A prospective, natural history study to assess the occurrence of HPA-1a alloimmunization in women identified at higher risk for Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT)

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TITLE PAGE

Protocol Title		A prospective, natural history study to assess the occurrence of HPA-1a alloimmunization in women identified at higher risk for Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT)	
Protocol Number		IPA2002	
Sponsor	**Name**	Rallybio IPA, LLC	
	Legal Registered Address	[redacted] New Haven, CT, USA 06510	
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Protocol Date		**Document Version**	**Date**
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This protocol was developed, reviewed, and approved in accordance with the Sponsor's standard operating procedures.

Confidentiality Notice

SPONSOR SIGNATORY

IPA2002: A prospective, natural history study to assess the occurrence of HPA-1a alloimmunization in women identified at higher risk for Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT)

I, the undersigned, have approved version 3.0 of the clinical trial protocol with the date of 20 May 2022.

Name and Title	Signature and Date
[redacted] Chief Medical Officer Rallybio IPA, LLC	DocuSigned by: [redacted]

INVESTIGATOR AGREEMENT

I have read the attached protocol entitled "A prospective, natural history study to assess the occurrence of HPA-1a alloimmunization in women identified at higher risk for Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT)", dated 20 May 2022, and agree to abide by all provisions set forth therein.

I agree to ensure that Financial Disclosure Statements will be completed by:

- me (including, if applicable, my spouse [or legal partner] and dependent children)
- my sub-investigators (including, if applicable, their spouses [or legal partners] and dependent children)

at the start of the study and for up to 1 year after the study is completed, if there are changes that affect my financial disclosure status.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

_____ _____

Principal Investigator Name (printed) Signature

_____

Date

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LIST OF ABBREVIATIONS

cffDNA	cell-free fetal DNA
CIOMS	Council for International Organizations of Medical Sciences
CRF	case report form
DNA	deoxyribonucleic acid
EDC	electronic data capture
FNAIT	fetal and neonatal alloimmune thrombocytopenia
GCP	Good Clinical Practice
HPA	human platelet antigen
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IQRs	interquartile ranges
IRB	Institutional Review Board
IVIG	intravenous immune globulin

1 PROTOCOL SUMMARY

Title: A prospective, natural history study to assess the occurrence of HPA-1a alloimmunization in women identified at higher risk for Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT).

Rationale: Data from this study will inform the frequency of women at higher FNAIT risk among pregnant women of different racial and ethnic characteristics obtaining pre-natal care at the involved institutions and consenting for the study and will assess the occurrence of human platelet antigen (HPA)-1a alloimmunization in these women. It is planned that data from this study be used as an external control for a future single arm registration study of an anti-HPA-1a antibody therapeutic for the prevention of FNAIT.

Research Objectives and Outcome Variables

Research Objectives	Outcome Variables
Primary	
To inform the frequency of women at higher FNAIT risk among pregnant women of different racial and ethnic characteristics who present for pre-natal care.	The number of participants that are determined to be at higher FNAIT risk compared to the total number of pregnant women assessed for higher FNAIT risk, with attention to self-characterized race and ethnicity.
Secondary	
To inform the frequency of HPA-1a alloimmunization among pregnant women identified at higher FNAIT risk.	Occurrence of anti-HPA-1a maternal alloimmunization at Week 10 postpartum[a]
To inform the frequency of pregnancy outcomes among pregnant women identified at higher FNAIT risk.	• Rate of spontaneous abortion, defined as non-deliberate fetal death which occurs prior to 19 weeks of gestation • Rate of elective abortion, defined as deliberate termination of pregnancy at any time in gestation • Rate of stillbirth, defined as non-deliberate fetal death anytime in gestation on or after 19 weeks of gestation • Rate of premature birth, defined as live birth prior to 37 completed weeks of gestation • Rate of live births (≥ 37 completed weeks of gestation)
To inform the frequency, where data are available, of neonatal thrombocytopenia in infants born to women who have alloimmunized, as determined by detectable anti-HPA-1a antibody at Week 10 postpartum.[a]	Neonatal thrombocytopenia and severe neonatal thrombocytopenia, as determined by a platelet count $< 150 \times 10^9$/L and $< 50 \times 10^9$/L respectively, within 72 hours of birth, where data are available

[a] For pregnancies that do not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

Hypothesis/Estimation: No hypothesis will be tested in this study.

Study Design: This is a prospective, non-interventional, natural history study.

Study Population: Pregnant women (≥ 18 years of age) who have provided informed consent for the study. Women with a history of FNAIT will not be included in the study.

Methodology: This study will be open to all pregnant women presenting at Gestation Week 10 to 14 pre-natal visit at the involved institutions.

At this pre-natal visit, the woman will be invited to provide informed consent for the study which includes collection of blood samples for the following tests:

- Maternal HPA-1 genotype: to identify women who are HPA-1b/b genotype (ie, HPA-1a negative)
- Maternal human leukocyte antigen (HLA)-DRB3*01:01 genotype: to identify women who are HLA-DRB3*01:01 positive
- Maternal anti-HPA-1a antibodies: to identify women who have no detectable anti-HPA-1a antibodies
- Fetal HPA-1 genotype: to inform on the presence of the antigenic stimulus (HPA-1a) for maternal alloimmunization

It is preferred that all samples be collected at the Gestation Week 10 to 14-visit. If all blood samples cannot be collected during one visit, the collection can be split into 2 timepoints. The first timepoint should include blood samples for maternal HPA-1 genotype, maternal HLA-DRB3*01:01 genotype and the maternal anti-HPA-1a antibodies and must be collected at the Gestation Week 10 to 14-visit. The second timepoint should include blood samples for the fetal HPA-1 genotype and should be collected by 18 weeks of gestation.

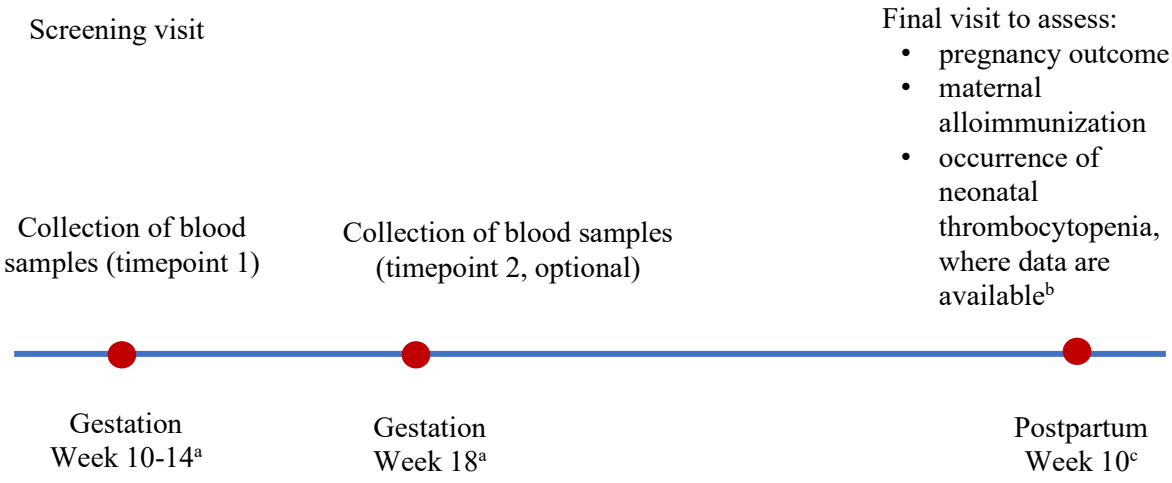
At the next pre-natal visit, for pregnant women who are HPA-1b/b genotype, HLA-DRB3*01:01 positive, and anti-HPA-1a negative, a second maternal blood sample will be requested to determine the fetal HPA-1 genotype.

At Week 10 postpartum, pregnant women who were assessed to be HPA-1b/b, positive for HLA-DRB3*01:01, negative for anti-HPA-1a antibodies, and positive fetal HPA-1a test result will be evaluated for the occurrence of HPA-1a alloimmunization, pregnancy outcome and, for those women who have alloimmunized, the occurrence of neonatal thrombocytopenia (where data are available). For pregnancies that do not result in a live birth, the assessment for alloimmunization will be performed at a visit scheduled 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

Study Sample Size: It is anticipated that approximately 20,000 to 30,000 participants will be enrolled with the goal of identifying approximately 30 to 50 pregnant women at higher FNAIT risk. Of the women identified at higher FNAIT risk, it is anticipated approximately 10 to 20 may alloimmunize.

Data Analysis: This study is intended to be descriptive in nature. Demographic and baseline characteristics will be summarized. Discrete variables will be summarized using numbers and percentages; continuous variables will be summarized using means and SD or medians and interquartile ranges (IQRs), as appropriate. Administrative tabulated summaries of study variables will be generated periodically.

Study Schema:



[a] It is preferred that all samples be collected at the Gestation Week 10 to 14-visit. If all blood samples cannot be collected during one visit, the collection can be split into 2 timepoints. The first timepoint should include blood samples for maternal human platelet antigen (HPA)-1 genotype, maternal HLA-DRB3*01:01 genotype and the maternal anti-HPA-1a antibodies and must be collected at the Gestation Week 10 to 14-visit. The second timepoint should include blood samples for the fetal HPA-1 genotype and should be collected by 18 weeks of gestation.
For pregnant women who are HPA-1b/b genotype (ie, HPA-1a negative), HLA-DRB3*01:01 positive, and anti-HPA-1a antibody negative, a second blood sample at the next pre-natal visit will be requested to determine the fetal HPA-1 genotype.

[b] Neonatal thrombocytopenia to be sourced from the neonate's medical records.

[c] For pregnancies that do not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

2 RATIONALE AND BACKGROUND

Of the approximately 2% of Caucasian pregnant women who are HPA-1a negative (ie, HPA-1b/b), approximately 85% will be carrying an HPA-1a positive fetus. Overall, approximately 10% of these pregnant women will become alloimmunized and among the fetuses or neonates of alloimmunized women, about 10% will have severe thrombocytopenia ($< 50 \times 10^9$/L; < 50,000 platelets/mL), many with bleeding sequelae; about 1% of the incompatible fetuses or neonates will suffer intracranial hemorrhage [1]. Among the women who become alloimmunized, 90% to 98% will come from among the approximately 27% who are HLA-DRB3*01:01 positive [2, 3], meaning that those women with this HLA allele face an alloimmunization risk of approximately 25% to 30% [4], vs 1% to 2% for those without the allele.

Human platelet antigen genotype frequencies vary by race, and an alloimmune response to HPA-1a, the immunodominant platelet antigen in Caucasians (~75% to 80% of cases), is implicated in the majority of FNAIT cases (ie, in HPA-1b/b homozygous pregnant women exposed to fetal platelets expressing HPA-1a derived from a HPA-1a positive father) [5-9]. Data reporting on the frequency of the HPA-1b/b genotype in non-Caucasian populations is limited, with reports showing a lower frequency in African populations and in Asians [8, 10, 11]. Data reporting on the frequency of a population reporting Hispanic ethnicity are sparse.

Data from this study will inform the frequency of women at higher FNAIT risk among pregnant women of different racial and ethnic characteristics obtaining pre-natal care at the involved institutions and consenting for the study and will assess the occurrence of HPA-1a alloimmunization in these women. It is planned that data from this study be used as an external control for a future single arm registration study of an anti-HPA-1a antibody therapeutic for the prevention of FNAIT.

Pregnant women at FNAIT risk will be those who are confirmed to be HPA1b/b (HPA-1a negative). This cohort will be enriched for higher FNAIT risk by establishing those HPA-1b/b women who are also HLA-DRB3*01:01 positive. A baseline negative anti-HPA-1a antibody test will establish the absence of detectable antibody and allow assessment of the occurrence of new alloimmunization at Week 10 postpartum (or 10 weeks from the date of the pregnancy-terminating event [ie, abortion (spontaneous/elective) or stillbirth] for pregnancies that do not result in a live birth). Assessment of fetal HPA-1 status will inform on the presence of the antigenic stimulus for maternal alloimmunization (cell-free fetal DNA [cffDNA] HPA-1a positive).

3 RESEARCH OBJECTIVES AND OUTCOME VARIABLES

Research Objectives	Outcome Variables
Primary	
To inform the frequency of women at higher FNAIT risk among pregnant women of different racial and ethnic characteristics who present for pre-natal care.	The number of participants that are determined to be at higher FNAIT risk compared to the total number of pregnant women assessed for higher FNAIT risk, with attention to self-characterized race and ethnicity.
Secondary	
To inform the frequency of HPA-1a alloimmunization among pregnant women identified at higher FNAIT risk.	Occurrence of anti-HPA-1a maternal alloimmunization at Week 10 postpartum[a]
To inform the frequency of pregnancy outcomes among pregnant women identified at higher FNAIT risk.	• Rate of spontaneous abortion, defined as non-deliberate fetal death which occurs prior to 19 weeks of gestation • Rate of elective abortion, defined as deliberate termination of pregnancy at any time in gestation • Rate of stillbirth, defined as non-deliberate fetal death anytime in gestation on or after 19 weeks of gestation • Rate of premature birth, defined as live birth prior to 37 completed weeks of gestation • Rate of live births (≥ 37 completed weeks of gestation)
To inform the frequency, where data are available, of neonatal thrombocytopenia in infants born to women who have alloimmunized, as determined by detectable anti-HPA-1a antibody at Week 10 postpartum.[a]	Neonatal thrombocytopenia and severe neonatal thrombocytopenia, as determined by a platelet count $< 150 \times 10^9$/L and $< 50 \times 10^9$/L respectively, within 72 hours of birth, where data are available

[a] For pregnancies that do not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

4 RESEARCH METHODS

4.1 Study Design

This is a prospective, non-interventional, natural history study that will be open to all pregnant women presenting at Gestation Week 10 to 14 pre-natal visit at the involved institutions. At this pre-natal visit, the woman will be invited to provide informed consent for blood samples to be collected. The blood samples will be tested to inform whether the woman is at higher FNAIT risk as described in Table 1.

Table 1 Pre-Natal FNAIT Laboratory Testing at Gestation Week 10 to 14

Test No.	Test	Description		Result	Protocol Specific Action
1	Maternal HPA-1 genotype	Test will identify women who are HPA-1b/b, indicating risk for HPA-1a sensitization and occurrence of FNAIT	1a	HPA-1b/b (HPA-1a negative)	Perform Test 2
			1b	HPA-1a/b HPA-1a/a	None – no further testing
2	Maternal HLA-DRB3*01:01 genotype	Test will identify women who are HLA-DRB3*01:01 positive, indicating higher FNAIT risk	2a	HLA-DRB3*01:01 positive	Perform Test 3
			2b	HLA-DRB3*01:01 negative	None – no further testing
3	Maternal anti-HPA-1a antibody	Test will establish women who have no detectable anti-HPA-1a antibody	3a	Anti-HPA-1a antibody negative	Perform Test 4
			3b	Anti-HPA-1a antibody positive	None – no further testing
4	Fetal HPA-1 genotype	A cell-free fetal DNA (cffDNA) test to inform on the presence of the antigenic stimulus for maternal alloimmunization	4a	HPA-1a/b	Will be followed for final assessments per Section 4.2.2 or Section 4.2.3
			4b	HPA-1b/b	None – no further testing

DNA = deoxynucleic acid; FNAIT = fetal and neonatal alloimmune thrombocytopenia; HLA = human leukocyte antigen; HPA = human platelet antigen

If the pregnant woman meets the study-specific blood test results, ie, genotype HPA-1b/b, genotype HLA-DRB3*01:01 positive, anti-HPA-1a antibody negative, and carrying an HPA-1a positive fetus (Table 1, Test Results 1a, 2a, 3a, and 4a), a follow-up determination will be made at Week 10 postpartum on the occurrence of HPA-1a alloimmunization, pregnancy outcome and, for women who have alloimmunized, the occurrence of neonatal thrombocytopenia (where data are available).

For women who meet the study specific tests results (ie, Test Results 1a, 2a, 3a, and 4a in Table 1) but the pregnancy does not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

4.1.1 Study Sample Size

It is anticipated that approximately 20,000 to 30,000 participants will be enrolled with the goal of identifying approximately 30 to 50 pregnant women at higher FNAIT risk. Of the women identified at higher FNAIT risk, it is anticipated approximately 10 to 20 may alloimmunize.

4.1.2 Selection and Number of Sites

The study will be conducted across multiple sites in the United States (approximately 10 sites) and Europe (approximately 10 sites).

4.1.3 Participant Eligibility

All participants must sign and personally date the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved informed consent before any study-specific procedures are performed.

4.1.3.1 Inclusion Criteria

Pregnant women (≥ 18 years of age) who have provided informed consent for the study.

4.1.3.2 Exclusion Criteria

Prior history of FNAIT.

4.2 Assessments

Data from the following sources (Section 4.2.1, Section 4.2.2, and Section 4.2.3) will be provided to the investigator for reviewing with the participant based on the assessment of the investigator and in accordance with local practices/guidelines.

4.2.1 Gestation Week 10 to 14 Pre-natal Visit Assessments

- Maternal demographics: year of birth, weight, height, and self-characterized race and ethnicity
- Gestational age in weeks as estimated by the principal investigator
- Obstetric history: mode of delivery, gestational age of delivery, live birth, abortion
- Blood samples will be collected (refer to the study Laboratory Manual for details):
  1. Maternal HPA-1 genotype
  2. Maternal HLA-DRB3*01:01 genotype
  3. Maternal anti-HPA-1a antibodies
  4. Fetal HPA-1 genotype

It is preferred that all samples be collected at the Gestation Week 10 to 14-visit. If all blood samples cannot be collected during one visit, the collection can be split into 2 timepoints. The first timepoint should include blood samples for maternal HPA-1 genotype, maternal HLA-DRB3*01:01 genotype and the maternal anti-HPA-1a antibodies and must be collected at the Gestation Week 10 to 14-visit. The second timepoint should include blood samples for the fetal HPA-1 genotype and should be collected by 18 weeks of gestation.

4.2.1.1 Fetal HPA-1 Genotype Retest

At the next pre-natal visit (ie, after the Gestation Week 10 to 14-visit), for pregnant women who are HPA-1b/b genotype, HLA-DRB3*01:01 positive, and anti-HPA-1a antibody negative, a second maternal blood sample will be requested to determine the fetal HPA-1 genotype.

4.2.2 Visit Assessments for Pregnancy Outcomes that are Live Births

For pregnancies that result in a live birth, the assessment of alloimmunization will be at 10 weeks postpartum.

- A blood sample will be collected for the assessment of maternal alloimmunization (refer to the study Laboratory Manual for details), as determined by the presence of detectable anti-HPA-1a antibodies.
- Pregnancy outcome (Section 3): live births, premature birth, mode of delivery including the date (based on medical records).
- Occurrence of neonatal thrombocytopenia and severe neonatal thrombocytopenia, as determined by a platelet count $< 150 \times 10^9$/L and $< 50 \times 10^9$/L, respectively, within 72 hours of birth where data are available (based on medical records).
- Interventions used: eg, intravenous immune globulin (IVIG), steroids, fetal platelet transfusions, newborn platelet transfusions.

4.2.3 Visit Assessments for Pregnancy Outcomes that are Not Live Births

For pregnancies that do not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

- A blood sample will be collected for the assessment of maternal alloimmunization (refer to the study Laboratory Manual for details), as determined by the presence of detectable anti-HPA-1a antibodies.
- Pregnancy outcome: spontaneous abortion, elective abortion, stillbirth, including the date (based on medical records)
- Interventions used: eg, IVIG, steroids

4.3 Data Management

The investigator is responsible for maintaining accurate, complete, and up-to-date records for each participant. The investigator is also responsible for maintaining any source documentation related to the study, including any films, tracings, computer discs, or tapes.

Site personnel will abstract data from the participant medical record directly into the electronic data capture (EDC) system.

The EDC system automatically generates queries resulting from computer checks embedded into the system to ensure accuracy, quality, consistency, and completeness of the database. Manual queries resulting from review by Data Management staff are also generated from within the EDC system, where they are tracked. Sites will resolve the queries and correct the entered data when necessary. Every change to data is captured in the EDC system audit trail. Upon completion of the study, or after reaching a pre-specified point in the study, Data Management will lock the database and the contract research organization will generate the SAS datasets necessary for data analysis and reporting.

4.4 Data Analysis

This study is intended to be descriptive in nature, no hypothesis will be tested in this study. Demographic and baseline characteristics will be summarized. Discrete variables will be summarized using numbers and percentages; continuous variables will be summarized using means and SD or medians and IQRs, as appropriate. Administrative tabulated summaries of study variables will be generated periodically.

5 PROTECTION OF HUMAN SUBJECTS

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

5.1 Informed Consent

Where an informed consent is required per local regulations, an initial sample informed consent form (ICF) is provided for the investigator or designee to prepare the informed consent document to be used at his or her site. Updates to the sample ICF are to be communicated formally in writing from the PPD Clinical Research Associate to the investigator or designee. The written ICF is to be prepared in the language(s) of the potential participant population and would be made available either electronically for subjects and investigator to sign or via paper.

Before a participant's participation in the study, the investigator or designee will explain to the subject the aims, methods, anticipated benefits, and potential hazards of the study, and answer all questions regarding the study.

The acquisition of informed consent is to be documented in the participant's medical records, and the ICF is to be signed and personally dated by the participant and by the person who conducted the informed consent discussion if a paper version is used, or electronically signed and dated through the electronic consenting system. For a paper process, the original signed ICF is to be retained in accordance with institutional policy and a copy of the ICF(s) must be provided to the participant. For an electronic process, the system would notify subject when a fully signed informed consent is received and would provide a copy of the informed consent for participant's records. Site personnel would have access to the fully signed informed consent PDF version and would be able to print it from the system to be documented in the participant's medical records.

If local regulations do not require an informed consent to be signed but mandate that the participant is notified about the study, the investigator or designee should document the notification process in the participant's medical record.

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

The protocol, protocol amendments, ICF, and other relevant documents must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

- The investigator will be responsible for:
  - Providing written summaries of the status of the study to the IRB/IEC in accordance with the requirements, policies, and procedures established by the IRB/IEC
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), European In Vitro Diagnostic Regulation 2017/746 for clinical device research (if applicable), and all other applicable local regulations

5.3 Participant Confidentiality

The investigator must ensure that the participant's confidentiality is maintained for documents submitted to the Sponsor.

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.

Documents that are not submitted to the Sponsor (eg, signed ICFs) are to be kept in confidence by the investigator, except as described below.

In compliance with governmental regulations/ICH GCP Guidelines, it is required that the investigator and institution permit authorized representatives of the company and the IRB/IEC direct access to review the participant's original medical records for verification of data. Direct access includes examining, analysing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The investigator is obligated to inform and obtain the consent of the participant to permit such individuals to have access to her study-related records, including personal information.

5.4 Subjects Decision to Withdraw

Participants have the right to withdraw from the study at any time and for any reason without prejudice to their future medical care by the physician or at the institution.

Withdrawal of consent for a study means that the participant does not wish to or is unable to continue further study participation. Participant data up to withdrawal of consent will be included in the analysis of the study and, where permitted, publicly available data can be included after withdrawal of consent. The investigator is to discuss with the subject appropriate steps for withdrawal of their consent from the study.

5.5 Sample Storage and Destruction

Samples will be anonymized and tracked using a unique identifier that is assigned to the samples for the study. Results will be stored in a secure database to ensure confidentiality.

If informed consent is provided by the subject, Rallybio may conduct additional testing on remaining samples to refine analytical methods to inform FNAIT risk.

Since the evaluations of analytical methods are not expected to benefit the participant directly, the results of these analyses will not be placed in the participant's medical record and will not be made available to the participant, members of the family, the personal physician, or other third parties, except as specified in the informed consent. Residual samples used for analytical method development can be retained for the duration of the study.

Genotyping samples will be destroyed once all protocol-defined procedures are completed at the end of the study. Serum samples from enrolled subjects, who are assessed for the occurrence of alloimmunization for the pregnancy under study, will be retained for 5 years following the study for assay development and validation purposes. The participant retains the right to request that the sample material be destroyed prior to the end of the study by contacting the investigator. Following the request from the participant, the investigator is to provide the Sponsor with the required study and participant number so that any remaining samples can be located and destroyed. However, information collected from samples prior to the request for destruction, will be retained by Rallybio.

6 SAFETY COLLECTION, RECORDING AND SUBMISSION REQUIREMENTS

This is a non-interventional natural history study in pregnant women at higher FNAIT risk. No investigational agent or treatment is being studied or supplied. Submission of reportable events to the study Sponsor is not required. Reportable events suspected to be related to any medicinal product a participant has received from their physician should be reported to the local authority in line with the local country requirements.

7 ADMINISTRATIVE AND LEGAL OBLIGATIONS

7.1 Protocol Amendments and Study Termination

The Sponsor may amend the protocol at any time. If the Sponsor amends the protocol, written agreement from the Investigator must be obtained where applicable per local governing law and/or regulations. The IRB/IEC must be informed of all amendments and give approval. The investigator must send a copy of the approval letter from the IRB/IEC to the Sponsor.

The Sponsor reserves the right to terminate the study at any time. Both the Sponsor and the Investigator reserve the right to terminate the Investigator's participation in the study according to the contractual agreement. The Investigator is to notify the IRB/IEC in writing of the study's completion or early termination and send a copy of the notification to the Sponsor.

7.2 Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the case report form (CRF) or entered in the electronic CRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in the Source Data Agreement (eg, Site Trial Binder, other site communication, etc.).

8 PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

The Sponsor will register and/or disclose the existence of, and the results of clinical studies as required by local laws and regulations.

8.1 Publication Policy

- The results of this study may be published or presented at scientific meetings in accordance with agreed upon procedures. If this is foreseen under agreed upon procedures, the investigator will submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments under the outlined procedures.
- In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. A coordinating investigator may be designated.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

9 REFERENCES

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10 APPENDIX: SUB-STUDY TO SUPPORT VALIDATION OF THE FETAL HPA-1 GENOTYPE ASSAY

10.1 Rationale and Background

IPA2002 is a prospective, natural history study to assess the occurrence of human platelet antigen (HPA)-1a alloimmunization in women identified at higher risk for Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT). It is planned that data from this study be used to contextualize results from a future single arm registration study of an anti-HPA-1a antibody therapeutic for the prevention of FNAIT.

In the IPA2002 study, assessment of fetal HPA-1 genotype is needed to confirm the presence of the antigenic stimulus (ie, fetal HPA-1a antigen) that may result in maternal alloimmunization. At the time of starting this natural history study, a validated assay to confirm fetal HPA-1 genotype using cell-free fetal DNA (cffDNA) is not available at the same central laboratory where all other FNAIT screen tests will be analyzed. The purpose of this sub-study is to collect an additional blood sample in a subset of women who provided their consent to participate in the IPA2002 study, to support validation of the fetal HPA-1 genotype/cffDNA test.

10.2 Sub-study Design

A total of 140 women who are HPA-1b/b is required for this sub-study. Two blood samples will be collected from each participant that can be used in the development and validation of a fetal HPA-1 genotype assay using cffDNA. The first of the 2 samples will be sourced from the blood sample collected at the Gestation Week 10 to 14 visit (Section 4.2.1). The second sample will be obtained as follows:

- Participants who are HPA-1b/b and human leukocyte antigen (HLA)-DRB3*01:01 negative at Gestation Week 10 to 14 will be asked to provide one additional blood sample at a subsequent pre-natal visit.
- Participants who are HPA-1b/b, HLA-DRB3*01:01 positive and anit-HPA-1a antibody negative at Gestation Week 10 to 14 will not be requested to provide an additional blood sample, since a second sample is already scheduled in the main study (Section 4.2.1.1).

A summary on how the 2 blood samples will be collected for all participants in the sub-study is described in the Table 2.

Written informed consent for the sub-study will be obtained from all participating women.

Table 2 Maternal Samples for Cell-free Fetal DNA HPA-1 Genotyping

Study/Visit	Population	Blood Sample Collected for cffDNA	
		Main study	Sub-study
Gestation Week 10-14/ pre-natal visit	All participants who are HPA-1b/b	X	
Fetal HPA-1 genotype re-test at a subsequent pre-natal visit/post Gestation Week 10-14	All participants who are HPA-1b/b and HLA-DRB3*01:01 negative genotype		X
	All participants who are HPA-1b/b, HLA-DRB3*01:01 positive, and anit-HPA-1a antibody negative	X	

cffDNA = cell-free fetal DNA; HLA = human leukocyte antigen; HPA = human platelet antigen

11 SUMMARY OF CHANGES

Version 3.0 (20 May 2022) superseding Version 2.0 (04 November 2021).

Rationale for Protocol Amendment 2:

- To provide clarification on blood sample collection for Gestation Week 10 to 14 Pre-natal Visit Assessments with the preferred approach to collect all samples at the Gestation Week 10 to 14-visit and an alternate approach to split blood collection into 2 timepoint along with the details of the timing and laboratory test(s) at each timepoint.
  - Targeted changes have been made in the protocol to reflect this clarification.
- Language under Section 4.2 regarding provision of data to the investigator has been updated for reviewing the laboratory test results with the participant.
- To establish a sub-study (Section 10) designed to collect an additional blood sample in a subset of women who provided their consent to participate in the IPA2002 study, to support validation of the fetal HPA-1 genotype/cffDNA test.
  - Targeted changes have been made in the protocol to reflect the addition of this sub-study.

Version 2.0 (04 November 2021) superseding Version 1.0 (13 April 2021).

Rationale for Protocol Amendment 1:

- The Week 10 Postpartum Visit has been clarified for where the pregnancy did not result in a live birth, a Week 10 visit should occur after the pregnancy event (ie, abortion, stillbirth).
- The repeat sample collection to determine HPA-1 status of the fetus has been clarified to apply only to the subset of pregnant woman who are determined to be HPA-1b/b genotype, HLA-DRB3*01:01 positive, and anti-HPA-1a antibody negative at Gestation Week 10 to 14.
- Rationale and Background (Section 2) has been updated to explain that data generated from this study is planned to be used as an external control for a future single-arm registration study of an anti-HPA-1a antibody therapeutic for the prevention of FNAIT. Additionally, this section has been updated for consistency with contemporary FNAIT literature.
- Inclusion Criteria (Section 4.1.3) have been clarified to accurately reflect the population who will be invited to consent and participate in screening for FNAIT risk.
- Details on the FNAIT blood tests to be performed have been moved to the Methodology section of the Protocol Summary and the Study Design (Section 4.1). A summary table which describes the Pre-Natal FNAIT Laboratory Testing (Table 1) has been added to Section 4.1 for the testing of blood samples for FNAIT risk.
- Section 5.5 (Sample Storage and Destruction) has been updated to permit remaining serum samples to be used for future assay development and validation.
- Administrative and editorial changes have been made throughout the protocol for consistent terminology use, to reduce redundant text, and for typographical, grammatical, and formatting changes.