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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STATISTICAL ANALYSIS PLAN

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, multicentre, open-label study

Statistical Analysis Plan Status: final version 2.0 (PK, Efficacy and Safety)
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Study Drug: Roledumab

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Updated with Amendment 10 and 11 (Protocol v12)

Covance Study Number: 8368060

Clinical Phase 2b

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1. STATISTICAL ANALYSIS PLAN APPROVAL SIGNATURES

By signing this page when the Statistical Analysis Plan (SAP) is considered final, the signatories agree to the statistical and pharmacokinetic (PK) analyses to be performed for this study, and to the basic format of the tables, figures, and listings (TFLs). Once the SAP has been signed, programming of the TFLs based upon this document can proceed. Any modifications to the SAP and TFLs made after signing may result in a work-scope change.

SAP final version 2.0 dated 15 March 2018: updates after protocol amendments 10 and 11

Function	Name	Date	Signature
Project Statistician	[redacted]	[redacted]	[redacted]
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2. TABLE OF CONTENTS

3. ABBREVIATIONS

Abbreviations pertain to the SAP only (not the TFLs).

Abbreviation or specialist term	Definition or explanation
ADA	Anti-Drug Antibody
ADaM	Analysis data model
AE	Adverse event
AIC	Akaike's information criterion
ALT	Alanine aminotransferase
APGAR	Appearance, Pulse, Grimace, Activity, and Respiration
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
ATC	Anatomical therapeutic chemical
AUC	Area under the plasma drug concentration-time curve
AUC_{0-t}	Area under the concentration-time curve from time 0 to time T_{last}
$AUC\%_{extrap}$	Percentage of AUC that is due to extrapolation from the time of the last quantifiable concentration to infinity
$AUC_{0-\infty}$	Area under the concentration-time curve from time zero to infinity
BLQ	Below the limit of quantification
BMI	Body mass index
CDISC	Clinical data interchange standards consortium
CL	Total serum clearance
CL/F	Apparent total serum clearance
C_{max}	maximum observed plasma concentration
CPAP	Clinical pharmacology analysis plan
CRCL	Creatinine clearance
CRF	Case report form
CRP	C-Reactive protein
CSR	Clinical study report
CV	Coefficient of variation
CWRES	Conditional weighted residuals
DAT	Direct antiglobulin test
DBL	Database lock
DSMB	Data safety monitoring board
DV	Dependent variable
EC	Early clinical
ENT	Ear–nose-throat
EOS	End of study
ETA or η	Random inter-individual effect that is normally distributed with mean 0 and variance-covariance matrix Ω
F	Bioavailability
FAS	Full analysis set
FMH	Fetomaternal hemorrhage
FO	First-Order estimation method

Abbreviation or specialist term	Definition or explanation
FOCE	First-order conditional estimation method
FOCEI	First-order conditional estimation with interaction method
GA	Gestational age
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IAT	Indirect antiglobulin test
ICH	International conference on harmonisation
IIV	Inter-individual variability
IM	Intramuscular
IMP	Investigational medicinal product
IPRED	Individual predicted concentration
ITT	Intent-To-Treat
IV	Intravenous
Ka	Absorption rate constant
KB	Kleihauer-Betke
λ_z	Apparent terminal elimination constant
LLOQ	Lower limit of quantification
LLR	Log likelihood ratio
LOCF	Last observation carried forward
LOESS	Locally weighed smoothed line
LRT	Likelihood ratio test
MCA-PSV	Middle cerebral arterial-peak systolic velocity
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MedDRA	Medical dictionary for regulatory activities
MOF	Minimum value of the objective function
MoM	Multiple of medians
NCA	Non-compartmental pharmacokinetic analysis
NM-TRAN	NONMEM translator
NONMEM	Non-linear mixed-effects modeling software
PAP	Population analysis plan
PD	Protocol deviation
PK	Pharmacokinetic or pharmacokinetics
PKS	Pharmacokinetics set
PopPK	Population pharmacokinetic
PRED	Population predicted concentration
PT	Preferred term
Q	Inter-compartmental clearance
Q1	First quartile
Q3	Third quartile
QQ	Quantile-quantile or QQ-plot

Abbreviation or specialist term	Definition or explanation
RBC	Red blood cells
RES	Residual
SAE	Serious adverse event
SAF	Safety analysis set
SAP	Statistical analysis plan
SD	Standard deviation
SDTM	Study data tabulation model
SOC	System organ class
SOJ	Site of injection
$t_{1/2}$	Apparent terminal elimination half-life
T_{last}	Last time point with a measurable concentration
TEAE	Treatment-emergent adverse event
TFLs	Tables, figures, and listings
TNF	Tumor necrosis factor
T_{max}	Time of maximum observed concentration
TVP	Typical value of a parameter P
ULOQ	Upper limit of quantification
V2 or Vc	Central compartment volume of distribution
V3 or Vp	Peripheral compartment volume of distribution
Vd	Volume of distribution
V_z/F	Apparent volume of distribution during the terminal phase after non-intravenous administration
V_z	Volume of distribution during the terminal phase
V_{ss}	Volume of distribution at steady-state (within the body)
WT	Body weight (kg)

4. INTRODUCTION

This SAP has been developed consistent with the clinical study protocol (final version 12.0 dated 27 October 2017) [1].

This SAP describes the planned analysis of the efficacy, safety and PK data from this study. A detailed description of the planned TFLs to be presented in the Clinical Study Report (CSR) is provided in the accompanying TFL shell document.

The intent of this document is to provide guidance for the statistical analyses of efficacy, PK and safety data. In general, the analyses are based on information from the protocol, unless they have been modified by agreement between LFB Biotechnologies and Covance Early Clinical (EC) Biometrics. A limited amount of information concerning this study (e.g. objectives, study design) is given to help the reader's interpretation. This SAP must be finalized prior to the database lock (DBL) for this study.

This SAP supersedes the statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified in the CSR. Any substantial deviations from this SAP will be agreed upon between LFB Biotechnologies and Covance EC Biometrics and identified in the CSR.

This SAP is written with consideration of the recommendations outlined in the International Conference on Harmonisation (ICH) E9 guideline entitled "Guidance for Industry: Statistical Principles for Clinical Trials" and the ICH E3 guideline entitled "Guidance for Industry: Structure and Content of Clinical Study Reports [2], [3]."

5. STUDY OBJECTIVES

Primary Objective

The primary objective is to assess the pharmacokinetic profile (PK) of roledumab 300 µg administered intramuscular (IM) or intravenous (IV) in RhD-negative pregnant women carrying an RhD-positive fetus.

Secondary Objectives

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

6. STUDY DESIGN

This is a phase 2b, interventional, multicenter, open-label study. The study is not randomized. When the target number of subjects was reached in IM part, the subsequent subjects were selected to participate in the IV part (sequential). It was initially planned to 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) (See Section 8 for the justification of the sample size).

6.1. Study plan and assessments

Figure 1: Study Plan (IM and IV arms)

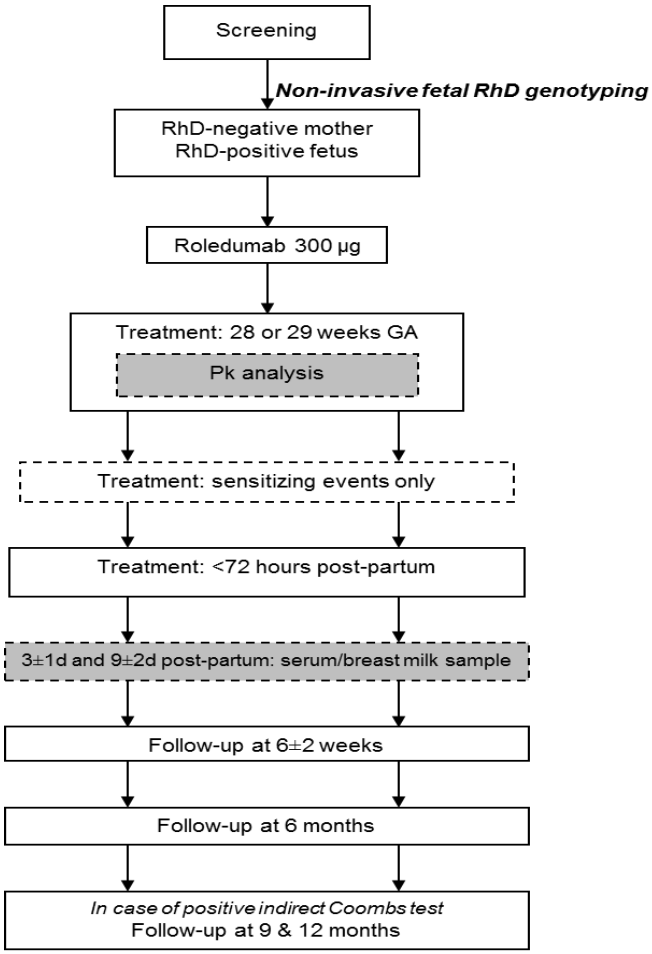

Figure 2: Visits schedule in the IM arm

Figure 3: Visits schedule in the IV arm

The subjects 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 IMP administration
- Potential RhD-alloimmunization requiring follow-up up to 12 months after the IMP administration.

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

- The screening period starting between 12 and 27 weeks gestation age (GA) with the screening visit performed as close as possible to the antenatal IMP administration, i.e. within 2 weeks
- The antenatal period comprises 6 visits (IM) and 7 visits (IV) and lasts about 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 when necessary (i.e. in case of a positive indirect Coombs test), 2 additional visits.

Each subject will participate in this study until 9.5 months if the Indirect Antiglobulin Test (IAT) result is negative at 6-month post-delivery. Otherwise, the maximum duration of the participation for a subject could go up to 18 months.

Table 1: Schedule of assessments 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			
KB test									x	x[11]							

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*							
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, Concomitant Medications
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 week.s

Table 2: Schedule of assessments 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
6 If IAT positive, IA characterization and Anti-D antibodies quantification
7 Collection of APGAR score, AEs, Concomitant Medications
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

Table 3: Study flow chart – 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	x
Partial physical examination	x		
IAT	x*		
Kleihauer-Betke test	x**		x***
Cytokines		x	
CRP			x
Adverse Events	x	x	x
Concomitant treatment	x	x	x

* *Perform an anti-D quantification by microtitration or 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 test results*

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

6.2. Pharmacokinetic Samplings

6.2.1. Maternal serum sampling

Roledumab in maternal serum will be measured using a flow cytometry PK method by Envigo (formerly Huntingdon Life Sciences), located at Huntingdon, UK. The method was validated based on FDA (2001) and EMA (2012) bioanalytical guidelines. The validated detection range for roledumab in whole serum is from 1 to 15 ng/mL and dilutional integrity was demonstrated, allowing pre-dilution of study samples when required. All analytical runs will include a calibration curve and three levels of quality control samples with predefined acceptance criteria.

PK parameters calculation (NCA) or estimates (population PK analysis) will be derived from roledumab measurements in serum samples of pregnant and post-delivery RhD-negative pregnant women carrying an RhD-positive fetus administered IM or IV with roledumab. All samples of a specific subject are to be assayed in the same assay run, except for those who will need to be rerun, such as results above the upper limit of quantification (ULOQ).

Samples of subjects enrolled in the ADNC-1301 clinical trial will be drawn within the 24 hours preceding injection of roledumab (pre-dose) and at the theoretical timepoints post-first and second administration described in Figure 2 and Figure 3 depending on the administration route.

It is of importance to note that this method of describing the scheduled sampling times is for information only. Calculation of the PK parameters will require the use of the actual elapsed time from the time of injection to the actual time of sampling.

6.2.2. Milk sampling

The validated lower limit of quantification (LLOQ) of roledumab in maternal serum is 1.5 ng/mL. Based on current product knowledge, it is expected that only very few of the clinical samples will have reported concentrations above the LLOQ.

If available the ratio between concentration of roledumab in breast milk and maternal serum concentration will be calculated.

6.2.3. Cord blood sampling

Cord blood at delivery (V8) will be collected for roledumab fetal/neonate concentration. The ratio between cord blood concentration of roledumab and maternal serum concentration will be calculated.

7. TREATMENTS

The following is a list of the study treatment abbreviations and ordering that will be used in the TFLs.

Study Treatment Name	Abbreviation	Treatment Order on TFLs
300 µg IM roledumab	IM	1
300 µg IV roledumab	IV	2

8. SAMPLE SIZE JUSTIFICATION

No statistical hypothesis will be tested. Therefore no formal sample size calculation is done. The expected number of subjects to be recruited was initially as follows:

- In the IM arm, thirty-five (35) subjects to be screened in order to get 30 evaluable subjects
- In the IV arm, twenty-five (25) subjects in order to get 20 evaluable subjects.

9. DEFINITION OF ANALYSIS POPULATIONS

The intent-to-treat (ITT) population:

The intent-to-treat (ITT) population includes all subjects who were enrolled in the study (i.e. who signed the ICF and who were not screening failures) and/or who did not complete the EOS form before the inclusion visit (V2) (See Section 18.1).

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.

Pharmacokinetics Sets (PKS):

PKS1: Analysis population 1 for population PK modeling
The PKS1 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 available concentration after administration (including antenatal and postnatal measurements after 1st or 2nd administration).

PKS2: Analysis population 2 for NCA IM arm

The PKS2 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 PKS3 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.

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

a. Exact 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 timepoint.

- Additionally, subjects who have complete dosing data but no concentrations associated to samplings will be excluded from all PK analysis
- If either a sampling time or its concentration is missing, then that sample data point will be excluded
- If preanalytical conditions are not met, sample results will be discarded from analysis.

All protocol deviations that occur during the study will be considered prior to DBL for their severity/impact and will be taken into consideration when subjects are assigned to analysis populations. Details of subject assignment to the analysis populations will be listed.

The complete specific criteria defining protocol deviations and their classification (major/minor) will be agreed by the study team during the Data Review or during Protocol Deviation discussion meeting prior to DBL. The protocol deviation specifications are described in a separate document (ADNC-1301 Protocol Deviation Specifications).

10. STUDY ENDPOINTS

10.1. Primary endpoints

Serum concentrations of roledumab will be measured at the defined time points as described in the schedule of assessments (See Table 1 and Table 2). The PK profile of roledumab will be described by:

1. A primary compartmental population PK model will be performed with 6 primary parameters: Central compartment clearance (CL), Central compartment volume of distribution (V2), Inter-compartmental clearance (Q), Peripheral compartment volume of distribution (V3), IM route bioavailability (F) and IM route Absorption rate constant (Ka); and derived parameter: half-life ($t_{1/2}$) (See Section 18.2 for changes from protocol).

2. A secondary PK analysis (NCA) will provide the following parameters: Vd, CL, C_{max}, T_{max}, AUC_t, $AUC_{0-\infty}$, 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.

10.2. 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.

The efficacy of roledumab 300 µg IM/IV to prevent RhD-alloimmunization in RhD-negative pregnant women carrying a RhD-positive fetus will be assessed by screening the blood sample collected at 6 months (and possibly at 9 and 12 months) after birth of the RhD-positive fetus using Indirect Antiglobulin Test (IAT). In case of a positive IAT, the anti-RhD antibodies will be quantified centrally by microtitration or ponderal dosage.

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. 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 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.

The immunogenicity of roledumab will be assessed during the course of the study using the results of ADA tests.

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

11. STATISTICAL METHODOLOGY

11.1. General

For continuous data, summary statistics will include the arithmetic mean, arithmetic standard deviation, median, minimum, maximum, Q1, Q3, number of observations and number of missing observations. For log-normal data (e.g. the PK parameters: areas under the concentration-time curve [AUCs] and maximum observed concentration [C_{max}]), the geometric mean and geometric coefficient of variation (CV%) will also be presented.

Categorical data (binary, nominal and ordinal) will be summarized by number of observations, number of missing observations, frequency count and percentages. Percentages will be calculated on the observed cases.

In general, missing values will not be imputed, otherwise it will be specified. Only descriptive statistics will be given, no confidence interval will be provided.

Individual data listings in Section 16.2 of the CSR will be provided for all subjects, including screen failures.

Summary statistics and statistical analyses will be performed for subjects included in the relevant analysis populations.

Data analysis will be performed using SAS® v9.4 or latest version in a secure and validated environment. All report outputs will be provided to the Sponsor in Microsoft Word version 10.

Analysis Data Model (ADaM) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) ADaM Version 2.1, and CDISC ADaM Implementation Guide Version 1.1. Pinnacle 21 Community Validator Version 2.1.2 will be used to ensure compliance with CDISC standards.

Study Data Tabulation Model (SDTM)) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) SDTM Version 1.4, and CDISC SDTM Implementation Guide Version 3.2 utilized.

11.1.1. Definition of Baseline and Change from Baseline

Baseline for each parameter is defined as the last value measured prior to the first IMP injection, including repeat (vital signs) and unscheduled (clinical laboratory parameters) readings (see Section 11.1.2 for definitions of repeat and unscheduled readings).

Mean change from baseline is the mean of all individual subjects' change from baseline values. Each individual change from baseline will be calculated by subtracting the individual subject's baseline value from the value at the timepoint. The individual subject's change from baseline values will be used to calculate the mean change from baseline.

11.1.2. Repeat and Unscheduled Readings

Repeat readings occur when the original vital signs result requires confirmation. Repeat readings are labelled as 'Repeat' in the listings and replace the original readings in all summaries and changes from baseline presentations and calculations. Prior to dosing, all readings taken in addition to the original reading are defined as pre-dose repeats. Post-dose repeat readings are defined as readings collected within 15 minutes of the actual time of the original reading.

With the exception of pre-dose results described above, unscheduled readings for vital signs are defined as readings collected >15 minutes from the actual time of the original reading. All results not taken at a scheduled timepoint for other data types (e.g. clinical laboratory parameters) are unscheduled. Unscheduled readings are labelled as 'Unscheduled' in the listings. Because unscheduled readings are not associated with any scheduled timepoint, they are excluded from all summaries (with the exception that they may be used as baseline, as stated in Section 11.1.1).

11.2. Study subjects

11.2.1. Subject disposition

The subject disposition will be summarized as follows:

- Number of screened subjects
- Number and percentage of screen failures
- Number of subjects enrolled into the study (who signed inform consent and not screening failure – see Section 18.1)
- Number and percentage of subjects who received study treatment
- Number and percentage of mothers treated with RhD-negative fetus
- Number and percentage of subjects who completed the study
- Number and percentage of subjects early withdrawn from the study and reasons for study discontinuation

The analysis populations will be summarized with the number and the percentage of subjects included in each analysis set with respect to the number of enrolled subjects:

- Number and percentage of subjects in ITT population
- Number and percentage of subjects in FAS
- Number and percentage of subjects in SAF
- Number and percentage of subjects in PKS1
- Number and percentage of subjects in PKS2

- Number and percentage of subjects in PKS3

For each analysis population, the number and the percentage of subjects who completed the study will be displayed.

A listing of subject disposition will be presented by subject. This listing will present for each subject the enrollment status (yes/no), the date of signature of the ICF, the date of screening visit, the date of inclusion visit, the date of last visit, date of completion (i.e. date of completion of the EOS form) the individual study duration (in days) and the final status of the subject (completed study or not).

A by-subject table of screen failures will be provided with the reason for excluding subjects during the screening phase. Another by-subject listing will provide the inclusion and exclusion criteria by subject for all screened subjects.

A listing of withdrawals will also be displayed with the reasons for discontinuation.

The disposition of mothers who delivered RhD-negative neonates will also be presented if any.

A by-subject listing will display the visits done by each subject during the study with the date associated to each visit. It will also be indicated if the visit was done at hospital or at home as well as the related day (from the first IMP administration).

In addition, a by-subject listing for each analysis population (ITT, FAS, SAF, PKS1, PKS2 and PKS3) will be provided with the reason for exclusion from the considered analysis population.

11.2.2. Protocol deviations

All protocol deviations (PD) will be listed in a by-subject listing and will also be summarized in a table displayingthe number and percentage of subjects with at least one PD for each protocol deviation category and classification (minor/major) on ITT population.

11.3. Demographic and baseline characteristics

11.3.1. Demographics

Summary statistics will be provided for demographic and other baseline characteristics by route of administration and overall for the SAF.

Demographic and other baseline characteristics will be qualitatively or quantitatively described (depending on the nature of data). No comparison will be performed between the groups of subjects based on the route of administration.

- The following demographic characteristics at baseline will be described in a summary table: age at enrolment (years), gender, race, weight before pregnancy (kg), weight at screening (kg) and BMI before pregnancy (kg/m²). These demographic characteristics will also be described for the FAS and PKS populations in addition to the SAF.

A by-subject listing will display some demographic data (age and race) as well as the date of screening, the date of signature of the ICF and the date of birth.

- Other baseline characteristics:
  - ✓ Laboratory data at baseline:

- Serology
  - HBV test (HBs Ag, Anti-HBc Ab, Anti-HBs Ab)
  - HCV test (Anti-HCV Ab)
  - HIV test (Anti-HIV 1+2)
- Immunogenicity assessment – ADA test
- Indirect Antiglobulin Test (IAT)

✓ Life habits: Substance use (Tobacco Consumption and Alcohol Consumption)

✓ Current pregnancy information

✓ ABO status for the mother and RhD status for the mother and the fetus

Laboratory data for serology (positive/negative/not done for each test) at baseline will be displayed in summary tables as well as in a by-subject listing.

The number of subjects with ADA tested at baseline and the associated result (positive/negative) will be qualitatively summarized.

The results of IAT at baseline will be qualitatively summarized (positive/negative).

Life habits will be summarized as frequency count and percentages. These data will also be listed in former and current smokers and/or in subjects with alcohol consumption. In addition, life habits will be listed for all subjects in another listing.

Current pregnancy information will be provided in a by-subject listing (first trimester (11-14 weeks) ultrasound performed (yes/no), date of the first trimester ultrasound, gestational age at the inclusion visit, type of pregnancy (single/multiple), maternal or fetal abnormality detected (yes/no), complication during the current pregnancy (yes/no), anti-RhD+ injection during the current pregnancy (yes/no)).

The ABO status and the RhD status of the mother as well as the RhD status for the fetus will be presented in a listing by subject idenfier of the mother.

11.3.2. Medical history and surgical history

Significant medical and surgical history

The MedDRA (V16.0) SOC in decreasing order (based on the overall column) and PT within SOC, for medical and surgical history will be summarized by frequency and percentages on the SAF by route of administration and overall.

Medical and surgical history data will also be listed by subject. This listing will contain the actual route of IMP administration, the verbatim text, SOC and PT associated to each individual surgical and medical history, the start date and the end date (or the on-going status) as well as the intake of a concomitant treatment if any (yes/no).

Obstetrical history

The obstetrical history will be summarized using appropriate statistics (depending on the nature of data) for the SAF by route of administration and overall:

- Previous pregnancy(ies) (Primigravidae subject: yes/no; if no, number of previous pregnancies) and delivery(ies) (number of deliveries >24 weeks of pregnancy; number of preterm deliveries (<37 weeks))

- Number of induced abortions
- Number of Spontaneous Miscarriages
- Number of live births
- Anti-RhD Injection during previous pregnancy(ies) (yes/no)
- Any complication during previous pregnancy(ies) (yes/no)
- Any complication post-partum (yes/no).

These data will also be presented in a by-subject listing. In addition, a by-subject listing will provide details on miscarriages and abortions (date, nature and reason of the event, gestation duration, type of delivery, anti-D injection (yes/no) and doses received if applicable). Another by-subject listing will provide information on complications during previous pregnancies (date of the event, complication post-event (yes/no) and details if any).

In addition, a by-subject listing will display information on newborn after previous pregnancies (date of delivery, gender, height, weight, gestation duration, mode of delivery, anesthesia (yes/no), instrumental extraction (yes/no) and details if any). Another by-subject listing will provide information on dose received for anti-D injection (anit-D injection (yes/no), dose, unit, antenatal dose, postnatal dose) and another listing will provide information on complications during previous pregnancies if any (description of the complications, start and end date).

11.3.3. Prior and concomitant therapies

Prior/Concomitant treatments will be summarized on the SAF by route of administration and overall.

Prior/Concomitant medications will be coded using WHO Drug Dictionary version 13.0. The number and percentage of subjects receiving at least one medication will be presented by generic name, ATC code (level 2 and 4).

A frequency table with number and percentage of subjects (a table for prior medications and another table for concomitants medications) with a least one medication will be provided. The results will be displayed in the alphabetic order (for the two considered levels).

Prior medication in this study refers to any medication received (within 1 month before ICF signature, 6 months before for blood transfusion (or blood derived products)) and stopped before signature of the informed consent.

Concomitant medication refers to any medication that the subject receives at any time from the signature of the informed consent to the last study visit. This includes the screening/baseline period, treatment period and follow-up period.

All prior and concomitant medications will be listed (based on coded drug names) for each subject with the associated ATC codes and will be identified as prior or as concomitant medications.

11.4. Measurement of treatment compliance

Summary statistics will be provided for treatment compliance for the SAF, by route of administration and overall.

Compliance to the administration schedule will be measured as follows:

- Number and percentage of subjects with IMP administration within the planned window of 28 or 29 weeks of gestation for the first administration (yes/no). In case of IMP administration outside the window, the number of days the administration is given outside the planned window of 28 or 29 weeks of gestation (first administration) will be quantitatively summarized
- Number and percentage of subjects with IMP administration within the planned window of delivery <72h (Yes/No). The number of hours the IMP administration given outside the planned window of delivery will be quantitatively summarized
- Number and percentage of subjects with IMP administration within the planned window of sensitizing event if any (yes/no). The number of hours the IMP administration given outside the planned window will be quantitatively summarized.
- Overall compliance will assess the number and percentage of subjects with all IMP administrations performed within the planned windows for the cases mentioned above.

All compliance data will also be displayed in a by-subject listing.

11.5. Pharmacokinetic Assessment

Pharmacokinetic assessment will be based on two PK analyses:

- Primary PK analysis will consist in a population PK analysis (PopPK analysis) that will be conducted on the analysis population PKS1 (See Section 9)
- Secondary PK analysis will be a non-compartmental PK analysis (NCA) that will be performed on the analysis populations PKS2 and PKS3 (See Section 9)

The PopPK analysis is detailed in Section 21.2. The non-compartmental PK analysis is presented below in Section 11.5.1.

11.5.1. Non-Compartmental Pharmacokinetic Analysis (NCA)

The following NCA PK parameters will be determined where possible from the serum roledumab concentrations following the first administrations using non-compartmental methods performed using Phoenix WinNonlin (Pharsight Corporation, Version 6.4 or higher):

IM Arm

Parameter	Definition
AUC_{0-t}	Area under the concentration-time curve from time 0 to time T_{last}, where T_{last} is the last time point with a measurable concentration, calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations
$AUC_{0-\infty}$	Area under the concentration-time curve extrapolated to infinity
$\%AUC_{extrap}$	Percentage of AUC that is due to extrapolation from the last measurable concentration to infinity
C_{max}	Maximum observed serum concentration
T_{max}	Time of maximum observed serum concentration
T_{last}	Time of last quantifiable serum concentration
$t_{1/2}$	Apparent terminal elimination half-life
λ_z	Apparent terminal elimination constant
CL/F	Apparent total serum clearance
V_z/F	Apparent volume of distribution during the terminal elimination phase after non-intravenous administration

IV Arm

Parameter	Definition
AUC_{0-t}	Area under the concentration-time curve from time 0 to time T_{last}, where T_{last} is the last time point with a measurable concentration, calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations
$AUC_{0-\infty}$	Area under the concentration-time curve extrapolated to infinity
$\%AUC_{extrap}$	Percentage of AUC that is due to extrapolation from the last measurable concentration to infinity
C_{max}	Maximum observed serum concentration
T_{max}	Time of maximum observed serum concentration
λ_z	Apparent terminal elimination constant
$t_{1/2}$	Apparent terminal elimination half-life
CL	Total serum clearance
V_z	Volume of distribution during the terminal elimination phase
Vss	Volume of distribution at steady state (within the body)

Additional PK parameters may be determined where appropriate.

PK analysis will be carried out using actual post-dose sampling times. C_{max} and T_{max} will be obtained directly from the serum concentration-time profiles following the first dose administration.

11.5.1. 1.Criteria for handling concentrations below the limit of quantification in NCA analysis

Concentration values that are below the limit of quantification (BLQ) will be set to zero, with defined exceptions as follows:

- Any embedded BLQ value (between 2 quantifiable concentrations) and BLQ values following the last quantifiable concentration in a profile will be set to missing for the purposes of PK analysis
- If there are late positive concentration values following 2 BLQ concentration values in the apparent terminal phase, these values will be evaluated to determine if these are plausible based on the PK characteristics of the drug. If these values are considered to be anomalous, they will be set to missing
- If an entire concentration-time profile is BLQ, the profile will be excluded from the PK analysis
- If a pre-dose concentration is missing, these values will be set to zero

11.5.1. 2.Criteria for the Calculation of an Apparent Terminal Elimination Half-Life

- At least three data points will be included in the regression analysis and should not include C_{max}
- When assessing terminal elimination phases, the R^2 adjusted value will be used as a measure of the goodness-of-fit of the data points to the determined line
- An elimination half-life will only be calculated if the R^2 adjusted value of the regression line is greater than or equal to 0.7
- Time period used for the estimation of apparent terminal elimination half-lives, where possible, will be over at least two half-lives
- Where an elimination half-life is estimated over a time period of less than two half-lives, it will be flagged in the data listings at the discretion of the pharmacokineticist, and the robustness of the value should be discussed in the study report

11.5.1. 3.Calculation of AUC

The minimum requirement for the calculation of AUC will be the inclusion of at least three consecutive serum concentrations above the LLOQ, with at least one of these concentrations following C_{max}.

- $AUC_{0-\infty}$ values where the percentage extrapolation is less than 20% will be reported.
  $AUC_{0-\infty}$ values where the percentage extrapolation is between 20 to 30% will be flagged and included in the descriptive statistics, whilst $AUC_{0-\infty}$ values where the percentage extrapolation is greater than 30% will be reported but excluded from descriptive statistics.

11.5.1. 4.Anomalous Values

If an outlier value is considered to be anomalous due to being inconsistent with the expected PK profile, it may be appropriate to exclude this point from the PK analysis. However, the exclusion of data must have strong justification and will be documented in the raw data and study report.

Embedded BLQ values may be considered anomalous depending on the route of administration and the characteristics of the drug.

11.5.2. Presentation of Pharmacokinetic Data

11.5.2. 1. Presentation of Pharmacokinetic Plasma Drug Concentration Data

The following rules will be applied if there are values that are BLQ or if there are missing values (e.g. no result [NR]) in a plasma concentration data series to be summarized.

- For the calculation of summary statistics, BLQ values will be set to zero
- If an embedded BLQ value is considered anomalous within the concentration-time profile, this value will be excluded from the summary statistics
- Where there is NR, these will be set to missing
- If there are less than three values in the data series, only the min, max and N will be presented. The other summary statistics will be denoted as not calculated (NC). BLQ is considered as a value.
- If all the values are BLQ, then the arithmetic mean, arithmetic SD, median, min and max will be presented as zero, and the geometric mean and geometric CV% will be denoted as NC
- If the value of the arithmetic mean or median is below the LLOQ, these values will be presented as zero and the geometric mean and geometric CV% will be denoted as NC.

11.5.2. 2. Presentation of NCA Parameters

- For the calculation of summary statistics of PK parameters, all NR and NC values in a data series will be set to missing
- The AUC values will be set to NC if they have been calculated using fewer than three concentrations, and/or three concentrations if the last is C_{max}
- Derived parameters will be summarized by the 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)

11.5.2. 3. Pharmacokinetic Report

A PK report will be written detailing all methodology followed during the Population PK (PopPK) process and will contain the results of both PopPK analysis (Section 21.2) and non-compartmental PK analysis (Section 21.3).

Primary and secondary PK parameters of roledumab in pregnant women will be given in tabular format. Results and interpretation of results will be discussed.

11.6. Efficacy Assessment

Efficacy analyses will be only descriptive, conducted on the FAS. The results will be displayed by route of administration and overall.

The number and the percentage of subjects with a positive and a negative anti-RhD IgG result (IAT) will be displayed by timepoints (V2/V8/V13 and possibly AV1 at 9 months post-delivery/AV2 at 12 months post-delivery and in case of sensitizing event). In addition, a by-subject listing wil display the sampling date and time as well as the corresponding study day.

In case of positive IAT, the results of the anti-RhD antibodies will be quantitatively analyzed. The number and percentage of subjects with passive anti-RhD will also be presented as well as the alloimmunization status of subjects. A by-subject listing will also be provided with the following information: visit, microtitration/ponderal dosage performed (yes/no), related day (from the first IMP administration), study day, sampling date and time, place where sampling was done, quantitative anit-RhD IgG result with the original unit, original normal ranges and investigator's assessment.

IAT results will be listed by visit. The anti-RhD quantification results will also be listed by visit.

11.7. Safety Assessment

Safety analyses will be conducted on the SAF. The results will be displayed by route of administration and overall.

Safety on the fetus/newborn will be presented by subgroups of their RhD status (positive / negative) as it may happen that child is observed as being RhD-negative at delivery. If all newborns are RhD status positive, the safety analyses will be presented on the whole SAF.

11.7.1. Extent of exposure

Subjects are planned to receive two injections of roledumab, one between at the 27th and the 29th week of gestation (depending on the version of the protocol – See Section 15) and one within 72h after delivery. If additional roledumab injection is administered (i.e. in case of sensitizing event), a description of the sensitizing event and doses received of roledumab or Rhophylac® will be provided.

The extent of exposure to roledumab will be summarized for:

- Total number of injections
- Actual cumulative roledumab dose (µg)
- Actual roledumab dose per injection (µg)

Total number of injections (including planned injections and additional injections in case of sensitizing event), actual cumulative dose and duration of roledumab exposure (in months) will be summarized using descriptive statistics. The duration of exposure will be calculated as shown in Section 16.

Actual IMP dose (per injection), administration duration in seconds, and administration site will be summarized using descriptive statistics by visit (V2, V8 and additional visit if applicable). The actual IMP dose will be calculated as shown in Section 16.

The number of subjects who received the antenatal dose, received the postnatal dose and who received extra doses of roledumab or Rhophylac® will be displayed and the doses (in mL) received will be quantitatively summarized.

A by-subject listing will display all exposure data by subject ID and visit: Related day (from first IMP administration), product injected, batch number, start date and time of injection, administration duration (in seconds), volume injected (mL), actual IMP Dose (µg), administration route, administration site, description of the sensitizing event if applicable.

The Rhophylac® doses received by the subject in case of sensitizing events will be listed by subject if any.

11.7.2. Adverse events

All adverse events (AEs) of the mother and/or the fetus/newborn/infants will be coded using the latest version of Medical Dictionary for Regulatory Activities (MedDRA) available at the beginning of the study at LFB Biotechnologies, i.e. Version 16.0.

All TEAEs (treatment-emergent adverse events), i.e. adverse events occurring between the first IMP administration and the end-of-study (EOS) visit will be described overall and by route of administration. In case the EOS visit did not take place as per protocol for any reason and in case of early withdrawal for any reason, the date of the EOS form in the e-CRF will be used to identify the end of treatment period.

A summary table will display the number of AEs as well as frequency counts and percentages of subjects with at least one AE. AEs will be divided into pre-treatment AEs, TEAEs, serious AEs, serious TEAEs and TEAEs leading to withdrawals. TEAEs will be split into severity categories (mild, moderate, severe), causal relationship with IMP (yes/no) and outcome (resolved without sequelae, resolved with sequelae, recovering, not recovered, fatal, unknown). A summary table will be provided for the mother, another one for both mother and fetus and another one for the newborn.

A summary table of all drug-related TEAEs will also be presented for the mother, another one for both mother and fetus and another one for the newborn. The number of TEAEs as well as the number and percentages of subjects with at least one drug-related TEAE will be displayed. The events will be divided into the following categories: seriousness, severity, outcome, action taken with IMP (none, dose reduced, dose increased, discontinuation of IMP, temporary interruption of IMP, not applicable), other action taken (none, procedure, withdraw from clinical trial due to AE, other), and corrective medication (yes/no).

Tables for the mother, for both mother and fetus, and for the newborn will list each TEAE, the number and percentage of subjects with at least one event by SOC and PT sorted by decreasing frequency of SOC and PT within SOC. The events will be classified in terms of severity and relationship to IMP (Related/Not related) as shown in Section 21.1.

Duration of TEAE and Time to onset of TEAE will be calculated as detailed in Section 16 and summarized using quantitative statistics. A table will be provided for the mother, another one for both mother and fetus, and another one for the newborn.

All AEs, including pre-treatment AEs (i.e. AEs occurring during the screening period and before the first injection of IMP), will be displayed in by-subject listings. For each AE, the following information will be given: who is concerned (mother/fetus/newborn), verbatim text, SOC, PT, start date and time, stop date and time or ongoing, duration in days, seriousness and seriousness criteria, severity, outcome, action taken, corrective medication, relationship with IMP. Among the AEs, the TEAEs will be clearly identified.

Serious AEs will also be listed. For each serious AE, the following information will be given: who is concerned (mother/fetus/newborn), PT, start date and time, stop date and time or ongoing, duration in days, seriousness (yes/no) and seriousness criteria, severity, outcome, action taken, corrective medication, relationship with IMP. The TEAEs will be clearly identified. AEs leading to withdrawals and deaths will also be displayed in by-subject listings containing the same information as the listing for serious AEs. A listing will also be specifically dedicated to displaying AEs

occurring in breastfed newborn with the same kind of information for each AE. In addition, the type of diet will be indicated (breast-milk/breast-milk and bottle-feeding).

11.7.3. Clinical laboratory evaluation

All laboratory data will be described using standard units.

- *Hematology*: Red blood cells (RBC), Hematocrit, Leucocytes, Neutrophils, Basophils, Eosinophils, Lymphocytes, Monocytes, Platelets, Hemoglobin, Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV), Activated Partial Thromboplastin Time (aPTT) (baseline only), Prothrombin Intl. Normalized Ratio (baseline only)
- *Biochemistry*: Creatinine, Blood Urea Nitrogen, Glucose, Total Bilirubin, ALT, AST, Alkaline Phosphatase, γGT, Elecrophoresis (Total proteins, Albumin, Alpha 1, Alpha 2, Beta 1, Beta 2, Gamma, Creatinine Clearance, Protein Peak)
- *Urinalysis*: Dipstick (Glucose, Nitrite, Occult Blood, Protein) and microscopy in case of positive dipstick (Erythrocytes, Leukocytes, Epithelial Cells, bacteria, casts, crystals)
- *Cytokines*: IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13 and TNF-α
- C-Reactive Protein (CRP)
- KB test
- *Laboratory parameters in cord blood*: DAT, hematocrit, hemoglobin, reticulocytes and total bilirubin performed in cord blood in order to detect fetal anemia

Normal ranges for SI units will be presented by subject in a listing for the parameters of hematology, biochemistry and urnialysis.

Abnormal laboratory values will be displayed in by-subject tables for hematology and biochemistry, including the subject ID, the actual route of administration, the age, the visit, the related day (from the first IMP administration), the sampling date and time, the place where sampling was done, the results in original unit and in SI unit, the original normal ranges, the measured value for each parameter at each visit and the absolute change from baseline in SI unit to each post-baseline time point for continuous parameters. In addition, each abnormal value will be qualified as abnormal without clinical significance or abnormal with clinical significance. For the biochemistry, the parameters included in the table will be the following ones: creatinine, blood urea nitrogen, glucose, total bilirubin, ALT, AST, alkaline phosphatase, γGT and electrophoresis (only an overall assessement of the electrophoresis).

Measured values and changes from baseline (for continuous parameters) will be quantitatively described for each parameter (except for protein peak, normal/abnormal) for each visit (including additional visits) in summary tables for hematology, biochemistry and for CRP.

The investigator's clinical assessment (normal, abnormal without clinical significance, abnormal with clinical significance, not reliable, not done) by visit will be displayed in a separate table. For the biochemistry, the parameters included in the table will be the following ones: creatinine, blood urea nitrogen, glucose, total bilirubin, ALT, AST, alkaline phosphatase, γGT and electrophoresis (only an overall assessement of the electrophoresis).

Shift tables for hematology and biochemistry will display for each parameter the number and the percentage of subjects with low, normal or high level over time with respect to baseline.

All laboratory data for hematology, biochemistry and CRP will be presented in by-subject listings containing the following information: subject ID, actual route of administration, age, visit, related day (from the first IMP administration), sampling date and time, the place where sampling was done, results in original unit and in SI unit, the orignal normal ranges, the measured value for each parameter at each visit and the absolute change from baseline in SI unit to each post-baseline time point for continuous parameters and the clinical assessment.

The parameters associated to cytokines will be displayed in a summary table by visit. For each parameter and each visit, the number and the percentage of subjects BLQ will be presented. In addition, boxplots for each parameter of cytokines by visit will be displayed by route of administration. In addition, a by-subject and by-time point listing will describe each parameter based on central laboratory with the following information: theoretical time, date and time of sampling, original result and unit, specimen type and method of analysis.

The results of KB test (positive/negative) will be presented in a summary table: at delivery before the IMP administration for all subjects and at delivery within 24 hours after the IMP administration in case the result obtained prior to the IMP administration was positive; after a sensitizing event, before the IMP administration and within 24h after the IMP administration only if the result of the first test was positive.

A by-subject listing will also be provided with the following variables: the route of administration, the age, the visit, the related day (from the first IMP administration), the sampling date and time, the KB test result as well as the FMH volume.

Results of urinalysis tests using dipstick will be displayed by visit (V1 and V13) in a summary table. The investigator's clinical assessment (normal, abnormal without clinical significance, abnormal with clinical significance, not reliable, not done) for each parameter and by visit will also be displayed in another table. These tables will also be presented for urinalysis by microscopy examination in case of postivie dipstick. A dipstick test was considered as positive when at least one of the four tested parameters was positive.

A by-subject listing for urinalysis (dipstick) will also be provided by subject ID, route of administration and visit, with the following information: age of the subject, study day, related day (from the first IMP administration), sampling date, result for each parameter and clinical assessment. The same listing will be provided for microscopy test in case of positive dispstick.

Laboratory parameters in cord blood

The investigator's clinical assessment for each parameter in cord blood (normal, abnormal without clinical significance, abnormal with clinical significance) will be displayed in a summary table by parameter.

In addition, a listing by route of administration and by subject will display the presence or not of an abnormality (yes/no) and the investigator's assessment (normal, abnormal without clinical significance, abnormal with clinical significance) for hemoglobin, hematocrit, reticulocytes, total bilirubin in cord blood as well as the associated measured value and lower and upper ranges. The results for DAT (anti-RhD IgG results, positive/negative/not done) will be displayed in a separate listing.

Another listing by-subject listing will detail for each parameter of cord blood the date and time of delivery, the date and time of sampling, where sampling was done, the name of parameters, the associated measured values in original and standard unit for hemoglobin, hematocrit, reticulocytes, total bilirubin well as the investigator's assessment. The results for DAT will be presented in separate listing with the anti-RhD IgG results (positive/negative) and the investigator's assessment.

11.7.4. Vital Signs

Vital signs parameters include: systolic and diastolic blood pressure, pulse rate, body temperature and weight.

These parmaters in addition to height and BMI will be described by time point in by-subject listings.

Measured values and changes from baseline for each parameter will be quantitatively described at each visit (including additional visits) in a summary table. In order to determine the changes from baseline, two baseline values will be considered: baseline 1 will refer to the last value prior to the first IMP administration (antenatal period) and baseline 2 will refer to the last value prior to the IMP administration after delivery (postnatal period). Within the antenatal (respectively postnatal) period, the change from baseline will be evaluated with respect to baseline 1 (respectively baseline 2).

Vital signs will also be analyzed and presented by time point graphically in boxplots, except for weight.

11.7.5. Delivery

Information pertaining to delivery and any associated complication will be described in a by-subject listing containing the following variables: subject ID, actual route of IMP administration, date and time of delivery, gestational age at birth (in weeks), duration of labor (in hours), anasthesia used (yes/no), mode of delivery, instrumental delivery (yes/no), any complication during delivery (yes/no), comment on delivery if any.

11.7.6. Characteristics and general status of the newborn

Information related to the newborn at birth will be described un a by-subject listing containing the following variables: subject id, date and time of birth, gestational age at birth (in weeks), gender of the newborn, Apgar score measured once at 1 minute after birth, and again at 5 minutes after birth.

General status of newborn will also be described in a by-subject listing with the following information: subject id, actual route of IMP administration, visit (V8, V9, V12), weight (kg) and height (cm) of the newborn, type of diet, first/breast milk sample (yes/no), any clinical significant abnormality in the newborn (yes/no), new concomitant treatment given to the newborn (yes/no), comment if any.

In addition, the ABO status and the RhD status of the newborn will also be described in a by-subject listing.

11.7.7. Other observations related to safety

11.7.7. 1.Anti-roledumab antibodies (immunogenicity)

Immunogenicity of roledumab (ADA) will be summarized using descriptive statistics by visit (screening, V2, V5, V6, V8, V11, V12, V13 and additional visits if applicable). A listing will

display results of ADA test at each visit for subjects with at least one positive ADA test during the course of the study.

In addition, a by-subject listing will show the ADA test resuls for all subjects by visit.

In case of retest, the last observed value will be used for the analysis.

11.7.7. 2.Obstetric and fetal Doppler ultrasound

Obstetric and fetal Doppler ultrasound results will be displayed in by-subject listings including the following variables: actual route of administration, visit (V2, V5, V6, V7 and additional visits if any), date of ultrasound examination, any abnormality detected (yes/no), normal fetal cardiac activity (yes/no), fetal hydrops detected (yes/no), fetal hydrops assessment, sign of polyhydro-amnios (yes/no), diameter of the largest pocket (cm), MCA-PSV (cm/s), Multiple of Medians (MoM), calculated MoM, MoM >1.5 (yes/no), Clinical assessment (by the investigator on MoM).

11.7.7. 3.Physical examination

A complete physical examination of the mother will be performed at the screening visit and includes the following assessments: height, weight, general appearance, skin, head, eye, Ear-Nose-Throat (ENT), lymph nodes, heart, lungs, abdomen, extremities/joints, neurological and mental status. A partial physical examination is performed at V2, V8, end of study visit and additional visits and includes weight, general appearance, skin, heart, lungs and abdomen.

A listing by patient and by visit will display when a physical examination was performed and if a clinically significant abnormalit was detected since the signature of ICF. Clinically significant physical examination abnormalities observed at screening will be reported as medical history and those at other visits will be reported as adverse events.

11.7.7. 4.Roledumab concentration in milk and cord blood

The concentrations of roledumab in first milk/breast milk and in maternal serum after postnatal administration as well as the ratio between these concentrations in milk and in blood will be presented using quantitative statistics at each visit (V10, V11) and a by-subject listing. This listing will display the following variables: actual route of administration, date and time of sampling, roledumab concententration in the milk (ng/mL), method of analysis, maternal concententration at V8 (ng/mL), value of the ratio between milk and maternal serum at V8.

The concentrations of roledumab in the cord blood and in the maternal serum as well as the ratio between these two concentrations will be presented using quantitative statistics and in a by-subject listing. This listing will display the following variables: actual route of administration, date and time of sampling, roledumab concententration in the cord blood (ng/mL), method of analysis, maternal concententration at V8 (ng/mL), value of the ratio between cord blood and maternal serum at V8.

12. STATISTICAL/ANALYTICAL ISSUES

12.1. Handling of Dropouts and Missing Data

12.1.1. Dropouts

No general/specific rules will be formed to handle missing data except for PK purposes. All missing data will be treated as missing. Every effort will be made to collect data on all study parameters even in case of dropout (up to EOS visit).

12.1.2. Missing data

In general, missing data will not be imputed. Exceptions will be made for data reported as ">" or "<" x; for quantitative statistics, these indeterminate values will be replaced by the x value itself.

If either a sampling time or its concentration is missing, then that sample data point will be excluded from PK sets as described in the CPAP.

12.1.3. Missing or incomplete dates

In general, missing data for dates will not be imputed except for concomitant medications or AEs for which they will be imputed to the earliest possible start date or the latest possible end date. If the first possible earliest start date (e.g. if only the year is available) is before the first infusion date then to be on a conservative side the start date of the AE will be imputed as the first infusion date.

12.1.4. Randomization

Not applicable.

12.1.5. Data safety monitoring board

An external independent 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. The reporting made for the DSMB is not discussed in the current SAP.

12.1.6. Blind Review

Not applicable as it is an open-label study.

12.1.7. Maintaining the blind

Not applicable as it is an open-label study.

12.1.8. Pooling of Centers

For the analysis, centers will be pooled by route of administration. The center will not be taken into account in the statistical description.

12.1.9. Multiplicity issues

No multiple testing will be performed in this study.

12.1.10. Subgroup analysis

No subgroup analysis is planned for the efficacy and safety. However all available data will be presented as follows:

- IM arm
- IV arm
- Total (if appropriate)

12.1.11. Other issues

12.1.11. 1. Significance testing and estimation

For the efficacy and safety, no formal statistical hypothesis testing will be performed.

12.1.11. 2. Experimental units

For this study the experimental unit is the mother and/or the newborn depending on examination.

13. INTERIM ANALYSES

No interim analysis will be performed. However and as the primary PK endpoint will be available before the last subject withdraws from study- the final analysis of the primary PK endpoint will be performed before the final DBL.

Consequently, all PK-related data will be frozen and this freezing will be documented.

14. SPECIFIC ANALYSIS RULES

For descriptive statistics, the following number of decimal places (dps) will generally be applied: arithmetic mean, SD, Q1, median and Q3 to 1 more dps than the raw data; minimum and maximum to the same number of dps as the raw data. Percentages should be presented to 1 dp.

The information and explanatory notes to be provided in the "footer" or bottom of each table will include the following information: date and time of output generation, SAS program name, any other output specific details that require further elaboration. All abbreviations used in the table will be defined.

After DBL if there is no change in the SDTM datasets used for the PK analysis (NCA and PopPK), the PK analysis done on freeze data will be considered as final.

15. VISIT AND TIME WINDOWS

Visit	Time windows
V1	> or = signature date of ICF and in the range of 12 to 27 weeks of GA (applicable from Protocol V8.0) > or = signature date of ICF and in the range of 12 to 26 weeks of GA (applicable from protocol V5.0 to protocol V7.0)
V2	> date of V1 and in the range of 28 to 29 weeks of GA
V2b	24h ± 2h after time of IMP injection IV arm only (from protocol V8.0)
V3	For IV: 48h ± 6h after time of IMP injection/ For IM: 48h ± 24h (applicable from Protocol V8.0) 2 days ± 1 day after time of IMP injection (applicable from protocol V5.0 to protocol V7.0)
V4	For IV: 96h to 120h after time of IMP injection/ For IM: 120h ± 24h (applicable from Protocol V8.0) 5 days ± 1 day after time of IMP injection (applicable from protocol V5.0 to protocol V7.0)
V5	9 days ± 3 days after time of IMP injection
V6	29 days ± 4 days after time of IMP injection
V7	59 days ± 4 days after time of IMP injection
SE	> date of V2 and within 72h after start of SE
SE-FU	24h after time of IMP injection (no time window)
V8	Within 72h after time of newborn birth
V9	48h ± 24h after date of newborn birth (applicable from Protocol V8.0) 2 days ± 1 days after date of newborn (applicable from protocol V5.0 to protocol V7.0)
V10	72h ± 24h after time of 1st Postnatal IMP injection (applicable from Protocol V8.0) 3 days ± 1 day after time of 1st Postnatal IMP injection (applicable from protocol V5.0 to protocol V7.0)
V11	9 days ± 2 days after day of 1st Postnatal IMP injection
V12	6 weeks ± 2 weeks after day of 1st Postnatal IMP injection
V13	6 months + 2 weeks after day of 1st Postnatal IMP injection
AV1	9 months ± 2 weeks after day of 1st Postnatal IMP injection
AV2	12 months ± 2 weeks after day of 1st Postnatal IMP injection

Timepoint	Time window
Measurements at screening V1	> or = date of ICF
H0 or Before start of injection	Before time of IMP administration
T 30 after IMP administration	30 min ± 5 min after end of injection (= time of IMP administration + 1 min)
T H1	1 hour ± 5 min after end of injection
T H6	6 hours ± 15 min after end of injection
After the end of IMP administration	> time of IMP administration + 1 min

IMP injection	Time window
V2	In the range of 28 to 29 weeks of GA (applicable from Protocol v6.0)
	In the range of 27 to 29 weeks of GA (applicable before Protocol v6.0)
SE: 1st injection	Within 72h after start of SE and > date of V2
SE-FU	24h after time of IMP injection (no time window)
V8	Within 72h after time of newborn birth

16. DEFINITION OF DERIVED VARIABLES

- *Body Mass Index (BMI, kg/m^2)*, will be derived as follows:
  BMI = (Weight / Height^2)
  with weight in kg and height in m
- *Actual IMP dose (µg)* = volume injected (mL) x 300 (concentration of 300 µg/mL)
- *Duration of IMP exposure (in months)* = (Date of last injection - Date of the first injection +1)/30.4375
- *Follow-up after first IMP injection (in months)* = (Date end of study assessment - Date of the first day of the IMP administration +1)/30.4375
- *Duration in the study (in months)* = (Date of end of study assessment - Date of the informed consent +1)/30.4375
- *Duration of an AE in days* = (AE end datetime - AE start datetime) +1 if duration >24h and /24 if duration ≤24h
- *Time to onset of an AE*=(AE start datetime after the last administration of IMP - Datetime of the last administration of IMP) in days (xx days) if duration >24h and /24 if duration ≤24h
- *Calculated MoM*
  MoMc= Observed MCA-PSV / Expected_MCA-PSV
  Where Expected_MCA-PSV = $e^{2\ 30921\ +\ 0\ 0463954*GA}$
  with GA=gestational age in weeks calculated as follows:
  GA=Integer ((datepart(Visit date)- datepart(Pregnancy date) +14 days)/7 days).
- The creatinine clearance (CRCL) will be calculated with the Cockcroft-Gault formula [4]
  – For female:

$$\text{Creatinine clearance (mL/min)} = \frac{(140 - \text{age}) \times \text{body weight (kg)}}{72 \times \text{creatinine (mg/dL)}} \times 0.85$$

- Ratio of roledumab in first milk/breast milk concentration to maternal serum concentration= Measurement of LFB-R593 in first milk/breast milk (ng/mL) at Vxx** / measurement of LFB-R593 in maternal serum (concentration at Vxx visit from PK sample*).

  *: sample coming from central measurement (Envigo)

  **: sample coming from central measurement (SGS)

  Vxx: measurement will be performed twice (at V10 and V11)

17. LABORATORY STANDARDIZATION (FOR LOCAL LABS)

No standardization of local labs will be done.

18. CHANGES FROM THE PROTOCOL SPECIFIED STATISTICAL ANALYSES

Not applicable.

18.1. Definition of analysis populations (Section 9)

In order to adopt a more standard definition, an enrolled subject will be a subject who signed the ICF and who was not screening failure.

The ITT population is then defined as follows: all subjects who were enrolled in the study (ICF signed and not screening failures) and/or who did not complete the EOS form before the inclusion visit (V2).

18.2. Primary endpoints (Section 10.1)

The population based models in previous Phases I and IIa, and current Phase IIb showed to be a two-compartment model instead of one-compartment model. Therefore, there should be two additional parameters Q and V3 due to the second compartment in two-compartment model, compared with only CL and V2 in one-compartment model.

In addition, only the derived parameter $t_{1/2}$ will be calculated. The parameters C_{max}, T_{max}, $AUC_{0-\infty}$ will be reported in secondary PK analysis (NCA) instead of population PK analysis.

18.3. Other observations related to safety (Section 11.1)

Except if stated in analyses sections above, standard error (Protocol §12.7.1) [1] will not be reported in descriptive tables.

18.4. Other observations related to safety (Section 11.7.5)

Physical examination findings: no specific listing will be provided for clinically significant abnormalities as they are reported either in medical history or adverse events and so will be analyzed in those sections.

19. DATA PRESENTATION

Some of the TFLs may not have sufficient numbers of subjects or data for presentation. If this occurs, the blank TFL shell will be presented with a message printed in the center of the table, such as, “NO DATA TO DISPLAY”.

20. REFERENCES

1 Clinical Study Protocol - ADNC-1301 (phase 2b) EUDRACT number 2013-000269-35 Pharmacokinetics and Safety of roledumab, a fully human recombinant monoclonal anti-RhD antibody in RhD-negative pregnant women carrying an RhD-positive fetus: a phase 2b, multicenter, open-label study.

2 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Statistical Principles for Clinical Trials (E9), 5 February 1998.

3 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Structure and Content of Clinical Study Reports (E3), 30 November 1995.

4 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16(1):31-41.

21. APPENDICES

21.1. Appendix 1 - Tables, figures and listings

21.1.1. Table of contents - Section 14 of CSR

TABLES, FIGURES AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT

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14.3.6.5 Listing: Description of sensitizing events

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14.3.6.7 Listing: Subjects with at least one positive ADA test result by visit

14.3.6.8 Table: Summary of roledumab concentration in cord blood

14.3.6.9 Table: Summary of concentration in first/breast milk and maternal serum concentration

14.3.6.10.1 Table: Exposure: summary of total number of injections and time between first and last administrations

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Listing 16.2.10.3: ABO status and RhD status of the newborn

21.1.3. Table shells

Tables will be presented using the following shell as an example:

Table x.x <title of the table>
<Population>

Characteristic	Statistics	Roledumab 300 µg IM	Roledumab 300 µg IV	Roledumab 300 µg Overall
Number of subjects				
<Numeric variable, unit>	N	xx	xx	xx
	Mean (SD)	xx.x (x.x)	xx.x (x.x)	xx.x (x.x)
	Median	xx.x	xx.x	xx.x
	Min - Max	xx - xx	xx - xx	xx - xx
	Missing	x	x	x
	Q1 - Q3	xx - xx	xx - xx	xx - xx
<Categorical variable>				
Category 1 …	n (%)	x (xx.x)	x (xx.x)	x (xx.x)
Category 2	n (%)	x (xx.x)	x (xx.x)	x (xx.x)
Category 3	n (%)	x (xx.x)	x (xx.x)	x (xx.x)
…	n (%)	x (xx.x)	x (xx.x)	x (xx.x)

Table x.x Summary of Adverse Events – Mothers
<Population>

Characteristic	Roledumab 300 µg IM		Roledumab 300 µg IV		Roledumab 300 µg Overall	
	E	**N (%)**	**E**	**N (%)**	**E**	**N (%)**
Number of subjects		xx		xx		xx
Any AE	xx	xx (xx.x%)	xx	xx (xx.x%)	xx	xx (xx.x%)
Any Pre-Treatment AE	xx	xx (xx.x%)	xx	xx (xx.x%)	xx	xx (xx.x%)
Any Serious AEs	xx	xx (xx.x%)	xx	xx (xx.x%)	xx	xx (xx.x%)
Any TEAEs	xx	xx (xx.x%)	xx	xx (xx.x%)	xx	xx (xx.x%)
Any Serious TEAEs	xx	xx (xx.x%)	xx	xx (xx.x%)	xx	xx (xx.x%)
Any TEAE	xx	xx (xx.x%)	xx	xx (xx.x%)	xx	xx (xx.x%)
Severity						
Mild	xx	xx (xx.x%)	xx	xx (xx.x%)	xx	xx (xx.x%)
Moderate	xx	xx (xx.x%)	xx	xx (xx.x%)	xx	xx (xx.x%)
Severe	xx	xx (xx.x%)	xx	xx (xx.x%)	xx	xx (xx.x%)
Causal relationship with IMP						
Yes	xx	xx (xx.x%)	xx	xx (xx.x%)	xx	xx (xx.x%)
No	xx	xx (xx.x%)	xx	xx (xx.x%)	xx	xx (xx.x%)

N: Number of subjects, E: Number of events

Study: ADNC-1301, Program: xxx.sas/ddmmmyyyy:hh:mm:ss

Same Table for Both Mother & Fetus and Fetus/Newborn

Table x.x Number of subjects with TEAE by intensity and relationship – Mothers
<Population>

Roledumab 300 µg IM (N*=)

SOC/PT	Mild						Moderate						Severe						Missing						Total					
	R		NR		Missing		R		NR		Missing		R		NR		Missing		R		NR		Missing		R		NR		Missing	
	E	N(%)	E	N(%)	E	N(%)	E	N(%)	E	N(%)	E	N(%)	E	N(%)	E	N(%)	E	N(%)	E	N(%)	E	N(%)	E	N(%)	E	N(%)	E	N(%)	E	N(%)
Any TEAE	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)
SOC 1	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)
Preferred term 1	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)
Preferred term 2	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)
SOC 2	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)
Preferred term 1	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)
Preferred term 2	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)	xx	xx(%)

Each AE is counted only once for each category
**: used as denominator for the calculation of percentages*
NR: Not Related, R: Related
SOC: System Organ Class, PT: Preferred Term, MedDRA (V16.0)
N: Number of subjects, E: Number of events

Study: ADNC-1301, Program: xxx.sas/ddmmmyyyy:hh mm:ss

Same Table for Both IV and Overall (IM + IV)

Listings shells

All individual data collected and derived will be listed in section 16.2 (to be in line with the ICH E3 CSR guideline).

Listings will be presented using the following shell as an example:

Listing x.x.x <title>
<Population>
Page x/y

Subject Identifier	Group	Class1	Var 1	Var 2	Var 3	Var 4	Var 5	Var 6	…
xxxx	IM/IV	Yes/No	xxxxx	xxxx	xxxxx	xxxx	xxxx	xxxx	…

21.2. Appendix 2 - Population analysis plan

BACKGROUND OF POPULATION PK ANALYSIS

The PK data for roledumab are available from previous two clinical trials. The PopPK analyses were conducted to evaluate PK and absolute bioavailability of roledumab in healthy RhD-negative subjects after IM and IV administration in Phase 1 study (ADNC-0701), and to identify the IV and IM effective dose of roledumab required to effectively clear 15 mL of RhD-positive RBCs pre-injected to healthy RhD-negative subjects in Phase 2 study (ADNC-0726). A two-compartment disposition model was used as with bolus input for the IV dose and first-order absorption for the IM dose with first-order elimination.

In the Phase 1 clinical trial ADNC-0701 on healthy RhD-negative subjects, the PK of roledumab was nonlinear at doses ranging from 30 to 3000 µg. Exposures (C_{max} and AUCs) increased more than proportionally with the dose. Clearance (CL) and volume decreased with the dose. At 300 µg, the typical values for central volume (Vc) and peripheral volume were and 3.66 L and 3.26 L with between-subjects variability of 35% and 13%, respectively; the typical value for CL at was 0.0113 L/h with between-subjects variability of 28%; the absorption rate constant (Ka) after IM was 0.00894 h^{-1} with large between-subjects variability of 78%; the elimination half-life after IV and IM was similar and estimated to be 17.9 days.

In the Phase 2 dose-finding study ADNC-0726, the dataset was enriched using 17 healthy RhD-negative subjects treated with IV or IM roledumab at doses from 100 to 300 µg from the Phase 1 study ADNC-0701 to reduce standard errors for all fixed and random effects parameters due to the limited time span for blood samples collection in study ADNC-0726. The PK of roledumab was nonlinear at doses ranging from 100 to 300 µg. The result showed that clearance and volumes of distribution or roledumab increased with subjects' body weight. The typical weight is 79 kg. The typical values for central volume (Vc) and peripheral volume at 300 µg were and 2.94 L and 3.10 L respectively, with between subjects variability of 24.9%. The typical value for clearance (CL) at 300 µg was 0.0113 L/h with between subjects variability 54.6%. The absorption rate constant (Ka) after 300 µg IM was 0.00890 h^{-1} with between subjects variability 80.6%. The elimination half-lives after IV and IM were 15.4 and 20.1 days.

These results confirmed the comparability of the PK parameters observed in the Phases 1 and 2. Although a difference in bioavailability between IM and IV exists (76% vs. a presumed 100%, respectively at dose of 300 µg), the exposure after 18 days was similar between the two routes of administration, indicating that the same dose of 300 µg IM and IV in this Phase 2b clinical study will lead to the same longer term protection against alloimmunization.

This Population Analysis Plan (PAP) contains a detailed description of the methodology and procedures that will be used in the planned Population Pharmacokinetic (PopPK) analysis using data from ADNC-1301 a phase 2b, multicenter, open-label study to evaluate PK and safety of roledumab, a fully human recombinant monoclonal anti-RhD antibody, in Rh-D negative pregnant women carrying an RhD-positive fetus.

The specific activity includes development and evaluation of a population PK model (PopPK). The objective of this PopPK modeling and simulation analysis is to obtain a quantitative understanding of the PK profile of roledumab 300 µg IV or IM in RhD-negative pregnant women carrying an RhD-

positive fetus in order to confirm the suitability of this dose to ensure adequate anti-D level coverage up to delivery.

This plan may be updated to reflect changes in the planned analysis which may arise due to unexpected issues during the study execution provided that the changes occur prior to database lock. Since PopPK analysis is fundamentally exploratory in nature, revisions may be data driven and based on results obtained during the course of the PopPK analysis; all revisions will be documented in the PopPK report.

OBJECTIVES OF POPULATION PK ANALYSIS

The objectives of PopPK analysis are:

- To develop and evaluate the PopPK model for roledumab in RhD-negative pregnant women carrying an RhD-positive fetus
- To confirm the suitability of dose for adequate anti-D level coverage up to delivery
- To quantify the variability in the PK of roledumab
- To identify significant covariate(s) in the PK of roledumab

SOURCE DATA AND STUDY DESIGN FOR POPULATION PK ANALYSIS

Plasma concentration data of roledumab from Phase 2b study ADNC-1301 will be used in this PopPK analysis.

Study No. 1 (Phase 2b)

This is a phase 2b, interventional, multicenter, open-label study of roledumab with IV and IM arms in RhD-negative pregnant women carrying an RhD-positive fetus.

It was planned that the first 35 subjects would be administered a first dose of roledumab 300 µg IM during the second visit (V2) at 28 or 29 weeks (gestational age) and a second dose of 300 µg IM within 72h of birth during V8. Additional administration according to Kleihauer-Betke chart should be applied with 72 hours of procedure in case of sensitizing event in the antenantal period. The following 25 subjects were allocated to the IV arm with same dosage regimen. More than one site took part in the study in order to include 35 subjects for 30 evaluable subjects in the IM arm and 25 for 20 evaluable subjects in the IV arm. The subjects should attend 13 visits for IM arm and 14 visits for IV arm, and further 1 or 2 follow-up visits if needed to document alloimmunization in both arms. A total of 10 blood samples in IM arm and 11 blood samples in IV arm were collected for PopPK analysis at the following time points:

- **IM arm**: Antenatal period: pre-dose (V2), 2±1 days (V3), 5±1 days (V4), 9±3 days (V5), 29±4 days (V6), and 59±4 days (V7)

  Postnatal period: pre-dose (V8, within 72 hours after delivery), 3±1 days (V10), 9±2 days (V11), and 6±2 weeks (V12)

- **IV arm**: Antenatal period: pre-dose (V2), 1 hour±5 min (V2), 24 hours±2 min (V2b), 48±6 hours (V3), 96-120 hours (V4), 29±4 days (V6), and 59±4 days (V7)

  Postnatal period: pre-dose (V8, within 72 hours after delivery), 3±1 days (V10), 9±2 days (V11), and 6±2 weeks (V12)

Roledumab first milk, breast milk, maternal serum concentration, and cord blood concentration for assessment of safety of the mother and the fetus/newborn were collected.

ANALYSIS POPULATION FOR POPPK ANALYSIS

PKS1: Analysis population 1 for popPK modeling

The PKS1 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 available concentration after administration (including antenatal and postnatal measurements after 1st or 2nd administration).

So all enrolled subjects who have received at least one dose of roledumab and have the following information entered will be included in PKS1:

- At least one measurable concentration with adequate information on the dose amount administered
- The date/time of drug administration, and
- The date/time of sample collection

DATA TRANSFER AND HANDLING

Dataset Preparation

Dataset preparation for PopPK analysis will be performed by Covance following the database lock. Concentrations, sampling times, dosing times, demographic and other covariate information supplied as SAS data transport files will be used to prepare PopPK analysis dataset. A SAS program (SAS version 9.3 or higher) will be used to assemble the analysis dataset from source data to be compatible with NONMEM translator (NM-TRAN). The analysis dataset will also be formatted for PDx-Pop (Version 5.2) and then saved as a comma delimited ASCII text file (*.csv file). Diagnostics will be performed to check for any gross errors during the compilation of the datasets.

Missing PK Data

If the date and/or time records of dosing are found to be missing at a specified scheduled visit, the drug level concentrations related to that time point will be excluded from analysis and an appropriate explanatory comment will be provided.

Drug level concentrations related to missing sample collection date and/or time records will be commented upon and excluded from the analysis. Missing drug concentrations will be excluded from the analysis.

Missing Covariate Data

For missing covariate values, the following methods may be used:

- Use baseline (last non-missing value prior to study drug administration) covariate value for all dosing and observation records for non-time varying covariates.
- Impute a value for a non-time varying missing covariate using
  - Median value of continuous covariate (i.e. body weight)
  - Mode of categorical covariate (i.e. route of delivery)
  - Stratification by study/treatment group for imputations will be considered
- Interpolation between time points may be considered for time varying covariates

BLQ Handling

No imputation will be performed for the concentrations that are missing or below the assay's quantification limit (BLQ). BLQ data at pre-dose will be set to zero. BLQ values at early time points, when appreciable absorption (IM) does not have occurred, will be set to zero. The BLQ data at the end of the PK profile will be set to missing. The one BLQ value occurring between two adjacent quantifiable values will be excluded from the analysis (treated as missing). When two consecutive BLQ values are encountered, all subsequent quantifiable values will be excluded from the analysis.

Outliers

Prior to any modeling, individual concentration-time profiles will be examined for any potential PK outliers (data points discordant from the bulk of the data within the same time interval) by visual inspection. Anomalous or extreme PK values (i.e. positive concentrations prior to the first dose, or concentrations that is outside of mean ±3 SD at each timepoint for rest of subjects via the same route) that have a disproportionate effect on parameter estimates will be explained with a comment and excluded from the analysis. All data exclusions will be documented in the analysis report.

Deleted Data

Drug concentration measurements from the samples that are documented to be processed, stored or shipped under inadequate conditions will not be included in the PopPK analysis.

Data Items Included in Datasets

The NM-TRAN dataset will include data items such as study number, subject identification, actual treatment, actual dose amount, actual dosing day/time, actual sampling day/time, plasma concentrations of study drug and baseline demographic, and creatinine clearance. The covariates listed in the following table (Table 4) may be tested in the PopPK analysis, depending on availability of data.

Table 4: Potential Covariates to be evaluated in the PopPK Analysis

Abbreviation	Covariate	Code/unit
AGE	Age	years
GA	Gestational age	weeks
SOJ	Site of injection	IV=0, IM=1

WT	Body weight	kg
CRCL	Creatinine clearance	mL/min if CRCL >150, CRCL=150
DLV	Route of delivery	Natural=0, C-section=1

Partially missing continuous covariates will be entered using the last observation carried forward (LOCF) approach and completely missing continuous covariates will be replaced with the population median values. In the case of missing weight, specific median values will be used.
Categorical covariates (e.g. route of delivery) missing for a subject will be coded as –6.

Software

The NONMEM software program (version 7.3 or higher, ICON Development Solutions, MD, USA) will be used for model fitting. NONMEM VI may be interfaced with PDxPop Version 3.0 Tools to allow for Expediting Population Analysis (ICON Development Solutions, MD, USA). The package is installed on a PC platform using Inter FORTRAN Standard Edition –Version 9 under Microsoft Windows 7.

Dataset preparation, exploration and visualization will be performed using SAS and S-PLUS version 8.2 Professional release 3 for Windows (Insightful, Seattle, WA, USA). The S-PLUS version 7.0 package, SAS, or R version 3.0.2 or higher may also be used for the statistical analysis and graphical display of all NONMEM output.

PHARMACOKINETIC ANALYSES

The popPK analysis will be performed using the non-linear mixed effects modeling approach. This approach estimates the typical (mean) value of parameters as well as associated inter-individual variances.

Estimation methods in NONMEM will be first-order conditional (FOCE) and first-order conditional with interaction (FOCEI). If supported by the data, in case of prohibitive computer intensive runs with FOCE, the first order (FO) estimation method may be used during model development. In such case, critical modeling steps will be reassessed using FOCE (with or without interaction). Alternative estimation methods implemented in NONMEM VII may be used, if needed.
Stability of NONMEM models will be assessed on the basis of:

- Acceptable goodness-of-fit via basic plots
- Number of significant digits >=3 for all Thetas
- Estimates of Theta's not close to boundary
- Condition number < 1000
- Correlation less than 0.95 between any parameters
- Stable parameter estimates with altering initial values to ensure global minimum being achieved

Model selection will be based on:

- The comparison of full vs. reduced models based on the Log-Likelihood Criterion: the difference in the minimum value of the objective function (MOF)
- Decrease in unexplained variability
- Change towards conditional weighted residuals over time randomly distributed around zero
- Scientific plausibility of the model

Exploratory Data Analysis

Prior to the PopPK analysis, the following pre-modeling analyses will be produced as part of the exploratory data analyses:

- Graphical presentation of the concentration-time data to examine the basic model structure and facilitate outlier identification. Pooled plots of study drug concentrations vs. elapsed time (all subjects in one panel) for each treatment will be presented in the linear and semi-logarithmic scale.
- Examination of covariate correlations which may help in building a covariate sub model and avoid confounds. For quantitative covariates, histograms of density versus residuals, box-whisker plots and plots of quartiles of covariate distribution versus quartiles of standard normal distribution (Quantile-Quantile plot) may be used in exploratory analysis. Moreover, scatter plots will be evaluated for potential correlations between quantitative covariate and box-whisker plots will be evaluated for every quantitative covariate against every categorical covariate.
- Descriptive statistics or noncompartmental analyses

  Depending on the nature of the study data, further exploratory analyses may be conducted. These will be fully described in the report.

Population Pharmacokinetic Model Development

- **Model Selection Criteria**

The following criteria will be evaluated to aid model selection:

- A significant reduction in the minimum values of the objective function (MOF) from the base model using the extended least squares method, equivalent to -2 times the maximum log of the likelihood of the data (-2LL). The log likelihood ratio (LLR) test will be used to compare between base and covariate model. This provides an overall summary of how closely the model predictions match the data. The Akaike information Criterion (AIC) may also be used to evaluate and rank models based on goodness-of-fit
- Goodness-of-fit measures including:

A uniform distribution (i.e. unbiased in the predictions) across the identity line in the diagnostic plots of observed vs. predicted concentration values: including both IPRED (individual predicted) vs. DV (observed), and PRED vs. DV (observed)

A more random (less systematic) distribution in the diagnostic plots of both residual (RES) and conditional weighted residuals (CWRES) against predicted concentration (PRED) and time after dosing to assess unaccounted heterogeneity in the data

A decrease in the inter-individual variability (IIV) of PK parameters

A decrease in the residual error

A decrease in the standard error estimate for model parameters

- **Base Model Development**

Structural Pharmacokinetic Model

Standard structural PK models will be explored, starting with first order absorption and elimination rate will be explored and compared to each other. Goodness-of-fit of the structural model will be assessed by diagnostic plots:

- Observed vs. population and individual predictions plots
- Population and individual conditional weighted residuals (CWRES) vs. time
- Plots of observations vs. time with population and individual fits

The model with best fit according to model selection criteria will be chosen as the final structural model.

Inter-individual Variability of Model Parameters

Initially, exponential error models will be utilized for between subject variability in all PK parameters and implemented as a diagonal variance matrix for random effects (ηp). PK parameters will be modeled assuming the log-normal distribution as follows:

$P_i = TVP * \exp(\eta_{Pi})$

Where P_i is a parameter of interest for individual i, TVP is the typical value (population estimate) of the parameter and ηPi is normally distributed with mean zero and variance ωP^2.

Residual Error

The residual error will start with a combined additive and proportional (constant coefficient of variation) error model.

$C_{ij} = \hat{C}_{ij} (1 + \varepsilon_{1ij}) + \varepsilon_{2ij}$

where C_{ij} is the measured concentration j in individual i, $\hat{C}_{ij}$ is the model predicted value corresponding to measurement j in individual i, ε_{1ij} is the proportional component of residual error for measurement j in individual i (normally distributed with mean zero and variance σ_1^2), ε_{2ij}, is the additive component of residual error for measurement j in individual i (normally distributed with mean zero and variance σ_2^2). Other residual error models: additive, log-additive, and proportional error models will also be explored.

Evaluation of Covariate Effects on model parameter estimates

The process of covariate screening will be started by visual inspection of posterior Bayes estimates of PK parameters' ηP plotted versus each of the covariates. For continuous covariates, scatter covariate-ηP plots will be overlaid with a non-parametric locally weighted smoothed line (LOESS)

to help identify any potential trend. For categorical covariates, box-whisker plots of ηP for each category will be compared side-by-side to identify any potential differences. Covariate-parameter relationships indicated by a clear trend will then be further tested.

Covariates	Reason for investigation	Parameter
WT	CL, and Vc are likely to be weight dependent	CL, Vc
CRCL	Creatinine clearance may impact CL and Vc	CL and Vc
GA	Gestational age may impact CL and Vc	CL, Vc and Vp
AGE	Age may impact CL and Vc	CL, Vc and Vp

Continuous covariates may be included in the model using a linear or multiplicative function after centering on the median:

$$TVP = \theta_x + \left(\frac{\text{Covariate}}{\text{Median}(\text{Covariate})} \right)^{\theta_y}$$

or

$$TVP = \theta_x \cdot \left(\frac{\text{Covariate}}{\text{Median}(\text{Covariate})} \right)^{\theta_y}$$

where TVP is the typical value of a PK parameter, θ_x is a to be estimated covariate independent fixed-effect component and θ_y is a covariate dependent fixed-effect component of the relationship.

Categorical covariates will only be tested if at least 5% (or 15 individuals – whichever is smaller) of subjects belong to that category. Categorical covariates will be incorporated into the model using index coding:

$$TVP = \theta x \cdot (1 + \theta y \cdot \text{Index})$$

where TVP is the typical value of a PK parameter, θx is a covariate independent fixed-effect component and θy is a covariate dependent fixed-effect component of the relationship. Index has a value of 1 in the presence of the covariate, otherwise it has a value of 0.

Assessment of the significance for covariate will use the forward addition and backward elimination approach. The MOF based on Likelihood ratio test (LRT) will be a primary evaluation for a covariate-parameter relationship to be considered statistically significant based on the alpha level specified prior to the analysis. For the addition of a covariate that decreases the MOF by more than 3.84 ($\chi 2$, $p < 0.05$; df=1) and the removal of a covariate that increases the MOF by more than 10.83 ($\chi 2$, $p < 0.001$; df=1) will be considered statistically significant. For the purposes of documenting the process of covariate screen, the MOF values will be tabulated in accordance with the covariates included in the models at each step.

Random Effect Correlations

Correlations between random effects will be tested after inclusion of significant fixed effects of covariates in the model. Pair-wise plots of posterior random effects produced by the POSTHOC step of a NONMEM run will be examined visually and correlation coefficients will be calculated. The cases with the highest correlations will be tested for significance by including such correlations in the population model. The LRT will be applied and the same criteria as for fixed effects will be used.

- **Final Model**

Key modeling results including summary of parameters' estimates from final model, description of covariate selection and Goodness of Fit information will be presented to show how well the model describe the data. Any limitation on the analysis conducted will be discussed. The assumptions during the modeling process will be justified.

- **Model Evaluation**
- A visual predictive check will be performed to evaluate performance of the final PopPK model. Simulation using final PK and variability parameters and dosing histories, sampling times and covariate information will be obtained for the observed study drug concentration. A 90% prediction interval (5^{th} to 95^{th} percentile) will be generated from the simulated values. The number of observed concentrations within the prediction intervals will be determined. If this number is greater than 80%, the model will be considered suitable for predictive purposes.
- The standard errors of the final model parameter values will be estimated using the non-parametric bootstrap approach. For the final model approximately 500-1000 bootstrap datasets will be generated. The mean and 95% confidence intervals for the PopPK parameter estimates will be calculated and compared with the estimates from the final model. The bootstrap 95% confidence interval will be calculated based on the percentile of the empirical distribution of the estimated parameters from the bootstrap runs.

Reporting of PopPK analysis

At the completion of the PopPK analysis, a report will be drafted, reviewed, and revised if necessary, and finalized. The final report along with the relevant models, datasets, and other related information will be archived. The final report will include a tabular summary of all models explored during model development. For the key models a complete set of goodness-of-fit plots will be included.

References

- Beal SL, Sheiner LB; NONMEM Project Group C255, University of California at San Francisco. NONMEM users guides – Part I-VIII. San Francisco; 1988-1998.
- Bachman W, et al. PDx Pop® Version 3.0 User Manual. 2007.
- Guidance for Industry PopPK, U.S. Department of Health and Human Services Food and Drug Administration, CDER and CBER, February 1999.

– Guideline on Reporting the Results of PopPK Analyses, European Medicines Agency, CHMP, 21 June 2007.

21.3. Appendix 3 - Tables, Figures, and Listings of PK report (NCA and POPPK)

Tables

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Figure 16: Visual predictive check

Listings

- Population PK Analysis Dataset

NONMEM Outputs

- NONMEM Control file and Output for Base PK Model
- NONMEM Control file and Output for Final PK Model
- Individual Panel Plots for Goodness of Fit