COVER PAGE

TITLE: Pharmacokinetics and Safety of Roledumab, a fully human recombinant monoclonal anti-RhD antibody, in RhD-negative pregnant women carrying an RhD-positive foetus: a phase IIb, multicenter, open-label study.

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Pharmacokinetics and Safety of Roledumab, a fully human recombinant monoclonal anti-RhD antibody, in RhD-negative pregnant women carrying an RhD-positive foetus: a phase IIb, multicenter, open-label study

CLINICAL STUDY PROTOCOL
No. ADNC-1301 (phase 2b)
EUDRACT number 2013-000269-35

SPONSOR:

LFB BIOTECHNOLOGIES
3, avenue des Tropiques
BP 40305
91958 COURTABOEUF Cedex - France

CLINICAL DEVELOPMENT DIRECTOR:

[redacted]

COORDINATING INVESTIGATOR:

[redacted]

CLINICAL PROJECT MANAGER:

[redacted]

MEDICAL CONTACT:

[redacted]

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADA	Anti-drug antibody
ADCC	Antibody-dependent cell mediated cytotoxicity
AE	Adverse Event
ALT	Alanine aminotransferase
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Curve
AV	Additional Visit
C_{max}	Maximum Concentration
CNGOF	Collège National des Gynécologues et Obstétriciens Français
CRF	Case Report Form
CRO	Contract Research Organization
CRP	C-Reactive Protein
CSP	Code de la Santé Publique
CV	With respect to staff: Curriculum Vitae With respect to PK: Coefficient of Variation
DAT	Direct Antiglobulin Test
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
e-CRF	Electronic Case Report Form
ENT	Ear-Nose-Throat
FAS	Full Analysis Set
FMH	Fetomaternal hemorrhage
FOCE	First Order Conditional Estimation
GA	Gestational Age
GCP	Good Clinical Practice
HDF	Hemolytic Disease of the Fetus
HDFN	Hemolytic Disease of the Fetus and Newborn
HDN	Hemolytic Disease of the Newborn
HIV	Human Immunodeficiency Virus
IA	Indirect Antibodies
IAT	Indirect Antiglobulin Test
ICF	Informed Consent Form

ICH	International Conference on Harmonisation
IEC/IRB	Independent Ethics Committee (IEC) or International Review Board (IRB)
Ig	Immunoglobulin
IM	Intramuscular
IMP	Investigational Medicinal Product
IV	Intravenous
KB	Kleihauer-Betke test
MAA	Marketing Authorization Application
mAb	Monoclonal Antibody
MCA-PSV	Middle Cerebral Artery - Peak Systolic Velocity
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medicinal Dictionary for Regulatory Activities
MoM	Multiple of Medians
NAB	Neutralizing Antibodies
NCA	Non Compartmental Analysis
NCR	None Carbon Required
NOAEL	No Observed Adverse Effect Limit
PK	Pharmacokinetic
PT	Preferred Terms
RBC	Red Blood Cells
RhD	Rhesus D
SAE	Serious Adverse Event
SAF	Safety set
SAP	Statistical Analysis Plan
SOC	System Organ Class
T_{max}	Time to reach maximum concentration
UCHP	Unité Centre d'Hémobiologie Périnatale (250 UCHP corresponds to 1 µg anti-D)
WBC	White blood cells

SYNOPSIS

STUDY No.	**ADNC-1301**
TITLE	Pharmacokinetics and Safety of roledumab, a fully human recombinant monoclonal anti-RhD antibody, in RhD-negative pregnant women carrying an RhD-positive foetus: a phase IIb, multicenter, open-label study.
SPONSOR	LFB BIOTECHNOLOGIES 3, Avenue des Tropiques, BP 40305 91958 COURTABOEUF Cedex
STUDY COORDINATOR	[redacted]
INVESTIGATIONAL MEDICINAL PRODUCT/ RULES FOR ADMINISTRATION	Investigational Medicinal Product Roledumab (human recombinant monoclonal anti-RhD immunoglobulin IgG1) – for intramuscular and intravenous administration, 0.3 mg/mL Rules for administration Antenatal prophylaxis • Planned antenatal prophylaxis: One single dose of 300 µg IM/IV of roledumab at 28 or 29 weeks of gestation • Antenatal prophylaxis following sensitizing events: One or more dose(s) of 300 µg IM/IV anti-RhD antibodies (Rhophylac® or roledumab) based on the Kleihauer-Betke (KB) test as soon as possible and no later than 72 hours after event occurrence. Sensitizing events include but are not limited to: – Obstetric complications (e.g. miscarriage, abortion, threatened abortion, FMH or invasive procedures during pregnancy (e.g. amniocentesis, chorionic biopsy) – Obstetric manipulative procedures (e.g. external version) or abdominal trauma. A KB test will be performed prior to Rhophylac® or roledumab administration in order to determine the volume of fetomaternal hemorrhage (FMH). If the result of the KB test is >4 mL, additional dose(s) of Rhophylac® or roledumab will need to be administered. A second Kleihauer-Betke test will be performed 24 hours after the injection and additional dose(s) of Rhophylac® or roledumab will be administered if needed.

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	Postnatal prophylaxis: Roledumab should be administered to the mother as soon as possible within 72 hours of delivery of an RhD-positive infant. The postnatal dose must still be given even when antenatal prophylaxis has been administered. Before roledumab 300 µg IM/IV postnatal administration, a KB test will be performed on a maternal blood sample taken no earlier than 30 min after delivery in order to determine the volume of fetomaternal hemorrhage (FMH). If the result of the KB test is >4 mL, additional dose(s) of roledumab will need to be administered. If the KB test before IMP administration is positive, a subsequent test should be done 24 hours after IMP administration. If the second KB test is positive, additional 300 µg dose(s) of roledumab will be administered according to the volume of FMH.
STUDY DESIGN	Phase 2b, interventional, multicenter, open-label, non-randomized, sequential
OBJECTIVES	Primary: • To assess the pharmacokinetic profile of roledumab 300 µg IM/IV in RhD-negative pregnant women carrying an RhD-positive fetus. Secondary: • To assess the safety of roledumab administered IM and IV in RhD-negative pregnant women and in RhD-positive fetus and newborns • To assess the efficacy of roledumab IM and IV to prevent RhD-alloimmunization in RhD-negative pregnant women carrying an RhD-positive fetus • To assess the immunogenicity of roledumab • To measure roledumab concentration in first milk and breast milk • To measure roledumab concentration in cord blood after delivery
NUMBER OF SUBJECTS	35 planned to reach 30 evaluable subjects in the IM arm 25 planned to reach 20 evaluable subjects in the IV arm
STUDY POPULATION	Pregnant RhD-negative women carrying an RhD-positive fetus

STUDY No.	ADNC-1301
MAIN INCLUSION/EXCLUSION CRITERIA	A subject is eligible when all inclusion and no exclusion criteria are met. Inclusion criteria • Signed and dated informed consent form provided by the subject prior to proceeding with any study-related procedure • At least 18 years old • Pregnancy between 12 and 27 weeks gestational age as confirmed by early ultrasound • Pregnant RhD-negative woman carrying an RhD-positive fetus confirmed by a non-invasive fetal RhD genotyping test • Negative serology: hepatitis B and C, HIV (1 and 2), except for positive results due to vaccinations • Covered by healthcare insurance in accordance with local requirements.
MAIN INCLUSION/EXCLUSION CRITERIA	Exclusion criteria • RhD-alloimmunized subject • Positive for ADA Test • Multiple fetuses • Occurrence of a documented potential sensitizing event in this pregnancy before the antenatal IMP administration • Prior administration of anti-RhD immunoglobulin during the current pregnancy • Known clinically relevant maternal or fetal abnormality (e.g. as determined by ultrasound or genetic testing), such as placenta previa • History of anaphylactic or severe systemic reaction to immunoglobulin of any origin • Current diagnosis of an immune disease which by itself or its treatment could impair the safety and/or efficacy evaluation of roledumab in this study. These diseases are: • All immune deficiencies, particularly those requiring IV-Ig supplementation or other systemic treatment • Connective tissue and autoimmune diseases (e.g. systemic lupus erythematosus, antiphospholipid syndrome, Sjögren's syndrome, rheumatoid arthritis, ankylosing spondylarthritis) requiring systemic immunosuppressive treatment • Allergic and inflammatory diseases requiring systemic immunosuppressive treatment. • Clinically significant medical history contraindicating the participation in the study according to the judgment of the Investigator or Sponsor • Clinically significant laboratory (hematology, blood chemistry, or urinalysis) parameters • Transfusion of RhD-positive blood or blood derived products within the 6 months prior to enrolment • Anticipated poor compliance with the study procedures

STUDY No.	ADNC-1301
	• Subject within exclusion period further to her participation in a clinical study • For the IM arm only, subject with coagulation disorders contraindicating intramuscular injection (subject will still be considered for the IV arm)
DURATION OF SUBJECT PARTICIPATION	Minimal duration: approximately 9 months Maximum duration: 18 months approximately
STUDY PLANNING	Expected start date: Q3 2013 Expected end date: Q4 2017
STUDY DESCRIPTION	Selection of country/site: France / multicenter Inclusion of subjects: subject information and written informed consent form must be obtained before any study procedure. Subjects will be included when they signed their informed consent form. The first 35 subjects will be enrolled in the IM arm; the subsequent 25 subjects will be enrolled in the IV arm. For the IM arm, the subject will attend 13 visits (9 visits will be performed at the hospital unit and possibly 4 at home). If further follow-up is needed to document alloimmunization, the subject may attend 1 or 2 additional visits. For the IV arm, the subject will attend 14 visits (9 visits will be performed at the hospital unit and possibly 5 at home). If further follow-up is needed to document alloimmunization, the subject may attend 1 or 2 additional visits. The study consists of a screening period, an antenatal period, a postnatal period and a follow-up period: • The screening visit will be performed as close as possible to the antenatal IMP administration, as long as all screening results are available prior to IMP administration. However, the screening period can occur between 12 and 27 weeks GA • The antenatal period comprises 6 visits for the IM route and 7 for the IV route and lasts approximately 12 weeks • The postnatal period comprises 2 visits and lasts 3 ± 1 days • The follow-up period comprises at least 4 visits (6 months ± 2 weeks) and possibly 2 additional visits. Clinical examinations and non-clinical tests will be performed as detailed in the flow chart.
ENDPOINTS AND EVALUATION PARAMETERS	<u>Primary endpoint</u> Serum concentrations of roledumab will be measured at the defined time points. The PK profile will be described by: 1) A compartmental population PK model with 3 primary parameters: Volume of distribution (Vd), Clearance (CL) and for the IM route absorption rate constant (Ka) and derived parameters: C_{max}, T_{max}, Area under the curve (AUC_{inf}), terminal half-life ($t_{1/2}$) and Elimination rate constant.

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	2) A secondary PK analysis (NCA), which will provide the following parameters Vd, CL, C_{max}, T_{max}, AUC_t, AUC_{inf}, terminal half-life and elimination rate constant. All samples (ante- and postnatal samples) will be used for population PK modeling whereas only samples post-first administration (ante-natal sample) will be analyzed by NCA for each study arm. <u>Secondary endpoints</u> – Safety of the mother and the fetus/newborn will be assessed throughout the study by the incidence, nature, severity, seriousness, relationship to the IMP of AEs and by changes in physical examination findings, vital signs, clinical laboratory parameters, and Doppler ultrasound. – Safety of newborns/infants up to 6 months age will be assessed by recording and reporting any AE and related concomitant medications.
	– RhD-alloimmunization rate will be assessed at 6 months and up to 12 months if applicable and described. – Roledumab first milk/breast milk concentration/maternal serum concentration ratio will be described. – Roledumab cord blood concentration/maternal serum concentration ratio after delivery will be described.
STATISTICS	The statistical analysis will be descriptive on all endpoints. No statistical hypothesis will be tested. There will be two PK analyses using two analysis populations for both the IM and IV arm: - The primary PK analysis (population PK modeling) will be performed on PK set 1 (PKS1). - The secondary pharmacokinetic analysis will be a non-compartmental analysis that will be performed on PK set 2 (PKS2) and PKS3

STUDY FLOW CHART FOR THE IM arm

Visit (V) Day (D) Week (W) Month (M)	Screening visit (V1) 12-27 GA	Antenatal period							Postnatal period			Follow-up period					
		V2 Week 28 or 29 GA (D1)		V3[10] 48h±24	V4[10] 120h±24	V5 9d±3	V6 29d±4	V7 59d±4	V8 Postnatal Treatment <72 hours of delivery		V 9 Discharge 48h±24	V10[10] 72h±24 after post-natal injection*	V11[10] 9d±2 after postnatal injection*	V12 6W±2W	V13 6M±2W	Additional AV1 9M± 2W	Additional AV2 12 M± 2W
		Inclusion	Injection						Before	Injection*							
Informed Consent	x																
Eligibility Criteria	x	x															
Demographics/ Medical History/ Life habits	x																
Serology: hepatitis B and C, HIV	x																
RhD status	x																
ABO status	x																
Non-inv. fetal *RhD* genotyping	x																
Vital Signs[1]	x	x	x 30 min and 6h after injection	x	x	x	x	x	x	x 30 min after injection	x	x	x	x	x	x	x
Physical Examination	x	x[2]							x[2]						x[2]	x[2]	x[2]
Laboratory Tests	x[3]							x[4]	x[4]					x[4]	x[12]	x[4]	x[4]
Cytokines[5]		x	x 6h after injection						x	x 6h after injection							
CRP		x		x					x		x						
Obstetric and fetal Doppler ultrasound		x				x	x	x									
IAT in mothers	x	x							x[6]						x[6]	x[6]	x[6]
Anti-D Quantification		x				x	x		x				x	x	x		
IM IMP administration			x							x							
PK sample		x		x	x	x	x	x	x			x	x	x			

Visit (V) Day (D) Week (W) Month (M)	Screening visit (V1) 12-27 GA	Antenatal period							Postnatal period			Follow-up period					
		V2 Week 28 or 29 GA (D1)		V3[10] 48h±24	V4[10] 120h±24	V5 9d±3	V6 29d±4	V7 59d±4	V8 Postnatal Treatment <72 hours of delivery		V 9 Discharge 48h±24	V10[10] 72h±24 after post-natal injection*	V11[10] 9d±2 after postnatal injection*	V12 6W±2W	V13 6M±2W	Additional AV1 9M± 2W	Additional AV2 12 M± 2W
		Inclusion	Injection						Before	Injection*							
KB test									x	x[11]							
Breast milk sample												x	x				
ADA tests	x[13]	x				x	x		x				x	x	x	x	x
Adverse events		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Concomitant treatment	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x
Delivery[7]									x								
Cord blood sample[8]									x								
Newborn RhD status[9]									x								
Newborn ABO status[14]									x								
Newborn general status									x		x			x			

1 Include blood pressure, heart rate and body temperature
2 Partial examination (Weight, General Appearance, Skin, Heart, Lungs, Abdomen)
3 Hematology, blood chemistry, aPTT, urinalysis
4 Hematology, blood chemistry
5 Cytokines: IFN- γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNF-α
6 If IAT positive, IA characterization and Anti-D antibodies quantification
7 Collection of APGAR score, AEs, CMs
8 Cord blood sample: DAT and hematocrit, hemoglobin, reticulocytes, total bilirubin including routine tests (RhD status), detection of roledumab
9 Only in case of a confirmed alloimmunization: RhD genotyping in cord blood will be performed to search for potential D-variants
10 Visit performed at home
11 Kleihauer-Betke test to be performed within 24h after IMP administration only if the one prior to the IMP administration was positive.
12 Hematology, blood chemistry, urinalysis
13 Evaluation of pre-existing antibody presence
14 If performed in routine practice during the delivery hospitalization of the mother

** Postnatal injection should be performed within 72h after delivery. All subsequent visits are based from this time point.*
GA: gestational age refers to the duration of the pregnancy since the 1st day of the last menstrual period and will be expressed in weeks

STUDY FLOW CHART FOR THE IV ARM

Visit (V) Hour (H) Day (D) Week (W) Month (M)	Screening visit (V1) 12-27 GA	Antenatal period											Postnatal period			Follow-up period					
		V2 Week 28 or 29 GA (D1)					V2b[10] 24h± 2h	V3[10] 48h ±6 h	V4[10] 96h-120h	V5 9d ±3	V6 29d±4	V7 59d±4	V8 Postnatal Treatment <72 hours of delivery		V 9 Discharge 48h±24	V10[10] 72h±24 after post-natal injection*	V11[10] 9d±2 after post-natal injection*	V12 6W±2W	V13 6M±2W	Additional AV1 9M± 2W	Additional AV2 12 M± 2W
		Inc.	Inj. (H0)	30 ±5min	H1 ±5min	H6 ±15min							Before	Injection*							
Informed Consent	x																				
Eligibility Criteria	x	x																			
Demographics/ Medical History/ Life habits	x																				
Serology: hepatitis B and C, HIV	x																				
RhD status	x																				
ABO status	x																				
Non-inv. fetal *RhD* genotyping	x																				
Vital Signs[1]	x	x	x	x	x	x	x	x	x	x	x	x	x	x 30 min after inj.	x	x	x	x	x	x	x
Physical Examination	x	x[2]											x[2]						x[2]	x[2]	x[2]
Laboratory Tests	x[3]											x[4]	x[4]					x[4]	x[12]	x[4]	x[4]
Cytokines[5]		x				x							x	x 6h after inj.							
CRP		x			x		x	x					x		x						
Obstetric and fetal Doppler ultrasound		x								x	x	x									
IAT in mothers	x	x											x[6]						x[6]	x[6]	x[6]
Anti-D Quantification		x								x	x		x				x	x	x		
IV IMP administration			x											x							
PK sample		x			x		x	x	x		x	x	x			x	x	x			
KB test													x	x[11]							

Visit (V) Hour (H) Day (D) Week (W) Month (M)	Screening visit (V1) 12-27 GA	Antenatal period											Postnatal period			Follow-up period					
		V2 Week 28 or 29 GA (D1)					V2b[10] 24h± 2h	V3[10] 48h ±6 h	V4[10] 96h-120h	V5 9d ±3	V6 29d±4	V7 59d±4	V8 Postnatal Treatment <72 hours of delivery		V 9 Discharge 48h±24	V10[10] 72h±24 after post-natal injection*	V11[10] 9d±2 after post-natal injection*	V12 6W±2W	V13 6M±2W	Additional AV1 9M± 2W	Additional AV2 12 M± 2W
		Inc.	Inj. (H0)	30 ±5min	H1 ±5min	H6 ±15min							Before	Injection*							
Breast milk sample																x	x				
ADA tests	x[13]	x								x	x		x				x	x	x	x	x
Adverse events		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Concomitant treatment	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x
Delivery[7]													x								
Cord blood sample[8]													x								
Newborn RhD status[9]													x								
Newborn ABO status[14]																					
Newborn general status													x		x			x			

1 Include blood pressure, heart rate and body temperature
2 Partial examination (Weight, General Appearance, Skin, Heart, Lungs, Abdomen)
3 Hematology, blood chemistry, aPTT, urinalysis
4 Hematology, blood chemistry
5 Cytokines: IFN- γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNF-α
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7 Collection of APGAR score, AEs, CMs
8 Cord blood sample: DAT and hematocrit, hemoglobin, reticulocytes, total bilirubin including routine tests (RhD status), detection of roledumab
9 Only in case of a confirmed alloimmunization: RhD genotyping in cord blood will be performed to search for potential D-variants
10 Visit performed at home
11 Kleihauer-Betke test to be performed within 24h after IMP administration only if the one prior to the IMP administration was positive
12 Hematology, blood chemistry, urinalysis
13 Evaluation of pre-existing antibody presence
14 If performed in routine practice during the delivery hospitalization of the mother

** Postnatal injection should be performed within 72h after delivery. All subsequent visits are based from this time point.*
Inc: Inclusion / Inj: Injection / h: Hours / Min: Minutes; GA: gestational age refers to the duration of the pregnancy since the 1st day of the last menstrual period and will be expressed in weeks

STUDY FLOWCHART – ADDITIONAL VISITS IN CASE OF SENSITIZING EVENT FOR IM/IV ARMS

Visit	Before injection	6 hours after IMP administration	24 hours after IMP administration
Sensitizing event (description)	x		
Vital signs	x	x (6h after injection)	x
Partial physical examination	x		
IAT	x*		
Kleihauer-Betke test	x**		x***
Cytokines		x (6h after injection)	
CRP			x
Adverse Events	x	x	x
Concomitant treatment	x	x	x

* Perform a ponderal dosage analysis if IAT is positive, to confirm the anti-D concentration

** Before and after (if the first one is positive) anti-D administration

*** The dose will be adapted to the KB results

The PK sampling schedule after the antenatal injection should stay the same in case of a sensitizing event treated with roledumab.

1. INTRODUCTION AND BACKGROUND INFORMATION

1.1. Disease and Context

1.1.1. Disease

The RhD (Rh) factor is a red blood cell (RBC) surface antigen that was named according to the monkeys in which it was first discovered. Rh incompatibility, also known as Rh disease, is a condition that occurs when a woman with Rh-negative blood type is exposed to Rh-positive blood cells, leading to the development of antibodies. Although the Rh blood group systems consist of several antigens (D, C, c, E, e), the D antigen is the most immunogenic and most commonly involved in Rh incompatibility.

Sensitization or alloimmunization can happen at any time during pregnancy, but is more common in the third trimester and during childbirth. The first exposure from an RhD-negative mother bearing an RhD-positive fetus does not generally affect the pregnancy during which it occurs, even if fetomaternal hemorrhage sensitizes the mother against the D antigen. Subsequent hemolytic disease, leading to morbidity and mortality, can affect the fetus or the newborn in future pregnancies [1], [2]. Hemolytic disease of the fetus and newborn (HDFN) can result in complications before birth, or anemia and hyperbilirubinemia after birth or both including intrauterine fetal death.

The risk of sensitization depends on the volume of transplacental hemorrhage, the level of the maternal immune response and the concomitant presence of ABO incompatibility. The risk and the severity of the sensitization response increase with each subsequent pregnancy involving a fetus with RhD-positive blood.

Routine antenatal care was first proposed in the 1970s to all RhD-negative pregnant women i.e. an anti-RhD IgG immunoglobulin injection at about 28 weeks of GA (with or without a booster at 34 weeks gestation). This reduces the effect of the vast majority of sensitizing events which mostly occur after 28 weeks of gestation.

Anti-RhD immunoglobulin is also given to non-sensitized Rh-negative women immediately (within 72 hours) after potentially sensitizing events occurring earlier in pregnancy.

Therefore the incidence of RhD-alloimmunization has significantly decreased from 13% to 0.35% of pregnancies, as a consequence of the preventive use of polyclonal human anti-RhD immunoglobulins [3].

1.1.2. Context

These anti-RhD immunoglobulins derived from human plasma of immunized RhD-negative donors exposed to RhD-positive RBC raise several concerns regarding i) the ethics of deliberately exposing healthy volunteers to human RBC, ii) the potential appearance in blood derived products concerning unknown transmissible agents, iii) the potential future shortage of donors and subsequent RhD immunoglobulin supply.

These concerns can be circumvented by the development of a human monoclonal recombinant anti-D antibody such as roledumab, which is manufactured with consistent quality, high purity as well as

with large-scale capacities, ensuring unlimited supplies. Thus, this process could enhance substantially the benefit-risk ratio of an anti-D treatment as not extracted from human plasma.

Roledumab is being developed to prevent RhD-alloimmunization in RhD-negative women carrying an RhD-positive fetus as well as treatment of RhD-negative individuals after incompatible transfusions with blood components containing RhD-positive RBCs.

1.2. Target

Roledumab is a human recombinant monoclonal antibody that specifically recognizes the RhD antigen on human RBC with high affinity.

Anti-D IgGs prevent alloimmunization by first binding to the RhD antigen of the RhD-positive RBC, resulting in the elimination of these RBCs from the circulation by mononuclear effector cells, mainly in the spleen. This clearance is associated with a dynamic suppression of the primary immune response, thus preventing immunization. The most serious clinical consequence of alloimmunization to fetal RhD-positive RBCs in pregnant RhD-negative females is HDFN during subsequent pregnancies.

1.3. IMP, Non-Clinical and Clinical Information

1.3.1. IMP and non-clinical information

Roledumab is a recombinant IgG1 deriving from a natural anti-RhD antibody isolated from an immunized donor. This IgG1 belongs to a new generation of monoclonal antibodies based on the proprietary EMABling® technology which confers to roledumab enhanced FcγRIIIa interaction. As a consequence, this anti-RhD antibody triggers in vitro Antibody Dependent Cell-mediated Cytotoxicity (ADCC) against RhD-positive RBC. In the absence of relevant in vivo efficacy animal models caused by a high level of species specificity for RhD antigen in mammals [4], extensive in vitro pharmacology studies comparative to the reference polyD antibodies have been performed. Roledumab affinity for the RhD antigen is similar to that reported for polyclonal anti-D antibody [5], [6].

Roledumab complies with global quality and safety criteria required at this stage of product development, considering that:

- The original clone, from which roledumab is produced, is genetically stable over time
- The global structure of the recombinant roledumab, especially of the N-terminal chains, is identical to the original human IgG1, with a voluntarily introduced mutation of a free cystein in phenylalanine at position 68 of the heavy chains
- The manufacturing of roledumab is compliant with cGMP

Although the mode of action of anti-RhD products is not completely known, yet binding of the mAbs to the RhD-antigen is important for the efficacy. Due to the monoclonal nature of mAbs, each mAb recognizes one specific epitope of the RhD-antigen and binds to it solely. RhD-variants which do not express this specific epitope will not be recognized by the individual mAb.

Roledumab is assumed to recognize epitope 13.1 that is not expressed by certain variants i.e. DIVb, DVI, DBT, DHAR and DHMi. Roledumab failed to bind to certain variants: Weak types: 10, 11, 18,

38 and 58; partial: DIV type 4 (DIVb) DV type 5 (also called DHK or DYO), DVI (type 1, 2 and 4), DBT (type 1 and 2), DHAR (ce-d (5)-ce (former ROHAR)), DAU4, DAR-E (also called DAR2) and DAR-A (DAR-E+silent mutation); not tested but probably not recognized on the basis of epitope 13.1 recognition profile: DHMi (epitope 13.1).

Roledumab did not induce any sign of toxicity, nor local intolerability at the injections site. No mutagenic activity was detected in the Ames test. Furthermore, non-specific cross reactivity was not observed. No differences were showed between roledumab and polyclonal D in *in vitro* and *ex vivo* experiments designed to compare the transplacental transfer of roledumab and poly-D and to assess the capacity of roledumab to cross react with fetal tissues.

In conclusion, no evidence of roledumab toxicity has been observed in the safety assessment experiment performed.

1.3.2. Clinical information

Further to pre-clinical evidence for activity, LFB BIOTECHNOLOGIES decided to start a clinical development program for a marketing authorization application (MAA) with these indications: prevention of RhD-alloimmunization in RhD-negative women with an Rh-incompatible pregnancy and treatment of RhD-negative individuals after incompatible transfusions with blood components containing RhD-positive RBCs.

The current guideline on the clinical investigation of human anti-D immunoglobulin for intravenous and/or intramuscular use (CPMP/BPWG/575/99 Rev.1) for the clinical investigation of plasma-derived polyclonal human anti-D IgG does not apply to a new product such as a recombinant monoclonal anti-D antibody. Consequently, a stepwise approach for the development of roledumab comprising two clinical studies in healthy male and female RhD-negative volunteers was adopted. Results from these clinical studies are summarized below.

1.3.2. 1. Phase 1 pharmacokinetic study (study ADNC-0701)

A phase 1, double-blind escalating single-dose design, randomized, placebo-controlled study in healthy RhD-negative volunteers was conducted to assess the safety and pharmacokinetic (PK) profile of roledumab after IM and IV administration (up to 3000 µg) in 46 subjects including 36 with roledumab.

The PK characteristics of roledumab (non-compartmental analysis) can be summarized as follows:

- The maximum concentration C_{max} (geometric mean = 24.5 ng/mL) was reached around 10 days after IM administration (300 µg dose). For IV administration the C_{max} (geometric mean) was 144.5 ng/mL reached theoretically just after administration
- The terminal elimination half-life was approximately 18 days with 29.9% CV
- The absolute bioavailability of a dose of 300 µg IM administration is estimated between 73% and 80%
- Exposure after IV and IM route was similar from 18 days after administration (300 µg dose)
- Inter-individual variability was relatively low (<25%)

The PK of roledumab was not significantly affected by the FCGRT polymorphisms.

Roledumab was well tolerated up to 3000 µg IV and 300 µg IM in healthy RhD-negative volunteers. No concern of site injection reactions was raised. And no immune responses were reported throughout the study (internal report [7]).

1.3.2. 2. Phase 2 dose-finding study (ADNC-0726)

This dose-finding study was designed to assess the ability of roledumab IV or IM to effectively eliminate 15 mL of exogenously-administered RhD-positive RBCs from the circulation of RhD-negative individuals (male and non-child bearing female), thereby preventing RhD-alloimmunization when compared to Rhophylac® 300 µg IM and IV.

The secondary objectives were to assess potential RhD-alloimmunization following RhD-positive RBCs administration, to evaluate the pharmacokinetic profile of roledumab and Rhophylac® in the presence of RhD-positive RBCs following IV and IM administrations and to assess the safety of roledumab in the presence of RhD-positive RBCs.

Roledumab 100, 200 and 300 µg IV were concluded to be similarly effective in terms of clearance of RhD-positive RBCs when compared to Rhophylac® 300 µg IV. Roledumab 300 µg IM was similarly effective in the clearance of RhD-positive RBCs when compared to Rhophylac® 300 µg IM.

The exposure to roledumab after IV administration of doses 100, 200 and 300 µg increased more than proportionally to the dose administered.

In healthy volunteers, the PK of roledumab was comparable to Rhophylac®.

The PK parameters determined from phase 2 study are summarized as follows:
- After IM injection median t_{max} of roledumab was 117 hours (~5 days) while that of Rhophylac® IM was 141 hours (~ 6 days)
- The absolute bioavailability of a dose of 300 µg IM administration was 76%

This study did not raise any significant safety concerns: no subject withdrawal for Adverse Event occurred and no immunization event was reported with roledumab. No difference in the nature and the incidence of AEs between roledumab and Rhophylac® was observed regardless of the route of administration (internal report [8]).

1.4. Rationale of the Study

The PK profile of roledumab has been studied, with doses ranging from 30 to 3000 µg (IV) and 300 µg IM in healthy volunteers without (phase 1 ADNC-0701) and with (phase 2 ADNC-0726) the target cells (RhD-positive RBCs). Conclusions are in favor of a comparable PK profile of roledumab with both IM and IV compared to a reference plasma derived polyclonal anti-D treatment: Rhophylac® IM and IV [9].

Roledumab has demonstrated its activity in non-clinical studies and in clinical experience in clearing RhD-positive RBCs in healthy volunteers. No relevant or significant safety issues were observed (up to 3000 µg IV) in subjects treated in the absence of target cells (RhD-positive RBCs) and up to 300 µg IV in the presence of the target cells.

These data from previous experience with roledumab support the conduct of this first study in pregnant woman to assess the PK profile of roledumab 300 µg IM/IV dose in the targeted population (i.e. pregnant women) and to assess the suitability of this dose in ensuring a sufficient residual level at delivery. This study will also document the safety of 300 µg IM/IV route in the mother, fetus and newborn. Data on the roledumab fetal concentration (cord blood at birth) and first milk/breast milk concentration will be also collected in the study.
The route of administration of anti-D has historically been through both IM and IV routes depending on different country's practices. The IM route is usually applied due to its convenience. The IV route, though, may be used in specific situations where, e.g. IM administration is contra-indicated (coagulation disorders), a larger volume of the drug is needed (large volume of FMH) or when an immediate bioavailability is warranted (after a sensitizing event) as well as according to local practice. Therefore, both administration routes (IM/IV) are being investigated in this phase 2b clinical study to allow further implementation of an international larger scale phase 3 pivotal clinical study required for roledumab regulatory registration.

1.4.1. Rationale of the dose

The current recommendations for Rhophylac® use in the prevention of alloimmunization are: a first dose of 300 µg around 28 weeks and a second dose of 300 µg within 72h after delivery (CNGOF, 2010). The objective of these recommendations for planned prevention is to administer a dose sufficient to clear potential fetal RBC in the maternal bloodstream during the third trimester of pregnancy and to ensure the coverage until delivery.

A phase 2 study (ADNC-0726) assessing the capability of roledumab to eliminate 15 mL of RhD-positive RBC from the circulation of healthy male and female RhD-negative volunteers showed that:
- 300 µg IM/IV dose of roledumab dose was as efficient as the recommended dose of Rhophylac® to clear 15 mL of RhD-positive RBC
- Roledumab pharmacokinetics IM/IV profile is comparable to that of Rhophylac®

These results confirmed the comparability of the PK parameters observed in the phase 1. Based on this comparability on the activity in term of RBCs clearance and in PK parameters, the dose of 300 µg IM/IV of roledumab is supported to ensure RhD-alloimmunization prevention until delivery. Although a difference in bioavailability between IM and IV exists (76% vs. a presumed 100%, respectively), the exposure after 18 days is similar between the two routes of administration, indicating that the same dose IM and IV will lead to the same longer term protection against alloimmunization. Hence the same dose of roledumab will be used in both the IM and IV arm.

Previous clinical experience with roledumab did not bring any safety concerns with doses up to 3000 µg in healthy volunteers.

All these results support the use of a dose of 300 µg IM/IV roledumab in this PK and safety study.

Furthermore, the repeated dose toxicity rat study showed that the NOAEL (No Observed Adverse Effect Limit) is equal or higher than 1650 µg/kg. This dose led to an exposure of more than 700 times of the exposure observed in healthy volunteers after IV administration of 300 µg of roledumab (AUC_{last} = 715.5 day µg/mL in rat vs. 0.995 day µg/mL in human). The maximum dose in this study in case of massive FMH is 1800 µg as per current practice with reference treatment (Rhophylac®) corresponding to 6 doses of 300 µg IM/IV. The exposure with this maximum dose is still approximately 30 fold lower than the exposure at the NOAEL.

All these data support further clinical investigation in pregnant women of 300 µg IM/IV for systematic prevention during third trimester and additional doses in case of sensitizing event as explained in Section 6.4.2. 2.2.

1.4.2. Appropriateness of this study

The primary objective of this study is to describe the pharmacokinetic profile of roledumab 300 µg administered in RhD-negative pregnant women in order to confirm the suitability of this dose to ensure adequate anti-D level coverage up to delivery.

The design of this study will allow the documentation of the PK profile and safety (for the mother, fetus and newborn) of roledumab 300 µg administered through both the IM and IV route in the target population. It will provide the basis for a phase 3 clinical study in a larger cohort of pregnant women.

2. STUDY OBJECTIVES

2.1. Primary Objective

The primary objective is to assess the pharmacokinetic profile of roledumab 300 µg IM and IV in RhD-negative pregnant women carrying an RhD-positive fetus.

2.2. Secondary Objective(s)

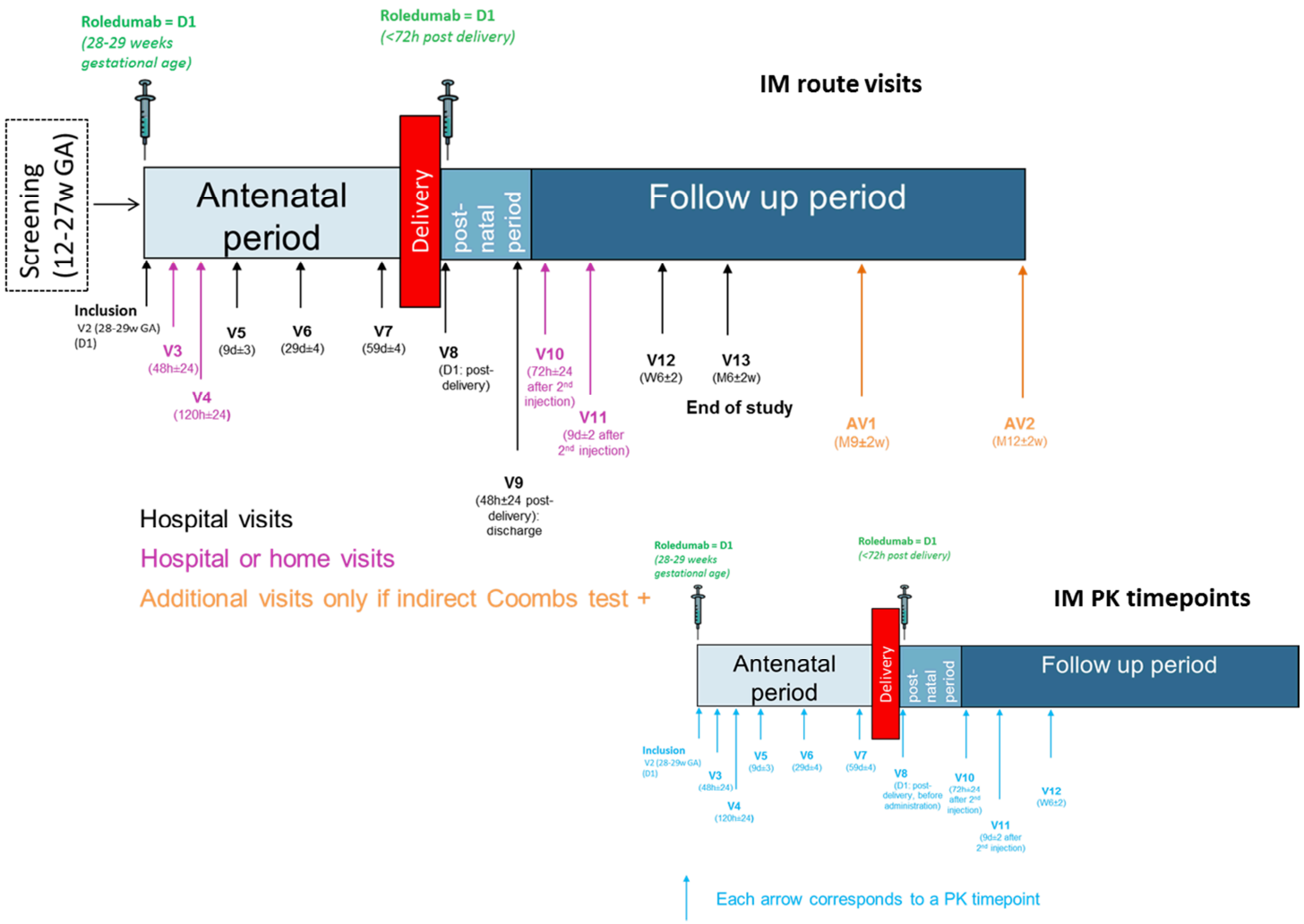
The secondary objectives are:

- To assess the safety of roledumab administered IM and IV in RhD-negative pregnant women and in RhD-positive fetus and newborn
- To assess the efficacy of roledumab 300 µg IM and IV to prevent RhD-alloimmunization in RhD-negative pregnant women carrying an RhD-positive fetus
- To assess the immunogenicity of roledumab
- To measure roledumab concentration in first milk and breast milk
- To measure roledumab concentration in cord blood after delivery

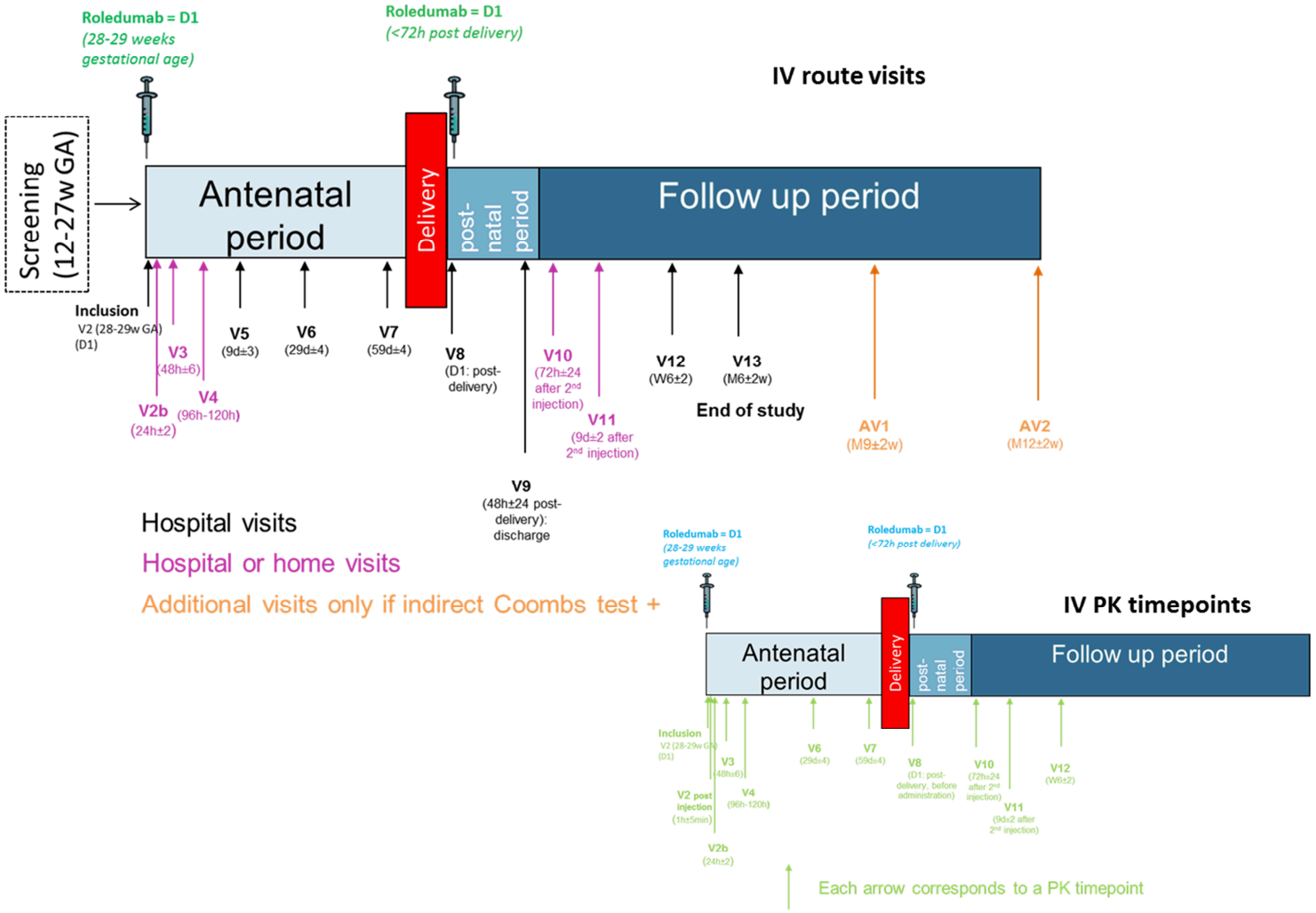
3. STUDY DESIGN

This is a phase 2b, interventional, multicenter, open-label, non-randomized, sequential study.

Visits schedule in the IM arm



Visits schedule in the IV arm



3.1. Design

The study will initially enroll 35 subjects to be treated with 300 µg IM (IM arm) and subsequently 25 subjects to be treated with 300 µg IV (IV arm).

The subject will attend at least 13 visits in the IM arm and 14 in the IV arm (9 will be performed at the hospital unit and 4 (IM) and 5 (IV) at home possibly). Additional visits may occur.

Additional visits will occur in case of:

- Sensitizing events requiring study treatment administration
- Potential RhD-immunization requiring follow-up up to 12 months after the study treatment administration.

A diagram of the study plan for both the IM and IV arm is given below in Figure 3–1 and Figure 3–2.

Figure 3–1: Study Plan for IM arm

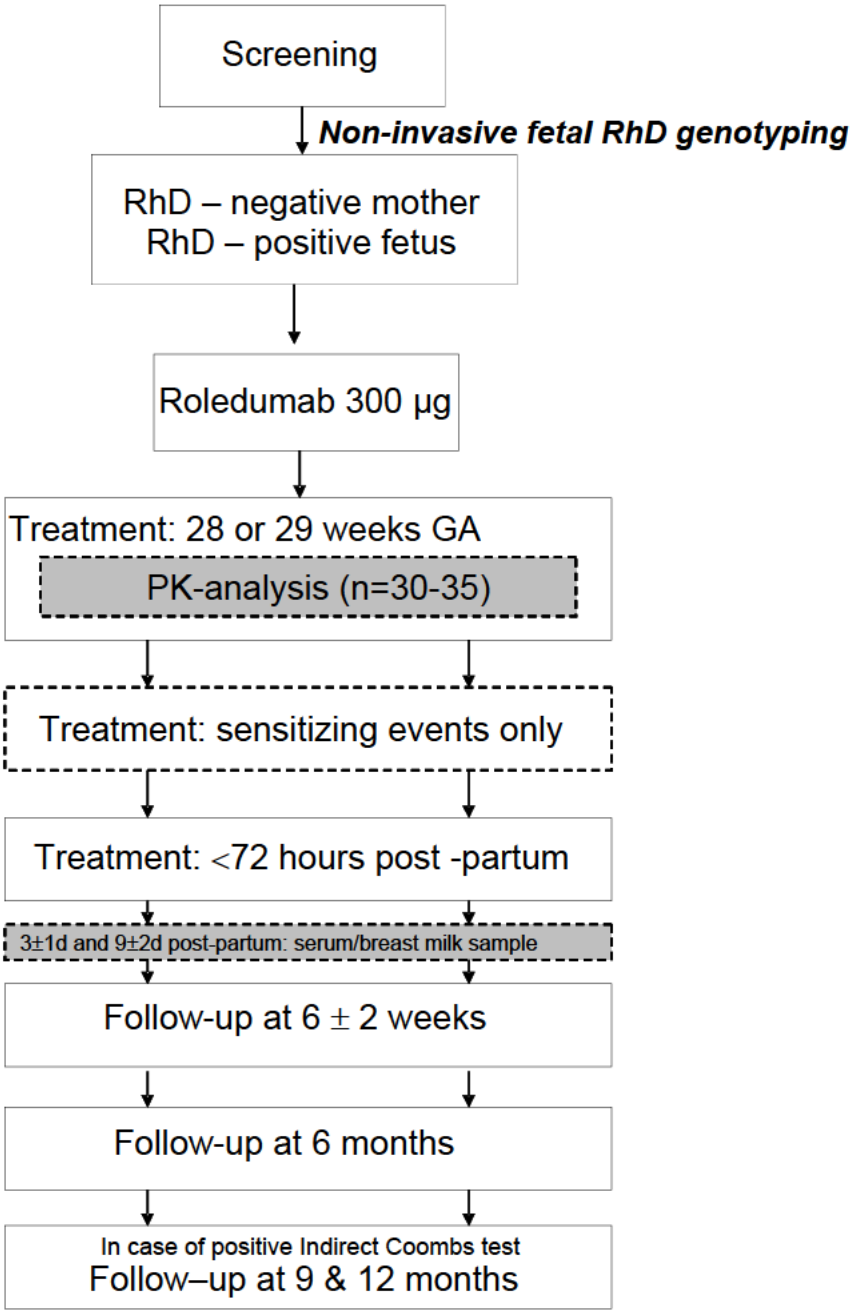
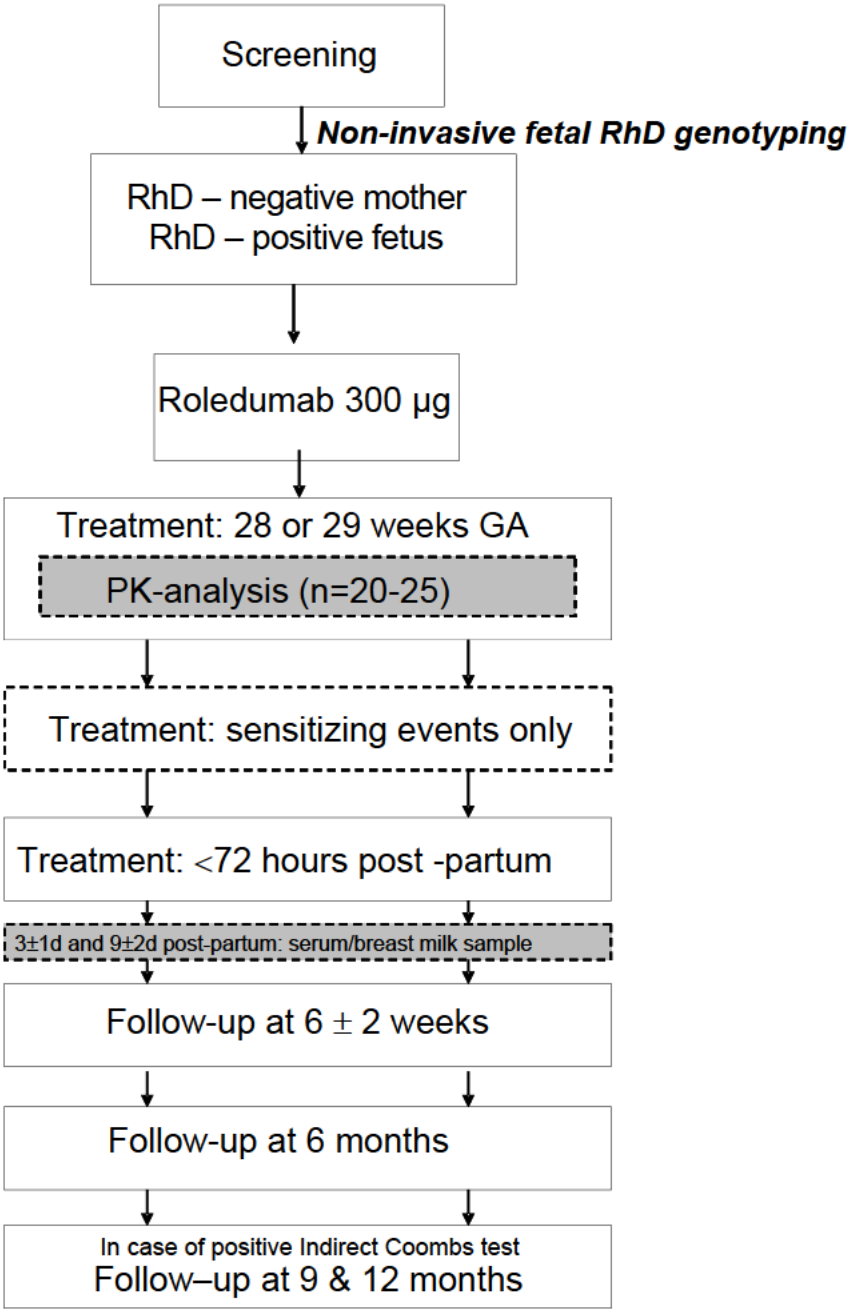


Figure 3–2: Study Plan for IV arm



The study consists of a screening period, an antenatal period, a postnatal period and a follow-up period:

- The screening visit will be performed as close as possible to the antenatal IMP administration, as long as all screening results are available prior to IMP administration. However the screening period could start within 12 and 27 weeks GA.
- The antenatal period comprises 6 visits (IM) or 7 visits (IV) and lasts about 12 weeks.
- The postnatal period comprises 2 visits and lasts 3 ± 1days.
- The follow-up period comprises at least 4 visits (6 months ± 2 weeks) and possibly 2 additional visits.

Screening period (from week 12 to week 27 GA)

Suitable subjects will be provided with appropriate information with regards to the study procedures and with an informed consent form. They will be given sufficient time to think about their participation and to raise questions. They will give signed written informed consent before any protocol related procedures.

Only RhD-negative women carrying an RhD-positive fetus are at risk for alloimmunization. Therefore the results of routine or not non-invasive fetal RhD genotyping test on maternal blood will be collected at screening for the subjects in the selected sites. The RhD fetus status will be determined by the genotyping test prior to the 29th week of gestation.

In addition, subjects will be screened for any previous sensitization (e.g. due to an incompatible transfusion or a previous pregnancy) or pre-existing antibodies against roledumab. The presence of anti-RhD IgG will be tested by an indirect antiglobulin test (IAT).

All screening results have to be available and verified before the treatment period: subjects who do not meet all inclusion criteria or who meet at least one exclusion criterion will not receive IMP administration and be excluded from the study.

Treatment period

This part will be described in Section 8.2.2 and following sections.

Follow-up period (from discharge to 6 months up to 12 months)

This period consists of 4 visits (6 months ± 2 weeks) and possibly 2 additional visits for safety assessment in the mother and collection of routine data for the newborn at the 6 month visit.

3.2. Endpoints

3.2.1. Primary endpoints

Serum concentrations of roledumab will be measured at the defined time points as described in the flowchart. The PK profile of roledumab will be described by:

1. A compartmental population PK model with 3 primary parameters: Volume of distribution (Vd), Clearance (CL) and for the IM route absorption rate constant (Ka); and derived parameters: C_{max}, T_{max}, Area under the curve (AUC_{inf}), terminal half-life ($t_{1/2}$) and Elimination rate constant.

2. A secondary PK analysis (NCA), which will provide the following parameters Vd, CL, C_{max}, T_{max}, AUC_t, AUC_{inf}, terminal half-life and elimination rate constant. Performing PK assessment after IV administration will further allow estimation of the bioavailability of roledumab and better accuracy of the parameters estimation.

All samples (ante- and postnatal samples) will be used for population PK modeling whereas only samples post-first administration (antenatal samples) will be analyzed by NCA for each study arm.

3.2.2. Secondary endpoints

Safety of the mother and the fetus/newborn will be assessed throughout the study by the incidence, nature, severity, seriousness, relationship to the IMP of AEs and by changes in physical examination findings, and vital sign measurements, clinical laboratory tests and Doppler ultrasound.

Safety of newborns/infants up to 6 months age will be assessed by recording and reporting any AE and related concomitant medications.

Roledumab breast milk/first milk concentration, maternal serum concentration and cord blood concentration will be also measured.

RhD-alloimmunization rate at 6 months and up to 12 months if applicable.

3.3. Study Investigational Site(s)

This study will be conducted in hospital's gynecology/obstetrics units. Their laboratory should perform non-invasive antenatal RhD genotyping test.

It is planned that more than one site will take part in the study in order to include 35 subjects and get 30 evaluable subjects in the IM arm and 25 to get 20 evaluable in the IV arm.

3.4. Data and Safety Monitoring Board

An external Data Safety Monitoring Board (DSMB) will monitor safety outcomes and study conduct and will provide the Sponsor with recommendations regarding continuing or stopping the study for all subjects or subgroups of subjects all along the study.

DSMB sessions could be regular sessions or ad-hoc sessions.

- DSMB sessions are planned to take place to review all safety data gathered, as below:
  - After the first subject receives roledumab, before administering subsequent subjects in the study
  - After 5 subjects administered at least one dose of roledumab IM
  - After 10 subjects administered at least one dose of roledumab IM, to allow treatment of sensitizing events with roledumab in subsequent subjects
  - After the last subject received the postnatal roledumab IM
  - After 10 subjects administered at least one dose of roledumab IV
  - After the last subject received the postnatal roledumab IV
- Ad-hoc sessions can be requested by the Sponsor or the DSMB as needed and at any time along the study.

Data summaries for the DSMB will be prepared by the Sponsor. The safety data will include, but not be restricted to (serious) adverse events (AEs) and the safety outcomes listed as secondary endpoints (laboratory data, Doppler Ultrasound results…).

DSMB sessions will contain an open part with DSMB members and Sponsor representatives to present data and answer any request from DSMB members, followed by second part with only DSMB members to analyze data and provide their decision and recommendations to the Sponsor in a written report.

If the DSMB recommends modification or cessation of the study protocol, this will be discussed with the Sponsor who will make the decision.

The DSMB will be composed of different individuals with different and complementary expertise: a neonatologist, two gynecologists-obstetricians who have experience with studies in pregnant women and some experience on previous DSMBs, an immunologist with extensive knowledge of the use of monoclonal antibodies and a toxicologist who have experience in immunotoxicology.

The Sponsor will propose a detailed mandate and review this with the DSMB, from the Outset (a detailed DSMB chart and operating rules will be prepared and signed by DSMB members before study start).

For ad-hoc DSMB sessions, a specific procedure will be set up and be used to monitor special conditions and acute situations that need the direct and immediate attention of the DSMB members.

The situations when this procedure must be used by the Sponsor are, but not limited to:

1. Any Serious Adverse Event suspected to be related to the IMP or to the study procedures
2. Any antenatal sensitizing event requiring additional doses
3. Suspicion of occurrence of anti-roledumab antibodies
4. Suspicion of any alloimmunization.

4. DURATION AND TIMELINES OF THE STUDY

The duration of this study is expected to be of approximately 39 months, with a First Patient In (FPI) expected in Q3 2013, and a Last Patient Out (LPO) in Q4 2017 if no positive IAT and alloimmunization confirmed by anti-D quantification at V13.

5. STUDY POPULATION

5.1. Number of Subjects

In the IM arm, thirty-five (35) subjects who pass the screening procedures will be included in order to get 30 evaluable subjects.

In the IV arm, twenty-five (25) subjects who pass the screening procedures will be included in order to get 20 evaluable subjects.

5.2. Eligibility Criteria

A subject is eligible when all inclusion and no exclusion criteria are met. These criteria will be checked during the screening/inclusion visit.

5.2.1. Inclusion criteria

a. Signed and dated informed consent form provided by the subject prior to proceeding with any study-related procedure

b. At least 18 years old

c. Pregnancy between 12 and 27 weeks gestational age as confirmed by early ultrasound

d. Pregnant RhD-negative woman carrying an RhD-positive fetus confirmed by a non-invasive fetal RhD genotyping test

e. Negative serology: HIV (1 and 2), hepatitis B, hepatitis C except for positive results due to vaccinations

f. Covered by healthcare insurance in accordance with local requirements.

5.2.2. Exclusion criteria

a. RhD-alloimmunized subject

b. Positive for ADA test

c. Multiple fetuses

d. Occurrence of a documented potential sensitizing event in this pregnancy before the antenatal IMP administration

e. Prior administration of anti-RhD immunoglobulin during the current pregnancy

f. Known clinically relevant maternal or fetal abnormality (e.g. as determined by ultrasound or genetic testing), such as placenta previa

g. History of anaphylactic or severe systemic reaction to immunoglobulin of any origin

h. Current diagnosis of an immune disease which by itself or its treatment could impair the safety and/or efficacy evaluation of roledumab in this study. These diseases are:

– All immune deficiencies, particularly those requiring IV-Ig supplementation or other systemic treatment

- Connective tissue and autoimmune diseases (e.g. systemic lupus erythematosus, antiphospholipid syndrome, Sjögren's syndrome, rheumatoid arthritis, ankylosing spondylarthritis) requiring systemic immunosuppressive treatment
- Allergic and inflammatory diseases requiring systemic immunosuppressive treatment.

i. Clinically significant medical history contraindicating the participation in the study according to the judgment of the Investigator or Sponsor

j. Clinically significant laboratory (hematology, blood chemistry, or urinalysis) parameters

k. For the IM arm only, subject with coagulation disorders contraindicating intramuscular injection (subject will still be considered for the IV arm)

l. Transfusion of RhD-positive blood or blood derived products within the 6 months prior to enrolment

m. Anticipated poor compliance with the study procedures

n. Subject within exclusion period further to her participation in a clinical study.

5.3. Duration of Subject Participation

For each subject, the participation will last 9 to 12 months if the IAT result is negative at 6-month delivery. Otherwise the maximum duration of the participation should be of 18 months approximately.

The screening visit will be performed as close as possible to the antenatal IMP administration. However the screening period can occur between 12 and 27 weeks GA.

The subject will be given an IMP injection within 72h after delivery and will be followed-up for 6 months or up to 12 months depending on the IAT results at 6 months or at 9 months.

5.4. Early Discontinuation Criteria / Stopping Rule(s)

All subjects are free to withdraw from participation in this study at any time, for any reason, and without prejudice. The Investigator must withdraw from the study any subject who requests to stop participating in the study.

The Investigator must withdraw a subject from the study at the request of the Sponsor or if the subject:

- Experiences any related serious or intolerable AE
- Develops a clinically significant laboratory abnormality that justifies early discontinuation (e.g. confirmed anti-roledumab antibodies i.e. occurring after IMP and absent before)
- Requires a medication that is prohibited by the protocol or that could impact fetal safety and interfere with the analyses (interpretation of the results)
- Is not compliant with the protocol
- Is lost to follow-up.

5.4.1. Handling of early terminations

If a subject is withdrawn before completing the study, the reason for withdrawal will be documented on the appropriate e-CRF. The specific reason for the withdrawal should be carefully documented on the e-CRF. For instance, rather than stating “withdrew informed consent”, the specific reason for withdrawing the informed consent should be stated. Whenever possible and reasonable, the evaluations that were to be conducted during the "Follow-up visit/End-of-study visit" should be performed at the time of premature discontinuation as follows:

- During the antenatal period (before delivery): the subject should have the evaluations planned at visit 7 (V7) completed with a physical examination and an ADA test. The Investigator should attempt to get an ADA test at 6 months ± 2 weeks after the last injection and collect all data on the subject and the newborn.
- During the postnatal period (after delivery): the subject should have the evaluations planned at visit 13 (V13) and information will be collected for the newborn as per protocol for a non-early study termination.

It is mandatory to obtain follow-up data on any subject who terminated prematurely the study because of an AE, abnormal laboratory test, or other finding related to the IMP administration.

In any case, every effort must be made to ensure safety follow-up procedures are completed.

5.4.2. Sponsor’s study termination

The Sponsor reserves the right to terminate the study at any time for clinical or administrative reasons. Such a termination must be implemented by the Investigator, if instructed to do so by the Sponsor, in a time frame that is compatible with the subject’s well-being.

5.4.3. Lost to follow-up

Investigators should attempt to minimize the number of lost to follow-up. Any attempt will be documented in the subject file including all the involved investigations.

6. INVESTIGATIONAL MEDICINAL PRODUCT(S) (IMP)

6.1. Description of IMP(s)

The Investigational Medicinal Product (IMP), roledumab is a recombinant fully human monoclonal antibody against RhD-positive RBCs produced by biotechnology.

Roledumab is a sterile, clear to slightly opalescent, colorless to slightly yellow, preservative-free solution for intra-muscular and intravenous administration. It is supplied at a concentration of 0.3 mg/mL in 0.9 mg (3.0 mL) single-use glass vials.

Further information such as ingredients is available in the current Investigator's Brochure.

6.2. IMP Packaging and Labelling

6.2.1. Roledumab packaging

Each vial of roledumab will be packaged in an individual box and will be supplied along with administration device including a combo syringe/safety needle:

- For IM administration: syringe of 3 mL and 22G 1 ½ needle
- For IV administration: syringe of 3 mL, 5 mL (for any administration of roledumab >3 mL), 21G x 11/2'' safety needle and catheter Vasofix safety 33 mm 20G (the choice of the needle/catheter is at investigator's discretion and documented in source documents).

6.2.2. Labelling

Each vial of roledumab will be identified by an individual label with two tear-off parts to be used for traceability. In addition, these tear-off label parts will be stuck on the NCR subject prescription, one will be kept in the subject file and the other at the pharmacy.

The labels will bear the required regulatory texts. Each vial will be labelled with at least the following information:

- Study no. ADNC-1301
- Sponsor details (LFB BIOTECHNOLOGIES): name, address and phone number
- Monoclonal Antibody anti-RhD - Roledumab (LFB-R593) - 0.3 mg/mL
- Batch number
- Re-test date MM/YYYY
- Subject number (to be filled in): |__|__|__| - |__|__|__|
  Site # - Subject #

Visit ☐ 2 ☐8 ☐ Other _____ (to be filled in)

- Investigator XXXXXXXX (to be filled in)
- For Intramuscular use or Intravenous use only
- Solution for injection
- Storage requirement:

- Between +2°C and +8°C
- Protect from light, do not freeze

- Standard caution statements:
  - For clinical use only
  - For use under medical supervision

6.3. Management of IMP

6.3.1. Shipment and receipt

Roledumab will be supplied to the Hospital Pharmacist of investigational sites by the Sponsor, free of charge for both hospital and subject.

Shipment will be organized by the drug distributor (under the Sponsor responsibility), at a temperature between +2°C and +8°C and with temperature monitoring device.

The frequency at which the IMP will be supplied to the site will be adapted to the enrolment rate in the site and will take into consideration the re-test date of the IMP.

Upon receipt of treatment supplies, the Pharmacist will inventory the study treatment and complete the acknowledgement of receipt. Should any abnormality of the supply boxes be observed, the Pharmacist must immediately inform the Monitor/Sponsor. The acknowledgement of receipt must be returned to the drug distributor as instructed.

6.3.2. Storage requirements

The Hospital Pharmacist will be responsible for the appropriate storage of the IMP at the study site. All IMPs must be stored in a safe and locked place with no access by unauthorized personnel.

Roledumab must be stored in a monitored refrigerator between +2°C and +8°C. Vials must be protected from light (kept in their boxes) and must not be frozen.

The Hospital Pharmacist must immediately inform the Monitor/Sponsor of non-respect of the required storage conditions. Any temperature deviation must be reported within 1 working day to the Monitor/Sponsor, using a temperature deviation form and the official written approval for the use of treatment must be obtained prior to any dispensation/administration. In the meantime, IMP under temperature deviation should be placed under quarantine at refrigerated conditions. In case of decision leading to not using the IMP, the vials will be stored under quarantine at ambient temperature until shipment back to the drug distributor using the ‘’Return Request form’’.

6.3.3. IMP re-supplying

The IMP re-supplying will be done according to the recruitment rate upon CRA’s or Investigator’s request and will take into consideration the re-test date of the IMP.

6.3.4. IMP accountability

The Sponsor will provide specific forms for drug accountability to the Hospital Pharmacists. These forms will allow following the reception, delivery, dispensation, use and return of the drug. Hospital Pharmacists will keep IMP accountability up-to-date throughout the study.

Used IMP vials should be kept at ambient temperature in a secure place at the Hospital Pharmacy until the records have been verified by the Monitor.

A Monitor delegated by the Sponsor will visit the Hospital Pharmacy and verify the drug accountability.

6.3.5. IMP return, destruction and recall

All used and unused IMP, including empty vials, must be returned to the drug distributor, preferably in their original package, as per instructions. When closing the investigational site, the Monitor should ensure all IMP vials (used and unused) have been returned to drug distributor.

The Investigator and/or Pharmacist must never destroy the used/unused treatment.

6.3.5. 1. Return

The return preparation of used/unused IMP will be performed by the Hospital Pharmacist and the Monitor. The return will be organized by the drug distributor. The person in charge of IMP at site level should fill in and send (e-mail or fax) to the drug distributor the IMP inventory form and the return request form in which the Hospital Pharmacist will set a date and time for pick-up of the vials by a courier service. The Hospital Pharmacist or a delegate should be available at the predetermined date and time when the boxes are collected from the investigational site.

6.3.5. 2. Destruction

After complete reconciliation, destruction of returned and stored (unshipped) vials will be performed by drug distributor upon Sponsor's request.

Destruction of syringes will be carried out on an on-going basis by the site under Investigator's responsibilities.

The Investigator and/or Pharmacist must never destroy the used/unused treatment. Unusually, it can be allowed destroying IMP after motivated written authorization from the Sponsor. In such case, destruction certificate should be issued.

6.3.5. 3. Recall

The Sponsor or its representative and the Investigator/Hospital Pharmacist will inform each other of any suspected or identified IMP defect. The concerned units of IMP stored at the investigational site must immediately be placed in quarantine and must not be administered to subjects until written instructions are given by the Sponsor.

The Monitor will organize with the Investigator/Hospital Pharmacist the return of the concerned batch(es) as per the return procedure. Depending on the study status, new batch(es) could be sent to the investigational site.

6.4. Treatment of Subjects

6.4.1. Methods for assigning subjects to treatment groups

Once the target number of subjects is reached in the IM arm, the subsequent subjects will be enrolled into the IV arm as per protocol.

All efforts must be taken for subjects to remain in the respective arms, which they were enrolled in (same antenatal and postnatal administration route).

6.4.2. Dispensing

Under no circumstances will the Investigator allow the IMP to be used differently than as directed in the protocol.

6.4.2. 1. Preparation

Roledumab will be supplied at a concentration of 300 µg/mL in a single use vial.

For administration, it will be prepared either at the Hospital Pharmacy or in the unit by the study nurse/midwife. In any case, the person in charge of IMP preparation should follow the different steps described below:

- Check the product appearance, by inspecting visually for particulate matter or discoloration. If the appearance is doubtful or diverts from the product description (roledumab is a clear to slightly opalescent and colorless to slightly yellow liquid preparation), the person in charge of IMP preparation should immediately alert the Monitor/Sponsor
- Retrieve more than 1 mL from the vial
- Adjust the volume to be injected to 1 mL (as precisely as possible)
- Pull the needle out of the vial
- If the preparation is performed by the Pharmacist:
  - The syringe must be recapped with a stopper and identified with an appropriate label, which specifies the time of the preparation.

The last step might be skipped if the IMP is prepared in the unit.

6.4.2. 2. Administration

6.4.2. 2.1. Handling

The materials provided by LFB for withdrawal and administration of the drug must in principal be used. The site may use other syringes, provided these are made of polypropylene. In such cases, LFB must approve these materials prior to actual use.

Roledumab can be administered up to 1 hour after filling up the syringe and having been at room temperature.

IM route: roledumab should be administered within one minute with a needle into the deltoids, the anterolateral upper thighs or in the buttocks.

IV route: roledumab should be administered as a bolus (within approximately one minute). Two ways of IV administration are currently supported by compatibility studies:

a. Directly into the vein with a needle
b. Use of an IV catheter of polyurethane. If this is the case, a flush with NaCl 0.9% should be performed to clear the catheter while still connected to the subject.

Roledumab should not be administered through a tube (e.g. butterfly needle, IV line).

The administration will be performed under close medical supervision.

6.4.2. 2.2. Dosage

The dosing guidelines for this protocol summarize the indication, the timing of administration and the dose in the table below.

Table 6–1: Dosing guidelines for administration

Indication	Timing of administration	Dose	Route of Administration
Planned antenatal prophylaxis	At week 28 or 29	300 µg*	IV or IM. *The same route of administration should be used throughout the subject's study participation*
Sensitizing event • Obstetric complications (e.g. miscarriage, abortion, threatened abortion, FMH) • Invasive procedures during pregnancy (e.g. amniocentesis, chorionic biopsy) • Obstetric manipulative procedures (e.g. external version) or abdominal trauma	Within 72 hours of procedure	300 µg* plus additional dose (s) if needed**	
Postpartum prophylaxis (only if RhD-positive)	Within 72 hours of birth	300 µg* plus additional dose (s) if needed**	

**A dose of 300 µg roledumab will suppress the immunizing potential of 15 mL of RhD-positive RBC. ** see Table 6–2.*

Antenatal prophylaxis

- Planned antenatal prophylaxis:

A single dose of 300 µg of roledumab at 28 or 29 weeks of gestation.

- Antenatal prophylaxis following sensitizing events:

* The first 10 subjects will be administered Rhophylac® in case of sensitizing event to enable the review of all safety data by the DSMB
* The subsequent subjects will be administered roledumab after the DSMB confirm this recommendation in a written report
* All subjects in the IV arm will receive roledumab and not Rhophylac® for treatment of a sensitizing event.

Sensitizing events include but are not limited to:

- Obstetric complications (e.g. miscarriage, abortion, threatened abortion, FMH)
- Invasive procedures during pregnancy (e.g. amniocentesis, chorionic biopsy)
- Obstetric manipulative procedures (e.g. external version) or abdominal trauma.

A Kleihauer-Betke test (KB1) will be performed prior to IMP administration in order to determine the volume of fetomaternal hemorrhage (FMH).

One dose of Rhophylac® (for the first 10 subjects) or roledumab 300 µg will be administered within 72 hours after the sensitizing event, usually before the KB results being available.

If the result of the KB1 shows that the volume of FMH is greater than 4 mL, additional doses will be administered (Table 6–2 and Figure 6–1). The total dose of roledumab as derived from Table 6–2 is inclusive of the first dose administered and should never exceed 1800 µg.

A second Kleihauer-Betke test (KB2) will be performed 24h after the last IMP administration to verify the clearance of fetal erythrocytes. If fetal erythrocytes are still present, additional doses will be administered according to Table 6–2 and Figure 6–1. All administered doses should not exceed a maximum of 1800 µg of roledumab, if more is required, another treatment should be provided.

Postnatal prophylaxis

Roledumab should be administered to the mother as soon as possible within 72 hours of delivery of an RhD-positive infant.

The postnatal dose must still be given even when antenatal prophylaxis has been administered and even if residual activity from antenatal prophylaxis can be demonstrated in maternal serum.

Before roledumab 300 µg IM/IV postnatal administration, a Kleihauer-Betke test (KB1) will be performed on a maternal blood sample taken no earlier than 30 min after delivery, in order to determine the volume of FMH.

If the result of the KB1 shows that the volume of FMH is greater than 4 mL, additional doses will be administered (Table 6–2 and Figure 6–1). The total dose of roledumab as derived from Table 6–2 is inclusive of the first dose administered and should never exceed 1800 µg.

A second Kleihauer-Betke test (KB2) will be performed 24h after the last IMP administration to verify the clearance of fetal erythrocytes. If fetal erythrocytes are still present, additional doses will be administered according to the standard reference algorithm used with polyclonal anti-RhD IgG referenced in Table 6–2 and Figure 6–1. All administered doses (IM or IV) should not exceed a maximum of 1800 µg of roledumab, if more is required, another treatment should be provided.

Table 6–2: Adapted dose according to the Kleihauer-Betke test CNGOF table

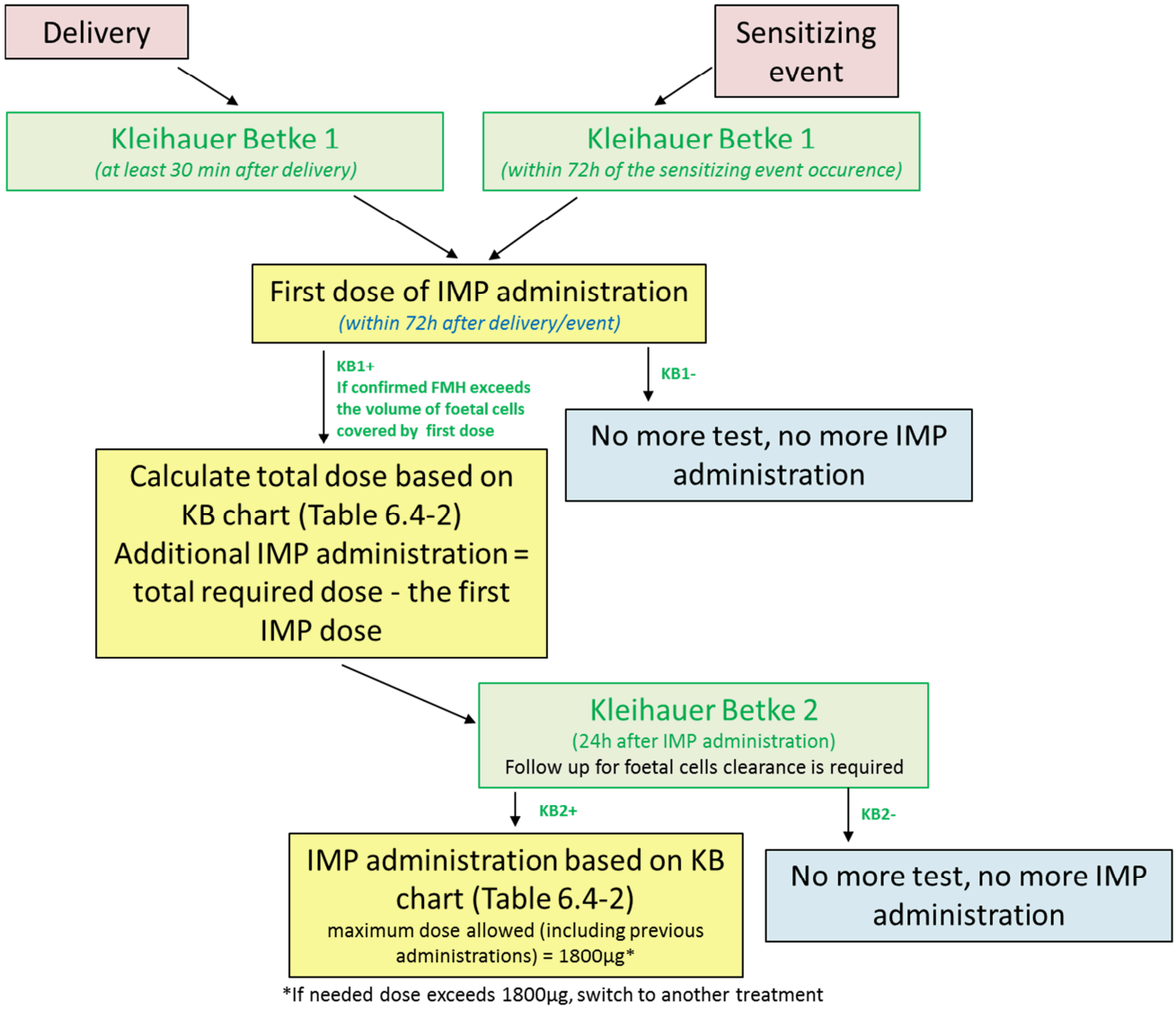
KLEIHAUER (FE / 10.000 ME)	Dose of 300 µg of Roledumab	
	Doses	**µg**
0-4	1	300
5-24	1	300
25-44*	1	300
45-64	2	600
65-84	2	600
85-104	2	600
105-124	3	900
125-144	3	900
145-164	3	900
165-184	4	1200
185-204	4	1200
205-224	4	1200
225-244	5	1500
245-264	5	1500
265-284	5	1500
285-304	6	1800

FE: fetal erythrocytes, ME: maternal erythrocytes
** 44 FE/10 000ME corresponds approximately to 4 mL of FMH*

This dose regimen is based on the CNGOF (Collège National des Gynécologues et Obstétriciens Français - Recommendations 2010 for alloimmunization prevention with 300 µg dose with current polyclonal anti-D (Rhophylac®)).

The exact dose, the date and time of administration, as well as the site and route of administration should be recorded in the e-CRF and subject's medical records.

Figure 6–1: Flow diagram for FMH testing and subsequent actions after delivery or sensitizing event



6.4.3. Compliance

Since the IMP will be only injected by the site staff trained to this sensitization and to the requirements of the study protocol, compliance should not be an issue. However any reason for non-compliance should be recorded in the e-CRF and the subject file.

6.5. Randomization Codes and Procedures for Unblinding

Not applicable.

7. PRIOR AND CONCOMITANT MEDICATION

7.1. Prior Medication

The term 'prior medication' refers to any medication given and stopped before study entry, i.e. before informed consent was signed.

All prior medications taken within one month before entering the study must be recorded in the subject's medical records and documented on the appropriate pages of the e-CRF.

However, any other relevant prior medication taken within more than 1 month before entering the study must be documented in the e-CRF. Careful interview should be done in order to ensure that none of the below treatments were administered to the subject before inclusion:

- Transfusion of RhD-positive blood or blood derived products within the 6 months prior to enrolment
- Therapy with an immunoglobulin, immuno-stimulating or -suppressive drug or any other therapy influencing the immune system, prior to IMP administration (flu vaccinations are allowed)
- And no anti-RhD immunoglobulin was given during the current pregnancy

7.2. Concomitant Medication

The term 'concomitant medication' refers to any medication that the subject receives from the study entry and at any time during the study, i.e. from informed consent signature to the last study visit. This includes the screening/baseline period, treatment period and follow-up as defined in the protocol. Concomitant medication should be kept to a minimum during the study. However, if it is considered to be necessary for the subject's welfare or well-being and is unlikely to interfere with the study assessments, it may be given at the discretion of the Investigator. All concomitant medication must be recorded in the subject's medical records and documented on the appropriate pages of the e-CRF.

8. STUDY PLAN

A study plan table outlining the study requirements by visit is presented below.

V2b, V3, V4, V10 and V11 can be done by a study nurse at the subject's home in agreement with the Investigator.

Study Period	Type of visit	Visit #	Visit timing
Antenatal	Screening	V1	12-27 weeks GA
	Inclusion Roledumab administration	V2	D1 (IM/IV)
	Only for the IV arm	V2b	D2
	Post-treatment	V3 to V7	D3, D6, D10, D30, D60
	Sensitizing events	Sx	Additional visit(s)
Postnatal	Roledumab administration	V8	within 72h post delivery
	Hospital discharge	V9	D3 (post-delivery)
	Sampling first milk/breast milk	V10,V11	D4, D10
	Follow-up	V12, V13	1.5M (6 Weeks), 6M
	If positive IAT and alloimmunization confirmed by anti-D quantification	AV1, AV2	9M, 12M

8.1. Subject Recruitment

8.1.1. Informed consent

The Investigator should provide each subject with relevant, comprehensive, verbal and written information regarding the objectives and procedures of the study as well as the possible risks involved.

The subject should have enough time and opportunity to inquire about study details. All his/her questions should be answered in a satisfying manner. The subject must be informed about her right to withdraw from the study at any time.

Signed informed consent must be obtained from the subject prior to undertaking any study-related procedure.

Two copies of the informed consent form will be signed and dated by both the subject and the Investigator, one will be given to the subject and the second kept in the subject's file. The process for obtaining consent will be documented in the subject's file.

The ICF will be in accordance with local regulatory requirements. The ICF signed by the subject and/or the designated person as described above will also apply for data collected on the newborn.

8.1.2. Subject enrolment

The subject is enrolled when having signed her consent form.

8.1.3. Subject allocation

All subjects fulfilling all inclusion and exclusion criteria will be considered as eligible. They will perform the screening/inclusion visit between 12 and 27 weeks (GA) and visit-related procedures. If the inclusion is confirmed, the first 35 subjects will be administered roledumab 300 µg IM during the second visit (V2) at 28 or 29 weeks (GA). The following 25 subjects will be allocated to the IV arm.

8.1.4. Replacement of early withdrawals

For the PK analysis, subjects who do not provide any blood samples prior to delivery will be replaced.

8.2. Schedule of Visits

8.2.1. Screening period: visit 1 (12-27 GA weeks)

At this visit the Investigator will:

- Obtain informed consent prior to any study-related procedures
- Check the eligibility criteria
- Collect demographics, medical history and life habits data

  An interview will identify specific information regarding previous pregnancies, abortion and other obstetric procedures, anti-RhD IgG administrations in the context of previous or current pregnancy.
  - Determine or record the RhD status of the mother
  - Record ABO status
  - Collect the vital signs
  - Perform a complete physical examination

    The complete physical examination will include Height, Weight, General Appearance, Skin, Head, Eye, Ear-Nose-Throat (ENT), lymph nodes, heart, lungs, abdomen, extremities/joints, neurological and mental status.

- Collect blood samples
  - For laboratory safety parameters (hematology, blood chemistry and aPTT) and serology (hepatitis B, hepatitis C, HIV)
  - For non-invasive fetal RhD genotyping (if not already available)
  - For indirect antiglobulin test (IAT)
  - For ADA test
- Collect urine (Section 8.3.1)
- Collect the information of the first or second trimester obstetric ultrasound (gestational age determination and any maternal or fetal abnormality detection) as well as significant events and complications of the pregnancy
- Collect prior and concomitant medication
- Record any AE which occurred between the ICF signature and inclusion visit
- Give a diary to the subject and explain her how to complete it
- Complete the e-CRF accordingly.

8.2.2. Antenatal period (from visit V2 to visit V7)

8.2.2. 1. Visit 2/D1 (28 or 29 GA)

This visit will be conducted in two parts: before and after IMP administration.

8.2.2. 1.1. Inclusion: prior to IMP administration

At this visit the Investigator will:

- Check inclusion/exclusion criteria in order to confirm the inclusion and particularly the results of the non-invasive fetal RhD genotyping and ADA tests
- Collect the vital signs
- Perform a physical examination (Weight, General Appearance, Skin, Heart, Lungs, Abdomen)
- Perform an obstetric and fetal Doppler ultrasound
- Collect blood samples for
  - PK assessment (baseline) for both arms IM/IV
  - Cytokines
  - CRP
  - Immunogenicity assessment – ADA test (baseline)
  - IAT
  - Anti-D quantification by microtitration or ponderal dosage
- Record any AE
- Record any concomitant medication
- Administer the IMP (IM or IV depending on the arm the subject is in)
- Complete the e-CRF accordingly.

8.2.2. 1.2. <u>After IMP administration</u>

At this visit the Investigator will:

IM route	IV route
• At 30 minutes ± 5 min after IMP administration – Vital signs – Record any AE and concomitant medication	
	• At 1 hour ± 5 min after IMP administration (IV arm only) – Collect vital signs – Blood sample for PK assessment – Blood sample for CRP – Record any AE and concomitant medication
• At 6 hour ± 15 min after IMP administration – Blood sample for cytokines – Collect vital signs – Record any AE – Record any concomitant medication • Complete the e-CRF accordingly.	

8.2.2. 2. Visit 2b (24 hours ± 2h after IMP administration) FOR <u>IV ROUTE</u> ONLY

IM route	IV route 24h ± 2h
Not applicable	This visit can be performed at the subject's home. At this visit the Investigator/study nurse will: • Collect a blood sample for PK assessment and CRP • Collect vital signs • Record any AE • Record any concomitant medication • Only the investigator will complete the e-CRF accordingly.

8.2.2. 3. Visit 3/D3 (48 hours)

IM route **48 hours ± 24h**	**IV route** **48 hours ± 6h**
This visit can be performed at the subject's home. At this visit the Investigator/study nurse will: • Collect vital signs • Collect a blood sample for PK assessment and CRP • Record any AE • Record any concomitant medication • Only the investigator will complete the e-CRF accordingly.	

8.2.2. 4. Visit 4/D6 (5 days after IMP administration)

IM route **5 days ± 1 day (120h ± 24h)**	**IV route** **At 4 or 5 days after injection (96h-120h)**
This visit can be performed at the subject's house. At this visit the Investigator/study nurse will: • Collect vital signs • Collect a blood sample for PK assessment • Record any AE • Record any concomitant medication • Only the investigator will complete the e-CRF accordingly.	

8.2.2. 5. Visit 5/D10 (9 ± 3 days after IMP administration)

IM route	**IV route**
9 days ± 3 days	
At this visit the Investigator will: • Collect the vital signs • Perform an obstetric and fetal Doppler ultrasound: in case of abnormality or in the Investigator's opinion the subject will be followed up once a week • Record any AE • Record any concomitant medication • Complete the e-CRF accordingly.	
• Collect blood samples for: – PK assessment – Immunogenicity assessment – ADA test – Perform an anti-D quantification by microtitration or ponderal dosage	• Collect blood samples for: – Immunogenicity assessment – ADA test – Perform an anti-D quantification by microtitration or ponderal dosage

8.2.2. 6. Visit 6/D30 (29 ± 4 days after IMP administration)

At this visit the Investigator will:

- Collect the vital signs
- Perform an obstetric and fetal Doppler ultrasound
  In case of abnormality or in the Investigator's opinion the subject will be followed up once a week
- Collect blood samples for:
  - PK assessment (for both IM and IV)
  - Immunogenicity assessment - ADA test
  - Perform an anti-D quantification by microtitration or ponderal dosage
- Record any AE
- Record any concomitant medication
- Complete the e-CRF accordingly

8.2.2. 7. Visit 7/D60 (59 ± 4 days after IMP administration, if not yet delivered)

At this visit the Investigator will:

- Collect the vital signs
- Perform an obstetric and fetal Doppler ultrasound: In case of abnormality or in the Investigator's opinion the subject will be followed up once a week
- Collect blood samples for:
  - PK assessment (for both IM and IV)
  - Laboratory safety tests (hematology, blood chemistry)
- Record any AE
- Record any concomitant medication
- Complete the e-CRF accordingly

8.2.3. Antenatal period - in case of a sensitizing event

In case of a sensitizing event, the subject should attend an additional visit and be administered an injection of Rhophylac® or roledumab. The first 10 subjects will be administered Rhophylac®, the subsequent subjects roledumab upon DSMB approval (for both arms).

8.2.3. 1. Additional visit (prior to IMP administration and 6h later)

The Investigator will:

- Collect the vital signs
- Perform a partial physical examination
- Check anti-roledumab antibodies result if available
- Collect blood sample before IMP administration for:
  - IAT testing, if the result is positive, an anti-D quantification will be performed by microtitration or ponderal dosage
  - Kleihauer-Betke test to determine the volume of the FMH
  - Administration of roledumab according to Section 6.4.2. 2
- Collect blood sample 6 hours after the last IMP administration for:
  - Cytokines testing

- Collect the vital signs 6 h after the IMP administration
- Record any AE
- Record any concomitant medication
- Complete the e-CRF accordingly

8.2.3. 2. Additional visit (24h after IMP administration)

The Investigator will
- Collect vital signs
- Collect blood sample for a Kleihauer-Betke test (in case the one prior to roledumab administration was positive) to determine the volume of the FMH within 24 hours after IMP administration, and CRP test
- If needed, administer roledumab according to Section 6.4.2. 2
- Record any AE
- Record any concomitant medication
- Complete the e-CRF accordingly

8.2.4. Postnatal period - visit 8 to visit 9

The postnatal treatment period will consist of:
- A postnatal visit: visit 8 - before and after IMP administration
- A visit at discharge from hospital: visit 9

8.2.4. 1. Postnatal period: within 72h after delivery - Visit 8

8.2.4. 1.1. Postnatal visit: prior to administration

At this visit the Investigator will record data on the mother and on the newborn.

Information on the mother
- Collect vital signs
- Record data on date and time of delivery, duration of parturition, type of delivery (spontaneous, induced, vaginal or C-section), any complications during delivery, instrumental delivery (spatula, forceps…)
- Perform a partial physical examination (weight, general appearance, skin, heart, lungs and abdomen)
- Collect blood samples for:
  - PK assessment (for both IM and IV arm)
  - Laboratory safety parameters (hematology, blood chemistry)
  - IAT
  - Anti-D quantification by microtitration or ponderal dosage
  - Immunogenicity assessment – ADA test
  - Kleihauer-Betke test
  - Cytokines
  - CRP
- Record any AE
- Record any concomitant medication

- Complete the e-CRF accordingly

Information on the newborn
- Collect APGAR scores, gender, weight,
- Collect cord blood sample for:
  - DAT, hematocrit, hemoglobin, reticulocytes, total bilirubin
  - RhD group, RhD genotyping in case of confirmed alloimmunization
  - Measurement of roledumab concentration
- Record any Adverse Event
- Record any concomitant therapy
- Complete the e-CRF accordingly

Prior to the postnatal administration (**within 72 hours of delivery**), a Kleihauer-Betke test will be performed in order to determine the volume of FMH. Administration of roledumab will be done according to Section 6.4.2. 2.2.

8.2.4. 1.2. Postnatal visit: after IMP administration

At this visit the Investigator will:
- Collect the vital signs 30 minutes after IMP administration
- Record any AE and concomitant medication
- Collect blood samples for cytokines testing 6h after the IMP administration
- Collect the vital signs 6h after the IMP administration
- Complete the e-CRF accordingly

8.2.4. 2. Postnatal period: discharge from hospital (visit 9 - D3 ± 1 after delivery)

The Investigator will:
- Collect vital signs
- Record newborn general status
- If done in routine practice, collect/record ABO status of the newborn, to assess the ABO incompatibility with the mother,
- Collect blood samples for CRP testing 24 to 48h after the IMP administration
- Record any AE
- Record any concomitant therapy
- Complete the e-CRF accordingly

8.2.5. Follow-up period (from visit 10 to visit 13, additional visits AV1 and AV2)

8.2.5. 1. Visit 10 - D4 ± 1 after postnatal administration

This visit can be performed at the subject's home.

The Investigator/study nurse will:
- Collect vital signs
- Collect a first milk sample (for consenting breastfeeding mother)
- Collect blood samples for PK assessment (for both IM and IV)
- Record any AE

- Record any concomitant therapy
- Only the investigator will complete the e-CRF accordingly

8.2.5. 2. Visit 11 - D9 ± 2 after postnatal administration

This visit can be performed at the subject's home.

The Investigator/study nurse will:
- Collect vital signs
- Collect a breast milk sample (for consenting breastfeeding mother)
- Collect blood samples for:
  - PK assessment (for both IM and IV)
  - Immunogenicity assessment – ADA test
  - Anti-D quantification by microtitration or ponderal dosage
- Record any AE
- Record any concomitant therapy
- Only the investigator will complete the e-CRF accordingly

8.2.5. 3. Visit 12 - at 6 ± 2 weeks after postnatal administration

The Investigator will:
- Collect vital signs
- Collect blood samples for:
  - PK assessment (for both IM and IV)
  - Laboratory safety parameters (hematology, blood chemistry)
  - Immunogenicity assessment – ADA test
  - Anti-D quantification by microtitration or ponderal dosage
- Record newborn general status
- Record any concomitant therapy
- Complete the e-CRF accordingly

8.2.5. 4. End of study visit - visit 13 (or visits AV1 or AV2)

In case of positive IAT and alloimmunization confirmed by anti-D quantification, the Investigator will ask the subject to attend an additional visit AV1. In case of premature discontinuation, the Investigator will perform the procedures described at visit 13.

8.2.5. 4.1. Visit 13 (6 months ± 2 weeks after postnatal administration)

At this visit, the Investigator will:
- Collect vital signs
- Perform a partial physical examination (weight, general appearance, skin, heart, lungs, abdomen)
- Collect blood samples and urines for:
  - Laboratory safety parameters (hematology, blood chemistry)
  - Urinary dipstick (glucose, nitrite, blood and protein) and complete urinalysis in case of positive dipstick
  - Immunogenicity assessment - ADA test

  - IAT
  - Anti-D quantification by microtitration or ponderal dosage
- Record any AE
- Record any concomitant therapy
- Complete the e-CRF accordingly

In case of positive IAT and alloimmunization confirmed by anti-D quantification, the subject will attend an additional visit (visit AV1).

8.2.5. 4.2. Additional visit AV1 (9 months ± 2weeks after postnatal administration)

The Investigator will:
- Collect vital signs
- Perform a partial physical examination (weight, general appearance, skin, heart, lungs, abdomen)
- Collect blood samples for:
  - Laboratory safety parameters (hematology, blood chemistry)
  - Immunogenicity assessment – ADA test
  - IAT
- Record any AE
- Record any concomitant therapy
- Complete the e-CRF accordingly

In case of positive IAT and alloimmunization confirmed by anti-D quantification, the subject will attend an additional visit (visit AV2) at 12 months ± 2 weeks. The procedures will be the same as those described for visit AV1.

8.2.6. Follow-up in case of alloimmunization

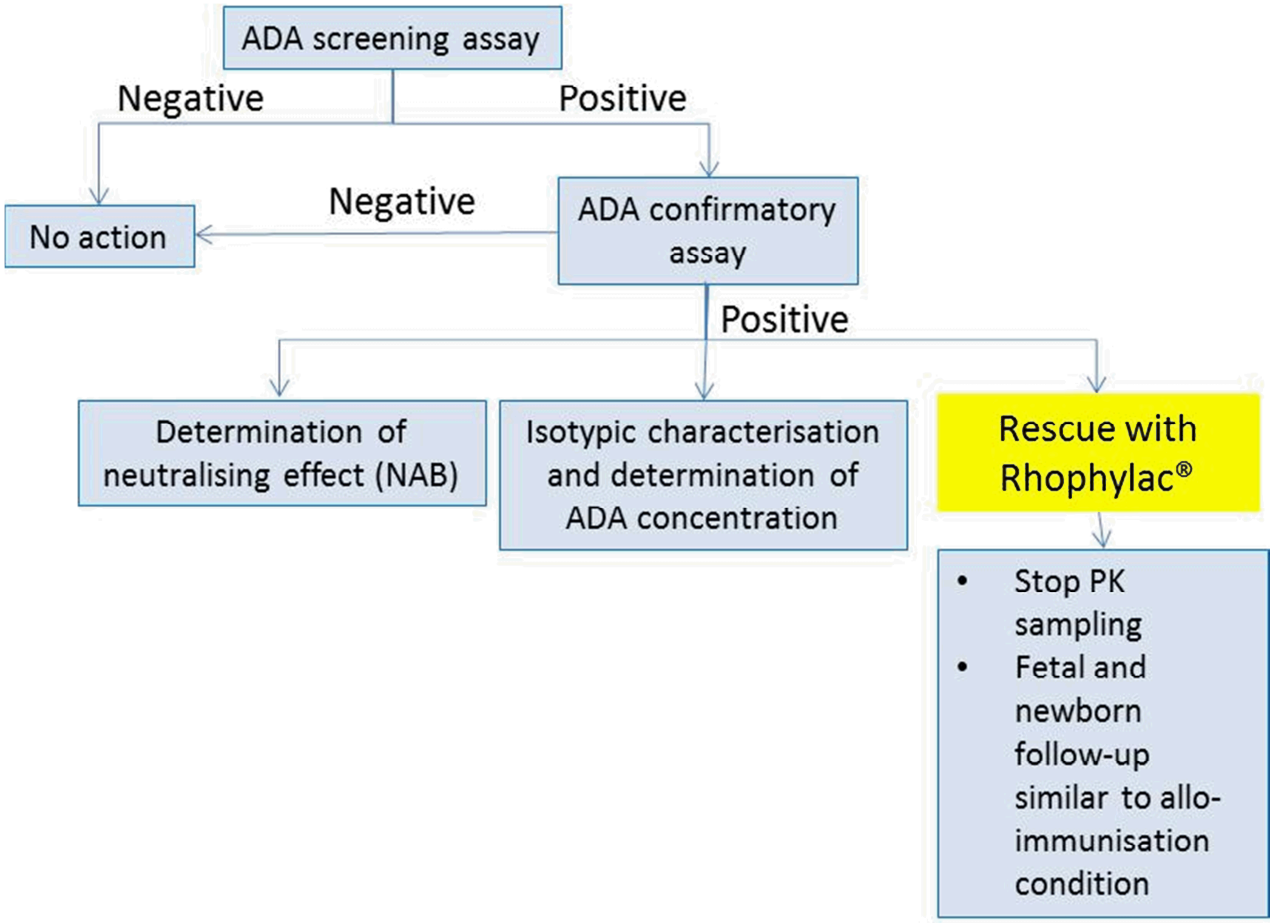
In case of confirmed alloimmunization, the following clinical, biological and ultrasound follow-up surveillance will be applied during pregnancy and after delivery:
- Irregular antibodies (IA) characterization and Anti-D antibodies titration and quantitation every 2 weeks
- If anti-D antibodies level is equal to, or higher than 1 µg/mL (or 250 UCHP/mL), weekly ultrasound examination will be performed, searching for fetal anemia (any signs of fetal hydrops, increased middle cerebral artery peak systolic velocity)
- In case of suspected fetal anemia, *in utero* blood transfusion and/or delivery in a reference center should be discussed
- Neonatal management will include clinical and biological follow up as well as adequate treatment in order to prevent or treat jaundice and anemia in a specialized center

In case of alloimmunization, there will be maternal anti-D generation, consequently the PK results will not be analyzed and the PK samples will not be drawn after the alloimmunization confirmation.

8.2.7. Follow-up in case of ADA production after IMP administration

An ad-hoc DSMB session will be held for these events and additional recommended tests will be strictly performed and adhered to.

In case of confirmed ADA, the following decision algorithm will be followed:



Anti-roledumab antibodies occurrence (induced by roledumab, i.e. detected after IMP and absent before IMP) will be systematically reported as a serious AE and in case of ADA confirmation in maternal plasma, rescue treatment (Rhophylac®) will be immediately administered and the same biological and ultrasound follow-up as for alloimmunization surveillance will be applied during pregnancy and after delivery:

- IA characterization and Anti-D antibodies titration and quantitation every 2 weeks
- If anti-D antibodies level is equal to, or higher than 1 µg/mL (or 250 UCHP/mL), weekly ultrasound examination will be performed, searching for fetal anemia (any signs of fetal hydrops, increased middle cerebral artery peak systolic velocity)
- In case of suspected fetal anemia, *in utero* blood transfusion and/or delivery in a reference center should be discussed
- Neonatal management will include clinical and biological follow up as well as adequate treatment in order to prevent or treat jaundice and anemia in a specialized center.

8.3. Assessments

8.3.1. Assessments performed on site

The assessments are described in Section 8.2 and summarized in the table below.

Assessment	Time points
RhD status	• At screening (V1) for the mother • At delivery (V8) for the newborn on cord blood
ABO status	• At screening (V1) for the mother • No later than at the discharge visit (V9) for the Newborn
Vital signs in mother	• At screening • At each visit throughout the study
Physical examination in mother	• At screening (complete one) • At Day 1 (V2) prior to antenatal administration • At delivery prior to postnatal administration (V8) • At the End of Study visit (V13) (6 months and up to 12 months after postnatal treatment, if applicable)
Laboratory safety parameters (hematology, blood chemistry, aPTT, urinalysis*) in mother	• At screening* (V1) • At V7 before delivery • At delivery prior to postnatal administration (V8) • At the End of Study visit* (V13) (6 months, and up to 12 months after postnatal treatment)
CRP	• At Day 1 prior and 1h, 24h and 48 hours after antenatal administration (depending of the administration route) • After delivery before and 24 to 48 hours after postnatal administration
Obstetric and fetal Doppler ultrasound	• At Day 1 prior to antenatal administration • At Day 10 (V5), D30 (V6) and Day 60 (V7) after antenatal treatment • Every week follow-up in the case of an abnormal result
Indirect Antiglobulin Test	• At screening (V1) • At Day 1 (V2) prior to antenatal administration • At delivery prior to postnatal administration • At the end of Study visit (V13) (6 months) • At the additional visits in case of a positive IAT and alloimmunization confirmed by anti-D quantification at V13 (9 or 12 months after postnatal treatment)
Kleihauer-Betke test	• Prior to and after IMP administration in case of a sensitizing event • At delivery prior to postnatal administration (and after when required)
Record APGAR score (at delivery) and newborn general status	• At delivery (+ APGAR score) • At discharge (V9) and 6 weeks ± 2 weeks (V12) after postnatal treatment

The methods of assessments are detailed below.

Non-invasive fetal RhD genotyping

Non-invasive fetal RhD genotyping based on polymerase-chain-reaction (PCR) is an accurate and validated technique. It will be performed at the screening visit (V1) on maternal blood sample.

Vital signs

Systolic and diastolic blood pressures (mmHg), heart rate (beats/minute), and temperature (°C) will be recorded in supine position. Any clinically significant abnormal findings will be recorded as AEs except for the screening visit.

Physical examination

A complete physical examination will be performed at the screening visit only. This will include the following observations/measurements: Height, Weight, General Appearance, Skin, Head, Eyes, Ears, Nose, and Throat (ENT), Lymph nodes, Heart, Lungs, Abdomen, Extremities/Joints, Neurological and Mental status. Any significant abnormalities observed at screening will be recorded as Medical History.

A partial examination will also be performed before the IMP administration at Day 1 (V2), before the postnatal administration (at V8), and at the End of Study Visit. In case of additional visit(s) AV1 and/or AV2, a partial examination will also be performed. The following body systems will be examined: General Appearance, Skin, Heart, Lungs and Abdomen. Any new clinically significant abnormalities will be reported as AEs.

Laboratory safety parameters

Laboratory safety parameters will be performed throughout the study:

- Hematology: RBC count, Hematocrit, Hemoglobin, Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV), platelet count, white blood cells (WBC) count with differential
- Blood chemistry: Albumin, Alkaline phosphatase, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Blood urea nitrogen, Creatinine, Gamma-glutamyl transferase (GGT), Glucose, Total bilirubin, Total protein including electrophoresis. C Reactive Protein (CRP) will be measured
- At screening only:
  - Activated partial thromboplastin time (aPTT)
  - Serology: Hepatitis C, Hepatitis B, HIV
- At screening (V1) and the end of study (V13) visits:
  - Urinalysis: Glucose, Microscopic examination of the sediment only in the case of a positive dipstick, Nitrite, Occult Blood, Protein.

Standard methods will be used for the measurement of hematology, blood chemistry and serology. Urinalysis will be performed using dipsticks.

The Investigator will be in charge to assess whether laboratory values out of normal range has to be considered as clinically significant or not. After ICF signature, any clinically significant out of range values will be reported as AEs.

Obstetric and fetal Doppler ultrasound

The Obstetric and fetal Doppler ultrasound will be performed prior to roledumab administration (V2) and 9 (V5), 29 (V6) and 59 days (V7) after roledumab administration. In case of abnormality or in the Investigator's opinion the subject will be followed up once a week.

Trained Investigator will perform the fetal Doppler ultrasound in order to measure the MCA - PSV (Middle Cerebral Artery - Peak Systolic Velocity) at the time points defined by the protocol. Frequency should be adapted in accordance with the Mari and Deter curves. The MCA-PSV values greater than 1.5 MoM (Multiples of Median) will be considered pathological [10].

Direct and Indirect Antiglobulin Test (DAT and IAT) / Direct and Indirect Coombs test

The Indirect Antiglobulin Test (IAT) will be performed locally using the standard ID-Card "LISS/Coombs" (BIORAD or a similar supplier) test at the screening visit (V1), prior to roledumab administration at Day 1 (V2), before postnatal administration (V8) and at the end of the study (V13 and the additional visit(s) AV1 and AV2 if applicable) in order to detect any alloimmunization during pregnancy.

The test consists of performing an antibody screening with the ready-to-use test cell reagent "ID-DiaCell". In the case of a positive reaction indicating the presence of irregular antibodies, antibody identification will be carried out using the ready-to-use test cell reagent "ID-DiaPanel".

The Indirect Antiglobulin Test (IAT), also known as Indirect Coombs Test, is a qualitative evaluation of RhD-positive RBC agglutination in the presence of anti-RhD antibodies. In the ADNC-1301 clinical study, IAT is performed right before the first and second IMP administrations to assess the alloimmunization status of subjects.

LFB conducted an internal preclinical study (report No. 14ENC005R) to compare monoclonal (roledumab) and polyclonal anti-RhD activity values obtained by two methods (A and C) from the European Pharmacopeia. Both methods were originally developed using polyclonal antibodies. It was observed that the specific activity of roledumab measured using method A (based on analysis of RhD-positive RBC agglutination on autoanalyser) was two times lower than the one obtained with polyclonal antibodies. The anti-RhD specific activity measured with method C (based on direct binding of antibodies to RhD-positive RBC and analysis by flow cytometry) was found to be in the same range for both roledumab and polyclonal antibodies. These results were in agreement with published data from Thorpe et al [11].

This suggested that not all methods are suitable to precisely measure monoclonal anti-RhD activity. In particular, methods based on the agglutination potency of anti-RhD antibodies potentially underestimate the specific activity of monoclonal antibodies like roledumab. Thus, a low IAT score for samples from roledumab treated subjects cannot be used to draw conclusions about a lack of protection by passive anti-RhD antibodies or the absence of IMP efficiency.

Consequently, IAT is only to be used to check the alloimmunization status of subjects by evaluating the production of polyclonal anti-RhD antibodies. Results from IAT must not be used to assess passive monoclonal anti-RhD protection after IMP administration.

The Direct Antiglobulin Test (DAT) will be performed in cord blood at delivery using the standard ID-Card "LISS/Coombs" (BIORAD).

Kleihauer-Betke (KB) test

- The Kleihauer-Betke test is the standard method for quantification of FMH
- Antenatal prophylaxis:
  In case of a sensitizing event, and before administration of 300 µg of roledumab, a KB test will be performed. If positive, a second KB will be performed 24h after the IMP administration.
- Postnatal prophylaxis:
  A KB test will be performed no earlier than 30 minutes after delivery and prior to administration of roledumab in order to measure the amount of fetal hemoglobin transferred from the fetus to the mother's bloodstream. If positive, a second KB test will be performed 24 hours after IMP administration.

General status of the newborn

APGAR (Appearance, Pulse, Grimace, Activity, and Respiration) score will be reported. The Apgar score is usually given to a baby twice: once at 1 minute after birth, and again at 5 minutes after birth.

The physical examination of the newborn is the routine clinical examination performed at birth, at discharge and at V12. This information will be collected.

Laboratory tests in cord blood

In order to detect any anemia in the newborn, the following laboratory parameters will be measured in cord blood according to the standard methods: hematocrit, hemoglobin, reticulocytes and total bilirubin and RhD status. If the last two parameters are not available on cord blood, they will be collected in the e-CRF if available as part of the standard practices.

The Investigator will be in charge to assess whether laboratory values out of normal range has to be considered as clinically significant or not. Any clinically significant out of range values will be reported as AEs.

The Investigator is responsible for reviewing all laboratory reports and evaluating any results that are outside the normal range. The Investigator must sign and date all laboratory reports.

8.3.2. Centralized assessments

Central Laboratories have been identified for the measurements of the following parameters listed in the table below.

PARAMETER	TIME POINTS
Roledumab serum concentration measurement (PK assessment)	• Before delivery – IM arm: prior to IMP administration at Day 1 (baseline), then at V3, V4, V5, V6 and V7 – IV arm: prior to IMP administration at Day 1 (baseline), then at V2 1h post-administration, V2b, V3, V4, V6 and V7 • IM and IV: at delivery, prior to second, postnatal IMP administration (V8), then at V10, V11 and V12
Roledumab measurement in cord blood and in first milk / breast milk	• At delivery in cord blood • At V10 and V11 in the first milk / breast milk
Anti-roledumab antibody detection (ADA)	• At screening visit (V1) • Prior to the IMP administration (V2) • Day 10 (V5) and Day 30 (V6) after antenatal IMP administration • At delivery, prior to postnatal IMP administration (V8) • Day 10 (V11) and 6 weeks (V12) after postnatal IMP administration • At the End of Study visit (V13), and if applicable at Month 9 (AV1), and Month 12 (AV2) after postnatal IMP administration
Cytokines	• At V2, V8 and in the case of a sensitizing event: before and 6 hours after the IMP administration.
Anti-D Quantification	• At V2, V5, V6, V8 (before postnatal administration), V11, V12, V13

Pharmacokinetic assessment: roledumab serum concentration measurement

Blood samplings will be drawn in all subjects once (6 mL) before the IMP administration, then (6 mL) five times (IM arm) or six times (IV arm) until delivery and (6 mL) four times (both IV and IM) after delivery. Two blood samplings will also be drawn during the postnatal follow-up period. The blood sampling will be performed either during a study visit or at the subject's home by a study nurse.

A PK sampling will be performed at study site:

- For the IM arm during the visits V2 (D1) (baseline value), V5 (9d ± 3), V6 (29d ± 4), V7 (59d ± 4), V8 (within 72h of delivery and before the second IMP administration) and V12 (week 6 ± 2w after the second IMP administration).
- For the IV arm during the visits V2 (D1) (baseline value), V2 1h post administration, V6 (29d ± 4), V7 (59d ± 4), V8 (within 72h of delivery and before the second IMP administration) and V12 (week 6 ± 2w after the second IMP administration).

And at subject's home or at the study site at the subject's preference:

- For the IM arm during the visits V3 (48h ± 24), V4 (120h ± 24) after the first IMP administration and for V10, V11 (respectively 3d ± 1, and 9d ± 2 after the second IMP administration).
- For the IV arm during the visits V2b (24h post-administration ± 1), V3 (48h ± 6), V4 (96-120h) after the first IMP administration and for V10, V11 (respectively 3d ± 1, and 9d ± 2 after the second IMP administration).

Roledumab serum concentrations will be measured by a validated flow cytometry assay with a lower limit of quantification of 1 ng/mL.

The analytical method for the quantification of roledumab in human RhD-negative serum consists of measuring the fluorescence intensity of RhD-positive RBCs previously incubated with the clinical sample containing roledumab. The complex RhD-positive RBC/roledumab is then revealed by a human Fab anti-IgG antibody coupled to a fluorochrome (FITC). Reading is done using flow cytometry. The recognition of variable regions of the immunoglobulin (roledumab) by RBCs expressing the RhD antigen corresponds to the activity of the compound. The couple human Fab-FITC anti light and heavy chains that reveals roledumab bound to RBCs corresponds to the structure of roledumab as human immunoglobulin.

After drawing biological samples and duplicating serum, the tubes in polypropylene should not remain at room temperature for a time-period greater than 12 hours, or for a time-period greater than 7 days at 4°C. Serum samples will be frozen at <-70°C at the investigational site then centralized to the Central Laboratory where analyses will be performed.

For each subject, 9 duplicated aliquots of serum will be prepared from the baseline sample because each assay by flow cytometry will have to be performed in comparison to the baseline value of the volunteer. For the remaining samplings, duplicate aliquots will be prepared.

The logistic provider will organize shipment of the serum samples from the investigational site to the Central Laboratory for testing, as instructed by LFB BIOTECHNOLOGIES. To secure biological samples, duplicated samples will be centralized at the Central Laboratory at separate occasion from the centralization of original samples.

<u>First milk/breast milk passage assessment: roledumab concentration testing in first milk/breast milk</u>

All consenting breastfeeding mothers will be asked to provide a breast milk sample 3 and 9 days after postnatal injection since it is predicted that C_{max} of roledumab after IM injection will occur during this period. For the IV arm, the C_{max} will be earlier even though the concentration is expected to be still higher or at the same level as for IM around these time points. Therefore, the probability to detect roledumab in breast milk is maximized. The ratio between the concentration in breast milk and blood after postnatal administration will then be calculated to assess the passage of roledumab into breast milk. For the determination of roledumab concentration in first milk/breast milk, a bridging ELISA method has been developed and will be validated using current bioanalysis guidelines. The method is based on a coated anti-idiotypic roledumab antibody using the electrochemiluminescent technology (from Meso Scale Discovery). After Quality Control and samples deposit, revelation is performed using the same anti-idiotypic antibody that is biotinyled and the Streptavidin-Sulfo-TAG as electrochemiluminescent material.

<u>Anti-RhD antibodies quantification by microtitration/ponderal dosage</u>

These tests will be performed centrally. In case of positive IAT, the characterization of the specificity of the antibodies will be done and, if anti-D alloimmunization is confirmed, the quantification of anti-RhD antibodies will be performed by microtitration or ponderal dosage (routine assessment in case of suspicion of alloimmunization).

Quantification of anti-D antibodies in subjects samples will be processed by a microtitration method if sample concentration is below 24 ng/mL or a ponderal dosage method if the sample concentration is above 24 ng/mL in accordance to the central laboratory SOPs.

Anti-Drug Antibodies (ADA) assessment: Anti-roledumab antibodies detection

Any biotechnology derived protein is potentially immunogenic.

Blood samples will be drawn at screening visit, before the antenatal IMP administration (baseline), at 9, 29 days after the first administration, after delivery before the postnatal administration, 9 days, 6 weeks and 6 months or up to 12 months after the postnatal administration.

Ten (10) mL of blood will be drawn at each time point, except at V2 and V8 where 15 mL will be drawn. The blood samples will be centrifuged and serum will be aliquoted. The aliquots will be immediately shipped to the Central Laboratory, at -70°C, for testing.

In case of symptoms of allergic reaction, an additional blood sample will be drawn.

LFB BIOTECHNOLOGIES has developed a specific assay based on ELISA method for the detection of anti-roledumab antibodies. In this assay, normal human serum samples have been used to determine a cut point corresponding to 95% of untreated human serum mean responses. This assay has been validated in a GCLP laboratory.

If ADA are detected at any time-point after IMP administration, the following assays will be implemented:

- ADA confirmatory assay
- Determination of neutralizing effect
- Isotypic characterization and determination of ADA
- Determination of ADA concentration
- Confirmatory assay: the same bridging ELISA is used to perform the confirmatory assay but in presence of roledumab in excess. This assay has been also fully validated in a GCLP laboratory
- Determination of neutralizing effect: neutralizing anti-drug antibodies (NAB) will be detected by an anti-D potency assay based on method C described in section 2.7.13. (method C) of the European pharmacopeia. Briefly the ability of roledumab to bind to RhD-positive RBCs will be checked in presence of serum containing ADA by a flow cytometric method. The method has been validated using a murine neutralizing anti-idiotype antibody specific from roledumab as positive control
- Isotypic Characterization: currently, the isotype characterization technique has not been developed. Since appropriate human anti-roledumab isotype controls are not easily available. It is foreseen to establish the isotype by surface plasmonic resonance (SPR) technology. Briefly roledumab will be fixed on a sensor chips then the serum containing ADA will be added and finally anti-IgM, IgG, IgE, IgG1, IgG2, IgG3 and anti-IgG4 will be used to detect the isotype of the ADA

- Determination of ADA concentration: Currently, a method to quantify anti-roledumab antibodies has not been developed. Since appropriate human anti-roledumab Ig reference standard controls are not easily available. It is foreseen to quantify ADA titles SPR technology. ADA quantification in serum could be achieved by a calibration-free concentration analysis SPR based approach. As for Isotypic characterization, roledumab will be fixed on a sensor chip and then the serum containing ADA will be added. Concentration will be calculated on the basis of antibody molecular mass after having characterized the isotype of the anti-roledumab antibody.

Results of screening and confirmatory tests will be available within 7 working days after blood withdrawal.

Cytokines

The following cytokines will be tested: IFN- γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNF-α. The specific time points are at V2, after a sensitizing event and V8: before and 6 hours after the IMP administration.

RhD genotyping on cord blood

Fetal full RhD genotyping will be performed on cord blood in case of RhD sensitization. The fetal RhD-variant will be identified on BioArray DNA chip and sequenced. One sample will be taken and frozen for later determination in case of alloimmunization.

8.4. Compliance with the Study Plan

The Investigator should make every effort to comply with the study plan. If the Investigator encounters difficulties in complying with the study plan, e.g. with regard to the schedule of visits or the required procedures, he/she must alert the Sponsor. The Sponsor may consider it relevant to generate an amendment.

The Investigator should make every effort to avoid the occurrence of deviations from the study plan. If any deviations occur or if the Investigator knows that a deviation will occur, he/she must promptly inform the Sponsor to determine how to handle the deviation.

9. SAFETY

9.1. Safety Reference Document

In this study, the roledumab Investigator's Brochure will be the Safety Reference Document for evaluation of the AE expectedness. AEs will be assessed for expectedness using the current Investigator's Brochure at the time of their notification.

9.2. Benefit / Risk Information

Alloimmunization of RhD-negative pregnant women carrying RhD-positive fetus can lead to HDFN resulting in complications before birth (HDF), and/or anemia and bilirubinemia after birth (HDN).

HDFN can range in severity from being detectable only in laboratory tests, through to stillbirth, birth of infants with severe disabilities or death of newborns from anemia and jaundice. In most severe form, HDF produces hydrops fetalis (characterized by total body edema, hepatosplenomegaly and heart failure) leading to intrauterine death.

Passive immunization with anti-D immunoglobulin can prevent the RhD-negative women from being actively alloimmunized, if given early after exposure to RhD-positive fetal RBC (within 72h). The introduction of routine postpartum prophylaxis in the early 1970s by administration of anti-D immunoglobulin to RhD-negative women delivering an RhD-positive fetus has reduced the alloimmunization of at-risk women from 16% to about 1.2%. A further reduction of alloimmunization was achieved by introducing antenatal prophylaxis during the third trimester of pregnancy, leading to a residual incidence of 0.35% [3].

Currently marketed human polyclonal anti-D IgG are produced by fractionation of pooled plasma obtained from RhD-negative plasma donors, immunized with RhD-positive RBCs. Although these products have proven safe and effective, it can be difficult to entirely meet the needs because of limitations in obtaining hyperimmune plasma from humans and because of the increasing prescriptions due to both ante-partum and postpartum prophylaxis. Indeed, as a result of the success of anti-D prophylaxis and the lower incidence of alloimmunization, fewer immunized women can donate plasma for anti-D fractionation.

Moreover, the practice of antigenic restimulation of the sensitized RhD-negative donors to maintain their anti-D titers has become ethically questionable due to the precautionary principle towards the risk of emerging pathogens (for which current tests are insensitive or inexistent) in RBCs used for sensitizing donors, and other complications of deliberate sensitization (e.g. immunogenicity).

Roledumab is being developed as a preventive treatment to avoid RhD-negative mothers to be sensitized by fetal RhD-positive RBCs after FMH and therefore to prevent HDFN in subsequent pregnancies.

The major benefits of developing a human recombinant monoclonal anti-D IgG such as roledumab over polyclonal anti-D IgGs currently on the market may be summarized as follows:

- Ability to provide virtually unlimited supplies of a standardized and safe product without the barriers associated with harvesting hyperimmune plasma from immunized humans
- Possibility to generate a product that minimizes the concerns of transmission of infectious diseases.

9.2.1. Potential risk(s) related to the IMP(s)

Potential risks of roledumab administration in the target population of pregnant women are based on pre-clinical studies program and the two clinical studies conducted with the IMP in healthy volunteers as well as on the comparison to polyclonal anti-D which is used and documented along the preclinical and clinical experiences.

Potential risks are assessed in target population (fetuses and pregnant women) in the line of this protocol ADNC-1301 in pregnant women to provide a benefit-risk assessment.

It is to note that in the two previous clinical studies with roledumab administration: the phase 1 which included RhD-negative healthy volunteers and the phase 2 which included RhD-negative healthy volunteers challenged with RhD-positive RBCs, no AE reported in both studies was associated to one of the potential risks described below for roledumab.

Potential risks associated to administration of roledumab in the target population could be:

- Potential risks associated to potential maternal effects of roledumab (mother & fetus)
- Potential risks associated to the transplacental barrier crossing of roledumab (fetal risks)
- Potential risk associated to a non-recognition of some rare RhD-variants.

9.2.1. 1. Potential risks associated to potential maternal effects of roledumab

9.2.1. 1.1. Allergic reactions

Allergic reactions can occur as with any biotechnology derived protein. Roledumab is a fully human IgG1 monoclonal antibody for which preclinical studies indicated neither local intolerance nor signal for expected high risk of allergic reactions. Humans exposed in the 2 previous clinical studies did not experience allergic reactions.

This rare potential risk will be closely monitored and the early signs of hypersensitivity reactions will be monitored after each administration (hypotension, dyspnea, cutaneous eruption...).

9.2.1. 2. Immunogenicity

Anti-roledumab antibodies appearance: as any therapeutic protein, roledumab has the potential to induce an undesirable immune response in subjects receiving this kind of product. Consequences of anti-roledumab responses could include lack of roledumab efficacy if they are neutralizing, alterations of pharmacokinetics or allergic reactions.

The risk of developing anti-drug antibodies (ADA) upon roledumab administration to pregnant women is considered to be low with the planned dose regimen since it is a natural fully human IgG1 with an amino-acid sequence derived from an antibody isolated form an RhD-positive immunized donor. Previous clinical experience did not show anti-roledumab antibodies occurrence in RhD-negative healthy volunteers following roledumab administration up to 3000 µg IV and up to 300 µg IM during the phase 1 and 2 clinical studies. The Sponsor will closely monitor the risk of roledumab antibodies formation and will perform required analysis to characterize them if any case of such undesirable immune response. A justified and detailed clinical and biological follow-up in case of ADA occurrence is provided in Sections 8.3.2 and 8.2.6 of this study protocol.

ADA could theoretically appear after the first administration of roledumab (W28 or 29).

When ADA are detected, the biological follow-up protocol will be implemented (refer to Section 8.3.2).

In case of ADA confirmation in maternal plasma (after IMP administration), rescue treatment (Rhophylac®) will be immediately administered and the same biological and ultrasound follow-up as for alloimmunization surveillance will be applied during pregnancy and after delivery:

- Anti-D antibodies titration and quantitation every 2 weeks
- If anti-D antibodies level is equal to, or higher than 1 µg/mL (or 250 UCHP/mL), weekly ultrasound examination will be performed, searching for fetal anemia (any signs of fetal hydrops, increased middle cerebral artery peak systolic velocity)
- In case of suspected fetal anemia, *in utero* blood transfusion and/or delivery in a reference center should be discussed
- Neonatal management will include clinical and biological follow up as well as adequate treatment in order to prevent or treat jaundice and anemia in a specialized center.

If ADA are not confirmed, no specific action is needed.

Breastfeeding

ADA assessment in newborn circulation is not foreseen since clinical undesirable effects of these induced antibodies are not predicted (if present, they are directed against roledumab idiotype, and not against any human somatic antigenic structures). Thus, breastfeeding will not be contra-indicated.

9.2.1. 2.1. Risk of cross-reactivity of anti-roledumab antibodies against marketed polyclonal anti-D

As polyclonal anti-D represent therapeutic alternative to treat pregnant women at risk of alloimmunization in case of potential anti-roledumab antibodies occurrence, a risk of cross-reactivity of potential anti-roledumab antibodies against marketed polyclonal anti-D could be evoked even if it does not represent an anticipated or a potential risk.

The RhD antigen includes at least 37 different epitopes [12]. Polyclonal antibody preparations are obtained by the immunization of donors and pooling of their anti-D immunoreactive plasmas. By definition, their individual anti-RhD immune responses are polyclonal and therefore diverse in terms of epitope recognition as well as VH and VL (paratope) repertoire for each recognized epitope. As a result, the different commercially available anti-D polyclonal antibodies have different patterns of recognition of the RhD antigen since they come from different pools of donors with different immune repertoires and immune responses [13].
To the best of our knowledge, immune responses against polyclonal anti-D antibodies have never been reported.

Roledumab is a fully human IgG1 monoclonal antibody recognizing the epitope epD13.1 [14] derived from a single B cell of one donor who had been immunized to obtain polyclonal anti-D plasma. Its paratope/idiotype is one of the multiple possibilities of recognizing RhD, which are represented in the polyclonal preparations. Therefore, in case of an immune response against the paratope/idiotype of roledumab (which has never been observed until now), the ADA would probably neutralize a negligible portion of antibodies constituting the diverse anti-RhD collection of antibodies contained in the marketed polyclonal anti-D drug.

In the context of the unlikelihood of anti-roledumab antibodies occurrence and the extremely rare probability of altering significantly the marketed polyclonal drug efficacy by the presence of this ADA, the treatment of a woman presenting an anti-roledumab positive confirmatory test with a marketed polyclonal anti-D associated to a careful follow-up of this woman as if she would be at risk of alloimmunization detailed in Section 8.2.6 (IAT testing every two weeks until delivery and anti-roledumab antibody follow-up and characterization until the response had come back to base line) represent a safe and justified therapeutic alternative.

9.2.1. 2.2. Cytokine release

Roledumab is a monoclonal antibody with improved biological activities and, in particular, enhanced affinity for FcGRIIIa (CD16). These properties could lead to undesired activation of CD16-expressing cells, which could potentially lead to cytokine-release syndromes [15], [16].

Non-clinical studies on the ability of roledumab to induce cytokines did not suggest enhanced cytokine secretion in comparison to polyclonal anti-D. It should be emphasized that the immunogenicity in animals is not predictive of immunogenicity in human.

Data from clinical studies in healthy volunteers, both in the presence and absence of RhD-positive RBC showed absent or very low cytokine secretion. The cytokine secretion profile in roledumab-treated healthy individuals in the presence of RhD-positive RBC was shown to be very similar (almost absent) to that observed in the polyclonal anti-D-treated subjects after an administration of 300 µg IV or IM dose. Given this similarity, it is not expected that roledumab will trigger significant cytokine release in pregnant women when administered for prevention of RhD-alloimmunization.

Since similar amounts of roledumab are expected to cross the placenta (similarly to polyclonal anti-D antibodies), roledumab is not expected to trigger significant cytokine release in the fetus also.

The risk of significant cytokine release leading to systemic inflammatory syndrome or more severe complication like a preterm birth is very unlikely. However, the Sponsor will continue to closely monitor the risk of cytokine release with adequate dosing of relevant cytokines and proinflammatory parameters when exposing pregnant women during this study.

9.2.1. 3. Specific potential risks associated to the transplacental barrier crossing of roledumab (fetal risks)

9.2.1. 3.1. Risk of hemolytic disease of the fetus/newborn

Polyclonal anti-D products have gained extensive clinical experience with administration of 300 µg around week 28, 29 of pregnancy as well as additional antenatal doses in case of a sensitizing event. The most common immunizing event, though, is delivery, whether this is vaginal or by Cesarean. Treatment with anti-D at that point in time is not a safety issue for the fetus anymore.

The French recommendations (CNOGF) have been revised (2006) in light of current scientific evidence, with regards to the immune-hematological and instrumental investigations that should be performed in the antenatal and postnatal periods and prophylaxis to prevent the HDFN due to RhD incompatibility. Polyclonal anti-D (Rhophylac®) is currently recommended to be used by the CNOGF.

The doses of anti-D reported in this recommendation are a standard of 300 µg for antenatal prophylaxis around week 28 of gestation. In addition, in case of a sensitizing event, a standard dose of 300 µg is (again) to be administered. However, depending on the amount of FMH, the dose needs to be adjusted. A dose of 300 µg is recommended for every 4 mL of FMH, with a maximum of 1800 µg.

In the large majority of cases one dose of 300 µg is considered sufficient to prevent active alloimmunization as the FMH usually does not exceed 4.0 mL. It is reported that in 99% of deliveries, FMH is below this value [17]. It is extremely rare to have massive FMH during the antenatal period.

Administration of 1 or 2 times 300 µg of polyclonal anti-D has been shown to be safe in the antenatal period, without any consequences to the fetus [18]. No reports of safety issues with the doses recommended by the CNOGF have been identified, assuming that it can be used safely for the mother, the fetus and the newborn.

It is known that in cases of an alloimmunized mother, only a level above 1 g/L there may be an issue for the fetus [19]. With the administration of 1800 µg roledumab it is expected that there may be a short peak potentially reaching this level, but the exposure over time is far less than a whole pregnancy with this level of anti-D present in an alloimmunized mother.

Non-clinical results do not suggest that roledumab could cross the placenta with a higher rate than Rhophylac® and is therefore not expected to lead to a higher concentration in the fetal circulation.

A tissue reactivity study was conducted with roledumab and RhoGAM® (PolyD). No unexpected specific reactivity was reported with roledumab or RhoGAM®. Roledumab and RhoGAM® react similarly regarding Tissue Cross Reactivity (TCR) with fetal tissues.

Based on this information, it can be concluded that there is no additional or increased harm expected with roledumab administration IM or IV in pregnant women regarding HDFN.

The protocol requires close and continuous monitoring of this risk when administering roledumab to pregnant women including adequate and regular fetal Doppler ultrasound monitoring to detect any hemolysis of the fetus.

9.2.1. 4. Potential risk associated to the non-recognition of some rare RhD-variants by roledumab

In RhD-negative Caucasians, the RhD gene is deleted while it is not expressed in certain populations such as Africans or Japanese. A large number of RHD alleles known as variants are described and characterized by the absence of some RhD epitopes and/or weak expression (weak D, partial D and Del phenotypes).

Roledumab is assumed to recognize epitope 13.1 that is not expressed by certain variants i.e. DIVb, DVI, DBT, DHAR and DHMi.

Roledumab failed to bind to certain variants:

- Weak: types, 10, 11, 18, 38 and 58
- Partial: DIV type 4 (DIVb), DV type 5 (also called DHK or DYO), DVI (type 1 and 2), DBT (type 1 and 2), DHAR (ce-d (5)-ce (former ROHAR)), DAU4; DAR-E (also called DAR2) and DAR-A (DAR-E+ silent mutation) not tested but probably not recognized on the basis of epitope 13.1 recognition profile: DHMi.

The most important potential risk of the non-recognition of these very rare variants by roledumab is the alloimmunization and its consequences, even if the ability of these rare variants to induce maternal alloimmunization is uncertain and the necessity of higher volumes of FMH in these cases to immunize the mother is likely.

Once this risk is evaluated and assessed as to its importance, it is also useful to place it in the context of acceptability by the subjects to be included in this study and by the anticipated target population considering the disease indication and the extreme rarity of the situations which it could occur.

This unsolved issue which brings uncertainties regarding potential risk of alloimmunization and the relevance of this finding to the use in pregnant women humans are discussed below.

For a quantitative approach to address this risk, estimations were done on the basis on the prevalence of weak and partial D-variants in the general population and on the estimated probability of carrying an RhD-variant fetus by an RhD-negative pregnant woman (refer to Report – Gabriele Dallman provided in the Investigator's Brochure).

The worst case scenario is taken for these estimations combining the facts that:

- We consider in the calculation that roledumab does not recognize all D-variants as a worst scenario even it does not recognize only a small part of these variants
- We consider that all D-variants can immunize an RhD-negative mother even if the immunogenicity of some of these variants such as DVI and DBT are commonly admitted to be lower than the wild type RhD.

The probability to have an RhD-negative mother at risk of carrying a fetus with D-variant antigen is estimated between 0.004% and 0.26% as a worst case scenario.

This potential risk related to extremely rare cases cannot be totally prevented as there is no available test to perform on mother blood sample before systematic prevention with roledumab to determine antenatally if the fetus is a D-variant. Differently, in case of sensitizing event which is at-risk situation of alloimmunization, a Kleihauer-Betke test will provide information on fetal RBCs clearance from maternal blood and will allow close monitoring of roledumab activity in case of presence of D-variant in the fetus. This will optimize management of this risk even it concerns only extremely rare at-risk situations.

According to these quantitative evaluations in the context of the disease indication and expected benefits of roledumab, these extremely rare situations in which this risk could occur bring an acceptable level of risk for the target population. The issue will continue to be addressed throughout the study and clinical development for further documentation, monitoring and minimization.

9.2.1. 5. Related to the study procedures

Blood sampling will be performed during this study in order to monitor the safety and to assess PK parameters. Although this procedure represents an inconvenience for the subject, it does not constitute an additional significant risk. There is a minor risk of hematoma from intramuscular and intravenous infusion of the IMP and from drawing blood. Slight pain at the injection site, feeling light-headed, bruising and, exceptionally, infection as well as bleeding from the site of the puncture may occur.

9.2.2. Benefit/risk balance

The assessment of the benefit-risk balance is based on the available relevant non-clinical and clinical tests and studies performed. All available data on roledumab were obtained out of the target conditions since no pregnant woman has been exposed yet to roledumab.

However, comparison to marketed polyclonal anti-D was performed in most of the tests and studies to provide relevant evaluations and conclusions.

The assessment of all relevant data gathered with roledumab together with all measures to be implemented to closely monitor important potential risks, provide objectively confidence that roledumab administration will have a favorable benefit/risk balance in pregnant women.

In conclusion, the Sponsor considers that these factors support a favorable benefit/risk assessment for the use of roledumab in pregnant women in this study.

9.3. Risk Minimization Actions/Monitoring Throughout the Protocol

Roledumab has not yet been administered to pregnant women, so the safety in this specific population is unknown.

Target population will be screened and included only if inclusion and exclusion criteria are met.

Allergic reactions may occur in humans. Symptoms of allergy or early signs of hypersensitivity reactions including generalized urticaria, tightness of the chest, wheezing, hypotension or/and anaphylaxis may occur. The treatment required depends on the nature and the severity of the AEs. If necessary, the current medical standards of care for shock treatment should be implemented.

As any therapeutic protein, roledumab antibody has the potential to induce an undesirable immune response in subjects receiving this kind of product. Consequences of anti-roledumab responses include lack of efficacy if they are neutralizing, alterations of pharmacokinetics or allergic reactions.

Cytokines panel will be tested at specific time points.

Close surveillance of fetal signs of anemia will be performed through regular fetal Doppler ultrasound (fetal well-being, mean MCA-PSV). This investigation will be repeated according to the MoM value.

The newborn will be examined as usual. Cord blood will be taken for DAT and other laboratory testing (hemoglobin, hematocrit, reticulocytes and bilirubin).

First milk / breast milk sampling will be also performed in order to measure roledumab concentrations although roledumab concentrations are expected to be extremely low as described for other monoclonal IgG1 antibodies [20].

Furthermore the DSMB will examine safety data at specific time-points during the study and give recommendations regarding any changes that may need to be made to the study, how it should be continued and if it should be stopped.

This summary of risks and benefits is not intended to be all inclusive, but is rather intended to highlight possible risks, benefits, and the potential important risks. The Investigator must become familiar with all sections of the IMP Clinical Investigator's Brochure provided by the Sponsor before the start of the study.

9.4. Alternative Therapeutic Management - Emergencies Handling

The subject may be administered Rhophylac® or any other anti-D immunoglobulin. In this case, the subject will be withdrawn from the study and followed as described in Sections 5.4.1, 8.2.6, 8.3.1 and 9.2.1. 2.

9.5. Definition and Reporting of (Serious) Adverse Events

9.5.1. Definition of adverse event and serious adverse event (see also Section 9.5.2: Definition of specific events in the study)

An adverse event (AE) is any untoward medical occurrence in a subject or clinical investigation subject administered, or intended for administration of, a medicinal product that occurs any time after the informed consent has been signed and until the final study visit or early termination and which does not necessarily 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. Adverse events include, but are not limited to: (1) a worsening or change in nature, severity, or frequency of conditions present at the start of the study; (2) subject deterioration due to primary illness; (3) intercurrent illness; (4) drug interaction; and/or (5) abnormal clinically significant laboratory values.

Subjects should be questioned in a general way, without asking about the occurrence of any specific symptom. The Investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and not the individual signs/symptoms.

Following questioning and evaluation, all AEs, whether believed by the Investigator to be related or unrelated to the study medication, must be documented in the subject's medical records, in accordance with the Investigator's normal clinical practice, and on the AE form in the e-CRF.

Each AE is to be evaluated for duration, intensity, seriousness, temporally association and causal relationship to the study medication.

A Serious Adverse Event (SAE) is an AE that, at any time, fulfils one or more of the following criteria:

- **Results in death**
- **Is life threatening**, i.e. the subject was at immediate 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 in-subject hospitalization or prolongation of existing hospitalization**
- **Results in persistent or significant disability/incapacity**, i.e. substantial disruption of a person's ability to carry out normal life functions
- **Is a congenital anomaly/birth defect**
- **Is any important medical event** that may not be immediately life threatening or result in death or hospitalization but, based upon appropriate medical judgment, may endanger the subject or may require intervention to prevent one of the above characteristics/consequences. Such events (subsequently referred to as 'important medical events') should also be considered 'serious' in accordance with the definition.

Medical and scientific judgement should be exercised in deciding whether an event is 'serious' in accordance with these criteria.

- Surgical intervention is not to be considered as SAE but the medical condition requiring the surgery is to be reported as such
- Events initially reported as an AE may become serious. For example, diarrhea may become debilitating and require hospitalization or prolongation of hospitalization and is then reported as SAE
- Distinction should be made between serious and severe AEs. Severity is a measure of intensity whereas seriousness is based on the seriousness criteria described above.

Occurrence of IMP overdose, abuse/misuse, drug dependency or any medication error, should be immediately reported to the Sponsor (via a dedicated email address: ADNC1301@lfb.fr) and considered as AEs. These special situations should be reported as SAEs only when fulfilling SAE criteria or associated with a SAE.

9.5.2. Definition of specific events in the study

- Hospitalisation for planned delivery should not be reported as a SAE. Occurrence of a FMH will not be considered as an SAE as it will be reported as targeted disease treated with roledumab. The sensitizing event at the origin of the FMH will be also described
- RhD-alloimmunization will be systematically reported as a SAE
- Anti-roledumab antibodies occurrence (after IMP and absent before IMP) will be systematically reported as a SAE
- Preterm delivery (<37 weeks of Gestational Age) will be systematically reported as a SAE.

9.5.3. Adverse events recording and reporting

Each individual AE is to be listed as a separate entry on the AE form in the e-CRF. The Investigator will provide information on dates of onset and resolution, seriousness, severity, action(s) taken, outcome and relationship to study medication.

Any AE collected for the newborn during a routine visit will be reported on a specific AE form in the e-CRF.

Documentation of immediately reportable events will follow procedures described in Section 9.5.4.

The Investigator must report to LFB BIOTECHNOLOGIES, or its designee all AEs that occur during the study from the time written informed consent is given until the final study visit or early termination, regardless of their relationship to IMP.

If the Investigator detects an (S)AE after the above-defined period of observation and considers the event as possibly related to the IMP, he should contact the Sponsor to determine how it must be documented and reported.

Apart from AEs clinically observed by the Investigator, or recorded on self-assessment forms (e.g. subject diary), the subject will be given the opportunity to report AEs spontaneously. A general prompt will also be given to detect AEs, e.g. "Did you notice anything unusual about your health since your last visit?"

It is the responsibility of the Investigator to record all the relevant information regarding the event.

The Investigator is requested to assess the relationship between the investigational product and the occurrence of each (S)AE. Alternatives causes, such as the underlying diseases, concomitant therapy or the temporal relationship of the event to the investigational product will be considered.

9.5.4. Procedures for reporting serious adverse events

At the occurrence of a subject event that fulfils one or more seriousness criteria, the Investigator must **immediately** forward to the Sponsor or its representative a duly completed "SERIOUS ADVERSE EVENT FORM" provided by the Sponsor, even if the data are incomplete, but as soon as the following minimum information is available:

- Identification of a subject (subject number and/or initials). In the context of this study, the subject experiencing the SAE (Mother, Newborn or Both) should be specified in the SAE Form.
- Description of the SAE.

The Investigator will ensure a follow-up of an initial SAE to elucidate the nature, the outcome or the causality of the SAE. Supporting documentation (discharge summaries, all examinations carried out, etc.) will not be sent systematically, as all relevant information must appear and be summarized in the narrative of the SAE report.

However if judged important, the Investigator could send these documents or LFB BIOTECHNOLOGIES could request them (all documents must be blinded with respect to the subject's name). Any follow up information must be reported within the same timelines.

TIMEFRAME

"Initial" SAE Form	**"Follow-up" SAE** form
IMMEDIATELY As of awareness of an SAE occurrence	**24 hours** As of availability of essential follow-up information

FAX TRANSMISSION

The SAE reports should be faxed:

FAX NUMBER: [redacted] ***(IMMUNO/NEURO)***

In rare circumstances, when fax transmission is not possible, reporting by telephone is acceptable. But this should be followed with a completed "SERIOUS ADVERSE EVENT FORM" signed and faxed by the Investigator as soon as possible.

LFB MEDICAL contacts

For urgent medical matters or questions, the Investigator may contact:
Head of Drug Safety:
[redacted]
Phone number: [redacted]
E-mail: [redacted]

9.5.5. Follow-up of adverse events

All (S)AEs assessed as not related to study medication, including clinically significant laboratory tests or physical examination findings, must be followed until the event resolves, the condition stabilizes, the event is otherwise explained, or the final study visit occurs, whichever comes first.

(S)AEs assessed as related to IMP must be followed for as long as necessary to adequately evaluate the subject's safety, or until the event stabilizes, or the subject is lost to follow up. If resolved, a resolution date should be provided.

The Investigator is responsible for ensuring that follow-up includes any additional investigations indicated to elucidate the nature and/or causality of the AE. This may include additional clinical laboratory testing or investigations, histo-pathological examinations, or consultation with other health care professionals as is practical.

If a subject is withdrawn from study due to safety reasons, the subject should be followed until the event disappears, is otherwise explained or the subject's condition has stabilized.

Any subject who voluntarily withdraws from the study should be carefully questioned for the possible occurrence of an AE. Whenever possible, a subject should be followed through the last scheduled visit.

If no follow-up information can be provided after all efforts and attempts, the Investigator must document the outcome as "unknown" and provide a justification.

9.6. Reporting of Pregnancies

In case a subject of this clinical study gets pregnant again during the follow-up period, the pregnancy will not be considered as an AE or an SAE. Nevertheless, as this pregnancy will require monitoring and follow-up, it must be notified to the Sponsor immediately and no later than 24 hours after the first site's awareness using the "PREGNANCY FORM" faxed at the following number:

[redacted] Any follow up information must be reported within the same timelines.

10. PARAMETERS AND ASSESSMENT CRITERIA

10.1. Assessment of Pharmacokinetics

The PK of roledumab will be assessed from serum concentrations of roledumab using all samples (ante- and postnatal samples) for the PK population approach and only samples after first infusion (antenatal samples) for NCA.

PK in alloimmunized subjects and in subjects with ADA rescued with Rhophylac® will not be analyzed, and no samples following the alloimmunization or ADA confirmation will be drawn.

10.2. Assessment of Safety

The safety of roledumab will be assessed by recording all AEs occurring during the study, i.e. after signature of the informed consent and until the end of the study. AEs in both mother and fetus/newborn will be collected and recorded.

Safety assessment during pregnancy will include but is not limited to a fetal Doppler ultrasound examination at 9, 29 and 59 days after the antenatal roledumab injection (see Section 8.2 for further details on safety assessment during pregnancy). If the Doppler ultrasound examination suggests a fetal hemolysis (e.g. a mean cerebral artery peak systolic velocity (MCA-PSV) showing a Multiple of Medians (MoM) >1.5) or other abnormalities, the subject will attend a follow-up visit weekly until resolution or delivery.

The safety of roledumab will be assessed in the RhD-negative pregnant women and in the RhD-positive fetus during the third trimester of pregnancy during the follow-up visits.

The safety of roledumab in the breastfed newborns will also include clinical follow-up of the newborn.

The safety will be assessed by the following variables:
- Incidence and severity of AEs
- Anti-roledumab antibodies
- Vital signs
- Physical examination.

10.2.1. Adverse events

Any abnormalities in vital signs, physical examination or laboratory safety parameters considered as clinically significant by the Investigator will be reported as an AE in the CRF.

The safety of roledumab will be assessed in the RhD-negative pregnant women and in the RhD-positive fetus during the third trimester of pregnancy and after birth in accordance with the study flowchart. Safety assessment during pregnancy will include but is not limited to a fetal Doppler ultrasound examination after the antenatal IMP administration. If the Doppler ultrasound

examinations suggest a fetal hemolysis (e.g. a mean cerebral artery peak systolic velocity (MCA-PSV) showing a MoM >1.5) or other abnormalities, subjects will attend a follow-up visit every week until resolution or delivery.
The immunogenicity of roledumab will be assessed by the detection of anti-roledumab antibodies before the antenatal IMP administration (baseline), at 9 and 29 days after IMP administration, at delivery before the postnatal administration, at 9 days, 6 weeks, 6 months or up to 12 months after the postnatal administration.

10.2.2. Physical examination

Vital signs including sitting systolic and diastolic blood pressure, pulse rate will be recorded in the e-CRF at each visit.

10.2.3. Laboratory data

Refer to Section 8.3.1.

10.3. Assessment of Efficacy: Occurrence of RhD-Alloimmunization

As recommended in the European guidelines for investigations on polyclonal anti-RhD antibodies [CPMP/BPWG/575/99 Rev.1], the efficacy of RhD-alloimmunization prophylaxis in clinical studies is usually assessed by screening the blood sample collected at 6 months after the birth of an RhD-positive fetus. However, it is well-known that in rare cases the passively administered anti-RhD IgG may remain detectable up to 6 months. Therefore, RhD-negative pregnant women will be followed-up until 6 months after delivery and in the case of a positive indirect antiglobulin test (IAT), an additional blood sample will be drawn and tested again at 9 and possibly 12 months post-delivery in order to estimate whether RhD-alloimmunization occurred or not.

The RhD-alloimmunization will be determined by a positive IAT (screening and identification of anti-RhD antibodies). In case of a positive IAT, the anti-RhD antibodies will be quantified centrally by microtitration or ponderal dosage in order to ascertain true alloimmunization from residual anti-RhD antibodies, depending on anti-RhD antibodies concentration levels. The serum concentration of anti-RhD IgG derived from RhD-alloimmunization is not expected to decline while passively acquired anti-RhD IgG should be completely eliminated 3 months later. Passively acquired anti-RhD IgG is usually weakly reactive while a high level of anti-RhD IgG is likely to be of maternal origin.

If the IAT is negative at 6 months post-delivery, the subject will be considered as negative at 9 and 12 months.

If the IAT is positive (i.e. confirmed by anti-D quantification) at 6 months but negative at 9 months post-delivery, the subject will be considered as negative at 12 months.

In the case of a sensitizing event occurring before delivery, additional follow-up visits will be performed according to the same scheme i.e. 2, 5, 9, 29 and 59 days after the additional IMP administration. For PK samplings, the schedule will stay the one initially scheduled after the first injection, and the data obtained will be used to perform the population PK model.

10.4. Other Assessments

A cord blood sample will be collected at delivery in order to perform a direct antiglobulin test (DAT) and to determine the fetal free serum concentrations of roledumab.

All breastfeeding mothers will be asked to provide a first milk/breast milk sample and a blood sample to assess the roledumab first milk or breast milk and maternal serum concentrations.

11. DATA MANAGEMENT

11.1. CRF Completion Guidelines

11.1.1. Introduction

All of the information that must be reported to the Sponsor as per the protocol will be entered in the electronic case report form (e-CRF) for each study subject. The e-CRFs must be completed by the Investigator or any study center staff designated by the Investigator. Access to the data at the clinical site will be restricted and monitored through the system-s software with its required logging, security procedures, and audit trail. The software used for this study will be Inform (Oracle). All the study data will be entered in the database using Inform and will be stored on an Oracle Server, based in the US. The study data will be transferred to the server of the contract research organization (CRO) in charge of data management and statistics, based in India. The database will be periodically transferred to the LFB server based in Lyon, in France.

The Investigator is responsible for ensuring the data recorded in the e-CRFs are accurate and complete. The e-CRFs should be completed within 5 working days after the subject's visit and the latest before review by the Monitor.

11.1.2. General instructions

11.1.2. 1. Overview

All data will be recorded in the e-CRF. The e-CRF is a web-based application which is a computerized system that allows users to create, modify, store, archive, retrieve, or transmit data.

Access to the e-CRF will be limited to authorized individuals. In this way, each Investigator and each person with authorized access to the e-CRF will receive an individual user name and unique password before the study starts. The different access will be limited according to the role of persons. Therefore users should only work using their own passwords and must not share these with other people. The attributed password should be changed when the user first logs in.

Audit trail

To ensure and protect the authenticity, integrity and confidentiality of electronic records an audit trail will be integrated into the e-CRF. All entries and changes will be tracked in an audit trail. The audit trail is a secure, computer-generated, time-stamped system which independently records the date and time of operator entries and actions that create, modify or delete electronic records.

Individual training

It will be the Sponsor's responsibility to organize individual training and certification for all the people who will use the computerized system. In order to obtain their log and password each person should have been performed this training. Each person who enters or processes data should have the necessary education, training and experience to perform the assigned functions. In addition the individuals responsible for monitoring the study should have the necessary education, training and experience in using the computerized system to enable them adequately monitor the study.

All data in the e-CRF must come from and be consistent with the source documents.

Source documents may be:

- The subject's medical file
- The hospital nurse's sheet
- Laboratory results logs
- The subject's diary
- Homecare nurse visit information
- Prescription form
- Central laboratory transmission form
- Any other document issued in the routine practice of healthcare and providing information on the subject's medical status.

Any discrepancy between the data in the e-CRF and those in the source documents should be documented by the Investigator.

Abbreviations should be avoided since they are often ambiguous.

11.1.2. 2.Subject identification

The information reported in the header will be automatically populated on all the e-CRF pages when the subject is created in the system.

Header information should include at least:

- The protocol number
- The name of the visit
- The subject's identification number according to local requirements: subject number is recorded with the center number provided by the Monitor at the set-up visit. The subject number is a sequential number incremented with each subject included in the order the informed consent forms are signed.

11.1.2. 3.Confidentiality

Subjects' names must be kept confidential and should not appear on any e-CRF page or study-specific documents.

11.1.2. 4.CRF completion in case of screening failure or premature withdrawal

For screening failure subjects, the Investigator or the study staff designated by the Investigator will complete the "End of study" section of the e-CRF.

If a subject has been included and prematurely withdrawn, the Investigator will perform the "End of study visit" and complete the "End of Study" Section in the e-CRF.

11.1.3. Completion of the e-CRF

The Investigator should complete the e-CRF within 5 working days of each subject's visit. He will complete all the corresponding pages and, for blank sections or pages, he will complete at least the status of these sections/pages.

11.1.4. Specific case report form instructions

Specific instructions for completing the e-CRF will be on line.

11.2. E-CRF and Data Handling

Data management will be carried out by a CRO from the electronic-based CRF in accordance with Good Clinical Practices (GCP, see ICH-E6, Section 5).

Data processing, from data capture through to database lock, will be carried out in accordance with GCP (see ICH-E6, Section 5).

The data handling documents, e.g. annotated CRFs, database structure, coding rules and computerized validation, are defined in a Data Management Plan. The database and data entry screens will be created in software specifically designed for clinical data management in compliance with ICH-E6 requirements.

Tracking of the e-CRFs will be integrated into the computerized system. As soon as a subject has signed their informed consent, the Investigator will create the subject in the e-CRF and complete the appropriate page(s).

Medical coding will be done using the MedDRA (Medical Dictionary of Regulatory Activities) for all medical terms (medical history, AEs) and the WHO Drug dictionary for all drug names (prior and concomitant medications).

The consistency of data will be checked by computerized programs and any related queries will be generated for resolution by the Investigator. The database will then be updated accordingly. Coding of medical terms and drug names, quality controls to ensure the overall quality and consistency of the database and reconciliation of SAE reports with the Pharmacovigilance database will also be carried out.

At the end of the data handling process, a data review meeting will be held to prepare the database lock.

12. STATISTICS

12.1. Study Objectives and Design

- The main objective is to assess the PK profile of roledumab 300 µg IM/IV in RhD-negative pregnant women carrying an RhD-positive fetus
- The design is an interventional, open-label study

12.2. Statistical Analysis Plan

The material of this section is the basis for the Statistical Analysis Plan (SAP) of the study.

The detailed technical aspects of the statistical analyses will be provided in the SAP. The SAP will possibly take protocol amendments into account and adapt to unexpected issues raised by the study running and/or data that affect planned analyses in the protocol.

Any changes from the protocol will be discussed in the study report.

Prior to locking the database, a data review meeting will be planned in order to review individual data and validate the SAP.

12.3. Sample Size Determination

No statistical hypothesis will be tested. Therefore no formal sample size calculation is done. However 30 subjects IM and 20 subjects IV should be sufficient to determine the PK parameters in pregnant women taking into account the moderate variability between subjects considering the route of administrations and the dose [9], [21].

12.4. Randomization

Not applicable.

12.5. Protocol Deviations and Analysis Sets

All the deviations from protocol definitions will be listed and defined as major or minor deviations in the protocol deviation specification document.

For the PK analysis, if any of the following 2 elements is missing, all of that subject's data will be excluded:

a. Date/time of IMP administration
b. Dose amount

Missing date and time of sampling for a subject will lead to a deviation for the specific PK time point.

The intent-to-treat (ITT) population
The intent-to-treat (ITT) population includes all subjects who were enrolled into the study.

The full analysis set (FAS)
The full analysis set (FAS) is a subset of ITT population and will be used for the efficacy analysis. It consists of all ITT subjects who have a negative IAT test at inclusion and who delivered an RhD-positive newborn.

The safety set (SAF)
The Safety Set (SAF) will be used for all safety analyses. It consists of all subjects who received at least one administration of IMP.

PK populations

PKS1: Analysis population 1 for population PK modeling
The PK set 1 consists of all ITT subjects enrolled in study ADNC-1301 (IM or IV arm), treated at least once with roledumab, and providing at least one roledumab evaluable concentration after administration (including antenatal and postnatal measurements after 1st or 2nd administration).

PKS2: Analysis population 2 for NCA IM arm
The PK set 2 consists of all ITT subjects enrolled in the IM arm of study ADNC-1301, who have at least one valid PK assessment after the first IMP administration, where valid PK assessment is defined as providing at least one evaluable PK parameter.

PKS3: Analysis population 3 for NCA IV arm
The PK set 3 consists of all ITT subjects enrolled in the IV arm of study ADNC-1301, who have at least one valid PK assessment after the first IMP administration, where valid PK assessment is defined as providing at least one evaluable PK parameter.

12.6. General Rules for Handling of Missing or Inconsistent Data

No general rule for the replacement of missing values is used. Any effort should be made to collect missing data.

12.7. Demographic and Baseline Characteristics

12.7.1. Demographic characteristics, medical history, diagnoses

Demographic and baseline characteristics of subjects will be summarized by route of administration and overall. This analysis will be performed in the SAF on all available variables including the following elements, but not limited to:

- Demographic characteristics
- Medical and surgical history
- Prior medication
- Prior pregnancies
- Information about the current pregnancy
- Physical examination
- Laboratory data

The distribution of parameters will be summarized using descriptive statistics depending on the characteristics of the variable:

- Categorical variables (binary, nominal and ordinal) will be summarized by number of missing observations as well as frequencies and percentages for each category. Percentages will be calculated on the observed cases
- Quantitative variables will be summarized by their number of missing observations, number of valid observations, mean, standard deviation, standard error, median, quartiles, minimum and maximum values.

12.7.2. Previous treatments

All previous treatments will be classified using anatomical therapeutic chemical (ATC) codes and listed by subject. The standard dictionary for coding treatments will be WHO Drug. Descriptive statistics will be produced to analyze the previous treatments by their associated ATC code.

12.8. Investigational Product (IMP) and Concomitant Treatments

The analyses described in this section will be performed on the SAF presented by route of administration and overall.

12.8.1. Extent of exposure

Subjects are planned to receive two injections of the IMP, one at the 28th week of gestation (or 29th week) and one within 72h after delivery. If other drug administration is given (i.e. in case of sensitizing event), a description of events and doses will be provided. Duration of exposure will be summarized as the time between the date of the first injection until the date of the last injection plus 1. The actual administered dose will be presented per injection as well as the number of injections and the cumulative dose of the administered IMP.

12.8.2. Treatment compliance

Compliance is defined as compliance to the administration regimen (dosage, schedule and administration route) as defined in Table 6–1.

12.8.3. Concomitant treatment

All concomitant treatment will be classified using ATC codes and listed by subject. The standard dictionary for coding treatments will be WHO Drug.

Descriptive statistics will be produced to analyze the concomitant treatments by their associated ATC code.

12.9. Efficacy Analysis

12.9.1. Primary efficacy variable(s)

The primary objective is to assess the PK of roledumab 300 µg IM and IV. Therefore, no primary efficacy variable is defined but only secondary efficacy variables.

12.9.2. Secondary efficacy variables

All efficacy analyses will be performed on the FAS set. Secondary efficacy variables are the following:

- RhD-alloimmunization status at 6, 9 and 12 months after delivery

RhD-alloimmunization will be assessed at 6 months post-delivery. In the case of a positive IAT and alloimmunization confirmed by anti-D quantification at 6 months, the subject will be followed until 9 and eventually 12 months in order to distinguish residual anti-RhD antibodies from anti-RhD

alloantibodies. If the IAT is negative at 6 months post-delivery, the subject will be considered as negative at 9 and 12 months. If the IAT is positive at 6 months but negative at 9 months post-delivery, the subject will be considered as negative at 12 months.

The number and percentage of subjects by alloimmunization status will be presented by route of administration and overall. A particular attention will be paid of the alloimmunization status in case of sensitizing events. No statistical hypothesis is tested.

12.10. Safety Analysis

All safety analyses will be performed on the SAF presented by route of administration and overall.

Despite the genotyping on maternal blood in order to include only women carrying an RhD-positive child it may happen that at delivery the child is observed as being RhD-negative. Therefore, safety on the fetus/infant will be presented by subgroups of their RhD-status (positive / negative).

12.10.1. Extent of exposure

Please refer to Section 12.8.1.

12.10.2. Adverse events

With respect to AEs, all AEs of the mother and/or the fetus/newborn/infants occurring during treatment and follow-up periods (up to 6 months age for the infant and up to End of Study visit for the mother) will be summarized using the Preferred Term (PT) and System Organ Class (SOC). AEs (occurring during screening period (between V1 and V2) before the first injection of the IMP) will only be listed. The duration of the related and specific AEs listed in Section 9.5.2 and the duration between the last dose of IMP and onset of these AEs will be calculated and summarized.

The number and percentage of AEs by SOC and PT on all AEs will be tabulated. SAEs, drug-related AEs and other significant AEs will be presented in the same way.

The number and percentage of subjects with at least one AE, at least one SAE, at least one drug-related AE or at least one other significant AE will be tabulated by SOC and PT.

The standard dictionary for coding events will be MedDRA.

12.10.3. Fetal anemia

The number and percentage of fetal anemia and other abnormalities detected by Doppler ultrasound and obstetric ultrasound will be summarized. Details are given in Section 10.2 of the protocol.

12.10.4. Vitals signs and physical findings

Vital signs and physical examination findings including APGAR score will be summarized using appropriate statistics. Any significant abnormalities will be listed.

12.10.5. Laboratory data

Laboratory values over time and changes in individual subjects will be summarized using appropriate statistics. All clinically significant abnormal values will be listed.

The immunogenicity of roledumab (ADA) will be assessed using descriptive statistics.

12.10.6. Other endpoints

The analysis of these endpoints will be performed on the SAF.

- Roledumab cord blood concentration

The endpoints will be presented using descriptive statistics. The ratios of roledumab in cord blood concentration to maternal serum concentration will be calculated.

- Roledumab first milk/breast milk concentration

The endpoints will be presented using descriptive statistics. The ratios of roledumab in first milk/breast milk concentration to maternal serum concentration will be calculated.

These endpoints will be only listed.

12.11. Pharmacokinetics, PK/PD Analysis

The PK analysis is detailed in the SAP.

12.11.1. Primary analysis

A population PK analysis will be performed as the primary PK analysis using nonlinear mixed effect modeling with both IM and IV data.

The model will be created using a two compartments disposition model with first order absorption and elimination. The effects of covariate body size will be evaluated on the PK parameters clearance and volume of distribution. Bioavailability of roledumab will also be estimated.

The effect of other covariates such as gestational age, glomerular filtration rate, effect of delivery, age and site of injection, will be explored using the likelihood ratio test. Given the small sample size of the study, no other covariate will be explored. The first-order conditional estimation (FOCE) method will be used throughout analyses.

Various random effects structures and residual errors approaches will be explored as needed.

Diagnostic plots of observed data versus population and individual predicted values and various residuals plots will be used to detect any significant deviation from the model fit.

A table of population PK parameters with confidence intervals will be produced.

Derived secondary parameters will be obtained from individual posterior estimates, including AUC_{inf}, C_{max}, T_{max}, $t_{1/2}$, elimination rate constant. Those derived parameters will be summarized by appropriate statistics (N, geometric mean, 90% confidence intervals, median, min and max, with the exception of T_{max} to be summarized by median, min and max only).

12.11.2. Secondary analysis

Two non-compartmental analyses will be used using Phoenix® software with IM and IV data.

- Volume of distribution (Vd)
- Clearance (CL)
- C_{max}
- T_{max}
- Area under the curve (AUC_t, AUC_{inf})
- Terminal half-life ($t_{1/2}$) and Elimination rate constant

Those derived parameters will be summarized by appropriate statistics (N, geometric mean, 90% confidence intervals, median, min and max, with the exception of T_{max} to be summarized by median, min and max only).

13. STUDY REPORT

A Clinical Study Report will be prepared in accordance with the ICH-E3 guidelines, by the Sponsor or subcontractor in collaboration with the coordinating Investigator and, if any, with the scientific committee.

Within 1 year after the end of the study (Last Visit Last Subject), the Sponsor will provide the Health Authorities with the full Clinical Study Report or summary. Only the Sponsor is entitled to make the study report available to the Authorities.

Neither the complete report nor any part of the study report may be used without the approval of the Sponsor.

14. CONFIDENTIALITY AND PUBLICATION

14.1. Subject Confidentiality

Subject data will be kept strictly confidential and subject anonymity will be protected by using number codes and /or initials.

The Sponsor or its representative(s) and the Health Authorities are obligated to respect medical secrecy and to refrain from divulging any personal subject information they might fortuitously be aware of.

14.2. Use of Information

The Investigator shall not divulge unpublished data or information related to the study provided by the Sponsor, including but not limited to the study product characteristics, the Investigator brochure, the study protocol, case report forms, assay methods and scientific data, to any third party without written approval from the Sponsor.

In addition, any new information that may become available during the course of the study shall be considered as confidential and shall not be used for any purpose other than the performance of the clinical study.

The study data are the property of the Sponsor. The Investigator and any of the research staff shall obtain written approval from the Sponsor prior to the publication/communication of the results of any work carried out during or in relation to the study.

Publication and/or communication of the results of the clinical study will be of a cooperative nature involving authors representing the Sponsor, the Study Coordinator, and the scientific committee, if any.

The Sponsor reserves the right to request modification of the content and/or timing of any publication or presentation if a patent application, an existing patent or other proprietary rights may be jeopardized.

Authorship of any publication related to the study and the order of presentation of the authors' names shall be approved by the Sponsor. The Sponsor shall not use an Investigator's name in any publication without his/her written permission and vice versa.

15. ARCHIVING

The Investigators should retain all essential study-related documents, i.e. documents which permit evaluation of the conduct of a study and the quality of the data produced, in accordance with the applicable regulatory requirements of his/her country. These essential documents include but are not limited to signed protocol, Investigator Brochure, e-CRFs, medical records, laboratory reports, informed consent forms, drug disposition records, safety reports, information regarding participants who discontinued, and other relevant documents and data.

The study-related documents should be kept together in the Investigator site file provided to the Investigator by the Sponsor.

Sufficient information about the identity of all study subjects, e.g. name, medical records number, subject number and study number, should be retained by the Investigator so that any Sponsor representatives, auditors or inspectors may access this information when required.

The Investigator must retain all records for 15 years or longer if required by specific local requirements.

The Investigator will contact the Sponsor for authorization prior to the destruction of any study records or in the event of accidental loss or destruction of any of them.

The Investigator will also notify the Sponsor should he/she relocate or move the study-related files to a location other than that specified in the Sponsor's Study Master File.

All records should be kept in a secure area, however in the cases of audit or inspection, they should be rapidly made available.

16. RESPONSIBILITIES OF PARTICIPANTS

16.1. Responsibilities of the Investigator(s)

The Investigators will conduct the study in accordance with ICH-E6, all applicable laws in the country where the study is conducted and in accordance with this study protocol.

The responsibilities of the Investigators are summarized below but not limited to:

- Subject information and consent

Prior to undertaking any study-related procedure, it is the responsibility of the Investigator, or a formal designee, to provide each subject and/or a legal representative/witness, with relevant, comprehensive, verbal and written information, including the written information which received approval or a favorable opinion from the IEC/IRB and the Health Authorities. Signed informed consent must be obtained prior to undertaking any study-related procedure. Obtaining of consent and how it was obtained must be described and documented in the subject's file.

- Information on the overall results of the study

Pursuant to the French "Subject's rights" law adopted on 09 August 2004, the Investigator must provide any subject who so requests it with the overall results of the study. The Sponsor will provide the Investigator with the overall results beforehand. The Investigator should document in the subject's file the fact that the information has been provided.

- Information to other practitioners (if relevant)

In agreement with the subject, the Investigator will formally inform other practitioners of the subject's participation in the study, to avoid any interference or bias in the conduct of the study.

- Institutional Review Board / Independent Ethics Committee (IRB/IEC)

In accordance with local regulations, the Investigator may be required to interface with the IRB/IEC.

- Adverse events

The Investigator is responsible for ensuring adequate safety monitoring and follow-up of the study subjects. The Investigator must report and handle any serious and non-serious AE, whether clinically observed or spontaneously reported by the subject, using concise medical terminology in accordance with Section 9 of the protocol.

- Data recording

It is the Investigator's responsibility to ensure, on an on-going basis, completion and validation of all case report forms as well as study-related supportive data. e-CRFs must be signed by the Investigator. If the Investigator formally delegates completion of the e-CRFs, the Investigator nevertheless has the final responsibility for signing the e-CRFs to certify the accuracy and reliability of the data recorded therein.

- Record retention

To enable inspections and audits from Health Authorities or the Sponsor, the Investigator agrees to keep records, including the identity of all participating subjects, i.e. sufficient information to link records, all original signed informed consent forms, copies of all CRFs and detailed records of treatment disposition. The Investigator should maintain a site file with all essential documents.

See also Section 15.

- Use of study-related information

The Investigator must provide the Sponsor with complete test results and all data derived from the study. Only the Sponsor may make information available to physicians, Health Authorities and/or subjects enrolled in the study (via the Investigators), except as required by local regulations.

- IMP

Responsibility for IMP accountability at the study site rests with the Investigator or with the institution, depending on local regulations.

- Quality control

The Investigator and the relevant personnel should be available during monitoring visits and possible audits or inspections and ensure that sufficient time is devoted to the process. The Investigator guarantees the Sponsor or its representative and appropriate Health Authorities direct access to source documents.

- Study discontinuation

Should the Health Authorities or the Sponsor decide to discontinue the study prematurely for any reason, the Investigator must promptly contact all participating subjects so they can be appropriately followed-up. All study supplies must be collected and all case report forms must be completed as fully as possible.

- Liability and insurance

Liability and insurance provisions for this study are set out in separate agreements.

- Delegation of Investigator duties

The Investigator can delegate tasks to the research team but he/she remains responsible for coordinating and informing his/her staff about the protocol and the possible changes made to it.

The Investigator should ensure that all persons assisting with the study are adequately qualified, and are informed about the study protocol, any amendments to the protocol, the study treatments, and their study-related duties and functions. The Investigator should maintain a list of appropriately qualified persons to whom significant study-related duties will be delegated. The list is to be kept up-to-date. The Investigator should supply an up-to-date curriculum vitae (CV) in English, dated and signed, together with a list of his/her collaborators responsible for the practical conduct of the study. These collaborators should also provide a recent English version of their CVs, dated and signed.

- Study agreement discontinuation

During the study, if events such as retirement, promotion or relocation prevent the Investigator from conducting the study as agreed, the Investigator should appropriately transfer his/her responsibilities, knowledge and documents to another willing individual, with the agreement of the Sponsor. Study specific contracts must be signed between the Sponsor and the newly assigned person.

16.2. Responsibilities of the Monitor

The responsibilities of the study Monitor are defined in ICH-E6, Chapter 5. The Monitor, who is mandated by the Sponsor, must ensure that the study is conducted in accordance with Good Clinical Practice guidelines and all applicable local laws, and that the rights, the security and the well-being of the subjects are respected.

During the conduct of the study, the Monitor reports any deviations or persistent poor compliance with the study requirements and the Sponsor makes decisions about appropriate corrective actions.

- Communication

The Monitor is the main line of communication between the Investigator and the Sponsor.

- Training

The Monitor must present the protocol and all procedures related to the study during the study set-up visit and provide the Investigator with CRF completion guidelines.

- Compliance

During periodic monitoring visits at mutually convenient times, the Monitor has the responsibility of assessing the progress of the study, of checking that the informed consent forms have been signed, of ensuring adhesion to and compliance with the study protocol and other study-related documents, and of ensuring the accuracy and completeness of the e-CRFs. Inconsistencies in the study records are to be resolved.

- Source data verification

The Monitor will perform source document verification and validation and request clarification to ensure the accuracy, completeness and reliability of data.

- IMP

The Monitor must ensure that IMP handling is properly carried out and documented.
He/she must ensure that the Investigator's file is up-to-date with regard to essential documents.

16.3. Responsibilities of the Data Manager

Cognizant will be in charge of the management of clinical data from data entry to database lock under the supervision of LFB Data Manager.

17. ETHICS AND REGULATORY CONSIDERATIONS

The current study is to be conducted in accordance with Good Clinical Practice (ICH-E6), European Directive 2001/20/EC, and the revised version of the Declaration of Helsinki set out in the European Directive, as well as with applicable local requirements.

In France, this study will be conducted in accordance with the CSP.

The protocol will be submitted to the Health Authorities and a properly constituted Ethics Committee (EC) for formal approval of the study conduct in accordance with local regulations. The study should not begin until the protocol has received written approval from the EC and Health Authorities in accordance with local requirements.

In accordance with specific local requirements, the Investigator may be responsible for submitting the protocol and any amendments to the local EC. A copy of the decision letter, a list and versions of documents submitted, the list of EC members and their affiliation should be provided by the Investigator to the Sponsor.

During the study, the Sponsor should promptly notify the Investigators, Health Authorities and EC of any relevant information that could affect the safety of subjects and could impact on the conduct of the study.

Personal Data Protection Committee

For biomedical research in France: the Sponsor attests his conformity regarding the Personal Data Protection French requirements ("Méthodologie de Réference MR001" dated 5 January 2006).

Insurance

The Sponsor will contract civil liability insurance to provide subjects with compensation for any injury, including the consequences of administration of the investigational product and of the study procedures. The insurance company is HDI Gerling and registration number is 01006575-14276.

In case of injury or disability resulting from participation in the study, the subject is requested to promptly inform the Investigator responsible for the study.

Indemnity

Subjects will receive compensation for their participation in this study for the following constraints imposed by the protocol:

- Participating to up to 16 study visits most of which will require the subject to come to the hospital, and some of which (V2 and V8) lasting more than several hours as the subject will have to wait 6 hours between the IMP administration and blood sample collection for cytokines, CRP analyses
- Having urine sample and several blood samples collected during these visits
- Having some first milk/breast milk sample collected for the breast feeding subjects.

The subjects will be indemnified based on the visits performed, with a total of 3000 € if completing the entire study (providing first milk/breast milk samples at V10 and V11, and participating to the additional visits AV1 and AV2). The indemnity per visit will be as follow:

- Screening visit V1: 200 €
- Antenatal IM roledumab administration V2: 375 € (200 € for the inclusion + 175 € for the IMP administration and follow-up sampling), or
- Antenatal IV roledumab administration V2: 275 € (100 € the for inclusion + 175 € for the IMP administration and follow-up samplings)
- V2b (IV only): 100 € per visit
- V3 and V4: 100 € (this PK sampling visit can be performed at home) per visit
- V5, V6, V7: 200 € per visit
- Postnatal IMP administration V8: 375 € (200 € before administration + 175 € for the IMP administration and follow-up sampling)
- V9: 100 €

- V10 and V11: 250 € (for first milk /breast milk sampling done at home) per visit
- V12: 200 €
- End of study visit V13: 150 €
- Additional visit AV1: 150 € and Additional visit AV2: 150 €

A subject participating to the screening visit but who will not meet all the inclusion criteria will then be indemnified 200 €, and a subject who for example will withdraw at V3 will receive 675 €.

Changes to the protocol

The Sponsor will not assume any responsibility or liability resulting from implementation of unapproved deviations or changes.

The only circumstance in which an amendment may be initiated prior to approval by the Health Authorities is where the change is necessary to eliminate apparent immediate hazards to the subjects. In this event, the Investigator must notify the Sponsor and if applicable the Ethics Committee, in writing within 5 working days after implementation.

18. AUDIT AND INSPECTION

An audit/inspection may be carried out by qualified Sponsor staff, by subcontracted auditors or by representatives of national or foreign Health Authorities to ensure that the study is conducted as per protocol and in accordance with regulatory requirements, and to ensure the validity of the data.

Participation in this study implies acceptance to cooperate in any potential audit/inspection.

The audit/inspection may consist of an inspection of the premises and equipment together with verification of the study documents and data. The investigational team must be available for inspection or audit.

When the Sponsor or the Investigator is informed that an inspection is to be performed, the other party must be informed immediately. Audits/inspection may take place after the end of the study.

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20. APPENDICES

Not applicable.