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Design of a Phase 3, Global, Multicenter, Randomized, Placebo-controlled, Double-blind Study of Nipocalimab in Pregnancies At-risk for Severe Hemolytic Disease of the Fetus and Newborn

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Conflict of Interest: YK, PA, E. Lam, JHL, LEL, RMN, VO, SSK, MLT, JZ, UA, and WS are employees of Janssen and hold stock/stock options from Johnson & Johnson. EV serves as the principal investigator of UNITY, CLARITY, and AZALEA studies in The Netherlands. ET, E. Lopriore, and DO received consulting fees for membership of steering committees and advisory boards for clinical studies from Momenta Pharmaceuticals, Inc, and Janssen Pharmaceuticals, Inc. KJM serves as the overall principal investigator for the phase 2 trial of nipocalimab (UNITY); received funding from Momenta Pharmaceuticals, Inc. paid on his behalf to the McGovern Medical School – UT Health; received funding from Janssen Pharmaceuticals, Inc, paid on his behalf to Dell Medical School at The University of Texas at Austin for a clinical trial on a monoclonal antibody for the treatment of HDFN; served on the steering committees and advisory boards for clinical studies for Momenta Pharmaceuticals, Inc, and Janssen Pharmaceuticals, Inc, but has not received funding for these activities; received royalty funding from UpToDate, Inc, for authorship of various chapters; received consulting fees from Health Management Associates, Inc, for consultation on the formation of fetal centers; received consulting fees from BillionToOne, Inc, paid on his behalf to Dell Medical School at The University of Texas at Austin; received honoraria from GLC Healthcare, Inc, for podcast content on HDFN; and serves as a nonpaid consultant for immunology at Janssen Pharmaceuticals, Inc.

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Trial registration: NCT05912517, ClinicalTrials.gov (http://www.clinicaltrials.gov/), global, multicenter, randomized, placebo-controlled, double-blind study

Abstract:
Objective: Nipocalimab is a neonatal Fc receptor (FcRn)–blocking monoclonal antibody that inhibits placental immunoglobulin G (IgG) transfer and lowers circulating maternal IgG levels. In an open-label, single-arm, phase 2 study, nipocalimab demonstrated evidence of safety and efficacy that support further investigation in a pivotal phase 3 trial of recurrent hemolytic disease of the fetus and newborn (HDFN). The phase 3 AZALEA study aims to evaluate the efficacy and safety of nipocalimab in a larger population at risk for severe HDFN, defined as HDFN associated with poor fetal outcomes or neonatal death.

Study design: AZALEA is a multicenter, randomized, placebo-controlled, double-blind, phase 3 study enrolling alloimmunized pregnant individuals (N≈120) at risk for severe HDFN based on obstetric history. Participants are randomized 2:1 to receive intravenous 45 mg/kg nipocalimab or placebo weekly from 13-16 to 35 weeks gestational age (GA). During the double-blind treatment period, participants receive standard-of-care weekly monitoring for fetal anemia until planned delivery at 37

to 38 weeks GA. Postnatal follow up periods are 24 weeks for maternal participants and 104 weeks for neonates/infants.

Results: The primary endpoint is the proportion of pregnancies that do not result in IUT, hydrops fetalis, or fetal loss/neonatal death from all causes. Key secondary endpoints include the severity of HDFN as measured by a composite HDFN severity index, the earliest time to occurrence of IUT or hydrops fetalis, the modified neonatal mortality and morbidity index in liveborn neonates, and the number of IUTs received. Other endpoints are safety, patient- and caregiver-reported outcomes, pharmacokinetics, pharmacodynamics (eg, IgG, FcRn receptor occupancy), and immunogenicity of nipocalimab.

Conclusion: AZALEA, the first placebo-controlled, randomized, multicenter, prospective trial in severe HDFN, is designed to evaluate the safety and efficacy of nipocalimab, a potential preventive and noninvasive intervention, in at-risk HDFN pregnancies.

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Design of a Phase 3, Global, Multicenter, Randomized, Placebo-controlled, Double-blind Study of Nipocalimab in Pregnancies At-risk for Severe Hemolytic Disease of the Fetus and Newborn

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Abstract

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Conclusion: AZALEA, the first placebo-controlled, randomized, multicenter, prospective trial in severe HDFN, is designed to evaluate the safety and efficacy of nipocalimab, a potential preventive and noninvasive intervention, in at-risk HDFN pregnancies.

Keywords: neonatal Fc receptor blocker (FcRn); nipocalimab; hemolytic disease of the fetus and newborn; HDFN; intrauterine transfusion; red blood cell alloimmunization; safety; efficacy; study design

Key Points:

- Severe HDFN leads to poor fetal/neonatal outcomes
- IUTs are associated with complications and fetal loss
- Nipocalimab blocks IgG recycling and placental transfer
- Nipocalimab reduces fetal anemia and IUTs in EOS-HDFN
- The Ph3 AZALEA study evaluates nipocalimab in severe HDFN

BACKGROUND

Hemolytic disease of the fetus and newborn (HDFN) is a rare, potentially life-threatening condition of progressive fetal/neonatal anemia due to the transplacental transfer of maternal anti-red blood cell (RBC) immunoglobulin G (IgG) alloantibodies.[1] In severe HDFN (defined as HDFN associated with poor fetal outcomes or neonatal death[2]), sufficient maternal IgG alloantibody transfer leads to fetal anemia or neonatal death. Despite the introduction of RhD IgG prophylaxis to prevent alloimmunization in RhD-negative pregnant individuals, the risk of developing severe HDFN remains.[3-7] Severe HDFN is associated with substantial fetal/neonatal morbidity and mortality.[8-13] The risk for recurrence of severe HDFN has been reported as 86% in subsequent pregnancies following previous severe HDFN, with a significant proportion developing HDFN at an earlier gestational age (GA) than in the prior severe HDFN pregnancy.[14,15]

Standard-of-care HDFN management involves noninvasive monitoring with middle cerebral artery (MCA) Doppler ultrasound for fetal anemia.[2] When MCA Doppler ultrasound results suggest moderate-to-severe fetal anemia, cordocentesis and intrauterine transfusion (IUT) of RBCs are performed to avoid hydrops fetalis and fetal loss.[2] However, the IUT procedure is an invasive intervention that is associated with procedural complications, including an increased risk of emergency cesarean delivery, premature or preterm birth, or fetal loss, as well as increased maternal alloimmunization.[9,11,16,17] Management of severe HDFN with standard-of-care monitoring and IUTs requires significant expertise and experience, including a maternal fetal medicine specialist, dedicated transfusion medicine unit, anesthesiologist, operating room with specialized staff, and/or on-call neonatal intensive care unit support.[18]

Intravenous immunoglobulin (IVIG), a human multidonor blood product, has been used with or without plasmapheresis aiming to delay the need for IUT in cases of severe HDFN.[15,19-24] However, in recent large, controlled, retrospective studies, IVIG exhibited a minimal effect on the development of fetal anemia or delay in the timing of an initial IUT.[15,23,24] IVIG and plasmapheresis are also associated with tolerability issues and substantial financial burden to patients and health care systems.[12] There remains a significant unmet medical need for an effective and safe intervention that can address the limitations of current treatments for pregnancies at risk for severe HDFN.

Nipocalimab is a high-affinity, fully human, effectorless IgG1 monoclonal antibody that is designed to selectively block the neonatal Fc receptor (FcRn; **Figure 1**),[25,26] the placental IgG transporter responsible for maternal-to-fetal IgG transfer and the IgG salvage receptor maintaining the long half-life of IgG in maternal circulation.[27] FcRn blockade by nipocalimab has been shown to rapidly and substantially decrease maternal circulating IgG concentrations through blocking IgG recycling and to reduce fetal/neonatal IgG concentration through blocking maternal placental IgG transfer and IgG recycling,[25,28] without affecting IgG production, other immunoglobulin levels (ie, IgM, IgA), or key humoral and cellular immune functions.[25,29]

Recently, nipocalimab demonstrated initial efficacy and safety in a more severe subset of recurrent, early-onset (≤24 weeks GA) severe HDFN (EOS-HDFN) in the phase 2, multicenter, open-label UNITY trial (ClinicalTrials.gov Identifier: NCT03842189). In this trial, 54% of pregnant participants treated with nipocalimab (30-45 mg/kg intravenous [IV] weekly) achieved the primary endpoint of live birth at ≥32 weeks GA without an IUT, compared with the 10% historical benchmark (95% CI, 25.1-80.8; P <0.001); the median GA at first IUT for those with IUT(s) was 27 weeks, compared with 22 weeks in published studies, and 46% of maternal/infant pairs required no antenatal or neonatal transfusions.[15,23,24,30] Nipocalimab also improved other antenatal and postnatal outcomes relative to previous EOS-HDFN management in the participants' most recent qualifying pregnancies.[30] During the phase 2 trial, the reported serious adverse events (AEs) were mainly related to HDFN or pregnancy-associated conditions and occurred with no apparent relationship to the nipocalimab dose or maternal/infant IgG level. These efficacy and safety data from the phase 2 UNITY trial support further investigation in a pivotal phase 3 trial of recurrent HDFN.

Here, we report the design of the phase 3, global, multicenter, randomized, placebo-controlled, double-blind AZALEA trial (ClinicalTrials.gov Identifier: NCT05912517), which aims to evaluate the efficacy and safety of nipocalimab in a larger population at risk for severe HDFN. Given the rarity of HDFN and significant unmet medical need for treatment, nipocalimab received fast-track designation from the US Food and Drug Administration (FDA) and orphan medicinal product designation from the European Medicines Agency for HDFN treatment in 2019, as well as orphan drug status from the FDA in 2020.[31,32] The FDA also granted a breakthrough therapy designation for nipocalimab for the treatment of HDFN in February 2024. The results from AZALEA will provide foundational evidence for the use of nipocalimab in HDFN as well as other serious alloantibody- and autoantibody-mediated perinatal diseases, where evidence-based treatments remain a considerable unmet need.[33,34]

METHODS/DESIGN

<u>Ethical and Study Oversight</u>

The AZALEA trial is being conducted in compliance with International Council for Harmonisation guidelines on Good Clinical Practice[35] and applicable regulatory and country- or territory-specific requirements. Independent Ethics Committee/Institutional Review Board approvals are obtained for each participating center according to applicable national regulations. All participants are fully informed of the risks and requirements of the study and receive any new information that may affect their decision to continue participation during the study. They are informed that their consent to participate in the study is voluntary and may be withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only participants who are fully able to understand the risks, benefits, and potential AEs of the study, and to provide their consent voluntarily, are enrolled.

<u>Participants</u>

Eligible participants are aged 18 to 45 years, pregnant at 13 to 16 weeks GA with a singleton fetus, have alloantibody titers for RhD, Rhc, RhE, RhC (≥16), or Kell antigens (≥4) with an antigen-positive fetus by cell-free fetal DNA analysis, and have a history of severe HDFN based on (1) a prior obstetric history of fetal anemia, defined as either hemoglobin level <0.84 multiples of the median (MoM) or requiring ≥1 IUT as a result of HDFN, or (2) fetal loss or neonatal death as a result of HDFN, with maternal alloantibody titers for RhD, Rhc, RhE, RhC (≥16), or Kell antigens (≥4), and evidence of an antigen-positive fetus (see complete inclusion criteria in **Table 1**).

Exclusion criteria include evidence of fetal anemia by ultrasound or repeated MCA peak systolic velocity (MCA-PSV) for a value ≥1.5 MoM prior to randomization; history of severe preeclampsia prior to GA Week 34 or severe fetal growth restriction; uncontrolled hypertension; history of myocardial infarction, unstable ischemic heart disease, or stroke; history of receiving anti-FcRn therapies; receiving systemic corticosteroids or immunosuppressants for disorders unrelated to the pregnancy; receiving or planning to receive plasmapheresis, immunoadsorption therapy, IVIG, or any IgG Fc-related protein therapeutics during the current pregnancy; or having a current severe or chronic infection (see complete exclusion criteria in **Table 1**).

Study Design

Approximately 120 eligible pregnant participants are planned to be recruited from multiple global study sites specializing in fetal therapy with the capability to perform IUTs on a regular basis and to provide access to a level 3 or 4 neonatal intensive care unit. The study includes a screening period (8-16 weeks GA), randomization (13-16 weeks GA), double-blind treatment (13-35 weeks GA) with anticipated delivery at 37 to 38 weeks GA, and postnatal follow-up periods of 24 weeks for the maternal participants and 104 weeks for neonates/infants (**Figure 2**). At 13 to 16 weeks GA (with early referral), pregnant participants are randomized 2:1 to receive weekly doses of 45 mg/kg IV nipocalimab or matching placebo until 35 weeks GA. The initiation of nipocalimab at 13 to 16 weeks GA was chosen to ensure placental FcRn blocking prior to the acceleration of placental transfer of maternal alloantibodies throughout the second trimester. The design includes stopping nipocalimab at 35 weeks GA, 2 to 3 weeks before planned delivery at 37 to 38 weeks GA. During the double-blind treatment period, weekly monitoring by MCA-PSV for a value ≥1.5 MoM informs the need for cordocentesis confirmation of fetal anemia and IUT. Subsequent IUTs are determined by MCA-PSV for a value ≥1.5 MoM, and/or time interval since the first IUT, and clinical judgment by the investigator. If an IUT is required, nipocalimab or placebo will continue until all fetal blood has been replaced by donor blood and laboratory tests confirm a lack of fetal RBCs.

Dose Selection Rationale

The nipocalimab dose regimen of 45 mg/kg IV weekly was selected based on the efficacy, safety, and pharmacokinetic (PK)/pharmacodynamic (PD) data from the phase 2 UNITY study in EOS-HDFN,[30] which was concordant with safety results and the PK/PD model developed in the phase 2 Vivacity-MG study (ClinicalTrials.gov Identifier: NCT03772587) in generalized myasthenia gravis,[36] and the phase 1 first-in-human study.[25] A mechanistic PK/receptor occupancy (RO)/PD model was constructed using data from the first-in-human study and phase 2 UNITY study[37] accounting for increase in maternal body weight and evaluated other pregnancy-related covariates,[27,38] with the goal of simulating the optimal dose regimen in the phase 3 study. The PK/RO/PD simulations suggested that the 45 mg/kg IV weekly dose regimen would be able to maintain full FcRn RO in ≥96% of participants for ≥8 days to ensure that unexpected dosing delays do not cause immediate loss of RO, placental transfer of alloantibodies, or IgG rebound. Simulated dose regimens <45 mg/kg IV weekly may lead to rapid loss of RO, potential placental transfer of alloantibodies, and subsequent rebound in IgG due to unexpected dosing delays.

Study Assessments

A listing of study endpoints is provided in **Table 2**. The primary efficacy endpoint is the proportion of pregnancies that do not result in fetal loss (due to any reason), IUT, hydrops fetalis, or neonatal death (due to any reason) through 4 weeks of age or 41 weeks postmenstrual age, whichever is later. Key secondary efficacy endpoints include the severity of HDFN as measured by a composite HDFN severity index (see definition in **Table 2**), the earliest time to occurrence of IUT or hydrops fetalis, the modified neonatal mortality and morbidity index in liveborn neonates, and the number of IUTs received. Safety outcomes, antenatal/pregnancy outcomes, neonatal outcomes, patient- and caregiver-reported outcomes (ie, Generalized Anxiety Disorder 7-item [GAD-7] scores, EuroQol 5-Dimension Questionnaire [EQ-5D-5L] Visual Analogue Scale [VAS] scores, and Short Form 36 Health Survey version 2 [SF-36 v2] Acute scores at baseline, GA Week 30, and postpartum Week 4; and Infant health-related Quality of Life Instrument [IQI] scores at Weeks 4, 8, and 52), as well as PK, PD, and immunogenicity of nipocalimab are also assessed.

<u>Statistical Analyses</u>

The sample size of approximately 120 evaluable participants will provide >95% power to detect an increase of 35% compared to placebo for the primary analysis (assuming a 2-sided test at the 0.05 level of significance and a randomization ratio of 2:1 for nipocalimab to placebo). The Cochran-Mantel-Haenszel test will be used for comparing treatment groups. A prespecified multiple comparison procedure to control the overall type 1 error rate (1-sided significance level of 0.025) for the primary and 4 key secondary endpoints will be used (**Figure 3**).

An independent, external Data Monitoring Committee (DMC) will monitor safety data on an ongoing basis throughout the study for maternal participants and neonates/infants and evaluate the benefit/risk of the study to stop the study early for futility or safety. An early safety analysis will be conducted by the DMC when ≥5 maternal participants have given birth. Initial enrollment will be limited to ≤35 participants until the DMC has made a recommendation about whether enrollment should continue after completion of the early safety analysis.

The first interim analysis for futility will be conducted to assess the treatment effect when approximately 50 maternal participants have given birth or terminated their pregnancy, have completed the Week 4 visit after delivery, and whose neonates have also completed the Week 4 visit (or 41 weeks postmenstrual age, whichever is later) or died prior to this time point. The unblinded DMC will review the efficacy and safety data and make recommendations regarding the continuation of the study to the sponsor committee, which will make the final decision regarding the conduct of the study.

DISCUSSION

For decades, IUT has served as the standard of care for treatment of severe HDFN and been associated with perinatal complications and fetal loss.[9,11,16] IVIG with or without plasmapheresis has been reported to delay the need for IUTs in some cases; yet, the vast majority of alloimmunized pregnancies at risk for severe HDFN resulted in recurrent severe fetal anemia requiring multiple IUTs, and a subset resulted in fetal/neonatal death.[15,23,24] These poor outcomes underscore a substantial unmet need for a new treatment option. Nipocalimab targets the underlying pathology of HDFN by binding to FcRn in the placenta, thereby blocking placental IgG transfer, and binding to FcRn in maternal endothelial cells, therefore blocking IgG recycling and lowering circulating maternal IgG alloantibodies, thus attenuating fetal anemia.[25,28] It represents the only noninvasive therapy currently in clinical development for the treatment of alloimmunized pregnant individuals at high risk for severe and EOS-HDFN.

As part of the drug-approval process, the FDA requires ≥1 randomized, placebo-controlled trial to be conducted, regardless of an orphan drug designation. In the AZALEA study, one-third of participants are planned to be randomized to the placebo arm and concurrent use of IVIG or plasmapheresis is prohibited due to the potential effect on the mechanism of nipocalimab.[25,30] To allow for the use of a placebo-controlled design, all participants receive standard-of-care monitoring for fetal anemia and IUT(s), if required, at centers experienced in the management of severe HDFN during the double-blind treatment period. Additionally, enrollment of alloimmunized participants with a variety of the most commonly implicated RBC antigens allows for the examination of efficacy of nipocalimab across different anti-RBC IgG-meditated HDFN.

Study evaluations, including both efficacy and safety assessments of nipocalimab, are consistent with the standard-of-care management of pregnant individuals at risk for fetal anemia to provide clinically meaningful information. A composite endpoint capturing fetal and neonatal death from all causes, IUT, and hydrops fetalis (the most important outcomes for severe HDFN) was chosen for the primary efficacy outcome as it is easy to interpret. Regarding the fetal/neonatal/infant safety assessments, monitoring for AEs, concomitant medications, clinical laboratory values, ultrasound monitoring of fetal growth and development, and neonatal/infant growth and immune development were planned, similar to the UNITY study, to further investigate safety-related and developmental outcomes in neonates/infants after maternal nipocalimab treatment. Regarding the PD outcomes, maternal serum IgG, alloantibody titers, and FcRn RO were selected as biomarkers for severe HDFN based on considerations of the anticipated mechanism of preventing placental IgG transfer and blocking maternal IgG recycling by nipocalimab and clinical evidence from UNITY[30] and other previous studies that support a correlation between IgG, alloantibody titers, and disease severity and clinical outcomes of HDFN.[2,39-43]

In previous phase 1 and 2 studies in nonpregnant and pregnant participants, nipocalimab showed rapid, substantial, recoverable, dose-dependent reductions in serum IgG concentrations at a maximum of –80% to –85% from baseline, as anticipated based on its mechanism of action.[25,30,36,44,45] Similarly, low serum IgG concentrations at or below the normal range were observed in neonates/infants born to maternal participants receiving nipocalimab for EOS-HDFN treatment.[30] Therefore, to mitigate the potential risk of infections due to decreased/low IgG, maternal participants who meet the following criteria are excluded from this study: (1) serum total IgG <6 g/L at screening, (2) severe infections requiring anti-infective(s), and (3) receiving or needing for a live virus vaccine during the study or within ≤8 weeks after the last dose. During the study, all infections will be monitored closely, and AEs of special interest (**Table 2**) must be reported to the sponsor within 24 hours. Concentrations of IgG, IgM, IgA, and IgE will be monitored throughout the study, and vaccine response to tetanus will be obtained to assess immune function in both maternal participants and neonates/infants. Neonates/infants who meet the following criteria will also be recommended to a pediatric immunologist: (1) IgG decreased with IgG <3.0 g/L at or after Week 52, (2) a nonprotective vaccine response to tetanus at Week 52, and (3) frequent, recurrent, or serious infections. However, it is important to note that no unexpected/unusual maternal or pediatric infections were observed with nipocalimab treatment in the UNITY study.[30] Studies in other autoantibody-mediated diseases show that, even when combined with glucocorticoids or other immunosuppressive agents, nipocalimab was not associated with an increased risk of infections.[36,45] Furthermore, nipocalimab treatment did not affect non-IgG immunoglobulins in participants with EOS-HDFN, myasthenia gravis, or rheumatoid arthritis enrolled in separate phase 2 studies,[30,36,46] nor did it impact key immune cell functions, IgG production, and the ability to mount immunization responses in nonhuman primates.[29] Treatment with FcRn blockers has generally been well tolerated and has allowed adequate immune responses to COVID and other vaccinations in participants with autoimmune diseases when concomitant with immunosuppressive agents.[47]

Potential limitations of this study include the small study size and 2:1 randomization, which may hinder data interpretation, such as the ability to identify drug-related AEs at a low frequency.

Additionally, there may be enrollment challenges due to the rarity of severe HDFN, requirements for the study design (eg, screening at ≤14 weeks of pregnancy, need for early referral to begin treatment, prohibition of IVIG/plasmapheresis), and potential geographic barriers. Therefore, approximately 50 global centers specializing in maternal-fetal medicine and the treatment of HDFN have been identified as study sites for the AZALEA trial in order to enroll a sufficient number of pregnant participants.

CONCLUSION

The AZALEA trial is the first global, multicenter, randomized, placebo-controlled, double-blind, prospective clinical trial in severe HDFN, designed to evaluate the safety and efficacy of nipocalimab as a potential preventive and noninvasive intervention for delaying or preventing the development of fetal anemia, the need for IUT in pregnant individuals, and the need for postnatal management in neonates/infants at risk for severe HDFN. Outcomes of this study will demonstrate the potential for a transformative treatment in HDFN and open a new potential frontier of investigation in other alloimmune or autoimmune diseases in pregnancy.

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CONFLICTS OF INTEREST

YK, **PA**, **E. Lam, JHL**, **LEL**, **RMN**, **VO**, **SSK, MLT**, **JZ, UA**, and **WS** are employees of Janssen and hold stock/stock options from Johnson & Johnson. **EV** serves as the principal investigator of UNITY, CLARITY, and AZALEA studies in The Netherlands. **ET**, **E. Lopriore**, and **DO** received consulting fees for membership of steering committees and advisory boards for clinical studies from Momenta Pharmaceuticals, Inc, and Janssen Pharmaceuticals, Inc. **KJM** serves as the overall principal investigator for the phase 2 trial of nipocalimab (UNITY); received funding from Momenta Pharmaceuticals, Inc. paid on his behalf to the McGovern Medical School – UT Health; received funding from Janssen Pharmaceuticals, Inc, paid on his behalf to Dell Medical School at The University of Texas at Austin for a clinical trial on a monoclonal antibody for the treatment of HDFN; served on the steering committees and advisory boards for clinical studies for Momenta Pharmaceuticals, Inc, and Janssen Pharmaceuticals, Inc, but has not received funding for these activities; received royalty funding from UpToDate, Inc, for authorship of various chapters; received consulting fees from Health Management Associates, Inc, for consultation on the formation of fetal centers; received consulting fees from BillionToOne, Inc, paid on his behalf to Dell Medical School at The University of Texas at Austin; received honoraria from GLC Healthcare, Inc, for podcast content on HDFN; and serves as a nonpaid consultant for immunology at Janssen Pharmaceuticals, Inc.

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Figure 1. Nipocalimab mechanism of action. (A) Illustration of nipocalimab preventing IgG-FcRn interaction. IgG binds with moderate strength to FcRn at the endosomal pH of 6.0, but not at extracellular pH of 7.4 (left). Nipocalimab binds strongly to FcRn under both conditions (pH 6.0 or 7.4), allowing rapid and complete blockade of FcRn. (B) Illustration of nipocalimab blocking transplacental IgG transfer and IgG recycling in maternal circulation. Placental IgG transfer (upper left panel) occurs when maternal IgG antibodies, including anti-erythrocyte IgG alloantibodies in EOS-HDFN, undergo pinocytotic uptake into syncytiotrophoblasts (the fetal-maternal barrier layer of the placenta), where they are bound to endosomal FcRn and undergo apical-to-basal transcytosis (transport and export) to enter the fetal vasculature. In maternal circulation, FcRn mediates IgG recycling in endothelial cells lining the maternal circulation (lower left panel), which functions to maintain high maternal serum IgG as well as anti-erythrocyte IgG alloantibodies available for placental transfer to the fetus. Nipocalimab is designed to block transplacental transfer of maternal IgG, including anti-erythrocyte alloantibodies (upper right panel), and to block FcRn-mediated IgG recycling to lower circulating maternal alloantibodies available for placental transfer (lower right panel). EOS-HDFN, early-onset severe hemolytic disease of the fetus and newborn; FcRn, neonatal Fc receptor; IgG, immunoglobulin G.

Figure 2. AZALEA study design.

GA, gestational age; HDFN, hemolytic disease of the fetus and newborn; IV, intravenous; MCA-PSV, middle cerebral artery peak systolic velocity; QW, weekly; R, randomization.

[a]Treatment can start any day from Weeks 13 to 16.

[b]R Day 1 (first dose of study intervention) can occur from GA Weeks 13 to 16.

Figure 3. Multiple testing procedure**.**

H, hypothesis; HDFN, hemolytic disease of the fetus and newborn; IA, interim analysis; IUT, intrauterine transfusion; PMA, postmenstrual age.

[a]One-sided level of significance.

Table 1. Inclusion and Exclusion Criteria

Inclusion criteria

• Pregnant individuals aged 18 to 45 years with singleton pregnancies and an estimated GA of between 13 and 16 weeks at randomization
• Previous pregnancy with severe HDFN that included ≥1 of the following:
  o Documented fetal anemia, defined as hemoglobin <0.84 MoM, or received ≥1 IUT as a result of HDFN
  o Fetal loss or neonatal death as a result of HDFN, with maternal alloantibody titers for RhD, Rhc, RhE, RhC (≥16), or Kell antigens (≥4), and evidence of an antigen-positive fetus
• Presence of alloantibody titers for RhD, Rhc, RhE, RhC (≥16), or Kell antigens (≥4), and an antigen-positive fetus in the current pregnancy based on the designated central laboratory results at screening
• cffDNA consistent with an antigen-positive fetus
• Screening laboratory results within the normal range for GA of pregnancy as follows:
  o Albumin ≥26 g/L

- Alanine aminotransferase ≤2 × ULN
- Aspartate aminotransferase ≤2 × ULN
- Creatinine ≤70.7 μmol/L
- IgG ≥6 g/L

- Healthy on the basis of physical examination, medical history, vital signs, 12-lead ECG, and clinical laboratory tests performed at screening
- Willing to receive standard of care with IUT if clinically indicated
- Agree to receive recommended vaccinations per local standard of care for both mother and child throughout the study
- Willing to forego collection of cord blood for stem cell storage or other nonstudy purposes
- For maternal participant and neonate, willing to forego participation in another clinical study of an investigational therapy for the duration of their participation in the current study
- Must sign an informed consent form indicating that she understands the purpose of, and procedures required for, the study and is willing to participate in the study and consents to a 24-week safety follow-up period. An additional consent may be obtained, if needed, according to local requirements. The parents/guardians of the neonates/infants must also sign an informed consent form (as per local requirements) to permit 104-week follow-up for the neonates/infants and agree to complete caregiver-reported outcomes for the infant
- Must be able to read and write
- Must agree not to donate blood through the final follow-up visit at Week 24 postpartum

Exclusion criteria

- Currently pregnant with a multiple gestation

- Evidence of fetal anemia by ultrasound or repeated MCA-PSV for a value ≥1.5 MoM prior to randomization
- History of severe preeclampsia prior to GA Week 34 or severe fetal growth restriction in a previous pregnancy
- Current uncontrolled hypertension
- History of myocardial infarction; unstable ischemic heart disease; stroke; severe and/or uncontrolled hepatic, gastrointestinal, renal, pulmonary, cardiovascular, psychiatric, neurologic, hypertension, or musculoskeletal disorder; and/or any other medical or uncontrolled autoimmune disorder(s)
- Having any confirmed or suspected clinical immunodeficiency syndrome or having a family history of congenital or hereditary immunodeficiency, unless confirmed to be absent in the participant
- History of solid organ or bone marrow transplantation, except for a corneal transplant performed >12 weeks before screening
- Having inflammatory or autoimmune diseases requiring immunosuppressive therapies that may jeopardize the safety of the participant
- Currently having a malignancy or has a history of malignancy within 3 years before screening (except for localized basal cell carcinoma and/or squamous cell carcinoma skin cancer that has been adequately treated with no evidence of recurrence for ≥3 months before the first study intervention, or cervical carcinoma in situ that has been treated with no evidence of recurrence for ≥3 months before the first study intervention)
- Known allergies, hypersensitivity, intolerance to excipients in the study intervention, or previous severe immediate hypersensitivity reaction, such as anaphylaxis to therapeutic

proteins

- History of receiving anti-FcRn therapies or receiving rituximab or eculizumab in the last 6 months
- History of receiving a BCG vaccination within 1 year prior to the first study intervention, or the need for a BCG vaccine during the study or within ≥8 weeks after the last study intervention
- Receiving a live virus vaccination during the current pregnancy or the need for a live virus vaccination during the study while receiving study intervention or ≥8 weeks after the last study intervention
- Receiving systemic corticosteroids or other immunosuppressants for disorders unrelated to the pregnancy
- Receiving or planning to receive plasmapheresis, immunoadsorption therapy, IVIG, or any IgG Fc-related protein therapeutics during the current pregnancy
- Receiving an investigational intervention within 3 months or 5 half-lives (whichever is longer) prior to the first study intervention or is currently enrolled or plans to enroll in an investigational study
- Having a severe infection, including opportunistic infections, chronic infection, or requiring chronic treatment with anti-infectives
- Active infection with Coxsackievirus, syphilis, cytomegalovirus, toxoplasmosis, or herpes simplex virus type 1 or 2, as evidenced by clinical signs and symptoms and serology results from the central laboratory
- History of severe or recurrent pyelonephritis or ≥4 lower urinary tract infections in the past year or in a previous pregnancy

- History of atypical mycobacterial disease or herpes zoster infection within the last 6 months
- History of being positive for HIV 1 or 2 antibodies or being positive for HIV at screening
- Positive for hepatitis B virus infection or seropositive for antibodies to hepatitis C virus
- COVID-19 infection during the 4 weeks prior to baseline
- Presence of abnormal hematologic laboratory values during screening
    - Hemoglobin <80 g/L
    - White blood cells <3.0 GI/L
    - Neutrophils <1.5 GI/L
    - Platelets <100 GI/L
- History of drug or alcohol abuse, according to *Diagnostic and Statistical Manual of Mental Disorders*, *Fifth Edition* criteria, within 1 year before screening
- Having any condition for which, in the opinion of the investigator, participation would not be in the best interest of the participant or fetus/neonate/infant or that could prevent, limit, or confound the protocol-specified assessment

BCG, Bacillus Calmette-Guérin; cffDNA, cell-free fetal deoxyribonucleic acid; ECG, electrocardiogram; FcRn, neonatal Fc receptor; GA, gestational age; HDFN, hemolytic disease of the fetus and newborn; IgG, immunoglobulin G; IUT, intrauterine transfusion; IVIG, intravenous immunoglobulin; MCA-PSV, middle cerebral artery peak systolic velocity; MoM, multiples of the median; ULN, the upper limit of normal.

Table 2. Study Objectives and Endpoints

Primary objective	**Primary endpoint**
To evaluate the efficacy of nipocalimab compared with placebo on reducing the risk of fetal anemia with liveborn neonates in pregnant participants at risk for severe HDFN	Proportion of pregnancies that do not result in fetal loss (due to any reason), IUT, hydrops fetalis, or neonatal death (due to any reason) through the neonatal period (4 weeks of age or 41 weeks PMA, whichever is later)
Secondary objectives	**Secondary endpoints**
To evaluate the efficacy of nipocalimab compared with placebo in reducing the severity of HDFN as measured by a composite severity index in pregnant participants and their neonates/infants at risk for severe HDFN	Number of participants with HDFN by severity. The severity of HDFN is measured by a composite HDFN severity index and defined as: • 5 (fatal): fetal or neonatal death due to any reason • 4 (severe): hydrops fetalis (in fetus or newborn) or receiving IUT during pregnancy as a result of HDFN, but not 5 (fatal) • 3 (moderate): neonatal exchange transfusions received as a result of HDFN-related hemolysis and jaundice, but not 4 (severe) or 5 (fatal) • 2 (mild): neonatal simple transfusions received due to HDFN after birth, with or without phototherapy, but not 3 (moderate), 4 (severe), or 5 (fatal)

	• 1 (minimal or none): not 2 (mild), 3 (moderate), 4 (severe), or 5 (fatal) as described above
To evaluate the efficacy of nipocalimab compared with placebo on delaying the onset of severe HDFN in pregnant participants at risk for severe HDFN	Time to first occurrence of IUT or hydrops fetalis
To evaluate the efficacy of nipocalimab compared with placebo on reducing the risk of mortality and morbidity in neonates born to participants at risk for severe HDFN	The modified NMMI in liveborn neonates through 38 weeks PMA or at discharge (if <38 weeks PMA)
To evaluate the impact of nipocalimab compared with placebo on antenatal HDFN management and outcomes in pregnant participants at risk for severe HDFN	Number of IUTs received during pregnancy
Other objectives	**Other endpoints**
To evaluate the impact of nipocalimab compared with placebo on other antenatal HDFN management and outcomes in pregnant participants at risk for severe HDFN	• Proportion of pregnancies with fetal loss • Proportion of pregnancies with fetal or neonatal death (through the neonatal period) as a result of HDFN • Proportion of pregnancies with hydrops fetalis • Proportion of pregnancies receiving IUT during pregnancy

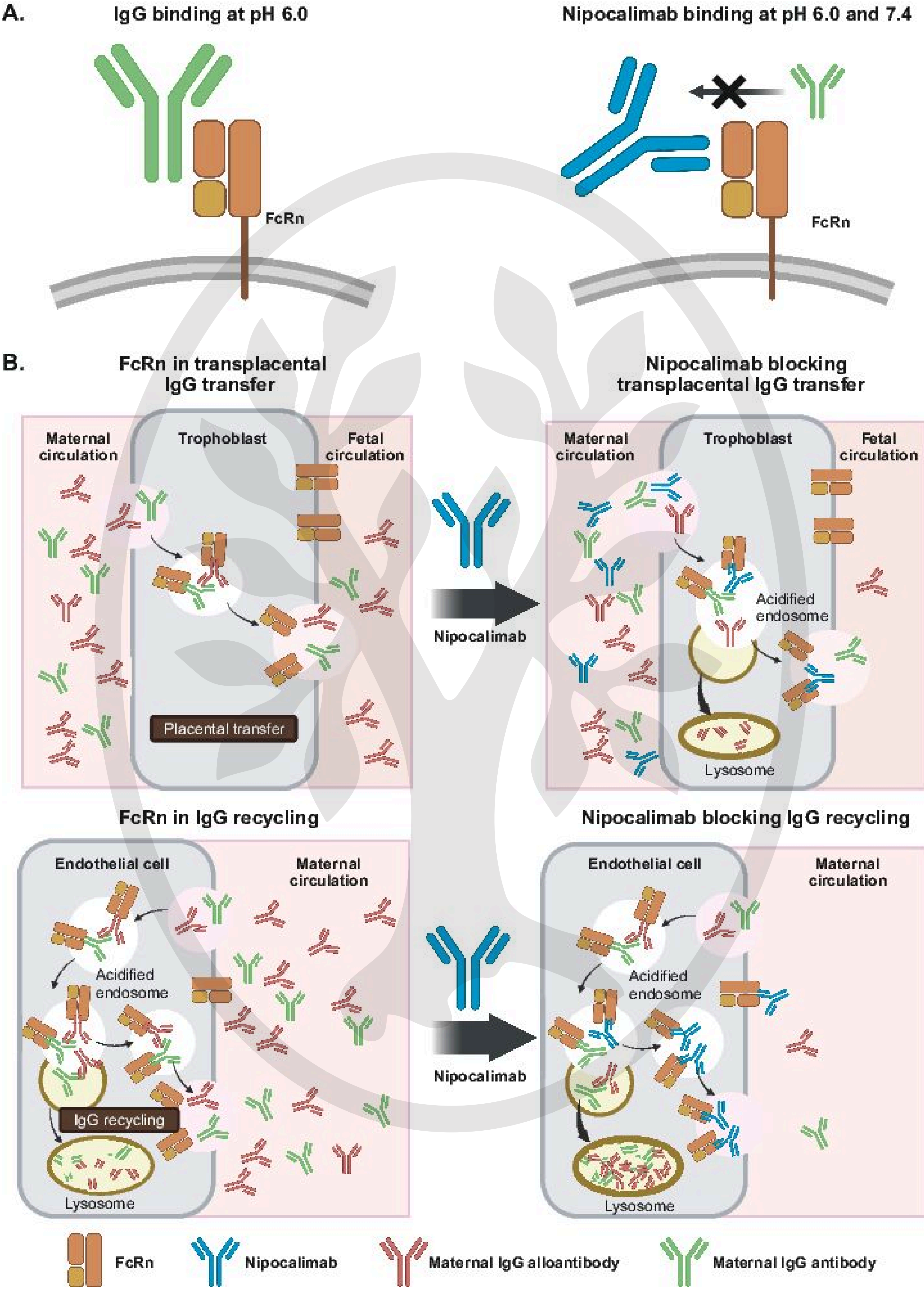
	• GA at first IUT • Proportion of pregnancies receiving >1 IUT during pregnancy • Proportion of pregnancies receiving IUT or HDFN resulting in fetal demise at <20 weeks GA • GA at delivery
To evaluate the impact of nipocalimab compared with placebo on neonatal HDFN management and other outcomes of HDFN in neonates/infants of pregnant participants at risk for severe HDFN	• Proportion of pregnancies with neonatal death through the neonatal period • Proportion of liveborn neonates with HDFN-related morbidities other than anemia and hyperbilirubinemia/jaundice • Absolute weight and weight changes over time from birth through Week 104 in liveborn neonates/infants • Length of stay in neonatal intensive care unit for liveborn neonates • Proportion of liveborn neonates receiving exchange transfusions for HDFN • Number of neonatal exchange transfusions per liveborn neonate • Proportion of liveborn neonates/infants with simple transfusions for HDFN through the neonatal period or 12 weeks

	after birth • Number of simple transfusions for HDFN per liveborn neonate/infant through the neonatal period or 12 weeks after birth • Proportion of liveborn neonates with hyperbilirubinemia treated with phototherapy • Number of days of phototherapy received for hyperbilirubinemia per liveborn neonate • Proportion of liveborn neonates/infants receiving IVIG for HDFN treatment

To evaluate the safety of nipocalimab in pregnant participants at risk for severe HDFN, including pregnancy outcomes, compared with placebo	**Maternal/fetal safety outcomes:** • Maternal death, AEs, serious AEs, AEs of special interest (infections requiring oral/IV anti-infective agents, maternal hypoalbuminemia with albumin <20 g/L), AEs leading to discontinuations, infections, serious infections, infusion reactions, and hypersensitivity reactions • Maternal pregnancy complications • IUT-related complications **Pregnancy outcomes:** • Proportion of pregnancies with cesarean delivery, cesarean delivery due to IUT complications, preterm birth, fetal growth restriction, and preeclampsia
To evaluate the safety of neonates/infants born to nipocalimab-treated participants compared with neonates/infants born to participants who received placebo	**Neonate/infant safety and development outcomes:** • Proportion of liveborn neonates/infants who died • Proportion of liveborn neonates/infants with AEs, serious AEs, AEs of special interest (infections requiring oral/IV anti-infective agents, infant IgG decreased with IgG <3.0 g/L at or after Week 52),

	infections, and serious infections • Proportion of liveborn neonates/infants receiving IVIG for non-HDFN indications • Proportion of liveborn neonates/infants with abnormal hearing • Bayley Scales of Infant and Toddler Development at Weeks 52 and 104 in infants
To evaluate the impact of nipocalimab on patient- and caregiver-reported outcomes in participants at risk for severe HDFN and their neonates/infants	**Maternal patient–reported outcomes:** • Change from baseline in GAD-7 over time during pregnancy (baseline and Week 30) and at postpartum Week 4 • Change from baseline in domain scores, physical component summary, and mental component summary in SF-36 v2 Acute over time during pregnancy (baseline and Week 30) and at postpartum Week 4 • Change from baseline in EQ-5D-5L VAS scores and in EQ-5D index scores by visit over time during pregnancy (baseline and Week 30) and at postpartum Week 4 **Neonate/infant caregiver–reported outcomes:** • Summary of IQI score over time (at Weeks 4, 8, and 52)

AE, adverse event; EQ-5D, EuroQol 5-Dimension Descriptive System; EQ-5D-5L, EuroQol 5-Dimension Questionnaire; GA, gestational age; GAD-7, Generalized Anxiety Disorder 7-item; HDFN, hemolytic disease of the fetus and newborn; IgG, immunoglobulin G; IQI, Infant health-related Quality of Life Instrument; IUT, intrauterine transfusion; IVIG, intravenous immunoglobulin; NMMI, neonatal mortality and morbidity index; PMA, postmenstrual age; SF-36 v2, Short Form 36 Health Survey version 2; VAS, visual analogue scale.


A.
IgG binding at pH 6.0
FcRn
Nipocalimab binding at pH 6.0 and 7.4
FcRn
B.
FcRn in transplacental IgG transfer
Maternal circulation
Trophoblast
Fetal circulation
Placental transfer
Nipocalimab
Nipocalimab blocking transplacental IgG transfer
Maternal circulation
Trophoblast
Fetal circulation
Acidified endosome
Lysosome
FcRn in IgG recycling
Endothelial cell
Maternal circulation
Acidified endosome
IgG recycling
Lysosome
Nipocalimab
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FcRn
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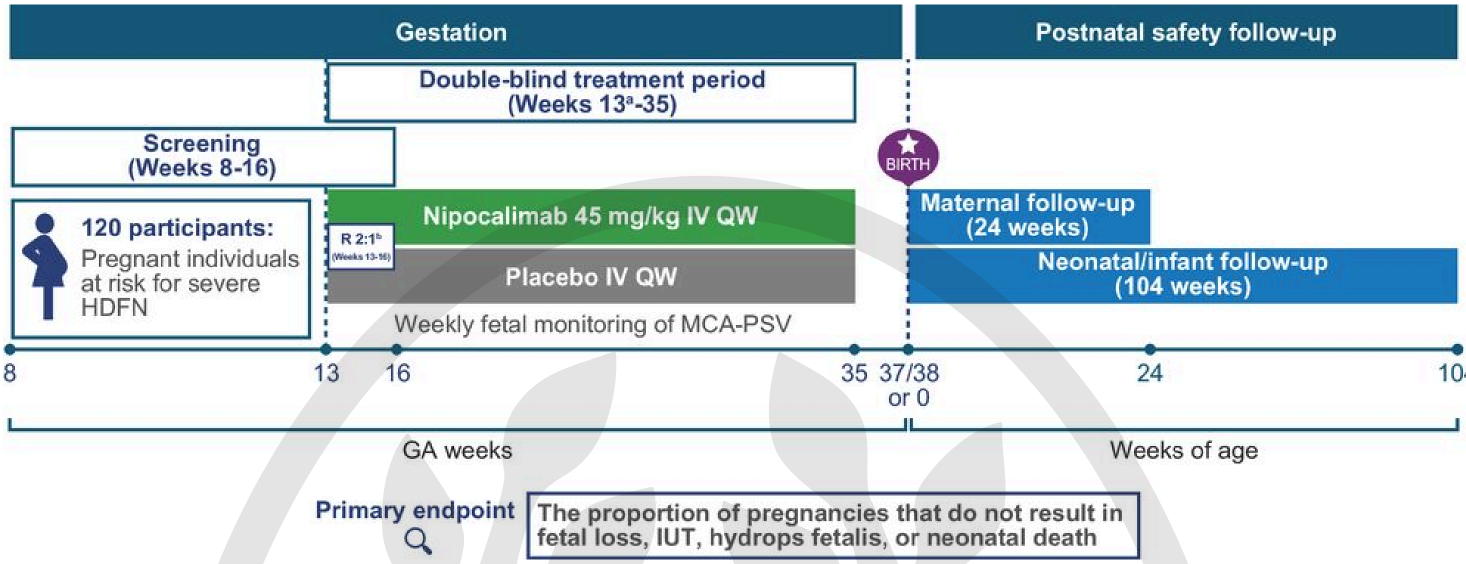
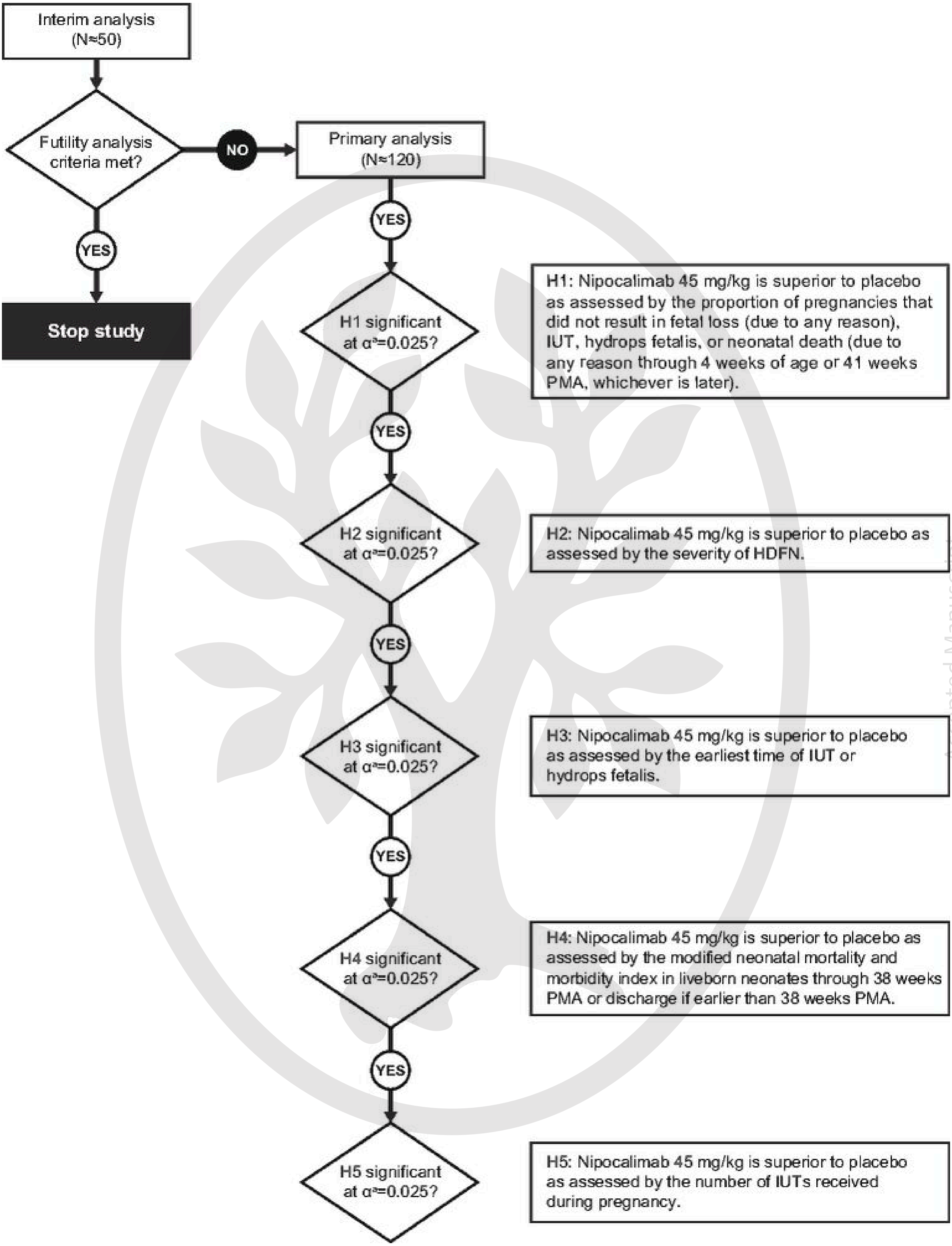
Maternal IgG alloantibody
Maternal IgG antibody


Gestation
Postnatal safety follow-up
Double-blind treatment period (Weeks 13a-35)
Screening (Weeks 8-16)
BIRTH
120 participants:
Pregnant individuals at risk for severe HDFN
R 2:1b
Nipocalimab 45 mg/kg IV QW
Placebo IV QW
Weekly fetal monitoring of MCA-PSV
Maternal follow-up (24 weeks)
Neonatal/infant follow-up (104 weeks)
8
13
16
35
37/38 or 0
24
104
GA weeks
Weeks of age
Primary endpoint
The proportion of pregnancies that do not result in fetal loss, IUT, hydrops fetalis, or neonatal death


Interim analysis (N≈50)
Futility analysis criteria met?
NO
YES
Stop study
Primary analysis (N≈120)
YES
H1 significant at α=0.025?
H1: Nipocalimab 45 mg/kg is superior to placebo as assessed by the proportion of pregnancies that did not result in fetal loss (due to any reason), IUT, hydrops fetalis, or neonatal death (due to any reason through 4 weeks of age or 41 weeks PMA, whichever is later).
YES
H2 significant at α=0.025?
H2: Nipocalimab 45 mg/kg is superior to placebo as assessed by the severity of HDFN.
YES
H3 significant at α=0.025?
H3: Nipocalimab 45 mg/kg is superior to placebo as assessed by the earliest time of IUT or hydrops fetalis.
YES
H4 significant at α=0.025?
H4: Nipocalimab 45 mg/kg is superior to placebo as assessed by the modified neonatal mortality and morbidity index in liveborn neonates through 38 weeks PMA or discharge if earlier than 38 weeks PMA.
YES
H5 significant at α=0.025?
H5: Nipocalimab 45 mg/kg is superior to placebo as assessed by the number of IUTs received during pregnancy.