BRISMA
Bendamustine and **R**ituximab for the treatment of **S**plenic **Ma**rginal Zone Lymphoma
The IELSG-36 phase II prospective study

EudraCT Number 2011-000880-28

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SYNOPSIS

Name	BRISMA
Protocol Title	**B**endamustine and **Ri**tuximab for the treatment of **S**plenic **Ma**rginal Zone Lymphoma. The **IELSG-36** phase II prospective study.
Protocol Version	VERSION 5.1 – 22 NOVEMBER, 2017
Investigational product	Bendamustine and Rituximab
Sponsor	IELSG
Study Phase	II
Study Design	Multicenter open label two stage phase II study
Principal Investigators	France: Catherine Thieblemont, Remi Gressin Italy: Alessandro Maggi, Emilio Iannito, Andres JM Ferreri, Massimo Federico
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Study Population	Previously untreated (except for splenectomy and/or antiviral therapy for HCV infection) and symptomatic Splenic Marginal Zone patients.
Objectives	**Evaluation of the efficacy and the safety of R-Bendamustine in symptomatic Splenic Marginal Zone Lymphoma patients.**
Primary Objective	**Efficacy of R-Bendamustine measured by Complete Response rate** Complete response rate defined as regression to normal size on CT of organomegaly (spleen, liver, lymph nodes); normalization of the blood counts and no evidence of circulating clonal cells, and no evidence or minor (≤ 5%) BM infiltration detected by immunohistochemistry. ** Analysis of minimal residual disease by Flow cytometry and by quantitative Taq Man PCR (RQ-PCR) targeting clonal IgVH antiCDR3 to evaluate response status and a potential impact on progression free survival and long-term remission of patients will be incorporated as research evaluation but will be not influence nor the response evaluation nor influence the management of the patient.*
Secondary Objectives	**Efficacy measured by** • **Overall Response Rate (ORR)** (Complete response + Partial response) • **Safety and tolerability measured by toxicities of R-Bendamustine** evaluated

by assessment of laboratory parameters and adverse events coded with NCI Common Toxicity Criteria, version 4.0 (Appendix F)

- **3-year Progression Free Survival (PFS)**, defined as the time from entry into the study until reappearance of cytopenia associated with disease progression (autoimmune, hypersplenism) or lymphoma relapse/progression with enlarged lymph node(s) or spleen if present, histologic transformation or death as a result of any cause
- **Duration Of Response (DOR)**, is defined for all patients who achieved a response (CR and PR) and is measured from the time of response until the date of first documentation of progression or relapse.
- **3-year Event Free Survival (EFS)**, measured from the time from study entry until appearance of any of the following event: any treatment failure including disease progression/relapse, or initiation of new anti-lymphoma therapy or death
- **Time to Next Treatment (TTNT)**, defined as the time from the end of the chemo-immunotherapy course to the day of next treatment commencement irrespective of cause
- **3-year Overall Survival (OS)**, defined as the time from date of treatment start into the study until the date of death irrespective of cause. Patients who have not died at the time of end of the whole study, and patients who are lost to follow up, will be censored at the date of the last contact
- **Risk of histological transformation**
- **5 year-PFS and - OS**

Study design

Multicentric Phase II study

Treatment

Immuno-Chemotherapy

All patients will be treated with 4-6 cycles of R-Bendamustine every 4 weeks, depending on the response after 3 cycles

Standard Doses:

R- Bendamustine		Dose (mg/m²)	Days
BENDAMUSTINE	I.V.	90	1 and 2*
RITUXIMAB	I.V.	375	1§

* Or days 2-3 according to institutional/patient/physician preference

§ Administration of Rituximab during cycle 1 and 2 can be postponed to day 8 or 14 in case of risk of tumor lysis syndrome e.g. marked splenomegaly or lymphocytosis above 10.000 lymphocytes/mm^3

The use of G-CSF for prophylaxis is recommended according to EORTC guidelines (appendix O); either filgrastim, lenograstim or peg-filgrastim can be used. It is recommended to prescribe anti-infectious agents for all patients with valaciclovir 500 mg/d and trimetroprim/cotrimoxazole 400mg/d during all the chemotherapy.

A centralized PET scan evaluation will be performed; the anonymized PET scan images will be collected and preserved for the entire duration of the study at the LYSARC (Resp. Romain Ricci, Coordinator Imaging LYSARC, CHU Henri Mondor Créteil, Service de Médecine Nucléaire). The images will be evaluated by a group of expert nuclear medicine (Dr. Salim Kanoun, Service de Medecine Nucleaire IUCT-ONCOPoLE, Institut C. Regaud, Toulouse, France and Dr. Michel Meignan, LYSA Imaging, Hopitaux Universitaires Henri Mondor, Créteil, France)

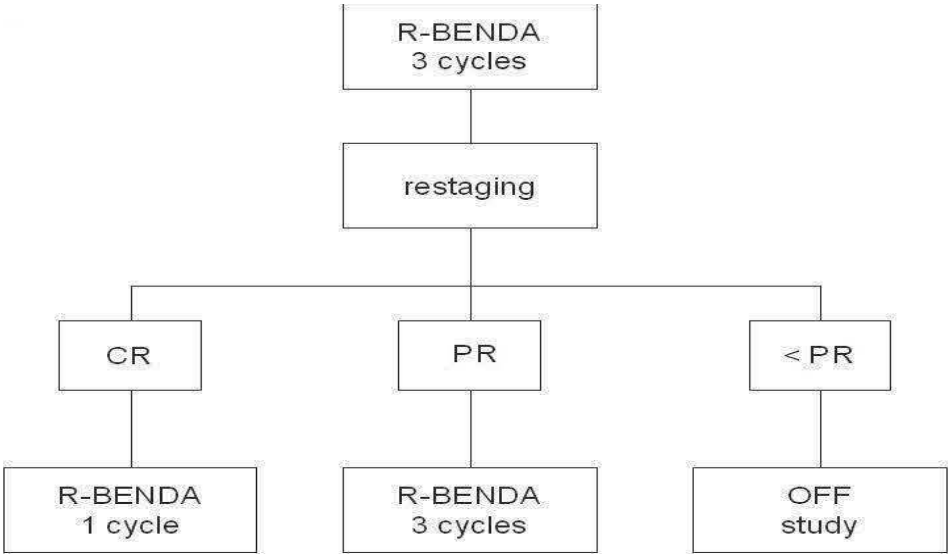
to analyze the clinical response of the patients.

Response Criteria (according to Matutes et al, Leukemia 2008)

- **Complete Response (CR)** all the following criteria must be fulfilled:
  1. Resolution of organomegaly
  2. Normalization of the blood counts (Hb >12 g/dL; Plt >100.000/mm^3; Absolute Neutrophils Count (ANC) > 1500/mm^3; no evidence of circulating clonal B-cells-
  3. No evidence of BM infiltration
- **Partial Response (PR)**

  ≥ 50% improvement in the disease manifestations that should include:
  1. Resolution or decrease in spleen size.
  2. Improvement on cytopenias.
  3. Resolution or decrease in lymphoadenopathy if present.
  4. BM should show a decrease in the level of lymphoid infiltration and improvement of the haematopoietic reserve.
- **No response (NR)** and **progressive disease (PD)**
  1. Less than 10% improvement on the disease manifestations or deterioration by increase >50% of measurable signs of the disease from nadir, respectively.

Study flow-chart



Inclusion criteria

- **Initial diagnosis of CD20+ Splenic Marginal Zone Lymphoma morphology confirmed by histology, cytology, immunophenotype (chromosomal abnormalities by quantitative multiplex PCR of short fluorescent fragments (QMPSF) is optional) according to WHO 2008 classification of Lymphoma criteria or according to the recommendation of the Splenic Lymphoma Group (Matutes et al. Leukemia 2008) for non splenectomized patient.**
  1. If patients **not splenectomised**: diagnosis on bone marrow biopsy (histology and immunohystochemistry), and blood (cytology, immunophenotype), chromosomal abnormalities by QMPSF optional.
  2. If patients **splenectomised** diagnosis on spleen, bone marrow biopsy (histology and immunohistochemistry), and blood (cytology,

immunophenotype) chromosomal abnormalities by QMPSF optional.

- **No previous treatment** with immunotherapy or chemotherapy or radiotherapy unless pretreatment by monocorticotherapy.

- **Patients requiring a treatment with at least one of the following situation:**
  1) **Symptomatic SMZL in not splenectomized patients**
     a) Bulky (arbitrarily defined as ≥6 cm below left costal margin) or progressive or painful splenomegaly, without enlarged lymphoadenopathy, with or without cytopenia, not eligible for splenectomy or not willing splenectomy
     b) One of the following symptomatic/progressive cytopenias: Hb <10 g/dL, or Plat <80.000/mm^3, or ANC <1.000/mm^3, whatever the reason (autoimmune or hypersplenism or bone marrow infiltration) not eligible for splenectomy or not willing splenectomy
     c) SMZL with enlarged lymphoadenopathy or involvement of extranodal sites with or without cytopenia
  2) **Symptomatic disease in SMZL splenectomised patients with rapidly raising lymphocyte counts, development of lymphadenopaty or involvement of extranodal sites.**
  3) **SMZL with concomitant hepatitis C infection who have not responded or are relapsed after Interferon and/or Ribavirin.**
- Clinically and/or radiologically confirmed measurable disease before treatment start.
- Aged ≥ 18 yo at time of initial diagnosis and ≤ 80 yo.
- Eastern Cooperative Oncology Group [ECOG] performance status 0-2 (Appendix C).
- Minimum life expectancy of >6 months.
- Voluntary signed informed consent before performance of any study related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.
- The following laboratory values at screening:
  1. Absolute neutrophil count (ANC) ≥1.000/mm^3 and Platelets ≥100.000/mm^3, unless these abnormalities are related to bone marrow infiltration or to hypersplenism.
  2. Aspartate transaminase (AST) ≤2 x ULN; Alanine transaminase (ALT) ≤2 x ULN; total bilirubin ≤1.5 x ULN.
  3. Creatinine clearance ≥ 10 ml/min (as calculated by the Cockcroft-Gault formula - Appendix I).
- **All patients must:**
  1. Agree to abstain from donating blood while taking study drug therapy and following discontinuation of study drug therapy.
  2. Agree not to share study medication with another person.
  3. Agree to use an adequate method of contraception for women of childbearing potential during the study treatment and until 12 months after the end of the study treatment.
  4. Agree to use an adequate method of contraception for men during the study treatment and until 6 months after the end of the study treatment

Exclusion criteria

- Any type of lymphoma other than SMZL.
- Patients with proven biopsy of histological transformation.
- Contraindication to any drug contained in the chemotherapy regimen.
- Myocardial infarction during last 3 months or unstable coronary disease or uncontrolled chronic symptomatic congestive heart insufficiency NYHA III – IV (Appendix H).
- Uncontrolled hypertension.
- Uncontrolled diabetes mellitus as defined by the investigator.
- Active systemic infection requiring treatment.
- Previously known HIV positive serology.
- Active hepatitis B virus infection (presence of antigen HBS+; in case of presence of antibody anti HBC+ and anti HBS+, controls should be organized according to guidelines of AASLD and l'EASL).
- Active and previously untreated HCV infection.
- Prior history of malignancies other than lymphoma within 3 years (except for complete resection of basal cell carcinoma, squamous cell carcinoma of the skin, or in situ malignancy). Patients previously diagnosed with prostate cancer are eligible if (1) their disease was T1-T2a, N0, M0, with a Gleason score $\leq$7, and a prostate specific antigen (PSA) $\leq$10 ng/mL prior to initial therapy, (2) they had definitive curative therapy (ie, prostatectomy or radiotherapy) $\geq$2 years before Day 1 of Cycle 1, and (3) at a minimum 2 years following therapy they had no clinical evidence of prostate cancer, and their PSA was undetectable if they underwent prostatectomy or <1 ng/mL if they did not undergo prostatectomy.
- Major surgery within 30 days before the inclusion in the study
- A positive Coombs test without haemolysis or an autoimmune hemolytic anemia is not an exclusion criterion.
- Impaired renal function with creatinine clearance <10 ml/min.
- Severe chronic obstructive pulmonary disease with hypoxemia.
- Medical condition requiring long-term use (>1 months) of systemic corticosteroids.
- Serious medical or psychiatric illness likely to interfere with participation in this clinical study.
- Prior participation in another study with experimental drug during the last 4 months.
- Pregnant or currently breast-feeding woman.

Procedures

At baseline

- **General baseline assessment:** Demographic (date of birth, gender, height, weight, body surface area). Relevant clinical history (general and disease-specific, including concurrent illnesses and therapies at the time of study entry). All patients will undergo a thorough scrutiny for the presence of comorbidity.
- **Complete physical examination:** (including peripheral lymphnodes, Waldeyer ring, size of liver and spleen measured as cm below costal margin). EGOG performance status. Laboratory: Complete blood count.
- **Complete biochemistry:** Complete blood count (haemoglobin, RBC, WBC

and differential), Coagulation assessment (PTT, PT, ATIII, XDP, fibrinogen) AST, ALT, serum albumin, serum alkaline phosphatase, GGT, total bilirubin, creatinine, creatinine clearance, Na, K, Ca, LDH, total serum protein with serum protein electrophoresis, Beta2 microglobulin. Serology for HIV, B and C Hepatitis. Coomb's test, Serum IgG, IgM, IgA (urine and serum Immunofixation if a monoclonal component is suspected), serum glucose, Mantoux screening test or QuantiFERON.

- **Tumor assessment:** Chest and abdomen CT-scan with iodine contrast (CT scan of head, neck, pelvis if clinically indicated); PET scan is highly recommended, to be performed according to local policies and to discretion of the physician in charge. Bone marrow trephine biopsy, bone marrow and peripheral blood smears to be centralized for central review (bone marrow if no blood infiltration). Results of flow cytometry on bone marrow and peripheral blood also to be centralized for central review. Biopsy of suspicious extranodal sites to be centralized for central review. If clinically indicated, abdomen ultrasounds with spleen measurement.
- **Any other exam if clinically indicated.** Cardiac function: 12 Lead ECG (only in case of abnormal ECG or history of cardiac disorder, LVEF by Echocardiography or Isotopic method if applicable to be performed).
- **Scoring through application of CIRS scale (Appendix F) for history and concomitant diseases.**

Are optional:

- ESR, Fe, BUN/urea, uric acid, transferrin, serum ferritin, urine analysis.
- Investigation for Minimal Residual Disease by Flow cytometry and by quantitative Taq Man PCR (RQ-PCR) targeting clonal IgVH, chromosomal abnormalities by QMPSF is centralized. Peripheral blood samples for molecular, genetic and cytogenetic investigations is also centralized (bone marrow if no blood infiltration)

During the study, please refer to the study procedure table.

Expected number of patients

N = 65

Statistical consideration

For the purposes of the study an efficacy and a safety population are defined. All patients receiving at least one dose of study medication will be considered for the safety population (SP). Only patients who receive at least two courses of RB combination and undergo procedures for response assessment will be considered for the efficacy population (EP). Sample size is calculated on the EP.

SAMPLE SIZE CALCULATION

We anticipated a CR rate of 80% and computed that, a sample size of 53 valuable patients would provide 90% power at the overall 5% (2-sided) significance level to detect a CR rate >60% (null hypothesis, 60%; alternative hypothesis, 80%) Assuming a drop-out of 10% and that about 10% of cases entered into the study could be refused after the final histologic revision a total of 65 patients will be included

ANALYSIS PLAN

INTERIM ANALYSIS at 19 valuable patients
FINAL ANALYSIS at 53 valuable patients

Estimated study duration

- Duration of recruitment is estimated at 2 years

- Duration of follow-up phase: 5 years after last patient treated
- Estimation of total duration: 7 years

Planned start of recruitment Second semester 2012

1 INTRODUCTION AND STUDY RATIONALE

1.1 Disease Background: Splenic Marginal Zone Lymphoma

Splenic Marginal Zone Lymphoma (SMZL) is a well defined low-grade B-cell lymphoma, ranking as a distinct entity in the WHO (World Health Organization) classification[1]. Although it is considered as a rare neoplasm accounting for about 2% of all non Hodgkin's lymphomas (NHL) it is estimated represents for most cases of otherwise unclassifiable chronic lymphoid B-cell CD5-lymphoprolipherative disorders[2]. The peak of incidence is in the sixth decade with an almost equal female/male ratio. SMZL is characterized by an almost exclusive involvement of the spleen and bone marrow[3-5]. Peripheral blood involvement is usually scanty and in about half of cases the circulating neoplastic lymphocytes display a characteristic villous appearance. Signs and symptoms are mostly related to the development of peripheral cytopenias and or to the abdominal discomfort associated with the presence of a huge splenomegaly[5-11]. The definite diagnosis rely on spleen histology which is considered the gold standard diagnostic assay[1,12]. Recently, to avoid a major surgical procedure for mere diagnostic purposes in otherwise healthy subjects, the Splenic Lymphoma Group proposed guidelines for the diagnosis of SMZL based on the merging of bone marrow and peripheral blood picture with immunophenotypic and cytogenetic data[13,14] (Appendix D). SMZL affects the elderly with a median age of 70 years, the median survival exceeds ten years and most patients can be effectively managed for many years with a watchful waiting policy. Yet in about 25% of cases the disease pursues an aggressive course and most patients die of lymphoma progression within 3-4 years. Some presenting features on diagnosis have been shown to have a significant prognostic value (extranodal involvement; high lymphocyte count; anemia; lymphonode involvement; thrombocytopenia) and when they develop during the course of the disease are harbinger of disease progression[6-8,11]. The Intergruppo Italiano Linfomi (IIL) have proposed a clinical score based on three parameters[15]: anemia, elevated LDH values and hypoalbuminemia (Appendix G). Patients showing two or more of the above adverse prognostic factors have a median life expectancy of less than five years. For the time being, there is not a prospectively validated therapy for SMZL and splenectomy has been deemed to be the therapeutic approach most effective in patients complaining signs and symptoms secondary to hyperpslenism. However, though almost all patients achieve a clinical response after splenectomy they eventually relapse or progress. Moreover, most of SMZL patients are elderly and show comorbities that classify them as poor surgical risk[16-18]. For these patient or those who show a spread of the disease to lymphonodes or other extranodal sites or others adverse prognostic factors, a systemic treatment may be appropriate. Retrospective studies have indicated that alkylating-agents monotherapy do not produce any clinical benefit while purine analogs (i.e. fludarabine, cladribine, and pentostatine) achieved very high response rates in both naïve and pre-treated patients[19-21]. Moreover, the introduction of the anti-CD20 humanized antibody rituximab, either used alone or in combination with chemotherapy has been reported to be very effective in producing a rapid clearance of neoplastic cells. Treatment of such patients with rituximab both alone or in combination with chemotherapy has shown remarkable responses[22-30].

In 2005 the IIL started a prospective phase II trial to verify the efficacy of rituximab in combination with COMP (Cyclophosphamide, Oncovin, Myocet, Prednisone) chemotherapy, as front-line therapy for SMZL patients, in terms of response, survival, and safety. To be included in the trial, patients should had a diagnosis of SMZL supported by spleen histology or by a combination of bone marrow and peripheral blood morphologic and immunophenotipic data; patients should also be untreated for lymphoma and have active disease defined by the presence of at least one of the following: Hb <10g/dl; plt

<100.000/mmc, symptomatic splenomegaly, elevated LDH, B symptoms, extrasplenic disease, LDT <12 months. The preliminary results of this study have been presented at 2007 ASH meeting[31]. The most important findings were an ORR of 100% with 63% of CR. The results have been recently update, confirming on 63 patients an 85% ORR with 63% CR rate, and a 82% PFS at three years. However, the toxicity profile showed a 24% haematological toxicity WHO 3-4 and one patients died of therapy-related cardiac failure while in CR.

1.2 Drug background

1.2.1 Bendamustine

Bendamustine was synthesized in 1963 in what was then the German Democratic Republic[32]. There, it was widely used, but not widely studied. When the Iron Curtain fell and the Federal Republic of Germany was reunited, investigators began to study bendamustine's mechanism of action and its clinical activity. Although the early studies were plagued with problems, clear evidence of activity against low-grade lymphoproliferative disorders previously treated with other alkylating agents began to emerge, largely in the absence of significant toxicity[33-38]. More recently, several large, well designed and well-conducted trials[39-41] have provided intriguing data that not only have allowed investigators outside of Germany to consider the potential role of bendamustine in the treatment of lymphoproliferative disorders in the future, but have also led the Food and Drug Adminstration (FDA) of the United States to approve bendamustine for the treatment of CLL and indolent B-cell non-Hodgkin lymphoma (NHL), thus providing clinicians a "new" treatment option for their patients right now[42].

Bendamustine Clinical Pharmacology

Mechanism of Action

The bendamustine molecule is composed of 3 structural elements: a mechlorethamine (nitrogen mustard) group, a benzimidazole ring, and a butyric acid side chain[42]. The mechlorethamine (nitrogen mustard) group is similar to other alkylators like cyclophosphamide and chlorambucil. The benzimidazole ring, which replaces the benzene ring present in chlorambucil, is unique and is similar in structure to some purine analogs such as 2-chlorodeoxyadenosine. This observation has led some to hypothesize that bendamustine may have purine analog activity as well, but no evidence for antimetabolite functionality has been confirmed. Although an alkylator itself, bendamustine displays distinct preclinical activity. DNA breaks induced by bendamustine are significantly greater in number than those produced by cyclophosphamide or carmustine and are more durable than those associated with melphalan, cyclophosphamide, or carmustine[34,43]. This extensive DNA damage leads to inefficient DNA repair mechanisms, resulting in inhibition of checkpoint control. The resultant cell cycle changes culminate in mitotic catastrophe, another pathway leading to cell death[44]. Furthermore, bendamustine activates a base-excision DNA-repair pathway, rather than an alkyltransferase DNA repair mechanism like other alkylating agents. This observation suggests that bendamustine may be less susceptible to alkylguanyl transferase expression–based drug resistance[45].

Bendamustine Pharmacokinetic Parameters And Drug Metabolism

Absorption

Not applicable, since bendamustine is administered by intravenous route.

Distribution

Bendamustine undergoes first-pass metabolism, primarily in the liver by the action of cytocrome p450 enzyme complex. Bendamustine is highly protein bound (> 95%), primarily to albumin. Protein binding is not affected by advanced age, low serum albumin levels or presence of advanced tumor. However,

only free, unbound bendamustine is active. After intravenous administration of bendamustine volume of distribution at steady state is 19,8 L.

Metabolism and Excretion
Elimination of bendamustine is rapid and occurs predominantly by the renal route, with peak metabolite concentrations found in the urine one hour after administration; only a small amount of the drug is eliminated by the liver. Mean total clearance was reported to be 49.6 L/h and to be independent of dosage over the range 0.5 to 5 mg/kg. Elimination is biphasic with a half-life (t1/2α) of 6 to 10 minutes, and a termination half-life (t1/2β) of approximately 30 minutes. Bendamustine is eliminated primary by renal route and therefore should not be given to patients in whom renal function is compromised (glomerular filtration rate <10ml/min). Bendamustine is also contraindicated in patients with severe hepatic damage, since primary metabolism occurs in the liver. There are at present no published data on placental transfer or excretion in breast milk of bendamustine.

Bendamustine Preclinical And Clinical Data

Preclinical studies demonstrate bendamustine's activity in cancer cells that are resistant to other alkylating agents. Alkylator-resistant human B-CLL cells are less resistant to bendamustine[46] and there is clinical evidence that bendamustine retains activity when other alkylators fail[47,48]. The overall remission rate was 56%, with a median duration of remission of 42.7 months. Several studies of bendamustine-based therapy in patients with relapsed and refractory low-grade lymphomas report overall remission rates from 48% to 97%[34,42-45]. Thus, both preclinical and clinical data suggest that bendamustine may have properties distinct from other alkylating agents. These observations informed the design of larger studies that extend these preliminary observations.

Several experiences have been published related to the use of bendamustine as a single or combined agent in low-grade lymphomas. As a single agent, bendamustine was administered to 52 evaluable patients with relapsed or refractory low-grade lymphomas at a dose of 120 mg/m2 on the first 2 days of a 21-day cycle[49]. The median age of the patients was 63 years (range, 36-82 years), and all patients had been previously exposed to alkylating agents. The overall response rate (ORR) was 73%, and 11% achieved complete remission (CR). The regimen appeared to be remarkably well tolerated, and only 3 patients developed grade 3 or 4 toxicity, both grade 3 neutropenia. This study and similar studies conducted in Europe suggest activity of bendamustine against low grade lymphoma in the absence of debilitating or unmanageable toxicity[39]. To help confirm these preliminary observations, a North American study was organized to test the safety and efficacy of bendamustine in rituximab refractory patients with indolent and transformed non-Hodgkin lymphoma. Patients enrolled on this study were defined as rituximab-refractory if they failed to respond or progressed within 6 months of previous treatment with rituximab. This multicenter trial enrolled 77 patients, and 76 patients were treated with bendamustine 120 mg/m2 on the first 2 consecutive days of a 21-day cycle. Nineteen patients required dose reductions. The incidence of grade 3-4 neutropenia was 54% and of grade 3-4 thrombocytopenia was 25%. Non-hematologic toxicities were common, but generally mild with grade 1-2 nausea in 68% and grade 1-2 fatigue in 42%. No patient developed alopecia or mucositis. Although these patients were frequently relapsed from combination chemotherapy regimens, and 20% had transformed disease, the ORR was 77%, and 34% achieved CR.41 Interestingly, the ORR in patients with alkylator-resistant disease was 61%, and it was 62% in the 8 patients with fludarabine-refractory disease. For all patients, the median progression-free survival (PFS) was 7.1 months. The results of the study performed by Friedberg et al confirm earlier findings and clearly establish bendamustine as a safe and effective treatment for relapsed and refractory low-grade lymphomas. Although remission rates were high, however, the duration of remission was relatively short. Thus, bendamustine has been combined with other active agents to determine whether results could be improved. Although its efficacy and safety

profile in the treatment of low-grade lymphoma would have been enough to consider rituximab in combination with bendamustine, preclinical studies also suggest a degree of synergism with the combination against lymphoma cell lines and in severe combined immunodeficient mice with Daudi xenografts[50]. Therefore, rituximab has been combined with bendamustine (BR) for the treatment of low-grade lymphoma in some recent clinical trials. A study from Germany accrued 63 patients with relapsed or refractory low-grade non-Hodgkin lymphoma for treatment with BR. Rituximab 375 mg/m^2 was administered on Day 1, and bendamustine was administered at a dose of 90 mg/m^2 on Days 2 and 3 of a 28-day treatment cycle[37]. Rituximab was also infused 1 week before the first treatment cycle and 4 weeks after the fourth and final treatment cycle. The median age of the patients was 64 years (range, 40 to 81 years). The beta-2 microglobulin level was >2 mg/dL in 48%, and the lactate dehydrogenase level was >240 U/L in 24% of assessable patients. Toxicity was graded according to World Health Organization (WHO) guidelines and was largely limited to myelosuppression (16% grade 3-4 leukopenia) and nausea (102 of 136 treatment cycles complicated by grade 1 nausea). Hematopoietic growth factors were allowed on the study, but none was used. The ORR rate to BR on this study was 90%, and 60% achieved a CR. Patients with relapsed mantle cell lymphoma had an ORR of 75%, and 50% achieved CR. These remissions were also reasonably durable, with a median progression-free survival of 24 months. The median PFS in patients with mantle cell lymphoma was 18 months. The remarkable results of the German study of BR prompted a confirmatory study in North America. The treatment regimen was identical to the German study, but toxicity was graded according to the National Cancer Institute's Common Terminology Criteria (NCI-CTC). Sixty-seven patients with relapsed or refractory low-grade non-Hodgkin lymphoma were enrolled in this trial, and 66 patients were treated with BR[41]. This population of patients had a median age of 60 years (range, 40-84 years) and was similar to the German cohort. The efficacy results of the North American Trial were also very similar to those of the German trial. Both studies demonstrate excellent activity against low-grade lymphomas across multiple histologic subtypes. The 2 studies differed in their assessment and reporting of toxicity, leading to apparent discrepancies in their respective toxicity results. In the German study, toxicity was reported as a function of events per treatment cycle, whereas in the North American study toxicity was reported as a function of study participants. Grade 3-4 leukopenia was reported in 16% of treatment cycles in the former study, and in 30% of study participants in the latter study. The discrepancy is more than just in the way toxicity was reported. Therefore, the toxicity in the 2 trials is not as easily compared as the response rates are. Nonetheless, severe adverse events were uncommon in both studies.

Considering this treatment option bendamustine is neither a new drug, nor a biologically targeted agent. Yet it has unique activity against lymphoproliferative disorders, and its favorable side-effect profile makes it amenable for use in combination with other agents.

1.2.2 Rituximab

Rituximab is a mouse/human chimaeric IgG1-k monoclonal antibody that targets the CD20 antigen found on the surface of malignant and normal B lymphocytes.[18]
Although not fully elucidated, the cytotoxic effects of rituximab on CD20-positive malignant B cells appears to involve complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity and induction of apoptosis.[19-20]
Rituximab has demonstrated efficacy in patients with various lymphoid malignancies, including indolent and aggressive forms of B-cell NHL and B-cell chronic lymphocytic leukaemia (CLL).[21-27] However few are the data on the use of rituximab alone or in combination in patients with Splenic Marginal Zone Lymphoma. Since approval of rituximab, treatment of such patients with rituximab both alone or in combination with chemotherapy has shown remarkable responses. In literature six retrospective studies adding up 52 patients are reported. The overall response rate both in refractory/relapsed and

treatment naive cases was rather high ranging from 88% to 100%. Worth noting the prompt and marked shrinkage of splenomegaly and the amelioration of the peripheral cytopenias, mostly registered within the third course of therapy. Treatment with rituximab is generally well tolerated, particularly in terms of adverse haematological effects and serious or opportunistic infections relative to standard chemotherapy.

1.3 Rationale

1.3.1 Rationale for combining drugs

Preclinical and recent clinical studies suggest a degree of synergism using the combination bendamustine and rituximab in the treatment of low-grade lymphoma demonstrating remarkable results.

1.3.2 Rationale for performing the study

To investigate the role of bendamustine in combination with rituximab in a peculiar subset of low-grade lymphoma.

2 OBJECTIVES

2.1 Primary Objective

To evaluate the complete response rate obtained with bendamustine in combination with rituximab in previously untreated splenic marginal zone lymphoma (SMZL).

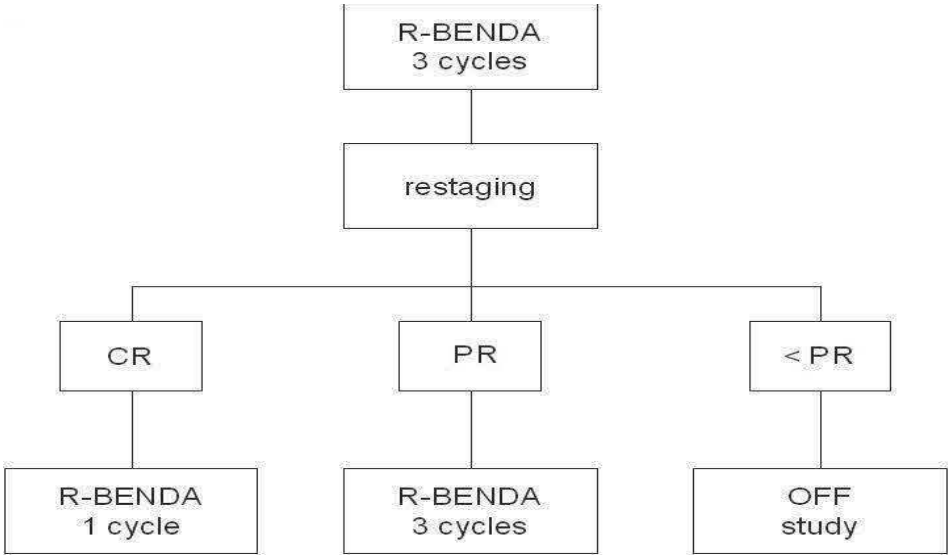
2.2 Secondary Objectives

- To evaluate **Overall Response Rate (ORR)** (Complete response + Partial response)
- To evaluate **safety and tolerability** measured by toxicities of R-Bendamustine (by assessment of laboratory parameters and adverse events coded with NCI Common Toxicity Criteria, version 4.0)
- To evaluate **3-year Progression Free Survival (PFS)**
- To evaluate **Duration Of Response (DOR)**
- To evaluate **3-year Event Free Survival (EFS)**
- To evaluate **Time To Next Treatment (TTNT)**
- To evaluate **3-year Overall Survival (OS)**
- To evaluate **risk of histological transformation**
- To evaluate **5 year-PFS and - OS**

3 INVESTIGATIONAL PLAN

3.1 Overall study design and plan of the study

This is a prospective, multicenter phase II trial designed to determine efficacy and safety of a Chemo-immunotherapy with the combination of bendamustine + rituximab in patients with splenic marginal zone lymphoma.
The study design is presented in the figure below:



3.2 Study population

Male and women patients (age ≥ 18 yo and ≤ 80 yo) are eligible for this clinical trial if they have B-cell splenic marginal zone lymphoma in first line treatment.
Specific inclusion and exclusion criteria for enrolling subjects in this study are described in the following sections.

3.2.1 Inclusion criteria

- Initial diagnosis of CD20+ Splenic Marginal Zone Lymphoma (According to WHO 2008 classification of Lymphoma) morphology confirmed by histology, cytology, and immunophenotype (Chromosomal abnormalities by QMPSF is optional) or according to the recommendation of the Splenic Lymphoma Group[13] for non splenectomized patient.
  1. If patients **not splenectomised**: diagnosis on bone marrow biopsy (histology and immunohistochemistry) and blood (cytology and immunophenotype - chromosomal abnormalities by QMPSF is optional)
  2. If patients **splenectomised** diagnosis on spleen, bone marrow biopsy (histology and immunophenotype), and blood (cytology and immunophenotype - chromosomal abnormalities by QMPSF optional)
- **No previous treatment** with immunotherapy or chemotherapy or radiotherapy unless pretreatment by monocorticotherapy.

- **Patients requiring a treatment with at least one of the following situation:**
  1) **Symptomatic SMZL in not splenectomized patients**
     a) **Bulky** (arbitrarily defined as ≥ 6 cm below left costal margin) or progressive or painful splenomegaly, without enlarged lymphoadenopathy with or without cytopenia, not eligible for splenectomy or not willing splenectomy.
     b) **one of the following symptomatic/progressive cytopenias**: Hb < 10 g/dL, or Plat < 80.000/mm^3, or neutropenia < 1.000/mm^3, whatever the reason (autoimmune or hypersplenism or bone marrow infiltration) not eligible for splenectomy or not willing splenectomy.
     c) **SMZL with enlarged lymphoadenopathy or involvement of extranodal sites, with or without cytopenia.**
  2) Symptomatic disease in SMZL splenectomised patients with rapidly raising lymphocyte counts, development of lymphadenopathy or involvement of extranodal sites.
  3) SMZL with concomitant hepatitis C infection who have not responded to or are relapsed after Interferon and/or Ribavirin
- Clinically and / or radiologically confirmed measurable disease before treatment start.
- Aged ≥ 18 yo at time of initial diagnosis and ≤ 80 yo.
- Eastern Cooperative Oncology Group [ECOG] performance status 0-2 (Appendix C).
- Minimum life expectancy of > 6 months.
- Voluntary signed informed consent before performance of any study related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.
- The following laboratory values at screening:
  1. Absolute neutrophil count (ANC) ≥1.000/mm^3 and Platelets ≥100.000/mm^3, unless these abnormalities are related to bone marrow infiltration or to hypersplenism.
  2. Aspartate transaminase (AST) ≤2 x ULN; Alanine transaminase (ALT) ≤2 x ULN; Total bilirubin ≤1.5 x ULN.
  3. Creatinine clearance ≥ 10 ml/min (as calculated by the Cockcroft-Gault formula – Appendix I).
- All patients must:
  1. Agree to abstain from donating blood while taking study drug therapy and following discontinuation of study drug therapy.
  2. Agree not to share medication with another person.
  3. Agree to practice an adequate method of contraception for women of childbearing potential during the study treatment and until 12 months after the end of the study treatment.
  4. Agree to practice an adequate method of contraception for men during the study treatment and until 6 months after the end of the study treatment.

3.2.2 Exclusion criteria

Potential patients who meet any of the following criteria will be excluded from participating in the study:

- Any type of lymphoma other than SMZL.
- Patients with proven biopsy of histological transformation.
- Contraindication to any drug contained in the chemotherapy regimen.
- Myocardial infarction during last 3 months or unstable coronary disease or uncontrolled chronic symptomatic congestive heart insufficiency NYHA III – IV (Appendix H).
- Uncontrolled hypertension.
- Uncontrolled diabetes mellitus as defined by the investigator.
- Active systemic infection requiring treatment.
- Previously known HIV positive serology.

- Active hepatitis B virus infection (presence of antigen HBS+; in case of presence of antibody anti HBC+ and anti HBS+, controls should be organized according to guidelines of AASLD and l'EASL).
- Active and previously untreated HCV infection.
- Prior history of malignancies other than lymphoma within 3 years (except for complete resection of basal cell carcinoma, squamous cell carcinoma of the skin, or in situ malignancy). Patients previously diagnosed with prostate cancer are eligible if (1) their disease was T1-T2a, N0, M0, with a Gleason score $\leq$ 7, and a prostate specific antigen (PSA) $\leq$ 10 ng/mL prior to initial therapy, (2) they had definitive curative therapy (ie, prostatectomy or radiotherapy) $\geq$ 2 years before Day 1 of Cycle 1, and (3) at a minimum 2 years following therapy they had no clinical evidence of prostate cancer, and their PSA was undetectable if they underwent prostatectomy or < 1 ng/mL if they did not undergo prostatectomy.
- Major surgery within 30 days before the inclusion in the study.
- A positive Coombs test without haemolysis or an autoimmune hemolytic anemia is not an exclusion criterion.
- Impaired renal function with creatinine clearance <10 ml/min.
- Severe chronic obstructive pulmonary disease with hypoxemia.
- Medical condition requiring long-term use (>1 months) of systemic corticosteroids.
- Serious medical or psychiatric illness likely to interfere with participation in this clinical study.
- Prior participation in another study with experimental drug during the last 4 months.
- Pregnant or currently breast feeding women.

4 STUDY TREATMENT

4.1 Clinical Trial Materials

4.1.1 Bendamustine

Bendamutine (Bendamustine Hydrochloride) is an antineoplastic agent available for I.V. use only. **Packaging -** Bendamustine will be provided as lyophilized powder. Two different packaging could be provided, depending on the calculated Body Surface Area (BSA) of the patient.
Two different packaging exist :
26 mL Type I brown glass vial with rubber stopper and aluminium flip-off cap for single use only contains 25 mg of bendamustine hydrochloride. Supplied in packs of 1 vial.
60 mL Type I brown glass vial with rubber stopper and aluminium flip-off cap for single use only contains 100 mg of bendamustine hydrochloride. Supplied in packs of 1 vial.
Shelf life - 3 years.

4.1.2 Rituximab

Rituximab is a mouse/human chimeric monoclonal antibody formulated for I.V. administration. The rituximab antibody is produced by a Chinese hamster ovary transfectoma.

4.2 Preparation, Handling and Storage of Study Drugs

Bendamustine: will be supplied in packs of 5 vials each.
Bendamustine may be stored up to 25°C with excursions permitted up to 30°C (see USP Controlled Room Temperature). Retain in original package until time of use to protect from light. Bendamustine

contains no antimicrobial preservative. The admixture should be prepared as close as possible to the time of patient administration. After reconstitution and dilution the chemical stability has been proved during 3,5 hours at temperature of 25°C and during 2 days in a range of temperature between 2°C and 8°C in polyethylene bags. Administration of bendamustine must be completed within this period.
Bendamustine solution should be prepared and handled according to the instructions provided in Appendix L.
Rituximab: for rituximab admixture preparation and handling please refer to the Standard Medical Practice.

4.3 Drug Supply and storage

As rituximab will be administrated according to the marketing authorization, it will not be supplied by the sponsor and will be provided by the local pharmacies.
Bendamustine will be supplied by Mundipharma.
At the study site, all investigational study drugs will be stored in a locked, safe area to prevent unauthorized access. The study drug should be stored at room temperature away from direct sunlight and protected from excessive heat and cold.

Packs of bendamustine vials shouldn't be stored at above 25°C.
The product is a protein - HANDLE GENTLY AND AVOID FOAMING. The avoidance of foaming during product handling, preparation and administration is important, as foaming may lead to the de-naturing of the product proteins. All transfer procedures require strict adherence to aseptic techniques, preferably in a laminar flow hood.

4.4 Drug Accountability

The Investigator or designee is responsible for taking an inventory of each shipment of study drug (Rituximab and Bendamustine in France, Bendamustine only outside France), and comparing it with the accompanying study drug accountability form. The Investigator will verify the accuracy of the information on the form, sign and date it, retain a copy in the study file.

4.5 Treatment Plan

4.5.1 Induction Phase

Rituximab – Bendamustine (R-B): cycles 1 to 3: (week 0, 4, 8)
Rituximab: 375 mg/sqm i.v. day 1*
Bendamustine: 90 mg/sqm iv days 1-2 or days 2-3 according to institutional/patient/physician choice
Treatment will be administered on a 28-day cycle basis.

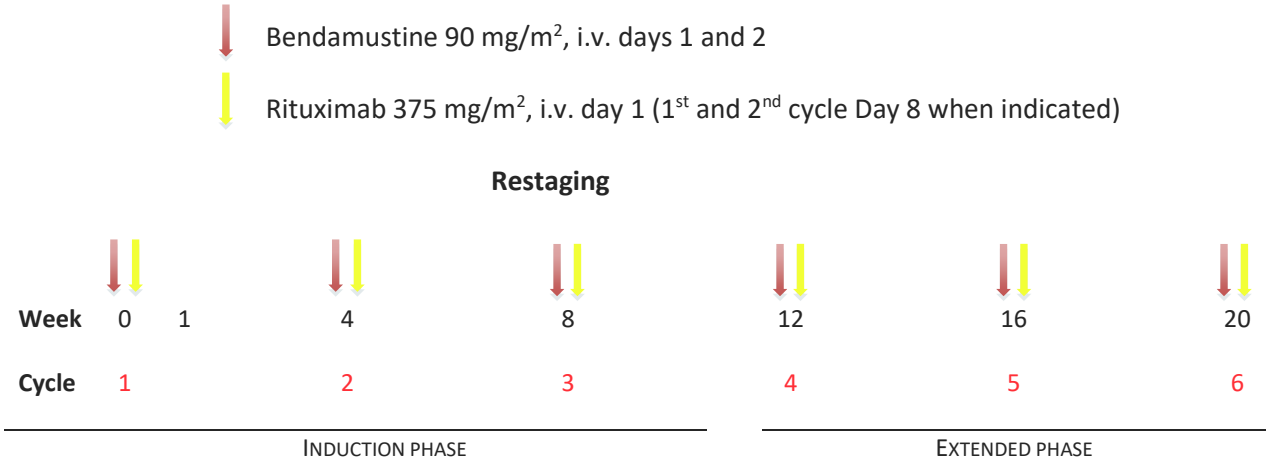
* Administration of rituximab during cycle 1 and 2 can be postponed to day 8 or day 14 in case of risk of tumor lysis syndrome: i.e. marked splenomegaly or lymphocytosis above 10.000 lymphocytes/µl.

4.5.2 Extended Phase

***Rituximab – Bendamustine (R-B)*:** cycles 4 to 6: (week 12, 16, 20)
Rituximab: 375 mg/sqm i.v. day 1
Bendamustine: 90 mg/sqm iv days 1-2 or days 2-3 according to institutional/patient/physician choice.
Treatment will be administered on a 28-day cycle basis.

Patients will be treated with **3 courses** of R-Bendamustine and then restaged. **If in PR** patients will proceed to the next extended phase with 3 more R-B courses. **If in CR** only one more R-Bendamustine course will be delivered. Patients with clinical response **less than PR** will go off the study. Patients in CR or PR at the end of the third course of R-Bendamustine and who proceeded to the extended phase will be considered to have completed the chemio-immunotherapy program and will undergo final restaging after **4 courses** or after **6 courses** (24 weeks) of R-Bendamustine treatment patients respectively.

Treatment plan schema



A centralized PET scan evaluation will be performed; the anonymized PET scan images will be collected and preserved for the entire duration of the study at the LYSARC (Resp. Romain Ricci). The images will be evaluated by a group of expert nuclear medicine to analyze the clinical response of the patients (See APPENDIX Q).

4.6 Dose modification or interruption

At the end of each course of R-B patients will be evaluated for possible toxicities that may have occurred during the cycle. Toxicities are to be assessed according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE), Version 4.0 (see Appendix J).

4.6.1 Bendamustine dose modification

Patients will start R-B every 28 days. Bendamustine administration should be delayed (after 7 days, on Day +35 of each cycle) in the event of Grade 4 hematologic toxicity or clinically significant ≥ Grade 2 non-hematologic toxicity.

For patients started on treatment with neutropenia or thrombocytopenia due to hypersplenism or to SMZL bone marrow infiltration (see section inclusion criteria 3.2.1) the R-B will be delivered every 28 days if neutrophils and platelet values match those at pre-treatment. If neutrophils and platelet values are lower than those at pre-treatment, R-B must be delayed (after 7days, on Day +35 of each cycle).

In case of neutropenia (<1.000/mm^3) at day +28, even if it is consequence of bone marrow infiltration and/or hypersplenism, subsequent cycles will be administered with G-CSF or PegGCSF support (see section 4.7.2).

Once non-hematologic toxicity has recovered to ≤ Grade 1 and/or the blood counts have improved [Absolute Neutrophil Count (ANC) ≥1.000/mm^3, platelets ≥75.000/mm^3], Bendamustine can be reinitiated at the discretion of the treating physician. In addition, dose reduction may be warranted.

Dosing will be delayed up to 4 weeks until the criteria for reinitiating therapy are met. Patients who did not meet these criteria after a 4-week delay will be removed from protocol therapy.

4.6.2 Rituximab dose modification

Rituximab administration and dose modification must follow labeling instructions and guidelines. Please refer to the approved product label for instructions.
Patients who develop severe infusion reactions should have rituximab infusion discontinued and supportive care measures as medically indicated (e.g. fluids, vasopressors, oxygen, bronchodilators, paracetamol, etc.). In most cases, the infusion can be resumed at 50% reduction rate (e.g. from 100 mg/hr to 50 mg/hr) when symptoms have completely resolved. Patients requiring close monitoring during first and all subsequent infusions include those pre-existing cardiac and pulmonary conditions, those with prior clinically significant cardiopulmonary adverse events, and whose with high numbers of circulating malignant cells (>25 x 10^9/l) with or without evidence of high tumor burden.

4.7 Concomitant Treatment

4.7.1 Recommended concomitant therapy

During treatment are recommended as concomitant therapy:
Patients will receive prophylactic treatment with valciclovir 500 mg/d and trimetroprim/Cotrimoxazole double strengths (one tablet twice daily, given three times per week, Monday, Tuesday, Wednesday) untill the completion of the last Rituximab-Bendamustine cycle.
If cutaneous toxicity appears, an amendment will be written in order to give Bactrim only in patients with low level of CD4.
In patients HBcAb+, prophylaxis against hepatitis B reactivation with Lamivudine 100 mg/die from the start of the treatment to one year after the end of the treatment.
All concomitant medications for medical conditions other than B-NHL are permitted, as clinically Indicated.
All supportive therapies other than anti-cancer treatment needed for the management of patients enrolled in this study are permitted.

4.7.2 Permitted concomitant therapy

The following medications and support therapies that may be used if needed during this study:
Prophylaxis with levofloxacine or ciprofloxacine and fluconazole/itraconazole will be administrated in case of neutropenia <1.000/mm^3. Platelets and red blood cell transfusion are allowed, if needed, and will be given in case of Hb <8 g/dl or Plt <10.000/mm^3.
Erytropoietin therapy is allowed according to ASH/ASCO guidelines. G-CSF or PegG-CSF Is allowed and will be given according to primary physician decision.
Immunoglobulin assay is advisable during induction therapy and follow-up; immunoglobulin replacement therapy is advisable in case of IgG level <0.3-0.5 gr/dl and frequent infectious events. Premedication for rituximab infusion with paracetamol and diphenhydramine should be considered before each infusion of rituximab, because it may reduce infusion reactions.

4.7.3 Prohibited concomitant therapy

The following medications and supportive therapies are prohibited at all times:

- Any antineoplastic agent other than those planned by the study program;

- Any experimental agent.
- Allopurinol.
- Vaccination against yellow fever.

5 REGISTRATION AND ENROLLMENT PROCEDURES

5.1 Patient registration

Following confirmation of eligibility and written informed consent, patients should be registered online at www.filinf.it, in the BRISMA dedicated section. An email of confirmation will be sent to the investigator and to each country coordination center, as well as the central labs that have on charge the diagnosis review.

5.2 Central review of diagnosis

Once the patient is registered into the trial he/she enters the ***Screening phase*** that consists in the central review of diagnosis. All patients will be centrally reviewed before starting therapy. For review procedures details refer to Appendix A and M.

5.3 Duration of Patients Participation

Patients will be treated for up to a total of 4-6 cycles R-B and unless removed from study for failure to respond after 3 courses or toxicity.
All responding patients will be followed-up until disease progression or death for a maximum of **60** months.

5.4 Discontinuation of Treatment

A patient should be discontinued from study treatment if:

- The investigator believes that for safety reasons (e.g., adverse event) it is in the best interest of the subject to stop treatment;
- The subject has disease progression at any time;
- The subject has grade 4 hematologic toxicity or clinically significant ≥ Grade 2 non-hematologic toxicity for > 4 weeks.

5.5 Completion of Treatment

A subject will be considered as having completed the study if he/she has completed all assessments of the treatment phase and follow-up.
Subjects who discontinue study treatment due to lack of efficacy are also considered to have completed the study.

5.6 Withdrawal from the Study

A subject will be withdrawn from the study for any of the following reasons:

- Lost at follow up
- Withdrawal of consent
- Discontinuation of study treatment (final assessments will be obtained) if applicable.

When a subject withdraws before completing the study, the reason for withdrawal is to be documented on the CRF and in the source document.

6 STUDY PROCEDURES

6.1 Disease Evaluation

6.1.1 Staging

Staging will be done according to the modified Ann Arbor staging system (see Appendix E)

6.2 Investigation at Baseline (following informed consent to trial entry)

All patients must satisfy all the inclusion criteria and none of exclusion criteria listed in section 3 and sign informed consent has to be obtained before any non routine baseline evaluation is conducted. Results of procedures performed as part of standard medical care before signing the informed consent may be used as part of the screening evaluation if performed within 2 months of beginning of therapy for laboratory tests, imaging studies, and bone marrow biopsy and aspirate and within 12 months for spleen histology.

All the following are required during screening phase
Diagnosis with:

- Spleen biopsy for splenectomized patients
- Bone marrow biopsy for immuno-histochemistry analysis (and optional bone marrow aspirate with cytology analysis and flow cytometry)
- Peripheral blood smears: WBC differential, identification and percentage of lymphoma cells with or without villous lymphocytes
- Flow cytometry peripheral blood (CD3, CD4, CD8, CD19, CD20, CD5, CD10, CD23, CD11c, FMC7, SIg, SIgM, SIgG, SIgD, K, L; CD22, CD43, CD103, CD25, CD123)

  For Central diagnosis review (Appendix M-N), the following material should be centralized in each country with local report.
  - Paraffin embedded tumour tissue will be sent (spleen and bone marrow biopsy).
  - Slides of peripheral blood smears (+/- bone marrow aspirate optional).
  - Flow cytometry results on Peripheral Blood and/or bone marrow.
- Relevant medical history
- Patient characteristics: age, gender, weight, height, body surface area, CIRS scale, history of the Lymphoma
- Concomitant diseases and treatment and use of an adequate method of contraception
- Recent clinical history (B symptoms)
- Physical examination (size of the spleen, of lymph nodes, sign of organ involvement)
- ECOG performance status
- Chest and abdomen CT scan; CT scan of the head, neck and pelvis at the discretion of the treating physician.
- FDG-PET; recommended (according to local policies and if clinically indicated at discretion of treating physician).
- ECG; echocardiogram or blood pool cardioscintigraphy are optional
- Hematology (hemoglobin, RBC, reticulocytes, WBC and differential, platelets)
- Serum LDH
- Beta-2-microglobulin
- Coombs test

- Serum protein electrophoresis and immunofixation
- Blood chemistry (AST, ALT, serum alkaline phosphatase, GGT, total bilirubin, creatinine, creatinine clearance, Na, K, Ca, uric acid, total protein, albumin, serum glucose)
- Coagulation assessment (PTT, PT, ATIII, XDP, fibrinogen)
- Blood serology (HIV, HBV, HCV)
- Mantoux screening test or QuantiFERON
- Blood sample and bone marrow for MRD
- Scoring through application of CIRS scale for history and concomitant disease (see Appendix F)

All the following are optional but strongly recommended

- ESR, Fe, BUN/urea, transferring serum ferritin, urine analysis.
- Investigation for chromosomal abnormalities by QMPSF. Peripheral blood samples for molecular, genetic and cytogenetic investigations to be centralized (bone marrow if no blood infiltration). Cytogenetics investigations should consist in evaluation of copy number abnormalities by QMPSF for patient without histological evaluation + IGVH status

6.3 Investigation before each course

- Physical examination (PS, spleen size).
- Use of an adequate method of contraception.
- Collection of adverse events during the month after the last dose of study drug administration.
- Hematology (Whole blood cell counts and differential).
- Blood chemistry (AST, ALT, serum alkaline phosphatase, total bilirubin, creatinine, Na, K).

6.4 Investigation at restaging after the third course (week 10-12)

- Physical examination (size of spleen, liver and lymph nodes, signs of organ involvement).
- Use of an adequate method of contraception.
- ECOG performance status.
- Chest and abdomen CT scan; CT scan of the head, neck and pelvis at the discretion of the treating physician.
- Hematology (hemoglobin, RBC, reticolocytes, WBC and differential, platelets.
- Peripheral blood smears: WBC differential, identification and percentage of lymphoma cells with or without villous lymphocytes.
- Serum LDH.
- Beta-2-microglobulin.
- Coombs test if documented as positive during the screening phase.
- Serum protein electrophoresis and immunofixation.
- Flow cytometry peripheral blood (CD3, CD4, CD8, CD19, CD20, CD5, CD10, CD23, CD11c, FMC7, SIg, SIgM, SIgG, SIgD, K, L; CD22, CD43, CD103, CD25, CD123).
- Bone marrow aspirate with cytology analysis and flow cytometry.
- Bone marrow biopsy (only if it was abnormal at baseline visit).
- Serum biochemistry (AST, ALT, serum alkaline phosphatase, total bilirubin, creatinine, Na, K, Ca, uric acid, total protein, albumin, serum glucose).
- Blood sample and bone marrow for MRD

6.5 Investigation after the fourth course (Early Complete Responders End of Treatment) (week 14-16)

The end treatment visit for those patients who turned out to be in complete remission after the third course, will include collection of adverse events during the month after the last dose of study drug administration; in this visit will be evaluated:

- Physical examination
- Use of an adequate method of contraception until 12 months for women and 6 months for men after the end of the study treatment
- ECOG performance status
- Hematology (hemoglobin, RBC, WBC and differential, platelets)
- Blood sample and bone marrow for MRD
- Blood chemistry (AST, ALT, serum alkaline phosphatase, GGT, total bilirubin, BUN, creatinine, Na, K, Ca, uric acid, total protein, albumin, serum glucose).
- FDG-PET; if documented as "positive" during the screening phase, after at least 45 day from the last dose of study administration.

6.6 Investigation after the sixth course (Late Responders End of Treatment) (week 22-24)

The end treatment visit for those patients who turned out to be in partial response after the third course, will include end of treatment procedures and collection of adverse events during the month after the last dose of study drug administration; in this visit will be evaluated:

- Physical examination (size of spleen, liver and lymph nodes, signs of organ involvement)
- Use of an adequate method of contraception until 12 months for women and 6 months for men after the end of the study treatment
- ECOG performance status
- Hematology (hematocrit, hemoglobin, RBC WBC and differential, Platelets.
- Flow cytometry peripheral blood (CD3, CD4, CD8, CD19, CD20, CD5, CD10, CD23, CD11c, FMC7, SIg, SIgM, SIgG, SIgD, K, L; CD22, CD43, CD103, CD25, CD123)
- Bone marrow aspirate with cytology analysis and flow cytometry
- Bone marrow biopsy (only if it was abnormal at baseline visit)
- Blood chemistry (AST, ALT, serum alkaline phosphatase, total bilirubin, BUN, creatinine, Na, K, Ca, uric acid, total protein, albumin, serum glucose)
- Serum LDH
- Beta-2-microglobulin
- Blood sample and bone marrow for MRD
- Chest and abdomen computer tomography; CT of the head, neck and pelvis at the discretion of the treating physician
- FDG-PET; if documented as "positive" during the screening phase, after at least 45 day from the last dose of study administration.
- Additional assessments if necessary according to the local standards and if clinically indicated at the discretion of the treating physician.

6.7 Investigation During Follow-Up

After the completion of the end of treatment evaluation all patients will enter in the follow up phase.
The follow-up phase will end in case of dead or progressive disease.
Follow-up clinical examination for all patients after the completion of the chemo-immunotherapy, will be scheduled once a year for 5 years.

- Hematology (hematocrit, hemoglobin, RBC WBC and differential, Platelets.
- Flow cytometry peripheral blood (CD3, CD4, CD8, CD19, CD20, CD5, CD10, CD23, CD11c, FMC7, SIg, SIgM, SIgG, SIgD, K, L; CD22, CD43, CD103, CD25, CD123)
- Blood chemistry (AST, ALT, serum alkaline phosphatase, total bilirubin, BUN, creatinine, Na, K, Ca, uric acid, total protein, albumin, serum glucose)
- Serum LDH
- Beta-2-microglobulin
- Blood sample and bone marrow for MRD
- Chest and abdomen computer tomography; CT of the head, neck and pelvis at the discretion of the treating physician
- Use of an adequate method of contraception during the 12 first months for women and 6 first months for men after the end of the study treatment
- Additional assessments if necessary according to the local standards and if clinically indicated at the discretion of the treating physician.

Patients will be evaluated through the study phases for possible toxicities and delays in dosing: dose modification will be made as required according to dose modification rules identified in section 4.6.
Patients who discontinued study drug due to toxicity will have end of treatment procedures completed and enter in the follow up phase.
Additional assessments have to be performed according to the local standards and if clinically indicated at the discretion of the treating physician.

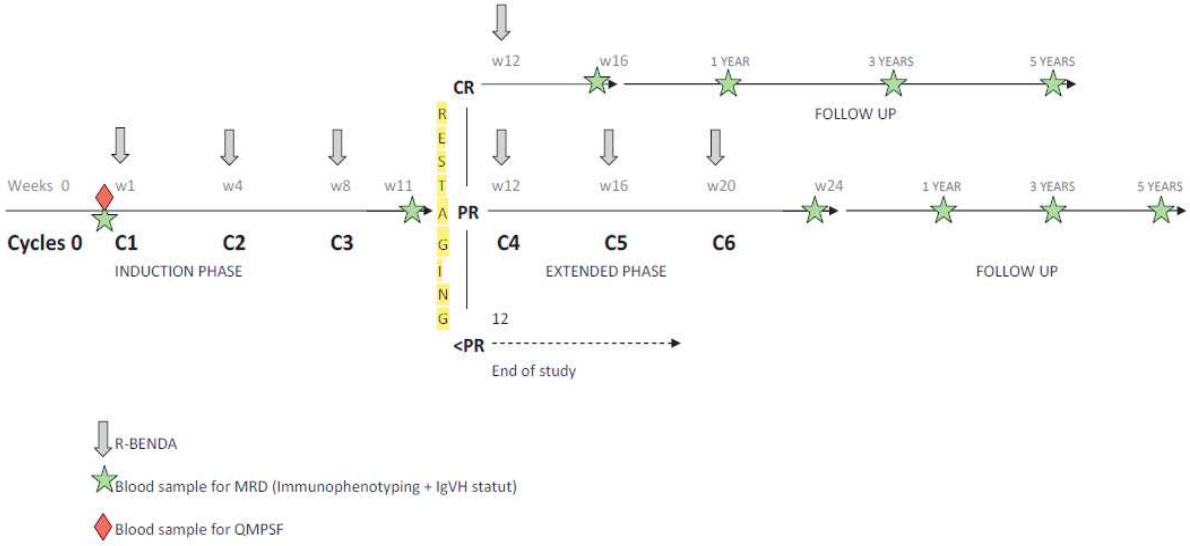
6.8 Summary tables of required investigations

	Before treatment start	First 3 cycles		After 4th cycles (only patients in CR after 3rd cycle)	After 6th cycles (only patients in PR after 3rd cycle)	Follow-up visits (once a year for 5 years)
		Before each cycle	After 3rd cycle			
Informed Consent	X					
Centr. Diagnostic review **(a, b)**	X					
Patient characteristics **(c)**	X					
Phys. Examination	X	X	X	X	X	X
ECOG PS	X	X	X	X	X	
Adverse events		Continuous report until one month after the end of treatment				
Electro-cardiogram	X					
Echocardiography or Isotopic method if applicable **(d)**	X					
Chest, abdomen +/- head, neck and pelvis CT scan **(e)**	X		X		X	
Abdominal Ultrasound **(e)**	X					X (every 6 months)
FDG-PET **(f)**	X			X	X	
Coombs D and I	X		X			
HIV, HBV, HCV serologies, Mantoux screening test or QuantiFERON	X					
Serum electrophoresis and Ig **(g)**	X		X		X	X (every 6 months)
Beta 2 microglobulin	X		X		X	
LDH	X		X		X	
Serum biochemistry **(h)**	X	X	X	X	X	
Haematology **(i)**	X	X	X	X	X	X
Coagulation assessment **(j)**	X					
Peripheral blood samples for QMPFS and IGVH status **(k)**	X					
Flow Cytometry (on peripheral blood) **(l)**	X		X		X	X (every 6 months
Peripheral blood smears **(m)**	X		X		X	X (every 6 months)
BoneMarrow Biopsy/aspirate **(n)**	X		X only if abnormal at baseline		X only if abnormal at baseline	X
Method of contraception **(o)**	X	X	X	X	X	X

a) Histological/cytological diagnostic. In France local histological diagnostic used for inclusion and central histological diagnostic review done post treatment start.
b) Biological material (Bone marrow biopsy and smears; peripheral blood smears; pathology review) + immunoistochemical on BM
c) Age, gender, weight, height, body surface area, relevant medical history, CIRS scale, history of the Lymphoma
d) In case of cardiac abnormality on ECG or history
e) If clinically indicated
f) Baseline PET is recommended. PET should be repeated, if positive at baseline, after the completion of chemoimmunotherapy
g) Serum and urine Immunofixation if a monoclonal component peak is suspected
h) Serum chemistry to include AST, ALT, serum alkaline phosphatase, GGT, total bilirubin, creatinine, creatinine clearance, Na, K, Ca, uric acid, total protein, albumin, serum glucose
i) hemoglobin, RBC, reticulocytes, WBC and differential, platelets
j) PTT, PT, ATIII, XDP, fibrinogen
k) Optional,

l) Flow cytometry investigation in peripheral blood and bone marrow samples (CD3, CD4, CD8, CD19, CD20, CD5, CD10, CD23, CD11c, FMC7, SIg, SIgM, SIgG, SIgD, K, L; CD22, CD43, CD103, CD25, CD123)
m) Every six months
n) Every 12 months for five years
o) Use of an adequate method of contraception during the 12 first months for women and 6 first months for men after the end of the study treatment

6.9 Schedule of blood samples for MRD and cytogenetics



7 EFFICACY MEASUREMENT AND PARAMETERS

7.1 Efficacy measurement

Patients receiving at least 2 courses of R-BENDA will be considered the *Efficacy Population (EP).*

7.2 Efficacy parameters

7.2.1 Primary endpoints

Efficacy of R-Bendamustine measured by **Complete Response rate.**
Complete response rate will be assessed by means of CT-scan, Immunophenotype in blood and bone marrow (PET-scan optional).

The recently published recommendations of an International experts panel[13] will be applied. Response criteria will be determined as follows:

Complete response (CR) requires the disappearance of all evidence of disease

a. Regression to normal size on CT of organomegaly (splenomegaly, hepatomegaly and lymphoadenopathies)
b. Normalization of the blood counts (Hb >12 g/dl; platelets >100.000/mm^3; neutrophils >1.500/mm^3 and no evidence of circulating clonal B-cells)

c. No evidence or minor (≤5%) BM infiltration detected by immunohistochemistry.

Partial response (PR) requires regression of 50% or greater in the measurable disease manifestations and no new sites of disease. This should include: resolution or decrease in spleen size, improvement on cytopenias and resolution or decrease in lymphadenopathy if present. Bone Marrow should show a decrease in the level of lymphoid infiltration and improvement of the haemopoietic reserve.

No response (NR) and progressive disease (PD) less than 10% improvement on the disease manifestations or deterioration, by increase >50%, of measurable signs of the disease from nadir-

Relapsed disease: Reappearance of any measurable sign of the disease.
A patient is defined as a *responder* if she/he has a complete or partial response. Patients without response assessment (due to whatever reason) will be considered as *non-responders*.

7.2.2 Secondary endpoints

- **Overall Response Rate (ORR)** (Complete response + Partial response)
- **Safety and tolerability measured by toxicities of R-Bendamustine** evaluated by assessment of laboratory parameters and adverse events coded with NCI Common Toxicity Criteria, version 4.0 (Appendix J).
- **3-year Progression Free Survival (PFS)**, defined as the time from entry into the study until reappearance of cytopenia or lymphoma relapse/ progression with enlarged lymph node(s) or spleen if present, histologic transformation or death as a result of any cause. Responding patients, patients who are lost to follow up, who withdrawal the consent or drop-out due to adverse event will be censored at their last assessment date. Patients died due to tumor will be considered in progression. Patients died for any other cause will be censored to the death date.
- **Duration of Response (DR)**, is defined for all patients who achieved a response (CR and PR) and is measured from the time of response until the date of first documentation of progression or relapse. Patients without relapse or progression will be censored at their last assessment date. Patients died due to tumor will be considered in progression. Patients died for any other cause will be censored to the death date.
- **3-year Event Free Survival (EFS)**, will be measured from the day of treatment start to the date of documentation of one of the following events: any treatment failure including disease progression, or discontinuation of treatment for any reason (eg, disease progression, toxicity, patient preference, initiation of new treatment without documented progression, or death). Responding patients, patients who are lost to follow up, who withdrawal the consent or drop-out due to adverse event will be censored at their last assessment date.
- **Time To Next Treatment (TTNT)**, defined as the time from the end of the chemo-immunotherapy course to the day of next treatment commencement irrespective of cause
- **3-year Overall Survival (OS),** defined as the time from the date of treatment start into the study until the date of death irrespective of cause. Patients who have not died at the time of end of the whole study, and patients who are lost to follow up, will be censored at the date of the last contact
- **Risk of histological transformation**
- **5-year -PFS and -OS**

The overall response rate and the rate of the single response type (CR and PR) will be estimated with the 95% confidence interval.
Time to event data (PFS, DR, TTNT, OS) will be estimated using the Kaplan-Meier method.
The curves will be plotted and the 95% confidence interval for median time will be calculated.

8 SAFETY MEASUREMENT AND PARAMETERS

8.1 Safety measurements

All patients who have received at least one dose of study medication will be considered the *Safety Population (SP)* and will be evaluated for toxicity from the time of their first drug administration. When toxicity occurs, it should be graded according to the NCI Common Toxicity Criteria, version 4.0 (Appendix J).
Any clinically significant abnormalities persisting at the end of the study will be followed by the investigator until resolution or until reaching a clinically stable endpoint.
The study will include the evaluations of safety and tolerability as described in the following sections.

8.2 Safety parameters

8.2.1 Adverse Events (AE)

Adverse events will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally acceptable representative) for the duration of the study.

Clinical Laboratory Tests
All laboratory tests should be performed at the laboratory of the investigational site or other certificate laboratories according to physician decision.
Any clinically significant abnormalities persisting at the end of the study will be followed by the investigator until resolution or until reaching a clinically stable endpoint.

8.2.1.1 Adverse Event reporting

Timely, accurate, and complete reporting and analysis of safety information from clinical studies are crucial for the protection of subjects and are mandated by regulatory agencies worldwide.

8.2.1.2 Definition of Adverse Event

An adverse event (AE) is any noxious, unintended, or untoward medical occurrence occurring at any dose that may appear or worsen in a subject during the course of a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject's health, including laboratory test values (as specified by the criteria below), regardless of etiology. Any medical condition that was present prior to study treatment and that remains unchanged or improved should not be recorded as an AE. If there is a worsening of that medical condition this should be considered an AE. A diagnosis or syndrome should be recorded on the AE page of the Case Report Form rather than the individual signs or symptoms of the diagnosis or syndrome.
All AEs will be recorded by the Investigator(s) from the time of signing the informed consent through the end of the designated follow-up period.

8.2.1.3 Abnormal laboratory values defined as Adverse Events

An abnormal laboratory value is considered to be an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study.

- Requires treatment, modification/interruption of study drug dose, or any other therapeutic intervention.
- Is judged by the Investigator(s) to be of significant clinical importance.

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page of the CRF. If the abnormality was not a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE.

8.2.2 Serious Adverse Events (SAE)

A serious adverse event (SAE) is any AE which:

- Results in death
- Is life-threatening (i.e., in the opinion of the Investigator(s) the subject is at immediate risk of death from the AE)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (a substantial disruption of the subject's ability to conduct normal life functions)
- Is a congenital anomaly/birth defect
- Constitutes an important medical event: important medical events are defined as those occurrences that may not be immediately life threatening or result in death, hospitalization, or disability, but may jeopardize the subject or require medical or surgical intervention to prevent one of the other outcomes listed above. Medical and scientific judgment should be exercised in deciding whether such an AE should be considered serious.

Events not considered to be SAEs are hospitalizations which: were planned before entry into the clinical study; are for elective treatment of a condition unrelated to the studied indication or its treatment; occur on an emergency outpatient basis and do not result in admission (unless fulfilling other criteria above); are part of the normal treatment or monitoring of the studied indication and are not associated with any deterioration in condition.
If an AE is considered serious, both the AE pages of the CRF and the SAE Report Form must be completed.
For each SAE, the Investigator(s) will provide information on severity, start and stop dates, relationship to study drug, action taken regarding study drug, and outcome.

8.2.2.1 SAE reporting to Regulatory Authorities and Ethic Committees

The pharmacovigilance team (see section 8.6) will inform relevant Regulatory Authorities and the Ethics Committee:

a) of all relevant information about serious unexpected adverse events suspected to be related to the study medication that are fatal or life threatening as soon as possible, and in any case no later than seven days after knowledge of such a case. Relevant follow-up information for these cases will subsequently be submitted within an additional eight days.
b) of all other serious unexpected events suspected to be related to the study medication as soon as possible, but within a maximum of fifteen days of first knowledge by the investigator(s).

8.3 Classification of severity

For both AEs and SAEs, the investigator(s) must assess the severity of the event. The severity of adverse events (AEs) will be graded on a scale of 1 to 5 according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events Version 4.0 (NCI CTCAE). The NCI CTCAE V4.0 can be viewed on-line at the following NCI web site (http://ctep.cancer.gov/reporting/ctc.html).

If a specific event is not included in the NCI CTCAE toxicity scale, the following scale should be used to grade the event:

1. **Mild:** Awareness of sign, symptom, or event, usually transient, requiring no special treatment and generally not interfering with usual daily activities.
2. **Moderate:** Discomfort that causes interference with usual activities; usually ameliorated by basic therapeutic maneuvers.
3. **Severe:** Incapacitating with inability to do usual activities or significantly affects clinical status and warrants intervention. Hospitalization may or may not be required.
4. **Life-threatening:** Immediate risk of death; requires hospitalization and clinical intervention.
5. **Death**.

8.4 Classification of relationship/causality of Adverse Events (SAE/AE) to study drug(s)

The Investigator(s) must determine the relationship between the administration of study drug and the occurrence of an AE/SAE as Not Suspected or Suspected as defined below:

- **Not suspected:** The temporal relationship of the adverse event to study drug administration makes a causal relationship unlikely or remote, or other medications, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event.
- **Suspected:** The temporal relationship of the adverse event to study drug administration makes a causal relationship possible, and other medications, therapeutic interventions, or underlying conditions do not provide a sufficient explanation for the observed event.

8.5 Monitoring of Adverse Event and period of observation

Adverse events, both serious and non–serious, and death that occur during the patient's study participation will be recorded in the source documents. All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

8.6 Safety contact information

PHARMACOVIGILANCE	Safety phone number	Safety Fax/e-mail
International Extranodal Lymphoma Study Group Oncology Institute of Southern Switzerland CH-6500 Bellinzona – Switzerland	Ph.: 0041 91 811 9040	E-mail: ielsg@ticino.com Fax: 0041 91 811 9182

Procedures for AE, SAE and SUSAR reporting are listed in Appendix P.

9 STATISTICAL METHODS

The primary objective of the study is to demonstrate a clinical benefit in Complete remission Rate (CR) with Rituximab-Bendamustine association in patients with splenic marginal zone lymphoma and splenic lymphoma. Complete Remission was used to determine the sample size of the study.
There are no established data in splenic marginal zone lymphoma patients in first line treatment. The CR rate with rituximab and different chemotherapy regimens in previous studies ranged from 40 to 80%. Comparison data for the study design and sample size calculations come from a median of those reported from different studies. The median expected CR rate for splenic marginal zone with the same characteristics indicated into the study and treated with standard rituximab + chemotherapy may be estimated to be approximately 60%. We would consider a positive result to increase CR rate from 60% to 80%.
This phase II study has been designed according to Simon's two-stage Optimal Design, since it is the design that minimizes the expected sample size given a 'bad' response rate.

9.1 Parameter specifications and sample size calculation

Parameters

- Standard proportion of CRR: p0 (null hypothesis) = 0.6
- Minimum required CRR for the new regimen: p1 (alternative hypothesis) = 0.80
- Alpha error (two sided) = 0.05
- Beta error (power) = 0.9

Sample size

- Potential recruitment = thirty-four patients/year
- # Valuable patients required = 53
- Drop out 10%
- Refused after central histological review 10%
- 65 subjects (total)
- Estimated study duration 7 years

9.2 Analysis plan

9.2.1 Analysis populations

The Safety Population consists of all enrolled patients who received at least one dose of study agent.
The Efficacy Population consists of enrolled patients who completed at least two cycles of treatment.
Number per protocol sample is defined.

9.2.2 Interim analysis

An interim analysis will be performed after recruitment of the first 19 valuable patients. Aim of this analysis is to determine preliminary the activity of the treatment.

9.2.3 Parameters of efficacy

The analysis of efficacy parameter will be performed according to the Intent to Treat principle.

9.2.4 Assessment of safety

Safety analysis will be based on all enrolled patients who received at least one dose of study agent. Adverse events, including toxicities, will be analyzed calculating the number and percentage of patients with event.

10 PUBLICATION POLICY

The results of this study will be submitted for publication in peer reviewed journals and for presentation at appropriate scientific meetings. In terms of the main clinical output the study chairs (Emilio Iannitto, Catherine Thieblemont) will be alternating the first the second and the last author; the pathological outputs will be led by the relevant pathologists with the study chairs alternating between last and penultimate author. Other contributors will be included as authors according to their input into the study. No publication of any results will occur without the agreement of the study chairs.

11 ETHICAL ASPECTS

11.1 Investigator Responsibilities

The investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory requirements.
GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki (Appendix B), and that the clinical study data are credible.

11.2 Ethical and Administrative considerations

The study will be submitted for approval to the relevant Ethical Committee in each country/institution. Copies of the approval letter kept on file at IELSG. Before entering patients into the study, clinicians must ensure that the protocol has received clearance from their Local Research Ethics Committee. The patient's consent to participate in the study should be obtained for all cases, after a written full explanation has been given of the treatment. PATIENT INFORMATION FORMS and INFORMED CONSENT FORMS must be prepared by the principal investigator in each country/institution following the requirements of the local Regulatory Authorities and ECs. The right of a patient to refuse to participate without giving reasons must be respected. After the patient has entered the part A of the study the clinician must remain free to give alternative treatment to that specified in the protocol at any stage if it is felt to be in the patient's best interests. The reason for giving such alternative treatment must be recorded and the patient should remain in the study for the purposes of follow-up and data analysis. Similarly, the patient must remain free to withdraw at any time from protocol treatment without giving reasons and without prejudicing further treatment. A clinical trial liability insurance which applies specifically to this study will be provided.

11.3 Independent Ethics Committee or Institutional Review Board (IEC/IRB)

Before the start of the study, the investigator will provide the IEC/IRB with current and complete copies of the following documents:

- Final protocol and, if applicable, amendments
- Informed consent form (and any other written materials to be provided to the subjects)

- Investigator's Brochure (or equivalent information) and amendments
- Information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by IEC/IRB)
- Information regarding funding, institutional affiliations, other potential conflicts of interest, and incentives for subjects
- Any other documents that the IEC/IRB requests to fulfill its obligation

During the study the investigator will send the following documents to the IEC/IRB for their review and approval, where appropriate:

- Protocol amendments
- Revision(s) to informed consent form and any other written materials to be provided to subjects
- If applicable, new or revised subject recruiting materials approved by the sponsor
- Revisions to compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- Investigator's Brochure amendments or new edition(s)
- Summaries of the status of the study (at least annually or at intervals stipulated in guidelines of the IEC/IRB)
- Reports of adverse events that are serious, unlisted, and associated with the investigational drug
- New information that may affect adversely the safety of the subjects or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the subjects
- Report of deaths of subjects under the investigator's care
- Notification if a new investigator is responsible for the study at the site.
- Any other requirements of the IEC/IRB

For protocol amendments that increase subject risk, the amendment and applicable informed consent form revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s).
At least once a year, the IEC/IRB will be informed about the clinical ongoing of this clinical study.
This request should be documented in writing.
At the end of the study, the investigator (or sponsor where required) will notify the IEC/IRB about the study completion.

11.4 Informed Consent

Each subject (or a legally acceptable representative) must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used must be approved by the reviewing IEC/IRB. The informed consent should be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.
Before entry into the study, the investigator or an authorized member of the investigational staff must explain to potential subjects or their legally acceptable representatives the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort it may entail.
Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive for the treatment of his/her disease. Subjects will be told that alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment.

The subject or legally acceptable representative will be given sufficient time to read the informed consent form and the opportunity to ask questions. After this explanation and before entry to the study, consent should be appropriately recorded by means of either the subject's or his/her legally acceptable representative's dated signature. After having obtained the consent, a copy of the informed consent form must be given to the subject.
If the subject or legally acceptable representative is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and personally date and sign the informed consent form after the oral consent of the subject or legally acceptable representative is obtained.

11.5 Privacy of Personal Data

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to investigate the efficacy, safety, quality, and utility of the investigational product(s) used in this study.
These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.
The investigator ensures that the personal data will be processed fairly and lawfully collected for specified, explicit, and legitimate purposes and not further processed in a way incompatible with these purposes adequate, relevant, and not excessive in relation to said purposes; accurate and, where necessary, kept current.
Explicit consent for the processing of personal data will be obtained from the participating subject (or his/her legally acceptable representative) before collection of data. Such consent should also address the transfer of the data to other entities and to other countries.
The subject has the right to request through the investigator access to his/her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps should be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

11.6 Data Quality Assurance

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study centers, review of protocol procedures with the investigator and associated personnel before the study. Written instructions will be provided for collection, preparation, and shipment of blood, plasma, and urine samples. CRF completion guidelines will be provided and reviewed with study personnel before the start of the study. Investigator will permit trial-related monitoring, providing direct access to source documents/data.

11.7 Record Retention

The results of the study will be reported in a Clinical study Report generated by the investigator and will contain all data from all investigational sites.
Data recorded on the CRFs (electronic record of data) on site www.filinf.it will be considered to be the source data for analyses.

11.8 On Site Audits

Investigator/institution will permit trial-related audits, providing direct access to source documents/data.

12 STUDY ACKNOWLEDGEMENT

PROTOCOL IELSG 36 (BRISMA)

BENDAMUSTINE AND RITUXIMAB FOR THE TREATMENT OF SPLENIC MARGINAL ZONE LYMPHOMA: THE IELSG36 PHASE II PROSPECTIVE STUDY.

As investigator for this study, I understand that this protocol contains information that is confidential and proprietary to IELSG. I have received and read the above mentioned protocol and agree that it contains all necessary details for carrying out the study as described; I will conduct this protocol as outlined therein.
I will provide copies of this protocol and access to all information furnished by IELSG to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the investigational product and the study. I agree to keep accurate records on all patients information (CRFs and Patients's informed consent statement) and all other information collected during the study for a minimum period of 10 years.
I agree not to publish all or any part of the results of the study carried out under this protocol, without the prior written consent of IELSG.
All parties agree to ensure direct access to examine, analyse, verify and reproduce source data / documents, and reports from all trial related sites for the purpose of monitoring and auditing, and inspection by domestic and foreign regulatory authorities.

Investigator (printed name) Signature Date

Prof. Dr. med. Franco Cavalli 

IELSG Representative Signature Date

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APPENDIX A - Guidelines for diagnosis review

In order to assure the high quality and consistent pathology diagnosis, a centralized diagnosis review is included in this trial, to be performed during the Screening phase of the study, i.e. before therapy start.
Paraffine embedded tissue block, bone marrow trephine biopsy, peripheral blood smears/bone marrow aspirate, flow-cytometry results on peripheral blood and marrow will be centrally reviewed separately for LYSA and FIL centers.
For timeline of review processes refer to group specific guidelines detailed in Appendix M.

Diagnosis will be established according to WHO classification for splenectomized cases[1] and according to the Splenic Lymphoma Group recommendations for non splenectomized cases[13], with full support of immunohistochemistry.

Where indicated, chromosomal translocation will be investigated using appropriate molecular genetic and cytogenetic methods. In suspected cases of SMZL with CD5 positivity, at (11;14) must be eliminated by FISH.

For each enrolled patient, the paraffin embedded tissue block should be sent to collecting centres (see contact addresses for the trial organization for LYSA and FIL centers) for processing; the block will be returned as soon as possible. If for local reasons, it is not possible to release the paraffin embedded tissue block, the following specimens should be submitted to collecting centres: one slide stained with H&E and 14 unstained sections (for immunohistochemistry and FISH), of each tumor bioptic sample (bone marrow, spleen, lymphonode).
For each enrolled patient, peripheral blood smears/bone marrow aspirate should be sent to collecting centres (see contact addresses for the trial organization for LYSA and FIL centers). The slides will be returned as soon as possible.

Summary Table

Flow-cytometry local results on peripheral blood and bone marrow aspirate will be reviewed during the screening phase (percentages and histograms; provide caryotype when performed locally).	**LYSA**			**FIL**		
	Spleen	Bone Marrow	Peripheral Blood	Spleen	Bone Marrow	Peripheral Blood
Histology	LYSA-P	LYSA-P		Palermo	Palermo	
Cytology		Slides of BM aspirate sent for review to Hôpital Lyon Sud or Hôpital Saint Louis - laboratoire hematologie	Slides of blood smears sent for review to Hôpital Lyon Sud or Hôpital Saint Louis - laboratoire hematologie		Slides of BM aspirate sent for review to Palermo	Slides of blood smears sent for review to Palermo
Immuno-phenotype			Local results sent for review to Lyon Sud or Hôpital Saint Louis - laboratoire hematologie	Palermo	Palermo	Local results sent for review to Palermo
Cytogenetics			Blood samples on paxgene sent to Lyon for DNA extraction further analysis of QMPFS and IGVH status (analysis post inclusion)		Blood samples in EDTA sent to Genova for DNA extraction and further analysis (FISH and IGVH status; (analysis post inclusion)	Blood samples in EDTA sent to Genova for DNA extraction and further analysis (FISH and IGVH status; analysis post inclusion)

APPENDIX B - World Medical Association

Declaration of Helsinki

Ethical Principles for Medical research involving Human Subjects

Adopted by the 18th WMA General Assembly,
Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.
   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.
9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.
17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.
26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
33. The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
34. Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
35. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
36. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.
37. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

APPENDIX C - WHO performance status scale

Grade	Performance scale
0	Able to carry out all normal activity without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out light work
2	Ambulatory and capable of all self-care but unable to carry out any work; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair

APPENDIX D - SMZL Diagnostic and Staging Work-Up

(Source: Matutes et al., Splenic marginal zone lymphoma proposals for a revision of diagnostic, staging and therapeutic criteria.
Leukemia, 22(3):487-495, 2008)

Diagnostic and staging workup

(1)	Full Blood count (FBC) with differential counts, reticulocytes, Coomb's test and autoimmune screen (ANA, anti-DNA, AMA, anti-thyroid, rheumatoid factor)
(2)	Renal and liver biochemistry including calcium levels and LDH
(3)	Serum and urine Igs and B-2 microglobulin
(4)	Serology for hepatitis C (if positive, reverse transcriptase-PCR for HCV RNA in the blood) and virus genotyping, when possible
(5)	HIV serology should be investigated, with the limitations due to specific countries policies
(6)	Review of blood morphology and flow cytometry (if circulating lymphoma cells)
(7)	BM aspirate with morphology and flow cytometry and trephine biopsy with immunohistochemistry (IHC). BM aspirate in the absence of trephine biopsy and immunohistochemistry may have a low diagnostic value.
(8)	Computerized tomography scan of the abdomen and chest

APPENDIX E
Ann Arbor staging* - Cotswolds recommendations**

(Sources: * Carbone PP, Kaplan HS, Musshoff K, et al. Report of the committee on Hodgkin's disease staging classification. Cancer. Res. 31:1860-1861, 1971. ** Lister TA, Crowther D, Sutcliffe SB, et al. Staging for Hodgkin's disease. Report of a committee convened todiscuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. J. Clin. Oncol. 7:1630-1636,1989 [Erratum J. Clin. Oncol. 8:1602, 1990])

Stage I: involvement of a single lymphatic region (I), or localized involvement of a single extralymphatic organ or site (IE).
Stage II: involvement of two or more lymphatic regions on the same side of diaphragm (II) or localized involvement of an extralymphatic organ or site and one or more lymph node regions the same side of diaphragm (IIE).
Stage III: involvement of two or more lymphatic regions on both sides of diaphragm (III) which may also be accompanied either by localized involvement of an extralymphatic organ or site (IIIE), or by involvement of the spleen (IIIS).
Stage IV: Diffuse or disseminated involvement of one or more extralymphatic organs or tissue, with or without associated lymph node involvement.
Bone marrow or liver involvement will always be considered as stage IV.

Lymphatic structures include lymph nodes, spleen, thymus, Waldeyer's ring including tonsils. The lymphatic regions are defined according to Ann Arbor as (a) clinical enlargement of a node when alternative pathology may reasonably be ruled out (suspicious nodes should always be biopsied if treatment decisions are based on their involvement) ; and (b) enlargement on plain radiograph, CT scan, or lymphography.
Localized involvement of an extralymphatic site (extranodal site or E-lesion) is diagnosed when the involvement is small enough to be in principle accessible for curative radiotherapy (thereby excluding diffuse organ involvement corresponding to stage IV).

Criteria for "B" symptoms
The presence of (a) unexplained weight loss of more than 10% of the body weight during the 6 months before initial staging investigation and/or (b) unexplained, persistent, or recurrent fever with temperatures above 38°C during the previous month and/or (c) recurrent drenching night sweats during the previous months is denoted by the suffix letter 'B'. 'A' indicates the absence of these symptoms.

Criteria for bulk disease
The bulk of palpable lymph nodes will be defined by the largest dimension (cm) of the single largest lymph node or conglomerate node mass in each region of involvement. A node or nodal mass must be 10 cm or greater to be recorded as "bulky".
A mediastinal mass will be defined as "bulky" on a postero-anterior chest radiograph, when the maximum width is equal or greater than one-third of the internal transverse diameter of the thorax at the T5-T6 level. The chest radiography should be taken with maximal inspiration in the upright position at a source-skin distance of 2 m.

Spleen bulky is arbitrarily defined as spleen ≥6 cm below left costal margin.

APPENDIX F - Cumulative Illness Rating Scale (CIRS)

(Source: Parmelee, P.A., et al., *Validation of the Cumulative Illness Rating Scale in a geriatric residential population.* J Am Geriatr Soc, 1995. **43**(2): p. 130-7)

Comorbidity indices	**CIRS**
Items	1. Cardiac (heart only) 2. Hypertension (rating is based on severity; affected systems are rated separately) 3. Vascular (blood, blood vessels and cells, marrow, spleen, lymphatics) 4. Respiratory (lungs, bronchi, trachea below the larynx) 5. EENT (eye, ear, nose, throat, larynx) 6. Upper gastrointestinal (esophagus, stomach, duodenum, biliary and pancreatic trees; do no include diabetes) 7. Lower gastrointestinal (intestines, hernias) 8. Hepatic (liver only) 9. Renal (kidneys only) 10. Other genitourinary (ureters, bladder, urethra, prostate, genitals) 11. Musculo-skeletal-integumentary (muscles, bone, skin) 12. Neurological (brain, spinal cord, nerves; do not include dementia) 13. Endocrine-Metabolic (includes diabetes, diffuse infections, infections, toxicity) 14. Psychiatric/Behavioral (includes depression, anxiety, agitation, psychosis, not dementia)
Weights	All systems weighted from 0 to 4:
0 = None	no impairment to that organ/system
1 = Mild	Impairment does not interfere with normal activity; treatment may not be required; prognosis is excellent (examples: skin lesions, hernias, hemorrhoids)
2 = Moderate	Impairment interferes with normal activity; treatment is needed; prognosis is good (examples: gallstones, diabetes, fractures)
3 = Severe	Impairment is disabling; treatment is urgently needed; prognosis is guarded (examples: respectable carcinoma, pulmonary emphysema, congestive heart failure)
4 = Extremely severe	Impairment is life threatening; treatment is urgent or of no avail; prognosis is grave (examples: myocardial infarction, cerebrovascular accident, gastrointestinal bleeding, embolus)
Final score	Sum of weights assigned to each system

APPENDIX G
Fondazione Italiana Linfomi Onlus (FIL) SMZL score

(Source: Arcaini et al., Splenic marginal zone lymphoma: a prognostic model for clinical use. Blood, 107:4643-4649, 2006)

Parameters	
a) Heamoglobin	< 12 gr/dL
b) LDH	> ULN
c) Albumin	< 3.5 gr/dL

Score	
Low Risk	non adverse factor
Intermediate Risk	1 adverse factor
High Risk	2-3 adverse factors

APPENDIX H
New York Heart Association Classification Of Cardiac Disease

(Source: The Criteria Committee of the New York Heart Association, Inc. : Diseases of the heart and blood vessels; Nomenclature and criteria for diagnosis, 6th Ed. Boston: Little, Brown; 1964)

The following table presents the NYHA classification of cardiac disease:

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the angina syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

APPENDIX I - Creatinine Clearance Calculation

(Source: Cockroft DW, Gault MH, Prediction of creatinine clearance from serum creatinine. Nephron 16, 31-41, 1976)

Creatinine clearance for men and women will be calculated according to the Cockcroft-Gault formula as follows:

$$\textit{In men} \qquad \frac{[(140 - age) \times weight\ (kg)]}{[72 \times creatinine\ (mg/dL)]}$$

$$\textit{In women} \qquad \frac{[(140 - age) \times weight\ (kg)]}{[72 \times creatinine\ (mg/dL)] \times 0.85}$$

Note
Age (in years), weight (in kg), serum-creatinine (in mg/dL)
72 (normalized to 72 kg body weight and a body surface of 1.72 m^2)

APPENDIX J
Common Terminology Criteria for Adverse Events

In the present study, adverse events and/or adverse drug reactions will be recorded according to the:

Common Terminology Criteria for Adverse Events (CTCA), version 4.0.

At the time this protocol was issued, the full CTC document was available on the NCI web site, at the following address: http://ctep.cancer.gov/reporting/ctc.html.

APPENDIX K - Suggested Body Surface Area Calculation

(Source: Mosteller RD., Simplified calculation of body-surface area. N Engl J Med, 317:1098, 1987)

BSA should be determinated using the appropriate following calculation:

$$\text{BSA} = \sqrt{\frac{Ht(inches)xWt(lbs)}{3131}}$$

OR

$$\text{BSA} = \sqrt{\frac{Ht(cm)xWt(kg)}{3600}}$$

APPENDIX L
Instruction for Preparation and Handling of Bendamustine

Refer to the clinical trial protocol for details about the dose and dose schedule.
Bendamustine should not be administered to patients with known hypersensitivity to bendamustine or mannitol.

A licensed pharmacist or a properly trained designee will prepare all doses of bendamustine. It is very important that the exact dose is accurately dispensed and administered. If possible, the same person should prepare all doses (on all dosing days). Bendamustine will be administered only to eligible subjects under the supervision of the investigator or identified subinvestigator(s).

Calculation of Dose
The amount (in mg) of bendamustine to be administered will be determined based on body surface area (BSA). BSA will be calculated based on body weight and height using a standard calculation (Appendix K, Suggested Body Surface Area Calculation). The bendamustine dose will not be corrected for obese subjects. The dose should be calculated on Day 1 of each cycle and remain consistent during an individual cycle. The dose administered should remain consistent across cycles unless a notable change in weight (e.g., loss or gain of $\geqslant$10%) is documented during the Day 1 weight assessment of the cycle, in which case the subject's dose should be recalculated at that time. In the event of bendamustine-associated toxicity during the study, the dose may be decreased according to the dose reduction schedules provided in Section 4.6.1.

Reconstruction and Dilution
Reconstruction
Reconstitute each vial containing 25 mg bendamustine hydrochloride in 10 ml water for injection by shaking;
Reconstitute each vial containing 100 mg bendamustine hydrochloride in 40 ml water for injection by shaking.
The reconstituted concentrate contains 2.5 mg bendamustine hydrochloride per ml and appears as a clear colourless solution
Dilution
As soon as a clear solution is obtained (usually after 5-10 minutes) dilute the total recommended dose immediately with 0.9% NaCl solution to produce a final volume of about 500 ml.
Bendamustine must be diluted with 0.9% NaCl solution and not with any other injectable solution.

Administration of bendamustine

The final admixture is stable 3.5 hours when stored at room temperature (25°C) and room light. Administration of bendamustine must be completed within this period.

The administration of bendamustine, reconstituted and diluted according to the above prescriptions will be accomplished by slow I.V. infusion in an out- or in-patient setting over approximately 30 to 60 minutes).

CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS

Discarding of Vials and Syringe

After administration all materials that have been used for preparation should be disposed of according to standard practices. Any unused solution should be discarded according to institutional procedures for antineoplastics.

All unused vials must be saved for reconciliation by the study monitor. A log must be kept of all disposed materials.

Procedures to follow in case of body contact

Inhalation: if inhaled, remove to fresh air. If breathing becomes difficult, call a physician.

Skin: in case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes.

Ingestion: if swallowed, wash out mouth with water provided person is conscious. Call a physician.

Eyes: in case of contact with eyes, flush with copious amount of water for at least 15 minutes. Assure adequate flushing by separating eyelids

Always contact the investigator after any form of body contact.

APPENDIX M
Guidelines for Management of Biological Material

The consent process allows for paraffin embedded blocks of tumour (bone marrow, spleen), peripheral blood smears, anticoagulated blood samples and anticoagulated bone marrow samples, serum (collectively defined in this protocol as study material) to be submitted for central histology review, cytogenetic analysis and immunoglobulin gene repertoire and mutation analysis.

1. LYSA Centers

SAMPLES FOR PATHOLOGY REVIEW OF SPLEEN/BONE MARROW

The pathological diagnosis of SMZL should have been first performed locally and will be used for the inclusion.

A central review of the diagnosis will be then organized by the LYSA-P Institute for each patient included in the trial. Diagnosis will be established according to WHO classification for splenectomized cases[1] and according to the Splenic Lymphoma Group recommendations for non splenectomized cases[13], with full support of immunohistochemistry. At patient screening, the investigator will be requested to join with the form of inclusion a copy of the histopathological report where the name and address of the pathologist having diagnosed the lymphoma will be easily identified. However, in the case where no histopathological report is available, the name and address of the initial pathologist as well as the report number will have to be indicated on the inclusion form.

The LYSA-P Institute will then organize and centralize the collection of the bone marrow biopsy and spleen (if available) material. At reception of the material, routinely stained sections will be performed and an appropriate panel of antibodies according to morphological aspects will be applied.

Tissue microarray (TMA) will also be constructed. Three tissue cylinders representative of tumor regions with a diameter of 0.6 mm will be punched from the paraffin embedded tissue and transferred into a recipient paraffin block using a manual tissue arrayer (Beecher Instruments). Reactive lymphoid tissues will also be included in the TMA blocks, as a control and two twin TMA blocks will be prepared, according to the procedure used in the LYSA-P.

The prepared stained slides will be reviewed by a panel of pathologist based on expert hematopathologists who will establish a consensus diagnosis. When the review process is achieved and the tissue array performed, a pathological report is sent to the initial pathologist as well as the investigator of the inclusion centre. The remaining material is sent back to the initial pathologist.

The LYSA-P will make the commitment to respect the legal requirements for the collection, preparation and storage of the samples.

SAMPLES FOR CYTOLOGY REVIEW ON BLOOD AND BONE MARROW ASPIRATE

At screening, peripheral blood smears (2 stained slides + 1 blank slide) and bone marrow aspirate (2 stained slides + 1 blank slide) will be shipped with the completed traceability form in a pre-paid enveloped by regular mail.

SAMPLES FOR MINIMAL RESIDUAL DISEASE (MRD)

Flow-cytometry immunophenotype and IgVH gene rearrangement (antiCDR3) analyses (molecular B cell clonality assay) will be performed on the peripheral blood and the bone marrow aspirate at baseline (screening phase), at each restaging phases, at end of the treatment and at intervals (one a year) during follow up.

For FACS analyses, 5 ml x 2 EDTA blood and 3 ml x 1 EDTA bone marrow will be required and will be sent by DHL to guarantee a delivery in 48h in centralized lab with a shipment form.

For IgVh gene rearrangement (antiCDR3) analyses, collection of blood samples and bone marrow for DNA preparation is required, with 10 ml x 1 on EDTA or on paxgene tube for blood at baseline (screening phase), at each restaging phases, at end of the treatment and at intervals during follow up. A special isolation tube (PAXgene tube) and labels will be provided by LYSARC. This PAXgene tube will be send on the day of collection by regular mail at room temperature.

The results of MRD will be not incorporated into the response evaluation nor influence the management of the patient.

SAMPLES FOR QMPFS

A specific informed consent form will be used to allow the collection of blood samples for DNA preparation. It is highly recommended to collect blood samples (10 ml on EDTA or on paxgene tube) at diagnosis in all patients of each LYSA center.
A special isolation tube (the same PAXgene tube used for IgVH at baseline) and labels will be provided by LYSARC. This PAXgene tube will be sent on the day of collection by regular mail at room temperature.

Address of shipping

Cytology review on blood and bone marrow aspirate
Centre Hospitalier Lyon Sud
Centre de Biologie et d'anatomie Pathologique Sud – Bât. 3D
Laboratoire d'hématologie cellulaire - Pascale Felman
F-69310 Pierre-Bénite

Hôpital Saint Louis
Laboratoire Central d'hématologie - Maria-Elena Noguera
Nouveau Saint-Louis – Secteur Vert Porte 1
Unité de cytologie immunologie – 1er étage
1, avenue C. Vellefaux - F-75010 Paris

DNA – MRD for molecular biology and QMPSF
Centre Hospitalier Lyon Sud
Laboratoire de biologie moléculaire et Hématologie
Centre de Biologie Sud – Espace Jacques Monot – Etage 1
Chemin du Grand Revoyet – F-69310 Pierre-Bénite
Please, complete the information sheet "BRISMA – Etude LYSARC – Envoi par la poste — tube PAXgene" and send this information sheet with PAXgene tube in Lyon Sud Hospital by post office.

All the samples will be immediately coded if they are not yet, or identification will be controlled at reception (date of sampling and number of inclusion in the study).
DNA will be extracted and provided to the concerned labs.

Flow cytometry - MRD
Maria-Elena Noguera
Hopital Saint-Louis
Laboratoire Central d'hématologie
Nouveau Saint-Louis – Secteur Vert Porte 1
Unité de cytologie immunologie – 1er etage

1, avenue C. Vellefaux - F-75010 Paris

🔔 Please, complete the information sheet "BRISMA – Etude LYSARC – Envoi par courrier — tube EDTA" and send this information sheet with EDTA tube in Saint-Louis.

2. FIL Centers

SAMPLES FOR PATHOLOGY REVIEW OF SPLEEN/BONE MARROW

A central review of the diagnosis will be organized by the co-ordinator for each patient included in the trial, in order to confirm the diagnosis by using the 2008 WHO classification.

The review of diagnosis will be performed up-front, during the screening phase, after patient's registration in the trial and before treatment start.

At patient screening, a copy of the histopathological report where the name and address of the pathologist having diagnosed the lymphoma will be easily identified. However, in the case where no histopathological report is available, the name and address of the initial pathologist as well as the report number will have to be indicated on the inclusion form.

The centralized collection of the tumor material will be routinely stained sections and an appropriate panel of antibodies will be applied.

The slides will be reviewed by a panel of pathologist who will establish a consensus diagnosis. When the review process is achieved, a pathological report is sent to the initial pathologist as well as the investigator of the inclusion centre. Once the study is completed, the stained sections and remaining material is sent back to the initial pathologist.

SAMPLES FOR CYTOLOGY REVIEW ON BLOOD AND BONE MARROW ASPIRATE

At screening, peripheral blood smears and bone marrow aspirate will be shipped with A SHIPMENT FORM provided to accompany the study material and a courier service (prepaid way-bill) will be activated to guarantee delivery of these within 48 hours.

SAMPLES FOR IGVH STATUS

If the blocks are available, tissue sections will be used from paraffin block for IgVh status.

Address of shipping
Dott. Claudio TRIPODO
ANATOMIA PATOLOGICA
Policlinico
Via del Vespro 129
I-90127 Palermo

APPENDIX N
Description of Minimal Residual Disease, and molecular, genetic and cytogenetic investigations

1. LYSA Centers

- **Minimal residual Disease (MRD)**

There are strong hints from recently published data that minimal residual disease (MRD) detection may play a role as a prognostic factor in patients with small B cell lymphomas such as mantle cell lymphoma. Nothing has been reported in Splenic Marginal Zone lymphoma. But this method could help to assess the status of complete response because evaluation of response in SMZL may not be easy even if criteria have been published (Matutes et al. 2008).

Aim of MRD analysis within this study protocol is the monitoring of MRD by Flow cytometry and by quantitative Taq Man PCR (RQ-PCR) targeting clonal IgVH (antiCDR3) to evaluate the prognostic impact of MRD on progression free survival and long-term remission of patients with splenic marginal zone lymphoma.

FACS analysis will be performed at baseline, after C4, after C6 and every year, to evaluate the B cell background during treatment with Rituximab. Therefore it is essential to send heparin blood/ bone marrow for FACS analysis together with EDTA blood/ bone marrow for FACS analysis at every time point of MRD investigation.

The real time PCR method is sensitive enough to detect a lymphoma cell in the background of 104-105 normal cells. PCR-analysis will be performed from peripheral blood (10 ml EDTA blood) and bone marrow (3-5 ml EDTA bone marrow aspirate). As a prerequisite for molecular assessment of MRD using RQ-PCR peripheral blood and bone marrow has to be sent before start of any treatment to determine the individual patient-specific DNA-sequence of the malignant clone. It will then realized at the timepoint than FACS analysis (C4, C6, and once a year).

Flow-cytometry immunophenotype and IgV_H gene rearrangement analyses (molecular B cell clonality assay) will be performed on the peripheral blood and the bone marrow aspirate at baseline (screening phase), at each restaging phases, at end of the treatment and at intervals during follow up.

The results of MRD will be not incorporated into the response evaluation nor influence the management of the patient.

Chromosomal abnormalities by QMPSF– C. Bastard

Analysis of chromosomal copy number abnormalities will be performed from DNA extracted from blood collected at baseline: +3 ; del7 ; +12 ; +18

In Centre Henri Becquerel - Laboratoire de Génétique Oncologique - rue d'Amiens - F-76038 Rouen - Telephone +33 2 32 08 25 77

Immunoglobulin heavy-chain variable-region (IgVH) gene mutation analysis – F. Davi

Analysis of IgVH genes expressed by tumor cells will be undertaken for both mutational status and repertoire evaluation. Nucleotide sequence of IgVH genes will be performed according to standardized protocols ***(Ghia et al., Leukemia, 2007, 21:1)***. Briefly, clonal IgVH rearrangements will be amplified from genomic DNA (0,5-2 micrograms) using 5' primers corresponding to the framework 1 (or peptide leader) region and 3' primers corresponding to the J genes. PCR products will be sequenced directly or after cloning if necessary. Sequence analysis including identification of the closest germline gene counterpart, distribution and characteristics of somatic mutations will be performed using dedicated bioinformatic tools (IMGT V-QUEST, IgBlast).

In Hôpital Pitié Salpêtrière- Inserm U 543, Service d'hématologie biologique - 47-83, boulevard de l'Hôpital – F-75651 PARIS Cedex 13

2. FIL Centers

Flow cytometry immunophenotype, Chromosomal abnormalities and Immunoglobulin heavy-chain variable-region (IgVH) gene mutation analysis – S. Ferrero/D. Drandi.

Heparined or EDTA peripheral blood (20 ml) and heparined or EDTA bone marrow aspirate (5-10 ml) will be sent by express shipment (24H delivery) to the Laboratory of hematology 1, U Prof. Boccadoro, Turin.

Address of shipping

Dott. Simone Ferrero/Dott.ssa Daniela Drandi
AOU "Città della Salute e della Scienza" di Torino, presidio "Molinette"
Laboratorio di ematologia 1 U Prof. Boccadoro
Via Genova 3, P.T. padiglione giallo, 10126 Torino

Flow-cytometry immunophenotype and IgV_H gene rearrangement analyses (molecular B cell clonality assay) will be performed on the peripheral blood and the bone marrow aspirate at baseline (screening phase), at each restaging phases, at end of the treatment and at intervals during follow up. Cytogenetic assessment and IgV_H mutation analyses will be performed at baseline only. Table 6.8 of the protocol summarises the time schedule of the required investigations (row g,h).

Samples

Mononuclear cells (MNC) will be isolated from peripheral blood and /or bone marrow aspirate by ficoll hypaque density gradient centrifugation. MNC will be splitted in three aliquots to perform the following different analysis: flow cytometry, cytogenetic assessment, molecular analysis (B cell clonality assay and IgV_H mutation analysis).

Flow cytometry

Immunophenotype analysis by flow cytometry will be performed on fresh MNC using the following monoclonal antibodies (moAb): CD3+, CD4+, CD8+, CD19+, CD20+, CD5+, CD10+, CD23+, CD11c+, FMC7+, CD103, CD25, SIg, SIgM, SIgG, SIgD, K, L). CD22+, CD43+, Cd103+, CD25+, CD123+. Four color stainings will be performed using moAb conjugated with four different fluorochromes and using FACSalibur cytometer (BD biosciences). The acquired data will be analyzed by Cell Quest software.

Chromosomal abnormalities

Analysis of chromosomal abnormalities will be performed from DNA extracted from MNC from periphera blood or bone marrow aspirates (only in the case of peripheral blood without neoplastic cells): +3 ; del7 ; +12 ; +18.

Molecular analysis

The detection of IgV_H gene rearrangements (B cell clonality assay) will be performed by fluorochrome labeled PCR as described in the protocol BIOMED 2 PCR (Leukemia 2003 17: 2257-2317). The fluorochrome labeled PCR products will be analyzed by GeneScanning analysis using ABI prism 3100 Avant Genetic Analyser. GeneScanning analysis of IgV_H rearrangements have been developed for B cell clonality studies with a sensitive of clonal populations of >1%, (not for detection of subclones <1%).

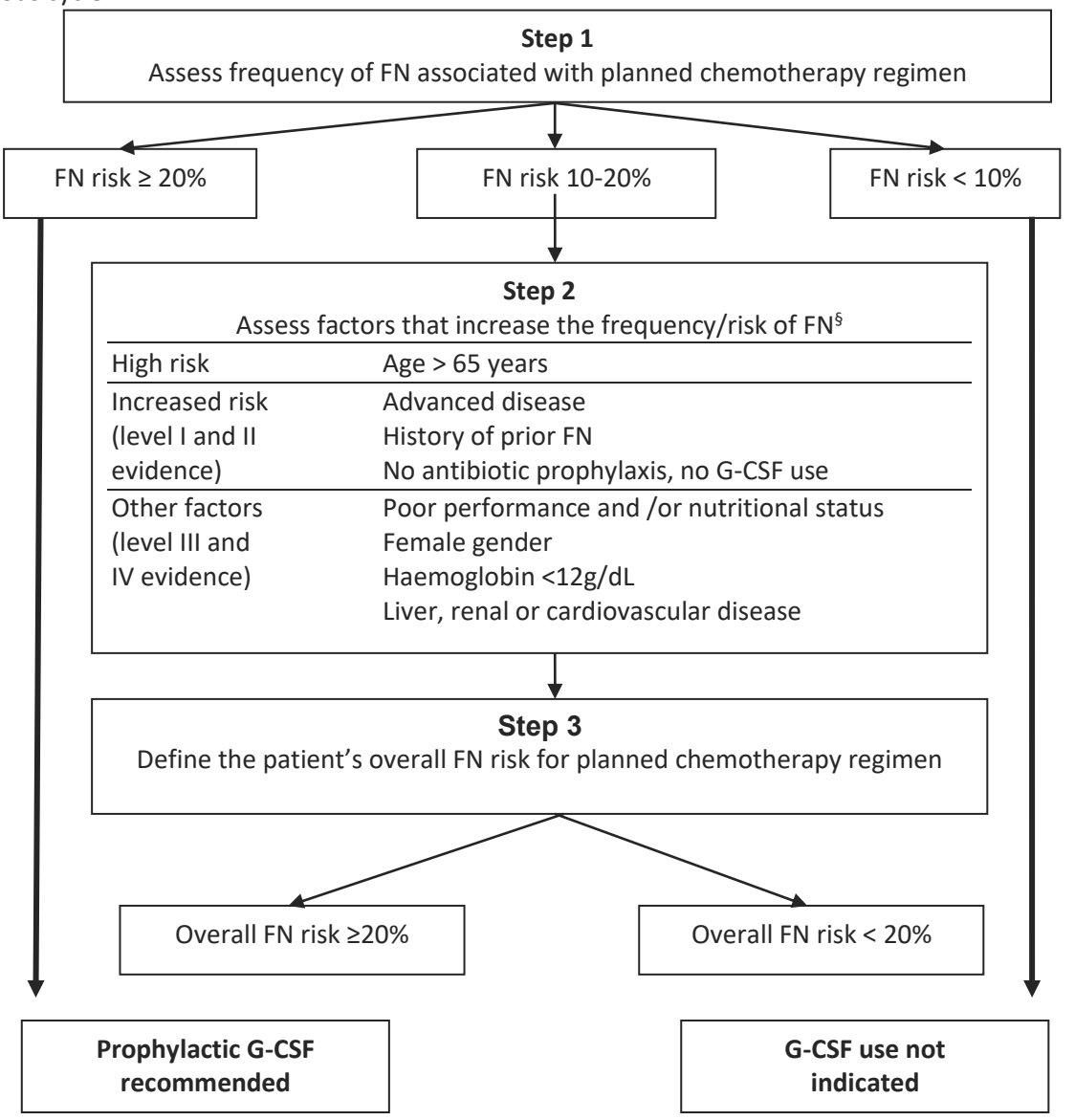
Immunoglobulin heavy-chain variable-region (IgV_H) gene mutation analysis will be performed as described in ***Dono M et al. J Immunol. 2000 Jun 1;164(11):5596-604***. Briefly, clonal IgVH rearrangements will be amplified from genomic DNA using 5' primers corresponding to the VH leaders or VH framework 1 region and 3' primers corresponding to the JH genes. PCR products will be sequenced directly by Sanger method using ABI prism 3100 Avant Genetic Analyser. Sequence analysis including identification of the closest germline gene counterpart, distribution and characteristics of somatic mutations will be performed using dedicated bioinformatic tools (IMGT V-QUEST, IgBlast).

APPENDIX O

Guidelines for the use of granulocyte colony stimulating factor

(source: Aapro MS, Bohlius J, Cameron DA, Dal Lago L, Donnelly JP, Kearney N, Lyman GH, Pettengell R, Tjan-Heijnen VC, Walewski J, Weber DC, Zielinski C: European Organisation for Research and Treatment of Cancer. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. Eur J Cancer. Jan;47(1):8-32 , 2011)

Algorithm to decide the use of primary prophylaxis of FN (Febrile neutropenia) with G-CSF (Granulocyte Colony Stimulating Factor). Start prophylaxis with G-CSF in the first cycle 24-72 hours after the end of the first cycle of chemotherapy and continue for subsequent cycles (when appropriate re-evaluated in each cycle). Secondary prophylaxis: start G-CSF if there was an event linked to neutropenia in the previous cycle.



§ The risk of febrile neutropenia associated with rituximab therapy bendamustine is considered 10%
(source: Knauf WU, Lissichkov T, Aldaoud A, Liberati A, Loscertales J, Herbrecht R,Juliusson G, Postner G, Gercheva L, Goranov S, Becker M, Fricke HJ, Huguet F, DelGiudice I, Klein P, Tremmel L, Merkle K, Montillo M: Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia. J Clin Oncol. Sep 10;27(26):4378-84, 2009)

APPENDIX P
Procedures for AE, SAE and SUSAR reporting

Patients will be instructed by the investigator to report the occurrence of any AE. The investigator assesses and records all AEs observed during the AE reporting period (i.e. from inclusion until 30 days after end of treatment).
AEs are coded with the NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.0 (appendix J) and assigned a grade (from 1 = mild to 5 = death related to AE) as well as a relationship (suspected vs not suspected) to trial treatment.
Any SAE must be reported within 24 hours (working days) by completing the "SAE Report form" and sending it by fax/email attachment to IELSG Study Coordination:

IELSG Study Coordination Oncology Institute of Southern Switzerland Ospedale San Giovanni CH - 6500 Bellinzona
Phone 0041 91 811 9040
Fax 0041 91 811 9182
E-mail ielsg@ticino.com

SAE reporting period is from inclusion until 30 days after end of treatment.
The SAE outcome must be reported within 2 weeks after definitive assessment by completing the "SAE Report form" and sending it by fax/email attachment again to IELSG Coordinating Center.

The investigator according to local regulations will inform local authorities (ethic committees). The physician responsible for patient care should organize any supplementary investigation of serious adverse events based on the clinical judgement on the likely causing factors. These means include seeking a further opinion from a specialist in the field of the adverse event. If a patient dies, any post mortem finding including histopathology must be provided.
The sponsor (IELSG) shall be responsible for ensuring that any SAEs are appropriately reported to the relevant health authorities according to applicable laws and regulations in each country where the Study will be conducted and to perform any additional activities.

APPENDIX Q

FDG-PET reviewing

In the literature, many published reviews and the guidelines by the main onco-hematological scientific associations consider it is still controversial to role the 18F-FDG PET in the staging/restaging of the MALT lymphomas, recommending a standard radiological approach with contrast enhanced CT.
However, recent clinical meta-analysis (Treglia G, Hematol Oncol 2015) and retrospective study (carrillo-Cruz E, Hematol Oncol) seems to indicate a potential clinical role of 18F-FDG PET or PET/CT in the initial evaluation of patients with SMZL.
In particular, the meta-analysis study adds significant novelty in this setting, underlining that MALT lymphomas are 18F-FDG-avid tumours in most of the cases, in particular in those with primary head-and-neck and bronchial locations.
Moreover, the retrospective study suggest that PET/CT has a high sensitivity in newly diagnosed patients with MZL and therefore can be useful in the staging of patients with nodal and extranodal subtypes. The technique is more sensitive than a CT scan at initial staging, mainly in cases with extranodal involvement. Although a larger number of patients will be required to further confirm these data, the study can conclude that PET/CT is a valuable imaging tool for both staging and response assessment in patients with NMZL and MALT lymphoma.

Also the analysis of clinical response conducted in IELSG36/BRISMA study has clearly demonstrated that the classic criteria based on physical examination and CT scan are far for being accurate. Particularly in patients showing a small splenomegaly as only sign of residual disease at the end of treatment it is very difficult differentiate complete responses from partial responses.

Due to these considerations, a centralized PET scan evaluation will be performed; the anonymized PET scan images will be collected and preserved for the entire duration of the study at the LYSARC (Resp. Romain Ricci, Coordinator Imaging LYSARC, CHU Henri Mondor Créteil, Service de Médecine Nucléaire).
The images will be evaluated by a group of expert nuclear medicine to analyze the clinical response of the patients (Dr. Salim Kanoun, Service de Medecine Nucleaire IUCT-ONCOPoLE, Institut C. Regaud, Toulouse, France and Dr. Luc Fornecker, Service d'Oncologie et d'Hématologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France).
The primary objective of this ancillary study was to evaluate the role of 18FDG/PET in SMZL at diagnosis for staging and as predictor of the treatment outcome including the response to treatment, the progression free survival and the time to next treatment.

The secondary objectives are the prognostic value of interim and post-R-benda PET and the prognostic value of the metabolic volume associated with biological markers.