



FLIRT

A RANDOMIZED PHASE III TRIAL EVALUATING TWO STRATEGIES OF RITUXIMAB ADMINISTRATION FOR THE TREATMENT OF FIRST LINE/LOW TUMOR BURDEN FOLLICULAR LYMPHOMA (FOLLICULAR LYMPHOMA IV/SC RITUXIMAB THERAPY)

A STUDY SPONSORED BY:

LYSARC

LYSARC: THE LYMPHOMA ACADEMIC RESEARCH ORGANISATION

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Version and date of Protocol: Version 2.2_201/07/2018

EudraCT number: 2014-000128-22

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PROTOCOL SIGNATURE PAGE

FLIRT

**A RANDOMIZED PHASE III TRIAL EVALUATING TWO ROUTES OF RITUXIMAB
ADMINISTRATION FOR THE TREATMENT OF FIRST LINE/LOW TUMOR BURDEN
FOLLICULAR LYMPHOMA**

(FOLLICULAR LYMPHOMA IV/SC RITUXIMAB THERAPY)

LYSARC



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1 SYNOPSIS

Study ID EudraCT N°	FLIRT 2014-000128-22
Title of the study	A RANDOMIZED PHASE III TRIAL EVALUATING TWO STRATEGIES OF RITUXIMAB ADMINISTRATION FOR THE TREATMENT OF FIRST LINE/LOW TUMOR BURDEN FOLLICULAR LYMPHOMA
Development phase of the study	III
Investigational products	Rituximab (Mabthera®) sub-cutaneous route and intravenous route
Protocol version	Version 2.2
Sponsor	LYSARC
Coordinating investigator Co-coordinating investigator	Pr Guillaume CARTRON Dr Delphine PRANGER
Centers	LYSA centers in France.
Study Objectives and endpoints	<p><u>Randomized cohort</u></p> <p><u>The primary objective</u> is to compare the efficacy of two therapeutic strategies (rituximab SC vs rituximab IV), based on the progression free survival (PFS) assessed according to response criteria for malignant lymphoma 1999 (Cheson 1999), in patients with previously untreated low tumor burden follicular lymphoma.</p> <p><u>Secondary objectives</u> are:</p> <ul style="list-style-type: none"> - To compare rituximab SC versus rituximab IV for the randomized cohort in terms of : <ul style="list-style-type: none"> • Overall survival • Response rate at M3 and M12 and best objective response (overall and complete response rates) • Molecular response (<i>BCL2-IGH</i> rearrangement) at M3 and M12 • Time to next anti-lymphoma treatment (TTNLT) - To describe the safety in both arms including cause of death and secondary cancers <p><u>Exploratory objectives</u> are:</p> <ul style="list-style-type: none"> • To analyze T-cell specific response against lymphoma • To evaluate the pharmacokinetics of rituximab and influence of tumor burden • To determine FcRn expression and polymorphism • To evaluate the predictive value of an integrated multi-parametric analysis including tumor genomic profiling, frequency of circulating t(14;18)^{pos} cells, genetic background (using GWAS analysis) and tumor microenvironment profiling (phenotype and function) in invaded lymph node and bone marrow • To correlate QPCR of circulating t(14;18) translocation and tumor burden assessed by CT scan or PET SCAN

	<p><u>Rituximab SC as of C1 cohort</u></p> <p>The exploratory objectives of the Rituximab SC as of C1 cohort are:</p> <ul style="list-style-type: none"> - to assess safety of rituximab given from the first administration - to evaluate pharmacokinetics of rituximab SC given from the first administration - to evaluate efficacy in terms of: <ul style="list-style-type: none"> • PFS • Overall survival • Response rate at M3 and M12 and best objective response (overall and complete response rates)
Study design	This study is a multicenter open-label randomized phase III trial
Duration of the study	The inclusion period is around 3 years. The patients will be followed at the latest 3 years after the last randomized patient. The total duration of the study is estimated to be 6 years at the latest.
Number of patients	<ul style="list-style-type: none"> - 202 subjects will be enrolled in the study in the Randomized cohort: 101 in rituximab IV arm (Arm A) and 101 in rituximab SC arm (Arm B). - 20 subjects will be enrolled in the Rituximab SC as of C1 cohort in few select centers.
Inclusion criteria	<p>Patients must satisfy all following criteria to be enrolled in the study:</p> <ul style="list-style-type: none"> • Histologically confirmed follicular lymphoma CD20+ grade 1, 2 and 3a by biopsy within 4 months before signing informed consent • Have a bone marrow biopsy within 4 months before the first study drug administration • Have no prior therapy except surgery for diagnosis • Aged 18 years or more with no upper age limit • ECOG performance status 0-2 • Ann Arbor Stage II, III or IV • Bi-dimensionally measurable disease defined by at least one single node or tumor lesion > 1.5 cm assessed by CT scan and/or clinical examination • With low-tumor burden defined as: <ul style="list-style-type: none"> - Nodal or extra-nodal mass with diameter less than 7 cm in its greater diameter - And involvement of less than 3 nodal or extra nodal sites with diameter greater than 3 cm - And absence of B symptoms - And no symptomatic splenomegaly - And no compression syndrome (ureteral, orbital, gastrointestinal...) - And no pleural or peritoneal serous effusion - And no cytopenia, with hemoglobin > 10 g/dL (6.25mmol/L), and absolute neutrophil count > 1.5 G/L and platelets > 100 G/L within 28 days before the randomization - And LDH < ULN within 28 days before the randomization - And $\beta 2$ microglobulin < ULN within 28 days before the

	<p>randomization.</p> <ul style="list-style-type: none"> • Have signed an informed consent • Must be covered by a social security system
Exclusion criteria	<p>Presence of any of the following will exclude a patient from enrollment:</p> <ul style="list-style-type: none"> • Grade 3b follicular lymphoma • Ann Arbor Stage I • Seropositive for or active viral infection with hepatitis B virus (HBV) defined as: <ul style="list-style-type: none"> - HBs Ag positive - HBs Ag negative, anti-HBs antibody positive and/or anti-HBc antibody positive and detectable viral DNA <p><i>Note:</i></p> <ul style="list-style-type: none"> - Patients who are HBs Ag negative, anti-HBs positive and/or anti-HBc positive but viral DNA negative are eligible. - Patients who are seropositive due to a history of hepatitis B vaccine are eligible. <ul style="list-style-type: none"> • Known seropositive for, or active viral infection with hepatitis C virus (HCV) • Known seropositive for, or active viral infection with Human Immunodeficiency virus (HIV) • Any of the following laboratory abnormalities within 28 days before the randomization: <ul style="list-style-type: none"> - Total bilirubin or GGT or AST or ALT > 3 ULN. - Calculated creatinine clearance (Cockcroft and Gault formula) < 60 mL /min • Presence or history of CNS involvement by lymphoma • Prior history of malignancies other than lymphoma (except for basal cell or squamous cell carcinoma of the skin or carcinoma in situ of the cervix or breast) unless the subject has been free of the disease for ≥ 3 years • Any serious medical condition, laboratory abnormality, or psychiatric illness that would prevent the subject from signing the informed consent form • Patient with mental deficiency preventing proper understanding of the informed consent and the requirements of treatment • Adult under law-control • Adult under tutelage • Contraindication to use rituximab or known sensitivity or allergy to murine products • Pregnant or lactating females • Concomitant disease requiring prolonged use of corticosteroids, or corticosteroids administration for lymphoma within 28 days before the first study drug administration • Male and female patients of childbearing potential who cannot or do not wish to use an effective method of contraception, during the study treatment and for 12 months thereafter

Study treatments	<p>✓ Randomized cohort:</p> <p>Arm A (Rituximab IV): Patient in arm A should receive:</p> <ul style="list-style-type: none"> • Rituximab (Mabthera®), 375 mg/m² at D1, D8, D15, D22 by intravenous route <p>Arm B (Rituximab SC): Patient in arm B should receive:</p> <ul style="list-style-type: none"> • Rituximab (Mabthera®): 375 mg/m² at D1 by intravenous route and 1400mg total dose at D8, D15, D22, M3, M5, M7, M9 by sub-cutaneous route <p>✓ Rituximab SC as of C1 cohort (in few selected centers):</p> <p>Patient should receive:</p> <ul style="list-style-type: none"> • Rituximab SC (Mabthera® SC): <ul style="list-style-type: none"> - For the patients 1 to 4 : 200mg at D1, 1200mg at D2, 1400mg total dose at D8, D15, D22, M3, M5, M7, M9 by sub-cutaneous route - For the patients 5 to 20 : 1400mg total dose at D1, D8, D15, D22, M3, M5, M7, M9 by sub-cutaneous route
Registration in the study / Randomization	<p>Once a patient signs informed consent, the subject may enter the screening period, which is permitted to last up to 28 days.</p> <p>During the screening period, the patient will undergo safety and other assessments to determine eligibility for the study and will be randomized to either experimental arm (rituximab SC) <i>versus</i> control arm (rituximab IV) or will be enrolled to rituximab SC as of C1 cohort.</p> <p>Randomization will be performed via a central randomization system.</p> <p>The stratification will be performed according to the following factor :</p> <p style="text-align: center;">FLIPI score at baseline (< 2 vs ≥ 2)</p>
Safety consideration	<ul style="list-style-type: none"> • AE of grade 2-5 for infections and neurological toxicities and AEs of grade 3-5 for other toxicities (CTCAE – v 4.03) regardless the relationship to IP occurring from the date of informed consent signature, during treatment administration period and up to 30 days after last drug administration, will be recorded on the AE pages of eCRF. <p>In addition, for the Rituximab SC as of C1 cohort the following will be recorded on the AE pages of the eCRF:</p> <ul style="list-style-type: none"> • all AE of grade 1-5 during cycle 1 and cycle 2 • Administration related reactions, ARR (any AE occurring during or within 24 hours of injection that were considered by the investigators to be related to rituximab) of grade 1-5 will be recorded for all cycles. • All events that meet one or more criteria of seriousness, regardless the relationship to IP, occurring from the date of the informed consent signature, during treatment administration period and up to 30 days after last drug administration, will be reported as SAE. <p>A SAE that occurs after this time, including follow up period, will be reported if considered related to IP.</p> <p>In a case of SAE the Investigator must immediately (within 24 hours):</p>

	<p>SEND the SAE pages to LYSARC Pharmacovigilance department: FAX: +33 (0) 3 59 11 01 86 OR EMAIL TO pharmacovigilance@lysarc.org</p> <p>Pregnancy and suspected pregnancy will be reported on Pregnancy Form to LYSARC PV from the date of informed consent signature and up to 12 months after the last study drug administration.</p>
<p>Statistical consideration</p>	<p style="text-align: center;">ANALYSIS SET</p> <p>The Intent-to-Treat (ITT) set includes all patients randomized regardless of study drug being received or not. Patients enrolled in the Rituximab SC as of C1 cohort won't be included in the ITT set.</p> <p>The Efficacy set includes all patients included in the ITT set with histopathologically confirmed follicular lymphoma by central review having:</p> <ul style="list-style-type: none"> - received at least one dose of rituximab <ul style="list-style-type: none"> o For patients randomized in the arm B, only patients who correctly switched to rituximab SC after one IV cycle will be included in this set - baseline tumor assessments - at least one post baseline tumor assessment <p>The Rituximab SC as of C1 set includes all patients treated in Rituximab SC as of C1 cohort.</p> <p>The safety set includes all patients who took at least one dose of study drug.</p> <p style="text-align: center;">SAMPLE SIZE CALCULATION</p> <p><i>Randomized cohort</i></p> <p><u>Hypothesis:</u></p> <ul style="list-style-type: none"> • alpha = 5% (two-sided), power = 90%, • an exponentially distributed PFS time with a constant hazard rate over the course of the study; • a median PFS of 23.5 months in the Rituximab IV arm (Colombat <i>et al.</i> 2012); • Hazard Ratio : 0.52 • an accrual rate of 70 patients/year; • an interim analysis for futility and efficacy when half of the events occurred • a total of 30-month enrolment period; • 5% drop-out rate / year; <p><u>Sample Size:</u></p> <p>Total patients to be randomized: 202 (101/arm), 102 events required.</p> <p><i>Rituximab SC as of C1 cohort</i></p> <p>No sample size calculation was done as no statistical hypotheses are tested. Descriptive statistics to be used for this cohort.</p>

ANALYSIS PLAN

The primary efficacy analysis will be based on the ITT set.

Treatment with rituximab sub-cutaneous (arm B) will be declared superior if the two-sided p-value from stratified log-rank test is < 0.05 . The stratification factor will be the one used for randomization (FLIPI score: < 2 vs ≥ 2). Supportive analyses will also be performed on unstratified log-rank test and on the Efficacy set.

Estimates of treatment effect will be expressed as hazard ratios including two-sided 95% confidence intervals. In addition Kaplan-Meier estimates of median progression-free survival as well as progression-free survival rates at different time points (1, 2, 3... years after randomization) with 95% confidence intervals will also be reported.

Secondary efficacy analyses will be conducted on the ITT set.

Secondary time to event endpoints will be analyzed by using a two-sided stratified log-rank test.

Response rates will be expressed with 95% confidence limits according to Pearson-Clopper method.

All efficacy analyses conducted on the Rituximab SC as of C1 set will be descriptive.

Randomized cohort**INTERIM ANALYSIS**

One interim analysis for futility and efficacy will be performed during the conduct of the study. The statistical evaluation will be done on the primary endpoint criterion. Futility and efficacy boundaries are based on O'Brien and Fleming alpha and beta spending function.

Stopping boundaries are based on O'Brien and Fleming alpha/beta spending function. See the following table for stopping boundaries details:

Look #	Info. Fraction (s/s_max)	Events (s)	Cumulative α Spent	Cumulative β Spent	Boundaries			
					Efficacy Z		Futility Z	
					Upper	Lower	Upper	Lower
1	0.5	51	0.003	0.011	2.963	-2.963	0.192	-0.192
2	1	102	0.05	0.098	1.969	-1.969	1.969	-1.969

- **Futility:** If the observed HR based on interim data is > 1.06 (rituximab SC vs rituximab IV – corresponding to $Z < -0.192$), the study will be recommended to terminate as it is highly unlikely to meet its primary objective.
- **Efficacy:** If the observed HR based on interim data is < 0.435 (rituximab SC vs rituximab IV – corresponding to $Z > 2.963$), the study will be recommended to terminate as it is statistically significant ($p < 0.003$).

The approximate timing of the interim analysis is after 50% (51 events in ITT set) of total number of planned events (102 events) which is projected to occur at about 35 months after the first patient is randomized.

	<p style="text-align: center;">PRIMARY ANALYSIS</p> <p>The primary PFS analysis will be realized when the number of progression/death events (102) has been reached in the ITT set or at the latest 3 years after last patient randomization. It is projected to achieve the 102 events at approximately 54 months after the first randomized patient.</p> <p><i>Rituximab SC as of C1 cohort</i></p> <p>The Rituximab SC as of C1 cohort will be analyzed at the same time as the randomized cohort. In addition, an interim analysis of Rituximab SC as of C1 cohort safety and pharmacokinetics data will be performed after 10 patients completed rituximab SC treatment in cycle 2.</p>
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3 LIST OF ABBREVIATIONS AND GLOSSARY OF TERMS

ABBREVIATION	TERM
AE	Adverse Event
AJCC	American Joint Committee on Cancer
ALT (SGPT)	ALanine Transaminase (Serum Glutamic Pyruvic Transaminase)
ANC	Absolute Neutrophil Count
ARR	Administration Related Reactions
AST (SGOT)	ASpartate Transaminase (Serum Glutamic Oxaloacetic Transaminase)
AUC	Area Under the Curve
βHCG	beta-Human Chorionic Gonadotropin
BLNI	British National Lymphoma Investigation
BSA	Body Surface Area
CBC	Complete Blood Cell
CD20	Antigen expressed on the surface of normal and malignant B lymphocytes
CFR	Code of Federal Regulations
CPC	Common Progenitor Cell
CR	Complete Response
CRF	Case Report Form
CRu	Complete Response unconfirmed
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
Ctrough	Trough concentration
D	Day
DSUR	Development Safety Update Report
ECOG	Eastern Cooperative Oncology Group
EFS	Event Free Survival
FCBP	Female of Childbearing Potential
FcRn	Neonatal Fc Receptor
FL	Follicular Lymphoma
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony-Stimulating Factor
GELF	Groupe d'Etude des Lymphomes Folliculaires
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
IP	Investigational Product
IRR	Infusion Related Reaction
IV	Intravenous

LDH	Lactic DeHydrogenase
LYSA	The Lymphoma Study Association
LYSARC	The Lymphoma Academic Research Organisation
M	Month
MDRD	Modification of Diet in Renal Disease
NCI	National Cancer Institute
NCIC CTG	National Cancer Institute of Canada - Clinical Trials Group
NHL	Non-Hodgkin's Lymphoma
ORR	Overall Response Rate
OS	Overall Survival
PD	Progressive Disease
PET	¹⁸ F-FDG Positron Emission Tomography
PFS	Progression Free Survival
PK	PharmacoKinetics
PR	Partial Response
PS	Performance Status
RR	Response Rate
SAE	Serious Adverse Event
SC	Sub-Cutaneous
SD	Stable Disease
SPM	Second Primary Malignancy
SUSAR	Suspected Unexpected Serious Adverse Reaction
SUVmax	Maximum Standardized Uptake Value
TMA	Tissue Micro Array
TTNT	Time To Next Treatment
ULN	Upper Limit of Normal
US	United States
WBC	White Blood Cell
WHO	World Health Organization
WW	Watch and Wait

4 RESPONSIBILITIES

4.1 Sponsor and program coordination center

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4.2 **Investigators**

All participating LYSA centers from France may include patients in this study. Before any inclusion, each center must be declared to the Ethical Committee and national competent authority according to each country regulations and have had the initiation visit. To be declared as a participating center, the principal investigator must send to the LYSARC all administrative documents required for regulatory submission (e.g. Curriculum vitae, for France the affiliation medical association number [CNOM or RPPS number] etc...).

4.3 **Laboratory sites**

Laboratories of each study center must provide their normal values and an updated accreditation for quality control.

5 **BACKGROUND AND STUDY RATIONALE**

5.1 **Feature of follicular lymphoma with low tumor burden**

Follicular lymphoma (FL) is the second most common non-Hodgkin's lymphoma. The majority of FL patients presents with advanced disease and many of them are asymptomatic at diagnosis. In contrast to advanced and symptomatic FL where the interest of immuno-chemotherapy (rituximab associated with chemotherapy regimen) followed by rituximab maintenance on survival has been demonstrated, the lack of interest for conventional chemotherapy in asymptomatic patients is well established. Thus, randomized trials conducted by GELF (Brice et al. 1997) and BNLI (Ardeshtna et al. 2003) clearly demonstrated no benefit for early treatment by chemotherapy compared to watch and wait strategy and treatment initiation when patients were symptomatic. Until the recent introduction of non-chemotherapy treatment, watch and wait policy was therefore considered as gold standard for asymptomatic FL patients; the criteria allowing to recognize patients with asymptomatic disease are well defined and used daily in clinical practice.

5.2 Genetic & microenvironment features in FL pathogenesis and response to treatment

The genetic hallmark of FL is the t(14;18) translocation associated with an overexpression of the anti-apoptotic protein BCL2 but this founder genetic event is detected at low frequency in the blood of most healthy individuals and additional driver genetic alterations are required to complete cell transformation (Roulland *et al* Adv Immunol 2011). Recently, genome-wide profiling has shed new lights on the mutational landscape in FL, highlighting FL clonal heterogeneity, and has delineated a hierarchical model of genetic events supporting FL tumorigenesis and transformation to an aggressive malignancy (Okosun *et al* Nat Genet 2014). Most importantly, the presence in “healthy” individuals/asymptomatic patients of common progenitor cells (CPCs) years before diagnosis has been also demonstrated (Roulland *et al* JCO 2014), CPCs that may reemerge and induce recurrent relapses under current therapeutic regimens. However, the very early steps of FL pathogenesis and the associated alterations remain poorly characterized, and no study has been designed to follow clonal selection during disease evolution and under treatment. Similarly, whereas susceptibility loci have been associated with the predisposition to FL or the transformation of FL into aggressive lymphomas (Vijai *et al* PLoS Genet 2013), such genome wide association studies (GWAS) should be extended to large series of patients with homogeneous treatment to further understand how these genetic factors could impact tumors and patient behavior.

Interestingly, several of the most common genetic alterations in FL are not oncogenic *per se* but are supposed to favor the crosstalk between malignant cells and their microenvironment (Ame-Thomas *et al* Semin Cancer Biol 2014). In agreement, FL B cells are found admixed with various non-malignant cell subsets, including stromal cells, macrophages and CD4^{pos} T cells, within invaded lymph nodes and bone marrow. Several studies have proposed a panel of prognostic biomarkers reflecting the number, activation and/or spatial distribution of infiltrating immune cells. Several recent studies have provided interesting cues illustrating the two faces of FL microenvironment; *i.e.* its capacity to exert anti-tumor activity by itself or by potentiating the efficacy of FL-targeting drugs, in particular rituximab, *versus* its capacity to favor directly and indirectly FL cell growth. A better understanding of how B cells co-opt their microenvironment to create conducive niches at the early stage of the disease, how this niche is modified after treatment and support FL relapse, and whether microenvironment-based biomarkers could predict patient behavior will be very useful to optimize patient treatment and follow-up.

5.3 Role of Rituximab in follicular lymphoma treatment

Rituximab (R) is an anti-CD20 monoclonal antibody (IgG1 κ) approved for the treatment of untreated and relapsed FL patients in association with chemotherapy (R-CHOP) and as maintenance therapy for those who respond to this immunochemotherapy. Rituximab has dramatically improved survival of patient in first line when associated with chemotherapy (Marcus *et al.* 2005, Hiddemann *et al.* 2005, Herold *et al.* 2007, Salles *et al.* 2008) and it has been demonstrated that rituximab maintenance every 2 months for 2 years increased significantly progression-free survival (Salles *et al.* 2010).

Rituximab has been tested as monotherapy in low-tumor burden FL. In a phase II study evaluating 50 low-tumor burden FL, the authors (Colombat *et al.* 2012) demonstrated that four weekly infusions of rituximab induced an high overall best response rate (ORR within a year) of 80% with 52% of patients in complete response/complete response unconfirmed (CR/CRu). With a median follow-up of 7 years, overall survival was 91.7% with a median progression-free survival (PFS) of 23.5 months. The quality of both molecular and clinical response seemed to influence PFS. In the RWW study, 463 patients were randomized to receive either four weekly infusions of rituximab (Rx4, arm stopped after 84 patients), four weekly infusions of rituximab followed by rituximab maintenance for 2 years (Rx4 +RM, 192 patients) or assigned to watch and wait approach (WW, 187 patients) (Ardeschna *et al.* 2010). Patients receiving rituximab exhibited significantly longer time to treatment initiation compared to patients receiving no initial treatment, with 48%, 80% and 90% of patients not requiring treatment at 3 years for WW, Rx4 and Rx4+RM arms, respectively. No influence on overall survival was observed even if the difference in term of time to

treatment initiation should translate into survival gain in future analysis. Nevertheless, previous phase II study and this randomized phase III study clearly demonstrated the interest of rituximab therapy, which did not modify quality of life in this later one. Low-tumor burden FL patients included in the RESORT trial received four weekly rituximab infusions (Khal *et al.* 2011). Responder patients were then randomized to receive either one rituximab infusion every 3 months for 2 years or rituximab four weekly infusions at relapse/progression. The authors demonstrated no advantage in terms of time to next treatment for the maintenance arm, whereas a significant advantage was found for time to initiation of cytotoxic treatment. These three studies using rituximab in low-tumor burden FL clearly demonstrated the interest of rituximab in terms of time to next treatment and progression-free survival and four weekly infusions of rituximab could be considered as a new standard of treatment for this population of patients.

5.4 Rituximab mechanisms of action

Mechanisms of rituximab action in humans remain largely unknown but experimental data clearly showed that rituximab is able to induce direct caspase-dependant cell death and Fc mediated mechanisms including complement dependent cytotoxicity, antibody dependent cellular cytotoxicity and phagocytosis (Cartron *et al.* Blood 2004). By pharmacogenomics approach the role of Fc γ R11a expressing cells in rituximab activity has been clearly demonstrated (Cartron *et al.* Blood 2002). Homozygous patients for Fc γ R11a-158V allele, exhibiting high affinity for the Fc portion, had higher response rate compared to Fc γ R11a-158F carrier patients, that allele having low affinity for the Fc portion. Because NK cells and macrophages expressed Fc γ R11a, we hypothesized that ADCC is one of the main mechanisms of rituximab action. More recently, T $\gamma\delta$ were reported to express high amount of Fc γ R11a in patients already infected by CMV, raising the question of their role in rituximab activity (Couzi *et al.* Blood 2012). It has been also suggested that rituximab was able to induce specific immunity against unknown lymphoma antigens in humans (Cartron *et al.* Blood 2004). Some recent results obtained in a murine model plaid also for such mechanism and identified CD20 itself as a potential immune target (Abes *et al.* 2010). These results are especially important because FL has a natural course characterized by remissions followed by relapses until the death of patient, emphasizing the need of a better long-term control of the disease. Dendritic cells (DC) are the main actors of adaptive immunity and they express Fc γ R11a receptor. Experimental data have demonstrated that anti-tumor monoclonal antibodies enhance cross-presentation of cellular antigen and the generation of tumor specific killer T-cells by dendritic cells (Dhodapkar *et al.* 2002). Monoclonal antibodies could therefore induced adaptive immunity against FL cells and the use of new modalities of rituximab administration or addition of anticancer therapies which potentially improve specific immunity induce by rituximab represents a great opportunity to avoid recurrent relapses.

A pivotal phase II study demonstrated that responses to rituximab (Mc Laughlin *et al.* 1998) observed in indolent lymphomas correlated with rituximab exposure. Patients experiencing the best response had the highest rituximab exposure (Berinstein *et al.* 1999). They demonstrated a large variability of rituximab exposure among a population of patients with indolent lymphoma receiving 4 weekly infusion of rituximab. Thus, a median rituximab concentration at 3 months of 25.4 μ g/mL (C_{trough}) was found in responder-patients and of 5.9 μ g/mL in non-responder patients. Authors have however failed to demonstrate an advantage for a PK-guided rituximab retreatment in order to optimize rituximab exposure (Gordan *et al.* 2005). Another approach is to increase rituximab dosing regimen for all patients making it possible to optimize rituximab concentrations in most of patients. Piro *et al.* (Piro *et al.* 1999) proposed 8 infusions of rituximab at standard dose in a phase II trial. Overall response rate was similar to the one obtained in pivotal phase II but complete response seemed to increase. The lack of a clear advantage of these two strategies is probably related to the fact that rituximab exposure is influenced itself by others factors such as histology, lymphocyte count or tumor burden and an individualized dosing regimen should take into account all these parameters and even those currently unknown. To include all these factors we have proposed a model-based design of rituximab dosage optimization in follicular non-Hodgkin's lymphoma. According to this model we clearly demonstrated that PFS at 2 years increased with the dose of rituximab

for both induction and maintenance with an optimal dose around 1500 mg/m² (Ternant *et al.* 2011). Thus both clinical and biological data show that current dose of 375 mg/m² is not adapted to obtain optimal response in all patients. There is a need to optimize rituximab dosing regimen in order to obtain optimal rituximab exposure for most of patients.

5.5 Subcutaneous administration of rituximab

Rituximab is the first anti-CD20 monoclonal antibody approved for the treatment of diffuse large B-cell lymphoma, follicular lymphoma and chronic lymphocytic leukemia. Rituximab is then used either alone or in combination with chemotherapy. The administration of rituximab was until recently performed only by intravenous route. This induces so-called "first infusion" or IRR side effects, most often associating fever, chill, and hypotension. These side effects are present almost exclusively during the first infusion and are usually grade 1-2 (5% of grade \geq 3; Marcus *et al.* 2017).

More recently, Roche has developed a formulation for a subcutaneous (SC) administration of rituximab (Davies A *et al.* 2017; Lugtenburg P *et al.* 2017, Salar 2014). The development was designed to simplify the administration of rituximab and could reduce the burden to patients and healthcare systems compared to rituximab IV. Nevertheless, in the development of the subcutaneous formulation of rituximab, the first administration is performed intravenously, followed by subcutaneous injections from second administration. The main reasons for having an intravenous infusion at the first cycle at time of the rituximab SC development program were:

- the well-known practice of managing IRRs by slowing down or interrupting the intravenous infusion
- the lack of experience with rituximab SC and the unknown PK profile
- Unknown profile of the administration-related reactions (ARRs) following Rituximab SC administration

The cause of IRR has long remained unrecognized, initially attributed to complement activation, it was originally described with other monoclonal antibodies such as alemtuzumab. Surprisingly, in this latter situation, one way to reduce IRR has been subcutaneous administration (Ludin *et al.* 2002; Patel K *et al.* 2016). More recently, the development of another anti-CD20 called obinutuzumab, which is associated with a higher frequency of IRR (10.6% of grade \geq 3; Marcus *et al.* 2017) has allowed to better understand the mechanism of occurrence of these IRR. Indeed, these appear to be directly related to the release of cytokines (IL6, IL10, TNF) and their intensity and frequency correlated with the level of expression of CD20 by tumor cells, and by the level of expression of CD16, in particular of its CD16-158V allotype by the effector cells (Freeman *et al.* 2015; Freeman *et al.* 2016). In other words, the IRR induced by obinutuzumab (glyco-modified antibody for a better affinity with CD16 and therefore a better ADCC) are related to activation of ADCC inducing cytokine release by the effector cells. In all, the data available today, whether historical (alemtuzumab) or more recent (with rituximab and obinutuzumab), suggest that the subcutaneous administration of a monoclonal antibody could potentially reduce the incidence and the severity of the IRRs

The pharmacokinetic analysis for subcutaneous administration showed a slow systemic uptake with C_{max} reached after ~72 hours compared to IV infusion where C_{max} is reached at the end of infusion, after

approximately 5 hours (Davies *et al.* 2017). This slow increase in rituximab concentration after SC administration is mimicking a very slow infusion of rituximab and is expected to allow management of ARRs. Equivalent area under the inter-cycle curve (AUC) between the two routes of administration is obtained (Salar *et al.* 2014).

The FLIRT trial, promoted by LYSA is including patients with asymptomatic follicular lymphoma, with a low tumor mass according to criteria established in our previous trials. In this trial, we are trying to demonstrate that the subcutaneous formulation of rituximab could have, in addition to an interest for the patient in terms of comfort, a potential interest in terms of activation of anti-lymphomatous immunity. An important biological study including pharmacokinetics and immunological analyses is an integrated part of the FLIRT study. This trial therefore randomized the standard regimen of 4 weekly injections at 375 mg/m² of rituximab iv versus an experimental treatment comprising 1 intravenous rituximab injection at 375 mg/m² followed by 3 subcutaneous injections at 1400 mg (ie a total of 4 weekly doses) followed by a short maintenance treatment consisting of 4 injections of 1400 mg subcutaneous rituximab at M3, M5, M7 and M9. To date the study has included 159 patients out of the expected 202.

The latest available data in the literature clearly show the preference of patients for the administration of the drug subcutaneously (Rummel *et al.* 2017). It also offers opportunities for treatment outsourcing, which is currently an important public health goal. We therefore believe that the FLIRT trial is an opportunity to evaluate the feasibility of subcutaneous administration already from the first cycle of treatment. We believe that the characteristics of patients (low tumor mass, indolent histology) will reduce any potential risks associated with rituximab SC administration from first treatment cycle. To ensure patient safety during the first SC administration, specific surveillance measures have been put in place and a pharmacokinetic analysis is planned. Finally, in order to better understand the clinical parameters that could modify the pharmacokinetics of rituximab during this first injection, the measurement of the tumor volume (reflection of the antigenic mass) will be evaluated. For methodological reasons and in order to not modify the main objective and the statistical power of the FLIRT study, introduction of a new cohort with additional patients will be a guarantee for the quality of the data obtained in FLIRT study ('Randomized cohort') and this additional arm ('Rituximab SC as of C1 cohort').

5.6 Hypothesis

We hypothesize that the use of rituximab by sub-cutaneous route could optimize rituximab exposure leading to improve response rate and that sub-cutaneous route as well as reinfusion every 2 months for 4 infusions could increase adaptive response and therefore improve long-term control disease.

6 STUDY OBJECTIVES

6.1 Primary Objective

The primary objective is to compare the efficacy of therapeutic strategies (rituximab SC vs rituximab IV), based on the progression free survival (PFS) assessed according to response criteria for malignant

lymphoma 1999 (Cheson 1999), in patients with previously untreated low tumor burden follicular lymphoma.

6.2 Secondary objectives

Secondary objectives are

- To compare rituximab SC versus rituximab IV for the randomized cohort in terms of:
 - Overall survival
 - Response rate at M3 and M12 and best objective response (overall and complete response rates)
 - Molecular response (*BCL2-IGH* rearrangement) at M3 and M12
 - Time to next anti-lymphoma treatment (TTNLT)
- To describe the safety in both arms including cause of death and secondary cancers

6.3 Exploratory objectives

Exploratory objectives are:

Randomized cohort

- To analyze T-cell specific response against lymphoma before and after rituximab treatment in 10 patients in arm B (proof-of-principle study), with a specific focus on the CD20 molecule itself as a potential target antigen.
- To evaluate the pharmacokinetics of rituximab and influence of tumor burden
- To determine FcRn expression on FL microenvironment *in situ* (bone marrow and lymph nodes) and in peripheral blood by flow cytometry and immunofluorescence studies and to analyze FcRn polymorphism and its correlation with pharmacokinetics study
- To evaluate the predictive value of an integrated multi-parametric analysis including tumor genomic profiling, frequency of circulating t(14;18)^{pos} cells, genetic background (using GWAS analysis), and tumor microenvironment profiling (phenotype and function) in invaded lymph node and bone marrow
- To correlate QPCR of circulating t(14,18) translocation and tumor burden assessed by CT scan or PET SCAN

Rituximab SC as of C1 cohort

- To assess safety of rituximab SC given from the first administration
- To evaluate pharmacokinetics of rituximab SC given from the first administration
- To evaluate efficacy in terms of PFS, OS, response rate at M3 and M12 and best objective response (overall and complete response rates) of rituximab SC given from the first administration

7 STUDY DESIGN

This study is a multicentric, open label trial.

Two types of cohort are analyzed in the study, one with a randomization (Randomized cohort) and another one without randomization (Rituximab SC as of C1 cohort).

Randomized cohort: phase III trial.

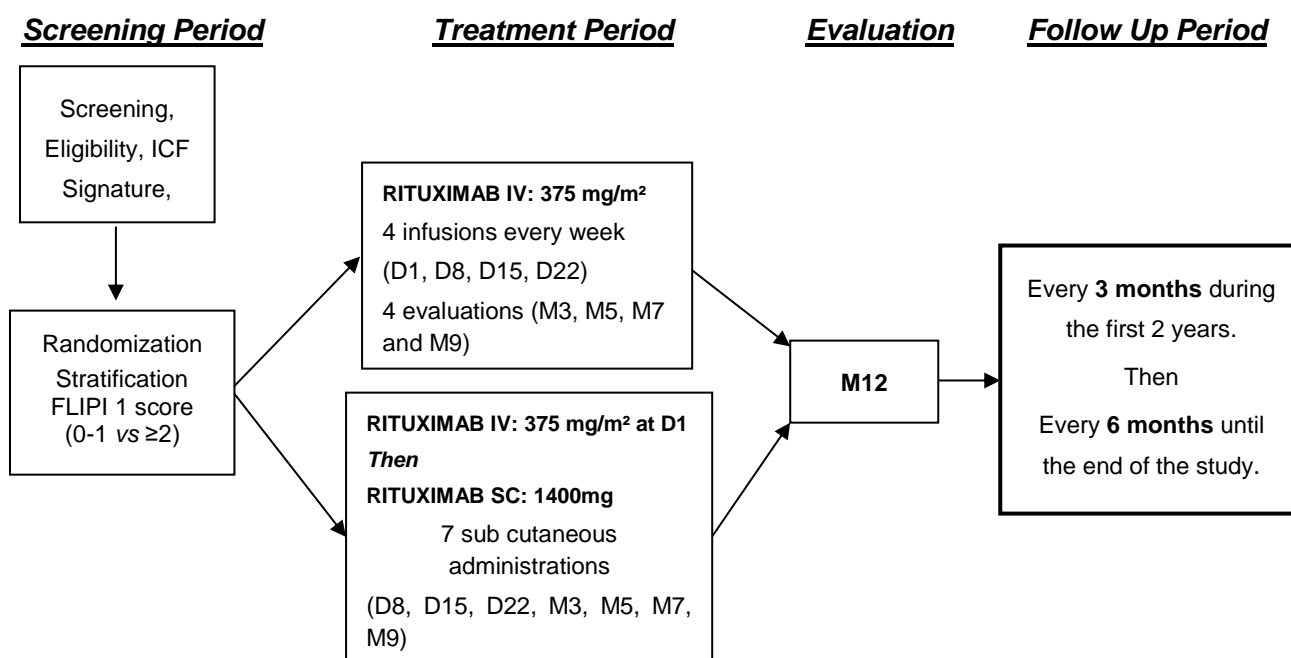
Patients will be recruited over 3 years and followed at the latest 3 years after the last patient is randomized or when 102 events (progressions/deaths from any cause) have been reached. The total duration of the study is around 6 years.

The anticipated study dates (start / end) are:

- 1st patient randomized (FPFV): Q1 2015
- Last patient randomized (LPFV): Q4 2018
- Last patient followed for primary analysis: Q4 2021

It is expected that a total of 202 patients will be randomized in the study.

The duration of the treatment period is approximately 3 weeks for arm A and 9 months for arm B.

**Rituximab SC as of C1 cohort:**

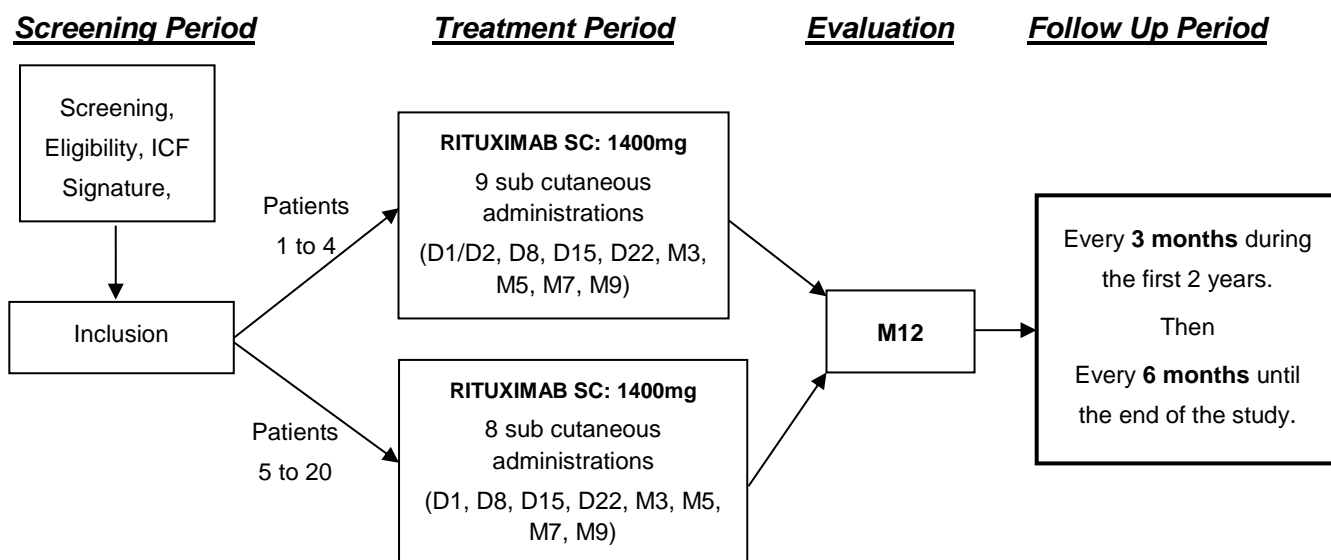
In few selected centers, patients are not randomized but are included in an additional Rituximab SC as of C1 cohort; patients will be enrolled over 6 months and followed for at the latest 3 years after the last patient is recruited.

The anticipated study dates (start / end) are:

- 1st patient enrolled (FPFV): Q1 2018
- Last patient enrolled (LPFV): Q4 2018
- Last patient followed: Q4 2021

It is expected that a total of 20 patients will be enrolled in the cohort.

The duration of the treatment period is approximately 9 months.



Enrollment of the first patients will be progressive until patient 4:

- Patient 2 could be enrolled from D6 of C1 of Patient 1 if safety allows
- Following patients will be enrolled from D2 of C1 of the previous patient if safety allows
- In case of related AE Grade >3 in patient 1-4, the next patient for first dosing will be delayed until the resolution of the AE in the previous patient

Enrollment of patients 5 to 20 depend on the AE profile observed in the first 4 patients (refer to paragraph 12)

In case of related Grade 5 AE, further enrollment of patients in this cohort will be suspended. The Safety Review Committee will review the case of related Grade 5 AE and will make a decision on further enrollment of patients in the experimental cohort.

End of study: End of study is defined by the last visit planned by the protocol of the last patient in follow-up.

8 STUDY POPULATION

Patients, aged at least 18 years, must have an investigator-assessed diagnosis of Ann Arbor stage II, III, IV follicular lymphoma grade 1 – 3a and have not been previously treated for their lymphoma.

8.1 Inclusion criteria

Patients must satisfy all of the following criteria to be enrolled in the study:

- Histologically confirmed follicular lymphoma CD20+ grade 1, 2 and 3a by biopsy within 4 months before signing informed consent
- Have a bone marrow biopsy within 4 months before the first study drug administration
- Have no prior therapy except surgery for diagnosis
- Aged 18 years or more with no upper age limit
- ECOG performance status 0-2
- Ann Arbor Stage II, III or IV
- Bi-dimensionally measurable disease defined by at least one single node or tumor lesion > 1.5 cm assessed by CT scan and/or clinical examination
- With low-tumor burden defined as:

- Nodal or extra-nodal tumor mass with diameter less than 7 cm in its greater diameter
- And involvement of less than 3 nodal or extra nodal sites with diameter greater than 3 cm
- And absence of B symptoms
- And no symptomatic splenomegaly
- And no compression syndrome (ureteral, orbital, gastrointestinal...)
- And no pleural or peritoneal serous effusion
- And no cytopenia, with hemoglobin > 10 g/dL (6.25mmol/L) and absolute neutrophil count > 1.5 G/L and platelets > 100 G/L within 28 days before the randomization
- And LDH < ULN within 28 days before the randomization
- And $\beta 2$ microglobulin < ULN within 28 days before the randomization
- Have signed an informed consent
- Must be covered by a social security system

8.2 Exclusion criteria

Presence of any of the following will exclude a patient from enrollment:

- Grade 3b follicular lymphoma
- Ann Arbor Stage I
- Seropositive for or active viral infection with hepatitis B virus (HBV)
 - HBs Ag positive
 - HBs Ag negative, anti-HBs antibody positive and/or anti-HBc antibody positive and detectable viral DNA

Note:

- Patients who are HBs Ag negative, anti-HBs positive and/or anti-HBc positive but viral DNA negative are eligible
- Patients who are seropositive due to a history of hepatitis B vaccine are eligible
- Known seropositive for, or active viral infection with hepatitis C virus (HCV)
- Known seropositive for, or active viral infection with Human Immunodeficiency Virus (HIV)
- Any of the following laboratory abnormalities within 28 days before the randomization:
 - Total bilirubin or GGT or AST or ALT > 3 ULN.
 - Calculated creatinine clearance (Cockcroft and Gault formula) < 60 mL /min
- Presence or history of CNS involvement by lymphoma
- Prior history of malignancies other than lymphoma (except for basal cell or squamous cell carcinoma of the skin or carcinoma in situ of the cervix or breast) unless the subject has been free of the disease for ≥ 3 years
- Any serious medical condition, laboratory abnormality, or psychiatric illness that would prevent the subject from signing the informed consent form.
- Patient with mental deficiency preventing proper understanding of the informed consent and the requirements of treatment.
- Adult under law-control
- Adult under tutelage
- Contraindication to use rituximab or known sensitivity or allergy to murine products
- Pregnant or lactating females.

- Concomitant disease requiring prolonged use of corticosteroids or corticosteroids administration for lymphoma within 28 days before the first study drug administration.
- Male and female patients of childbearing potential who cannot or do not wish to use an effective method of contraception, during the study treatment and for 12 months thereafter.

9 STUDY FLOW CHART AND SCHEDULE OF ASSESSMENTS

9.1 Study flow chart

See on **Appendix 1**.

9.2 Informed consent

To participate in this study and before any baseline or screening evaluation (except biopsies and imaging done in routine), a specific written informed consent must be signed by each patient, either for the randomized cohort or for the rituximab SC as of C1 cohort.

An additional written informed consent for biological studies (biology and genetics), only applicable for randomized patients, must be signed before any sample procedure.

These consents are signed after the investigator gave to the patient all required information.

The patient and the investigator will date and sign 2 original copies of each informed consent form, one will be provided to the patient and one will be maintained in the Investigator's Study File.

The investigator will attest on eCRF that the patient has signed and dated informed consent forms.

9.3 Baseline examination

The subject's eligibility is evaluated during the baseline period prior to the first administration of the study drug. The assessments are conducted **within 28 days** (except for biopsies) before the first administration of the study treatment:

- Tumor biopsy, assessed within 4 months before signing Informed Consent Form, available for centralized pathology review
- Age, gender, weight, height, body surface area (BSA) (**see Appendix 6: Mosteller Formula**)
- ECOG Performance Status (**see Appendix 7 "Performance Status Criteria"**)
- B symptoms (**see Appendix 4**)
- Clinical examination (including tumor assessment)
- Staging (**see Appendix 3 "Ann Arbor staging"**) including FLIPI 1 and FLIPI 2 (**see Appendix 5**)
- Relevant medical history
- Concomitant medication at inclusion
- History of the NHL. A tumor biopsy and a bone marrow biopsy must be available during the trial for confirmation of the disease (see section 11.3)
- Hemoglobin, WBC count with differential, platelets count
- Biochemical tests:

✓ Serum creatinine and creatinine clearance according to Cockcroft-Gault formula (**CrCl**):

Serum creatinine units mg/dL => for females, the formula is multiplied by 0.85.

➤ $CrCl \text{ (mL/min)} = [(140 - \text{age (years)}) \times (\text{weight [kg]})] / [72 \times (\text{serum creatinine [mg/dL]})];$

Serum creatinine units $\mu\text{mol/L}$ => A = 1.23 for men and A = 1.04 for females.

➤ $\text{CrCl (mL/min)} = [(140 - \text{age (years)}) \times (\text{weight [kg]}) \times \text{A}] / (\text{serum creatinine } [\mu\text{mol/L}]);$

Creatinine clearance should be determined utilizing actual body weight or ideal body weight, whichever is less (Cockcroft 1976, Luke 1990).

- ✓ AST, ALT, GGT, total and indirect bilirubin and alkaline phosphatase
- Lactate dehydrogenase (LDH) and β_2 microglobulin levels
- Serum electrophoresis, serum levels of IgG, IgM, IgA.
- HIV (1-2), HBV (Ag HBs, Ac anti-HBs, Ac anti-HBc and viral DNA) and HCV (Ac anti-HCV) serologies
- Pregnancy test (β HCG) for female with childbearing potential (FCBP)
- Whole-Body CT scan (neck, thorax, abdomen, pelvis) (with IV contrast) with bi-dimensionally tumor assessment. In case of contraindication of IV contrast, MRI is accepted.
- Cerebral CT scan if clinically indicated
- FDG-PET scan (PET0) performed within 28 days before the first study drug administration
- Bone marrow biopsy and bone marrow aspiration (*BCL2-IGH* rearrangement) within 4 months before the first study drug administration for local diagnosis
- Samples for biological studies will be collected after signature of a specific biological consent (for randomized cohort only, not for rituximab SC as of C1 cohort) (**see Appendix 11**) :
 - ✓ Blood samples and bone marrow for biological studies: flow cytometry, plasma storage, cell freezing for further functional studies.
 - ✓ Blood and bone marrow samples for genetic analyzes (specific genetic consent): analysis of susceptibility loci, t(14;18) quantification, FcRn polymorphism, HLA typing, and tumor cell genetic heterogeneity.

9.4 Evaluation during treatment for arm B and during treatment and first year of follow up for arm A (Randomized cohort):

D1, D8, D15, D22, M3, M5, M7, M9: Before each administration of rituximab or visit (for A arm):

Assessments should be performed as follows (except for pharmacokinetics):

- D8, D15, D22: +/- 1 day
 - Physical examination, ECOG Performance Status, B symptoms
 - Hemoglobin, WBC count with differential, platelets count
- M3, M5, M7 and M9: +/- 1 week
 - Physical examination, ECOG Performance Status, B symptoms, weight
 - Hemoglobin, WBC count with differential, platelets count
 - Creatinine and creatinine clearance according to Cockcroft-Gault formula (**CrCl**)
 - Total and indirect bilirubin, AST, ALT, GGT
 - LDH and β_2 microglobulin levels
- Pharmacokinetics : Blood sample BEFORE rituximab (H0) and 2 HOURS AFTER the end of rituximab administration (H2)
 - Arm A: D1, D8, D15, D22 (H0 and H2) and M3 (H0 only)

- Arm B: D1, D8, D15, D22 (H0 and H2)
M3, M5, M7 and M9 (H0 only)
- Adverse events and Serious Adverse Events
- Concomitant therapy related to AE

At M3 (+/- 1 week):

- Whole-Body CT scan (neck, thorax, abdomen, pelvis) (with IV contrast) with bi-dimensionally tumor assessment (MRI if performed at baseline.)
- Evaluation of the disease response (Cheson 1999)
- Bone marrow trephine biopsy for local diagnosis in all patients in CR/CRu assessed by CT scan (if bone marrow involved or not done or unspecified at baseline). Bone marrow biopsy can be performed until M4.
- Blood samples for biological studies: flow cytometry, plasma storage, cell freezing for further functional studies.
- Blood sample for genetic analysis (specific genetic consent): t(14;18) quantification, and tumor cell genetic heterogeneity.

9.5 Evaluation during treatment for rituximab SC as of C1 cohort

Patients 1 to 4 have to be monitored for 24h after the first injection with an observation overnight. The patients will be monitored for at least 4 hours after the second split dose before patient can return home.

A safety review committee meeting will be scheduled after the 4th patient has completed cycle 2. The split dose over D1 and D2 and the additional observations (hospitalization and FU phone calls) will be removed if safety profile of the first 4 patients allows. Thereafter patients 5 to 20 will be monitored 12 hours after the first injection (1400mg).

D1, D2 (patients 1 to 4), D8, D15, D22, M3, M5, M7, M9: Before each administration of rituximab:

Assessments should be performed as follows:

- D2, D4, D5 (only for patients 1 to 4)
 - Physical examination, ECOG Performance Status, B symptoms
- D3 (only for patients 1 to 4)
 - Additional follow up performed by phone call
- D8, D15, D22: +/- 1 day
 - Physical examination, ECOG Performance Status, B symptoms
 - Hemoglobin, WBC count with differential, platelets count
- M3, M5, M7 and M9: +/- 1 week
 - Physical examination, ECOG Performance Status, B symptoms, weight
 - Hemoglobin, WBC count with differential, platelets count
 - Creatinine and creatinine clearance according to Cockcroft-Gault formula (CrCl)
 - Total and indirect bilirubin, AST, ALT, GGT
 - LDH and β_2 microglobulin levels

- **Pharmacokinetics** : Blood sample BEFORE rituximab (H0)
 - **Patients 1 to 4 (split dose)**: D1, D2, D4, D5, D8, D11, D15, D22, M3, M5, M7 and M9 (all samples to be taken pre-dose rituximab except D4, D5 and D11)
 - **Patients 5 to 20 (full dose)**: D1, D4, D8, D11, D15, D22, M3, M5, M7 and M9 (all samples to be taken predose except D4 and D11)
- Adverse events and Serious Adverse Events (refer to paragraphs 14.2 and 14.3)
- Concomitant therapy related to AE

At M3 (+/- 1 week):

- Whole-Body CT scan (neck, thorax, abdomen, pelvis) (with IV contrast) with bi-dimensionally tumor assessment (MRI if performed at baseline.)
- Evaluation of the disease response (Cheson 1999)
- Bone marrow trephine biopsy for local diagnosis in all patients in CR/CRu assessed by CT scan (if bone marrow involved or not done or unspecified at baseline). Bone marrow biopsy can be performed until M4.

9.6 M12 and permanent treatment discontinuation evaluations

Patients, who have completed the treatment, will be evaluated at M12 (between 2 and 4 months after M9).

Patients who have withdrawn from the study will be evaluated within 4 weeks at the latest after the withdrawal.

- Physical examination, ECOG Performance Status, B symptoms
- Concomitant therapy related to AE
- Hemoglobin, WBC count with differential, platelets count
- LDH and β_2 microglobulin levels
- Serum electrophoresis, serum levels of IgG, IgM, IgA
- Whole-Body CT scan (neck, thorax, abdomen, pelvis) (with IV contrast) with bi-dimensionally tumor assessment (MRI if performed at baseline)
- FDG-PET scan
- Evaluation of the disease response (Cheson 1999 and 2014)
- Bone marrow trephine biopsy and aspiration for local analysis in CR/CRu patients assessed by CT scan if bone marrow histologically involved (or not done or unspecified) at baseline and/or M3 and bone marrow aspiration for local analysis only in CR/CRu /PR patients assessed by CT scan without histological bone marrow involvement at baseline but with *BCL2-IGH* rearrangement in bone marrow
- Any other evaluations or procedures performed at baseline for evaluation of the disease response
- Adverse events and Serious Adverse Events
- Blood samples for biological studies at M12 and at permanent treatment discontinuation in case of relapse or progression: flow cytometry, plasma storage, cell freezing for further functional studies (for randomized cohort only, not for rituximab SC as of C1 cohort)

- Blood sample for genetic analysis at M12 and at permanent treatment discontinuation in case of relapse or progression (specific genetic consent): t(14;18) quantification, and tumor cell genetic heterogeneity (for randomized cohort only, not for rituximab SC as of C1 cohort)
- Bone marrow sample in all patients in CR/CRu assessed by CT scan (if bone marrow involved or not done or unspecified at baseline) and in CR/CRu /PR patients assessed by CT scan without histological involvement at baseline but with *BCL2-IGH* rearrangement in bone marrow, for both: flow cytometry, cell freezing for further functional studies, t(14;18) quantification, and tumor cell genetic heterogeneity.
- Blood samples for pharmacokinetics for patient who withdraw from the study in case of relapse or progression.

9.7 Follow-up assessments

The patients will be followed at the latest for 3 years after the last randomized patient. Thereafter, the long term follow-up of patients will be organized for further analysis.

During the first 2 years of follow-up: (Year 2 and Year 3):

Every 3 months:

- Physical examination, ECOG Performance Status, B symptoms
- Hemoglobin, WBC count with differential, platelets count
- LDH and β_2 microglobulin levels
- Serum electrophoresis, serum levels of IgG, IgM, IgA

Every 6 months:

- Whole Body CT scan (neck, thorax, abdomen, pelvis), with IV contrast in absence of contraindication (MRI if performed at baseline)

During the 3 following years: (Year 4, Year 5 and Year 6): Every 6 months

- Physical examination, ECOG Performance Status, B symptoms
- Whole Body CT scan (neck, thorax, abdomen, pelvis), with IV contrast in absence of contraindication (MRI if performed at baseline)

Any other evaluations or procedures for evaluation of the treatment response

Patients who withdraw from the study treatment or patients who progress or receive new treatment after treatment period should be followed according to protocol requirements.

9.8 Progression/relapse

Progression/relapse will be determined as per Cheson 1999 criteria (**see Appendix 8** “Response criteria for lymphoma”).

Progressive disease should be based on CT scan and/or clinical examination. After progression, patients will be followed every 6 months during the first 2 years and yearly thereafter.

A pathological confirmation by biopsy of the lesion should be done if possible.

10 TREATMENTS

10.1 Drugs description, storage and handling

Rituximab IV and rituximab SC will be supplied by Roche and labeled as IP.

10.1.1 **Description**

Rituximab is a highly purified 1328–amino acid antibody with an approximate molecular weight of 145 kd. The chimeric mouse/human anti-CD20 antibody is a glycosylated IgG1 κ immunoglobulin containing murine light- and heavy-chain variable regions and human γ 1 heavy-chain and κ light-chain constant regions.

Rituximab IV: Rituximab is formulated for intravenous administration as a sterile product in 9.0 mg/mL sodium chloride, 0.7 mg/mL polysorbate 80, 7.35 mg/mL sodium citrated hydrate, and Sterile Water for Injection (pH 6.5). Rituximab will be supplied in 100 mg (10-mL) or 500 mg (50-mL) vials. The rituximab IV dose will be fixed at 375 mg/m² for all patients (depending on the patient's body surface area [BSA]).

Rituximab SC: Rituximab is formulated for subcutaneous administration. Rituximab SC contains rituximab at a concentration of 120 mg/mL and human recombinant hyaluronidase (rHuPH20), as an excipient at a concentration of 2000 U/mL. The drug product is a sterile, colorless to yellowish, clear to opalescent liquid supplied in colorless 15-mL vials (extractable volume 11.7 mL, 1400 mg antibody).

The rituximab SC dose will be fixed at 1400 mg for all patients (i.e., independent of the patient's body surface area [BSA]), except for patients 1 to 4 of the rituximab SC as of C1 cohort at C1 (see below).

10.1.2 **Packaging and labeling**

Rituximab IV: Rituximab for IV administration will be provided by Roche. It is supplied at a concentration of 10 mg/mL in either 100 mg (10 mL – packs of 2 vials) or 500 mg (50 mL – packs of 1 vial) single-use vials.

The vials containing the investigational product and the packaging containing vials in will be labeled according to Good Manufacturing Practice guidelines and the local requirements.

Rituximab SC: Rituximab for SC administration will be provided by Roche. It is supplied as a ready-to-use liquid formulation (1400mg / 11.7mL).

The vials containing the investigational product and the packaging containing vials in will be labeled according to Good Manufacturing Practice guidelines and the local requirements.

10.1.3 **Storage conditions**

Rituximab IV: No preservative is used in Rituximab IV; therefore, the vial is intended for single use only. Rituximab IV is biologically and chemically stable between +2°C and +8°C (36°F–46°F) and has a proposed shelf-life stability of 30 months. Once reconstituted into IV bags, Rituximab IV is chemically stable for up to 24 hours between +2°C and +8°C (36°F–46°F), followed by up to 12 hours at room temperature (+23°C). However, because rituximab solutions do not contain a preservative, diluted solutions should be stored +2°C and +8°C (36 °F–46 °F).

Rituximab IV vials should be protected from direct sunlight.

Rituximab SC: The recommended storage conditions for the rituximab SC drug product are between +2°C and +8°C, protected from light. Rituximab SC has a proposed shelf-life stability of 30 months. Chemical and physical in-use stability after withdrawal into the syringe with the subcutaneous needle infusion set attached has been demonstrated for 6 hours at ambient temperature under diffuse daylight, but should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user.

10.1.4 **Handlings**

Rituximab IV should be reconstituted by appropriately trained person using an aseptic technique.

The dosage calculation for rituximab will be based on the patient's body surface area, using Mosteller formula, using actual weight for calculation. This will be determined on the first day of study drug administration of cycle 1. The same dose should be infused during the treatment period whatever weight fluctuations.

The same lot number must be used when combining reconstituted vials for dose preparation.

Withdraw the necessary amount of rituximab and dilute to a final concentration of 1 to 4 mg/mL into an infusion bag containing either 0.9% Sodium Chloride, USP, or 5% Dextrose in Water, USP. It is recommended that a dilution of 2 mg/mL be used for ease in calculating dose. Gently invert the bag to mix the solution. Discard any unused portion left in the vial. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS. Do not infuse the rituximab concomitantly with another IV solution or other IV medications.

The first rituximab infusion should be administered IV at an initial rate of 50 mg/hr. If no hypersensitivity or infusion-related reactions occur, the rate of the infusion can be increased by 50 mg/hr increments at 30-minute intervals to a maximum rate of 400 mg/hr. If hypersensitivity (non-IgE-mediated) or an infusion-related reaction develops, the infusion should be temporarily slowed or interrupted. The infusion can continue at one-half the previous rate upon improvement of the patient's symptoms.

If the patient tolerated the first infusion well, subsequent rituximab infusions can be administered at an initial rate of 100 mg/hr and increased by 100 mg/hr increments at 30-minute intervals to a maximum of 400 mg/hr as tolerated. If the patient did not tolerate the first infusion well, the above guidelines for the first infusion should be followed for the subsequent rituximab infusion. All transfer procedures require strict adherence to aseptic techniques, preferably in a laminar flow hood. **DO NOT USE** evacuated glass containers that require vented administration sets because this causes foaming as air bubbles pass through the solution.

Diluted solution of rituximab is compatible with PVC, PE infusion bags.

Rituximab SC: Rituximab for SC administration is supplied as a ready-to-use liquid formulation. The formulation must not be diluted prior to administration. The product should be handled gently and foaming avoided. It should not be shaken.

10.2 Treatment schedule and design

Upon completion of the required assessments in the screening phase and fulfillment of the eligibility criteria, eligible patients will be randomized to arm A or B for the randomized cohort or enrolled into the rituximab SC as of C1 cohort and will enter in the treatment phase.

Treatment must start as soon as possible after randomization/enrollment, no later than 2 weeks after randomization/enrollment.

Treatment period for each patient starts with first administration of investigational product, which is defined as Study Day 1 Cycle 1.

Investigational products will be administered only to eligible patients under the supervision of the investigator or identified sub-investigator(s).

10.2.1 **Experimental Arm: Rituximab SC**

Patients randomized in experimental arm B will receive 8 cycles of rituximab: Rituximab IV (375 mg/m²) at the first cycle and then, in absence of grade 3-4 infusion related reaction (IRR) during cycle 1, rituximab SC (1400mg) at D8, D15, D22, M3, M5, M7 and M9.

Rituximab for SC subcutaneous formulation should be administered as subcutaneous injection only, over approximately 5 minutes.

The syringe should be prepared at the time of administration. The hypodermic injection needle must only be attached to the syringe immediately prior to administration to avoid potential needle clogging.

Rituximab for SC subcutaneous formulation should be injected subcutaneously into the abdominal wall and never into areas where the skin is red, bruised, tender, hard or areas where there are moles or scars.

If an injection is interrupted it can be resumed at the same site or another location may be used, if appropriate.

It is permissible for the subject to ambulate after the injection is completed.

10.2.2 **Control Arm: Rituximab IV**

Patients randomized in control arm (Arm A) will receive 4 cycles of rituximab IV (375 mg/m²) at D1, D8, D15 and D22.

10.2.3 **Rituximab SC as of C1 cohort**

Patients 1 to 4:

In cycle 1, the administration will be split to 200mg at D1 and the second part of the split dose (1200mg) will be given on the following day, at least 12 hours after the first administration in absence of Rituximab related grade 3-4 AE

Patients have to be monitored for at least 12h after the first administration with an observation overnight. The patients will be monitored for at least 4 hours, up to 12 hours, after the second split dose before patient can return home.

Additional follow up until D5 can be performed at time for PK sampling or by daily phone calls.

In case of related AE Grade >3 in patient 1-4, start of next patient's dosing will be delayed until the resolution of the AE in the previous patient

The full dose of rituximab SC (1400mg) will be administered at D8, D15, D22, M3, M5, M7 and M9.

Patients 5 to 20:

Patients will receive 8 cycles of the full dose of Rituximab SC (1400mg) at D1, D8, D15, D22, M3, M5, M7 and M9.

After administration of the first full dose in cycle 1, the patients have to be monitored 12 hours.

Rituximab SC administration

Rituximab for SC subcutaneous formulation should be administered as subcutaneous injection only, over approximately 5 minutes.

The syringe should be prepared at the time of administration. The hypodermic injection needle must only be attached to the syringe immediately prior to administration to avoid potential needle clogging.

Rituximab for SC subcutaneous formulation should be injected subcutaneously into the abdominal wall and never into areas where the skin is red, bruised, tender, hard or areas where there are moles or scars.

If an injection is interrupted it can be resumed at the same site or another location may be used, if appropriate.

It is permissible for the subject to ambulate after the injection is completed.

10.2.4 **Dose adjustments and cycles postponements**

No dose reduction is planned.

In case of concomitant event, the rituximab administration can be postponed as follows:

- D8 : up to 8 days
- D15 and D22 : up to 15 days
- M3, M5, M7 and M9: up to 1 month

If rituximab administration cannot be performed after these delays, the study treatment must be stopped and this will be considered as treatment discontinuation.

10.3 Drug Dispensation and accountability

10.3.1 *Responsibilities*

All investigational product packages have to be inspected upon receipt at the study site prior to being drawn up. If any particulate matter is detected, the investigational product has not to be used. Damaged packaging is reported to the sponsor and stored until instructions have been given.

The Investigator, the Hospital Pharmacist, or other personnel allowed to store and dispense Investigational Products (Rituximab IV and Rituximab SC) are responsible for ensuring that the Investigational Products used in the clinical trial are securely maintained as specified by the Sponsor and in accordance with the applicable regulatory requirements. All Investigational Products are stored in accordance with labeling and shall be dispensed in accordance with the Investigator's prescription. The Investigator is in charge of ensuring that an accurate record of Investigational Product issued and returned is maintained. Any quality issue noticed with the receipt or use of an Investigational Product (deficient IP in condition, appearance, pertaining documentation, labeling, expiry date, etc.) should be promptly notified to the Sponsor, who will initiate a complaint procedure to Roche. Under no circumstances will the Investigator supply Investigational Product to a third party, allow the Investigational Product to be used other than as directed by this Clinical Trial Protocol, or dispose of Investigational Product in any other manner.

10.3.2 *Retrieval or destruction*

All partially used or unused treatments will be verified by the Sponsor. A detailed treatment log of the returned Investigational Product will be established with the Investigator (or the pharmacist) and countersigned by the Investigator (or the Pharmacist) and the Sponsor representative. The Investigator (or the pharmacist) will not destroy the unused Investigational Product unless the Sponsor provides written authorization. In case of a potential defect in the quality of Investigational Product, the Sponsor may initiate a recall procedure. In this case, the Investigator will be responsible for promptly addressing any request made by the Sponsor, in order to recall Investigational Product and eliminate potential hazards.

10.3.3 *Accountability and compliance*

The investigator or pharmacist will inventory and acknowledge receipt of all shipments of the investigational product. The investigator or pharmacist will also keep accurate records of the quantities of the study treatments dispensed and used for each patient, to assess the patient treatment compliance. The sponsor representative will periodically check the supplies of investigational products held by the investigator or pharmacist to verify accountability of all investigational products used. All unused investigational products and all medication containers will be destroyed by the study sites. A certificate of destruction will be provided by the site to the sponsor. The Sponsor will verify that a final report of drug accountability at the unit dose level is maintained and archived in the investigator study file. Administration of the study treatment will be supervised by the investigator or sub-investigator.

10.4 Prophylactic measures

Premedication consisting of acetaminophen and an antihistamine should be administered before each rituximab infusion.

Premedication with glucocorticoids should be also considered.

11 STUDY PROCEDURES

11.1 Registration and randomization/inclusion procedure

A patient will be registered after verification of eligibility directly on the data capture system by the investigators through the internet network with the address below. To access the interactive registration program, the investigator needs to record the study name (FLIRT), a username and a password.

Internet: <http://study.lysarc.info>

Registration must be done before the start of the protocol treatment. The study site will receive back the registration number and the arm of treatment for the registered patient.

The investigator must fax to **LYSARC (+33 (0)4 26 07 40 13)** a copy of the pathological reports (nodal or extranodal biopsy and bone marrow biopsy), the patient inclusion form and the biological traceability forms.

It will be the contact for any request (**Tel: +33 (0)4 72 66 93 33**).

Stratification: Patients will be stratified according to **FLIPI score at baseline (< 2 versus ≥ 2)** and randomly assigned to treatment arms (A or B).

Patients enrolled in Rituximab SC as of C1 cohort are included directly without randomization.

11.2 Pathological diagnosis

The pathological diagnosis of follicular lymphoma should have been performed locally before the randomization of each patient.

Histopathology central review process has become in the last years a common and prerequisite procedure for clinical trials in the field of lymphomas. A mandatory pathological review will therefore be organized for all patients randomized in the trial at diagnosis. The goal of this central review will be to confirm the diagnosis and to precise its classification according to the WHO classification 2008.

The pathological review will be centralized at the LYSA-Pathology institute, Hôpital Henri Mondor, Créteil - France. For each patient, the investigator will be requested to join a copy of the anonymized histopathological report where the name and address of the pathologist having diagnosed the lymphoma will be easily identified as well as a copy of the bone marrow report.

All requested tumor paraffin embedded blocks from the formalin fixed sample (that have been used for diagnosis), or 12 unstained slides will be sent to the LYSA-Pathology institute, Hôpital Henri Mondor, Créteil - France, according to the process described in Appendix 10 "Pathological samples review". At reception, routinely stained sections will be performed and an appropriate panel of antibodies according to morphological aspects will be applied. A pathological review, performed by at least 2 expert hematopathologists will be organized at the LYSA-P and a consensus diagnosis will be established. A LYSA-P pathological report will then be sent to the clinical coordinator and to the initial pathologist.

Bone marrow trephine biopsy from patients assessed with a complete response (CR and CRu) by CT scan and presenting an involved bone marrow at baseline will also have to be reviewed.

The initial tumor block will be used to study the surrounding microenvironment of the tumor known to influence the development and progression of follicular lymphoma and biomarkers known to influence the prognosis of follicular lymphoma using either full slides or tissue microarray (TMA). For further ancillary study, blocks will be kept temporarily to avoid a second request. In the meanwhile, the block will be at the entire disposition of the initial pathology laboratory under request if they need it.

At the end of the inclusions, frozen tumor tissue will be requested for all randomized patients. The collection of the frozen tumor specimen will be organized and centralized at the LYSA-P. On frozen tissue,

gene and protein expression **analysis** will be performed to assess the level of expression of genes/proteins known to influence the outcome of follicular lymphoma patients.

11.3 Biological study/ samples

Minimal residual disease as detected by circulating t(14,18) QPCR is of clinical value in high tumor burden follicular lymphoma patients (Ladetto *et al.*, *Blood* 2013) and has been used for preemptive treatment protocol design (Ferrero *et al.*, *Ann Hematol* 2013). In low tumor burden, data are missing. The relationship between quantitative t(14,18) PCR in blood and tumor burden as evaluated by CT-Scan or PET scan at diagnosis has not been reported. Is there a simple parallelism or can we define patients with and patients without minimal circulating disease independently of tumor mass and is it of prognostic value? Are circulating tumor cells correlated with bone marrow histological involvement?

Only samples from patients will be collected (M3 and M12) and kinetic of blood clearance will be compared in the two arms.

The objectives of the ancillary study are:

- To analyze T-cell specific response against lymphoma before and after rituximab treatment in 10 patients in arm B (proof-of-principle study), with a specific focus on the CD20 molecule itself as a potential target antigen
- To evaluate the pharmacokinetics of rituximab and influence of tumor burden
- To determine FcRn expression and polymorphism on FL microenvironment *in situ* (bone marrow and lymph nodes) and in peripheral blood by flow cytometry and immunofluorescence studies and to analyze FcRn polymorphism and its correlation with pharmacokinetics study
- To evaluate the predictive value of an integrated multi-parametric analysis including tumor genomic profiling, frequency of circulating t(14;18)^{pos} cells, genetic background and tumor microenvironment profiling. Whole exome sequencing will be performed on a discovery set at diagnosis (10 LN biopsies in each arm), followed by backtracking using targeted deep-sequencing on paired BM biopsies and/or blood when frequency allows. A validation set will be subsequently performed in an extension cohort of LN biopsies and available paired BM/blood, and on relapses when occurring. t(14;18) quantification in blood and tissues will be performed as previously described. GWAS analysis will be performed using the Illumina Human Core Exome array on blood obtained at diagnosis and/or M3 (M3 samples may be used in case of overt lymphoma blood involvement at diagnosis to avoid mixed population of tumor/normal cells). LN, BM (all available patients), and peripheral blood (90 patients including 45 in each arm) will be extensively characterized for classical lymphoid and myeloid immune cell subsets by multicolor flow cytometry. Moreover, we will quantify and detail the phenotype (flow cytometry and multicolor immunofluorescence), heterogeneity/polarization, and function of infiltrating stromal cells and CD4^{pos} T cells that play a key role in FL pathogenesis and drug resistance.
- To correlate QPCR of circulating t(14,18) translocation and tumor burden assessed by CT scan or PET SCAN

Sampling of blood will be encouraged for participation to the pharmacokinetics, biological and genetic studies. The specific informed consent for these studies must be signed before any sample procedure. For genomic studies, a specific informed consent is required and mandatory.

The samples processing is described on **the Appendix 11**.

11.3.1 Genomic Study

Biological and genetic informed consent must be signed before any procedure.

All study sites (202 patients enrolled in the randomized cohort only, not applicable for rituximab SC as of C1 cohort) will participate to this study:

- Collect 8mL of blood from each patient on PAXgene DNA tube at 3 time points:
 - Before study treatment
 - At M3
 - At M12 or permanent treatment discontinuation in case of relapse or progression.

Then the PAXgene DNA tube as well as the filled in traceability form, provided by the LYSARC, are sent, according to the procedures indicated by LYSARC, by regular mail, at room temperature, to either CHU Pontchalliou – Rennes - France or CHU Henri Mondor – Créteil - France.

- 3ml to 5ml of bone marrow will also be collected on a heparin tube at the following time points:
 - Before study treatment
 - At M12 in all patients in CR/CRu assessed by CT scan (if bone marrow involved or not done or unspecified at baseline) and in CR/CRu /PR patients assessed by CT scan without histological involvement at baseline but with *BCL2-IGH* rearrangement in bone marrow

The heparin tubes are sent, with the corresponding traceability form according to the procedures indicated by LYSARC, by a designated carrier at room temperature, to Laboratoire SITI - BMT-HC 1^{er} étage - CHU Pontchaillou - Rennes.

11.3.2 *Immunological Studies*

For the randomized cohort only, not applicable for Rituximab SC as of C1 cohort

Biological informed consent must be signed before any procedure.

11.3.2.1 Flow cytometry – plasma storage

Only part of French LYSA centers will participate in this biological study performed on a restricted number of patients (**90 patients with an objective of 45 in each arm**)

Collect 15mL of blood on heparin tubes at 3 time points:

- Before study treatment
- At M3
- At M12 or at permanent treatment discontinuation in case of relapse or progression.

The heparin tubes are sent on the day of sampling with the corresponding traceability form according to the procedures indicated by LYSARC, by carrier at room temperature, to Laboratoire SITI – BMT - HC 1^{er} étage - CHU Pontchaillou - Rennes.

11.3.2.2 Immunogenicity

Only a **few centers** will participate in the immunogenicity study performed on a restricted number of patients (**10 patients in arm B**).

- Collect 15mL of blood on heparin tubes at 3 time points:
 - Before study treatment
 - At M3
 - At M12 or permanent treatment discontinuation in case of relapse or progression.
- Send the heparin tubes, on the day of sample collection, with the appropriately filled traceability form according to the procedures indicated by LYSARC, by carrier at room temperature, to Laboratoire SITI - BMT- HC 1^{er} étage - CHU Pontchaillou - Rennes.

11.3.3 *Pharmacokinetics analysis*

The pharmacokinetics of rituximab will be assessed using compartmental pharmacokinetic modeling. Pharmacokinetic parameters will be estimated using a population approach. The population approach allows describing the interindividual distribution of parameters of interest by computing simultaneously data from all individuals in a given population. This interindividual distribution allows the quantification of (i) the “mean” (referred as “typical”) value of each parameter, (ii) the interindividual variability (referred as “interindividual variance”) and (iii) the influence of individual factors on the interindividual distribution (referred as “covariates”). The influence of tumor burden will be tested as a covariate on pharmacokinetic parameters.

In the rituximab SC as of C1 cohort the pharmacokinetic data will be listed individually with summary statistics per timepoint.

Biological informed consent for patients enrolled in the randomized cohort and specific informed consent for rituximab SC as of C1 cohort must be signed before any procedure.

For all the participating patients (140 patients of the randomized cohort – 20 patients of the rituximab SC as of C1 cohort), the following time points will be analyzed:

For randomized patients

- Arm A: Blood sample before rituximab (H0) and 2 hours after the end of rituximab infusion (H2) at:
D1, D8, D15, D22 (H0 and H2) and M3 (H0 only).
At permanent treatment discontinuation in case of progression or relapse
 - Arm B: Blood sample before rituximab (H0) and 2 hours after the end of rituximab administration (H2) at:
D1, D8, D15, D22 (H0 and H2)
M3, M5, M7 and M9 (H0 only).
At permanent treatment discontinuation in case of progression or relapse
- Collect 10mL of blood on dry tubes
 - After each blood sample collection, centrifuge the tubes at 1400g for 10 to 15 minutes.
 - Collect the serum in 2 cryotubes of 2mL provided by LYSARC.
 - Keep the serum aliquots at -80°C (a collection will be organized by LYSARC at the end of follow up period for sending tubes to CHU Bretonneau – Tours.).
 - Fill in the traceability form “ PROTOCOLE FLIRT – Etude pharmacocinétique – Analyse des échantillons à Tours ” after each blood sample and fax it to LYSARC biological department or email fax.bio@lysarc.org)

For rituximab SC as of C1 cohort:

- Patients 1 to 4: Blood sample before rituximab (except D4, D5 and D11) at:
D1, D2, D4, D5, D8, D11, D15, D22, M3, M5, M7 and M9
- Patients 5 to 20: Blood sample before rituximab (except D4, D11) at:
D1, D4, D8, D11, D15, D22, M3, M5, M7 and M9
- All patients: PK should also be taken at the time of an IRR with Gr. 2-4

- Collect 5mL of blood on 1 dry tube
- At the latest 30 minutes after blood sampling, centrifuge the dry tube at 4°C, at 1500g to 2000g during 15 minutes
- Quickly after centrifugation, collect 1,5ml of serum in 1 cryotube
- Within 2 hours after blood sampling, keep the serum aliquots at -80°C (a collection will be organized by LYSARC for sending tubes to QPS)
- Fill in the traceability form “ PROTOCOLE FLIRT – Etude pharmacocinétique – Cohorte Rituximab SC ” after each blood sampling and fax it to LYSARC biological department email fax.bio@lysarc.org).

12 SAFETY REVIEW COMMITTEE

For the Rituximab SC as of C1 cohort only, not applicable for randomized cohort.

A Safety Review Committee (SRC) will review the safety profile of SC administration in cycle 1 after 4 patients have completed cycle 2 or permanently discontinued treatment.

Enrollment will be suspended once 4 patients have been included to allow data review by SRC.

If one or more Gr>3 AE considered related to Rituximab is observed in C1 among the first 4 patients, additional monitoring (one night hospitalization and daily follow up) will be extended to patients 5-8. An additional safety review will take place after the 8th patient has completed cycle 2 or permanently discontinued treatment. .

Additional safety review committees could be implemented.

13 CRITERIA FOR PREMATURE DISCONTINUATION OF THE STUDY

13.1 Permanent treatment discontinuation

Circumstances that lead to permanent treatment discontinuation of a patient from the trial must be reported by the investigator on the appropriate eCRF.

Criteria for permanent treatment discontinuation include (but are not limited to):

- death,
- toxicity,
- lymphoma progression,
- concomitant disease,
- noncompliance (including loss of subject to follow-up),
- consent withdrawal,
- and major protocol violation, including initiation of alternate anti-neoplastic therapy.

Even in case of permanent treatment discontinuation, all patients alive (except consent withdrawal) should however remain in the trial for the purposes of follow-up and data analysis.

Any patient who discontinues before completing the study will be encouraged to return to the study site between 3 and 5 weeks after the last study drug administration for an evaluation.

13.2 Withdrawal of Consent

Patients are free to withdraw from the study at any time without prejudice to their treatment. When a patient decides to withdraw from the study, she/he should always be contacted in order to obtain information about the reason for withdrawal and to record any adverse events. When possible, the patient should return for a study visit at the time of, or soon after withdrawal, and the relevant assessments should be performed.

If the patient explicitly states his/her wish not to contribute further data to the study, the relevant LYSARC contact should be informed and the withdrawal of consent should be documented by the investigator in the patient's case report form. However, data up to the time of consent withdrawal will be included in the data reported for the study.

13.3 Patients Lost to Follow up

Every effort will be made to contact patients who fail to return for scheduled visits. A patient is considered lost to follow-up if no information has been obtained when the last patient has completed the clinical phase of the study. During this time site investigator must document attempts to contact the patient either by phone or letter.

13.4 Premature discontinuation of the study

The sponsor reserves the right to stop the trial at any time. The investigators will be informed of this decision in writing.

The same applies to any investigator wanting to discontinue his/her participation to the trial. The investigator must immediately inform the sponsor in writing of this decision.

14 SAFETY PARAMETERS

14.1 Definitions

14.1.1 Adverse Events

An **adverse event** (AE) is any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition.

In the Rituximab SC as of C1 cohort, an administration-related reaction (ARR) is defined as any AE which occurred during or within 24 hours of injection that were considered by the investigators to be related to rituximab. As these events are of special interest, each symptom is to be reported as a separate adverse event in order that the intensity of each symptom may be individually assessed.

14.1.2 Serious Adverse Events

A **serious adverse event** (SAE) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (*the term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event ; it does not refer to an event which hypothetically might have caused death if it were more severe*)

- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a medically significant event:

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriated in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above.

The term “severe” is a measure of intensity, thus a severe AE is not necessarily serious. For example, “nausea of several hours” duration may be severe but may not be clinically serious.

14.1.3 *Intensity*

The **intensity of the AE or SAE** will be graded by the investigator according to the Common Terminology Criteria for Adverse Events (CTCAE) grading system v4.03 in the toxicity categories that have recommended grading (see Investigator’s file or online at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

AEs not listed on this grading system will be graded according to the five-point system below:

- Mild (grade 1) Discomfort noticed but no disruption of normal daily activity
- Moderate (grade 2) Discomfort sufficient to reduce or affect normal daily activity
- Severe (grade 3) Incapacitating with inability to work or perform normal daily activity
- Life-threatening (grade 4) Substantial risk of dying at time of event
- Death (grade 5)

14.2 Adverse Events reporting rules

For patients in the randomized cohort:

AE of grade 2-5 for infections and neurological toxicities and AEs of grade 3-5 for other toxicities (CTCAE – version 4.03) regardless of their relationship to investigational product occurring from the date of informed consent signature, during treatment administration period and up to 30 days after last drug administration will be recorded in the AE pages of the eCRF.

For patients in the rituximab SC as of C1 cohort:

AE, regardless of their relationship to investigational product occurring from the date of informed consent signature, during treatment administration period and up to 30 days after last drug administration will be recorded in the AE pages of the eCRF (CTCAE – version 4.03):

- Cycle 1 and cycle 2: all AE of grade 1-5 including ARR reported by symptom (any AE which occurred during or within 24 hours of injection that were considered by the investigators to be related to rituximab)

- Cycle 3 to cycle 8:

- **ARR of grade 1-5** reported by symptom (any AE which occurred during or within 24 hours of injection that were considered by the investigators to be related to rituximab)
- **AE of grade 2-5 for infections and neurological toxicities**
- **AE of grade 3-5 for other toxicities**

- Arm A of the randomized cohort: AE and SAE:

Reporting from signature of ICF and up to 30 days after D22 administration (and regardless the time of occurrence for related SAE and the corresponding AE).

- Arm B of the randomized cohort or Rituximab SC as of C1 cohort: AE and SAE

Reporting from the signature of ICF, during treatment administration period and up to 30 days after M9 administration (and regardless the time of occurrence for related SAE and the corresponding AE).

- Premature treatment discontinuation: all arms

AE and SAE have to be reported from signature of ICF and up to 30 days after last study drug administration (and regardless the time of occurrence for related SAE and the corresponding AE).

When associated to a SAE and regardless the time of occurrence and the grade, the AE must be reported as “Adverse Event” in the appropriate eCRF pages.

Whenever possible, symptoms should be grouped as a single syndrome or diagnosis. The investigator should specify the date of onset, intensity, action taken regarding trial medication, corrective therapy given, outcome of all AEs and his opinion as to whether the AE can be related to the study drugs.

All events that meet one or more criteria of seriousness (see Section 12.1.2.) will be reported as SAE (see Section 12.3).

General AE reporting rules:

- Non-serious AE will be reported through eCRF.
- Any episode of any grade of toxicities, related to a SAE must be reported as “Adverse Event” in the appropriate eCRF pages regardless the time of occurrence.
- Signs, symptoms and physical findings indicative of lymphoma or progression of lymphoma are not to be reported as “Adverse Event”.
- “Alopecia” toxicity (any grade) will never be reported as “Adverse event”.
- At baseline, any abnormal medical condition which is not reported in laboratory tests (biochemistry, hematology) should be declared on medical history page, whether due to lymphoma or not. This medical condition should be followed at each clinical examination: if it worsens (transition to grade 3), it should be declared as an AE.

Abnormal laboratory values reporting rules:

An abnormal laboratory value is considered to be an AE if the abnormality:

- results in discontinuation from the study;
- requires treatment, modification/ interruption of study drugs dose, or any other therapeutic intervention; or
- is judged to be of significant clinical importance.

Regardless of severity grade, only laboratory abnormalities that fulfill a seriousness criterion need to be documented as a serious adverse event.

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/screen of the eCRF. If the abnormality was not part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as AE (if grade 3-5).

14.3 Serious Adverse Events reporting rules

All events that meet one or more criteria of seriousness (see Section 13.1.2.) occurring **after the informed consent signature, during treatment administration period and up to 30 days after last drug administration**, regardless the relationship to the study treatment will be reported as SAE.

A SAE that occurs after this time, including during the follow-up period, **if considered related to the study medication**, will be reported.

Whenever possible, symptoms should be grouped as a single syndrome or diagnosis. The investigator should specify the date of onset, intensity, action taken regarding trial medication, corrective therapy given, outcome of all SAEs and his opinion as to whether the SAE can be related to the study drugs.

General SAE reporting rules:

- Any episode of any grade of toxicities, which meets one of the seriousness criteria, must be reported as “Serious Adverse Event” in the appropriate SAE form.
- Signs, symptoms and physical findings indicative of lymphoma or progression of lymphoma are not to be reported as “Serious Adverse Event”.
- “Alopecia” toxicity (any grade) will never be reported as “Serious Adverse Event”.
- Planned hospital admissions or surgical procedures for an illness or disease which existed before the patient was enrolled in the study or before study drug was given are not to be considered SAEs unless the condition deteriorated in an unexpected manner during the study (eg. surgery was performed earlier than planned).
- SPM have to be reported in the eCRF (SPM pages). During the SAE reporting period, serious SPM have to be reported in SAE pages. If a serious SPM occurs after this time including in the follow-up period, and if considered related to the study medication, SPM have to be reported in SAE pages (with the corresponding AE page).

14.3.1 *Obligations of the Investigator*

In a case of SAE the Investigator must immediately (within 24 hours):

- **SEND the SAE pages to LYSARC Pharmacovigilance department:**

FAX: +33 (0) 3 59 11 01 86 OR EMAIL TO pharmacovigilance@lysarc.org

All SAE forms must be dated and signed by the responsible Investigator or one of his/her authorized staff Members.

- May attach the photocopy of relevant examinations carried out and the dates on which these examinations were performed. Care should be taken to ensure that the patient’s identity is protected and the patient’s identifiers in the Clinical study are properly mentioned on any copy of source document. For laboratory results, include the laboratory normal ranges.
- Follow up of any SAE that is fatal or life-threatening should be provided within one calendar week.

For SAEs, the following must be assessed: relationship to each study drug, action taken, and outcome to date. The assessment of whether there is a reasonable possibility of a causal relationship is usually made by the investigator and it can be one of two possibilities:

- Unrelated
- Related

14.3.2 *Obligations of the Sponsor*

During the course of the study, the Sponsor will report in an expedited manner all SAEs that are both unexpected and at least reasonably related to study drugs, to the EMA, Health Authorities, Ethic Committees in each country in accordance with international and local regulations, and to the Investigators. The causality assessment given by the investigator should not be downgraded by the

sponsor. If the sponsor disagrees with the investigator's causality assessment, the opinion of both the investigator and the sponsor should be provided with the report.

The expectedness of a serious adverse reaction will be determined by the Sponsor according to the reference safety information (Investigator's Brochure) of the study drugs.

The LYSARC Pharmacovigilance department will report all safety information from the trial in the Development Safety Update Reports and will notify the reports to the Health Authorities and Ethics Committees in accordance with international and local regulations.

14.4 Pregnancy

14.4.1 *Females of Childbearing Potential*

This protocol defines a female of childbearing potential as a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy or 2) has not been naturally postmenopausal (amenorrhea following cancer therapy does not rule out childbearing potential) for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on study drug, or within 12 months of the subject's last dose of study drug, are considered events to be reported immediately to LYSARC Pharmacovigilance on the appropriate Pregnancy Form.

LYSARC fax number +33 (0) 3 59 11 01 86 or email to pharmacovigilance@lysarc.org

If the subject is on study drug, the study drug is to be discontinued immediately and the subject instructed to return any unused portion of the study drug to the Investigator.

The exposure of any pregnant female (e.g. caregiver or pharmacist) to study drug is also an immediately reportable event.

The female should be referred to an obstetrician/gynecologist preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the female subject until completion of the pregnancy, and must notify LYSARC immediately about the outcome of the pregnancy (either normal or abnormal outcome).

If the outcome of the pregnancy was abnormal (i.e., spontaneous or therapeutic abortion) the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the Investigator(s) suspects is related to the in utero exposure to the study drug should also be reported to LYSARC within 24 hours of the Investigator's knowledge of the event using SAE form.

14.4.2 *Male patients*

If a female partner of a male patient taking study drug becomes pregnant, the male patient taking study drug should notify the Investigator, and the pregnant female partner should be advised to call her healthcare provider immediately.

If a pregnancy related event is reported in a female partner of a male subject, the investigator should determine whether the female partner is willing to release her medical information to LYSARC Pharmacovigilance and allow the pregnancy related event to be followed-up to completion.

14.5 Follow up of AEs and SAEs

Any SAE should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or underlying condition. Any additional information known after the event has been initially reported should be sent to the LYSARC as soon as information become available.

All AEs must be documented and the outcome must be followed-up until the return to normal (grade 0) or consolidation of the patient's condition.

Subjects withdrawn from the study due to any AE will be followed at least until the outcome is determined even if it implies that the follow-up continues after the patient has left the trial.

15 STATISTICAL CONSIDERATIONS

15.1 Study design

This study is a multicenter, phase III, open-label, randomized (1:1) trial evaluating two routes of rituximab administration (IV vs SC) for the treatment of first line/low tumor burden follicular lymphoma.

15.2 Primary endpoint

The primary endpoint of the randomized cohort is the Progression Free Survival (PFS).

The PFS is defined as the time from randomization into the study to the first observation of documented disease progression or death due to any cause. If a subject has not progressed or died, PFS will be censored at the time of last visit with adequate assessment.

15.3 Secondary endpoints

15.3.1 Secondary efficacy endpoints

Secondary efficacy endpoints will include:

- Overall Survival (OS)

Overall survival will be measured from the date of randomization to the date of death from any cause. Alive patients will be censored at their last follow-up date.

- Response Rates according to the response criteria for malignant lymphoma at M3 (1999) and M12 (1999 and 2014 criteria)

Disease response evaluation at M3 and M12 will be used to determine the Response Rate at M3 and M12.

Assessment of response will be based on the International Workshop to Standardize Response criteria for NHL (Criteria for evaluation of response in Non-Hodgkin's lymphoma) according to Cheson 1999 (M3 and M12) and according to Cheson 2014 (M12 only).

The response rates will be described for each modality (CR, CRu, PR, SD and PD) and the Overall response rates (CR+CRu+PR) will also be described at the two time points (M3 & M12).

- Best Response Rate according to the response criteria for malignant lymphoma 1999 during the study

Disease response evaluation at M3, M12 and Follow-Up visits will be used to determine the Best Response Rate. Assessment of response will be based on the International Workshop to Standardize Response criteria for NHL (Criteria for evaluation of response in Non-Hodgkin's lymphoma (Cheson, 1999)).

The response rates will be described for each modality (CR, CRu, PR, SD and PD) and the Overall response rates (CR+CRu+PR) will also be described at different time points (M3, M12, M15...).

- Time to Next Anti-Lymphoma Treatment (TTNLT)

TTNLT is defined as the time from randomization to the date of first documented administration of any new anti-lymphoma treatment (chemotherapy, radiotherapy, radio-immunotherapy, immunotherapy...). Patients continuing in response or who are lost to follow-up will be censored on their last visit date. Patients who died (due to any cause) before having received a new anti-lymphoma treatment will be included in the statistical analysis with death being counted as an event.

- Molecular Response (Bcl-2-IgH rearrangement) at M3 and M12

15.3.2 Safety endpoints

Considering that we are evaluating two different regimens with different numbers of administrations, the safety endpoints will be described on the two following time windows:

- From C1 to end of C4
- From C1 to end of Maintenance

Summary of study drug administration including treatment duration, average dose will be displayed by treatment arm.

Number, frequency and reasons for treatment discontinuation and study discontinuation will be summarized by treatment arm.

Adverse events, clinical laboratory measurements, and concomitant medications at baseline and concomitant therapy related to AE will be described according to treatment arm.

AEs will be classified using the latest version of Medical Dictionary for Drug Regulatory Activities (MedDRA) coding system at the time of database lock. The severity of the toxicities will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) whenever possible. Subsets of AEs to be summarized include serious, all NCI CTCAE grade severities, suspected treatment-related, and events that resulted in withdrawal of investigational product. The most severe grade of each preferred term for a patient will be utilized for summaries of adverse events by NCI CTCAE grade.

All AEs will be described by system organ class, preferred term according to treatment arm (a patient having the same event more than once will be counted only once). AEs of special interest will also be displayed in a separate table.

AEs of special interest will be:

- AE leading to death or to treatment discontinuation
- Serious adverse event

All AEs will be displayed in a by-patient listing and AEs of special interest also in a separate by-patient listings.

Other malignancies will also be summarized and displayed in a by-patient listing.

All deaths will be listed and also summarized by cause of death.

As the treatment exposure period is different between the two treatment arms, incidence rate of specific AEs will also be displayed. More information will be detailed in the Statistical Analysis Plan.

Clinical laboratory tests will be normalized and their change from baseline will be summarized in terms of mean, standard deviation, median, minimum and maximum values by visit. Clinical laboratory results will be summarized in terms of mean, standard deviation, median, minimum and maximum values by visit. Clinical laboratory results will be also displayed taking into account laboratories Upper/Lower Limit of Normal (ULN/LLN).

Graphical displays may be provided when useful to assist in the interpretation of results.

15.4 Exploratory endpoints

Randomized cohort

All exploratory endpoints will be described and the correlation with survival endpoints (PFS, OS) will also be evaluated.

Pharmacokinetic parameters of rituximab will be used to estimate individual area under the concentration curves of rituximab (AUC). The AUC will be used to describe the relationship between rituximab pharmacokinetics and clinical response (objective response, survival).

Rituximab SC as of C1 cohort

Same endpoints as for randomized cohort will be used for the Rituximab SC as of C1 cohort (PFS, see 15.2; OS and response rates, see **Erreur ! Source du renvoi introuvable.**; safety, see **Erreur ! Source du renvoi introuvable.**).

In addition, the ARRs will be summarized and displayed in by-patient listings.

During first two cycles, the AEs will be displayed as follow:

- all AEs
- specific AEs including AEs of grade 2-5 for infections and neurological toxicities and AEs of grade 3-5 for other toxicities

15.5 Analysis population

15.5.1 ***Intent-to-Treat Set (ITT Set)***

The ITT set includes all patients randomized regardless of study drug being received or not. Patients will be analyzed based on the assigned treatment group at the time of randomization.

As patients enrolled in the Rituximab SC as of C1 cohort won't be randomized, they won't be included in the ITT set.

15.5.2 ***Efficacy Set (Efficacy set)***

The Efficacy set includes all patients included in the ITT set with histopathologically confirmed follicular lymphoma by central review having:

- received at least one dose of rituximab
 - o For patients randomized in the arm B, only patients who correctly switched to rituximab SC after one IV cycle will be included in this set
- baseline tumor assessments
- at least one post baseline tumor assessment

15.5.3 ***Rituximab SC as of C1 set***

The **Rituximab SC as of C1 set** includes all patients treated in Rituximab SC as of C1 cohort.

15.5.4 ***Safety Set***

Safety set includes all patients who took at least one dose of study drug. Patients will be analyzed according to the actual treatment received ("as treated").

In Rituximab SC as of C1 cohort, patients will be analyzed according to the number of administrations.

15.6 Statistical methods

Continuous variables will be summarized in tables displaying sample size, mean, standard deviation, median, range; quartiles will also be presented when considered relevant.

Categorical data will be described in counts and percentages (of non-missing data).

Censored data will be presented as Kaplan-Meier plots of time to first event and summary tables of Kaplan-Meier estimates for criterion rates at fixed time points, with 95% CIs. The median time to event will be calculated (if reached) with 95% confidence intervals.

Response rates will be expressed as percentages with their 95% Exact Clopper Pearson Confidence Interval limits.

15.6.1 Efficacy Analysis

Randomized cohort

The primary efficacy analysis will be based on the ITT set.

Treatment with rituximab Sub-Cutaneous will be declared superior if the two-sided p-value from stratified log-rank test is < 0.05 . Stratification factor will be the one used for randomization (FLIPI score: < 2 vs ≥ 2). Hazard ratio with two-sided 95% confidence intervals will be estimated using the Cox proportional hazards model if proportional hazard assumption holds. Graphical display of Kaplan-Meier curves will be provided to aid data interpretation visually.

Sensitivity analysis will be performed on the primary endpoint:

- Based on Efficacy set.
- Based on an unstratified log rank test.

All secondary efficacy analyses will be performed on the ITT set.

Rituximab SC as of C1 cohort

Efficacy analyses will be based on the Rituximab SC as of C1 set. Summary descriptive statistics will be provided.

15.6.2 Safety Analysis

Randomized cohort

All safety analyses will be performed on Safety set and according to the actual treatment received ("as treated").

Rituximab SC as of C1 cohort

Safety and pharmacokinetics analyses will be based on the Rituximab SC as of C1 set. Summary descriptive statistics will be provided.

15.7 Hypothesis testing

A two-sided log-rank test will be used for testing the difference in progression free survival between the two treatment arms. The significance level for the primary analysis will be 0.05.

The hypothesis will be:

H_0 : PFS (rituximab SC) = PFS (rituximab IV)

Versus

H_A : PFS (rituximab SC) \neq PFS (rituximab IV)

Where PFS denotes the survival distribution of the parameter time to progression free survival.

For secondary parameters, statistical tests will be two-sided and performed using a 5% level of significance.

15.8 Sample size calculation

Randomized cohort

Sample size determination is based on progression free survival (PFS) which serves as the primary efficacy endpoint in this study.

To provide 90% power to detect Hazard Ratio (HR) of 0.52 (hazard of rituximab SC *versus* rituximab IV) with two-sided alpha (type I error) of 0.05, a total of 102 events from both arms will be required. HR of 0.52 corresponds to an increase of the median PFS from 23.5 months for the rituximab IV arm (control arm) (Colombat *et al.* 2012) to 45 months for the rituximab SC arm (experimental arm).

The calculation takes into account the following assumptions:

- an exponentially distributed PFS time with a constant hazard rate over the course of the study;
- a median PFS of 23.5 months in the rituximab IV arm;
- a Hazard Ratio : 0.52
- an accrual rate of 70 patients/year;
- an interim analysis for futility and efficacy when half of the events occurred
- a total of 30-month enrolment period;
- 5% drop-out rate/year;

These results in a total of 102 events required which implies 202 patients to be randomized in the two treatment arms (101 in each arm).

It is projected to achieve the 102 events at approximately 54 months after the first patient randomization.

Rituximab SC as of C1 cohort

All the analyses of Rituximab SC as of C1 cohort will be exploratory in nature and there will be no specific hypothesis testing. Therefore, sample size is not based on formal calculations. As a proof of concept methodology we anticipate that 20 patients are sufficient to explore the safety, the pharmacokinetics and the efficacy of SC dosing from 1st cycle.

15.9 Interim analysis

It was planned to perform one interim analysis on PFS at 50% of the total number of planned events (51 cumulative progression/death events in ITT set) for futility and efficacy purpose. The interim analysis is projected to occur at about 35th months from the first randomized patient.

Futility boundaries are based on O'Brien and Fleming beta spending function. The futility boundaries are non-binding (see Table 14.9.1 for stopping boundaries).

If the observed HR based on interim data is > 1.06 (Rituximab SC vs Rituximab IV – corresponding to $Z < -0.192$), the study will be recommended to terminate as it is highly unlikely to meet its primary objective.

Efficacy boundaries are based on O'Brien and Fleming alpha spending function (see Table 14.9.1 for stopping boundaries).

If the observed HR based on interim data is < 0.435 (Rituximab SC vs Rituximab IV – corresponding to $Z > 2.963$), the study will be recommended to terminate as it is highly significant and therefore that it is highly likely to meet its primary objective.

Table 14.9.1: Stopping boundaries

Look #	Info. Fraction (s/s_max)	Events (s)	Cumulative α Spent	Cumulative β Spent	Boundaries			
					Efficacy Z		Futility Z	
					Upper	Lower	Upper	Lower
1	0.5	51	0.003	0.011	2.963	-2.963	0.192	-0.192
2	1	102	0.05	0.098	1.969	-1.969	1.969	-1.969

An interim analysis of Rituximab SC as of C1 cohort safety and pharmacokinetics data will be performed after 10 patients completed rituximab SC treatment in cycle 2.

15.10 Final analysis

The final analysis will be realized when the number of events (102) has been reached in the ITT set or at the latest 3 years after last patient randomization. The approximate schedule of the final analysis will be approximately 54 months after first enrollment and at the latest 5.5 years after the first patient randomization.

16 STUDY MONITORING

16.1 Responsibilities of investigators

The investigator(s) undertake(s) to perform the study in accordance with Good Clinical Practice and specifically either good clinical practice for trials on medicinal products in the European Community (ISBN 92 - 825-9563-3) and guidelines for the monitoring of clinical investigations.

The investigators ensure compliance with respect to the investigational drug schedule, visit schedule and procedures required by the study. The investigators agree to provide all information requested in the case report form in an accurate and legible manner according to instructions provided.

As may be required by the local legislation, the investigators will check that the patients are directly or indirectly affiliated to the national health insurance or coverage system if there is any.

16.2 Responsibilities of the sponsor

The sponsor (LYSARC) of this study has responsibilities to health authorities to take all reasonable steps to ensure the proper conduct of the study as regards ethics, study adherence, integrity and validity of the data recorded on the case report forms. Thus, the main duty of the sponsor project leader and of the Sponsor clinical research support team (LYSARC) is to help the investigator maintaining a high level of ethical, scientific, technical and regulatory quality in all aspects of the study.

At regular intervals during the study, the center will be contacted, through site visits, letters or telephone calls, by a representative of the monitoring team (LYSARC) to review study progress, investigator and subject adherence to study requirements and any emergent problems.

During monitoring visits, the following points will be assessed with the investigator for each patient selected and randomized: subject informed consent signature, patient eligibility (inclusion and exclusion criteria), subject recruitment and follow-up, subject compliance to the study treatment, study treatment accountability, concomitant therapy use, evaluations of response, serious/non serious adverse event documentation and reporting, and quality of data. Sections of Case Report Forms may be entered on a visit per visit basis.

16.3 Source document requirements

According to the guidelines on Good Clinical Practice, the sponsor representative will check the case report form entries against the source documents as described in the monitoring plan. These personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information.

16.4 Use and completion of electronic case report form (eCRF)

An electronic Case Report Form (eCRF) will be completed for each study subject. It is the investigator's responsibility to ensure the accuracy, completeness, legibility and timeliness of the data reported in the subject's eCRF available at the following website:

<http://study.lysarc.info>.

Source documentation supporting the eCRF data should indicate the subject's participation in the study and should document the dates and details of study procedures, adverse events and subject status.

The investigator, or designated representative, should complete the eCRF pages as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination.

All data entry and corrections are recorded in the audit trail (date of data entry/correction, name of person, type of action).

17 ETHICAL AND REGULATORY STANDARDS

17.1 Ethical principles

This study is in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and subsequent amendments and will be conducted according to ICH/GCP guidelines.

17.2 Laws and regulations

This study is performed also in accordance with applicable laws and regulations of each country involved in the trial, as well as any applicable guidelines.

All data of the patients collected by the sponsor will be anonymized.

17.3 Informed consent

It is the responsibility of the investigator to obtain informed consent in compliance with national requirements from each subject prior to entering the trial or, where relevant, prior to evaluating the patient's suitability for the study.

The informed consent document used by the investigator for obtaining subject's informed consent must be reviewed and approved by LYSARC prior to Ethics Review Committee submission.

The investigator must explain to potential patient the aims, methods, reasonable anticipated benefits and potential hazards of the trial and any discomfort it may entail. Patients will be informed that they are free not to participate in the trial and that they may withdraw consent to participate at any time. They will be told which alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment.

The consent form will include a statement by which the patients allow the sponsor's duly authorized personnel (trial monitoring team) to have direct access to source data which supports data on the case report forms (e.g. patient's medical file, appointment books, original laboratory records, etc...).

The patient and the investigator should sign and date 3 original copies of informed consent form and the patient should receive the patient information leaflet. The randomization process will be documented in

each patient's medical records. At the end of the recruitment, an original copy of informed consent form will be recovered by the Sponsor in a sealed envelope.

For biological studies, a specific informed consent form will be signed and dated by patients and investigators.

17.4 Ethics Review Committee and competent authorities submission

The sponsor must submit this study to country central ethics review committee, and to competent authorities and it is required to forward a copy of written approvals / advices signed to the investigators.

18 ADMINISTRATIVE PROCEDURES

18.1 Curriculum vitae

An updated copy of the *curriculum vitae* of each investigator and sub-investigator will be provided the LYSARC prior to the beginning of the study.

18.2 Confidentiality agreement

All goods, materials, information (oral or written) and unpublished documentation provided to the investigators (or any company acting on their behalf), inclusive of this study, the patient case report forms are the exclusive property of LYSARC.

They may not be given or disclosed by the investigator or by any person within his authority either in part or in totality to any unauthorized person without the prior written formal consent of LYSARC.

It is specified that the submission of this study and other necessary documentation to the Ethics Review Committee or a like body is expressly permitted, the Ethics Committee members having the same obligation of confidentiality.

The investigator shall consider as confidential and shall take all necessary measures to ensure that there is no breach of confidentiality in respect of all information accumulated, acquired or deduced in the course of the trial, other than that information to be disclosed by law.

18.3 Record retention in investigating center(s)

The investigator must maintain all study records, patient files and other source data for the maximum period of time permitted by the hospital, institution or private practice.

However national regulations should be taken into account, the longest time having to be considered.

For trials performed in the European Community, the investigator is required to arrange for the retention of the patient identification codes for at least 15 years after the completion or discontinuation of the trial.

Any center will notify the sponsor before destroying any data or records.

18.4 Ownership of data and use of the study results

The sponsor has the ownership of all data and results collected during this study. In consequence the sponsor or any third Party either appointed by the Sponsor or having concluded a specific agreement with the Sponsor, reserves the right to use the data of the present study, either in the form of case report forms (or copies of these), or in the form of a report, with or without comments and with or without analysis, in order to submit them to the health authorities of any country.

The Investigator is committed to give his support to any requests for a patent or any property title based on, or illustrated with the results of the present Study for any country.

18.5 Publication

The results of the trial will be published after complete data collection and evaluation. Partial or preliminary results can be published beforehand. Publication is to be initiated by the two coordinating investigators in charge of the study with approval of partner if applicable.

Any publication in the form of a lecture, poster or article must be basically approved by the Scientific Committee of the LYSA.

The authors will be proposed (according to the updated LYSA publication rules) by the coordinating investigators in charge of the study, and finally endorsed by the Steering Committee of the LYSA.

All study data and publications are the property of LYSARC/LYSARC.

18.6 Insurance compensation

The sponsor certifies having taken out appropriate liability insurance policy which covers the Sponsor, the investigator and his co-workers and which is in accordance with the local laws and requirements. Specific statements will be contained in appendix where needed.

A certificate of insurance will be provided to the investigator in countries in which this document is required.

The Investigator(s) will remain responsible towards the Sponsor of any fault or misconduct regarding the performance of the Study.

18.7 Company audits and inspections by regulatory agencies

For the purpose of ensuring compliance with good clinical practice and regulatory agency guidelines it may be necessary to conduct a site audit or an inspection.

By signing this study, the investigator agrees to allow LYSARC and its representative, and drug regulatory agencies to have direct access to his study records for review. These personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information.

These audits involve review of source documents supporting the adequacy and accuracy of data gathered in eCRF, review of documentation required to be maintained, and checks on drug accountability.

LYSARC will in all cases help the investigator prepare for an inspection by any regulatory agency.

18.8 Clinical study report

The sponsor will inform of the end of the trial the Competent Authorities and Ethics Committees during the 3 months following the end of the study. A publication as a study report will be prepared under the responsibility of the sponsor, less than one year after the end of the study and forwarded to the Competent Authorities and Ethics Committees.

18.9 Protocol amendments

It is specified that the appendices attached to this study and referred to in the main text of this study, form an integral part of the study.

No changes or amendments to this study may be made by the investigator or by the sponsor after the study has been agreed to and signed by both parties unless such change(s) or amendment(s) have been fully discussed and agreed upon by the investigator and the LYSARC.

Any change agreed upon will be recorded in writing, the written amendment will be signed by the investigator and by the sponsor and the signed amendment will be appended to this study.

Approval / advice of amendments by Ethics Review Committee and Competent Authorities are required prior to their implementation, unless there are overriding safety reasons.

If the change or deviation increases risk to the study population, or adversely affects the validity of the clinical investigation or the subject's rights, full approval / advice must be obtained prior to implementation. For changes that do not involve increased risk or affect the validity of the investigation or the subject's rights, approval / advice may be obtained by expedited review, where applicable.

In some instances, an amendment may require a change to a consent form. The investigator must receive approval / advice of the revised consent form prior to implementation of the change. In addition, changes to the case report forms, if required, will be incorporated in the amendment.

Prior to initiating the changes, study amendment must be submitted to regulatory agencies, where applicable, except under emergency conditions.

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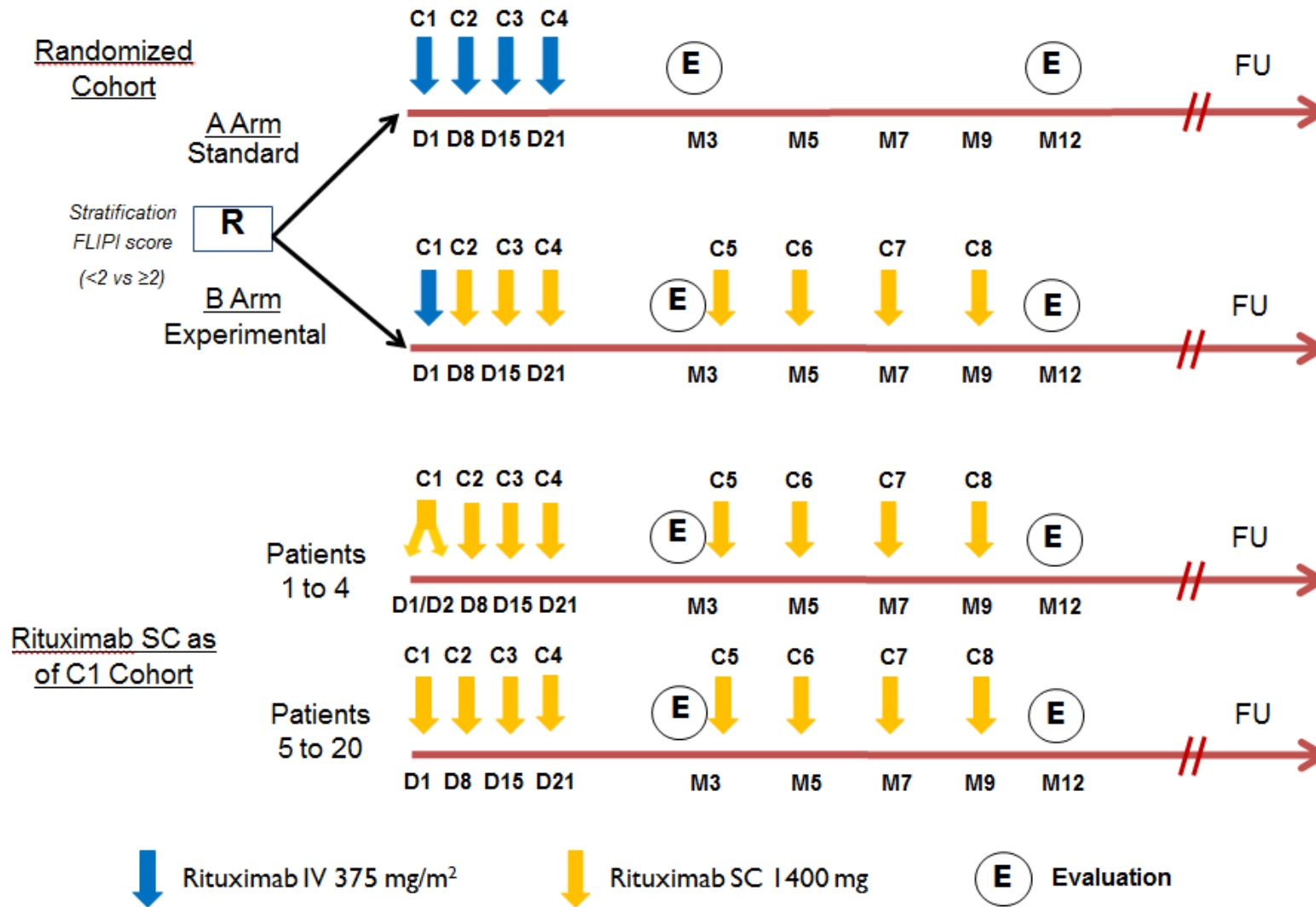
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20 APPENDIXES

20.1 Appendix 1: Study Flow Chart



20.2 Appendix 2: Schedule of Study Assessments

Table 1: Schedule of Study Assessments during treatment period

Procedure	Screening	D1, D8, D15, D22 (+/- 1 day)	M3 (+/- 1 week)	M5 (+/- 1 week)	M7 (+/- 1 week)	M9 (+/- 1 week)	M12 (+/- 1 month)	PW (within 4 weeks)
Informed Consent	X							
Inclusion/Exclusion Criteria	X							
Complete Medical History	X							
Concomitant Medications	X	X ²	X ²	X ²	X ²	X ²	X ²	X ²
Vital signs ¹	X							
ECOG Performance Status	X	X	X	X	X	X	X	X
B Symptoms	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X
Tumor biopsy ³	X							
Bone Marrow biopsy ⁴ , aspiration	X		X ⁵				X ^{5,6}	X ^{5,6}
Hematology lab tests ⁷	X	X	X	X	X	X	X	X
Creatinine Clearance (Cockcroft-Gault estimation)	X		X	X	X	X		
Serum Chemistry ⁸	X		X	X	X	X		
β ₂ microglobulin	X		X	X	X	X	X	X
Lactate dehydrogenase (LDH)	X		X	X	X	X	X	X
Serum electrophoresis Serum levels IgG, IgM, IgA	X						X	X
HBV screening ⁹	X							
HIV / HCV Serologies	X							
Pregnancy test (βHCG) ¹⁰	X							
CT scan of Neck, Thorax, Abdomen and Pelvis ¹¹	X		X				X	X
Response Assessment (Cheson 1999)			X				X	X
FDG-PET Scan	X						X	X
Response Assessment (Lugano Classification)							X	X
Blood sample for PK (Arm A)		X ¹²	X ¹³					X ¹⁵
Blood sample for PK (Arm B)		X ¹²	X ¹³	X ¹³	X ¹³	X ¹³		X ¹⁵
Blood sample for PK (SC Cohort)		X ¹⁴	X ¹³	X ¹³	X ¹³	X ¹³		
Blood sample for genomic and genetic studies	X		X				X	X ¹⁵
Bone marrow sample for genetic and immunological studies	X						X	
Blood sample for flow cytometry and plasma storage ¹⁵	X		X				X	X ¹⁵
Blood sample for immunogenicity ¹⁶	X		X				X	X ¹⁵
Adverse Events ¹⁷	X	X	X	X	X	X	X	

- ¹ Weight, height, body surface area according Mosteller formula (see Appendix 6)
- ² Only if related to a recorded AE.
- ³ Paraffin fixed tumor block of diagnostic tumor tissue has to be done within 4 months before signing the informed consent and must be submitted for review and storage to the LYSA-P.
- ⁴ Paraffin fixed bone marrow biopsy has to be done within 4 months before the first study drug administration and must be submitted for review and storage to the LYSA-P.
- ⁵ In all patients in CR/CRu assessed by CT scan when bone marrow involved (or not done or unspecified) at baseline.
- ⁶ Marrow aspiration only in CR/CRu /PR patients assessed by CT scan without histological bone marrow involvement at baseline but with *BCL2-IGH* rearrangement in marrow.
- ⁷ Hemoglobin, WBC count with differential and platelets count
- ⁸ AST, ALT, GGT, Total and indirect bilirubin, alkaline phosphatase (only at baseline)
- ⁹ AgHBs, Ac anti-HBs, Ac anti-HBc (and viral DNA if applicable)
- ¹⁰ For female with childbearing potential (FCBP)
- ¹¹ CT scan must be performed with IV contrast. In case of contra indication to IV contrast, MRI is accepted.
- ¹² Blood sample for pharmacokinetic must be performed **before rituximab (H0) and 2 hours** after the end of rituximab administration (**H2**) (see Appendix 11).
- ¹³ Blood sample **only before rituximab administration (H0)**
- ¹⁴ Blood sample for pharmacokinetic must be performed **before rituximab (H0)** at D1, D2, D4, D5, D8, D11, D15, D22 for patients 1 to 4 and D1, D4, D8, D11, D15, D22 for patients 5 to 20. For all patients: PK should also be taken at the time of an ARR with Gr. 2-4
- ¹⁵ Blood samples in case of permanent treatment discontinuation due to relapse or progression.
- ¹⁶ Flow cytometry, plasma storage and immunogenicity will be assessed as part of exploratory analysis and will be performed only by selected sites
- ¹⁷ AE of grade 2-5 for infections and neurological toxicities and AE of grade 3-5 for other toxicities. For rituximab SC as of C1 cohort all AE of grade 1-5 at cycles 1-2 and ARR of grade 1-5 at cycles 1 to 8 (CTCAE – version 4.03) regardless relationship to investigational product occurring from the date of informed consent signature, during treatment administration period and up to 30 days after last drug administration will be recorded.

Table 2: Schedule of Study Assessments during follow up period

Year	1				2				3		4		5	
Months	M12	15	18	21	24	27	30	33	36	42	48	54	60	66
	PW													
Physical examination	End of treatment assessments	X	X	X	X	X	X	X	X	X	X	X	X	X
ECOG PS		X	X	X	X	X	X	X	X	X	X	X	X	X
B symptoms		X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology Lab Tests ¹		X	X	X	X	X	X	X	X					
LDH		X	X	X	X	X	X	X	X					
β ₂ microglobulin		X	X	X	X	X	X	X	X					
Serum electrophoresis Serum levels IgG, IgM, IgA		X	X	X	X	X	X	X	X					
CT scan of Neck, Thorax, Abdomen and Pelvis ²			X		X		X		X	X	X	X	X	X
Assessment of Second Primary Malignancy (SPM)		X	X	X	X	X	X	X	X	X	X	X	X	X
Response Assessment (Cheson 1999)			X		X		X		X	X	X	X	X	X
All subsequent anti-lymphoma Therapy		X	X	X	X	X	X	X	X	X	X	X	X	X

¹ Hemoglobin, WBC count with differential and platelets count

² CT scan must be performed with IV contrast. In case of contra indication to IV contrast, MRI is accepted.

20.3 Appendix 3: Ann Arbor staging

- **Stage I:**
 - I: Involvement of a single lymph node region
 - IE: Localized involvement of a single extra lymphatic organ or site.
- **Stage II:**
 - II: Involvement of 2 or more lymph node regions on the same side of the diaphragm
 - IIE: Localized involvement of a single associated extra lymphatic organ or site and its regional lymph nodes with or without other lymph node regions on the same side of the diaphragm
- **Stage III:**
 - III: Involvement of lymph node regions on both sides of the diaphragm
 - IIIE: Involvement of lymph node regions on both sides of the diaphragm accompanied by localized involvement of an extra lymphatic organ or site
 - IIIS: Involvement of lymph node regions on both sides of the diaphragm accompanied by involvement of the spleen
 - IIIS+E: Both IIIS+IIIE
- **Stage IV:**
 - IV: Disseminated (multifocal) involvement of 1 or more extra lymphatic sites with or without associated lymph node involvement or isolated extralymphatic organ involvement with distant (non regional) nodal involvement
 - IVE: Extranodal lymphoid malignancies arise in tissues separate from, but near, the major lymphatic aggregates.

Source: American Joint Committee on Cancer. Non Hodgkin's lymphoma. In: AJCC Staging Manual. 5th ed. Philadelphia, PA: Lippincott-Raven;1997:289-294.

20.4 Appendix 4: B symptoms

- Fever (>38C) of unclear etiology
- Night sweats
- Weight loss greater than 10% within the prior 6 months

20.5 Appendix 5: FLIPI for Follicular Lymphoma

FLIPI (Solal-Celigny *et al.*, 2004)

Score 1 point for each of the following risk factors:

Hemoglobin, g/dL < 12 g/L

Number of nodal areas > 4

(The spleen is considered as an extranodal site and not a nodal area)

Age, years > 60

LDH level > normal

Ann Arbor Stage III/IV

<u>RISK GROUPS</u>	<u>Number of Factors</u>
Low	0-1
Intermediate	2
High	3-5

FLIPI 2 (Federico *et al.*, 2009)

Score 1 point for each of the following risk factors:

β 2 microglobulin > ULN (upper limit of normal)

Bone marrow involvement yes

Hemoglobin, g/dL < 12 g/L

LoDLIN, cm > 6

(Longest diameter of the largest involved lymph node)

Age, years > 60

<u>RISK GROUPS</u>	<u>Number of Factors</u>
Low	0
Intermediate	1-2
High	3-5

20.6 Appendix 6: Body Surface Area calculation

The algorithm to be used in this study is Mosteller formula (1987):

$$\text{BSA} = \sqrt{[(\text{Height (cm)} \times \text{Weight (kg)})/3600]}$$

20.7 Appendix 7: Performance Status Criteria

The following table presents the ECOG performance status scale:

ECOG Performance Status Scale	
Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction
1	Symptoms but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (eg, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982; 5 (6):649-55.

20.8 Appendix 8: Response criteria for lymphoma – Cheson 1999

Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. *J Clin Oncol* 1999;17:1244–53.

Complete Response (CR)

A complete response requires the following:

- Complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms if present before therapy, and normalization of those biochemical abnormalities [e.g., lactate dehydrogenase (LDH)] definitely assignable to NHL
- All lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in their greatest transverse diameter for nodes ≥ 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their greatest transverse diameter before treatment must have decreased to < 1 cm in their greatest transverse diameter after treatment, or by more than 75% in the sum of the products of the greatest diameters (SPD).
- The spleen, if considered to be enlarged before therapy on the basis of a CT scan, must have regressed in size and must not be palpable on physical examination. Similarly, other organs considered to be enlarged before therapy due to involvement by lymphoma, such as liver and kidneys, must have decreased in size.
- Bone marrow, if positive at baseline, must be histologically negative for lymphoma.

Complete Response, unconfirmed (CRu)

CRu includes those patients who fulfil criteria 1 and 3 above, but with one or more of the following features:

- A residual lymph node mass greater than 1.5 cm in greatest transverse diameter that has regressed by more than 75% in the SPD. Individual nodes that were previously confluent must have regressed by more than 75% in their SPD compared with the size of the original mass.
- Indeterminate bone marrow (increased number or size of aggregates without cytologic or architectural atypia).

Partial Response (PR)

A partial response requires the following:

- $\geq 50\%$ decrease in SPD of the six largest dominant nodes or nodal masses. These nodes or masses should be selected according to the following features: (a) they should be clearly measurable in at least two perpendicular dimensions, (b) they should be from as disparate regions of the body as possible, and (c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.
- No increase in the size of the other nodes, liver, or spleen
- Splenic and hepatic nodules must regress by at least 50% in the SPD.
- With the exception of splenic and hepatic nodules, involvement of other organs is considered assessable and not measurable disease.
- No new sites of disease

Stable Disease (SD)

Stable disease is defined as less than a PR (as described above) but not progressive disease (see below).

Relapsed disease (CR, CRu)

- Appearance of any new lesion or increase by $\geq 50\%$ in the size of previously involved sites.
- $\geq 50\%$ increase in greatest diameter of any previously identified node greater than 1 cm in its short axis or in SPD of more than one node.

Progressive Disease (PD)

Progressive Disease is defined as follows:

- $\geq 50\%$ increase from nadir in the SPD of any previously identified abnormal node.
- Appearance of any new lesion during or at the end of therapy

20.9 Appendix 9: Recommendations for Initial Evaluation, Staging, and Response Assessment of Hodgkin and Non-Hodgkin Lymphoma : The Lugano Classification

Bruce D. Cheson, Richard I. Fisher, Sally F. Barrington, Franco Cavalli, Lawrence H. Schwartz, Emanuele Zucca, and T. Andrew Lister. *J Clin Oncol.* 2014 Sep 20;32(27):3059-68

Revised Criteria for Response Assessment		
Response and Site	PET-CT–Based Response	CT-Based Response
<p>Complete</p> <p>Lymph nodes and extralymphatic sites</p> <p>Nonmeasured lesion</p> <p>Organ enlargement</p> <p>New lesions</p> <p>Bone marrow</p>	<p>Complete metabolic response</p> <p>Score 1, 2, or 3_ with or without a residual mass on 5 Point Scale†</p> <p>It is recognized that in Waldeyer’s ring or extranodal sites with high physiologic uptake or with activation within spleen or marrow (eg, with chemotherapy or myeloid colony-stimulating factors), uptake may be greater than normal mediastinum and/or liver. In this circumstance, complete metabolic response may be inferred if uptake at sites of initial involvement is no greater than surrounding normal tissue even if the tissue has high physiologic uptake</p> <p>Not applicable</p> <p>Not applicable</p> <p>None</p> <p>No evidence of FDG-avid disease in marrow</p>	<p>Complete radiologic response (all of the following)</p> <p>Target nodes/nodal masses must regress to ≤ 1.5 cm in LD</p> <p>No extralymphatic sites of disease</p> <p>Not applicable</p> <p>Regress to normal</p> <p>None</p> <p>Normal by morphology; if indeterminate, IHC negative</p>
<p>Partial</p> <p>Lymph nodes and extralymphatic sites</p> <p>Nonmeasured lesion</p> <p>Organ enlargement</p> <p>New lesions</p> <p>Bone marrow</p>	<p>Partial metabolic response</p> <p>Score 4 or 5† with reduced uptake compared with baseline and residual mass(es) of any size</p> <p>At interim, these findings suggest responding disease</p> <p>At end of treatment, these findings indicate residual disease</p> <p>Not applicable</p> <p>Not applicable</p> <p>None</p> <p>Residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy or an interval scan</p>	<p>Partial remission (all of the following)</p> <p>$\geq 50\%$ decrease in SPD of up to 6 target measurable nodes and extranodal sites</p> <p>When a lesion is too small to measure on CT, assign 5 mm x 5 mm as the default value</p> <p>When no longer visible, 0 x 0 mm</p> <p>For a node > 5 mm x 5 mm, but smaller than normal, use actual measurement for calculation</p> <p>Absent/normal, regressed, but no increase</p> <p>Spleen must have regressed by > 50% in length beyond normal</p> <p>None</p> <p>Not Applicable</p>
<p>No Response or</p>	<p>No metabolic response</p>	<p>Stable Disease</p>

<p>stable disease</p> <p>Target nodes/nodal masses, extranodal lesions</p> <p>Nonmeasured lesion</p> <p>Organ enlargement</p> <p>New lesions</p> <p>Bone marrow</p>	<p>Score 4 or 5 with no significant change in FDG uptake from baseline at interim or end of treatment</p> <p>Not applicable</p> <p>Not applicable</p> <p>None</p> <p>No change from baseline</p>	<p>< 50% decrease from baseline in SPD of up to 6 dominant, measurable nodes and extranodal sites; no criteria for progressive disease are met</p> <p>No increase consistent with progression</p> <p>No increase consistent with progression</p> <p>None</p> <p>Not applicable</p>
<p>Progressive disease</p> <p>Individual target nodes/nodal masses</p> <p>Extranodal lesions</p> <p>Nonmeasured lesion</p> <p>New lesions</p> <p>Bone Marrow</p>	<p>Progressive Metabolic Response</p> <p>Score 4 or 5 with an increase in intensity of uptake from baseline and/or</p> <p>New FDG-avid foci consistent with lymphoma at interim or end-of-treatment assessment</p> <p>None</p> <p>New FDG-avid foci consistent with lymphoma rather than another etiology (eg, infection, inflammation). If uncertain regarding etiology of new lesions, biopsy or interval scan may be considered</p> <p>New or recurrent FDG-avid foci</p>	<p>Progressive disease requires at least 1 of the following:</p> <p>PPD progression</p> <p>An individual node/lesion must be abnormal with:</p> <p>LDi > 1.5 cm and</p> <p>Increase by \geq 50% from PPD nadir and</p> <p>An increase in LDi or SDi from nadir</p> <p>0.5 cm for lesions \leq 2 cm</p> <p>1.0 cm for lesions > 2 cm</p> <p>In the setting of splenomegaly, the splenic length must increase by > 50% of the extent of its prior increase beyond baseline (eg, a 15-cm spleen must increase to > 16 cm). If no prior splenomegaly, must increase by at least 2 cm from baseline</p> <p>New or recurrent splenomegaly</p> <p>New or clear progression of preexisting nonmeasured lesions</p> <p>Regrowth of previously resolved lesions</p> <p>A new node > 1.5 cm in any axis</p> <p>A new extranodal site >1.0 cm in any axis; if < 1.0 cm in any axis, its presence must be unequivocal and must be attributable to lymphoma</p> <p>Assessable disease of any size unequivocally attributable to lymphoma</p> <p>New or recurrent involvement</p>

20.10 Appendix 10: Pathological Samples Review

General principles and organization of the pathological review:

The FLIRT study requires a histological review of all cases included in the trial at diagnosis. The aims of the centralized pathological review will be to **confirm the diagnosis of follicular lymphoma**, according to the criteria of the updated WHO classification 2008 (S. Swerdlow *et al.*) for each patient in the FLIRT study. Histological criteria of inclusion and exclusion have been detailed in the current protocol.

The review process will be organized by the LYSA-Pathology institute, Hôpital Henri Mondor, Créteil (LYSA-P).

Therefore for each randomized/included patient, tumor tissue blocks or - only when not possible - unstained slides will have to be sent for analysis and confirmation of diagnosis to LYSA-P.

Practical aspects of the LYSA review:

1. Information on patient inclusion

At patient inclusion, the investigator must fax (+33 (0)4 26 07 40 13) to LYSARC a copy of the anonymized histopathological report on which the name and address of the pathologist having diagnosed the follicular lymphoma will be easily identified as well as the bone marrow report.

2. Sample request

At reception of the pathological report and inclusion form, the LYSA-P will send to the initial pathologist a letter requesting:

- The paraffin block from the formalin fixed sample that was used to set the diagnosis and/ or 10 unstained Superfrost + slides
- A copy of the bone marrow pathological report if not sent previously
- To notify the LYSA-P of the presence of frozen tissue

For patient assessed with a complete response or unconfirmed complete response by CT scan and presenting an involved bone marrow at baseline, the bone marrow trephine biopsy will also be requested for central review.

3. Sample centralization at LYSA-P

All these requirements (excluding frozen tissue) will be sent in prepaid envelopes and centralized by the LYSA-P at the following address:

**LYSA-P, LYSA – FLIRT study,
Hôpital Henri Mondor
51, avenue de Lattre de Tassigny, 94010 Créteil France**

4. Sample review

At sample reception, routinely stained sections will be performed and an appropriate panel of antibodies according to morphological aspects will be applied. When sufficient slides are available, a pathological review will be organized at the LYSA-P with the designated panel of pathologists for this study. All the cases will be reviewed by at least 2 expert hematopathologists and a consensus diagnosis will be set and registered in the LYSA-P data base. This consensus diagnosis will then be sent to the clinical investigator and to the initial pathologist. For the need of the ancillary study, blocks will be kept temporarily to avoid a second request. In the meanwhile, the block is at the entire disposition of the initial pathology laboratory under request.

20.11 Appendix 11: Biological samples for further ancillary studies

A) RATIONALE

The FLIRT study represents a unique opportunity to collect biological samples from patients with follicular lymphoma at diagnosis that can be used to improve comprehension of the disease, better define the prognostic criteria and identify new factors that influence treatments results and outcome of this disease. These scientific studies will be performed as ancillary studies based on the FLIRT protocol. The general objective is to determine the prognosis value of an integrated multi-parametric analysis including tumor genomic profiling, frequency of circulating t(14;18) (a predictive factor of pre-FL to FL development), genetic background (using GWAS analysis), tumor microenvironment profiling, histopathology, imaging and documented clinical data, in low-tumor burden patients treated with front-line rituximab.

A specific informed consent form will be used to allow the collection of blood samples for genomic DNA preparation and storage and to analyze markers known to influence disease outcome and response to therapy

B) TUMOR BIOPSY: MICROENVIRONMENT AND MALIGNANT B CELL HETEROGENEITY

Several of the most common genetic alterations in FL are not oncogenic *per se* but are supposed to favor the crosstalk between malignant cells and their microenvironment (Ame-Thomas et al. Semin Cancer Biol 2014). In agreement, FL B cells are found admixed with various non-malignant cell subsets, including stromal cells, macrophages and CD4^{POS} T cells, within invaded lymph nodes and bone marrow. Several studies have proposed a panel of prognostic biomarkers reflecting the number, activation and/or spatial distribution of infiltrating immune cells. Moreover, recent studies have provided interesting cues illustrating the two faces of FL microenvironment; *i.e.* its capacity to exert anti-tumor activity by itself or by potentiating the efficacy of FL-targeting drugs *versus* its capacity to favor directly and indirectly FL cell growth. A better understanding of how B cells co-opt their microenvironment to create conducive niches at the early stage of the disease, how this niche is modified after treatment and support FL relapse, and whether microenvironment-based biomarkers could predict patient behavior will be very useful to optimize patient treatment and follow-up.

First, the surrounding microenvironment of the tumor known to influence the development and progression of follicular lymphoma will be studied on full slides looking at markers such as PD1, FOXP3, CD3, CD4, CD8, ICOS, Granzyme B, CD68, CD163.

Other biological biomarkers known to influence the prognosis of follicular lymphoma will also be analyzed on tissue microarray (TMA) performed from the paraffin embedded tissue block received.

For the need of ancillary study, blocks will be kept temporarily to avoid a second request. Meanwhile, the block will be at the entire disposition of the initial pathology laboratory under request if they need it.

At the end of the inclusion, frozen tumor tissue will be requested for all included patients. The collection of the frozen tumor specimen will be organized and centralized at the LYSA-P. On frozen tissue, gene and protein expression analysis will be performed to assess the level of expression of genes/proteins known to influence the outcome of follicular lymphoma patients.

Stromal cells and CD4^{POS} T cells play a key role in FL pathogenesis and drug resistance and their phenotype will be further analyzed by multicolor immunofluorescence on frozen tissue in a set of 40 FL patients. In parallel, localization, proliferation (Ki67), and activation (STAT and NF-κB pathways) of malignant B cells will be studied in order to correlate B cell and microenvironment features.

It is therefore highly important to consider at diagnosis all the possibilities to freeze the tumor sample.

The LYSA-P Institute will make a commitment to stock the frozen samples in a tumor library or a biological center insuring the respect of a convention which declines commitments of LYSARC relating to collection, conservation and use of frozen biological samples

All the samples will be stored in:

Hôpital Henri Mondor
Département de Pathologie
Plateforme de Ressources Biologiques : Tissuthèque
51 av du Mal de Lattre de Tassigny
94010 CRETEIL – France

Moreover, when possible, viable tumor cells will be frozen and stored in one of the 4 already set up CeVi platforms (Rennes, Créteil, Lyon, Marseille) in order to be used to perform wide multicolor flow cytometry studies on lymphoid, myeloid, and stromal cell compartments (analysis of cell subsets, polarization/activation, expression of immune modulatory molecules) and dedicated gene expression profile/functional studies on purified microenvironment cell subsets, with a specific interest for stromal cells and CD4^{pos} T cells. In addition, DNA from lymph node biopsies will also be used for an extensive analysis of genetic heterogeneity of FL malignant cells by whole exome sequencing (10 lymph node biopsies in each arm) followed by backtracking using targeted deep-sequencing on paired bone marrow biopsies and/or blood when frequency allows. A validation set will be subsequently performed in an extension cohort (n>30) of lymph node biopsies and available paired bone marrow/blood, and on relapses when occurring.

C) BONE MARROW ASPIRATE: MICROENVIRONMENT AND MALIGNANT B CELL HETEROGENEITY

Bone marrow (BM) infiltration, found in up to 70% of patients at diagnosis, is characterized by an ectopic differentiation of lymphoid-like stromal cells and local enrichment in CD4^{pos} T cells suggesting a critical dependence of malignant B cells to this specific supportive cell niche. Despite these similarities, some differences in cell composition and organization exist between LN and BM niches. In agreement, different subclones can be detected within BM and LN, and BM FL cells are characterized by a lower cytological grade and proliferation. These data support the hypothesis that trafficking in various specific microenvironments could contribute to FL clonal selection and molecular heterogeneity. We plan here to characterize in detail bone marrow microenvironment (lymphoid, myeloid, stromal cell subsets) by multicolor flow cytometry and to evaluate the differential genomic landscape in nodal *versus* medullar tumors in relation with specific micro-environmental niches with a specific interest for the dynamics of subclonal evolution and microenvironment plasticity in response to Rituximab treatment.

- After each inclusion in French sites, the LYSARC will provide to the center the consumables required to perform these studies:
 - 1 heparin tube sample for each time point (before study treatment, M12 in all patients in CR assessed by CT scan) with labels
 - 2 kits for the shipment of heparin tube to CHU Pontchaillou – Rennes
 - Traceability forms for biological studies.
- Sample processing: Before treatment and at M12 for patients in CR/CRu assessed by CT scan when bone marrow involved at baseline (or not done or unspecified) and for patients in CR/CRu/PR assessed by CT without histological bone marrow involvement at baseline but with *BCL2-IGH* rearrangement in bone marrow:
 - Collect 3ml to 5ml of bone marrow on heparin tube provided by LYSARC.

- Identify the tube with label provided.
- Fill in the traceability form « *PROTOCOLE FLIRT – Etude génomique Moelle et Sang – Envoi des échantillons à Rennes* »
- **The day of bone marrow sample collection:** Send the tube and the filled in traceability form within the shipment kit provided by LYSARC, by the designated carrier, at room temperature to CHU Pontchaillou – Rennes.

Address of shipping: Pr Karin TARTE/Cédric MENARD
 Laboratoire SIT1
 BMT-HC 1er étage
 CHU Pontchaillou
 2 rue Henri Le Guilloux
 35033 RENNES

D) PERIPHERAL BLOOD SAMPLES: SUSCEPTIBILITY LOCI, FCRN POLYMORPHISM, IMMUNOLOGICAL STUDIES AND MALIGNANT B CELL HETEROGENEITY

GENOMIC STUDY FOR ALL PARTICIPATING LYSA CENTERS

The presence in “healthy” individuals/asymptomatic patients of circulating common progenitor cells (CPCs) years before diagnosis has been demonstrated (Roulland et al JCO 2014), CPCs that may reemerge as recurrent relapses under current therapeutic regimens. However, the very early steps of FL pathogenesis and the associated alterations remain poorly characterized, and no study has been designed to follow clonal selection during disease evolution and under treatment. Similarly, whereas susceptibility loci have been associated with the predisposition to FL or the transformation of FL into aggressive lymphomas, such genome wide association studies (GWAS) should be extended to large series of patients with homogeneous treatment to further understand how these genetic factors could impact tumors and patient behavior. Moreover, some polymorphisms, such as this affecting FCGR3A, are associated with response rate to Rituximab. In this study, we plan to evaluate the role of FcRn polymorphism on rituximab pharmacokinetics and on response to treatment. Interestingly, such approach underlines the role of the microenvironment in the efficacy of Rituximab. We plan here to identify some circulating biomarkers that could predict response to treatment. This will include i) multicolor flow cytometry on fresh samples (T cell subsets including circulating naive/memory T cells, TFh cells, T $\gamma\delta$, NK cells, myeloid cell subsets, dendritic cells); ii) analysis of immunomodulatory molecules in frozen plasma (at least sPD-L1, sCD163, IDO, sIL2R, CCL22); iii) analysis of CMV serology to confirm that CMV^{POS} patients display RCRgIII^{POS} T $\gamma\delta$ T cells and to evaluate how this could be correlated with response to rituximab.

After each inclusion in FLIRT study, the LYSARC will provide to the center the consumables required to perform the genomic studies:

- **For genetic studies (202 patients):**
 - 1 PAXgene DNA tube for each time points (before study treatment, M3 and M12 or permanent treatment discontinuation in case of progression or relapse) with labels
 - 3 kits for the shipment of PAXgene DNA tubes to either CHU Pontchaillou – Rennes or CHU Henri Mondor – Créteil
 - Traceability forms for genetic studies.

- For immunological studies (90 patients)
 - Heparin tubes for blood samples at each time points (before study treatment, M3 and M12 or permanent treatment discontinuation in case of progression or relapse) with labels.
 - 3 kits for the shipment of heparin tubes to CHU Pontchaillou – Rennes.
 - Traceability forms for biological studies.

Sample processing:

- **GENETIC STUDIES: 202 PATIENTS**

At each time-point (before study treatment, M3, M12 or permanent treatment discontinuation in case of progression or relapse):

- Collect 8mL of blood on PAXgene DNA tube. Identify the tube with LYSARC provided label.
- Fill in the traceability form :
 - Centers, which send the PAXgene tube to Créteil should use the traceability form “*Protocole FLIRT – Etude génomique sur sang – Envoi des échantillons à Créteil*”
 - ⇒ Send the tube and filled in traceability form within the shipment kit provided by LYSARC, by **regular mail** at room temperature CHU Henri Mondor – Créteil.
 - Centers, which send the PAXgene tube to Rennes (centers selected), should use the traceability form « *PROTOCOLE FLIRT – Etude génomique Moelle et Sang – Envoi des échantillons à Rennes* »
 - ⇒ Send the tube and filled in traceability form within the shipment kit provided by LYSARC, by **the designated carrier** at room temperature to CHU Pontchaillou – Rennes

Address of shipping: Pr Karin TARTE / Cédric MENARD
 Laboratoire SIT1
 BMT-HC 1er étage
 CHU Pontchaillou
 2 rue Henri Le Guilloux
 35033 RENNES

Address of shipping: Pr Marie-Hélène DELFAU
 Service d'Immunologie Biologique
 INSERM U617
 CHU Henri Mondor
 51, avenue de Lattre de Tassigny
 94010 CRETEIL

Extracted DNA will be further used for study of FcRn polymorphism (Tours), identification of susceptibility loci (Lyon), analysis of frequency and genetic instability of t(14;18)^{pos} cells (Creteil and Marseille), and, for the 10 patients involved in immunogenicity study, for HLA typing (Paris).

- **IMMUNOLOGICAL STUDIES: 90 PATIENTS**

A restricted number of French LYSA centers will participate to this study, involving 90 patients.

At each time-point (before study treatment, M3, M12 or permanent treatment discontinuation in case of progression or relapse):

- Collect 15mL of blood on heparin tubes provided by LYSARC.
- Identify the tube with labels provided.

- Fill in the traceability form « *PROCOLE FLIRT – Etudes biologiques (cytométrie en flux + immunogénicité) – envoi des échantillons à Rennes* »
- **The day of blood sample:** Send the tube and the filled in traceability form within the provided shipment kit, by the designated carrier, at room temperature to CHU Pontchaillou – Rennes.

Address of shipping: Pr Karin TARTE / Cédric MENARD

Laboratoire SITI
BMT-HC 1er étage
CHU Pontchaillou
2 rue Henri Le Guilloux
35033 RENNES

D) IMMUNOGENICITY (PROOF-OF-PRINCIPLE STUDY): 10 PATIENTS RANDOMIZED IN B ARM

Rituximab is supposed to exert its anti-tumor activity at least in part through the dynamic induction of delayed but long-lasting specific immune response. Recently, CD20 has been suggested to be immunogenic by itself (Abes et al Blood 2010). This hypothesis will be evaluated here on 10 patients included in Arm B in few LYSA centers. Blood cells will be frozen and T cells will be further analyzed for their response to classical vaccinal antibodies and to selected CD20-derived peptides.

At each time-point (before study treatment, M3 and at M12 or permanent treatment discontinuation in case of progression or relapse):

- Collect 15mL of blood on heparin tubes
- Identify the tube with labels provided.
- Fill in the traceability form « *PROCOLE FLIRT – Etudes biologiques (cytométrie en flux + immunogénicité) – envoi des échantillons à Rennes* »
- **The day of blood sample collection:** Send the tube and the filled in traceability form within the provided shipment kit, by the designated carrier, at room temperature to CHU Pontchaillou - Rennes.

Address of shipping: Pr Karin TARTE / Cédric MENARD

Laboratoire SITI
BMT-HC 1er étage
CHU Pontchaillou
2 rue Henri Le Guilloux
35033 RENNES

E) PHARMACOKINETICS ANALYSIS FOR ALL PARTICIPATING LYSA CENTERS: 140 RANDOMIZED PATIENTS

After each inclusion in FLIRT study, the LYSARC will provide to the center the required consumables to perform the pharmacokinetics (PK) analysis:

- Cryotubes 2ml with labels for serum storage at -80°C (or -20°C if not available).
- Traceability forms for PK study.

Sample processing:

- Collect 10mL of blood on serum tube without gel at the following time-points:
 - **Arm A:** Blood sample before rituximab (H0) and 2 hours after the end of rituximab infusion (H2) at:

- ✓ D1, D8, D15, D22 (H0 and H2) and M3 (H0 only)
- ✓ At permanent treatment discontinuation in case of progression or relapse
- **Arm B:** Blood sample before rituximab (H0) and 2 hours after the end of rituximab administration (H2) at:
 - ✓ D1, D8, D15, D22: H0 and H2
 - ✓ M3, M5, M7 and M9: H0 only
 - ✓ At permanent treatment discontinuation in case of progression or relapse
- After each blood sample, centrifuge the tubes at 2000g for 10 minutes.
- Collect the serum in 2 cryotubes of 2mL provided by LYSARC.
- Keep the serum aliquots at -80°C (or -20°C if not available), until sending of the tubes to CHU Bretonneau -Tours (a collection will be organized by LYSARC, at the end of the follow up period).
- Fill in the traceability form “ PROTOCOLE FLIRT – Etude pharmacocinétique – Analyse des échantillons à Tours ” after each blood sample and fax it to LYSARC biological department (+33 (0)4 72 66 93 71).

All PK samples will be collected by LYSARC at the end of treatment of the last randomized patient and sent to CHU Bretonneau – Tours to perform analyzes.

CHRU Bretonneau
Céline Desvignes
Laboratoire de Pharmacologie – Toxicologie
Bât B2A – 4^{ème} étage
2 bd Tonnelé
37044 TOURS cedex 9

F) PHARMACOKINETICS ANALYSIS FOR THE RITUXIMAB SC AS OF C1 COHORT: 20 PATIENTS

- Blood sample before rituximab (H0)
 - Patients 1 to 4: D1, D2, D4, D5, D8, D11, D15, D22, M3, M5, M7 and M9 (all samples to be taken pre-dose rituximab except D4, D5 and D11)
 - Patients 5 to 10: D1, D4, D8, D11, D15, D22, M3, M5, M7 and M9 (all samples to be taken pre-dose rituximab except D4 and D11)
 - All patients: PK should also be taken at the time of an ARR with Gr. 2-4
- Collect 5mL of blood on 1 dry tube
- At the latest 30 minutes after the blood sampling, centrifuge the dry tube at 4°C, at 1500g to 2000g during 15 minutes
- Quickly after centrifugation, collect 1,5ml of serum in 1 cryotube
- Within 2 hours after blood sampling, keep the serum aliquots at -80°C (a collection will be organized by LYSARC for sending tubes to QPS)
- Fill in the traceability form “ PROTOCOLE FLIRT – Etude pharmacocinétique – Cohorte Rituximab SC dès le C1 ” after each blood sampling and fax it to LYSARC biological department (+33 (0)4 72 66 93 71 or email fax.bio@lysarc.org).

All PK samples will be collected by LYSARC at 3 time points for each patient, first quickly after DAY5, the second quickly after DAY22, the last after M9, and set to QPS