone: 33 (0)1 42 11 54 99) if necessary.

The system will check the eligibility criteria and will register the stratification status. If the patient is eligible, the randomisation by minimization will be done. A registration number and randomisation assignment (with or without rituximab) will be sent by return by email to the physician, to the national datacenter, to the pharmacist of the center and to the relevant address for drug supply.

For the COG centers, COG will use its existing randomization module in its eRDE system. The treatment assignment will be done by stratified blocked randomization (block of 6). Patients are first registered with COG and obtain a COG ID number. Then they attempt to enrol by completing the study eligibility CRF. If all eligibility questions are answers successfully, then the patient is enrolled, a treatment assignment is made and a confirmation of study enrolment is returned to the institution. No information on patients who have been screened locally but for whom a study enrolment has not been attempted are maintained.

Blocked randomisation will not be used in Europe because, as the number of strates will be high (8x2x3=48) with some of them including very few patients, the risk of imbalance will be high.

IGR will be informed in real time of the COG randomizations and COG will be informed in real time of the IGR randomization. This will allow closely monitoring of the number of randomized patients in order to be sure to have the right total number of required patients.

The following data will be asked for registration and randomisation:

- 0 to 3 first letters of surname (according to local regulation),
- 0 to 2 first letters of first name (according to local regulation),
- date of birth,
- sex,
- pathology with histological subtype (Burkitt or B-AL (=L3AL), DLBCL, aggressive B-cell not otherwise sub classified),
- Murphy stage (stage III, stage IV, B-AL, CNS involvement, CSF blasts),
- LDH level and adult upper limit norm of the institution,
- treatment group (B, C1, C3),
- all eligibility criteria (see section 6),
- height, weight (the body surface will be automatically calculated (according to the Dubois formula except for patients with weight<=10kg, see*) and printed in the mail of randomization confirmation). Body surface based on weight is acceptable also depending on National practice (UK).
- signed informed consent.

* Dubois formula =

[Weight>10kg] Body area (m²) = 0.007184 * Height(cm)^{0.725} * Weight(kg)^{0.425}
[Weight <=10kg] Body area (m²) = (Weight(kg) * 4 + 7)/(Weight(kg)+90)

November 2015: The randomization was halted on November 20th 2015. The same data are asked for the registration of the patients who will be treated on a single arm with rituximab.

9.3. PHASE II STUDY

Registration in the phase II trial must be done before start of chemotherapy.

For the European countries, registration will be done using TenAlea software (NKI, Amsterdam) via internet. Modalities of the registration may depend on the administrative organisation of each national group. The investigators can perform registration directly by internet (username and password will be given to the investigators) or by faxing the registration form to the Biostatistic Unit of the Gustave Roussy Institut, France or to their national data center. The modality of registration chosen by each national group must be defined before the start of the trial in the country. In case of problem with web connection, the investigator must fax the registration form to the Biostatistic Unit of the Gustave Roussy Institut and can contact Gisèle GOMA (phone: 33 (0)1 42 11 54 72 or Beep 33 (0)1 42 11 49 00) or Anne Aupérin (phone: 33 (0)1 42 11 54 99) if necessary.

The system will check the eligibility criteria. If the patient is eligible, the registration will be done. A registration number will be sent by return by email to the physician and to the national data center, to the pharmacist of the center and to the relevant address for drug supply.

For the COG centers, COG will use its existing registration module in its eRDE system.

IGR will be informed in real time of the COG registrations and COG will be informed in real time of the IGR registrations. This will allow closely monitoring of the number of registered patients.

The following data will be asked for registration:

- 0 to 3 first letters of surname (according to local regulation),
- 0 to 2 first letters of first name (according to local regulation),
- date of birth,
- sex,
- Pathology with histological subtype,
- Murphy stage (stage III, stage IV, CNS involvement status),
- all other eligibility criteria (see section 6),
- height, weight (the body surface will be automatically calculated (according to the Dubois formula except for patients with weight ≤ 10kg, see*) and printed in the mail of registration confirmation),
- signed informed consent.

* Dubois formula =

[Weight > 10kg] Body area (m²) = 0.007184 * Height(cm)^{0.725} * Weight(kg)^{0.425}

- [Weight ≤ 10kg] Body area (m²) = (Weight(kg) * 4 + 7) / (Weight(kg) + 90)

10. DRUGS AND TREATMENT PLAN

10.1. RITUXIMAB

The rituximab will be provided to the participating centres for the patients assigned by randomisation to the treatment with rituximab.

Rituximab is given at the dose of 375 mg/m² I.V. See annexe A for the details of the administration and the premedication. If corticosteroids are part of the course (COPADM), start steroids before rituximab administration.

In the **phase III study**, the patients randomised to receive rituximab will receive a total of 6 injections of the antibody: 2 at 48h interval at D-2 and D1 of the 2 COPADM courses and one injection at D1 of the 2 consolidation courses either CYM (B) or CYVE (C1 or C3).

Patients randomised to receive rituximab and who are switched to group C after CYM1, will receive rituximab only with the first CYVE in order to receive a total of 6 courses.

November 2015: all patients receive rituximab according to the same scheme as previously.

In the **phase II study**, all PLMLBL patients will receive rituximab: one injection at the beginning of each of the 6 courses of DA-EPOCH-R.

10.2. TREATMENT GENERALITIES

10.2.1. Phase III study

GROUP B: prephase followed by 4 courses: 2 induction courses [COPADM] and 2 consolidation courses (CYM)

Randomisation to rituximab vs no rituximab.

November 2015: the randomization is halted: all patients receive rituximab

(see section 10.3)

GROUP C: prephase followed by 6 courses: 2 induction courses (COPADM) with HDMTX 8g/m², 2 consolidation courses (CYVE) and 2 maintenance courses (M1 & M2).

CNS positive patients will also receive 1 IT before each CYVE course and one HDMTX between the 2 CYVE courses. HDMTX is given in 4 h infusion except for the patients with blasts in the CSF who will receive it in 24h infusion starting at the 2nd COPADM.

Randomisation to rituximab vs no rituximab.

November 2015: the randomization is halted: all patients receive rituximab

➤ **C CSF neg (C1): HDMTX 8g/m² in 4h infusion (rescue at H24)(section 10.4)**

➤ **C CSF pos (C3): HDMTX 8g/m² in 24h infusion(rescue at H36), except in first course (1st COPADM) (section 10.5)**

Important note: Consecutive courses should be given as soon as blood count recovery and patient's condition allows except for the maintenance courses which are given at 28 day intervals.

For patients younger than one year (this situation is exceptional in this disease), see annexe C3

10.2.2. Phase II study

PMLBL: All patients receive rituximab.

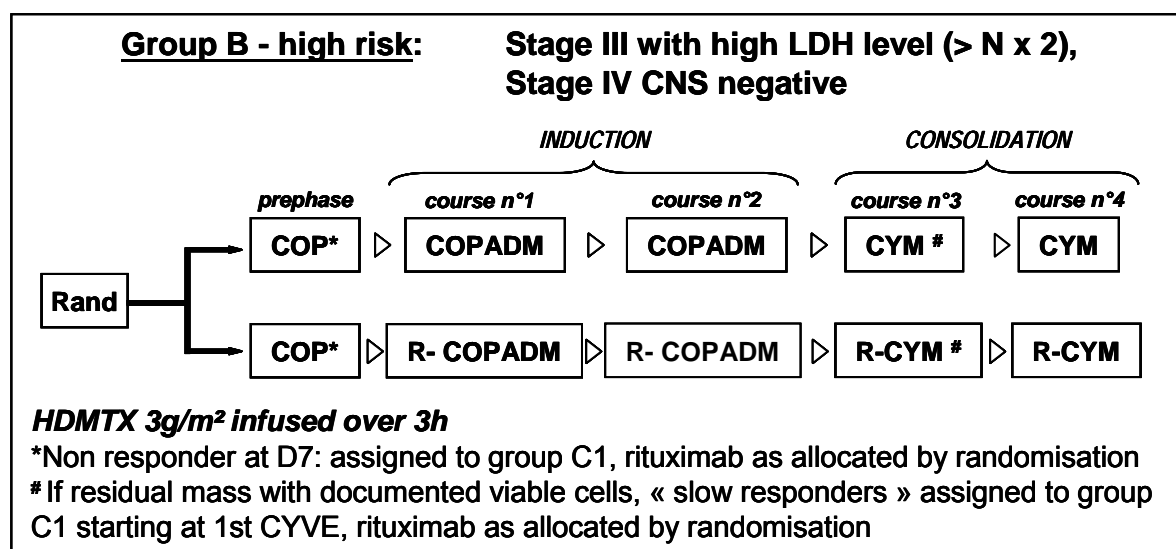
- 6 courses of the DA-EPOCH-R regimen, with rituximab at the beginning of each course, infusional administration of doxorubicin, vincristine and VP16, and dose adaptation based on previous cycle ANC nadir.

(section 10.6.3)

10.2.3. Tables of investigations at diagnostic and during treatment: (section 10.7)

10.3. GROUP B HIGH RISK: TREATMENT SCHEME AND DETAILS

▪ SCHEME



#“Slow responders” randomised to receive rituximab and who are switched to group C after CYM1, will receive rituximab only with the first CYVE in order to receive a total of 6 courses.

November 2015: the randomization is halted: all patients receive the arm of treatment with rituximab

▪ TABLES OF INVESTIGATIONS at diagnosis and during treatment

See section 10.7.

▪ TREATMENT DETAILS (sections 10.3.1 to 10.3.3)

Patients fulfilling the criteria for Group B high risk (**stage III with high LDH level**, i.e. above twice the normal value of the institution, and **stage IV CNS neg**, i.e. with bone marrow involvement below 25%) will receive their treatment according to the standard **B** regimen, i.e., a prephase followed by four courses including two induction courses (COPADM) with doxorubicin in **1h** infusion followed by 2 consolidation courses (CYM) with two double IT per course.

Patients are randomised to receive or not receive rituximab in combination with this chemotherapy.

November 2015: the randomization is halted: all patients receive rituximab

Intervals between courses should be **as short as possible**, and the subsequent course given as soon as recovery allows. In case of severe complication (such as gut perforation, fungal infection) which necessitates to delay chemotherapy and does not allow to start the next course on time, a course of COP (“**waiting COP**” course) should be considered in order not to let the patient without any chemotherapy.

Contact the study national co-investigator for further advice if necessary.

10.3.1. B High Risk – Pre Phase: COP

Prevention or treatment of the tumor lysis syndrome must be started before the administration of the chemotherapy and be continued as long as necessary the following days (see annexe D2)

Vincristine	1.0 mg/m ² (max single dose 2.0 mg) IV bolus on Day 1
Cyclophosphamide	300 mg/m ² as an infusion over 15 minutes on Day 1
Predniso(lo)ne	60 mg/m ² /day (divided into two doses) orally on Days 1 – 7 Methylpredniso(lo)ne IV may be used at the same dose of predniso(lo)ne if unable to take PO.
Methotrexate (MTX) IT*	8 - 15 mg by IT injection on Day 1 (dose varies with age, see annexe B)
Hydrocortisone (HC) IT*	8 - 15 mg by IT injection on Day 1 (dose varies with age, see annexe B)

B-high: prephase COP

Days	1	2	3	4	5	6 = D-2 of 1 st R-COPADM	7 tumour evaluation
Vincristine	•						
Cyclophosphamide	•						
Predniso(lo)ne	• •	• •	• •	• •	• •	• •	• •
IT MTX & HC*	•						

Note: *Omit D1 intrathecal if given previous intrathecal therapy (with the exploratory lumbar puncture)

Evaluation of tumour response should be performed on day 7. Patients who have no tumour response at D7 (“non responder to COP”, see section 11.1 and 11.2.1) should be treated according to C1 arm without or with rituximab as assigned by randomisation.

November 2015: the randomization is halted: all patients receive rituximab

PATIENTS RANDOMISED TO (nov 2015: ALL PATIENTS) RECEIVE RITUXIMAB (AND) SHOULD RECEIVE IT AT D-2 OF COURSE N°1, ie, AT D6 OF COP, one day before the D7 COP evaluation.

There may be situations where it does not seem possible to proceed with course n°1 (R)COPADM) at D8, then a **second course of COP** should be considered:

- the patient is in too critical condition to receive a COPADM, i.e. patients with renal failure, creatinine clearance <60 ml/min, septicaemia or other sepsis or grade 3/4 organ toxicity, then a second course of COP will have to be considered.
- In the case of significant effusion at diagnosis (with a risk of MTX accumulation):
 - If the effusion is small at D7 assessment, ignore and continue course n°1.
 - If significant effusions but overall response then give next course with MTX at day 5 instead of day 1 or consider to give a second course COP
 - If significant effusions and no response, then move to Group C and give next course with MTX at day 5 instead of day 1 (as long as effusions small).
- elevation of transaminases, sometimes with an increase in liver size, can be seen after COP and is probably due to a combination of corticosteroids and poor nutrition status (where this was present at outset). In the case of transaminases > 10x ULN it is preferable to wait 48 hours and proceed to course n°1 if transaminases decrease. If not, give a second course of COP.

In any case when course n°1 ([R-] COPADM) has to be postponed, **rituximab will not be given with course n°1 ([R-] COPADM) but at the planned time, ie at D6 and D8 of 1st COP, if the clinical situation is controlled.**

Contact the study national co-investigator for further advice if necessary.

10.3.2. B-high- INDUCTION: Two courses of COPADM or R-COPADM (November 2015: all patients receive R-COPADM) = Course n°1 and course n°2

1st (R-)COPADM (course n°1) starts on day 8 after COP, (may be delayed for up to 3 days if continuing metabolic or other problems).

Note: Control renal function before administering methotrexate. If GFR is significantly reduced do not give the full dose of MTX, see annexe C.4.4 for dose modifications and consult trial national co-investigator.

Rituximab	if assigned by randomisation Nov 2015: all patients receive rituximab 375 mg/m ² on D-2 (=D6 after COP) and on D1, before starting chemotherapy (see annexe A for details of administration)
Vincristine	2.0 mg/m ² (max dose 2 mg) as IV bolus on day 1.
Predniso(lo)ne	60 mg/m ² /day (divided into two doses) orally on Days 1 - 5 inclusive then reduced to zero over 3 days. Methylpredniso(lo)ne IV may be used at the same dose of predniso(lo)ne if unable to take PO.
Methotrexate	3 g/m ² in 500 ml/m ² dextrose 5% as IV infusion over 3 hours on Day 1. See annexe B.1 for further details.
Folinic acid	15 mg/m ² orally every 6 hours until MTX level is below 0.15 µmol/L (1.5x10 ⁻⁷ M), see annexe B.3 for further details. This begins at 24 hours from the start of the methotrexate infusion.
Cyclophosphamide	250 mg/m ² /dose every 12 hours as an infusion over 15 mins on days 2-4 (total of 6 doses). Continue hydration at a rate of 3000 ml/m ² /day until 12 hours after the last dose of cyclophosphamide.
Doxorubicin	60 mg/m ² /day in 1 hour infusion on Day 2,
2 drugs IT	Methotrexate & Hydrocortisone 8-15 mg IT injection on Day 2 & 6 dose varies with age: see annexe B for details. On D2, should be given before folinic acid is started.

B-High: (R-)COPADM November 2015: all patients receive R-COPADM

Days	-2 (=D6 COP)	-1	1	2	3	4	5	6
[Rituximab]	[.]		[.]					
Vincristine			.					
Predniso(lo)ne			Tail to zero over 3 days
Methotrexate			.					
Folinic acid			(..)		
Cyclophosphamide					
Doxorubicin				.				
IT MTX & HC				.				.

2nd course of COPADM (course n°2): it is identical to course n°1. It should start as soon as peripheral blood counts have recovered (ANC ~ 1.0 x 10⁹/l and platelets ~ 100 x 10⁹/l) (but not less than D16) generally around D18–21 and should be no more than 21 days after D1 of the first course.

If allocated by randomisation (November 2015: for all patients), **Rituximab at D-2** should be given as soon as peripheral blood count is starting to recover (ANC ~ 0.5x10⁹/l) and fever has resolved, so that course n°2 can start as soon as peripheral count has recovered (ANC ~ 1.0 x 10⁹/l and platelets ~ 100 x 10⁹/l). **It is very important not to delay the start of course n°2**, as delay between the 2 courses has been shown to be of adverse prognostic significance.

Note: G-CSF is not recommended after these courses. If G-CSF is given after the first COPADM course it should have been discontinued for at least 48 hours prior to start the second course of COPADM.

10.3.3. B-high - CONSOLIDATION: 2 courses of CYM or R-CYM (November 2015: all patients receive R-CYM)

= Course n°3 and course n°4

First (R-)CYM (= course n°3) should start when the peripheral counts have recovered following 2nd course of COPADM with ANC ~ $1.0 \times 10^9/l$ and platelets ~ $100 \times 10^9/l$ (but not less than D16).

Rituximab if assigned by randomization. **November 2015: all patients receive rituximab:** 375 mg/m² on D1, before starting chemotherapy (see annexe A for the details of the administration)

Methotrexate 3 g/m² in 500 ml/m² dextrose 5% as IV infusion over 3 hours, on Day 1. See annexe B.1 for further details.

Folinic acid 15 mg/m² orally every 6 hours until MTX level is below 0.15 µmol/L ($1.5 \times 10^{-7}M$), see annexe B.3 for further details.
This begins at 24 hours from the start of the methotrexate infusion

Cytarabine 100 mg/m² in 1000 ml/m² dextrose saline as infusion over 24 hours. Repeat daily from day 2-6 inclusive (5 days total, last day continues into day 7).

IT Methotrexate 8 - 15 mg by IT injection on Day 2 (dose varies with age, see annexe B), should be given before folinic acid rescue is started.

IT Cytarabine 15 - 30 mg by IT injection on Day 7 (dose varies with age, see annexe B)

IT Hydrocortisone 8 - 15 mg by IT injection on Day 2 & 7 (dose varies with age, see annexe B)

B-High: course n°3 and course n°4 [(R-)CYM] **November 2015: all patients receive R-CYM**

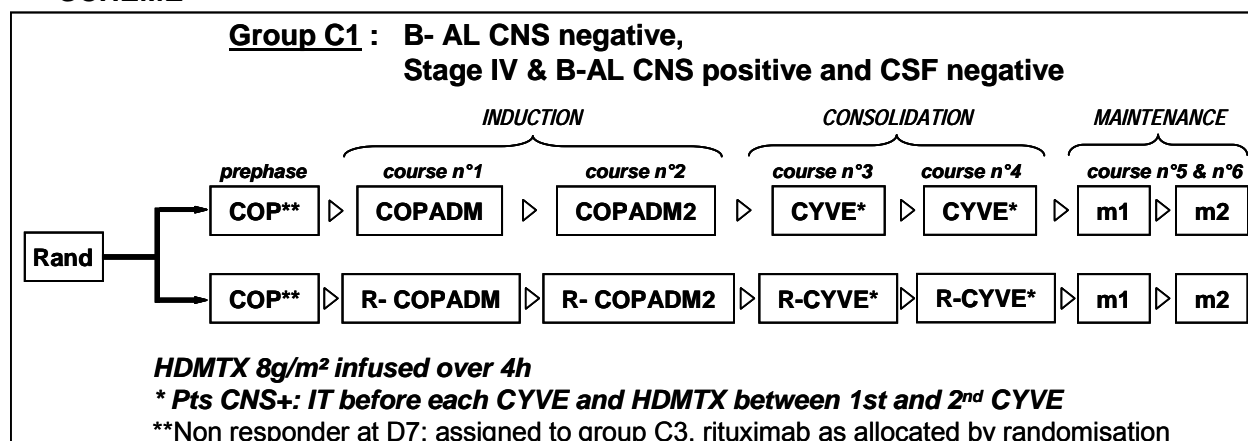
Days	1	2	3	4	5	6	7
[Rituximab]	[•]						
Methotrexate	•						
Folinic acid		• • • •	• • (• •	• • • •)			
Cytarabine							
IT MTX		•					
IT HC		•					•
IT cytarabine							•

Following recovery from 1st (R-)CYM a full assessment of response should be carried out. If complete remission is not obtained with histological confirmation, the patient ("slow responder") should be switched to C1 regimen starting at (R-)CYVE (see section 10.4.3 and 10.4.4). If they were randomised to receive rituximab, they will receive rituximab only with the first R-CYVE in order to receive a total of 6 courses.

Second (R-)CYM (course n°4) (**November 2015: all patients receive R-CYM which**) is identical to course n° 3. It should start as soon as peripheral counts have recovered (ANC ~ $1.0 \times 10^9/l$ and platelets ~ $100 \times 10^9/l$)

10.4. GROUP C1 (CSF NEG) : TREATMENT SCHEME AND DETAILS

▪ SCHEME



November 2015: the randomization is halted: all patients receive the arm of treatment with rituximab

▪ TABLES OF INVESTIGATIONS at diagnosis and during treatment

See section 10.7.

▪ TREATMENT DETAILS (sections 10.4.1 to 10.4.5)

Patients fulfilling the criteria for Group C without blasts in the CSF (**B-AL CNS negative, stage IV and B-AL with CNS involvement except blasts in the CSF**) will receive their treatment according to the standard **C1** regimen, ie, a prephase followed by 6 courses (2 courses of induction with HDMTX at 8g/m² in **4h** infusion followed by 2 courses of consolidation (CYVE) and 2 maintenance courses. Patients with CNS involvement will also receive 1 IT before each CYVE course and one HDMTX+ IT between the 2 CYVE courses.

Patients are randomised to receive or not receive rituximab (**November 2015: all patients receive rituximab**) in combination with this chemotherapy.

Intervals between courses should be **as short as possible**, and the subsequent course given as soon as recovery allows. In case of severe complication (such as gut perforation, fungal infection) which necessitates to delay chemotherapy and does not allow to start the next course on time, a course of COP ("**waiting COP**" course) should be considered in order not to let the patient without any chemotherapy.

Contact the study national co-investigator for further advice if necessary.

10.4.1. C-CSF neg - (C1) Pre-phase: COP

Prevention or treatment of the tumor lysis syndrome must be started before the administration of the chemotherapy and be continued as long as necessary the following days (see annexe D2).

Vincristine	1 mg/m ² (max single dose 2.0 mg) IV bolus on Day 1
Cyclophosphamide	300 mg/m ² as an infusion over 15 minutes on Day 1
Predniso(lo)ne	60 mg/m ² /day (divided into two doses) orally on Days 1 – 7 Methylpredniso(lo)ne IV may be used at the same dose of predniso(lo)ne if unable to take PO.
3 drugs IT	Methotrexate 8-15 mg & Hydrocortisone 8-15 mg & cytarabine 15-30mg IT injection on Days 1*, 3 & 5 (dose varies with age, see below).
Folinic acid	15 mg/m ² orally 12 hly (= twice the day at 12h interval) on D2 and D4

Intrathecal drugs doses (mg)	Age (years)	Methotrexate	Hydrocortisone	cytarabine
	< 1	8	8	15
	>1 - <2	10	10	20
	>2 - <3	12	12	25
	> 3	15	15	30

C: prephase COP

Days	1	2	3	4	5	6 = D-2 of 1 st R-COPADM	7 tumour evaluation
Vincristine	•						
Cyclophosphamide	•						
Predniso(lo)ne	• •	• •	• •	• •	• •	• •	• •
3 drugs IT *	•		•		•		

Note: *Omit D1 intrathecal if given previous intrathecal therapy (with the exploratory lumbar puncture)

Evaluation of tumour response should be performed on day 7. Patients who had a no tumour response at D7 (“non responder” to COP, (see section 11.1. and 11.2.1) should be treated according to C3 arm without or with rituximab as assigned by randomization- November 2015: all patients receive rituximab

PATIENTS RANDOMISED TO (November 2015: ALL PATIENTS) RECEIVE RITUXIMAB (AND) SHOULD RECEIVE IT AT D-2 OF COURSE N°1 (COPADM), ie, AT D6 OF COP, one day before the D7 COP evaluation.

There may be situations where it does not seem possible to proceed with course n°1 (COPADM) at D8, then a **second course of COP** should be considered:

- the patient is in too critical condition to receive a COPADM, ie patients with renal failure, creatinine clearance <60 ml/min, septicaemia or other sepsis or grade 3/4 organ toxicity, then a second course of COP will have to be considered.
- In the case of significant effusion at diagnosis (with a risk of MTX accumulation):

If the effusion is small at D7 assessment, ignore and continue course n°1.

If significant effusions but overall response then give next course with MTX at day 5 instead of day 1 or consider to give a 2nd COP.

If significant effusions and no response, then move to Group C and give next course with MTX at day 5 instead of day 1(as long as effusions small).

- elevation of transaminases, sometimes with an increase in liver size, can be seen after COP and is probably due to a combination of corticosteroids and previous poor nutrition status. In the case of transaminases > 10x ULN it is preferable to wait 48 hours and proceed to course n°1 if transaminases decrease. If not, give a second course of COP.

In any case when course n°1 ([R-] COPADM) has to be postponed, **rituximab will not be given with course n°1 ([R-] COPADM) but at the planned time, ie at D6 and D8 of 1st COP, if the clinical**

situation is controlled. Contact the study national co-investigator for further advice if necessary.

10.4.2. C-CSF neg (C1) - INDUCTION: (R)-COPADM followed by (R)-COPADM2 (November 2015: all patients receive R-COPADM1 and R-COPADM2)

= Course n°1 and course n°2

The difference between COPADM and COPADM2 is the dose of cyclophosphamide which is doubled in COPADM2.

First (R)COPADM (course n°1) starts on day 8 after COP (may be delayed for up to 3 days if continuing metabolic or other problems)

Note: Control renal function before administering methotrexate, If GFR is significantly reduced, do not give methotrexate, see annexe C.4.4 for dose modifications and consult trial national co investigators.

Rituximab	if assigned by randomisation (November 2015: all patients receive rituximab): 375 mg/m ² on D-2 (=D6 after COP) and on D1, before starting chemotherapy (see annexe A for the details of the administration)
Vincristine	2 mg/m ² (max dose 2 mg) as IV bolus on D 1
Predniso(lo)ne	60 mg/m ² /day (divided into two doses) orally on D 1 to 5 inclusive then reduced to zero over 3 days. Methylpredniso(lo)ne IV may be used at the same dose of predniso(lo)ne if unable to take PO.
Methotrexate HD	8 g/m ² in 500 ml/m ² dextrose 5% IV infusion over 4 hours on D1. See annexe B.1 for further details.
Folinic acid	15 mg/m ² orally every 6 hours for a total of 12 doses (or as adapted until MTX level is below 0.15 µmol/L (1.5x10 ⁻⁷ M), see annexe B.3 for further details. This begins at 24 hours from the start of the MTX infusion.
Cyclophosphamide	250 mg/m ² /dose (will be 500 mg/m ² in course n°2), every 12 hours as an infusion over 15 mins on D 2-4 (total 6 doses). Continue hydration at rate of 3000 mls/m ² /day until 12 h after last dose of cyclophosphamide.
Doxorubicin	60 mg/m ² /day in 1 hour infusion on D 2.
3 drugs IT	Hydrocortisone, Methotrexate and cytarabine by IT injection on D 2, 4 & 6. on D2 should be given before start of folinic acid rescue. Doses same as in COP course

C1:(R)-COPADM (November 2015: all patients receive R-COPADM)

Days	- 2 (=D6 COP)	- 1	1	2	3	4	5	6
[Rituximab]	[•]		[•]					
Vincristine			•					
Predniso(lo)ne			• •	• •	• •	• •	• •	Tail to zero over 3 days
HD Methotrexate			•					
Folinic acid				• • • •	• • • •	• • • •		
Cyclophosphamide (double dose in course n°2)				• •	• •	• •		
Doxorubicin				•				
3 drugs IT				•		•		•

COPADM2 (course n°2) is identical to COPADM1, EXCEPT cyclophosphamide: 500 mg/m²/dose, every 12 hours on D 2-4 (total 6 doses).

It should start as soon as peripheral counts have recovered (ANC ~ 1.0 x 10⁹/l and platelets ~ 100 x 10⁹/l), (but not less than D16), generally around D18 – 21 and should be no more than 21 days after D1 of the first course. If allocated by randomisation (November 2015: all patients receive rituximab), **Rituximab of D-2** should be given as soon as peripheral is starting recovering (ANC ~ 0.5x10⁹/l) and fever has resolved, so that COPADM2 can start as soon as peripheral count has recovered. Note: If G-CSF is given after the first (R-)COPADM course it should have been discontinued for at least 48 hours prior to start (R-)COPADM2.

10.4.3. C- CSF neg (C1) - CONSOLIDATION: 2 courses of (R-)CYVE (November 2015: all patients receive R-CYVE)

= course n°3 and course n°4

First (R-)CYVE +/- IT&HDMTX (course n°3) should start after COPADM2 when peripheral counts have recovered with ANC > $1.0 \times 10^9/l$ and platelets > $100 \times 10^9/l$ (usually by day 18, but not less than D16).

Rituximab if assigned by randomisation: (November 2015: all patients receive rituximab) 375 mg/m² on D1, before chemotherapy (see annexe A for the details of the administration)

2 drugs IT if **CNS positive**: Hydrocortisone and methotrexate should be given on Day 1 at least 6 hours before the beginning of the cytarabine infusion.
NB No intrathecal cytarabine is given. Doses as in COP course

Cytarabine 50 mg/m² by continuous infusion over 12 hours in dextrose saline. This should start at 8.0 pm and run till 8.0 am the following day. Repeat daily x 5. (D1 to D5)

Cytarabine HD[^] 3 g/m² in 375 mls/m² dextrose saline as IV infusion over 3 hours, to start at the end of the 12 hour infusion of cytarabine on D 2 to 5 (from 8.0 am to 11 am).

Etoposide* 200 mg/m² in 500 mls/m² dextrose saline as IV infusion over 2 hours daily x 4, D 2 to 5 inclusive. Etoposide starts at 2.0 pm, 3 hours after end of high dose cytarabine (Ara-C HD).

If **CNS positive**, the **HDMTX+TIT course is given only after 1st (R)CYVE (course n°3), about D18** when ANC > $0.5 \times 10^9/l$ and platelets > $50 \times 10^9/l$ and 48 hrs after any G-CSF injections or platelet transfusions. Transaminases should be < Nx10.

Methotrexate HD 8 g/m² as infusion over 4 hrs. See annexe B for further details of administration.

3 drugs IT Hydrocortisone, methotrexate, cytarabine by IT injection should be given the day after HDMTX, before folinic acid rescue is started. Doses as in COP course.

Folinic acid 15 mg/m² orally every 6 hrs for a total of 12 doses (or as adapted until MTX level is below $0.15 \mu\text{mol/L}$ ($1.5 \times 10^{-7}\text{M}$), see annexe B.3 for further details. This begins at 24 hours from the start of the methotrexate infusion.

C1 (R-)CYVE 1 and 2 for B-AL CNS negative (November 2015: all patients receive R-CYVE)

Days	1	2	3	4	5
[Rituximab]	□				
Cytarabine Continuous, from 8 pm to 8 am	□	□	□	□	□
cytarabine HD [^] from 8 am to 11 am		•	•	•	•
Etoposide*, from 2 pm to 4 pm		•	•	•	•

[^]See annexe D.9. for steroid eye drops with HD cytarabine.

* See annexe C.4.3. for patients intolerant of etoposide or for longer infusion for etoposide induced hypotension.

10.4.3 Continues on Next page.

C1: (R-)CYVE 1 + IT & HDMTX for stage IV & B-AL CNS positive CSF negative (November 2015: all patients receive R-CYVE1 + IT & HDMTX)

Days	1	2	3	4	5	18	19 to 21
[Rituximab]	[•]						
Cytarabine Continuous, 8 pm to 8 am	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
cytarabine HD [^] 8 am to 11 am		•	•	•	•		
Etoposide, 2 pm to 4 pm		•	•	•	•		
Methotrexate HD						•	
IT (D1=2 drugs, D19=3drugs)	•						•
Folinic acid						

[^] See annexe D.9. for steroid eye drops with HD cytarabine.

* See annexe C.4.3. for patients intolerant of etoposide or for longer infusion for etoposide induced hypotension.

The 2nd (R-)CYVE+/- IT (Nov 2015: The 2nd R-CYVE +/- IT) (course n°4) is identical to course n°3, (but without HDMTX&IT of D18-19 for CNS positive patients) and should start as soon as peripheral counts have recovered (ANC ~ $1.0 \times 10^9/l$ and platelets ~ $100 \times 10^9/l$) (usually by days 25 to 28).

Following recovery from course n°4 [2nd (R-)CYVE], a full assessment of response should be carried out. The patient should be in CR to continue on protocol. Only progressive disease or incomplete remission with histological confirmation of presence of viable cells in the residue will be considered as treatment failure. A decision should not be taken only on the basis of a positive PET(-CT).

In case of treatment failure, one option can be the R-ICE regimen, to be discussed according to sites of the disease.

Contact the national co-investigator for further advice if necessary.

10.4.4. C-CSF neg: MAINTENANCE (C1): courses m1, m2

= Course n°5 and course n°6

Course m1 (course n°5) starts when peripheral counts have recovered from course n°4 [(R)-CYVE 2] with ANC > $1.0 \times 10^9/l$ and platelets > $100 \times 10^9/l$ (usually by day 25 to 28).

Vincristine	2.0 mg/m ² (max dose 2 mg) as IV bolus on D 1
Predniso(lo)ne	60 mg/m ² /day (divided into two doses) orally on D 1 -5 inclusive then reduced to zero over 3 days. Methylpredniso(lo)ne IV may be used at the same dose of predniso(lo)ne if unable to take PO.
Methotrexate HD	8 g/m ² in 500 mls/m ² dextrose 5% IV infusion over 4 hours on D 1. (See annexe B.1)
Folinic acid	15 mg/m ² orally every 6 hours for a total of 12 doses (or as adapted until MTX level is below 0.15 µmol/L ($1.5 \times 10^{-7}M$), see annexe B.3 for further details. This begins at 24 hours from the start of the methotrexate infusion.
Cyclophosphamide	500 mg/m ² /day given daily as IV infusion over 30 minutes on D 2 and 3.
Doxorubicin	60 mg/m ² on D 2 in 1h infusion
3 drugs IT	Hydrocortisone, methotrexate, cytarabine by IT injection on Day 2, should be given before folinic acid rescue is started. (dose same as in COP course).

Maintenance m1

Days	1	2	3	4	5	6
Vincristine	•					
Predniso(lo)ne	• •	• •	• •	• •	• •	Tail to zero over 3 days
Methotrexate HD	•					
Folinic acid		••••	••••	••••		
Cyclophosphamide		•	•			
Doxorubicin		•				
3 drugs IT		•				

Course m 2 (course n°6) starts at D28 of course n°5

Cytarabine	50 mg/m ² as subcutaneous injection every 12 hours, D 1 - 5
Etoposide	150 mg/m ² IV infusion over 90 minutes, D 1-3

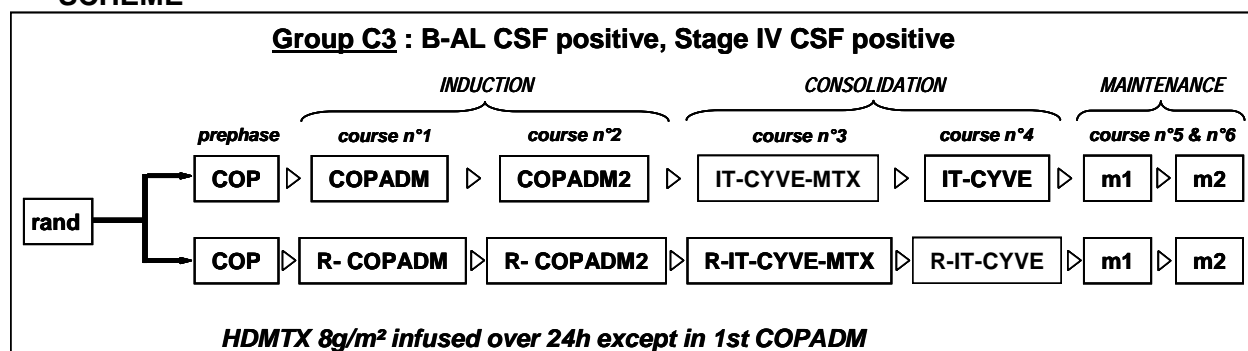
Maintenance m2

Days	1	2	3	4	5
Cytarabine	• •	• •	• •	• •	• •
Etoposide*	•	•	•		

* See annexe C.4.3. for patients intolerant of etoposide or for longer infusion for etoposide induced hypotension.

10.5. GROUP C: CSF POS (C3): TREATMENT SCHEME AND DETAILS

▪ SCHEME



November 2015: the randomization is halted: all patients receive the arm of treatment with rituximab

▪ TABLES OF INVESTIGATIONS at diagnosis and during treatment

See section 10.7.

▪ TREATMENT DETAILS (sections 10.5.1 to 10.5.5)

Patients fulfilling the criteria for Group C-CSF pos with blasts in the CSF (stage IV & B-AL **with blasts in the CSF**) will receive their treatment according to the standard **C3** regimen, ie, a prephase followed by two induction courses with HDMTX at 8g/m² in 4h and in 24h infusion in first and second course respectively followed by 2 consolidation courses with additional IT and HDMTX and 2 maintenance.

Intervals between courses should be **as short as possible**, and the subsequent course given as soon as recovery allows. In case of severe complication (such as gut perforation, fungal infection) which necessitates delaying chemotherapy and does not allow to start the next course on time, a course of COP ("**waiting COP**" course) should be considered in order not to let the patient without any chemotherapy.

Contact the study national co-investigator for further advice if necessary.

10.5.1. C-CSF pos (C3) Pre-phase: COP

Prevention or treatment of the tumor lysis syndrome must be started before the administration of the chemotherapy and be continued as long as necessary the following days (see annexe D2)

Vincristine	1 mg/m ² (max single dose 2.0 mg) IV bolus on D1
Cyclophosphamide	300 mg/m ² as an infusion over 15 minutes on D1
Predniso(lo)ne	60 mg/m ² /day (divided into two doses) orally on D1 to 7 Methylpredniso(lo)ne IV may be used at the same dose of predniso(lo)ne if unable to take PO.
3 drugs IT	Methotrexate 8-15 mg & Hydrocortisone 8-15 mg & cytarabine 15-30mg IT injection on Days 1*, 3 & 5 (dose varies with age, see below).
Folinic acid	15 mg/m ² orally 12 hrly (= twice the day at 12h interval) on D2 and D4

Intrathecal drugs doses (mg)

Age (years)	Methotrexate	Hydrocortisone	Cytarabine
< 1	8	8	15
≥1 - <2	10	10	20
≥2 - <3	12	12	25
≥ 3	15	15	30

C-CSF pos: COP (C3)

Days	1	2	3	4	5	6 = D-2 of 1st R-COPADM	7 tumour evaluation
Vincristine	•						
Cyclophosphamide	•						
Predniso(lo)ne	• •	• •	• •	• •	• •	• •	• •
3 drugs IT*	•		•		•		

Note: *Omit D1 intrathecal if given previous intrathecal therapy (with the exploratory lumbar puncture)

Evaluation of tumour response should be performed on day 7. However there is no treatment modification depending on COP response.

PATIENTS RANDOMISED TO (November 2015: ALL PATIENTS) RECEIVE RITUXIMAB (AND) SHOULD RECEIVE IT AT D-2 OF COPADM, ie, AT D6 OF COP, one day before the D7 COP evaluation.

There may be situations where it does not seem possible to proceed with course n°1 (COPADM) at D8, then a **second course of COP** should be considered:

- the patient is in too critical condition to receive a COPADM, ie patients with renal failure, creatinine clearance <60 ml/min, septicaemia or other sepsis or grade 3/4 organ toxicity, then a second course of COP have to be considered.
- In the case of significant effusion at diagnosis (with a risk of MTX accumulation):
 - If the effusion is small at D7 assessment, ignore and continue course n°1.
 - If significant effusions but overall response then give next course with MTX at day 5 instead of day 1 or consider to give a 2nd COP
 - If significant effusions and no response, ~~then move to Group C and give next course~~ with MTX at day 5 instead of day 1 (as long as effusions small)
- elevation of transaminases, sometimes with an increase in liver size, can be seen after COP and is probably due to a combination of corticosteroids and previous poor nutrition status. In the case of transaminases > 10x ULN it is preferable to wait 48 hours and proceed to course n°1 if transaminases decrease. If not, give a second course of COP.

In any case when course n°1 ([R-] COPADM) has to be postponed, **rituximab will not be given with course n°1 ([R-] COPADM) but at the planned time, ie at D6 and D8 of 1st COP, if the clinical situation is controlled.**

Contact the study national co-investigator for further advice if necessary.

10.5.2. C-CSF pos: INDUCTION (C3): (R)-COPADM1 followed by (R)-COPADM2 (November 2015: all patients receive R-COPADM1 and R-COPADM2)

= course n°1 and course n°2

The difference between COPADM1 and COPADM2 is the duration of **infusion of MTX** and the **dose of cyclophosphamide which is doubled in COPADM2**.

The first (R)-COPADM (November 2015: R-COPADM) (course n°1) starts on day 8 after COP (may be delayed for up to 3 days if continuing metabolic or other problems).

Note: Control renal function before administering methotrexate. If GFR is significantly reduced, do not give methotrexate, see annexe C.4.4 for dose modifications, and consult trial coordinators. **In C-CSF pos, HDMTX 8g/m² is given in 24h infusion (C3 arm) except in the first course where it is given in 4h,** (see annexe B for details of administration).

Rituximab	if assigned by randomisation: (November 2015: all patients receive rituximab) 375 mg/m ² on D-2 (=D6 after COP) and on D1, before starting chemotherapy (see annexe A for the details of the administration)
Vincristine	2 mg/m ² (max dose 2 mg) as IV bolus on D1
Predniso(lo)ne	60 mg/m ² /day (divided into doses) orally on D1 to 5 inclusive then reduced to zero over 3 days. Methylpredniso(lo)ne IV may be used at the same dose of predniso(lo)ne if unable to take PO.
Methotrexate HD	8 g/m ² in IV infusion over 4 hours on D1. See annexe B.1
Folinic acid	15 mg/m ² orally every 6 hours for a total of 12 doses (or as adapted until MTX level is below 0.15 µmol/L (1.5x10 ⁻⁷ M), see annexe B.3 for further details. This begins at 24 hours from the start of the methotrexate infusion.
Cyclophosphamide	250 mg/m ² /dose, every 12 hours as an infusion over 15 min on D 2 to 4 (total 6 doses). Continue hydration at rate of 3000 mls/m ² /day until 12 hours after last dose of cyclophosphamide.
Doxorubicin	60 mg/m ² /day in 1 hour infusion on D 2.
3 drugs IT	Hydrocortisone, Methotrexate and cytarabine by IT injection on D 2, 4 & 6 dose same as in COP course

C-CSF pos: C3- 1st (R)-COPADM (November 2015: all patients receive R-COPADM)

Days	-2 (=D6 COP)	-1	1	2	3	4	5	6
[Rituximab]	[•]		[•]					
Vincristine			•					
Predniso(lo)ne			• •	• •	• •	• •	• •	Tail to zero over 3 days
HD Methotrexate (4h)			•					
Folinic acid				• • • •	• • • •	• • • •		
Cyclophosphamide				• •	• •	• •		
Doxorubicin				•				
3 drugs IT				•		•		• *

* check MTX level (see annexe C)

Note: If G-CSF is given after the first (R)-COPADM course it should have been discontinued for at least 48 hours prior to start of the course of (R)-COPADM2.

Section.10.5.2. continues on next page

(R-)COPADM2 (November 2015: all patients receive R-COPADM2) (course n°2) is identical to course n°1, EXCEPT infusion time of MTX which is 24h (rescue at H36) and cyclophosphamide: 500 mg/m²/dose, every 12 hours on D 2-4 (total 6 doses).

It should start as soon as peripheral blood count have recovered (ANC ~ 1.0 x 10⁹/l and platelets ~ 100 x 10⁹/l) (but not less than D16), generally around D18 – 21 and should be no more than 21 days after D1 of the first course.

If allocated by randomisation (**November 2015: for all patients**), Rituximab of D-2 should be given as soon as peripheral blood count is starting recovering (ANC ~ 0.5x10⁹/l) and fever has resolved, so that course n°2 can start as soon as peripheral count has recovered.

Rituximab	if assigned by randomisation: (November 2015: all patients receive rituximab) 375 mg/m ² on D-2 (=D6 after COP) and on D1, before starting chemotherapy (see annexe A for details of administration)
Vincristine	2 mg/m ² (max dose 2 mg) as IV bolus on D 1
Predniso(lo)ne	60 mg/m ² /day (divided into two doses) orally on D1 to 5 inclusive then reduced to zero over 3 days. Methylpredniso(lo)ne IV may be used at the same dose of predniso(lo)ne if unable to take PO.
Methotrexate HD	8 g/m ² in IV infusion over 24 hours on D 1 (1.6 g/m ² in 30' then 6.4 g/m ² during the following 23h30). See annexe B.1
Folinic acid	15 mg/m ² orally every 6 hours for a total of 12 doses (or as adapted until MTX level is below 0.15 µmol/L (1.5x10 ⁻⁷ M), see annexe B.3 for further details. This begins at 36 hours from the start of the methotrexate infusion.
Cyclophosphamide	500 mg/m ² , every 12 hours as an infusion over 15 min on D 2 to 4 (total 6 doses). Continue hydration at rate of 3000 mls/m ² /day until 12 hours after last dose of cyclophosphamide.
Doxorubicin	60 mg/m ² /day in 1 hour infusion on D 2.
3 drugs IT	Hydrocortisone, Methotrexate and cytarabine by IT injection on D 2, 4 & 6. Dose same as in COP course

C-CSF pos: C3-(R-)COPADM2 (November 2015: all patients receive C3-R-COPADM2)

Days	- 2	-1	1	2	3	4	5	6
[Rituximab]	[.]		[.]					
Vincristine			.					
Predniso(lo)ne			Tail to zero over 3 days
HD Methotrexate (24h)			.					
Folinic acid					
Cyclophosphamide					
Doxorubicin			.					
3 drugs IT			.			.		. *

* check MTX level (see annexe C)

10.5.3. C- CSF pos:(C3) CONSOLIDATION: 2 courses of (R-)IT-CYVE (November 2015: all patients receive 2 courses R-IT-CYVE)

=Course n°3 and course n°4

(R-)IT-CYVE- 1+MTX course (course n° 3) should start after (R-)COPADM2 when peripheral counts have recovered with ANC > $1.0 \times 10^9/l$ and platelets > $100 \times 10^9/l$ (usually by day 18).

Rituximab	if assigned by randomisation: (<u>November 2015: all patients receive rituximab</u>) 375 mg/m ² on D1 before chemotherapy (see annexe A for details of administration)
2 drugs IT	Hydrocortisone and methotrexate should be given on Day 1 at least 6 hours before the beginning of the cytarabine infusion. NB No intrathecal cytarabine is given. Doses as in COP course
Cytarabine	50 mg/m ² by continuous infusion over 12 hours in dextrose saline. This should start at 8.0 pm and run till 8.0 am the following day. Repeat daily x 5. (D1 to D5)
HD Cytarabine [^]	3 g/m ² in 375 mls/m ² dextrose saline as IV infusion over 3 hours, to start at the end of the 12 hour infusion of cytarabine on D 2 to 5 (from 8.0 am to 11 am).
Etoposide [*]	200 mg/m ² in 500 mls/m ² dextrose saline as IV infusion over 2 hours daily x 4, D 2 to 5 inclusive. Etoposide starts at 2.0 pm, 3 hours after end of HD cytarabine.
Methotrexate HD	This is given only after course n°3, about D18 when ANC > $0.5 \times 10^9/l$ and platelets > $50 \times 10^9/l$ and 48 hrs after any G-CSF injections or platelet transfusions. Transaminases should be < Nx10. 8 g/m ² as infusion over 24 hrs (1.6 g/m ² in 30' then 6.4 g/m ² during the following 23h30). See annexe B for further details of administration.
3 drugs IT	Hydrocortisone, methotrexate, cytarabine by IT injection the day following HDMTX Should be given before folinic acid rescue is started. Doses as in COP course.
Folinic acid	15 mg/m ² orally every 6 hours for a total of 12 doses (or as adapted until MTX level is below $0.15 \mu\text{mol/L}$ ($1.5 \times 10^{-7}\text{M}$), see annexe B.3 for further details. This begins at 36 hours from the start of the methotrexate infusion.

C-CSF pos : (R-)IT-CYVE 1+MTX (course n°3) (November 2015: all patients receive R-IT-CYVE 1+MTX)

Days	1	2	3	4	5	18	19 to 21
[Rituximab]	[•]						
Cytarabine Continuous, 8 pm to 8 am	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
HD cytarabine [^] 8 am to 11 am		•	•	•	•		
Etoposide [*] , 2 pm to 4 pm		•	•	•	•		
Methotrexate HD						•	
IT drugs (D1=2, D19=3)	•						•
Folinic acid						

[^]See annexe D.9. for steroid eye drops with HD cytarabine.

^{*} See annexe C.4.3. for patients intolerant of etoposide or for longer infusion for etoposide induced hypotension.

The 2nd (R-)IT-CYVE (November 2015: for all patients: R-IT-CYVE 1+MTX) (course n°4) is without HD MTX& IT of D18-19. It should start as soon as peripheral counts have recovered (ANC ~ $1.0 \times 10^9/l$ and platelets ~ $100 \times 10^9/l$) (usually by days 25 to 28).

Following recovery from 2e (R-)IT-CYVE a full assessment of response should be carried out. The patient should be in CR to continue on protocol. Only progressive disease or incomplete remission with histological confirmation of presence of viable cells in the residue will be considered as treatment failure. A decision should not be taken only on the basis of a positive PET(-CT).

Contact the national co-investigator for further advice if necessary.

2nd (R-) IT-CYVE (course n°4) (November 2015: all patients receive R-IT-CYVE)

Days	1	2	3	4	5
[Rituximab]	[•]				
Cytarabine Continuous, 8 pm to 8 am	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
HD cytarabine [^] 8 am to 11 am		•	•	•	•
Etoposide [*] , 2 pm to 4 pm		•	•	•	•
2 drugs IT	•				

[^]See annexe D.9. for steroid eye drops with HD cytarabine

^{*} See annexe C.4.3. for patients intolerant of etoposide or for longer infusion for etoposide induced hypotension.

10.5.4. C-CSF pos: = MAINTENANCE (C3): courses m1, m2

= course n°5 and course n°6

Course m1 (course n° 5) starts when peripheral counts have recovered from course n°4 [(R)-CYVE-IT 2] with ANC > $1.0 \times 10^9/l$ and platelets > $100 \times 10^9/l$ (usually by day 25 to 28).

Vincristine	2.0 mg/m ² (max dose 2 mg) as IV bolus on D 1
Predniso(lo)ne	60 mg/m ² /day (divided into two doses) orally on D 1 to 5 inclusive then reduced to zero over 3 days. Methylpredniso(lo)ne IV may be used at the same dose of predniso(lo)ne if unable to take PO.
Methotrexate HD	8 g/m ² in IV infusion over 24 hours on D 1 (1.6 g/m ² in 30' then 6.4 g/m ² during the following 23h30). (See annexe B.1)
Folinic acid	15 mg/m ² orally every 6 hours for a total of 12 doses (or as adapted until MTX level is below 0.15 µmol/L ($1.5 \times 10^{-7}M$), see annexe B.3 for further details. This begins at 36 hours from the start of the methotrexate infusion.
Cyclophosphamide	500 mg/m ² /day given daily as IV infusion over 30 minutes on D 2 and 3.
Doxorubicin	60 mg/m ² on day 2 in 1h infusion on D2
3 drugs IT	Hydrocortisone, methotrexate, cytarabine by IT injection on Day 2, should be given before folinic acid rescue is started. (dose same as in COP course).

Maintenance m1

Days	1	2	3	4	5	6
Vincristine	•					
Predniso(lo)ne	• •	• •	• •	• •	• •	Tail to zero over 3 days
Methotrexate HD	•					
Folinic acid		• • • •	• • • •	• • • •		
Cyclophosphamide		•	•			
Doxorubicin		•				
3 drugs IT		•				

Course m2 (course n°6) starts at D28 of course n°5

Cytarabine	50 mg/m ² as subcutaneous injection every 12 hours, D 1 – 5
Etoposide	150 mg/m ² IV infusion over 90 minutes, D 1-3

Maintenance m2

Days	1	2	3	4	5
Cytarabine	• •	• •	• •	• •	• •
Etoposide	•	•	•		

* See annexe C.4.3. for patients intolerant of etoposide or for longer infusion for etoposide induced hypotension.

10.6. TREATMENT PLAN FOR PMLBL - PHASE II STUDY

▪ SCHEME

PMLBL: 6 courses of EPOCH with rituximab, with dose adaptation at each course based on previous course ANC nadir



Doxorubicin, VCR, VP16 infused over 96h, no IT, no HDMTX

▪ TABLES OF INVESTIGATIONS at diagnosis and during treatment

See section 10.7

▪ TREATMENT BASIC PRINCIPLES (sections 10.6.1 to 10.6.5)

- Patients with PMLBL will receive six courses of chemotherapy associated with six injections of Rituximab.
- If $ANC \geq 1 \times 10^9/l$ and platelets $\geq 100 \times 10^9/l$ on day 21, begin next course.
- There is a dose adaptation at each course based on previous ANC nadir (see following pages).
- Some of these patients with bulky disease may be at risk from metabolic complications secondary to tumour lysis syndrome, although it is less frequent than in Burkitt lymphoma. Adapted measures should be taken to minimise this risk (see annexe G).
- In the case of difficulty to obtain the definitive histopathological diagnosis and necessity to start a treatment rapidly, a COP course is allowed, one week before starting the 1st course of DA-EPOCH-R.

10.6.1. DA-EPOCH-R: Course n°1

See annexe C6 for the infusion modalities of Etoposide, Doxorubicine, Vincristine together during 4 days.

Starting Dose Level (Level 1)

Begin the infusion of Etoposide, Doxorubicin and Vincristine immediately after Rituximab is completed. The infusion should be administered through a central venous access device.

Administer Cyclophosphamide immediately after infusions of Etoposide, Doxorubicin and Vincristine are completed.

Rituximab	375 mg/m ² IV on day 1 before starting chemotherapy (see <i>annexe A</i> for details)
Predniso(lo)ne	120 mg/m ² /day (divided into 2 doses) orally (or IV) on days 1, 2, 3, 4 and 5 Methylpredniso(lo)ne IV may be used at the same dose of predniso(lo)ne if unable to take PO.
Etoposide	50 mg/m ² /day continuous infusion over 24 h days D1, D2, D3, and D4 (→ 200 mg/m ² over 96 h); begin the infusion immediately after Rituximab is completed
Doxorubicin	10 mg/m ² /day continuous infusion over 24 h days D1, D2, D3, and D4 (→ 40 mg/m ² over 96 h); begin the infusion immediately after Rituximab is completed
Vincristine	0.4 mg/m ² /day continuous infusion over 24 h days D1, D2, D3, and D4 (→ 1.6 mg/m ² over 96 h); begin the infusion immediately after Rituximab is completed. No dose limitation.
Cyclophosphamide	750 mg/m ² IV bolus on day 5; administer Cyclophosphamide immediately after infusions of Etoposide, Doxorubicin and Vincristine are completed

EPOCH-R

Days	1	2	3	4	5	6
Rituximab	•					
Predniso(lo)ne	• •	• •	• •	• •	• •	
VP16*						
Doxorubicin						
VCR						
Cyclophosphamide					•	
G-CSF						•

* See annexe C.4.3. for patients intolerant of etoposide or for longer infusion for etoposide induced hypotension.

Start **G-CSF** subcutaneous injection at the dose of 5 µg/kg/day at D6 (24 hours after the end of cyclophosphamide) until ANC > 5 x 10⁹/l past nadir (this is usually for 9/10 days). The ANC may rise the very large values but G-CSF is not stopped until the nadir has been reached and passed.

10.6.2. DA-EPOCH-R: Courses n°2 and following

Repeat courses every 3 weeks (21 days) for a total of 6 courses.

- If ANC $\geq 1 \times 10^9/l$ and platelets $\geq 100 \times 10^9/l$ on day 21, begin treatment.
- If ANC $< 1 \times 10^9/l$ or platelets $< 100 \times 10^9/l$ on day 21, delay up to 1 week. G-CSF may be started for ANC $< 1 \times 10^9/l$ and stopped 24 hours before treatment. If counts still low after 1 week delay, reduce 1 dose level below last course.

10.6.3. Dose-Adjustment Paradigm for the following DA-EPOCH-R course

Basic principles of treatment regulation

- Dose adjustments above starting dose (level 1) apply to Etoposide, Doxorubicin and Cyclophosphamide.
- Dose adjustments below starting dose (level 1) apply to Cyclophosphamide only.
- Doses of drugs are based on the previous course ANC nadir according the following two tables:

ANC nadir after previous course	dose level for next course
$\geq 0.5 \times 10^9/l$ on all measurements	increase 1 dose level above last course
$< 0.5 \times 10^9/l$ on 1 or 2 measurements	same dose as last course
$< 0.5 \times 10^9/l$ on ≥ 3 measurements	decrease 1 dose level below last course

OR

platelet nadir after previous course	dose level for next course
$< 25 \times 10^9/l$ on ≥ 1 measurement	decrease 1 dose level below last course

- If ANC $\geq 1 \times 10^9/l$ and platelets $\geq 100 \times 10^9/l$ on day 21, begin next course.
- If $1 \times 10^9/l$ or platelets $< 100 \times 10^9/l$ on day 21, delay up to 1 week. G-CSF may be started for ANC $< 1 \times 10^9/l$ and stopped 24 hours before treatment. If counts still low after 1 week delay, ↓ 1 dose level below last course.

Important: Measurement of ANC nadir is based on twice weekly blood counts only (3 days apart). Only use twice weekly blood counts for dose adjustment, even if additional blood counts are obtained.

- If a patient has severe life-threatening complications, such as infection requiring intubation or pressor support, the responsible physician has the option not to escalate or to reduce doses.

- In the absence of severe complications, the dose-adjusted principles should be followed.

Table of doses per level for adjusted agents:

Drugs	drug doses per dose levels							
	-2	-1	1 "starting dose"	2	3	4	5	6
Doxorubicin (mg/m ² /day)			10	12	14.4	17.3	20.7	24.8
Etoposide (mg/m ² /day)			50	60	72	86.4	103.7	124.4
Cyclophosphamide (mg/m ² /day)	480	600	750	900	1080	1296*	1555*	1866*

* These levels of doses should be accompanied by at least 12h hydration. Mesna may also be added

10.6.4. Dose modifications due to complications during DA-EPOCH-R

- In the absence of severe complications, the dose-adjusted principles should be followed.
- If a patient has severe life-threatening complications, such as infection requiring intubation or pressor support, the responsible physician has the option not to escalate or to reduce doses.

- **Ileus:** Constipation commonly occurs in patients receiving Vincristine, so patients should receive stool softeners as indicated. Occasionally, symptomatic ileus may occur and this should be treated with a Vincristine dose reduction. Because the severity of ileus is dose related, it is usually unnecessary to stop the Vincristine altogether. Furthermore, because the therapy administered is curative, every effort should be made to not unnecessarily reduce Vincristine doses. The following guidelines for symptomatic ileus on a previous course should be followed:

- **Clinical ileus < 8 days** with abdominal pain requiring narcotics and / or persistent nausea / vomiting > 2 days: Reduce Vincristine dose 25 %.
- **Clinical ileus 8-12 days** with abdominal pain requiring narcotics and / or persistent nausea / vomiting > 2 days: Reduce Vincristine dose 50 %.
- **Clinical ileus >12 days** with abdominal pain requiring narcotics and / or persistent nausea / vomiting > 2 days: Hold Vincristine on next course. May restart at 50 % reduction on subsequent course.

- Neurological and Hepatic Toxicity:

Toxicity	Grade	% dose of Vincristine
Sensory neuropathy	2	100
	3	50
Motor neuropathy	1	100
	2	75
	3	25
	4	0
Hepatic dysfunction	Bilirubin on day 1	
	1.5-3.0	75
	> 3.0	50

- **Rituximab infusion-related toxicity:** Infusion-related side effects of Rituximab may often be reduced by slowing the rate of infusion or by administration of medication (see annexe A for administration instructions and treatment of severe infusion-related toxicity, including anaphylaxis). Rituximab infusions may be slowed or discontinued completely, but no dose reductions of Rituximab are allowed on the protocol. Rituximab will be discontinued in patients with grade 4 allergic reactions.

10.6.5. Remission

Complete remission is assessed after the 6th course.

In case of positive PET(-CT) scan, or of a huge residual tumor, a biopsy/removal of the residual mass should be performed. Only in case of viable cells in the residue, the situation will be considered as an event.

The salvage treatment is not part of the study and is left to the decision of the national co-investigator. However a second line treatment is recommended (with the R-ICE or R-DHAP regimen or with another drug association) and a high-dose chemotherapy is preferred to the radiotherapy, although radiotherapy may have a place in some situations. Whatever is the chosen salvage treatment, the patient will be followed for survival status.

10.7. TABLES OF INVESTIGATIONS AT DIAGNOSIS AND DURING TREATMENT

For safety reasons, all laboratory parameters relevant for the application of chemotherapeutic drugs should be measured before start of every new cycle of chemotherapy. Additionally, blood counts should be measured at least twice during every cycle, especially at time of nadir. The patient's general condition (Lansky or Karnofsky index) should be documented before therapy and regularly during therapy. All these clinical and laboratory data must be available in the patient's medical record which will serve as the source document for verification if necessary. Only parameters of the investigations useful for endpoints will be recorded in the CRF.

See also paragraph 11.2

10.7.1. Phase III Group B (November 2015: all patients receive rituximab)

Observation	Pre and during COP	Pre 1 st R-COPADM & response to prephase	Pre R-COPADM2	Pre 1 st R-CYM	CR assessment	Pre 2 nd R-CYM
Physical exam	X	X	X	X	X	X
Performance status	X				X residue	
Histological diagnosis	X					
Blood counts	X	X	X	X		X
Biochemistries		X	X	X		X
Complete biology	X					
LDH	X					
Immunity status	X					
HBV screening	X					
HIV test	^					
Bone marrow bilateral aspirates	X	X if involved	^ if involved		X if involved	
BM Biopsy (2 sites)	X if DLBCL				X if involved	
Lumbar Puncture	X	X with IT drugs	X with IT drugs	X with IT drugs		X with IT drugs
Chest XR	X	X if involved	X if involved	X if involved	X if involved	
Abdo U/S	X	X if involved	X if not in CR	X if not in CR	X if involved	
CT or MRI of any area clinically involved	X	X fully assess involved areas	^	^	X fully assess involved areas	
bone scan	^				^	
PET(-CT)	o		o		o	
MDD/MRD	^		^ if BM pos		^	
Cardiac echo		X				
Sperm banking (pubertal boy)	^					
Pregnancy test (pubertal girl)	^					

X = mandatory investigation

^ = mandatory if clinically indicated, or part of an ancillary study, otherwise optional

o = optional

Biochemistries = urea, creatinine, electrolytes, Ca, PO₄, SGOT, SGPT, proteinemia, gamma GT

Complete biology = biochemistries + uric acid, albumin, bilirubin, alkaline phosphatases, haemostasis/coagulation

10.7.2. Phase III Group C (November 2015: all patients receive rituximab)

Observation	Pre Tt	Pre 1 st R-COPADM & response to prephase	Pre R-COPADM2	Pre 1 st R-CYVE	Pre HD MTX (in C3)	Pre 2 nd R-CYVE	CR assessment	Pre course m1 and m2
Physical exam Performance status	X	X	X	X	X	X		X
Histological diagnosis	X						X residue	
Blood count	X	X	X	X	X	X		X
Biochemistries		X	X	X	X	X		X
Complete biology	X							
LDH	X							
Immunity status	X							
Hepatitis B screening	X							
HIV test	^							
Bone marrow (2 aspirates) BM Biopsy (2 sites)	X ^ if DLBCL	X if involved	^ if involved	 ^			X if involved X if involved	
LP	X	X with IT drugs	X with IT drugs	X with IT drugs (if CNS+)	X with IT drugs	X with IT drugs (if CNS+)	X	X with IT drugs (m1)
Chest XR Abdo U/S	X	X if involved	X if involved	X if involved			X if involved	
CT or MRI of any area clinically involved	X	X Assess all involved areas		^ Involved areas if not in CR			X involved areas	^
Bone scan	^						^	
Biopsy	X						X if residual mass	
PET(-CT)	o		o				o	
MDD/MRD	^		^ if BM pos				^	
Cardiac echo		X						X
Sperm banking (pubertal boy)	^							
Pregnancy test (pubertal girl)	^							

Notes

X = mandatory investigation

^ = mandatory if clinically indicated, or part of an ancillary study, otherwise optional

o = optional

Biochemistries = urea, creatinine, electrolytes, Ca, PO₄, SGOT, SGPT, proteinemia, gamma GT

Complete biology = biochemistry + uric acid, albumin, bilirubin, alkaline phosphatases, haemostasis/coagulation

10.7.3. Phase II: PMLBL (DA-EPOCH-R)

Observation	Pre treatment	Before course n°2	Before course n°3	Before courses n°4,5,6	CR Assessment (after course n°6)
Physical exam / Performance status	X	X	X	X	X
Histological diagnosis	X				X if residual mass
Blood count	X	X	X	X	
Biochemistries		X	X	X	
Complete biology	X				
LDH	X				
Immunity status	X				X
Hepatitis B screening	X				
HIV test	^				
Bone marrow (aspirate + biopsy) x 2	X				X if involved
LP	X				
Chest XR Abdo U/S	X X	X X if involved	X X if involved	X	
Chest CT	X		X	X before course n°5	X
Abdomen CT	X		X if involved		X if involved
CT or MRI of any area clinically involved	X		X Assess all involved areas		X involved areas
PET(-CT)	o		o		O
Cardiac echo	X		X	X	X
Sperm banking (pubertal boy)	^				
Pregnancy test (pubertal girl)	^				

Notes

X = mandatory investigation

^ = mandatory if clinically indicated, or part of an ancillary study, otherwise optional

o = optional

Biochemistries = urea, creatinine, electrolytes, Ca, PO₄, SGOT, SGPT, proteinemia

Complete biology = biochemistry + uric acid, albumin, gamma GT, bilirubin, haemostasis/coagulation

After each course:

Twice weekly (3 days apart): cell blood count/differential.

11. RESPONSE CRITERIA AND EVALUATION DURING AND AFTER TREATMENT

11.1. RESPONSE CRITERIA

Definition of evaluable disease

All abnormal sites at diagnosis should be evaluated using whatever modality is most appropriate and taking into account the possible context of emergency treatment to do the minimal necessary work-up. This will include Chest X-Ray, ultrasound, CT or MRI scans for solid masses.

Response Post COP

The response following COP should be recorded as:

Complete remission	Complete disappearance of all measurable or evaluable lesions (except bone), no blasts in the bone marrow nor in the CSF.
Incomplete response	20-99% reduction in the product of the two largest diameters of measurable lesions (as long as no regrowth occurred by day 7 after earlier shrinkage)
Non-response	<20% tumor reduction of the product of the two largest diameters of measurable lesions, or Tumour progression, or Tumour regrowth after initial shrinkage

Response status prior to each subsequent course

The response status prior to each subsequent course should be classified as follows:

Complete remission	Complete disappearance of all measurable or evaluable lesions (except bone), no blasts in the bone marrow nor in the CSF.
Tumour regression	Persistence of tumour, but tumour volume in aggregate decreasing
Stable disease	Persistence of tumour with tumour volume unchanged or with increase insufficient to classify as progression (see below)
Disease progression	Any progression of more than 25% in the product of the two largest diameters of any measurable lesion, appearance of new lesions, appearance or reappearance of Burkitt cells or tumoral large cells in bone marrow or CSF

Response status after 1st (R-)CYM (B arm) or 2nd (R-)CYVE (C1 or C3 arm) or the 6th EPOCH course in PMLBL, or after end of treatment

Complete remission	Complete disappearance of all measurable or evaluable lesions (except bone), no L3 blasts in the bone marrow nor in the CSF.
Considered as CR	<p>Patients may still be considered to be in CR even if bone lesions have not completely normalised on X ray, if all other evidence of disease has disappeared. Similarly a patient can still be in CR in the presence of a residual mass, providing large biopsies show no viable cells. A small residual mass (<2 cm) not accessible to surgery can also be considered to be in CR (providing all other disease sites are in complete remission).</p> <p>When disease affects the liver or kidneys it may be the case that residual abnormalities are seen following treatment. These generally measure < 1 cm diameter. In this case, wait and watch shortly.</p> <p>Any second look surgery should be carefully planned so that it can be done as soon as haematological recovery following the 1st (R-)CYM (group B) or 2nd (R-) CYVE course (group C) occurs, in order not to delay the next course of chemotherapy.</p>
Persistent disease	Existence of histologically proven residual disease.
Disease progression or relapse	<p>Any progression of more than 25% in the product of the two largest diameters of any measurable lesion</p> <p>Appearance of new lesions</p> <p>Appearance or reappearance of Burkitt cells or tumoral large cells in bone marrow or CSF</p>

11.2. EVALUATION DURING TREATMENT

See Tables in Section 10.7

11.2.1. Response after COP at D7

The response evaluation after COP is only required for the phase III study (non PMLBL patients). Areas of tumour involvement should be evaluated by clinical, radiological and/or ultrasound examination to determine if there was any tumour reduction. In case of B-AL with only bone marrow involvement, a bone marrow aspirate is necessary to assess the response.

In case of no response, the treatment should be switched to a more intensive treatment as indicated in each treatment details in section 10.3.1 and 10.4.1, except patients with CSFpos. No response to COP is not an event

11.2.2. Evaluation before each chemotherapy course

For safety reasons, the following evaluations relevant for the application of chemotherapeutic drugs should be measured before start of every new cycle of chemotherapy. All these clinical and laboratory data must be available in the patient's medical record which will serve as the source document for verification if necessary. Only parameters of the investigations useful for endpoints will be recorded in the CRF.

- The patient's general condition (Lansky or Karnofsky index) should be documented
- Full blood count, differential, and serum biochemistry (urea, creatinine, calcium, phosphorous, SGOT, and SGPT, gamma GT, electrolytes)
- In case of tumour lysis or evidence of renal dysfunction during initial chemotherapy glomerular filtration rate (GFR) should be determined by DTPA or EDTA excretion before proceeding with HD MTX of the first course.
- All known tumour sites easily evaluable clinically, by standard X-Rays and by abdominal ultrasonography should be evaluated before each course of chemotherapy until complete regression.
- CSF should be examined at time of lumbar puncture for IT therapy
- For patients with initial BM involvement included in the MDD/MRD study, besides the cytological examination of the bone marrow, bone marrow and peripheral blood samples should also be sent for MRD study before the second cycle of chemotherapy COPADM (see annexe G).
- Cardiac echography :
 - Phase III study - non PMLBL patients: after COP if not done prior to start of treatment and every 2 administrations of doxorubicin in group C,
 - Phase II study - PMLBL patients: before each chemotherapy except before course number 2.

11.2.3. Evaluation during each chemotherapy course

Blood counts should be measured at least twice weekly after every cycle, generally three times weekly at time of nadir. The patient's general condition (Lansky or Karnofsky index) should be documented regularly during therapy.

In PMLBL phase II of DA-EPOCH-R, only use twice weekly blood counts (3 days apart) for dose adjustment, even if additional blood counts are obtained.

11.2.4. Evaluation of remission

Remission must be evaluated at a specified time point.

For evaluation of complete remission, all the sites with initially tumour involvement must be fully evaluated. For patients in the MDD/MRD study, blood and bone marrow samplings should be done in addition to the standard cytological examination. PET(-CT) scan is recommended and the result will be registered. **But no decision of changing treatment must be taken only on the result of the PET(-CT) scan.** It is only in case of histological documentation of viable cells in the residue, or progression, that the absence of complete remission can be declared. Histological documentation is recommended in case of

positivity of PET (-CT) scan or large residual mass. This necessitates to remove or largely biopsy the residue (needle biopsy is not sufficient in this situation, unless it shows tumoral cells).

Patients treated in **group B** must be in complete remission after course n°3 [first (R-)CYM]. In case of residual mass with documented viable cells, patients must be treated more intensively in C1 group starting at consolidation CYVE n°1 (details in section 10.3.3 and 10.4), rituximab as allocated by randomisation. This is not considered as an event. Only progression or need for another line treatment in case of residue with positive histology after CYVE2 will be considered as an event.

For the patients treated in **groups C1 and C3**, remission has to be obtained after course n°4 [2nd (R-)CYVE]. Presence of viable cells in the residue at this evaluation or progression is considered as an event. The 2nd line treatment is left to the decision of the national coordinator and the patient will be followed for survival status.

For patients with **PMLBL**, remission will be assessed after the end of the 6th DA-EPOCH course. Presence of viable cells in the residue at this evaluation or progression is considered as an event. The 2nd line treatment is left to the decision of the national coordinator and the patient will be followed for survival status.

11.2.5. Tumour investigations at completion of treatment

At completion of treatment all initially positive sites should be investigated to confirm remission. This should include radiological examinations, bone marrow aspirate and lumbar puncture examination when appropriate.

11.3. FOLLOW-UP OF IMMUNE STATUS

Immune functions will be assessed:

- at diagnosis prior to treatment (section 7.2),
- 1 month after the end of treatment, i.e. 2 months after the start of the last course of chemotherapy
- prior to immunoglobulin substitution if decided,
- 1 year following study entry in all patients
- and yearly, if not normalized at 1 year.

This immune function evaluation is only for patients without event or before event (event as defined in EFS)

The immune functions evaluation includes:

- the total peripheral blood lymphocyte count and CD19⁺CD20⁺ B cells evaluated using flow cytometry,
- the serum levels of IgG, IgA and IgM,
- serum antibodies to polioviruses and tetanus and diphtheria toxoids will be measured using enzyme-linked immunosorbent assays, as well as serum antibodies to pneumococcus and hemophilus influenza
- optional evaluation: peripheral blood counts of various lymphocytes subsets [CD3⁺, CD3⁺CD4⁺, CD3⁺CD8⁺, as well as, CD3⁻CD16⁺ (or CD3⁻CD56⁺, or CD3⁻CD56⁺ CD16⁺) NK cells] using flow cytometry.

The reason for immunoglobulin infusions and the number of immunoglobulin infusions will be recorded. Clinicians are reminded that the occurrence of any unusual infections and/or infections requiring hospitalizations, any unusual neurological complications, or any unusual events during treatment and during the follow-up has to be reported in the CRF and as Serious Adverse Event if necessary (definition of serious adverse event in chapter 16).

Not only a significant low level of immunoglobulins but also the clinical situation with severe or repeated infections should be taken into consideration before deciding immunoglobulin substitution.

Please contact the national co-investigator to take the decision or follow your national guidelines on Ig substitution if any.

Physicians should not administer immunizations with live vaccines at any age until the B-cell population has returned. Vaccinations with non-live vaccines can proceed per normal schedule. It is however possible that the efficacy of such vaccines may be decreased.

11.4. FOLLOW UP AND LATE EFFECTS MONITORING

Patients will be examined at monthly intervals during the first year after start of treatment. A clinical examination should be carried out and any supplementary tests done depending on initial tumor localisation and clinical signs. It is not necessary to repeat lumbar punctures or bone marrow aspirates off treatment in asymptomatic patients. Abdominal ultrasonography and thorax X-Ray are generally sufficient workup to follow abdominal or mediastinal initially involved tumor sites. Routine CT or RMI imaging or PET(-CT) imaging of these children at follow-up visits in the absence of symptoms or signs of relapse is not standard of care and would expose the children to an unnecessary amount of radiation exposure.

After the first year patients with Burkitt will be seen at 3 monthly intervals for 6 months, then at 6 monthly intervals to 3 years off treatment. Patients with DLBCL will be seen at 3 months intervals to 3 years off treatment. Thereafter they will be seen at annual intervals. Relapse is unlikely after 15 months from diagnosis in Burkitt. It may occur until 36 months in DLBCL.

Follow up is important for the monitoring of possible late effects which include surveillance of the cardiac function, especially for the patients treated with the DA-EPOCH-R regimen, evaluation of the pubertal development and the fertility after puberty (evaluation of puberty and fertility is not part of the study and data will not be registered, but it is good clinical practice). For any patient receiving anthracyclin, the general recommendation is to perform echocardiogram (or other exploratory methods according to local practice) once during the year following the end of treatment and every 5 years. For the patients receiving more than 300mg/m² of doxorubicin, the echocardiogram monitoring will be closer, every 3 years. This frequency is recommended when there is no detected abnormality. In case of abnormality in one echocardiogram, the next one should be performed between 6 to 12 months later to confirm or not the alteration and evaluate the progressiveness. The further frequency will be adapted to the situation and planned with the cardiologist. Follow-up will be recorded in this study for a minimum of 5 years.

11.5. FOLLOW-UP AFTER DISEASE PROGRESSION OR RECURRENCE

Patients with disease progression or recurrence will be treated off protocol therapy.

Any recurrence of the disease, either progression during treatment (except progression during COP), or after treatment or relapse after a complete remission was obtained, is considered as an event and must be rapidly declared to the data centre. These patients are discontinued from the protocol but will be followed for survival.

Patients with:

- No response to initial COP therapy
- Biopsy positive residual disease following (R-) CYM1 in Group B

must be treated as specified in the protocol. **These are not considered as events.**

12. STATISTICAL CONSIDERATIONS

12.1. RITUXIMAB RANDOMIZED TRIAL IN HIGH RISK PATIENTS (NOVEMBER 2015: ALL PATIENTS RECEIVE RITUXIMAB)

12.1.1. Endpoints

Primary endpoint:

Event Free Survival (EFS): Minimum time to death from any cause, presence of viable cells in residue after [2nd (R-)CYVE], relapse, progressive disease, or second malignancy measured from randomization.

No response to initial COP therapy and biopsy positive residual disease following (R-)CYM1 in Group B are not considered as an event.

Secondary endpoints:

- Survival (S): Time to death from any cause, measured from the time of randomization

- Complete Remission Rate at the assessment time

- For group B patients: response in 3 categories:

- CR at assessment time (after CYM1)
- slow responder = CR at CYVE2 but not after CYM1
- no CR

- Acute (at each course) and long term toxicity:

toxic deaths, adverse events of NCI-CTC V4 (non haematological toxicity grade ≥ 3 , infections grade 3 to 5), cardiac toxicity (CTC grade 2-5 and abnormal left ventricular ejection fraction (LV-EF) or abnormal left ventricular shortening fraction (LV-SF)), number of platelets transfusion and of red cells transfusion, intensive care unit admission, rituximab infusion reactions.

According to the recommendations of several authors (Steinherz 1992, Kremer-Van Dalen 2006) the cardiotoxicity is defined as following:

LV-EF < 55 % or LV-SF < 28% or a fall > 20 % of baseline for one of these two criteria.

- Immune reconstitution assessed by Ig (G, A and M) level and lymphocyte counts at 1 year and every year during follow-up until normal level, post vaccination antibody levels (tetanus, polio, diphtheria, haemophilus influenza and pneumococcus) and need for immunoglobulin infusion.

12.1.2. Objective

To determine the efficacy and the safety of Rituximab antibody in children/adolescents with high risk mature B cell lymphoma/leukemia in combination with LMB based chemotherapy.

EFS will be used as the primary analysis endpoint for final and interim analyses. Survival, complete remission, response in group B, toxicity and rate of patients with Ig level abnormally low, lymphocyte count abnormally low, post vaccination antibody levels and Ig infusion during follow up will be used as secondary analysis endpoints.

12.1.3. Number of required patients

The 3-year EFS for these patients was estimated as about 84% with the standard chemotherapy FAB-LMB-96.

As this is a study comparing "standard" versus "standard+new" and as only definitive determination that the addition of rituximab improves outcome is of interest, the design employed is a **one-sided test with futility monitoring**.

Assuming one-sided 5% level of statistical significance, observing 72 events will provide 90% power to detect an increase in the EFS long-term from 84% to 92% (corresponding to a 50% reduction in the risk of failure [hazard ratio = 0.5]). 72 events are expected out of a total of 600 eligible randomized patients (300 per treatment arm). The enrolment rate for these patients is expected to be 130-150

patients per year, suggesting a total enrolment period (including 6 months to get to steady-state) of 4-5 years.

November 2015: The randomization is halted after the first interim analysis that was based on 27 events.

12.1.4. Interim monitoring for efficacy and futility

The interim monitoring for efficacy and futility will be based on the Event Free Survival. The trial can be stopped at interim analysis for efficacy or for futility. The results of the efficacy and futility interim analyses will be given only to the IDMC members. Steering committee and investigators will be kept blinded to the interim analysis results.

12.1.4.1. Interim monitoring for efficacy

The interim analyses for efficacy will be done using the Lan-DeMets alpha-spending function approach applied to an O'Brien-Fleming boundary (truncated at 3 standard deviations). The overall type I error is set at 5% (1-sided). The type I error for the interim analyses will depend on the available information fraction at the timing of the interim analyses. This information fraction will be calculated as the ratio of the number of events observed at the time of the interim analysis on the 72 total events expected for the final analysis. For example, if 54 total events are observed, the available information fraction at this interim analysis will be $54/72=0.75$. The interim analysis boundary values will be determined using the software for Computing Group Sequential Boundaries Using the Lan-DeMets Method (Version 2.1) developed by DM Reboussin, DL DeMets, KM Kim et KKG Lan (University of Wisconsin) (<http://www.biostat.wisc.edu/landemets/>).

The first analysis will be done at about the time 24 total events have been observed (one-third of the expected information) and the following analyses will be done yearly after that, using the alpha-spending function approach to determine the boundaries. We estimated that the first interim analysis will occur at approximately 2.5 years after the beginning of the study. With the subsequent interim analyses occurring at 12 month intervals, 3 interim analyses will probably be performed: at 2.5 years, 3.5 years, 4.5 years (approximately at the end of accrual). The final analysis will be performed when all patients will have been followed for at least 18 months.

If, for instance, the interim analyses were to be performed at exactly 33%, 55% and 75% of the expected information, then the nominal type I error for these looks would be 0.00135 at 33%, 0.00764 at 55%, 0.02099 at 75% and 0.04249 at 100% of the expected information, with the overall cumulative type I error set at 5%.

The results of efficacy interim analyses will be given only to the IDMC members.

According to the IDMC recommendations and to the sponsor decision, the randomization is halted after the first interim analysis for efficacy that was based on 27 events.

12.1.4.2. Futility monitoring

The trial can be stopped for futility if the alternative hypothesis (i.e. HR of 0.50) is rejected. Monitoring for futility will be done using the Fleming, Harrington and O'Brien approach (repeated testing of the alternative hypothesis at a p-value of 0.005), considering stopping for futility if the alternative hypothesis is ever rejected at a p-value below 0.005. Futility analyses will be performed at the same times as the efficacy interim analyses and the results will be given only to the IDMC members.

12.1.4.3. Monitoring of efficacy of Group C treatment standard arm

Monitoring to ensure that Group C patients treated on the Inter-B-NHL standard arm do not have an inferior event-free survival (EFS) compared to previous LMB-treated patients because of the deletion of two last maintenance courses:

The results for the FAB group C patients receiving standard therapy was a long-term EFS of 84% with the distribution of events for those experiencing events approximately exponential(2). This implies about 40% of the events observed by 3 months; 63% by 6 months and 86% by 1 year.

One sample monitoring will be performed using the Woolson 1-sample log-rank test, comparing the observed outcome to a fixed distribution $S(t) = 0.84 + 0.16 \{ \exp(-2t) \}$. If K is the number of failures observed in the available follow-up, and R is the sum of the null cumulative hazards to time t_i where t_i

is the follow-up for patient i , then $T = K - R$ is approximately normal with independent increments and may be used for interim monitoring using standard group sequential boundaries.

It is estimated that about 60% of all randomized patients will be in Group C and that the standard therapy regimen will generate about 29 events. Testing at the 10% level of statistical significance (1-sided), there will be 88% power to detect a reduction in the long-term EFS to 77% (relative risk: 1.5:1.0 compared to the results expected with FAB group C therapy).

12.1.5. Safety analysis

Acute toxicity will be analysed per arm every 6 months during the trial and the results will be available for the IDMC and for the steering committee. The final analysis of acute toxicity will be done at the same time than the final analysis of efficacy. The analysis of 1-year immune reconstitution will also be done at the same time.

Analysis of late toxicity will be done after 5-year follow-up of the last treated patient.

See also paragraph 12.1.6.4.

12.1.6. Statistical plan

12.1.6.1. Analyzed population

Patients with positive HBV serology known after randomisation are not eligible to receive rituximab. These patients will be excluded from the trial as soon as the serology positivity will be known whatever the allocated arm. They will not be included in the principal analyses and will not be counted to reach the requested number of 600 patients. However for sensitive analysis of efficacy endpoint, treatment and follow-up data will be collected for these patients.

Analyses of efficacy and futility will be realised on the intent-to-treat population: all patients, except those with HBV positivity, will be taken into account in the treatment group allocated, including those of group B or CSF negative who had no response to COP and who will be switched to C1 or C3 arm, those who were wrongly enrolled and those who did not fully comply with the protocol. A sensitivity analysis will be done in a modified intent to treat population with exclusion of patient ineligible because of wrong diagnosis (for example, patients with other lymphoma or other malignancy after pathological review, patients with CD20 negativity after review...).

The safety analysis will be based on patients who have received at least one drug administration (chemotherapy or rituximab).

The immune reconstitution analysis will be based on patients who have received at least one drug administration and who have not experienced an event (residue after 2nd (R-)CYVE, relapse, progressive disease, or second malignancy). Patients with event will be included in the immune reconstitution until the event occurrence.

November 2015: The final comparative analyses between the two treatment arms (for efficacy, safety, immune reconstitution, economic evaluation) will be based on patients randomized between the two arms but will not include patients randomized after the date of randomization of the first patient of the control arm who will be switched to receive rituximab according to the sponsor decision following the first interim analysis. This date is approximately 01/09/2015. Thus, the final comparative analyses will be based on around 331 patients randomized before September 2015, 166 allocated to the control arm and 165 allocated to the arm with Rituximab. "An intent-to-treat analysis based on all randomized patients and a rituximab-as-treated analysis based on all randomized patients will also be done as sensitivity analyses."

The final comparative analyses will not include the patients registered in the single arm study following the randomization stop.

However, the description of the efficacy, safety (acute and late) and immune reconstitution of the LMB regimen with Rituximab will be performed on all patients included in the Rituximab arm (i.e; those allocated to the Rituximab arm during the randomized part of the trial and those registered during the single arm part of the trial).

The MDD/MRD study and the PET study will be based on all patients included in the study (i.e. those randomized during the randomized part of the trial and those registered during the single arm part of the trial).

12.1.6.2. Efficacy analysis

Survival functions for the time-to-event endpoints (EFS and OS) will be estimated with the Kaplan Meier method. The 95% confidence intervals (95% CI) of the actuarial rates will be calculated with the Rothman method.

The comparisons between the two arms for each endpoint will be done by Cox's models that included the stratification factors (national group, histology, prognostic group). P-values will be one-sided. Two-sided 90% bounds of the hazard ratio will also be reported.

The trial is underpowered to test interaction between clinical characteristics and rituximab. However, some sensitivity analyses of rituximab effect on EFS will be done according to histology (large cell, not large cell), age (<12 years versus ≥12 years) and prognostic group (B versus C CSF negative, C CSF positive).

The rate of complete remission will be compared between the 2 arms by logistic regression taking into account the stratification factors (national group, histology, prognostic group).

The response in group B patients (CR, slow response, No CR) will be compared between the two arms.

12.1.6.3. Response to COP

As the response to COP will be evaluated at D7, i.e. 24h after the first rituximab administration for the patients allocated in the rituximab arm, the response to COP (complete, incomplete or no response) will be compared between the two arms to evaluate whether the first rituximab administration modifies it and modifies the rate of patients early switched to more intensive therapy. Even if the rate of patients early switched to more intensive therapy is different between the two arms, it will not jeopardized the trial because the main objective of the trial is to compare two strategies, one with rituximab and one without rituximab, on EFS.

12.1.6.4. Safety analysis

Acute toxicity:

The rate of non haematological toxicity (grade ≥ 3 altogether, and grade 3 and grade 4 separately) will be compared between the two arms at each course and globally for all courses.

Specific toxicity will be analysed separately: The rate of infections (CTC-V4 grade 3 to 5) and of severe unusual infections during treatment and until 3 months after the end of treatment will be compared between the two arms. The rate of cardiac toxicity (CTC-V4 grade 2 to 5) and the rate of patients with abnormal left ventricular ejection fraction or abnormal left ventricular shortening fraction at any time during treatment will be compared between the two arms. The rate of neurological toxicity (CTC-V4 grade 3 to 5) will be compared between the two arms.

The number of platelets transfusion and of red cells transfusion will be compared between the two arms. The analyses will be done without and with adjustment for G-CSF administration.

The rate of intensive care unit admission will be compared between the two arms.

The rate of rituximab infusion reactions (total and by severity of reactions) will be estimated.

We will monitor the duration between courses, the number of patients with courses postponed after 21 days, in particular between the two first induction courses COPADM1 and COPADM2 and the number of patients who have courses with chemotherapy dose reduction higher than 25%. These parameters will be presented per arm in the IDMC reports every 6 months.

Monitoring the toxic death rate for Group C patients receiving 24 hour methotrexate (C3 treatment):

We will apply a specific monitoring rule to assess the rate of treatment-related mortality associated with Group C patients receiving 24 hour methotrexate. The COG rituximab pilot had an overall treatment related mortality of 2% (2/90). Enrollment of a total of 80 Group C patients receiving 24 hour methotrexate is expected. These patients will provide about 89% power (testing at a 8% level of statistical significance) to detect a true death rate of 8%. Interim monitoring for an elevated treatment-related death rate will be performed using an O'Brien-Fleming boundary truncated at 3 standard deviations, with monitoring beginning after the first 20 patients have been enrolled. In the event that a treatment-related death occurs in the first 20 patients, a subcommittee of the study committee will review the circumstances of the death and make recommendations to the study committee and the DSMC regarding any action to be taken. Crossing the boundaries will generate the review of the cases with the IDMC and a discussion whether a protocol change is warranted.

Monitoring the toxic death rate for patients randomized to receive rituximab:

We will also apply a specific monitoring rule to assess the rate of treatment-related mortality associated with patients randomized to receive rituximab. The COG rituximab pilot had an overall treatment related mortality of 2% (2/90). Enrollment of a total of 300 patients to rituximab is expected. These patients will provide about 94% power (testing at a 8% level of statistical significance) to detect a true death rate of 5%. Interim monitoring for an elevated treatment-related death rate will be performed using an O'Brien-Fleming boundary truncated at 3 standard deviations. Until a hundred of patients have been treated with rituximab, we will monitor the treatment-related deaths in real time. The boundaries will be crossed and we will ask the IDMC to review the treatment-related deaths if there are:

3 treatment-related deaths out of ≤ 11 rituximab-treated patients (3.7%) (for 3/11, one-sided p-value = 0.00117)

4 treatment-related deaths out of ≤ 24 rituximab-treated patients (8.0%) (for 4/24, one-sided p-value = 0.001234)

5 treatment-related deaths out of ≤ 41 rituximab-treated patients (13%) (for 5/41, one-sided p-value = 0.001317)

6 treatment-related deaths out of ≤ 60 rituximab-treated patients (20%) (for 6/60, one-sided p-value = 0.001272)

7 treatment-related deaths out of ≤ 82 rituximab-treated patients (27%) (for 7/82, one-sided p-value = 0.001314)

8 treatment-related deaths out of ≤ 105 rituximab-treated patients (36%) (for 8/105, one-sided p-value = 0.001279)

After the inclusion of the first 105 patients in the rituximab arm, we will report the treatment-related deaths to the IDMC yearly (around 60-75 patients randomized per year in the rituximab arm). For instance, the boundary will be crossed in case of: 9 or more treatment-related deaths among 180 rituximab-treated patients ($p = 0.010837$), 10 or more treatment-related deaths among 240 rituximab-treated patients ($p = 0.023854$) and 11 or more treatment-related deaths among the total of 300 rituximab-treated patients ($p = 0.040962$).

Monitoring the toxic death rate for Group B patients:

We will also apply a specific monitoring rule to assess the rate of treatment-related mortality associated with patients in Group B.

The COG rituximab pilot had an overall treatment related mortality of 2% (2/90). The FAB-LMB96 study had an overall treatment related mortality of 0.5% (4/771). We will assume that the "null" rate for Group B patients is 1.5%. Enrollment of a total of about 300 patients to Group B is expected. These patients will provide about 85% power (testing at a 5% level of statistical significance) to detect a true death rate of 4%. Interim monitoring for an elevated treatment-related death rate will be performed yearly using an O'Brien-Fleming boundary. (This rule is added in the protocol on March 2015, when around 130 group B patients are included in the trial.)

Late toxicity (after 3 months after treatment end and during at least 5 years):

This analysis will be done among patients who have not received any other treatment for relapse or second malignancy.

The rate of infections (CTC-V4 grade 3 to 5) and of severe unusual infections will be compared between the two arms.

The rate of cardiac toxicity (CTC-V4 grade 2 to 5) and the rate of patients with abnormal left ventricular ejection fraction or abnormal left ventricular shortening fraction at any time during follow-up will be compared between the two arms.

Immune reconstitution:

The rate of patients with Ig level abnormally low at 1 year will be compared between the two treatment arms without and with rituximab. This analysis will be adjusted for the baseline level of Ig.

It is hypothesized that rituximab will increase the percentage of patients with Ig level abnormally low at 1 year. Assuming chemotherapy alone will result in 50% of patients with Ig level abnormally low, approximately 270 alive patients per arm at 1 year, with a two-sided test at a 0.05 significance the following differences and power can be detected: 50% vs 55% (21% power), 50% vs 60% (64% power), 50% vs 62% (power 80%), 50% vs 65% (power 94%).

The recovery of Ig normal level in the following years will be studied and compared between the two arms.

The need for immunoglobuline infusions, the number of immunoglobuline infusions and the number periods with immunoglobuline infusions will also be compared between the two arms.

The rate of patients with lymphocyte level (global and B lymphocyte subset) abnormally low at 1 year will be compared between the two treatment arms without and with rituximab. The recovery of lymphocyte normal level in the following years will be studied and compared between the two arms. The levels of post vaccination antibodies (tetanus, polio, diphtheria, haemophilus influenza and pneumococcus) will be compared between the two arms at one year.

12.1.6.5. Handling of missing data:

Patients with Burkitt lymphoma lost to follow-up after 18 months and patients with DLBCL lost to follow-up after 36 months will be considered as having full information concerning the efficacy endpoints (EFS, OS) because very few events occurred after these times. Patients lost to follow-up before these thresholds will be considered as having incomplete efficacy data.

The data endpoint availability will be described by treatment arm: duration of follow-up, rate of patients with incomplete efficacy data (as defined above), rate of cardiac echocardiogram done at each planned cardiac evaluation, rate of immunological evaluation at each planned evaluation.

The rate of missing data will be presented to the IDMC. In case of high rate of missing data or of imbalance between the two arms at the final analysis, the trial results and their interpretation will be discussed with the IDMC members.

12.2. PHASE II TRIAL IN PMLBL

12.2.1. Endpoints

Primary endpoint:

Event Free Survival (EFS):	Minimum time to death from any cause, presence of viable cells in residue after 6 th DA-EPOCH course, relapse, progressive disease, or second malignancy measured from registration.
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Secondary endpoints:

- Survival (S):	Time to death from any cause, measured from the time of registration
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- Complete Remission Rate at the assessment time

- Acute (at each course) and long term toxicity:

toxic deaths, adverse events of NCI-CTC V4 (non haematological toxicity grade ≥ 3 , infections grade 3 to 5), cardiac toxicity (CTC grade 2-5 and abnormal left ventricular ejection fraction (LV-EF) or abnormal left ventricular shortening fraction (LV-SF)), number of platelets transfusion and of red cells transfusion, intensive care unit admission, rituximab infusion reactions.

According to the recommendations of several authors (Steinherz 1992, Kremer-Van Dalen 2006) the cardiotoxicity is defined as following:

LV-EF < 55 % or LV-SF < 28% or a fall > 20 % of baseline for one of these two criteria.

- Immune reconstitution assessed by Ig (G, A and M) level and lymphocyte counts at 1 year and every year during follow-up until normal level, post vaccination antibody levels (tetanus, polio, diphtheria, haemophilus influenza and pneumococcus) and need for immunoglobulin infusion.

12.2.2. Objective

Primary objective: To determine the efficacy of DA-EPOCH-R in children and adolescent PMLBL

Secondary objective: To determine the safety of DA-EPOCH-R in children and adolescent PMLBL

12.2.3. Trial design

Event-free survival is defined as the time to the first occurrence of progression, relapse after response, death from any cause or second malignancy. Historically, the outcome for patients with pediatric primary mediastinal large B-cell lymphoma (PMLBL) is a long-term event-free survival of 67% with most events occurring in the first two years (1-year rate of 75% and 2-year rate of 69%), with few events after that. Recent reports from the US National Cancer Institute (Dunleavy, ASH 2006; ASH,

2009) suggest that the outcome for adult patients with PMLBL improved with DA-EPOCH-R therapy, with 100% event-free survival for 35 patients with PMLBL.

We will assess the efficacy of DA-EPOCH-R therapy by comparing the event-free survival (EFS) for a sample of children and adolescents with PMLBL to a fixed outcome, reflecting the historical pediatric PMLBL experience.

The null hypothesis is that the EFS for these patients is $S(t) = 0.67 + 0.33\{\exp(-1.5t)\}$, versus the alternative that the EFS is $S(t) = [0.67 + 0.33\{\exp(-1.5t)\}]^R$, where R is less than 1.0.

A total of 40 patients will be enrolled on this study over a period of approximately 3.5 years.

A one-sample log-rank test (Finkelstein, Muzikansky and Schoenfeld, JNCI, 95:1434-39, 2003) will be used to compare the EFS experience to the fixed null outcome. Testing will be done at the 10% level of statistical significance (1-sided). The sample of 40 patients will provide 90% power to detect a true long-term EFS of 84.6% and 85% power to detect a true long-term EFS of 83.4%. Power would be 80% to detect a true long-term EFS of 82.4%.

A single futility analysis, testing the hypothesis that the true event-free survival is $S^*(t) = [0.67 + 0.33\{\exp(-1.5t)\}]^{0.406}$ (corresponding to a long-term EFS of 85%) will be performed once 6 events (half of the expected events under the null hypothesis) have been observed. If this alternative hypothesis is rejected at a significance level of 0.025, consideration will be given to suspending enrolment and concluding that DA-EPOCH-R is insufficiently active for children and adolescents with PMLBL.

12.2.4. Statistical plan

12.2.4.1. Analyzed population

Analyses of efficacy: all registered eligible patients will be taken into account, including those who did not fully comply with the protocol. The patients who were wrongly enrolled (not eligible for the trial) will not be analysed and will be replaced to have the right number of 40 eligible patients.

The safety analysis will be based on patients who have received at least one drug administration (chemotherapy or rituximab).

The immune reconstitution analysis will be based on patients who have received at least one rituximab administration and who have not experienced an event (presence of viable cells in residue after 6th DA-EPOCH course, relapse, progressive disease or second malignancy). Patients with event will be included in the immune reconstitution until the event occurrence.

Modification following the recommendations of the IDMC:

Seven patients received only half dose of prednisolone because of an error in the first version of the protocol. The IDMC recommended that seven patients be added in order that the efficacy and safety of the treatment strategy can be evaluated as originally planned on 40 patients. Thus, 47 patients will be included in the trial. The seven patients who received half dose of prednisolone will continue to be followed and reported. The primary analyses of efficacy, toxicity and immune reconstitution of the DA-EPOCH-R regimen will be done without these 7 patients. Analyses of efficacy, toxicity and immune reconstitution based on all patients (47) will be done as sensitivity analyses.

12.2.4.2. Efficacy analysis

Survival functions for EFS (primary endpoint) and overall survival (OS, secondary endpoint) will be estimated with the Kaplan Meier method. The 95% confidence intervals (95% CI) of the actuarial rates will be calculated with the Rothman method.

The observed EFS experience will be compared to the null distribution, $S(t) = 0.67 + 0.33\{\exp(-1.5t)\}$, using the one-sample log-rank test (Woolson, Biometrics, 37:687-96, 1981). The analysis will be done when all patients will be followed for at least 18 months.

Survival functions for EFS and OS will also be estimated when all patients will have been followed for 5 years.

12.2.4.3. Safety analysis

Acute toxicity:

Report on the acute toxicity will be done every 6 months. The number of patients who will have received more than 350 mg/m² of doxorubicin will be closely monitored.

The rate of rituximab infusion reactions (total and by severity of reactions) will be estimated at each course.

The rate of non haematological toxicity (grade ≥ 3 altogether, and grade 3 and grade 4 separately) will be estimated at each course and globally for the whole DA-EPOCH-R regimen.

The rate of infections (CTC-V4 grade 3 to 5) and of severe unusual infections during treatment and until 3 months after the end of treatment will be estimated.

The rate of cardiac toxicity (CTC-V4 grade 2 to 5) and the rate of patients with abnormal left ventricular ejection fraction or abnormal left ventricular shortening fraction at any time during treatment will be estimated among all patients and according to the doxorubicin dose received (<350 mg/m² versus ≥ 350 mg/m²).

The number of platelets transfusion and of red cells transfusion will be given (means, standard error, median and range).

The rate of intensive care unit admission will be given.

Late toxicity (after 3 months after treatment end and during at least 5 years):

This analysis will be done among patients who have not received any other treatment for relapse or second malignancy.

The rate of infections (CTC-V4 grade 3 to 5) and of severe unusual infections will be estimated.

The rate of cardiac toxicity (CTC-V4 grade 2 to 5) and the rate of patients with abnormal left ventricular ejection fraction or abnormal left ventricular shortening fraction at any time during follow-up will be estimated. Evolution of left ventricular ejection fraction and left ventricular shortening fraction will be studied according to the doxorubicin dose received (<350 mg/m² versus ≥ 350 mg/m²).

Immune reconstitution:

The rates of patients with Ig level abnormally low each year between 1 and 5 years of follow-up will be estimated. The need for immunoglobuline infusions, the number of immunoglobuline infusions and the number periods with immunoglobuline infusions will be estimated.

The rate of patients with lymphocyte level (global and B lymphocyte subset) abnormally low at 1 year will be estimated. The recovery of lymphocyte normal level in the follow-up will be described.

The levels of post vaccination antibodies (tetanus, polio, diphtheria, haemophilus influenza and pneumococcus) at one year will be described.

The 95% confidence intervals of these rates will be given. For rare events, the exact binomial confidence intervals will be calculated.

13. ANCILLARY STUDIES

November 2015: The MDD/MRD study and the PET study will be based on all patients included in the study (i.e. those randomized during the randomized part of the trial and those registered during the single arm part of the trial).

The cost effectiveness study will be based on patients randomized between the two arms but will not include patients randomized after the date of randomization of the first patient of the control arm who will be switched to receive rituximab according to the sponsor decision following the first interim analysis. This date is approximately 01/09/2015.

13.1. BIOLOGICAL STUDIES, ESPECIALLY MDD AND MRD

Parallel biological studies will be performed, on tumour cells, and/on characteristics of the patients which might modulate response to treatment with or without rituximab. These studies will not concern all the patients of the trial and will be organized by country, some studies being done in several countries altogether.

Minimal disseminated disease and minimal residual disease study

Minimal disseminated disease and minimal residual disease will be studied on bone marrow (BM) and in peripheral blood (PB) at different time points: before treatment, after COPADM1 (only for B-AL patients and if possible at the same day as the PET(-CT) scan performed to evaluate early response if this PET(-CT) scan is done) and at remission assessment time (see annexe G).

The prevalence of minimal disseminated disease in bone marrow and in peripheral blood at diagnosis will be estimated in all patients and according to the clinical prognostic groups (stage, level of pathological bone marrow involvement). The relation between the presence of minimal disseminated disease in BM and in PB will be studied.

The prognostic value of minimal disease will be evaluated on EFS in univariate analysis (logrank test) and in multivariate analysis (by Cox models) taking into account the other prognostic factors to estimate the additional prognostic impact of these biological factors.

The prognostic value of initial minimal disseminated disease will be evaluated. The patients will be classified according to the pathological involvement of bone marrow and to the MDD evaluation:

- patients with pathological involvement of bone marrow,
- patients without pathological involvement of bone marrow but with MDD in BM and not in PB,
- patients without pathological involvement of bone marrow but with MDD in PB,
- patients without pathological involvement of bone marrow and without MDD.

The prognostic value of minimal residual disease in BM and PB before the second course will be evaluated in B-AL patients.

The prognostic value of minimal residual disease in BM and PB at the remission assessment time will be evaluated in patients in clinically complete remission at that time.

13.2. COST EFFECTIVENESS ANALYSIS (EICNHL ONLY) (NOVEMBER 2015: THIS STUDY IS HALTED PARALELY TO THE HALT OF THE RANDOMISATION)

Objective

The aim of the economic study is to compare the cost-effectiveness ratio between two therapeutic strategies: chemotherapy with anti-CD20 antibody Rituximab (arm 2) versus chemotherapy without Rituximab (arm 1).

Method

The economic study will be performed only in case of statistically significant difference in effectiveness. Costs data will be collected only in the French centres. Three cost-effectiveness ratios will be computed: the cost per event free surviving year, the cost per carcinologic event avoided, the cost per life year gained. A confidence interval of these ratios will be computed using the Bootstrap method.

Effectiveness

Effectiveness will be measured by three endpoints: EFS, carcinologic event avoided (main end-points) and survival (secondary end-point).

Costs

Mean inpatient costs will be assessed from the payer perspective. Cost computation will be focused on inpatient resource use. Data on hospital stays for chemotherapy (including the cost of Rituximab), toxicities and relapses will be collected on the sub-sample of French patients. We will use costs per diagnosis-related groups (DRG) to compute the cost of hospital stays. If the DRG is a missing data on case report form, we will use the duration of the stay and the type of wards to estimate indirectly the cost of the hospital stay.

Incremental cost effectiveness ratio

The number of subjects was calculated to observe a significant difference in efficacy between the two arms of the trial. The difference will unlikely be significant in the French subset. We will thus use the results of efficacy observed on the whole population of the trial.

Costs data will be collected in the French centres. A mean cost per French patient will be computed in the 4 following groups:

- Cost per patient in arm 2 (with Rituximab) for patients with at least one carcinologic event.
- Cost per patient in arm 2 (with Rituximab) for event-free patients.
- Cost per patient in arm 1 (without Rituximab) for patients with at least one carcinologic event.
- Cost per patient in arm 1 (without Rituximab) for event-free patients.

A total cost per arm will be computed using these mean French costs and the repartition of patients among the above groups observed in the whole trial population.

Analysis

Costs will be compared in the two arms using the Wilcoxon test. Median costs and interquartile interval will be computed in each arm. For each cost-effectiveness ratio, a 95% confidence interval and an acceptability curve will be estimated using the non-parametric Bootstrap method.

13.3. PET(-CT) SCAN USE ANALYSIS (NOVEMBER 2015: THE STUDY CONTINUES)

In the Inter-B-NHL protocol, possibility to perform PET(-CT) scan is not required for study participation. Indeed, as long as the value of PET(-CT) scan has not been clarified, it cannot be mandatory for every patient and must not be used for therapeutic decisions.

However, this protocol provides the opportunity to collect data on PET(-CT) scan in a large series of children/adolescents with high risk B NHL. Results of the PET(-CT) scans which are performed, especially in the countries where PET(-CT) scan is performed as standard procedure in lymphoma, will be prospectively registered. In centers where PET(-CT) scan is available, investigators are encouraged to perform PET(-CT) scan in all patients included in the inter-B-NHL protocol in order to be able to study consecutive series of patients without selection bias. PET-CT scan is preferred but PET scan (without CT) is also acceptable.

13.3.1. Objectives of the study

Primary: Evaluation of the value of PET(-CT) to remission assessment in standard clinical practice.

Secondary: Evaluation of the prognostic value of early PET-CT.

The participation in the PET(-CT) scan study is optional and depends of the availability of PET(-CT) scan in the centers. Centers can participate only to the primary objective of the PET(-CT) study.

13.3.2. Recommendations for PET(-CT) exams in the Inter-B-NHL protocol

PET(-CT) is optional, but results will be reported if performed.

- At diagnosis: PET(-CT) will be performed if possible, but it **MUST NOT** postpone the beginning of the treatment.
- Early PET(-CT): In PMLBL, PET(-CT) scan will be performed after 2nd course of EPOCH-R. In the other cases, PET(-CT) scan will be performed just before COPADM2 (as long as it does not delay the start of the second COPADM). PET(-CT) scan should be performed at least 14 days after the last chemotherapy injection (in any case, PET(-CT) scan must not be performed less than 10 days after the end of the previous course). In patients with B-AL who participate in the MRD study, it is recommended to perform the PET(-CT) scan and bone marrow and peripheral blood samples for MRD evaluation at the same time to be able to correlate their results.
- PET(-CT) at remission assessment: in PMLBL, PET(-CT) will be performed after the 6th EPOCH-R course. In the other cases, PET(-CT) scan will be performed after (R-)CYM1 in group B and after (R-)CYVE2 in group C. PET(-CT) scan must be performed as closer as possible to the next course but without postponing the beginning of this course. PET(-CT) scan should be performed at least 14 days after the last chemotherapy injection of the previous course. In case of residual mass at the time of remission assessment, there will be a comparison of the results of the PET(-CT) scan and of the histology of the residue. As precised in the protocol, no decision of intensifying treatment should be taken only on PET(-CT) scan results.

In PMLBL, the 3 PET(-CT) scans are strongly recommended. However, in PMLBL and also in the other cases, if only one PET(-CT) scan is allowed, the single PET(-CT) scan at the remission assessment time is strongly recommended.

13.3.3. Analysis of PET(-CT) data for remission assessment (primary objective)

Sensitivity, specificity, positive and negative predictive values of the PET(-CT) scan for the remission evaluation will be estimated. As the aim of the study is to evaluate the value of PET(-CT) scan in standard clinical practice, the results of the PET(-CT) scan evaluated locally by the nuclear physician will be used for the analysis and there will not be a review of the PET(-CT) scan. The gold standard will be the histology result and the follow-up in case of residual disease and only the follow-up in case of remission according to the imaging.

Determination of gold standard:

			Conventional imaging workup			
			Negative	Positive		
				Histology negative	Histology positive	Histology not done
PET/ CT	Negative		Negative	Negative	Positive	Positive if early event / else negative
	Positive	Histology negative	Negative	Negative		
		Histology positive	Positive		Positive	
		Histology not done	Positive if early event, else negative			Positive if early event / else negative

As around only 5-7% of residual disease is expected, even if all patients are evaluated by PET(-CT) scan at remission assessment time, the accuracy of sensitivity estimation will not be very good. However, if 200 patients are evaluated by PET(-CT) scan at remission assessment time, with a hypothesis of a PET(-CT) negative predictive value (NPV) of around 98%, the accuracy estimate of the NPV that is an important parameter will be good: size of the 95%CI interval of the NPV of 5%.

13.3.4. Evaluation of the prognostic value of the early metabolic response on remission and EFS (secondary objective)

The secondary objective of the PET(-CT) study is to evaluate the prognostic value of PET(-CT) scan early metabolic response after 2 courses. A central review of the PET(-CT) scan will be done and analysis will be based on the results of this central review. The sensitivity and the specificity of the early PET(-CT) scan to predict remission (at the remission assessment time, complete remission versus no complete remission) will be estimated. Its prognostic value on EFS will be studied using log rank test and Cox regression.

As there is no data suggesting that the meaning of an early response in terms of remission can be different between patients treated with or without rituximab, PET(-CT) scan can be done in all patients whatever is the received treatment.

The results of early PET(-CT) scan will be classified as positive (pathological (malignant) FDG uptake), doubtful, negative. The SUV will also be studied (values at early evaluation and evolution between baseline PET(-CT) if done and early evaluation).

No treatment decision or investigation decision must be taken according to the results of this early PET(-CT) scan.

The number of patients who will benefit from an early PET(-CT) scan during the Inter B-NHL study is unknown. If the number of patients with an early PET(-CT) scan and, among them, the number of patients with no complete remission at remission assessment time or the number of patients with an event are sufficient to perform an analysis with enough power, the analysis will be done inside this protocol. If the numbers are too small, a relevant analysis will only be possible by pooling the data collected in these patients with the data recorded in the current French study named "Assessment of the value of Positron Emission Tomography scan (PET(-CT)) in the evaluation of response to treatment in childhood non-Hodgkin's lymphoma (NHL)".

13.4. PHARMACOKINETICS STUDY (NOVEMBER 2015: THE STUDY CONTINUES)

A pharmacokinetics (PK) study PK will be performed in at least 34 patients split into three age ranges: 4 patients age 6 months - < 3 years, 15 patients age 3-11 and 15 patients age 12-18. The recommended sample timepoints and the logistic modalities are given in annexe I. This study will be performed only in selected European centres.

14. DRUG MANAGEMENT

14.1. RITUXIMAB SUPPLY

Rituximab will be provided by Roche. Rituximab will be packaged, labelled and distributed directly to the sites by Roche.

14.2. RITUXIMAB ACCOUNTABILITY, RECONCILIATION AND RETURN

The investigator must maintain a complete and current dispensing and inventory record that has been supplied by the sponsor. All unused rituximab must be returned in the original containers. At the end of the study, rituximab may be destroyed on site when the site has documented procedures and the accountability has been defined.

14.3. OTHER CHEMOTHERAPY DRUGS

All other chemotherapy drugs that constitute the LMB protocol and the DA-EPOCH protocol will not be provided by the trial sponsor. They will be provided by each treatment site.

15. ADMINISTRATIVE ORGANISATION

This is a collaborative trial between the European participating national groups, organised within the EICNHL (affiliated with SIOP-E) and the COG of the US.

15.1. TRIAL PROTOCOL

- i) One common protocol will be used by the national groups. The finalised master protocol is in the English language. Each national group will translate a summary or a part of the protocol in its own language according to its local regulation.
- ii) Each national group will be responsible for distribution of protocols to centres within that national group.
- iii) Any modification to the final protocol must be agreed by all the national groups, and significant changes to the protocol will require a protocol amendment and approval by the Health Authorities prior to implementation.

15.2. STUDY CRF

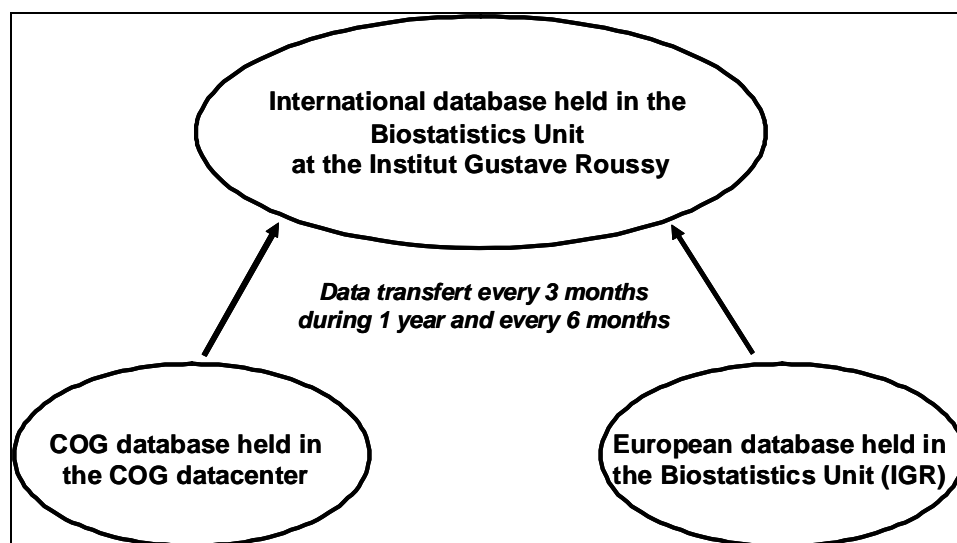
- i) One common set of CRF will be used by the national groups. The English language master version of the study CRF will be held at the Biostatistics Unit of the Gustave Roussy Institut, France.

- ii) Each national group Data Centre will be responsible for distribution of CRF to centres within that national group.
- iii) Subsequent to finalisation, amendments to the CRF must be agreed by all the national groups. The Biostatistics Unit of the Gustave Roussy Institut will be responsible for the issue of amended forms.

15.3. DATABASE AND DATA COLLECTION

There will be a single definitive international study database held by the Biostatistics Unit of the Institut Gustave Roussy, France.

As it is not possible for the COG centers to enter their data directly in this international database, an intermediate step with 2 separate but similar databases, one for European centers and one for COG centers, will be necessary. All data from these two databases will be included in the international database every 3 months during the first year of the study and then every 6 months and before each interim analysis.



For the European centers, the database will be an exact copy of the structure (variables, verification programs...) of the international database developed with the same system. Data from the European centers will be transferred to the international study database at the previously defined times of transfer.

For the COG center, a database will be developed by the COG datacenter under their own system. COG will assemble the data for its patients using its existing clinical data management system (eRDES). This database will contain the same variables and the same data verifications than the international database. The COG Statistics and Data Center (SDC) will create SAS save files of data collected for each of the CRFs and will transmit these files to the IGR. The data will then be transferred to the international database. For the first year of study enrollment, data will be transferred to IGR every 3 months (corresponding to routine data freezes done on January 1, April 1, July 1 and October 1). After the first year, transfers will be done every 6 months and before each interim analysis. Data will be extracted from the databases in SAS files. The SAS files will be imported in the main database and again extracted in SAS files. The original SAS files from local database and the SAS files from the main database will be compared using proc compare to study the integrity of the transfer.

The variable characteristics and the data verification programs will be defined by the European and American data centers together before the development of the databases.

15.4. DATA ENTRY AND DATA MANAGEMENT

The main principle is that each national datacenter is responsible for the data entry and for the data management of the data of the patients of its national centers. A data management plan will be established before the study start and distributed to each national data center.

The datacenter of the Institut Gustave Roussy where the international main database will be held, will check that the data are correct at each data exchange and in case of problems, this datacenter will contact the national datacenter to verify that the national datacenter has asked investigators for data clarification.

For European centers

There will be a common central database with web data entry (exact copy of the international main database) and held in the Biostatistics Unit of the Institut Gustave Roussy.

Paper CRF or e-CRF can be used for recording patient data. The data entry can be done by each national datacenter if the investigators fill in "paper" CRF or it can be done directly in the database by each treatment center.

As to technical architecture, there are a database server and a web server allowing simultaneous accesses via secure connection. Communications between database server and web server are filtered by the firewall. External users only need a browser.

Each datacenter will be responsible of the data management of the data of the patients included in its own country or group. The system will allow each national datacenter to do the data management of its own patients (editing and sending queries). The datacenter will also be able to extract the data of its own patients.

For COG centers

The COG will use its existing clinical data management system (eRDES). However the same variables and the same data verification rules than in the European database will be implemented. The data management of the data of patients included by the COG centers will be done according to the COG practice.

Each patient will have a unique identification number in the trials.

15.5. STUDY APPROVAL

This protocol will open in the national groups after approval of the study according to their local regulation and ethics committee approvals.

16. COMMITTEES AND PANEL REVIEW

16.1. STEERING COMMITTEE

The Steering Committee will meet as appropriate, at least once a year, to consider patient treatment, eligibility and outcome to ensure the smooth running of the study.

COG and SFCE will perform one report with an overview of the whole study twice a year. The information given to the steering committee is:

- accrual rate
- group allocation,
- toxicity data,
- description of the events,
- survival curves (overall and EFS) of the whole population.

The clinical members of the steering committee will be blinded to efficacy by arm.

Each event (no CR at assessment time for group C and PMLBL patients, progression, relapse, toxic death, death from other cause, secondary malignancy) will be reviewed by the steering committee. All unexpected SAEs and all patients who will be switched from group B to group C will be also reviewed by the steering committee. A summary of the clinical report and appropriate imaging if applicable will be asked to the physician. The demand will be done by the national investigator.

All scientific decision concerning the study can be made by the Steering Committee, possibly after discussion with the Independent Data Monitoring Committee (IDMC).

The final decision concerning premature study termination or protocol amendment will be taken by the sponsor.

November 2015: the members of the steering committee had reviewed blindly all the events. On november 17th they agreed to follow the recommendations of the IDMC and to recommend to the sponsors to halt the randomisation and to treat all patients with rituximab). The sponsors halted the randomization on November 20th, 2015.

16.2. STUDY COMMITTEE

One or two paediatric oncologists of each national group, and possibly their statistician and a representative of the coordinating sponsor will participate in the study committee at least one yearly. As the steering committee, they will receive information on accrual rate, group allocation, toxicity data, survival curves (overall and EFS) of the whole population. At the study meeting committee will be discussed specific questions on the running of the study, on toxicity, the difficulties encountered, etc, and in case of problems, how to improve or to solve them.

16.3. INDEPENDENT DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (IDMC) composed of 3 international experts (including one statistician) will monitor the progress of the study on ethical and scientific grounds. The Committee will meet approximately every 6 months (by meeting or conference call).

The role of the IDMC will be:

a) To review accrual rate

b) To monitor toxicity

Every 6 months the statistician(s) of the trial will circulate a report to the members of the DMC about toxicity. The IDMC will review these interim toxicity data although this is primarily the responsibility of the study committee. This biannual procedure prevents against problems of major toxicity.

c) To examine interim analyses

The interim analyses will be unblinded to the treatment groups. These interim analyses will remain confidential. The results of interim analyses will be shared by the three statisticians and sent only to the IDMC members. The statisticians commit to keeping the interim analyses results confidential except for the IDMC members.

On the basis of these analyses, the IDMC may recommend to the sponsor whether the study should continue or whether it should be changed or terminated prematurely. The sponsor will take the final decision.

d) To examine other trials

The IDMC will review reports of related studies performed by other groups or organisations to determine whether such information materially affects the aims or preliminary findings of the trial.

e) Other

The IDMC may be asked to review a major modification to the study proposed by the steering committee prior to its implementation as a study amendment.

November 2015: The members of the IDMC met in October 2013, in April 2014 and in December 2014 for the monitoring of the study. In August 2015, they receive the results of the first interim analysis. After 3 conference calls (21 August 2015, 7 October 2015, 5 November 2015) and complementary analyses, they give their recommendations on November 9th: "The randomization in the Rituximab randomized trial in children/adolescents with high risk B cell lymphoma has to be halted. If enrollment to the trial is to continue, future patients enrolled on the trial should receive the rituximab-containing regimen. Patients already enrolled who are currently receiving treatment without rituximab should have rituximab added during subsequent courses at points in therapy where they would otherwise receive it."

16.4. CENTRAL HISTOLOGY REVIEW

A central histology review will be organized by country and internationally.

16.5. RADIOLOGY REVIEW

There will not be systematic review of the radiological work up or evaluations, except in the situations described in paragraph 16.1.

17. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

17.1. DEFINITIONS

17.1.1. Adverse Event (AE)

An Adverse Event (AE) is any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product. An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings for example), symptom, or disease temporally associated with the use of a medical product, whether or not a causal relationship (i.e. related/not related) with the treatment is suspected.

17.1.2. Serious Adverse Event (SAE)

A **Serious Adverse Event (SAE)** is any untoward medical occurrence that at any dose:

- is fatal (results in death)
- is life-threatening
- requires or prolongs in-patient hospitalization
- results in persistent or significant disability / incapacity
- is a congenital anomaly / birth defect
- is medically significant (defined as any clinical event or laboratory result that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above. Examples of such events include but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasia or convulsions that do not result in inpatient hospitalization, development of drug dependency or drug abuse).

Although overdose and cancer are not always serious by regulatory definitions, these events should be reported on a SAE report form and sent to the sponsor in an expedited manner.

An SAE judged as potentially related to a study drug qualifies as **Serious Adverse Drug Reaction (SADR)**. Events exclusively related to tumour relapse / progression or treatment of tumour relapse / progressions are not considered as SAE.

NOTE:

The following hospitalizations are **not** considered SAEs:

- A visit to the emergency room or other hospital department for less than 24 hours that does not result in admission (unless considered an "important medical event" or a life-threatening event)
- Outpatient or same-day or ambulatory procedures
- Observation or short-stay units
- Hospitalization due to diagnostic procedures or standard supportive care (e.g. implant of central venous catheter)
- A pre-planned hospitalization for a condition which existed at the start of study drug and which did not worsen during the course of study drug treatment
- Social admission (e.g., subject has no place to sleep; hospice facilities)
- Administrative admission (e.g., for yearly physical examinations)
- Protocol-specified admission during a clinical trial (e.g., for a procedure required by the study protocol or for clinical research)
- Optional admission not associated with a precipitating clinical AE (e.g., for elective cosmetic surgery)

17.2. REPORTING

17.2.1. Reporting of Adverse Events (AEs)

All grades 3-5 Non-Hematologic toxicities and all Grade 3-5 infections and grade 2-5 cardiac events will be recorded on the CRF. Abnormal laboratory values will not be considered adverse events unless they are associated with clinical symptoms or actions.

AEs will be graded using NCI-CTAE v4.0.

The causal relationship of the AE to the treatment should also be assessed as related (possibly or probably related) or unrelated to study medication. When assigning relatedness, all of the chemotherapy components or the rituximab mAB are considered study medication.

17.2.2. Reporting of Serious Adverse Events (SAE)

Any SAE which occurs or comes to the attention of the investigator at any time during the study since consent is given and within 30 days after the last administration of study drugs (i.e. Rituximab and/or Chemotherapy treatments), independent of the circumstances or suspected cause, must be reported immediately, within 24 hours of knowledge (at latest on the next working day).

Any events where the event is considered possibly or probably related to trial treatment and occurring after these time periods, should be reported to the sponsor, regardless of time elapsed since last study drug dose.

The following Serious Adverse Events are excluded from the above mentioned time lines – unless being life threatening or fatal (in that case immediate SAE reporting is needed)

- neutropenia
- febrile neutropenia
- and mucositis

These are expected events with this chemotherapy regimen, thus they do not need urgent reporting. They will be reported in the CRF only.

Information collected in the SAE form is crucial to assess the case. For this reason diligence in collecting as much verifiable and reliable information is needed: both, quality and timelines are key factors. If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all SAEs: onset, duration, intensity, seriousness, relationship to study drugs, action taken and treatment required.

The investigator must also attach the following wherever possible:

- a copy of the summary of hospitalization or prolongation of hospitalization
- a copy of the post-mortem report (if applicable)
- a copy of all relevant laboratory examinations and the dates on which these examinations were carried out, including relevant negative results as well as normal laboratory ranges
- all other document that are judged useful and relevant

All these documents will remain anonymous.

The investigator shall supply the principle sponsor or his designated representative, the clinical monitor, the corresponding study centre, the national study chairperson, the coordinating investigator and the Ethical Review Board(s) and the national authorities with any additionally requested information. Further information can be requested by fax, letter, e-mail, telephone, the web database or when visiting.

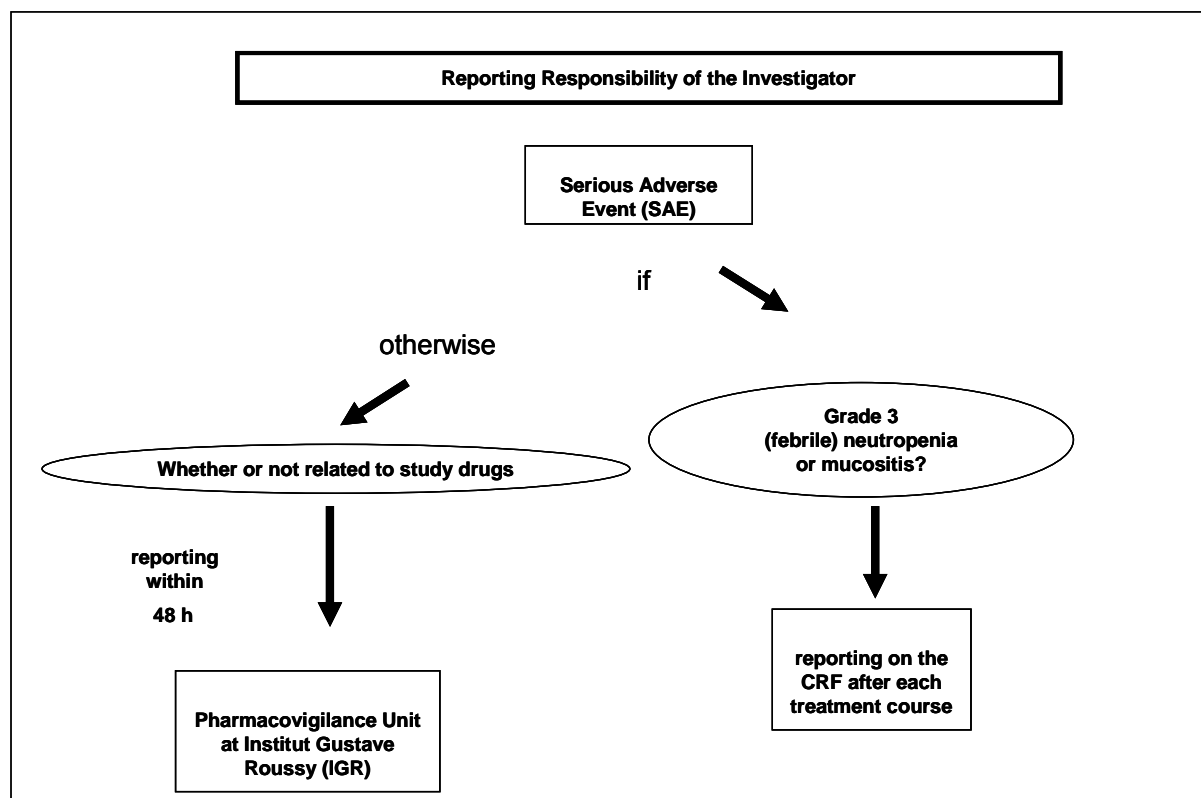
Follow-up information

The investigator is responsible for the appropriate medical follow-up of patients until resolution or stabilization of the adverse event or until the patient's death. This may mean that follow-up should continue once the patient has left the trial. Follow up information about a previously reported SAE must be reported by the investigator to the Pharmacovigilance Unit within 48 hours of receiving it (at latest on the next working day), on the serious adverse event report form, by ticking the box marked "Follow-up N°...". The investigator also transmits the final report at the time of resolution or stabilization of the SAE. The investigator retains the documents concerning the supposed serious adverse event so that previously transmitted information can be completed if necessary.

Patients on observation / Patients not evaluable for therapeutic questions

Some national study groups may register patients who fulfill one or more of the exclusion criteria as "patients on observation" or "patient not evaluable for therapeutic questions". These patients will be registered in the database, but their data will be used by the responsible national data centre only. Their data will not be exchanged with the COG nor merged with the COG data nor used for the analyses of the trials. Thus, for these patients, the SAEs have to be reported to national study centre only (not to the Pharmacovigilance Unit at IGR), but within the above mentioned time lines.

The figure below summarizes the ways and time lines for SAE reporting by the investigator.



The Investigator has to report any SAE within the appropriate time limit on the SAE form via fax to the Pharmacovigilance Unit at Institut Gustave Roussy (IGR): Fax: +33 (0) 1 42 11 61 50

17.3. ASSIGNMENT OF ADVERSE EVENT INTENSITY AND RELATIONSHIP TO STUDY DRUGS

All adverse events, including those that are serious, will be graded according to the National Cancer Institute CTCAE version 4.0 (NCI-CTCAE v.4.0).

All serious adverse events judged by either the reporting investigator or the pharmacovigilance unit as having a reasonable causal relationship to a medicinal product quality as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or arguments to suggest a causal relationship. The relationship is assessed as below:

- Related
- Not related

17.4. RESPONSIBILITIES OF THE COORDINATING SPONSOR

The Pharmacovigilance Unit at IGR will assess the SAE in terms of seriousness, severity (NCI-CTCAE v4.0), relationship to the study drugs and expectedness (see also other chapters). All SAEs will be coded using medRA v13.0. Assessment of causality of SAEs may be reviewed during the study by the study coordinators.

Suspected Unexpected Serious Adverse Reactions (SUSARs)

To comply with regulatory requirements, the coordinating sponsor will notify all national sponsor of all SAEs that are related to the investigational medicinal product and unexpected (ie, not previously described in the investigator brochure or in the Summary of Product Characteristics). In the European

Union, an event meeting these criteria is termed as Suspected Unexpected Serious Adverse Reaction (SUSAR).

In case of a SUSAR, a CIOMS-1 form will be sent by the Pharmacovigilance Unit of IGR to each national sponsor within 72 hours.

Each national sponsor is responsible for the declaration to the National Ethic committee (if required according to local regulation) and to the national competent authority.

All SUSAR reports and all reports involving expected SADR that are fatal will additionally be forwarded to all study investigators and to the Independent Data Monitoring Committee. The involved study offices are responsible for information of the Ethical Review Board(s) concerned as well as of the respective investigators. The reporting procedure has to comply with the national legislation.

18. ETHICAL AND REGULATORY ASPECTS

This study will be conducted in accordance with the Declaration of Helsinki and will be consistent with Good Clinical Practice (GCP), ICH, FDA and applicable regulatory requirements.

18.1. INVESTIGATOR / SPONSOR OBLIGATIONS

Institut Gustave Roussy, France is the sponsor and is the coordinating sponsor for the European countries. One co-sponsor will be identified in each European participating country.

COG will be the coordinating sponsor for the North American countries and other COG centers outside of USA including Canada, Australia, New Zealand, Switzerland, Israel, and Mexico.

Each coordinating sponsor will take care of its own regulatory submissions, its own products logistic organization, and its own safety surveillance.

For Europe, co-sponsors will be in charge of the patient information notice translation, insurance contracting or indemnity of the included patients in accordance with the applicable regulatory requirements.

All protocol amendments must be agreed upon by the Coordinating/ co-sponsor and must be written with input from the investigators.

The Coordinating/co-sponsor has the right to prematurely discontinue the study for significant safety or efficacy reason and will notify all Investigators in writing.

Coordinating/ co-sponsor and investigational sites must archive the entire documents for 15 years.

A co-sponsorship agreement will be signed between the coordinating sponsor and each co-sponsor with the list of the responsibilities for each party.

Any investigator or co-investigator who signed this protocol will agree to carry out this clinical study in accordance with the study protocol approved by the ethic committee, GCP and regulatory requirements.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s).

The investigator should provide his national Sponsor with his CV and the co-investigator's ones.

18.2. PATIENTS INFORMED CONSENT

Written Informed Consent is required according to local regulation and in accord with GCP and the Clinical trials directives 2001/20/EC. The informed consent form used during the informed consent process must be approved by the IEC/IRB and available for inspection.

The rights, safety and well being of the trial patients are the most important considerations and should prevail over interests of science and society.

Before agreeing to participate in this trial, all patients will be provided with sufficient information in the Informed Consent Form prepared in the local language.

Because this study includes minor subjects who can only be enrolled with the consent of the subject's legally acceptable representative, the subject must be informed about the study to the extent compatible with the subject's understanding and, if capable, personally sign and date the consent form or Patient Information Sheet according to local regulations.

The Investigator or a person designated by the Investigator must provide the subject with a copy of the consent form and full written information about the study in language that is non technical and easily understood. The Investigator should allow time necessary for subject or subject's legally acceptable representative to inquire about the details of the study, then informed consent must be freely signed and personally dated by the subject and by the person who conducted the informed consent discussion before commencement of the study. The subject should receive a copy of the signed informed consent and any other written information provided to study subjects prior to subject's participation in the trial.

The Investigator, or a person designated by the Investigator should inform the subject of any new information relevant to the subject's willingness to continue participation in the study.

During a subject's participation in the trial, any updates to the consent form and any updates to the written information will be provided to the subject.

18.3. PROTOCOL AMENDMENT

Any significant change in the study requires a protocol amendment. A protocol intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, but the change must be documented in an amendment, reported to the IRB/IEC, and submitted to the appropriate regulatory agency in the required time frame. All protocol amendments must be reviewed and approved following the same process as the original protocol.

18.4. QUALITY CONTROL

Quality control and assurance checks will be performed by the sponsor. The phase II and phase III trials will be conducted according to regional regulations and according to quality and conduct standard procedures of the participating study groups IGR and COG. All study group procedures must be in accord with ICH, GCP and in compliance with Directive 2001/20/EC.

Before including any patients in the study, the investigator will review the protocol, the investigator's brochure of Rituximab, the CRFs and their completion instructions, the procedure for obtaining signed informed consent, and the procedure for reporting AEs and SAEs.

18.5. AUDIT / INSPECTION

The investigator will permit sponsor audits, IRB/IEC review, and regulatory inspection by providing direct access to data source and documents.

18.6. CASE REPORT FORMS

Paper case report forms (CRF) or e-CRF will be used for recording all data for each patient. It is the responsibility of the Investigator to ensure that the CRF / e-CRF are properly and completely filled in. CRF / e-CRF must be completed for all patients who have given informed consent and have been enrolled in the study.

Source documentation for patients should be the physician's patient records, and as such, will be maintained at the study site.

18.7. RECORDS RETENTION / PATIENTS IDENTIFICATION

It is the Investigator's responsibility to ensure that sufficient information appertaining to the identity of the patients will be retained.

Copies of all pertinent information, including patient identity, allocation number and individual patient data records, will be retained in a confidential manner by the Investigator for a minimum period of 15 years from study completion.

18.8. STUDY TERMINATION

The end of study will be the last visit of the last subject undergoing the study, i.e. after 5-year follow-up of the last treated patient. This study will be completed in approximately 10 years (accrual and treatment: around 5 years, follow-up after treatment end: 5 years).

The sponsor may suspend or terminate the study or part of the study at any time for any reason.

If the investigator suspends or terminate his participation the study, the investigator will promptly inform the sponsor and the IRB/IEC.

18.9. CLINICAL STUDY REPORT

A final report, including a review of the objectives and methods, a presentation and discussion of the results are drawn up according to ICH guidelines. The coordinating investigator is the signatory for the clinical study report.

18.10. DATA OWNERSHIP

Study results are the coordinating Sponsor's property, shared with the co-sponsors according to the contract. They will be communicated to health authorities and ethic committees by the Sponsor and the co-sponsors according to each national law. All information related to the study is confidential at

least until appropriate analysis and control have been made by the coordinating Sponsor and the steering committee of the study.

18.11. PUBLICATION POLICY

Results from the study may not be published or presented orally without the authorization of the sponsor and the national investigators. The study results shall be presented and published in collaboration with the coordinating Sponsor. Any publication or abstract shall be submitted to the coordinating Sponsor or national coordinating investigators for approval.

Authorship for publications will be defined according the “International Committee of Medical Journal Editors” criteria NEJM, 1997 ».

The responsibility of main publications of each sub-study will be distributed among the members of the steering committee. Other members of the study committee will be in charge of additional publications.

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20. ANNEXES

ANNEXE A: GENERAL INFORMATION ON THE ADMINISTRATION OF RITUXIMAB

The antibody will be provided to the participating centres for the patients assigned by randomisation to the treatment with rituximab. Please refer to the package label information for further information and see in annexe C4 the summary of this information with particular attention to be paid to PML and infection.

A.1. DOSAGE

The dose of rituximab will be calculated by the physician on the basis of 375 mg per m² body weight (no maximum dose).

A.2. PREPARATION

Use appropriate aseptic technique. Dilute rituximab to a final concentration of 1 to 4 mg/mL in 0.9% NaCl, USP, or 5% Dextrose in Water, USP to a final concentration 1 to 4 mg/mL. Gently invert the bag to mix the solution. Do not mix or dilute with other drugs. Discard any unused portion left in the vial. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. No incompatibilities between rituximab and polyvinylchloride or polyethylene bags have been observed.

A.3. ADMINISTRATION

Prior to the first antibody application, sufficient administration of fluids and pretreatment with rasburicase or allopurinol if indicated should be performed. Metabolic status should be controlled.

Caution: Rituximab must not be administered as an intravenous bolus injection.

Prior pretreatment with acetaminophen (= paracetamol) (10-15 mg/kg, in COG: maximum 650 mg*) and antihistaminic H1 (=diphenhydramine or equivalent) will be administered 30 to 60 minutes before the start of the infusion of rituximab. During the 1st COPADM courses, patients will also receive prednisone as part of the course. But in the 2nd COPADM and R-CYVE courses, prednisone is not given at the time of rituximab administration. Unknowing if corticosteroids may interfere with rituximab activity, premedication with corticosteroids should not be systematic, but given as clinically indicated.

The administration of rituximab should be carried out via a peripheral or central line. Prior to infusion, epinephrine for subcutaneous injection and antihistaminic H1 for intravenous injection must be available for the case of allergic or anaphylactic reactions. Facilities for immediate emergency intervention including resuscitation in case of an anaphylactic reaction must be available.

First infusion: Begin infusion at an initial rate of 0.5 mg/kg/hour (maximum 50mg/hour). If no hypersensitivity or infusion-related events, increase infusion rate by 0.5 mg/kg/hour every 30 minutes to a maximum rate of 400 mg/hour. If a hypersensitivity or infusion-related event develops, the infusion should be slowed or interrupted. The infusion can continue at one half the previous rate, upon improvement of symptoms.

Subsequent infusions: If the patient tolerated the first infusion well, begin infusion at an initial rate of 1 mg/kg/hour (maximum 50mg/hour). If no hypersensitivity or infusion related events, increase infusion rate by 1 mg/kg/hour every 30 minutes as tolerated to a maximum rate of 400 mg/hour. If a hypersensitivity or infusion-related event develops, the infusion should be slowed or interrupted. The infusion can continue at one half the previous rate upon improvement of symptoms.

Follow the initial infusion guidelines if the patient did not tolerate the first infusion.

Vital signs (blood pressure, heart rate, respiration rate, and temperature) are monitored every 15 minutes during the first infusion of rituximab. For all subsequent infusions, these parameters may be

monitored every 30 minutes at the discretion of the treating physician if there were no complications during the first infusions.

During the infusion of rituximab, the occurrence of fever and chills and/or hypotension is possible as well as other infusion related symptoms. In case of these adverse events, the rituximab infusion must be interrupted and the patient treated appropriately. After the symptoms have disappeared, the infusion may be restarted at half the initial infusion rate.

Infusion rate	Fever	Chills	Mucosal swelling	Hypotension
in case of adverse events, cut the rate of infusion by one half	> 38.5° Celsius	+	+	Drop in B.P. by > 30 mm Hg

After the end of infusion, the intravenous line should remain in situ for at least 1 hour in order to be able to administer drugs intravenously if necessary.
(see table A.3. below)

** Per COG recommendations, the maximum dose of acetaminophen is 650 mg. Explanation: "this would reflect the FDA's strategy of limiting the risk of acetaminophen daily doses exceeding 4000 mg/day. Given that acetaminophen is often prescribed at intervals as short as every 4 hours, individual doses exceeding 650 mg are inappropriate in this setting. The FDA already made manufacturers change all prescription products containing acetaminophen 500 mg to 325 mg, with a max of 2 tablets/dose."*

Table A.3.: Guidance on the Management of Infusion-Related Symptoms (from Roche)

Infusion-related symptoms ^a	Guidance
Grade 1–2	Grade 1–2- Slow or hold infusion. Give supportive treatment ^b . Upon symptom resolution, may resume infusion rate escalation at the investigator's discretion (reduced rate) ^c .
Grade 3	Discontinue infusion. Give supportive treatment ^b . Upon symptom resolution, may resume infusion rate escalation, at investigator discretion (reduced rate) ^c . Note: If the same adverse event recurs with same severity, treatment must be permanently discontinued.
Grade 4	Discontinue infusion immediately, treat symptoms aggressively, and do not restart drug.
^a Refer to National Cancer Institute Common Terminology Criteria for Adverse Events, v4.0, for the grading of symptoms. This table does not refer to management of IgE-mediated allergic reactions, which should be managed as directed in the protocol. ^b Supportive treatment: Patients should be treated with acetaminophen/paracetamol and an antihistamine such as diphenhydramine if they have not been received in the last 4 hours. Intravenous saline may be indicated. For bronchospasm, urticaria, or dyspnea, patients may require antihistamines, oxygen, corticosteroids (e.g., 1 mg/kg or 2 mg/kg (max of 100 mg of predniso(lo)ne) or equivalent), and/or bronchodilators. For hypotension, patients may require vasopressors. ^c Infusion rate escalation after re-initiation: Upon complete resolution of symptoms, the infusion may be resumed at 50% of the rate achieved prior to interruption. In the absence of infusion-related symptoms, the rate of infusion may be escalated in increments of 0.5 mg/kg/hr (maximum of 50 mg/hr every 30 minutes to a maximum rate of 400 mg/hr).	

ANNEXE B – HD METHOTREXATE ADMINISTRATION AND IT

B.1. HD METHOTREXATE HYDRATION AND ADMINISTRATION

Prehydrate with 125 ml/m²/hr (dextrose saline with sodium carbonate (NaHCO₃) 50 mmol/l) for a minimum of 2 hours in order to achieve a pH of ≥ 7 and a urine output of ≥ 100 ml/m²/hr

MTX is administered in dextrose (around 125 ml/m²/h) at a dose of 3 g/m² over 3 hours in group B, and at the dose of 8g/m² in 4 hours in arm C1.

In arm C3: MTX is administered at the dose of 8 g/m² in 24 hours infusion except in 1st (R-)COPADM:

- 1.6 g/m² in 30 minutes in 100 to 250 ml dextrose, then
- 6.4 g/m² in 23h 30 min in 500 to 1000 ml/m² dextrose (depending on the weight of the child and considering around 125 ml/m²/h). During the 24 h infusion of MTX there is the alkaline hyperhydration as described below. Consider the amount of dextrose used for the administration of MTX for the calculation of the hyperhydration.

During and after MTX infusion, continue hydration at a rate of 3000 ml/m²/day with dextrose 5% with added NaHCO₃ (50 mmol/L) and KCl (20 mmol/L) to maintain urinary pH ≥ 7 for a further 48 hours with normal rate of hydration the following 24 hours. Strict attention should be paid to fluid balance.

If no central venous line is available, oral alkalinization is an alternative. Give 1 mmol/kg NaHCO₃ every 6 hours and increased as necessary to maintain the urine pH at ≥ 7 is an alternative. It is important to check that the urine pH is maintained ≥ 7 .

During COPADM course, be aware that prolonged contact of doxorubicin with solutions of alkaline pH should be avoided as this will result in hydrolysis of the drug. Doxorubicin should therefore be infused via a separate lumen from alkaline solutions following MTX administration.

NB: For UK sites, the composition of the fluids may be adapted according to local practice, as long as the rules of the hydration are respected: hyperhydration of 125 ml/m, alkalinisation of urine before start of HDMTX and after administration.

B.2. DRUG INTERACTIONS

Drugs which compromise renal function eg aminoglycosides can decrease clearance of MTX and lead to systemic toxicity. Avoid concurrent use of NSAIDs including salicylates and sulphonamides. Penicillin may interfere with the active renal tubular secretion of MTX. Prophylactic co-trimoxazole (if given) should be stopped 1 week prior to MTX administration.

B.3. FOLINIC ACID

In all cases where Folinic acid is specified in the protocol, the racemic form is intended. In case of use of **levofolinate**, the dose is **divided by two**.

15 mg/m² should be given orally every 6 hours for a total of 12 doses or until MTX level is below 0.15 μ mol/L.

The dose should be rounded up to the nearest 5mg. **Intrathecal** drugs should be given **before** rescue starts. Other oral drugs should be avoided within 30 minutes of folinic acid administration. If vomiting occurs within 30 minutes, repeat the dose. If persistent vomiting or diarrhoea occurs then give folinic acid by IV injection. MTX levels, creatinemia, urea and electrolytes should be measured **daily for 3 days** after MTX infusion. Strict attention should be paid to fluid balance. The folinic acid dose should be modified as required based on the MTX level. The level should be measured until completely rescued ie plasma MTX level $< 0.15 \mu$ mol/L (1.5×10^{-7} M).

The rescue begins **24 hours** from the start of MTX infusion when HDMTX is given in 3h (group B) or 4h (group C1 or 1st COPADM of C3) and begins **36 hours** from the start of MTX infusion when HDMTX is

given in 24h (2nd COPADM, HDMTX between CYVE and m1 in C3). It **stops when MTX level is below the limit of the detection**. This may be 0.1 µmol/l (1×10^{-7} M) to 0.2 µmol/l (2×10^{-7} M) depending on the laboratory, but it is generally 0.15 µmol/l (1.5×10^{-7} M), this limit will be used in the general recommendations. This level should be achieved with fewer than 12 doses. In this situation stop folinic acid after this time.

If 48 hour MTX level is > 20 µmol/L ($> 2 \times 10^{-5}$ M) then increase the folinic acid dose.

If 72 hour MTX level is > 2 µmol/L ($> 2 \times 10^{-6}$ M) then increase the folinic acid dose.

If 72 hour MTX level is > 0.15 µmol/L ($> 1.5 \times 10^{-7}$ M) then continue folinic acid 15 mg/m² (7.5 mg/m² if levofolinate) every 6 hours until level < 0.15 µmol/L ($< 1.5 \times 10^{-7}$ M).

NB: A further check on the MTX level should be done at 120 hours in arm C1 and C3 COPADM courses to ensure that the level does not increase again after IT MTX, and to alter the folinic acid rescue as required

Hydration should continue beyond 72 hours in the following situations:

If there is still evidence of tumour lysis

If cyclophosphamide infusion is still in process

If the MTX level is still > 0.15 µmol/l (1.5×10^{-7} M)

If the level does not fall as expected, increase the folinic acid as shown below:

Schedule for calculation of folinic acid rescue on basis of plasma MTX levels

Time from start of MTX	MTX plasma concentration (µmol/l)				
	(and Molar concentration)				
	< 0.15 1.5×10^{-7} M	0.15 - 2 1.5×10^{-7} M - 2×10^{-6} M	2 - 20 2×10^{-6} M - 2×10^{-5} M	20 - 100 2×10^{-5} M - 1×10^{-4} M	> 100 $> 1 \times 10^{-4}$ M
48 hrs	None ^a	15 mg/m ² q6h ^b	15 mg/m ² q6h	10 mg/m ² q3h	100 mg/m ² q3h
72 hrs	None	15 mg/m ² q6h	10 mg/m ² q3h	100 mg/m ² q3h	1000 mg/m ² q3h
96 hrs	None	15 mg/m ² q6h	10 mg/m ² q3h	100 mg/m ² q3h	1000 mg/m ² q3h
120 hrs ^c	None	15 mg/m ² q6h	10 mg/m ² q3h	100 mg/m ² q3h	1000 mg/m ² q3h

Notes

The doses of folinic acid are given for the racemic form.

a. No extra folinic acid is required, providing MTX levels are < 0.15 µmol/l (1.5×10^{-7} M) at 48 hrs

b. Dose and schedule of folinic acid: q6h = every 6 hours

c. At time beyond 120 hrs, folinic acid should be continued as recommended for 120 hrs

B.4. DOSE OF INTRATHECAL INJECTIONS ACCORDING TO AGE

Intrathecal injection of D2 after HDMTX should be given before start of folinic acid rescue.

Intrathecal drugs doses (mg)

Age (years)	Methotrexate	Hydrocortisone	cytarabine
< 1	8	8	15
≥1 - <2	10	10	20
≥2 - <3	12	12	25
≥ 3	15	15	30

B.5. GLUCARPIDASE (FORMELY CARBOYPEPTIDASE) = VORAXASE®

From: S. Demirdjian, S. Laghouati, G. Vassal. Glucarpidase: a method of rescue from HD MTX, European Journal of Oncology Pharmacy. 2008: 20-22.

MTX is hydrolysed to 7-OH MTX and is cleared primarily by renal excretion. High doses of MTX cause acute renal dysfunction: MTX and its metabolites precipitate in the renal tubules. The results of this lead to delayed MTX elimination and life-threatening toxicity. An elevation of plasma MTX concentrations increases other MTX-related side effects such as myelosuppression, hepatitis, dermatitis, and orointestinal mucositis.

Nephrotoxicity is prevented by alkaline hydration. If overexposure is found by monitoring MTX serum levels, the dose of leucovorin is increased over a prolonged period of time, but it does not completely reverse the toxicity of MTX.

Glucarpidase (formerly known as carboxypeptidase-G2 (CPDG2) and marketed as Voraxaze®) is a recombinant bacterial enzyme that rapidly hydrolyses MTX to an inactive metabolite, DAMPA (2, 4-diamino-N10-methyptericoic acid). It may be used as a rescue agent for methotrexate-induced nephrotoxicity.

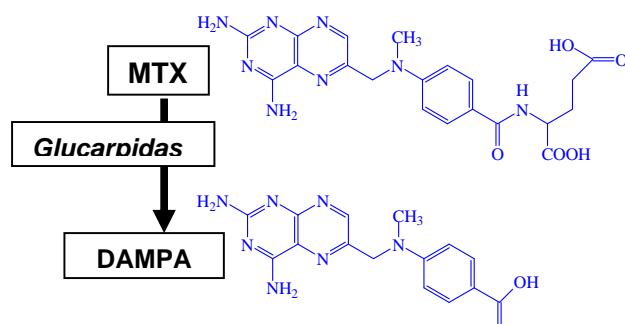
Leucovorin (LV) rescue prevents the nephrotoxicity due to HDMTX but has no effect on delayed MTX excretion. Glucarpidase decreases extracellular MTX concentrations. A slow process leads to an efflux of intracellular MTX back into the serum and MTX concentrations rise again some hours after infusion of glucarpidase. For this reason LV is always administered in combination, after glucarpidase.

Glucarpidase rapidly hydrolyses MTX into DAMPA. This is less soluble than MTX in acidic pH but is eliminated by an extra-renal route. This is the reason why with glucarpidase, patients continue to need hydration and alkalisation.

Haemofiltration or haemodialysis might be used when the patients are oliguric or anuric.

B.5.1.MECHANISM OF ACTION

Glucarpidase hydrolyses MTX and 7-OH MTX into DAMPA, a non-toxic metabolite (figure 1). Glucarpidase is restricted to the extracellular compartment due to its molecular size and does not hydrolyse intracellular MTX.



This hydrolysis results in the plasma MTX concentration decreasing by 99% within 15 minutes (figure 2).

Concentration of MTX in the plasma (µM)

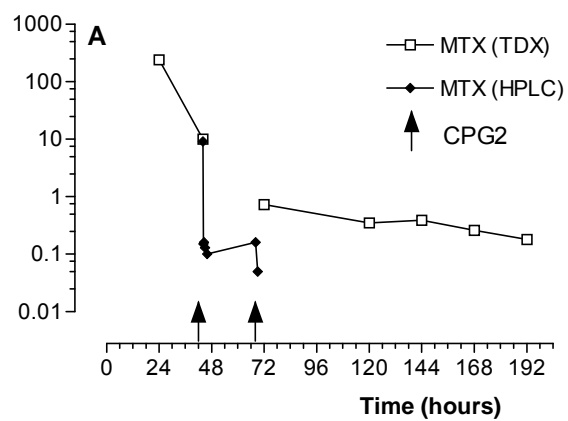


Figure 2: decrease in the plasma MTX concentration after glucarpidase infusion

B.5.2. INDICATIONS

- plasma MTX concentration > 10 µM more than 42 hours after start of HDMTX infusion
or
- renal insufficiency [serum creatinine $\geq 1.5 \times$ basal values or creatinine clearance < 60 ml/min/m²] with delayed elimination of MTX [plasma levels >mean +2 standard deviations within 12 hours of MTX administration]
and
- availability to infuse glucarpidase within 96 hours of starting HDMTX infusion

B.5.3. POSOLOGY AND ADMINISTRATION

Glucarpidase is given at 50 Units/Kg in an intravenous (IV) infusion over 5 min.

It is presented in a lyophilised form. The lyophilised powder is reconstituted with 1 ml of NaCl 0,9%. The solution obtained contains 1000 units of glucarpidase [7].

Hyperhydration (3L/m²), alkalinisation and leucovorin rescue are required when using glucarpidase

As LV is a competitive substrate of glucarpidase, a study of the interaction between Voraxaze® and LV has shown that glucarpidase increases the clearance of LV and reduce its efficacy.

Thus LV should be administered at least 4 hours prior or 4 hours after Glucarpidase infusion, to replete intracellular reduce folate pools.

B.5.4. UNDESIRABLE EFFECTS

Glucarpidase is well tolerated. Anaphylactic reactions could theoretically occur.

Side effects are infrequent, reversible and minor: paraesthesia, feeling of warmth, tingling in the fingers, flushing, shaking, and headache.

B.5.5. SPECIAL WARNINGS AND SPECIAL PRECAUTIONS FOR USE

The patients must be evaluated for signs and symptoms toxicity: complete blood counts, liver function and serum creatinine level.

To evaluate the efficacy of glucarpidase, plasma MTX concentrations are measured, but using High-Pressure Liquid Chromatography (HPLC) because this technique can quantify both MTX and its metabolite DAMPA. The commercially available immunoassays as Fluorescence Polarisation Immunoassay (FPIA with TDX) is not appropriate to determine MTX concentrations after glucarpidase administration because DAMPA, the metabolite of MTX, cross-reacts with MTX. The concentrations of MTX are thus overestimated.

The samples for determination of MTX concentrations are obtained before and 30 minutes, 24 hours after glucarpidase is given: the blood samples are placed on ice and rapidly centrifuged. To inactive glucarpidase, the serum samples are heated to above 80°C for 5 min in a water bath or treated with 1N HCl to obtain a final concentration of 0.1N of HCl.

The results of this monitoring show that glucarpidase leads to decrease the plasma MTX concentrations of 99%.

A second dose maybe necessary in some patients.

B.5.6. PHARMACEUTICAL PROBLEMS FOR THE MANAGEMENT OF GLUCARPIDASE

The number of glucarpidase infusions depends on the concentration of MTX .

Voraxaze® is supplied in packs of 2 vials. Each vial contains 1000 Units and each pack costs 14076 euros (October 2010)

B.5.7. HOW TO OBTAIN GLUCARPIDASE IN EUROPE

The manufacturer of Voraxaze® is Protherics, which has contracted with their distributor in the European countries to respond to the request for products. Voraxaze® will normally be delivered within 24 hours of receipt of the order, with a shipment the next day.

- The investigator fills in a “named patient basis” form from Prostherics

- The pharmacist faxes this form to the national competent authority
- When the competent authority authorizes the request for the product, the hospital pharmacy can order Voraxaze® from their distributor

B.5.8. FOR U.S. COG INVESTIGATORS:

For patients who have markedly delayed MTX clearance secondary to renal dysfunction, consider using glucarpidase (carboxypeptidase G2). Glucarpidase (carboxypeptidase G2, VoraxazeTM) is available in the US under a Treatment Protocol for patients receiving high dose methotrexate. To obtain supplies of glucarpidase in the US contact AAIPharma 24-Hour Access Call Center at 877-398-9829. COG ex-US members (such as New Zealand and Australia) the access number is 44 (0) 20 7575 0000. Additional information can be found at <http://www.btgplc.com/products/voraxaze-us-treatment-ind>. Patients requiring glucarpidase rescue may remain on study, and may receive further courses of IDMTX at investigator discretion if renal function is adequate as per parameters above.

ANNEXE C: GENERAL INFORMATION ON THE ANTINEOPLASIC DRUGS AND RITUXIMAB USED IN THE PROTOCOL AND DOSE MODIFICATIONS

C.1. INTRAVENOUS AND ORAL DRUGS

VINCRIStINE

<i>Formulation</i>	1mg/1ml, 2mg/2 mL vials in solution (1 mg/mL)
<i>Storage</i>	In refrigerator, between 2° and 8°C
<i>Stability</i>	After dilution stable for 21 days when stored at 4°C
<i>Administration</i>	1 to 2 mg/m ² given as an intravenous bolus. Max dose 2mg.
<i>Toxicity</i>	Abdominal pain, constipation, myelosuppression, peripheral neuropathy syndrome of inappropriate ADH. Note: given as a 24 hr i.v. infusion in PMLBL. Vincristine must be diluted in bags. This recommendation is to avoid intrathecal injection of Vincristine when there is an intrathecal injection and a vincristine injection on the same day (in COP courses).

CYCLOPHOSPHAMIDE

<i>Formulation</i>	100 mg, 200 mg, 500 mg and 1 g vials for reconstitution.
<i>Storage</i>	At room temperature.
<i>Stability</i>	Unreconstituted vials stable for 5 years at room temperature. A solution of cyclophosphamide appears to be chemically stable for at least 28 days when stored at 4°C. Reconstituted solution (20 mg/ml) should be used within 8 hours when stored at room temperature.
<i>Administration</i>	The dose is 300 mg/m ² in the COP phase, 250 mg/m ² (or 500 mg/m ²) in the COPADMs and m1 courses, and 750 mg/m ² (starting dose) in the DA-EPOCH-R regimen, given by intravenous infusion over 15-60 minutes. Mesna is not required (may be added in higher doses > 1g/m ² in DA-EPOCH-R), but hydration is given (see individual course details for more information).
<i>Toxicity</i>	Myelosuppression, nausea, vomiting, alopecia, haemorrhagic cystitis, sterility, second malignancies including leukaemia or bladder cancer.

DOXORUBICIN

<i>Formulation</i>	Vials containing 10 mg, 50 mg, 100 mg and 200 mg in solution (2 mg/ml) Vials containing 10 mg, 50 mg as powder.
<i>Storage</i>	Solution at 2°C - 8°C in refrigerator Powder can be stored at room temperature.
<i>Stability</i>	Powder 3 years at room temperature. Solution 18 months at 2-6°C in refrigerator. Reconstituted solution (100 µg/ml) in 5% dextrose or 0.9% saline is stable for at least 28 days when stored in refrigerator. Solutions should be protected from light during storage and administration unless concentration is > 500 µg/ml and freshly prepared. Photodegradation may be substantial at concentrations below 100 µg/ml if solution is exposed to light.
<i>Administration</i>	In the LMB protocol 60 mg/m ² is given as an infusion over 1 hour. The drug should be mixed with 0.9% saline. <u>Prolonged contact with solutions of alkaline pH should be avoided as this will result in hydrolysis of the drug.</u> Doxorubicin should therefore be infused via a separate lumen from alkaline solutions following MTX administration.
<i>Toxicity</i>	It is given as a 24 hr i.v. infusion in PMLBL. Local necrosis if extravasation occurs. Cardiotoxicity. Bone marrow suppression, mucosal ulceration, nausea, vomiting, alopecia.

METHOTREXATE

<i>Formulation</i>	Ready mixed vials in following strengths: 2.5 mg in 1 ml 100 mg in 4 ml 5 g in 200 ml
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	50 mg in 2 ml 200 mg in 8 ml 25 mg in 1 ml 500 mg in 20 ml 50 mg in 2 ml 1 g in 40 ml 1 g in 10 ml, and 5 g in 50 ml (hypertonic requires dilution) The vials contain sodium chloride and sodium hydroxide adjusted to a pH of approx 8.5; there is no preservative present.
<i>Storage</i>	Room temperature
<i>Stability</i>	25 mg/ml solution 3 years, other strengths 2 years at room temperature. The manufacturers do not recommend re-use of MTX injection, but providing it is manipulated in aseptic conditions it is stable for 28 days at 2-6°C in refrigerator (protected from light). Solutions of 1-10 mg/ml in 5% dextrose or 1.25-12.5 mg/ml in 0.9% saline are stable for 28 days at 4°C in PVC bags.
<i>Administration</i>	Dose in this protocol is 3g/m ² given as a 3 hr infusion, 8g/m ² over 4 or 24 hours.
<i>Toxicity</i>	Neurotoxicity, mucositis, liver dysfunction, bone marrow depression, renal failure, mucosal membrane inflammation, ulceration, and bleeding. In addition to the side effects associated with this agent, the effects of intrathecal administration include headache, stiff neck, lethargy, nausea and vomiting, confusion, and seizures.

ETOPOSIDE (VP 16)

<i>Formulation</i>	Vials containing 100 mg etoposide in 5 ml, 200 mg in 10 ml or 1000 mg in 50 ml.
<i>Storage</i>	At room temperature.
<i>Reconstitution</i>	In general dilute to a concentration of not more than 0.4 mg/ml etoposide in 0.9% sodium chloride (or 5% dextrose + electrolytes).
<i>Stability</i>	Vials are stable for 5 years at room temperature. At concentrations of 0.4 mg/ml in 0.9% saline solutions are stable for 48 hours at room temperature in normal fluorescent lighting.
<i>Administration</i>	100 mg/m ² by intravenous infusion over 1 hour - protected from light. Note: it given as a 24 hr i.v. infusion in the R-DA-EPOCH protocol for PMLBL Caution: Anaphylactic reaction usually manifested as severe hypotension may occur if infusion given too rapidly. Avoid extravasation.
<i>Toxicity</i>	Bone marrow suppression. Alopecia, headache, fever, hypotension, nausea, vomiting, anaphylactic reactions, second malignancies, including leukaemia. (See C.4.3 in case of hypotension or allergic reaction)

ETOPOSIDE PHOSPHATE (ETOPOPHOS) can substitute Etoposide at the same dose, for local facilities or in case of allergic reaction to etoposide (does not contain polysorbate 80 and polyethyleneglycol, the solubilizing agent in etoposide that may induce allergic reactions and hypotension).

CYTARABINE

<i>Formulation</i>	Vials containing freeze-dried powder of 100 mg, 500 mg, 1000 mg or 2000 mg cytarabine. Other preparations available. Solution containing 20 mg/ml, 100 mg/ml.
<i>Storage</i>	At room temperature. (Alexan product should be stored below 15°C)
<i>Stability</i>	Alexan is stable for 3 years below 15°C. Cytosar vials are stable for 3 years at room temperature. Reconstituted solution (5% dextrose or 0.9% saline) is stable for 7 days.
<i>Administration</i>	Dose 100mg/m ² given in a 24hr infusion in (R-)CYM course. Dose 50 mg/m ² in a 12h infusion followed by 3g/m ² in 3h infusion in (R-)CYVE

<i>Toxicity</i>	<p>Bone marrow suppression, nausea, vomiting, oral ulceration, fever and arthralgia, diarrhoea, mucosal membrane inflammation, ulceration and bleeding, alopecia and flu-like syndrome.</p> <p>For intrathecal administration, reconstitute with preservative-free lactated Ringer's or normal saline.</p> <p>Cytarabine is compatible with KCl and NaHCO₃.</p> <p>At higher doses (3000 mg/m²) cerebellar toxicity may occur. Gastrointestinal toxicity with diarrhoea, mucositis and vomiting may also be more severe. Pulmonary toxicity is uncommon, but may present with unexplained breathlessness. Conjunctivitis can be distressing, but it may be prevented by the regular use of steroid eye drops (see annexe D9).</p>
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FOLINIC ACID (LEUCOVORIN CALCIUM, CITROVORUM FACTOR) = RACEMIC FORM

<i>Formulation</i>	Lyophilized powder, 3 mg, 5 mg, 25 mg, 50 and 100 mg per vial. Tablets of 5 mg, 10 mg, 15 mg, and 25 mg.
<i>Storage</i>	Room temperature.
<i>Reconstitution</i>	Reconstitute each vial with bacteriostatic or sterile water for injection, to achieve a final concentration of 10 mg/ml. No data is available concerning compatibility with KCl or NaHCO ₃ .
<i>Stability</i>	Reconstituted solution should be and discarded after eight hours.
<i>Administration</i>	15mg/m ² given iv or orally. Follow details of administration in annexe B.
<i>Toxicities</i>	Allergic reactions (rash, pruritus and erythema).

C.2. INTRATHECAL DRUGS

Please note details of the suitable solvent for each of the drugs for intrathecal use.

Please pay attention that in COP courses there is an intrathecal injection and a IV Vincristine injection planned on the same day. As indicated above, vincristine has to be prepared in bags to avoid an intra thecal injection de vincristine.

HYDROCORTISONE SODIUM SUCCINATE

<i>Formulation</i>	Lyophilized powder, 100 mg vials.
<i>Storage</i>	Room temperature. Protect vials from light.
<i>Reconstitution</i>	For intrathecal use, reconstitute with preservative-free Lactated Ringer's, or Normal Saline.
<i>Stability</i>	Discard IT solutions after 8 hours, since they contain no preservatives.
<i>Toxicities</i>	Salt or fluid retention, hypertension, potassium loss. Muscle weakness, loss of muscle mass, severe arthralgia, aseptic necrosis of femoral/humeral heads, osteoporosis. Peptic ulcer, pancreatitis, abdominal distention, ulcerative oesophagitis. Impaired wound healing, thin fragile skin, striae, bruises, facial erythema. Convulsion, headache, increased intracranial pressure. Cushingoid state, suppression of growth in children. Posterior subcapsular cataracts, increased intraocular pressure. Fatigue, psychosomatic complaints.

METHOTREXATE

<i>Formulation</i>	Ready mixed vials 50mg/2ml, 25mg/ml or 5mg/2ml without preservative The vials contain sodium chloride and sodium hydroxide adjusted to a pH of approx 8.5; there is no preservative present.
<i>Storage</i>	Room temperature
<i>Stability</i>	25 mg/ml solution 3 years, Other strengths 2 years at room temperature.
<i>Administration</i>	Dose is age dependent. Please refer to the protocol
<i>Toxicity</i>	The effects of intrathecal administration include headache, stiff neck, lethargy, nausea and vomiting, confusion, and seizures.

CYTARABINE

<i>Formulation</i>	Vials containing freeze-dried powder of 100 mg cytarabine.
<i>Storage</i>	At room temperature. (<i>Alexan</i> product should be stored below 15°C)
<i>Stability</i>	<i>Alexan</i> is stable for 3 years below 15°C. <i>Cytosar</i> vials are stable for 3 years at room temperature. For intrathecal administration, reconstitute with preservative-free lactated Ringer's or normal saline.
<i>Administration</i>	Dose is age dependent. Please refer to the protocol
<i>Toxicity</i>	The effects of intrathecal administration include headache, stiff neck, lethargy, nausea, and vomiting.

C.3. DOSES REDUCTIONS BECAUSE OF AGE < ONE YEAR

Systemic drugs should be given at 2/3 dose. If well tolerated increase doses for subsequent course (particularly HD MTX).

Intrathecal doses are:

MTX= 8 mg, HC = 8 mg, cytarabine= 15 mg (see annexe B4)

C.4. TOXICITY RELATED DRUG DOSE MODIFICATIONS (FROM COG RECOMMENDATIONS)

For patients included in the phase II, see also specific recommendations in section 10.6.4.

C.4.1. CYCLOPHOSPHAMIDERenal toxicity

If creatinine clearance <10 ml/min/1.73m², reduce dose by 50%

C.4.2. DOXORUBICIN- Cardiotoxicity

For fractional shortening <28%, hold doxorubicin until fractional shortening is ≥ 28%. If there is other evidence of cardiac dysfunction consult a cardiologist and the Study Chair.

- Hyperbilirubinemia

Direct bilirubin <1.2 mg/dL (<20.5 µmol/l) give full dose

Direct bilirubin 1.2 -3.0 (20.5 – 52 µmol/l) give 50% of dose

Direct bilirubin 3.1 -5.0 (53-85 µmol/l) give 25% of dose

Direct bilirubin > 5 mg (> 85 µmol/l) withhold dose for the cycle of chemotherapy

C.4.3. ETOPOSIDE

If hypotension occurs, first slow rate of administration.

In case of allergic reaction: Administer an antihistaminic H1 (=diphenhydramine or equivalent). If symptoms persist, add IV corticosteroids such as hydrocortisone. Act also according to local practice for allergic reactions. Continue to use premedication before etoposide in future. Also consider substituting an equimolar amount of etoposide phosphate, in the face of significant allergy and/or hypotension. Etoposide phosphate is a water soluble prodrug that does not contain polysorbate 80 and polyethyleneglycol, the solubilizing agent in etoposide that may induce allergic reactions and hypotension.

In case of grade IV allergic reaction with etoposide or allergic reaction persisting after the use of etoposide phosphate, consider no further administration of etoposide

C.4.4. HIGH-DOSE METHOTREXATENephrotoxicity

General recommendation: For patients with creatinine clearance <60 ml/min/ 1.73m^2 , delay further courses of high-dose methotrexate until creatinine clearance > 60 ml/min/ 1.73m^2 . But other modifications (concerning MTX dose and infusion duration, folinic acid rescue dose and timing) can be discussed with the study chair

Hepatic Toxicity

ALT or AST < 20 times normal give full dose

ALT or AST ≥ 20 times normal of upper limits, check every 48 hours until $< 10 \times$ normal, then give full dose

Direct bilirubin >2.0 mg/dL (>34 $\mu\text{mol/l}$) hold high-dose methotrexate and discuss with Study Chair.

Other supportive care guidelines for methotrexate administration, see annexe B.

C.4.5. HIGH-DOSE CYTARABINE

Neurotoxicity

For \geq Grade 3 neurotoxicity associated with high-dose cytarabine, no further cytarabine should be given.

C.4.6. VINCRISTINE

- Hyperbilirubinemia

Direct bilirubin <3.1 mg/dL (< 53 $\mu\text{mol/l}$) give full dose

Direct bilirubin $3.1 - 5.0$ ($53 - 85$ $\mu\text{mol/l}$) give 50% of dose

Direct bilirubin $5.1 - 6.0$ ($85 - 100$ $\mu\text{mol/l}$) give 25% of dose

Direct bilirubin > 6 mg (>100 $\mu\text{mol/l}$) withhold dose for the cycle of chemotherapy

- Neurotoxicity (including constipation)

If \geq Grade 3 toxicity, hold dose for the cycle of chemotherapy, and give 50% dose on next cycle and escalate to full dose thereafter if no further toxicity.

C.5. RISKS ASSOCIATED WITH THE USE OF RITUXIMAB

Please see rituximab label (Mabthera SmPC in Europe; USPI in USA) for further safety information.

C.5.1. RISK OF PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY (PML)

Rituximab administration may increase risks of developing PML. PML is a rare and frequently fatal demyelinating disease of the CNS that primarily affects patients whose immune systems are compromised by disease or medical treatments. It is caused by reactivation of the JC virus, which remains latent in up to 80% of healthy patients. There are no known effective treatments for PML.

Investigators are advised to maintain a high index of suspicion for PML in patients receiving rituximab therapy, particularly those who develop new neurologic signs or symptoms.

Clumsiness may be the 1st symptom. Hemiparesis is the most common finding. Aphasia, dysarthria, and hemianopia are also common. Multifocal cortical damage produces cognitive impairment in $\frac{2}{3}$ of patients. Sensory, cerebellar, and brain stem deficits may be present. Headaches and seizures are rare. In case of suggestive symptoms of PML, investigator's must:

- suspend treatment with rituximab
- Perform repeated neurological examinations and additional tests including MRI and JC virus polymerase chain reaction (PCR) assay

The treatment can be resumed only after having excluded the diagnosis of PML.

Any PML or suspicion of PML must be reported to the Pharmacovigilance Unit within 48 hours.

C.5.2. INFUSION-RELATED REACTIONS

Rituximab can cause severe, including fatal, infusion reactions. Severe reactions typically occurred during the first infusion with time to onset of 20-120 minutes. Incidence of infusion related reactions decreases substantially with subsequent infusions. Rituximab-induced infusion reactions and sequelae include

urticaria, hypotension, angioedema, hypoxia, bronchospasm, pulmonary infiltrates acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, cardiogenic shock, anaphylactoid events, or death. Other symptoms of infusion reactions include fever, chills, rigors, nausea, fatigue, headache, throat irritation, rhinitis, vomiting and tumor pain.

C.5.3. TUMOR LYSIS SYNDROME (TLS) IN CLL

Rituximab mediates the rapid lysis of benign and malignant CD20-positive cells. Signs and symptoms (e.g. hyperuricemia, hyperkalemia, hypocalcaemia, hyperphosphataemia, acute renal failure, elevated LDH) consistent with tumour lysis syndrome (TLS) have been reported to occur after the first rituximab infusion in patients with high numbers of circulating malignant lymphocytes (CLL). Prophylaxis for TLS should be considered for patients at risk of developing rapid tumor lysis (e.g. patients with a high tumor burden or a high number of circulating malignant cells). These patients should be followed closely and appropriate laboratory monitoring performed. Appropriate medical therapy should be provided for patients who develop signs and symptoms consistent with rapid tumor lysis.

C.5.4. INFECTIONS

Serious infections, including fatalities, can occur during therapy with rituximab. Rituximab should not be administered to patients with an active and/or severe infection (eg. tuberculosis, sepsis and opportunistic infections).

Cases of hepatitis B reactivation have been reported in subjects receiving rituximab including fulminant hepatitis with fatal outcome. The majority of these subjects were also exposed to cytotoxic chemotherapy. Patients with a history of hepatitis B infection or with positive serology are excluded from this study.

Infections reported to occur in higher incidence in NHL patients treated with rituximab in addition to chemotherapy include bronchitis, herpes zoster, sinusitis, and respiratory tract infections. Very rare cases of Progressive Multifocal Leukoencephalopathy (PML) have been reported during post-marketing use of rituximab in NHL and CLL.

C.5.5. CARDIOVASCULAR

Angina pectoris, or cardiac arrhythmias such as atrial flutter and fibrillation heart failure or myocardial infarction have occurred in patients treated with rituximab. Therefore patients with a history of cardiac disease and/or cardiotoxic chemotherapy should be monitored closely. Since hypotension may occur during rituximab infusion, consideration should be given to withholding anti-hypertensive medicines 12 hours prior to the rituximab infusion.

C.5.6. BOWEL OBSTRUCTION AND PERFORATION

Abdominal pain, bowel obstruction, and perforation, in some cases, leading to death were observed in patients receiving rituximab in combination with chemotherapy for DLBCL. Complaints of abdominal pain, especially early in the course of treatment, should prompt a thorough diagnostic evaluation and appropriate treatment.

C.5.7. HEMATOLOGICAL SIDE EFFECTS

B-cell depletion after monotherapy generally persists for 6 months post treatment, with recovery by 12 months. B cell depletion after rituximab in combination with chemotherapy can result in longer periods of B cell depletion. The incidence of neutropenia and febrile neutropenia is higher in NHL patients treated with rituximab and chemotherapy than with chemotherapy alone.

C.5.7. IMMUNIZATION

Live vaccines should not be administered to patients while B cell depleted. The response to killed vaccines is reduced and the protective effect of vaccination is not known.

C.5.8. REPRODUCTIVE TOXICITY

The long-term effects of rituximab are unknown.

Contraception is required until 12 months after stopping treatment with rituximab for women with childbearing potential.

C.6. RECOMMENDATIONS FOR DA-EPOCH ADMINISTRATION

Stability studies conducted by the Pharmaceutical Development Service, Pharmacy Department, NIH Clinical Center, have demonstrated that admixtures of vincristine, doxorubicin, and etoposide in 0.9% Sodium Chloride Injection, USP at concentrations, respectively, of 1, 25 and 125 mcg/mL; 1.4, 35 and 175 mcg/mL; 2, 50 and 250 mcg/mL; and 2.8, 70 and 350 mcg/mL are stable for at least 36 hours at room temperature when protected from light. Also admixtures containing vincristine, doxorubicin and etoposide concentrations of 1.6, 40 and 200 mcg/mL are stable for at least 30 hours at 32 degrees C.(reference: J L Wolfe, L A Thoma, C Du, et al. Compatibility and stability of vincristine sulfate, doxorubicin hydrochloride, and etoposide in 0.9% sodium chloride injection Am J Health-Syst Pharm. 1999; 56:985-9)

Some pharmacists may be reluctant to provide admixture of the 3 drugs together. It is permissible for the 3 drugs to be provided separately.

ANNEXE D: SUPPORTIVE CARE GUIDELINES

Supportive Therapy is primarily the responsibility of the physician in charge. In this chapter only general recommendations are given for the patient management in certain situations. The national study coordinators are available for consultation.

D.1. IN CASE OF ORGAN DYSFUNCTION AT DIAGNOSIS

At diagnosis, patients may present with organ dysfunction due to organ tumour infiltration or consequence of a spontaneous tumour lysis syndrome. This is not a contraindication for inclusion in the study, but may necessitate special appropriate measures before or at start of treatment.

Liver involvement at diagnosis may occur. Vincristine dose should be reduced at least 50% for bilirubin > 10 times normal.

In case of renal dysfunction at diagnosis, precise the mechanism of this renal dysfunction. If organic renal dysfunction, the 3 usual aetiologies are:

- obstruction of the urinary tract, generally due to pelvic tumour. It may be necessary to derivate urine by transcutaneous pyelostomy, or if not available, by ureter catheter (JJ catheter). These catheters are usually needed for a short period of time, the time that the tumour decreases.
- uratic nephropathy already present before diagnosis. Consider alkaline hyperhydration until normalisation of uric acid level and orate oxidase. If correct diuresis cannot be obtained rapidly, eventually helped by furosemide, consider dialysis. See also Tumor lysis syndrome section (annexe D2).
- diffuse bilateral kidney infiltration by the lymphoma/leukemic cells. If the correct diuresis cannot be obtained rapidly, eventually helped by furosemide, rapidly consider dialysis.

D.2. TUMOR LYSIS SYNDROME

The recommendations for the management of tumour lysis vary slightly from country to country. Rapid neoplastic cell lysis may occur in lymphoma and B cell leukaemia and can produce metabolic complications including hyperuricaemia and hyperphosphataemia. This may be present at diagnosis due to rapid cell turnover or occur during induction.

Primary complications of tumour lysis include hyperkalaemia and hyperphosphataemia with secondary hypocalcaemia. Secondary renal dysfunction occurs as the result of a combination of hyperuricaemia and hyperphosphataemia with intrarenal deposition of calcium. Renal failure leads to a further rise in serum potassium, urea and creatinine.

A central venous line should be inserted for fluid administration and monitoring.

See review: Coiffier B, Altman A, Pui CH, Younes A, Cairo MS : Guidelines for the management of pediatric and adult tumor lysis syndrome: an evidence-based review. J Clin Oncol, 2008 Jun 1;26(16):2767-78.

For classification of risk and general guidelines for prevention and treatment of the tumor lysis syndrome

D.2.1. PRE TREATMENT INVESTIGATIONS SHOULD INCLUDE :

- 1] Renal ultrasound - to assess parenchymal infiltration. Occasionally there may also be an obstructive element due to tumour pressure.
- 2] Chest X ray
- 3] Baseline serum urate, urea, creatinine, electrolytes, Ca, Mg, PO₄
- 4] Weight
- 5] Blood pressure
- 6] +/- ECG

D.2.2. PREVENTATIVE MEASURES

- 1] If available, Rasburicase (Fasturtec® in Europe) is preferred to allopurinol. The recommended dose is 0.15 or 0.20 mg/kg IV infusion over 30 minutes once daily, for up to 5 days. The dose can be increased

as necessary depending on the uric acid level. Administration beyond 5 days treatment or more than one treatment course is not recommended. Do not give allopurinol at same time.

- 2] If rasburicase is not available (or contraindicated) then use allopurinol (300 mg/m²/dose) daily (IV or orally) to reduce renal tubule urate precipitation.
- 3] Start intravenous fluids at least 6-12 hours before chemotherapy. Give at a rate of 3000 mls/m²/day (125 mls/m²/hr). Do not add potassium to IV fluids.
- 4] Other additives such as calcium may be required as clinically indicated, see below. Fluids with low NaCl content reduce the risk of urate supersaturation.
- 5] Close observation is essential as the main period of lysis occurs 8 - 24 hours after the start of chemotherapy.
- 6] It is not necessary to alkalinise urine if fluid input is adequate, it carries a risk of increased phosphate and xanthine precipitation in the renal tubules. If the pH remains very low it may be helpful to add alkali to achieve pH 7 when rasburicase is not given.

D.2.3. MONITORING DURING INDUCTION CHEMOTHERAPY

- 1] Strict attention to fluid balance is essential.
- 2] Blood pressure should be checked hourly.
- 3] Weight should be checked twice daily.
- 4] Use a cardiac monitor: to detect early signs of hyperkalaemia, elevation of T waves, widening of QT interval
- 5] Measure electrolytes, calcium and phosphate at least 8 hourly.

D.3. VENOUS ACCESS

Insertion of a central venous line is recommended because of the repeated courses of chemotherapy with prolonged hydration and the use of doxorubicin. In PMLBL, it may have to be delayed after start of treatment because of the large mediastinal mass.

D.4. PROPHYLACTIC ANTIBIOTICS

Co-trimoxazole prophylaxis against pneumocystis carinii is strongly recommended particularly in group C and PMLBL regimen, as well as in group B with rituximab. It should be started after the 2nd course of COPADM (at the 2nd course of CYVE in CSF pos) and to continue during the following courses and for 3 months after the end of treatment. The recommended dose is 25 mg/kg of Septrin (Bactrim) 3 days a week. Co-trimoxazole should be **omitted for at least 3 days before HD MTX** and during the following week.

D.5. MUCOSITIS

Mucositis is expected after COPADM and M1 courses. The use of mouthcare should be encouraged to reduce oral infections and prevent mucositis. Appropriate analgesics and nutrition should be given during the period of mucositis.

D.6. FEVER AND NEUTROPENIA

Patients will experience significant haematologic toxicity resulting in a high risk of bacterial infection. Experience with LMB protocols suggests that COPADM and CYVE courses are followed by febrile neutropenia in the majority of the cases, requiring hospitalisation. Patients experiencing fever should be promptly assessed. If the absolute neutrophil count is $< 0.5 \times 10^9/l$ then the patient will be hospitalised, and broad spectrum antibiotics started after appropriate cultures, according to the clinical situation of each individual patient, to the antibiotics local policy and to subsequent bacteriology results. If no cause is found for the fever and if and the neutropenia persist greater than five days then consideration should be given to antifungal therapy. It is recommended that broad spectrum antibiotics be carried on until the absolute neutrophil count has risen above $0.5 \times 10^9/l$ and the patient has been afebrile for at least 24 hours.

D.7. G-CSF

Following the French randomised GL93 study, systematic prophylactic G-CSF is not recommended in the phase III, but this attitude is not followed in US. If required therapeutically it must have been stopped for at least 48 hours prior to the next course of chemotherapy.

In the phase II, G-CSF is a critical part of the DA-EPOCH-R regimen and is started on day 6 of all cycles - 24 hours after the end of cyclophosphamide - and continued once daily past the neutrophil nadir (checking labs at least twice weekly) until the ANC is $> 5 \times 10^9/l$ (this is usually for 9/10 days). G-CSF might be allowed if the ANC on day 1 of treatment is not $> 1 \times 10^9/l$ – this is occasionally needed to do that where a patient is due to start a new cycle (day 1) and their ANC is $< 1 \times 10^9/l$. Try to never delay cycles with these patients.

D.8. BLOOD PRODUCT SUPPORT

Substitution of blood products should be performed according to local/national standards.

Preventing GVHD (graft versus host disease): Irradiation of all blood products with a minimum of 25 Gy (use of irradiated blood according to national policy).

Experience with the LMB protocols shows around 50% of children will require red cell transfusion. Significant thrombocytopenia is rare after the COPADM courses except in cases with initial bone marrow involvement, but is frequent after the CYVE courses.

To note

The CYM courses are much better tolerated than COPADM and CYVE courses, so it is reasonable to discharge patients from hospital following these courses.

D.9. EYE CARE CONJUNCTIVITIS PROPHYLAXIS (ACCORDING TO COG GUIDELINES)

Administer steroid eye drops (0.1% dexamethasone or 1% prednisolone ophthalmic solution), 2 drops each eye q 6 hours beginning immediately before the first dose of high dose cytarabine ($> 1000 \text{ mg/m}^2/\text{dose}$) and continuing 24 hours after the last dose. If the patient does not tolerate steroid eye drops, the physician may administer artificial tears on a q 2 to 4 hour schedule to prevent conjunctival and corneal pain.

ANNEXE E: STAGE AND GROUP CLASSIFICATION

E.1. ST JUDE'S (MURPHY'S) STAGING

(Murphy, 1980)

Stage 1: A single tumor (extranodal) or a single anatomical site (nodal) with exclusion of the mediastinum or abdomen.

Stage 2: A single tumor (extranodal) with regional involvement

Two or more nodal areas on the same side of the diaphragm

Two single (extranodal) tumors with or without regional node involvement on the same side of the diaphragm

A primary gastrointestinal tract tumor, usually in the ileocecal area, with or without involvement of associated mesenteric nodes only, grossly completely resected

Stage 3: Two single tumors (extranodal) on opposite sides of the diaphragm

Two or more nodal areas above and below the diaphragm

All primary intrathoracic tumors (mediastinal, pleural, thymic)

All extensive primary intra-abdominal disease, unresectable

All paraspinal or epidural tumors, regardless of other tumor sites

Stage 4: Any of the above with initial CNS and/or bone marrow involvement

E.2. PROPOSAL OF REVISED PEDIATRIC NHL STAGING SYSTEM

(A Rosolen et al)

Updated (June, 2009) revised pediatric NHL staging system (Based on the classification proposed by Murphy.(Murphy 1980)

Stage I

A single tumor with the exclusion of the mediastinum and abdomen. (N: nodal; EN: extra-nodal; bone or skin: EN-B, EN-S).

Stage II

A single extranodal tumor with regional node involvement.

Two or more nodal areas on the same side of the diaphragm.

A primary gastrointestinal tract tumor (usually in the ileocecal area), with or without involvement of associated mesenteric nodes, that is completely resectable. (if ascites or extension of the tumor to adjacent organs it should be regarded as stage III).

Stage III

Two or more extranodal tumors (including bone or skin: EN-B, EN-S).

Two or more nodal areas above and below the diaphragm.

Any intrathoracic tumor (mediastinal, hilar, pulmonary, pleural, or thymic).

Incompletely resected intra-abdominal disease, including liver, spleen and/or kidney localisations.

Any paraspinal or epidural tumor, whether or not other sites are involved.

Single bone lesion with concomitant involvement of extra-nodal and/or non-regional nodal sites.

Stage IV

Any of the above findings with initial involvement of the CNS (IV CNS), bone marrow (IV BM), or both (IV combined).

SUPPLEMENTAL DATA

Bone Marrow Involvement

Stage IV disease, due to BM involvement, is currently defined by morphologic evidence of lymphoma cells. This applies to any histological subtypes.

Independently of stage, type and degree of BM involvement should be specified, using the abbreviations below:

BMm = BM positive by morphology (specify % lymphoma cells)

BMi = BM positive by immunophenotypic methods (histochemical/flow-cytometric analysis) (specify % lymphoma cells)

BMc = BM positive by cytogenetic/FISH analysis

BMmol= BM positive by molecular techniques (PCR-based)

Examples: "stage III (BMi)" (stage III disease with BM positive by flow-cytometric analysis);
"stage III (BMmol)" (stage III disease with BM positive by PCR analysis).

Same approach should be used for peripheral blood (PB) involvement (i.e.: PBm; PBi, etc.)

Central Nervous System (CNS) Involvement

CNS is considered involved in case of:

- 1) any CNS tumor mass (identified by imaging techniques, i.e. CT, MRI);
- 2) in case of cranial nerve palsy that cannot be explained by extra-dural lesions
- 3) in case of blasts morphologically identified in the CSF

Condition that define CNS positivity should be specified: CNS positive/mass; CNS positive/palsy; CNS positive/blasts

CSF status: CSF positivity is based on morphologic evidence of lymphoma cells.

CSF should be considered positive when any number of blasts is detected.

CSF unknown

Similarly to BM, type of CSF involvement should be described whenever possible.

CSFm= CSF positive by morphology (specify the number of blasts/ μ L)

CSFi= CSF positive by immunophenotype methods (histochemical/flow-cytometric analysis)

CSFc= BM positive by cytogenetic/FISH analysis

CSFmol= CSF positive by molecular techniques (PCR-based)

Note

Until sufficient data are available, Positron Emission Tomography (PET(-CT)) should not be used for staging. A PET(-CT) positivity should not be interpreted as tumor localization unless proven by other imaging approaches (Echo, CT, MRI) or, ideally, by biopsy.

E.3. GROUP CLASSIFICATION IN THE LMB STUDIES

- Group A** Completely resected stage I or completely resected abdominal stage II lesions.
- Group B** All cases not eligible for Group A or Group C. (Unresected stage I and non abdominal stage II, Murphy Stage III and non-CNS Stage IV)
- Group C** Any CNS involvement and/or BM involvement $\geq 25\%$ blasts.
For CNS involvement one or more of the following applies:
- Any L3 blasts in CSF
 - Cranial nerve palsy (if not explained by extracranial tumor)
 - Clinical spinal cord compression
 - Isolated intracerebral mass
 - Parameningeal extension: cranial and/or spinal

Pathology Staging Criteria as in FAB LMB96 study:

1. Cerebrospinal fluid: The presence of **any** blasts or lymphoma cells represents involvement of the CNS.
2. Bone marrow: The presence of **any** blasts or lymphoma cells in a BM aspirate represents involvement of the marrow by lymphoma. This applies to all three types of lymphoma included in this study.

E.4. ANN ARBOR CLASSIFICATION OF DLBCL PATIENTS

Stage I: involvement of a single lymph node area or of a single extralymphatic organ or site

Stage II: involvement of 2 or more lymph node regions or lymphatic structures on the same side of the diaphragm alone or with involvement of limited, contiguous extralymphatic organ or tissue

Stage III: involvement of lymph node regions on both sides of the diaphragm which may include the spleen or limited, contiguous extralymphatic organ or site or both

Stage IV: Diffuse or disseminated foci of involvement of one or more extralymphatic organs or tissues, with or without associated lymphatic involvement

ANNEXE F: GUIDELINES FOR PATHOLOGY AND CYTOGENETICS STUDIES (EICNHL ONLY)

F.1. PATHOLOGY GUIDELINES

(reviewed by W Klapper, nov 2009 and C Copie, march 2010)

F.1.1. PATHOLOGY GOALS

Provide quality control by pathological review with accurate diagnosis of paediatric B-cell lymphoma included in this treatment protocol. The diagnosis to be based on both morphological and immunophenotypic criteria. The classification will be according to the WHO classification published in 2008.

F.1.2. REQUIREMENTS FOR HANDLING TISSUE SPECIMENS

Tissue should preferentially, whenever possible, be obtained fresh and delivered immediately to the Pathology laboratory for optimal handling and distribution (touch imprint, fixation, snap freezing, cytogenetics, etc.)

Representative tissue sections should be submitted for fixation in 10% Formalin. Generally, different fixatives e.g. B5 (France), Bouins fixative, AFA, Zenker, alcohol based Bouin are not recommended. The fixative utilised and length of time in fixative before processing should be indicated for each respective paraffin block. Fixation of tissue for over 24 hours should be avoided to allow optimal antigen preservation and immunophenotypic analysis.

If sufficient tissue is available, representative fresh tumour tissue should be snap frozen at -70°C and placed in a plastic tube.

Besides touch imprints for standard cytology, touch imprint preparations should be made on ten (10) sialinised glass microscope slides, fixed in 100% methanol for at least three minutes and air dried completely. These slides should be stored frozen at -20 to -70°C. These preparations can be used for fluorescence in situ hybridisation studies.

If sufficient tissue is available, representative fresh tissue should be submitted for cytogenetic analysis.

F.1.3. DIAGNOSTIC CRITERIA

- Morphology

Morphologic evaluation and classification of the study cases will utilize the criteria described in the WHO Lymphoma Classification 2008. Eligible paediatric lymphomas are those classified into categories as follows:

1. Burkitt lymphoma and atypical Burkitt lymphoma
2. Diffuse large B-cell lymphoma
3. Primary mediastinal large B-cell lymphoma
4. "high grade" non classifiable B-cell lymphoma, essentially due to technical problem

Are non includable in this study the other B-cell lymphoma, such as the (immature) B-cell lymphoblastic lymphoma, the marginal zone lymphoma, the mantle cell lymphoma, the Malt lymphom follicular lymphoma . These patients might be registered as observation patients in some participating study groups.

- Immunophenotyping

Determination of (i) aggressive nature (ii) mature phenotype and (iii) B cell lineage and thus entry into the study might be performed by paraffin section, immunohistochemistry, frozen section immunohistochemistry, and cytopsin immunocytochemistry cytology and FACS. The diagnosis has to be

confirmed by a national reference pathology center parallel or immediately after inclusion of a patient into the trial.

Mandatory antibodies to be stained for each B-cell lymphoma

- anti-B antibodies (CD20 and if negative other B-cell antibodies: CD19, Pax5, CD79a, surface light chain expression),
- CD3
- Ki-67
- TdT
- BCL2, BCL6, MUM-1, CD10

Additional mandatory antibodies to be stained for PLMBL:

CD30
CD23

Optional antibodies in cases of other aggressive large B-cell lymphomas (if not available locally immunohistochemical studies can be performed at the National Central Review Panel)

- CD30, CD79a, ALK-1, EBER in situ Hybridisation

FISH Analyses

In paediatric NHL, FISH analysis for diagnostic reasons is recommended for all cases but is mandatory only in doubtful cases and includes detection of breaks in the *MYC* gene and *MYC* partner.

F.1.4. MATERIAL TO SUBMIT FOR CENTRAL PATHOLOGY REVIEW

Paraffin blocks of tissue have to be submitted to the National Central Review Laboratory. If paraffin blocks cannot be submitted, then submit 20 unstained sections on silane-coated slides.

A copy of all pathology reports for each case should be added to each submission including the final pathology diagnosis report, immunophenotyping report and any results of genomic studies or cytogenetic analysis. If immunophenotyping studies are performed at the primary institution, a report of the results should be included along with the methodology specified. Paraffin blocks will be retained at the National Central Review Laboratory.

In pediatric NHL, FISH analysis for diagnostic reasons will only be performed in doubtful cases and includes detection of breaks in the *MYC* gene. If such analysis are performed in a primary pathology center, the pictures and reports (including the information of probes used) have to be submitted to the reference pathologist.

Paraffin blocks will be retained at the National Central Review Laboratory at least until the pathology review panel diagnosis is completed. For cases requiring urgent return of paraffin blocks or cytologic slides to the primary institution, the referring pathologist should contact the Central Review laboratory in order that the initiation of the review process can be expedited or blocks returned immediately after performance of immunophenotypic studies.

F1.5. DIAGNOSIS IN LIQUID MATERIAL

In cases with only liquid material is assessable for diagnosis (e.g. effusions), the diagnosis of a mature, aggressive B-cell lymphoma must be based on flow cytometry or cytology, immunohistochemistry on cytologic smears and cytogenetics following the four steps outlined below.

1) Proof of mature phenotype:

Lack of TdT, CD34 expression. Detection of surface light chain restriction

2) Proof of B-cell phenotype and CD20 expression:

Expression of CD20, CD19, Pax5, CD79a and surface light chain restriction are considered as proof of B-cell lineage in mature lymphomas. However, CD20 expression should be assessed in all cases.

3) Proof of aggressive nature:

Blastic cytomorphology and/or high Ki-67 expression will proof the aggressive nature.

4) Further subclassification

To further subclassify the mature, aggressive B-NHL matching the criteria outlined above, cytogenetic information and cytomorphology will have to be combined for a final diagnosis as outlined below:

- L3-morphology and karyotype or *MYC*-FISH or LD-PCR for t(8;14) suggestive of Burkitt lymphoma: Burkitt lymphoma
- L3-morphology and **NO** karyotype or *MYC*-FISH or LD-PCR for t(8;14) suggestive of Burkitt lymphoma: DLBCL
- **NO** L3-morphology and **NO** karyotype or FISH or LD-PCR for t(8;14) suggestive of Burkitt lymphoma: DLBCL
- **NO** L3-morphology and karyotype or FISH or LD-PCR for t(8;14) suggestive of Burkitt lymphoma: Mature aggressive B-NHL, unclassifiable

F.2. GENETICS GUIDELINES

(H Poirel, W Siebert, June 2009):

F.2.1. KARYOTYPE

Conventional cytogenetics is recommended on invaded liquid samples (bone marrow, blood, pleural/peritoneal effusion...) whenever possible, and, if available material for a cytogenetic laboratory, on fresh biopsies once enough fixed and frozen samples were taken off for pathology.

At least 2 representative karyotype of each clones should be reviewed by 2 different cytogeneticists from 2 different centres. The discordant cases will be centrally reviewed.

F.2.2. FISH

A panel of loci will be centrally characterized by FISH with the same probe sets:

- *MYC* (8q24) rearrangements : the different type of *Ig-MYC* translocations and if non *Ig-MYC*, *MYC* insertions;
- *REL* (2p12) and *JAK2* (9p24) with a control of the ploidy in all lymphomas with mediastinal localization;
- 7q and 13q alterations in all mature B-cell lymphomas : clinical research as a confirmation of the previous cytogenetics FAB/LMB96 study.

FISH will be performed by a panel of genetic reference laboratories in the different participating countries. 6 slides have to be sent to the reference panel from the solid biopsies (imprints or 4µ cryo-sections > 4µ FPET) or the liquids (bone marrow smears or fluid cytopins). The very same probe sets and evaluation criteria will be used in all reference laboratories. At least 10% of the slides of each reference laboratory will be sent to another genetic reference laboratory in the trial to control for inter-laboratory variation.

If some FISH experiments are locally performed (for diagnostic purpose by the pathology panel and/or for cytogenetic characterization), it should be done by the reference probe set(s) and the hybridized slide(s) have to be sent to one of the reference laboratory for central review.

F.2.3. CONTROL CELLS:

Normal cells should be taken off for germline DNA at diagnosis: PBL if not invaded or buccal swap (or cutaneous biopsy) ...

DNA extraction: centralized in reference laboratory.

F.2.4. LIST OF REFERENCE LABORATORIES

HA Poirel, Brussels (B / F)

A Bernheim, Villejuif (F)

WG Sanger, Omaha (USA)

CJ Harisson, Southampton (UK)
 L Mussolin, Padova (I)
 I Wlodarska (B)
 Berna Beverloo (NL)

F.2.5. REFERENCE FISH PROBES

MYC BA, IGH BA, IGK BA, IGL BA, MYC-IGH
 REL/CEP5
 JAK2/CEP10
 7q22/7q35 or 7q21/7q31
 13q31/13q34

F.3. PROPOSAL FOR BIOLOGY STUDIES AND TUMOUR TISSUE BANKING IN PMLBL (EICNHL ONLY)

(C Copie, June 2010)

If sufficient tissue is available, it should be stored at -70° for subsequent molecular and cytogenetic studies (according to national policy). The minimal biological project should be to precisely describe the characteristics of this PMLBL:

- morphology and immunohistochemistry characteristics (GC or non GC)
- expression of specific markers: MAL, CD23, IL411, STAT6
- cytogenetic characteristics: gain of 9p, rearrangements of *BCL2*, *BCL6* +/- *c-MYC*.
- FISH studies on paraffin sections are being developed to search these abnormalities.

If more tissue is available, especially frozen tissue, more advanced studies will be performed as optional research project, especially concerning JAK2 pathway.

F.4 PROPOSAL FOR STEM CELL BIOLOGY STUDY IN PHASE II AND III STUDY (EICNHL ONLY)

(S TURNER, A BURKE FEB 2013)

Evaluation of cancer stem cell profile and distribution in paediatric Non-Hodgkin Lymphoma

REQUIREMENT: Fresh biopsies in transport medium (protocol for tissue collection in Appendix 1).

Cancer stem cells (CSC) are responsible for tumour propagation and are defined by their self-renewal and differentiation capabilities. It is likely that the CSC should be the target of cancer therapy. We have shown that T-LBL contain a small percentage of CSC; these cells are identifiable by variable cell surface expression of CD7 and CD34 and by the presence of the transcription factor Oct4, an indicator of pluripotency (Turner et al., unpublished observations). We have also demonstrated the presence of CSC in Anaplastic Large Cell Lymphoma (ALCL) but in this case using the side population (SP) technique as opposed to cell surface immunophenotype (which to date has not distinguished CSC; Moti et al., Submitted). We will extend our findings to B-NHL employing existing techniques and the experience we have gained with the other paediatric lymphoma sub-types.

Firstly, we will analyse the tumour populations by flow cytometry for their cell surface immunophenotype employing a panel of markers typical to B-cells at all stages of development, including primitive and memory cells at either end of the spectrum. We will also assess the tumour populations for the presence of SP cells by flow cytometry following incubation with Hoechst dye. The identified cellular subsets will be further analysed following RNA extraction and expression array profiling and for their functional capacity to produce tumours following injection into immunodeficient NSG mice. qPCR will be performed to validate the presence of gene transcripts identified in the expression array study. If the tumour cell subsets propagate well *in vivo* and there is sufficient material, we will also assess their sensitivity to a range of chemotherapeutic agents including rituximab.

TUMOUR PROCESSING

Tumour samples should be sent fresh in travel medium or alternatively, processed samples (as detailed below) can be stored in liquid nitrogen and sent in batches on dry ice.

On receipt in the lab, sample details are checked and given a new patient number, if a previous sample has been received from the same patient we retain the original patient number.

All relevant patient details are recorded on our computer database.

Method – sample processing

1. Mash tissue through a 50µm cell strainer rinsing with Minimacs buffer to obtain a single cell suspension.
2. Layer the single cell suspension over a density gradient – usually lymphoprep.
3. Spin 15 mins at 2700rpm, brake off. Remove cell layer from the interface; approximately 2mls.
4. Set up Trypan Blue viability cell count.
5. Freeze cells viably in 92% Fetal Bovine Serum/8% DMSO in 1×10^7 cells/ml aliquots. Store in liquid nitrogen.

STORAGE

All samples will be stored in liquid nitrogen (viably in 92% Foetal Bovine Serum/8% DMSO in 1×10^7 cells/ml aliquots) at the Division of Molecular Histopathology, Lab Block Level 3, Box 231, Addenbrooke's Hospital, Cambridge.

Buffers*Travel Medium*

500ml RPMI 1640
5ml Glutamine (200mM stock)
20ml Pen/Strep
100ml FBS
7.5ml 10mg/ml Gentamicin
12.5ml 1M HEPES

Sample Delivery

Samples should be sent by overnight or same day courier to:

FAO: Dr Suzanne Turner
Division of Molecular Histopathology
Lab Block Level 3, **Box 231**
Addenbrooke's Hospital
Hills Road
Cambridge CB20QQ

ANNEXE G: MINIMAL DISSEMINATED DISEASE AND MINIMAL RESIDUAL DISEASE STUDY (PHASE III ONLY). (Specific recommendations for COG in a specific COG annexe)

To be organized by country

(A Rosolen, L Mussolin, Nov 2009)***

Background

Burkitt lymphoma (BL) and B-ALL are characterized by the presence of chromosomal translocations, including t(8;14)(q24;q32) and its variant forms. Mature B-cell malignancies are also characterized by specific immunoglobulin (Ig) gene rearrangements. Both the chromosomal translocations and the idiotypic Ig rearrangements represent tumor specific markers and, as such, can be used for the study of minimal disseminated disease.

Since the majority of BL and B-ALL are characterized by the presence of the chromosomal translocation t(8;14), which is detectable by LD-PCR, and more than 95% of all mature B-cell malignancies possess a clonotypic Ig-rearrangement, it is important that every single case be characterized by molecular analysis of tumor markers.

Rationale for the study

Recently it was demonstrated that minimal disseminated disease, determined by molecular methods, was positive in at least 30-35% of children with BL. In addition it was suggested that minimal disseminated disease is an independent prognostic marker among high risk BL patients and that persistence of minimal residual disease in the BM of B-ALL patients have a negative prognostic impact.

L. Mussolin et al. *Prospective Analysis of Minimal Bone Marrow Infiltration in Pediatric Burkitt's Lymphomas by Long-Distance Polymerase Chain Reaction for t(8;14)(q24;q32)*. Leukemia, 2003 March; 17(3): 585-9

L. Mussolin et al. *Prognostic role of minimal residual disease in mature B-cell acute lymphoblastic leukemia of childhood*. Journal of Clinical Oncology, 2007; Vol 25 (33): 5254-5261

L. Mussolin et al. *Minimal Disseminated Disease is a poor prognosis marker among high risk Burkitt's lymphoma patients*. Journal of Clinical Oncology, 2011; Vol 29 (13): 1779-1784

F. Lovisa et al. *IgH and IgK gene rearrangement as PCR targets for pediatric Burkitt's lymphoma and mature B-ALL MRD analysis*. Laboratory Investigation 2009; 89: 1182-1186

Specimens

Fresh tumor tissue (or neoplastic ascites or pleural effusions), along with bilateral bone marrow aspirates in Sodium Citrate (3-5 ml) and peripheral blood in Sodium Citrate (5 ml) should be sent to the reference Laboratory for molecular analysis **at diagnosis**.

In case of B-NHL with initial BM involvement and of B-ALL bone marrow and peripheral blood samples should also be sent **before the second cycle of chemotherapy COPADM**.

An additional BM and PB sample should be sent at the time of **CR evaluation** both for BL/B-cell lymphoma and B-ALL.

	Diagnosis	Before 2 nd COPADM cycle	At CR evaluation*
BL/B-cell lymphoma	X	-	X
B-ALL or BM involvement < 25%	X	X	X

* After the first CYM cycle in Group B and after the second CYVE cycle in Group C patients.

Peripheral Blood Stem Cell (PBSC) Harvest

The approach used to study MDD/MRD can be applied to detect possible lymphoma cell contamination in PBSC harvests in patients undergoing intensification treatment with autologous PBSC rescue, provided that a marker has been identified in the tumor cells at diagnosis.

Summary of Methods and Materials used for MRD assessment

LD-PCR assay

Diagnostic tissue is incubated overnight at 56°C with proteinase K solution; high molecular weight genomic DNA is prepared using the QIAamp Tissue kit (Qiagen, Hilden, Germany), following manufacturer's instructions. From each BM sample, nucleated cells are isolated by differential lysis and processed for DNA extraction.

To detect c-Myc/IgH rearrangements one primer for the c-Myc gene combined alternatively with one of four primers specific for the IgH locus (3 primers for the constant region and 1 for the joining region) are used. Quality of genomic DNA is assessed in each case by using a combination of primers of the Human tPA Control Primer set (Roche Diagnostics, Milan, Italy). Each reaction mixture (50µl) contains 250 ng of DNA, 60 pmol of each primer, and amounts of dNTPs, buffer III°, oligonucleotides and a mix of Taq and Pwo polymerases (Expand Long template PCR System, Roche Diagnostics), as suggested by the manufacturer. PCR is performed in a Thermal Cycler, using cycle characteristics specified in the detailed technical protocol. PCR products are analyzed by agarose gel electrophoresis and visualized under UV illumination after ethidium bromide staining.

Detailed technical protocol of the LD-PCR assay has been made available to the laboratories identified by each national group for the MRD studies.

IgH and IgK rearrangement assay

Seven to 10 µg of tumor DNA (lymphoid tissue or BM in case of NHL or B-ALL, respectively) and 4–5 µg of DNA of follow-up samples are required for reliable MRD analysis based on Ig rearrangements. A set of PCR reactions is performed on DNA from diagnostic tumor tissue or BM to identify complete and incomplete IGH rearrangements, IGKV deletional rearrangements and IGKV-J rearrangements. IGH rearrangements are identified using five VH and seven DH family primers, in combination with one JH consensus primer, as previously reported. If no clonal IGHV-D-J band is detected, the sample is subjected to amplification by using three multiplex primer sets, corresponding to the three IGHV FR regions, according to the BIOMED-2 guidelines. BIOMED-1 and BIOMED-2 primer sets are used for the amplification of IGK deletional and IGKV-J rearrangements, respectively. To define whether an amplified gene rearrangement is clonal, PCR products are subjected to a heteroduplex analysis. In case a single homoduplex band is identified, the PCR product is directly sequenced, whereas in cases of heteroduplex bands, the PCR product is cloned and at least 10 colonies are sequenced. Alternatively, the PCR products are excised from agarose gel, eluted and re-amplified before sequencing. Sequences of complete IGH and IGKV-J rearrangements are aligned to the IMGT database (<http://imgt.cines.fr/>), while IGH incomplete rearrangements and IGK deletional rearrangements are compared with the Nucleotide Blast directory (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and manually inspected.

At least one allele-specific oligonucleotide (ASO) primer was designed complementary to the junctional region of each potential MRD PCR target, either manually or using Primer Express software (Applied Biosystems, Foster City, CA). Each target was tested for specificity and sensitivity. RQ-PCR analysis was performed by using sequence-specific TaqMan hydrolysis probe. A standard curve was made by serially diluting the diagnostic DNA specimen in DNA obtained from mononuclear cells (MNCs) from a pool of four healthy donors (HDs). To determine the background of the RQ-PCR assay (ie, the amplification of comparable Ig gene rearrangements in normal cells), HD-MNC DNA samples were run in six-fold per experiment. The quantitative range and sensitivity of each MRD PCR target were determined according to the guidelines of the European Study Group on MRD detection in ALL (ESG-MRD-ALL). Detailed technical protocol of the IgH and IgK rearrangement assays has been made available to the laboratories identified by each national group for the MRD studies.

Non exhaustive list of reference Laboratories

- **Italy**
Lara Mussolin
Laboratorio Biologia Tumori Solidi
Clinica di Oncoematologia Pediatrica
Azienda Ospedaliera-Università di Padova

via Giustiniani 3, 35128 Padova, Italy
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- **UK**

Dr Anthony Bench
Inter B-NHL ritux 2010 MDD/MRD study
Haemato-Oncology Diagnostic Service
Department of Haematology
Box 234
Addenbrooke's Hospital
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Phone: 01223 586926
Fax : 01223 217017
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- **SFCE**

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Département de Génétique, UF de Biochimie et Génétique Moléculaire
Hôpital Robert Debré
48 Bd Sérurier
75019 PARIS
France
Tel : 33 (0)1-40-03-57-11 (Secretary) –
Fax : 33 (0)1-40-03-22-77
Email : emmanuelle.clappier@rdb.aphp.fr

- **COG**

Bruce Shiramizu
University of Hawaii
John A. Burns School of Medicine
Specimen Processing Laboratory
3675 Kilauea Ave., Young Bldg., Basement
Honolulu, Hawaii, USA 96816
Attn: Dr. Melissa Aagsalda/Kawakahi Amina
Phone : 808-285-6794/808-554-5044/808-386-3312
Fax: 808-441-1597
e-mail: magsalda@hawaii.edu/kawakahi@gmail.com

ANNEXE H: TOXICITY CRITERIA (CTCAE)

Use the CTC NCI which is available on the web site below :



<http://ctep.cancer.gov/>

**Common Terminology Criteria for Adverse Events v4.0 (CTCAE)
(Publish Date June 2010)**

ANNEXE I: PHARMACOKINETICS OF RITUXIMAB (EICNHL ONLY)

I.1 STUDY DESIGN

Pharmacokinetics (PK) analysis will be performed in some European centers on a subset of at least 34 patients randomized to the treatment rituximab arm from selected sites. The subset must include at least 15 patients in each of two age ranges; 3 to 11 years and 12 to 18 years of age and at least 4 in the age range 6 months to < 3 years. Centers who will participate to this PK study will receive specific instructions for blood specimen collection, processing and shipping. Specific tubes to collect blood samples will be provided by Roche.

In the 3 to 11 and 12 to 18 age range samples are to be taken to determine C_{max} and C_{min} on treatment i.e. pre-dose and post-dose samples on the days of dosing and at least four samples after the last dose to determine a terminal half-life (see Table 1). This assumes two doses are given in Cycle 1 and Cycle 2, within 48 hours of each other, and two additional doses in Cycles 3 and 4.

For the 6 month to < 3 year age range 4 samples should be taken at pre-dose and post-dose on the day of dosing in Cycles 3 and 4 (see Table 2).

I.2 PK SAMPLING

On dosing days, post-dose samples must not be taken from the same arm (appendage) as that used for the infusion of rituximab. On the days of dosing pre-dose samples should be taken within 2 hours of the start of the infusion and 15 min (\pm 15 minutes) after the end of infusion.

In case of double lumen central line use for chemotherapy infusion, PK post-infusion sampling has to be between 30 min (\pm 15 minutes) after the end of infusion.

On all other days samples can be taken any time during that visit day. As a minimum, approximately 0.5 mL of blood should be taken for analysis of rituximab level at each time point. All infusion times and dates and times of PK blood draws must be recorded accurately on the CRF.

I.3 PK SAMPLE ANALYSIS

Serum concentrations will be measured by specific and validated method.

I.4 PHARMACOKINETIC ANALYSIS

Analysis will be performed on all patients using nonlinear mixed effect modelling (NONMEM) to produce parameter estimates of AUC, clearance (CL), volume of distribution (V) and terminal half life ($t_{1/2}$). Descriptive statistics will be generated on these data not limited to but including mean, min, max, standard deviation, standard error, median, geometric mean and CV% and including those for the observed data on the pre-dose (C_{max}) and post-dose (C_{min}) values. In addition, these analyses will be compared with historical PK data obtained from adult NHL patients.

Table 1 Schedule of PK Sampling for subset of patients from Phase III study given 6 doses of rituximab in the 3 to 11 year old and the 12 to 18 year old groups

Cycle	Day of Cycle	Time-point
1	-2	Pre-dose
	-2	Post-dose
	1	Pre-dose
	1	Post-dose
2	-2	Pre-dose
	-2	Post-dose
	1	Pre-dose
	1	Post-dose
3	1	Pre-dose

4	1	Pre-dose
	1	Post-dose
	6	Post dose
	29 (1 mo)	Post-dose
	57 (2 mo)	Post-dose
	85 (3 mo)	Post-dose

Table 2 Schedule of PK Sampling for subset of patients from Phase III study given 6 doses of rituximab in the 6 month to < 3 year old group.

Cycle	Day of Cycle	Time-point
3	1	Pre-dose
	1	Post-dose
4	1	Pre-dose
	1	Post-dose