A Prospective, Randomized, Clinical Trial to Compare Adverse Birth Outcomes in Pregnant Women Receiving Quadrivalent Recombinant Influenza Vaccine (RIV4) versus Quadrivalent Inactivated Influenza Vaccine (IIV4)

Short Title: Safety of RIV4 versus IIV4 in Pregnant Women

Centers for Disease Control and Prevention
Clinical Immunization Safety Assessment (CISA) Project

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STATEMENT OF COMPLIANCE

- This trial will be conducted in compliance with the protocol, the International Conference on Harmonization (ICH) Guideline E6-Good Clinical Practice (GCP), and the applicable guidelines and regulatory requirements from the United States (US) Code of Federal Regulations (CFR), 45 CFR Part 46.
- All study personnel with subject contact have completed Human Subjects Protection Training.

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PROTOCOL SUMMARY

Title:	A Prospective, Randomized, Clinical Trial to Compare Adverse Birth Outcomes in Pregnant Women Receiving Quadrivalent Recombinant Influenza Vaccine (RIV4) versus Quadrivalent Inactivated Influenza Vaccine (IIV4)
Phase:	Phase 4
Population:	430 adult pregnant women ≥ 18 years at ≤ 34 weeks gestation who plan on receiving RIV4 or IIV4 during the current pregnancy in accordance with the Advisory Committee on Immunization Practices (ACIP) and American College of Obstetricians & Gynecologists (ACOG) national recommendations.
Clinical Sites:	Three: Duke University (Lead); Cincinnati Children's Hospital Medical Center (Contributor); Boston Medical Center (Contributor)
Study Duration:	60 months (approximately 12 months to recruit/enroll), maximum of 24 months to follow, 24 months to perform analysis and laboratory assays
Participant Duration:	Up to 12 months depending upon gestational age at enrollment and delivery. Will be followed for 90 days postpartum.
Description of Study Procedures:	This is a prospective, randomized clinical trial. During the study, pregnant women will be randomized (1:1) to receive RIV4 or IIV4. Vaccines will be administered by licensed providers. Prior influenza vaccine history will be verified by medical record review when possible. Injection-site (local) and systemic reaction data will be assessed on vaccination day and during the 8 days following vaccination using either identical web-based or paper diaries, depending on study participant preference. Maternal serum samples will be collected for antibody titers relevant to Influenza at time points that include: prior to vaccination and ~29 days post vaccination. At Duke University, maternal and infant cord blood will be collected at delivery and analyzed for the same antibody titers. At other clinical sites, these delivery samples will only be collected if feasible.

	Pregnant women will be followed through 90 days postpartum. with comprehensive obstetric and neonatal outcomes obtained from medical record review.
Objectives:	Primary Objectives: • To compare the proportions of adverse birth outcomes in pregnant women vaccinated with RIV4 versus IIV4 Secondary Objectives: • To compare proportions of preterm birth after RIV4 versus IIV4 vaccination • To compare proportions of combined fetal and neonatal death after RIV4 versus IIV4 vaccination • To compare proportions of spontaneous abortion after RIV4 versus IIV4 vaccination • To compare proportions of moderate/severe solicited reactogenicity events in pregnant women vaccinated with RIV4 versus IIV4 Exploratory Objectives: • To compare and describe serious adverse events (SAE) in pregnant women vaccinated with RIV4 versus IIV4 • To compare proportions of adverse pregnancy and birth outcomes of clinical interest after RIV4 versus IIV4 vaccination • To compare and describe health outcomes through 90 days of life in infants born to women after RIV4 versus IIV4 vaccination • To compare maternal immune responses to influenza antigens after RIV4 versus IIV4 vaccination • To compare cord blood antibody levels for influenza antigens after RIV4 versus IIV4 vaccination
Outcome Measures:	Primary: • Proportions of adverse birth outcomes in pregnant women vaccinated with RIV4 versus IIV4 Secondary: • Proportions of preterm birth after RIV4 versus IIV4 vaccination • Proportions of combined fetal and neonatal death after RIV4 versus IIV4 vaccination • Proportions of spontaneous abortion after RIV4 versus IIV4 vaccination

	• Proportions of pregnant women with moderate/severe solicited reactogenicity events (local and systemic) within 8 days after vaccination with RIV4 versus IIV4 Exploratory: • Frequency and descriptions of serious adverse events in pregnant women vaccinated with RIV4 versus IIV4 during the 42 days after vaccination • Frequency and descriptions of serious adverse events in pregnant women vaccinated with RIV4 versus IIV4 after vaccination during the study • Proportions of infants born small for gestational age after RIV4 versus IIV4 vaccination • Proportion of pregnant women with clinical chorioamnionitis, after RIV4 versus IIV4 vaccination • Proportion of women with preeclampsia or eclampsia after RIV4 versus IIV4 vaccination • Proportion of infants with medically-attended adverse events through 90 days of life after maternal RIV4 versus IIV4 vaccination • Frequency and description of SAEs in infants through 90 days of life after maternal RIV4 versus IIV4 vaccination • Proportion of infants with neonatal death after maternal RIV4 versus IIV4 vaccination • Proportion of pregnant women achieving seroconversion at day 29 after RIV4 versus IIV4 vaccination [a Hemagglutination Inhibition Assay (HAI) titer $\geq$ 1:40 at day 29 if the baseline titer is < 1:10 or a minimum four-fold rise in HAI titer if the baseline titer is $\geq$ 1:10) for each influenza vaccine antigen] • Proportion of pregnant women with a seroprotective HAI titer (≥ 1:40) post-immunization at day 29 after RIV4 versus IIV4 vaccination for each influenza vaccine antigen • The maternal geometric mean HAI titer (GMT) pre and post-vaccination at day 29 after RIV4 versus IIV4 for each influenza vaccine antigen and the post/pre-vaccination ratio • Proportion of infants with cord blood seroprotective HAI titer (≥ 1:40) for each influenza vaccine antigen after maternal RIV4 versus IIV4 vaccination • Infant cord blood geometric mean HAI titer (GMT) for each influenza vaccine antigen after maternal RIV4 versus IIV4 vaccination • Ratio of cord blood to maternal influenza antibody titers at time of delivery for each influenza vaccine antigen after maternal RIV4 versus IIV4 vaccination

Estimated Time to Complete Enrollment:	Approximately 6 months for enrollment for the 2019-20 influenza season and 6 months for enrollment during the 2020-2021 influenza season.

1 BACKGROUND

1.1 Background

Pregnant women are at an increased risk for influenza-related morbidity due to changes in immunology and cardiorespiratory physiology.[1] Influenza-infected pregnant women have higher rates of hospitalization and cardiopulmonary complications during influenza seasons, compared to the general public.[2] Further, some studies suggest influenza can be associated with adverse birth outcomes such as preterm birth and fetal demise.[3] Complications of influenza during pregnancy can be even higher during pandemics, as seen in the 2009 H1N1 pandemic when the preterm birth rate rose threefold among infected pregnant women and 5% of all related deaths reported to CDC occurred in pregnant women, yet they only encompass 1% of the total population.[4] The Advisory Committee on Immunization Practices (ACIP) and the American College of Obstetricians & Gynecologists (ACOG) have recommend an age-appropriate inactivated influenza vaccine (IIV) for all women who are or will be pregnant during influenza season.[5,6] Initial recommendations for IIV administration during pregnancy were extrapolated from the documented efficacy and safety of IIV in non-pregnant adults. Until the 2009 pandemic, there were only a few small-scale studies on the safety and immunogenicity of maternal influenza vaccination.[7] Recent large cohort studies and randomized controlled trials have supported the safety and effectiveness of IIV in pregnancy.[8-12]

Due to continuous evolution of influenza glycoproteins, the World Health Organization (WHO) meets to consider and make recommendations for updates in influenza vaccine composition twice a year. For the northern hemisphere, the WHO generally makes these recommendations in February. The U.S. Food and Drug Administration (FDA) Vaccines and Related Biological Products Advisory Committee (VRBPAC) subsequently meets in late February or early March to determine composition of the vaccines to be produced, distributed, and administered in the United States starting late summer of the same year. Since the 1940s, standard IIV manufacturing has involved the use of embryonated hen eggs. While effective, this technology is hampered by the requirement for pathogen-free eggs and specialized manufacturing facilities in which throughput can only be amplified by increasing the number of eggs. This process is also vulnerable to avian influenza viruses. Finally, the lengthy production process hinders potential for rapid scale-up in response to emerging needs such as a pandemic or supply shortage.

HHS has supported development of alternate influenza vaccine technology as a component of influenza preparedness.[13] In January 2013 the U.S. FDA approved Flublok®, the first recombinant influenza vaccine (RIV), RIV contains influenza virus antigens manufactured using an insect baculovirus expression system and recombinant technology. RIV does not contain ovalbumin. FDA licensed a quadrivalent formulation of RIV, Flublok® Quadrivalent (RIV4) in 2016.[14] RIV may offer some advantages to egg-based vaccines. Production time during a pandemic may be shorter, and permit earlier availability of vaccine (though this has not yet been demonstrated). In addition, it is not subject to egg-adaptation mutations that can occur in influenza vaccine virus strains as they are passaged through eggs. Some evidence suggests that such mutations may affect vaccine effectiveness.[15] However, specific effectiveness data for RIV are currently limited due to its relatively limited use to date.

In 2017, ACIP expanded its influenza vaccine recommendation for pregnant women to include RIV with no stated preference between RIV and IIV products.[6] Data on uptake of RIV in pregnant women following the 2017 ACIP recommendations to include this vaccine in its maternal influenza recommendations are not available. Data from CDC's Vaccine Safety Datalink (VSD) suggest that use of RIV in the adult population has been

limited (VSD, unpublished data, 2019). However, it is possible that use among pregnant women might increase during a pandemic or a season of vaccine shortage, as RIV might be available earlier than other vaccines, and pregnant women are among the populations recommended to be a focus for vaccination efforts when vaccine supply is limited. Furthermore, use of RIV may become more widespread in future seasons due to concerns about decreased vaccine effectiveness related to egg adaptation.

Although approved for adults ≥ 18 years, prelicensure studies for RIV specifically excluded pregnant women. To date, safety data on RIV use during pregnancy are limited to manufacturer registry data, regulatory data that describes incidental pregnancies that occurred during the course of clinical trials, and passive surveillance data, including data obtained from a large influenza vaccination campaign in Mongolia.[6] These data are reassuring, with no indication of adverse reactogenicity or obstetric events. While the Vaccine Adverse Event Reporting System (VAERS) can provide information on safety of licensed vaccines, there are inherent limitations of this passive surveillance system. In the VSD, the number of RIV doses administered to pregnant women is expected to be insufficient for comparative studies. ACIP stated during their June 2017 meeting that safety data on RIV in pregnant women are limited.[6] Understanding whether benefit is conferred with adequate protection against influenza illness in pregnant is also important. Given the obligatory changes in maternal immunology to allow for fetal tolerance, the immunogenicity of RIV during pregnancy should also be assessed.

1.2 Summary & Rationale

ACIP currently recommends that pregnant women aged ≥ 18 years receive an age-appropriate IIV or RIV (Flublok® Quadrivalent. While there is no specific reason to expect RIV to be unsafe during pregnancy, the lack of systematically collected data on the safety of RIV in pregnancy places a burden on obstetric providers and pregnant women to assess risk-benefit balance in the context of limited data. Establishing the safety of RIV is imperative to support current use among pregnant women during seasonal vaccination efforts and in the event that RIV is widely used during a pandemic or supply shortage when pregnant women may be prioritized for vaccination. Systematically collected data from a rigorous randomized controlled trial of RIV in pregnant women will provide much needed information on the overall safety for pregnant women and their infants, including adverse pregnancy outcomes, local and systemic reactogenicity, and vaccine-related adverse events. Additionally, assessing immunogenicity following maternal RIV will add to the evidence base for maternal vaccination.

2 STUDY OBJECTIVES

Primary Objective:

- PO1: To compare the proportions of adverse birth outcomes in pregnant women vaccinated with RIV4 versus IIV4.

  Research hypothesis: The proportion of pregnant women with adverse birth outcomes will be noninferior (not higher) after receipt of RIV4 compared to IIV4

Secondary Objectives:

- SO1: To compare proportions of preterm birth after RIV4 versus IIV4 vaccination
- SO2: To compare proportions of combined fetal and neonatal death after RIV4 versus IIV4 vaccination
- SO3: To compare proportions of spontaneous abortion after RIV4 versus IIV4 vaccination
- SO4: To compare proportions of moderate/severe solicited reactogenicity events in pregnant women vaccinated with RIV4 versus IIV4

Exploratory Objectives:

- EO1: To compare and describe serious adverse events (SAE) in pregnant women vaccinated with RIV4 versus IIV4
- EO2: To compare proportions of adverse pregnancy and birth outcomes of clinical interest after RIV4 versus IIV4 vaccination
- EO3: To compare and describe health outcomes through 90 days of life in infants born to women after RIV4 versus IIV4 vaccination
- EO4: To compare maternal immune responses to influenza antigens after RIV4 versus IIV4 vaccination
- EO5: To compare cord blood antibody levels for influenza antigens after RIV4 versus IIV4 vaccination

2.1 Study Outcome Measures

2.1.1 Primary Outcome Measure:

POM1: Proportions of adverse birth outcomes in pregnant women vaccinated with RIV4 versus IIV4
Adverse birth outcome is a composite of occurrence of at least one of the following: preterm birth, spontaneous abortion, fetal death, or neonatal death.

2.1.2 Secondary Outcome Measures:

- SOM1: Proportions of preterm birth after RIV4 versus IIV4 vaccination
- SOM2: Proportions of combined fetal and neonatal death after RIV4 versus IIV4 vaccination
- SOM3: Proportions of spontaneous abortion after RIV4 versus IIV4 vaccination
- SOM4: Proportions of pregnant women with moderate/severe solicited reactogenicity events (local and systemic) within 8 days after vaccination with RIV4 versus IIV4

2.1.3 Exploratory Outcome Measures:

- EOM1.1: Frequency and descriptions of serious adverse events in pregnant women vaccinated with RIV4 versus IIV4 during the 42 days after vaccination
- EOM1.2: Frequency and descriptions of serious adverse events in pregnant women vaccinated with RIV4 versus IIV4 during the study
- EOM2.1. Proportions of infants born small-for-gestational age after RIV4 versus IIV4 vaccination.

- EOM2.2: Proportion of pregnant women with clinical chorioamnionitis after RIV4 versus IIV4 vaccination
- EOM2.3: Proportion of women with preeclampsia or eclampsia after RIV4 versus IIV4 vaccination
- EOM3.1: Proportion of infants with medically-attended events through 90 days of life after maternal RIV4 versus IIV4 vaccination
- EOM3.2: Frequency and description of SAEs in infants through 90 days of life after maternal RIV4 versus IIV4 vaccination.
- EOM3.3 Proportion of infants with neonatal death after maternal RIV4 versus IIV4 vaccination
- EOM4.1: Proportion of pregnant women achieving seroconversion at day 29 after RIV4 versus IIV4 vaccination [a Hemagglutination Inhibition Assay (HAI) titer ≥ 1:40 at day 29 if the baseline titer is < 1:10 or a minimum four-fold rise in HAI titer if the baseline titer is ≥ 1:10) for each influenza vaccine antigen]
- EOM4.2: Proportion of pregnant women with a seroprotective HAI titer (≥ 1:40) post-immunization at day 29 after RIV4 versus IIV4 vaccination for each influenza vaccine antigen
- EOM4.3: The maternal geometric mean HAI titer (GMT) pre and post-vaccination at day 29 after RIV4 versus IIV4 for each influenza vaccine antigen and the post/pre-vaccination ratio
- EOM5.1: Proportion of infants with cord blood seroprotective HAI titer (≥ 1:40) for each influenza vaccine antigen after maternal RIV4 versus IIV4 vaccination
- EOM5.2: Infant cord blood geometric mean HAI titer (GMT) for each influenza vaccine antigen after maternal RIV4 versus IIV4 vaccination
- EOM5.3: Ratio of cord blood to maternal influenza antibody titers at time of delivery for each influenza vaccine antigen after maternal RIV4 versus IIV4 vaccination

3 STUDY DESIGN

3.1 Main study design

This study is a prospective, randomized clinical trial of approximately 430 pregnant women enrolled at Duke University Medical Center (Lead Contractor), Cincinnati Children's Hospital Medical Center (Contributing Contractor), and Boston Medical Center (Contributing Contractor). Pregnant women ≥ 18 years of age at ≤ 34 weeks gestation, will be enrolled and randomized 1:1 to receive either quadrivalent recombinant influenza vaccine (RIV4) or quadrivalent inactivated influenza vaccine (IIV4). The designated vaccine brands for this study are Flublok® quadrivalent (Sanofi) and quadrivalent Flulaval® (GSK) (comparator IIV4). However, the choice of IIV4 vaccine administered will be based on availability from manufacturers prior to the 2019-2020 and 2020-2021 influenza seasons.

After vaccination, participants and their infants will be followed to assess adverse pregnancy and birth outcomes, maternal vaccine reactogenicity, immunogenicity, serious adverse events, and short-term infant outcomes. Maternal and infant outcomes will be collected by medical record review from enrollment through 90 days postpartum.

3.2 Laboratory studies

3.2.1 Serologic studies – We will evaluate pre- and post-vaccination serologic responses in both IIV4 and RIV4 groups. IgG levels will be measured for the

specific viral strains included in the 2019-2020 and 2020-2021 quadrivalent influenza vaccines using hemagglutination inhibition assays at the Duke Regional Biocontainment Laboratory (RBL) Virology Unit. Venous blood (approximately 15 mL of blood) will be collected from each participant before and 29 (-7 to +14) days after vaccination. At Duke, maternal and cord blood (approximately 15mL of blood each) collection will be performed at delivery for serological analysis in both RIV4 and IIV4 vaccination groups. At the other sites, these samples (maternal and cord blood) will be collected during delivery only if feasible for similar serological analysis. An assessment of placental antibody transfer (maternal:cord antibody ratio) will be determined.

3.2.2 Future studies - In addition to the specified analyses described thus far, there may be other tests or assays that have yet to be identified that may be important for interpreting our study findings or of relevance to maternal-infant health outcomes. Therefore, participants will be offered, through an opt-in/opt-out strategy to allow for the storage of any remaining blood (serum/plasma) after all specified analyses have been completed. Additional laboratory assays may test for antibodies against other bacteria or viruses, markers of inflammation, or used in research on the health of mothers and infants. Specimens banked for use in other studies will be linked to information (including identifying information) that participants provided to the study. Because it is unknown if future testing will be of any utility, results of future testing will not be provided.
In addition, participants will be offered, again through an opt-in/opt-out strategy, to allow study staff to contact them in the future to take part in other research studies.

4 STUDY ENROLLMENT AND WITHDRAWALS

4.1 Participant Inclusion Criteria

Participants who meet all of the following criteria will be eligible to participate in this interventional study.

1. Pregnant, as determined by medical history
2. Age ≥ 18 years of age at enrollment
3. Intention of receiving influenza vaccine based on ACIP-CDC guidelines
4. Willing to provide written informed consent prior to initiation of any study procedures
5. Gestational age at vaccination ≤ 34 weeks 0 days based on reconciliation of last menstrual period and ultrasound dating. Estimated due date (EDD) and Gestational Age (GA-EDD) will be based on reconciliation of “sure” first day of the last menstrual period (LMP) and earliest dating ultrasound. If the LMP is uncertain, then the

Table 1. Ultrasound Parameters for Using Sure LMP to Determine Gestational Age	
Gestational age at first ultrasound by LMP	**Ultrasound agreement with LMP**

earliest dating ultrasound will be used to determine EDD and GA. If the ultrasound derived-EDD is in agreement with sure-LMP derived EDD (**Table 1**), then the LMP-derived EDD is used to determine GA. If the ultrasound derived EDD is not in agreement with the LMP-derived EDD, the ultrasound-derived EDD is used to determine GA.

8 6/7 wk or less	± 5 days
9 0/7 wk to 13 6/7 wk	± 7 days
14 0/7 wk to 15 6/7 wk	± 7 days
16 0/7 wk to 21 6/7 wk	± 10 days
22 0/7 wk to 27 6/7 wk	± 14 days
28 0/7 wk and beyond	± 21 days

6. English or Spanish literate
7. Intention of being available for entire study period and complete all relevant study procedures, including follow-up phone calls and collection of delivery information.

4.2 Participant Exclusion Criteria

Participants who meet any of the following criteria will not be eligible to participate in this study:

1. Influenza vaccine receipt during 2019-2020 or 2020-2021 influenza season prior to study enrollment.
2. Participation in this study in 2019-2020 influenza season
3. Any condition that may interfere with assessment of local injection site reactions, e.g. obscuring tattoos
4. Known or suspected immunosuppression as a result of an underlying illness or treatment
5. Use of anti-cancer chemotherapy or radiation therapy within the preceding 36 months
6. Use of oral or parenteral corticosteroids (≥ 20mg/day prednisone equivalent) or high-dose inhaled glucocorticoid for ≥ 14 consecutive days within the preceding 30 days
7. Has an active neoplastic disease (excluding non-melanoma skin cancer), a history of any hematologic malignancy, current bleeding disorder, or taking anticoagulants (a daily aspirin is acceptable)
8. Has a history of receiving immunoglobulin or other blood product (with exception of Rh immunoglobulin) within the 3 months prior to study vaccination.
9. History of febrile illness (≥ 100.4°F or 38°C) within the past 24 hours prior to study vaccination
10. Contraindication to IIV or RIV receipt including history of severe allergic reaction after a previous dose of any influenza vaccine; or to a vaccine component, including egg protein
11. History of Guillain-Barré syndrome within 6 weeks of a prior dose of any influenza vaccine
12. Receipt of any licensed vaccine within 7 days prior to study vaccination or intention of receiving any vaccines during 8-day post-vaccination period
13. Receipt of live vaccine during current pregnancy
14. Signs or symptoms of active preterm labor, defined as regular uterine contractions with cervical change (dilation/effacement)
15. Known multi-fetal gestation or fetal congenital anomaly, e.g. genetic abnormality or major congenital malformation based on antenatal ultrasound
16. Anyone who is already enrolled or plans to enroll in another randomized clinical trial with any drug, vaccine or medical device. Co-enrollment in observational or behavioral intervention studies are allowed at any time

17. Any condition which, in the opinion of the investigators, may pose a health risk to the participant or interfere with the evaluation of the study objectives.
18. Anyone who is a relative of any research study personnel or is an employee supervised by study staff

4.3 Recruitment

Pregnant women at ≤ 34 weeks, ≥ 18 years old, who are planning to receive influenza vaccination during their current pregnancy will be recruited at prenatal clinics whose patients deliver at Duke University Hospital (Duke), University of Cincinnati Medical Center (Cincinnati), or Boston Medical Center (Boston). Pregnant women will be pre-screened for eligibility via medical record review. The study will be introduced to a potential participant by a member of their care team. Potential participants that fall within the ≤ 34 weeks gestation range will be approached by a member of the research team for study enrollment. Medical and obstetric history, including prior influenza vaccine history, will be obtained via participant self-report with verification by chart review whenever feasible (including medical records, employee health records, immunization registry records, and pharmacy records).

4.4 Reasons for and Handling of Withdrawals

The following may be reason for study withdrawal:

- As deemed necessary by the principal investigator (PI).
- Participant withdrawal of consent.
- Loss to follow-up.
- Termination of the study by the sponsor.

Participants may withdraw their consent for study participation at any time and for any reason, without penalty. Participants who withdraw from the study prior to receiving study vaccine will be replaced. Participants who withdraw from the study after receiving vaccine will not be replaced. Every attempt should be made to collect all data specified by the protocol, including collection of pregnancy outcome/safety data via medical record review for participants who request withdrawal from study interventions/procedures.

4.5 Termination of Study

This study may be terminated for safety concerns of the principal investigators from the Lead or Contributing sites, CDC, or participating IRBs.

5 STUDY SCHEDULE, PROCEDURES, & EVALUATIONS

5.1 Schedule of events

Pregnant women meeting the proposed eligibility criteria will be recruited. Written informed consent (will be obtained from study participants prior to conducting any study procedures. **Table 1** describes the proposed schedule of study visits with further details below.

Table 1. Study Visit Schedule									
Procedure	Screening Visit* Day -30 to 1	Visit 1 Day 1	Visit 2 Day 4 ± 1	Visit 3 Day 9 + 3	Visit 4 Day 29 -7/+14	Visit 5 Day 43 + 7	Visit 6 Delivery	Visit 7 Postnatal Day 90 + 14	Unscheduled Visit
Type of contact	Chart Abstraction/ Clinic	Clinic	Phone/Text/ Email/Data Review	Phone/Text /Email/Data Review	Clinic	Phone/Text/ Email/Chart Abstraction	Hospital/ Chart Abstraction	Phone/Text/ Email/Chart Abstraction	Clinic
Informed consent & Medical Release of Information	X								
Review Eligibility Criteria	X	X							
Demographic, Medical, and obstetric history	X	X							
Influenza Vaccination History	X	X							
Vital signs (temperature, blood pressure, and heart rate)		X							X
Blood Sample Collection		X			X		X[1]		
Randomization and Vaccination		X							
Obtain immediate adverse event information		X							
Dispense memory aid		X							
Dispense study supplies		X							
Complete Memory aid form (REDCap or paper)		X	X	X					
Concomitant medications/vaccinations	X	X	X	X	X	X	X	X	X
Obtain solicited adverse events		X	X	X					
Obtain unsolicited adverse events		X	X	X					X
Obtain serious adverse event information		X	X	X	X	X	X	X	X
Infant data collection								X	

[1]Cord blood and maternal blood collection at Visit 6 will be optional and collected when feasible by the site.

Screening Visit, Study Day -30 to 1 (Chart Abstraction & Clinic Visit – may occur simultaneously with Visit 1)

- Obtain written informed consent and release of medical record information
- Review and confirm study eligibility
- Obtain information on preferred method of contact for follow-up (telephone or email reminder or text reminder)
- Obtain demographic data
- Obtain influenza vaccination history
- Obtain medical history (e.g. chronic hypertension, diabetes, autoimmune disorder), obstetric history (e.g. parity, prior preterm birth, low birthweight, small for gestational age), and current pregnancy status (e.g. gestational diabetes, gestational hypertension, placenta previa, estimated due date)
- Obtain concomitant medication use

Visit 1, Study Day 1 - Screening, Enrollment, and Vaccination (Clinic Visit)

- If Screening Visit occurred prior to Visit 1, review and confirm eligibility and all data collected during Screening Visit
- Obtain vital signs including oral temperature prior to vaccination
- Obtain 15mL (~2 tubes) blood sample prior to vaccination for serologic analysis
- Randomize study participant to RIV4 or IIV4
- Administer assigned study product – trained, licensed staff will serve as unblinded vaccinators to administer either RIV4 or IIV4 as described in Section A.4.3. Participants and all other study personnel involved in collection of memory aids, follow-up phone calls, data collection, etc. will be blinded to randomization arm.
- Ensure participants receive the CDC Influenza Vaccine Information Statement (VIS) during the visit.
- Observe participants for at least 20 minutes after vaccination and record any immediate adverse events (AEs)
- Dispense oral thermometer, ruler (in order to standardize measurements) and memory aid. Review instructions for use of thermometer, ruler, and memory aid completion.
- Participants will be given the choice of completing the memory aid electronically or on paper. Participants who select the electronic method will enter their data into a REDCap web-based system
- Confirm preferred method of contact for follow-up (telephone, email, or text reminder)
- Confirm date of study Visit 4 (attempt to coincide with regularly scheduled prenatal visit as feasible)
- Review concomitant medication use
- Study staff will instruct participants to
    - Notify the study staff if they are hospitalized or have a severe adverse event
    - Follow up with their healthcare provider if they have any symptoms they find concerning
    - Notify study staff if they enroll in another research study

Visit 2, Study Day 4 (window Days 3 – 5) Phone/Text/Email/Online Memory Aid review

- Participants will complete their memory aid via paper or electronic entry from Day 1-8.
- Participants using paper diary:
    - Study staff will contact participants using paper memory aid to collect and record memory aid data including AEs (solicited and unsolicited), serious AEs (SAEs), pregnancy status, and concomitant medications.
    - Participants will be
        - Asked to notify the study staff if they are hospitalized or have a severe adverse event,
        - Follow up with their healthcare if they have symptoms they find concerning
        - Reminded that they will be contacted again between Day 9 and Day 12 for Visit 3

- Participants using REDCap web-based system:

- Study staff will review REDCap system to confirm data capture and assess for any AEs, SAEs, pregnancy status, and concomitant medications.
- The study team will contact participants if they have any missing information. The study team may also contact participants if more information is needed to better describe AEs [including SAEs and severe (Grade 3) events] reported in the REDCap web-based system.
- All participants using REDCAP web-based will be
  - Reminded to continue data entry into REDCap web-based system
  - Asked to notify the study staff if they are hospitalized or have a severe adverse event,
  - Follow up with their healthcare provider if they have symptoms they find concerning

Visit 3, Study Day 9 (window Days 9 – 12) Phone/Text/Email/Online Memory Aid Review

- Participants will complete their memory aid via paper or electronic entry from Day 1-8.
- Participants using paper diary:
  - Study staff will contact participants using paper memory aid to collect and record memory aid data including AEs (solicited and unsolicited), serious AEs (SAEs), and concomitant medications.
  - Remind participants to
    - Notify the study staff if they are hospitalized or have a severe adverse event,
      Follow up with their healthcare if they have symptoms they find concerning
  - Confirm date of next scheduled study visit (attempt to coincide with regularly scheduled prenatal visit as feasible)

- Participants using REDCap web-based system:
  - Study staff will review REDCap system to confirm data capture and assess for any AEs, SAEs, and concomitant medications.
  - The study team will contact participants if they have any missing information. The study team may also contact participants if more information is needed to better describe AEs [including SAEs and severe (Grade 3) events] reported in the REDCap web-based system.
  - All participants using REDCAP web-based will be
    Asked to notify the study staff if they are hospitalized or have a severe adverse event,
    - Follow up with their healthcare if they have symptoms they find concerning
    - Have their date for next scheduled study visit confirmed (attempt to coincide with regularly scheduled prenatal visit as feasible).

Visit 4, Study Day 29 (window Days 21 – 42) Clinic Visit

- Study staff will review the memory aid data (paper or electronic) and record any SAEs and concomitant medications
- Obtain 15mL (~2 tubes) blood sample for serologic analysis
- Confirm preferred method of contact for follow-up (telephone, email, or text

reminder)
- Remind participants
    - They will be contacted between Day 43 – 50
    - To notify the study staff if they enroll in another research study or are hospitalized
    - To follow up with their healthcare if they have symptoms they find concerning

Visit 5, Study Day 43 (window Days 43 - 50) Phone Call/Text/Email Follow-Up/Chart Abstraction

Study staff will contact study participants to record any SAEs and concomitant medications. Medical records will be reviewed to obtain more information about any SAEs. Participants will be reminded that they may be contacted again after delivery and that they will be contacted ~ 90 days after delivery to obtain maternal and infant health data.

Visit 6, Birth Outcomes Visit, Medical Record Review/Phone Call/ Hospital

Study staff will screen the inpatient census for the study site Birthing Centers on a daily basis to identify study participants admitted for delivery. Medical records will be reviewed for maternal and infant outcomes as well as maternal Tdap receipt. At Duke, maternal and cord blood collection will be performed at delivery. At the other sites, these samples (maternal and cord blood) will be collected during delivery if feasible. Study participants who are not identified via review of inpatient census records will be contacted approximately 2 weeks after their estimated due date to determine if delivery occurred elsewhere. If so, maternal and infant medical records will be requested from the delivery hospital for data collection.
For the pregnancy outcomes abortion (spontaneous or elective termination) or fetal death, medical records will also be reviewed. Study participants with these outcomes will be contacted only if more information is needed for data collection.

Visit 7, Postnatal Day 90 (window Postnatal Days 90 - 104) Phone Call/Text/Email Follow-Up/Chart Abstraction

Study staff will contact study participants to record any maternal or infant SAEs, including neonatal/infant death and re-hospitalization, and concomitant medications. Information will also be collected on emergency room visits and unanticipated visits to the primary care pediatrician or a specialist. Data will be verified via review of medical records. Sites will have subjects sign medical records release forms for both themselves and their infants at the time of informed consent. This is done so that in the event subjects and/or their infants are seen outside of the site's medical coverages, that medical records regarding their emergency room or unanticipated visits can be requested.
For neonatal deaths identified by the study team prior to Visit 7, study participants will contact participants only if more information is needed for data collection.

***Unscheduled Visits**

- Obtain vital signs including oral temperature for the pregnant women
- Record any solicited and unsolicited AEs through Day 8, maternal and infant SAEs and concomitant medications through 90 days after delivery
- Confirm preferred method of contact for follow-up (telephone, email, or text reminder)

End of Study
After unblinding, study participants will be given the opportunity to learn which vaccine they received. For interested participants, study staff will contact subjects with an IRB-approved letter by either mail, email or patient portal message (e.g., MyChart) to inform them which vaccine they received.

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Treatment Assignment Procedures
This is a randomized, controlled, double-blinded study involving pregnant women who are to receive the influenza vaccine. The designated vaccine brands for this study are Flublok® quadrivalent (Sanofi) and quadrivalent Flulaval® (GSK) (IIV4). However, the specific comparator IIV4 vaccine administered will be based on availability from manufacturers prior to the 2019-2020 and 2020-2021 influenza seasons. To the extent feasible, the same influenza vaccine brand will be used as the comparator vaccine during both influenza seasons at all recruiting sites.

5.1.1 Randomization
Participants will be randomized (1:1) to receive either RIV4 (Flublok® quadrivalent recombinant influenza vaccine) or IIV4 (quadrivalent inactivated influenza vaccine) using a permuted block randomization scheme stratified by study site (Duke, Cincinnati, Boston). The project statistician will generate permuted block randomization schemes which will be uploaded to REDCap (section 7.2.1). The randomization schedule will not be available to the study staff, so the next randomization allocation will not be known before randomization occurs. Following confirmation of study eligibility criteria during Visit 1, participant randomization will be through REDCap with treatment allocation recorded on the case report form (CRF).
In the event that REDCap is unavailable, manual randomization will occur through the use of envelopes. The project statistician will prepare 20 envelopes per gestational age group per site (total of 40 per site) that will use the same randomization strategy as the primary scheme embedded in REDCap. When an unblinded team member is informed of the gestational age group, he/she will pull the next envelope in order. In order to capture the allocation per subject, a separate form in REDCap will be used by the unblinded personnel to add the assignment. A log will need to be kept at the site capturing these instances.

5.1.2 Blinding
This study is double-blinded, therefore study staff and participants will be blinded to randomization assignments. Trained, licensed staff will serve as unblinded vaccinators to administer either RIV4 or IIV4. All other study personnel involved in collection of memory aids, follow-up phone calls, and data collection, will be blinded to randomization arm. Laboratory staff will be blinded to randomization arm assignment.

The participant will receive documentation of receipt of influenza vaccine without specification of whether it was RIV4 or IIV4 vaccine to preserve blinding. In the event of individual participant clinical safety issues or overall study safety concerns, then blinding may be broken.

5.1.3 Vaccine Supply, Storage, and Administration
In order to ensure adherence to study randomization assignment, licensed RIV4 and IIV4 vaccines will be administered as study procedures. Licensed RIV4 and IIV4 will be

purchased for study administration and will be stored at 2° to 8°C in a research-specific medication refrigerator. While research staff maintain daily temperature logs for the medication refrigeration, it is also monitored 24/7 with alarm activation if out of range. Research staff are notified of any alarm activations and have an on-call system in place to report to the research center for further investigation. Flublok® quadrivalent (Sanofi) and quadrivalent Flulaval® (GSK) will be the designated study products.

A single administration of both RIV4 and IIV4 comprises intramuscular delivery of 0.5mL total volume of each vaccine for adults. Unblinded, licensed providers will prepare the RIV4 or IIV4 by using opaque tape to cover the syringe before vaccine administration. Vaccine lot numbers will be recorded in the study accountability log available only to unblinded licensed staff or the investigational pharmacist. Dose and site of vaccine administration will be recorded by unblinded licensed staff. RIV4 and IIV4 will be administered in the deltoid of the patient's preferred arm by an unblinded licensed provider. After administration, used study syringes will be disposed of according to standard operating procedure.

A licensed provider who will be trained on the study protocol, will be present at the time of vaccine administration along with emergency management supplies available for initial treatment of an allergic reaction if needed. Additionally, a blinded post-assessment team will be present to assist study participants following vaccine administration; they will not be in the room at the time of vaccination.

5.2 Reactogenicity and Safety Assessments

Participants will be observed for at least 20 minutes post-vaccination and queried for any adverse events immediately following vaccination (e.g. wheezing, rash, dizziness, fainting) and recorded.

Frequency and occurrence of local and systemic reactogenicity, unsolicited adverse events (AEs), serious AEs (SAEs), concomitant medication use, and unscheduled medical care will be assessed through post-vaccination Day 8 using a standard memory aid. At the time of study enrollment, participants will be given a thermometer and instructed on using the memory aid to document oral temperatures and post-vaccination symptoms. Beginning on the evening of Study Visit 1 (Day 1) following influenza vaccination, participants will record their oral temperature using the study-supplied thermometer for the next 8 days (Day 1 – 8). Temperature will be recorded at roughly the same time each day or when a participant feels feverish. If a temperature ≥ 100.4°F (38°C) is recorded, a second measurement will be taken. If more than one temperature is taken on the same day, the highest temperature should be recorded. Fever will be defined as a measured temperature ≥ 100.4°F (38°C). Participants will classify local and other systemic reactogenicity events as mild, moderate, or severe as described in **Table 4 and 5**.

During Visits 2, 3, and 4 information reported by participants will be reviewed for accuracy and completion. Participants who report severe solicited adverse events or express any concern about symptoms/unsolicited events will be encouraged to follow up with their obstetrician or primary care provider. Study staff will assist with coordination of referral appointments as necessary. Medical records will be obtained and reviewed for any unscheduled medical appointment through post-vaccination Day 43.

Table 4: Injection-site Reactogenicity*			
Symptom	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**

Pain	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and resulted in medical visit or absenteeism
Tenderness	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and resulted in medical visit or absenteeism
Induration/ Swelling	25 to ≤50 mm	51 to ≤100 mm	>100 mm
Erythema	25 to ≤50 mm	51 to ≤100 mm	>100 mm

**Injection-site criteria being used in the CISA Study on Safety of RIV4 versus IIV4 in Pregnant Women*

Table 5: System Reactogenicity*			
Systemic	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Fever **	≥100.4 to ≤101.1° F	≥101.2 to ≤102.0° F	≥102.1° F
Malaise (Fatigue)	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and resulted in medical visit or absenteeism
Myalgia (Body aches)	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and resulted in medical visit or absenteeism
Arthralgia (Joint pain)	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and resulted in medical visit or absenteeism
Nausea	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and resulted in medical visit or absenteeism
Vomiting	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and resulted in medical visit or absenteeism
Diarrhea	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and resulted in medical visit or absenteeism
Abdominal pain	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and resulted in medical visit or absenteeism
Headache	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and resulted in medical visit or absenteeism
Chills/shivering	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and resulted in medical visit or absenteeism

*** Oral temperature, no recent hot/cold beverages or smoking*

Administration of influenza vaccines including IIV and RIV is routine clinical care during pregnancy such that we do not anticipate having a significant issue with serious adverse events (SAEs). However, we will monitor study participants and their infants for SAEs during the protocol-defined surveillance period [i.e. from enrollment through 90 days postpartum].

An SAE is defined as an AE that meets one of the following conditions:

- Results in death (including SAB and fetal death) during the period of protocol-defined surveillance
- Is life-threatening (defined as immediate risk of death at the time of the event)
- Requires inpatient hospitalization or prolonged hospitalization during the period of protocol-defined surveillance (other than routine hospital admission such as for labor & delivery)
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity
- Any other important medical event that may not result in death, be life threatening, or

require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Reporting Adverse Events

SAEs and unanticipated problems will be reported to the CDC and all participating IRBs according to institutional requirements. Given that this study involves vaccines that are included as part of routine clinical care, there will not be a designated data safety monitoring board. If deemed necessary, we will designate an independent safety monitor with relevant expertise, in collaboration with the CDC.

If indicated, adverse events (AEs) will be reported to the CDC's Vaccine Adverse Event Reporting System (VAERS). The National Childhood Vaccine Injury Act requires healthcare providers to report:

- Any adverse event listed by the vaccine manufacturer as a contraindication to further doses of the vaccine; or
- Any adverse event listed in VAERS Table of Reportable Events following Vaccination (accessed at https://vaers.hhs.gov/docs/VAERS_Table_of_Reportable_Events_Following_Vaccination.pdf) that occurs within the specified time period after vaccination.

In addition, CDC encourages reporting of any clinically significant adverse event that occurs in a patient.

Study site investigators will assess relatedness to vaccine or study procedures (related, possibly related, unlikely related, or not related) for all AEs including SAEs. The study investigators will use their clinical judgement to make causality assessments and the final causality assessment decision is the responsibility of the site principal investigator where the subject was enrolled.

5.3 Biospecimens Collection & Handling

5.3.1 Serum

Maternal and cord blood specimens will be collected during study visits as described in **Table 1**. All blood samples will be collected, processed, and frozen according to the standard operating procedures and not contain personal identifiers. Serum aliquots will be frozen and stored at each site until planned laboratory analyses.

The Study site(s) will process and ship these samples to Duke using standard procedures, in accordance with specimen transport policies.

Any unused serum samples will be returned to the study site(s) after all assays have been completed.

6 LABORATORY ANALYSES

6.1 Influenza Laboratory Analysis

Flublok® quadrivalent (Sanofi) and quadrivalent Flulaval® (GSK) will be the designated study products. An alternate licensed IIV4 may be used in place of Flulaval® if there are unforeseen issues.

As such, reference wild-type, reassortant, or vaccine virus strains representative of the specific viral antigens included in the 2019-2020 or 2020-2021 influenza vaccines will be used to evaluate the relative levels of all four influenza strain-specific antibodies in maternal serum (pre- and post-vaccination) and infant cord blood. To accomplish these activities all patient samples will be interrogated for influenza antibodies against the strains of interest using the influenza hemagglutination inhibition assay (HAI). This assay is considered the "gold-standard" measure by which to evaluate seroconversion/seroprotection in response to seasonal influenza vaccination. This assay will be performed in accordance with the Duke RBL Virology Unit's fully optimized and approved SOP (RVUSOP004 Influenza HI of Serum Samples). Briefly, test samples will be assayed by HAI as duplicate 2-fold dilution series starting at 1:10. Serum dilutions are then incubated with a concentration of virus verified to possess a known potential for red blood cell (RBC) agglutination. The presence of virus-specific antibodies is visualized via incubation of the virus-serum mixture with a RBC solution; the endpoint titer for a given dilution series is then expressed as the reciprocal of the final dilution in which complete HAI is observed. By convention, seronegative samples are defined as having an endpoint HAI titer < 1:40 and seropositive samples as having an endpoint titer of ≥ 1:40; and seroconversion as a HAI titer ≥ 1:40 at day 29 if the baseline titer is < 1:10 or a minimum four-fold rise in HAI titer if the baseline titer is ≥ 1:10 for each influenza vaccine antigen.

7 STATISTICAL CONSIDERATIONS

In collaboration with the Boston Medical Center, Cincinnati Children's Hospital Medical Center, and CDC the research team at Duke will oversee the statistical analysis. Data will reside on a secure Duke server maintained by Duke Health Technology Solutions (DHTS). For the study, a database will be developed and a data set for the study without personal identifiers will be made available to the CDC upon request. Duke statisticians will develop a comprehensive Statistical Analysis Plan. The summary points of the analysis plan are presented below.

7.1 Definitions

Pregnancy and birth outcomes in this study will be defined by the American College of Obstetrics and Gynecology's REVITALIZE Obstetric Data Definitions[16], CDC national Vital Statistic System[17] or World Health Organization[18] if available. Definitions for the component of the primary outcome measures are as follows:

- Preterm birth- born alive at less than 37 weeks and 0 days gestation
- Spontaneous abortion (SAB)- pregnancy loss prior to 20 weeks 0 days
- Fetal death- intrauterine death of fetus at or after 20 weeks 0 days
- Neonatal death- infant death within first 28 days of life

7.2 Sample Size and Power Estimation

Allowing for a 5% drop out rate and given an initial N=430, there should be approximately 408 participants for evaluation. We assume that 15% of pregnant women will have adverse birth outcomes after vaccination with RIV4 or IIV4, based

on national and CISA site estimates of preterm birth rates. We have selected a clinically meaningful noninferiority margin of 10%. Statistical calculations show that with a one-sided alpha of 0.025 and 204 subjects in each group across all study sites, there is at least 80% power to reject the null hypothesis that RIV4 is noninferior to IIV4 in the proportion of adverse birth outcomes.

7.3 Analysis Plan

7.3.1 Study Populations **–** There will be three study populations the modified Intent-to-treat (mITT), Per Proocol (PP), and Immunogenicity populations. The mITT Population includes any participant that was enrolled, randomized into the study, and received vaccine. The PP Population is a subset of the ITT Population excluding those participants with major protocol violations. The Immunogenicity Population is a subset of the mITT Population that includes only subjects who received vaccine, provide baseline and Visit 4 blood draws available for analysis within the protocol-defined time frame, and with no protocol violations affecting immunogenicity. These protocol violations will be listed in the Statistical Analysis Plan. Statistical analyses of the safety outcomes will be performed for both mITT and PP populations, and immunogenic outcomes will be analyzed for the Immunogenicity Population.

7.3.2 Alpha Level - No adjustment to the alpha level will be made for the secondary and exploratory objectives.

7.3.3 Primary Objective - To compare the proportions of adverse birth outcomes in pregnant women vaccinated with RIV4 versus IIV4.

- Research hypothesis: The proportion of pregnant women with adverse birth outcomes will be non-inferior (not higher – based on the noninferiority margin) after receipt of RIV4 compared to IIV4.

  This objective will be assessed using a one-sided noninferiority test with the alpha level set at 0.025 (1-sided) and a noninferiority margin of 10%.

  The null hypothesis assumes that RIV4 is inferior to IIV4 in regards to the proportion of pregnant women with adverse birth outcomes.

  $$Ho: RIV4 - IIV4 \geq 0.10 \ (10\%)$$

  The alternative hypothesis states that RIV4 is noninferior to IIV4 in regards to the proportion of pregnant women with adverse birth outcomes.

  $$Ha: RIV4 - IIV4 < 0.10 \ (10\%)$$

  The upper bound of a stratified (by study site) Newcombe binomial confidence interval (Yan and Su 2010) with Cochran-Mantel-Haenszel (CMH) weighting of the difference will be used to make this assessment.

  A composite outcome for adverse birth outcome [defined by the occurrence of at least one of the following events: preterm birth, spontaneous abortion, fetal death or neonatal death] will be used as the primary study endpoint.

7.3.4 Secondary Objective 1 - To compare proportions of preterm birth after RIV4 versus IIV4 vaccination. This proportion will be compared between the RIV4 group and the IIV4 group using an exact Mantel-Haenszel statistic (calculated in Proc Logistic in SAS) in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level. The site adjusted relative risk and corresponding 95% confidence interval for the proportion of preterm birth will also be calculated.

7.3.5 Secondary Objective 2 - To compare proportions of combined fetal death and neonatal death after RIV4 versus IIV4 vaccination. These proportions will be compared between the RIV4 group and the IIV4 group using an exact Mantel-Haenszel statistic (calculated in Proc Logistic in SAS) in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level. The site adjusted relative risk and corresponding 95% confidence interval for the proportions of combined fetal death and neonatal death will also be calculated.

7.3.6 Secondary Objective 3 - To compare proportions of spontaneous abortion after RIV4 versus IIV4 vaccination. This proportion will be compared between the RIV4 group and the IIV4 group using an exact Mantel-Haenszel statistic (calculated in Proc Logistic in SAS) in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level. The site adjusted relative risk and corresponding 95% confidence interval for the proportion of spontaneous abortion after vaccination will also be calculated. This will be a subgroup analysis of only those participants vaccinated at less than 20 weeks gestational age.

7.3.7 Secondary Objective 4 - To compare proportions of moderate/severe solicited reactogenicity events in pregnant women vaccinated with RIV4 versus IIV4. Reactogenicity will be assessed for 8 days following the vaccination at Visit 1. These proportions will be presented by treatment group, symptom, and grade level (moderate/severe) for women with injection site reactogenicity within 8 days post-vaccination. These proportions will be compared between the RIV4 group and the IIV4 group, within symptom, using an exact Mantel-Haenszel statistic (calculated in Proc Logistic in SAS) in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level. Systemic reactogenicity will be presented in a similar fashion.

7.3.8 Exploratory Objective 1: To compare and describe serious adverse events (SAE) in pregnant women vaccinated with RIV4 and IIV4. The proportion and 95% exact binomial confidence interval of serious adverse events (SAEs), as well as the total number of events, will be presented by site, vaccine group, severity, and relatedness. Listings of these SAEs will also be presented. Non-overlapping confidence boundaries will be an indication of a statistical difference between the groups. These presentations and listings will be provided for the participant's study duration and for the first 42 days after vaccination.

7.3.9 Exploratory Objective 2**:** To compare proportions of adverse birth outcomes of clinical interest after RIV4 versus IIV4 vaccination. These proportions (i.e., infants born small-for-gestational age, pregnant women with clinical chorioamnionitis, and pregnant women with preeclampsia or eclampsia) will be compared between the RIV4 group and the IIV4 group using an exact Mantel-Haenszel statistic (calculated in Proc Logistic in SAS) in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level.

7.3.10 Exploratory Objective 3: To compare and describe health outcomes through 90 days of life in infants born to women after RIV4 versus IIV4 vaccination. The proportion of infants with medically-attended events through 90 days of life and the proportion of infants with neonatal death will be compared between the RIV4 group and the IIV4 group using an exact Mantel-Haenszel statistic (calculated in Proc Logistic in SAS) in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level. The proportion and 95% exact binomial confidence interval of SAEs in infants through 90 days of life, as well as the total number of events, will be presented by site, vaccine group, severity, and relatedness. Listings of these SAEs will also be presented. Non-overlapping confidence boundaries will be an indication of a statistical difference between the groups.

7.3.11 Exploratory Objective 4: To compare maternal immune responses to influenza antigens and calculate the post and pre-vaccination ratio after RIV4 versus IIV4 vaccination. The proportion of pregnant women achieving seroconversion at day 29 and the proportion pregnant women with a seroprotective HAI titer (≥ 1:40) pre- and post-immunization at day 29 will be compared using a Mantel-Haenszel statistic in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level. The geometric mean HAI titer (GMT) pre- and post-immunization at day 29 after RIV4 versus IIV4 vaccination for each influenza vaccine antigen will be compared (within vaccine arm pre versus post and within timeframe RIV4 versus IIV4) using a regression model with the log transformed titer value at the two-sided alpha 0.05 level.

7.3.12 Exploratory Objective 5: To compare cord blood antibody levels for influenza antigens after RIV4 versus IIV4 vaccination. The proportion of infants with cord blood seroprotective HAI titer will be compared using a Mantel-Haenszel statistic in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level. Infant cord blood geometric mean HAI titer (GMT) for each influenza vaccine antigen will be compared using a regression model with the log transformed titer value at the two-sided alpha 0.05 level. The ratio and 95% confidence interval for cord blood to maternal influenza antibody titers at time of delivery for each influenza vaccine antigen after maternal RIV4 versus IIV4 vaccination will be presented. Non-overlapping confidence boundaries will be an indication of a statistical difference between the groups.

7.3.13 Sensitivity analysis

If there are any participants who receive the incorrect treatment (i.e., treatment other than what they were randomized to receive), then the primary and

secondary objectives will be analyzed based on the treatment the participant received not what the participant was randomized to receive.

7.3.14 Interim Safety Data Review

Given that this study involves administering U.S.-licensed vaccines recommended by the ACIP for use in pregnant women, and is included as part of routine clinical care, there will not be a designated data safety monitoring board for this study. However, because RIV4 safety in pregnancy has not been systematically studied, an interim safety data review of all serious adverse events (SAEs) will be performed with the goal of identifying unexpected safety concerns of clinical importance. The safety data review will provide the study the opportunity to make changes to the protocol, if needed, prior to enrollment in the 2020-2021 influenza season. The interim safety data review will be performed by an independent panel with relevant expertise who are not investigators on the study. The independent safety review panel will assess the clinical narratives of SAEs for all participants who were randomized and vaccinated in 2019-2020 influenza season. If the CDC and study investigators determine additional analyses or reviews are needed, efforts will be made to conduct additional analyses or reviews that will not include analyzing the primary endpoint as a first step. This is to avoid introducing bias or increasing sample size needs for statistical power.

7.3.16 Further analysis

Prior to the second season of enrollment, randomization was stratified by gestational age (<20 weeks versus 20-34 weeks) and by site. Stratification by gestational age was removed from Version 2.1 due to an error in the randomization schema noted after the first season. The overall randomization to RIV4 vs IIV4 was maintained but the gestational age stratification was unbalanced due to the error.

If the error in stratification by gestational age results in an imbalance in gestational age between the treatment groups, evaluation of secondary outcomes by treatment group will be done using gestational age as a covariate if specified analyses can adjust for this factor. To have reasonable results, each cell count needs to be 5 or greater.

7.4 Data Management Plan

The amount of data that will be collected for the proposed project will be substantial and will require a sophisticated, practical and flexible system that can accommodate different modes of data collection and several separate linked surveys. The novel Vanderbilt-designed resource developed specifically for online collection of research information, the Research Electronic Data Capture (REDCap) platform, will be used to design study forms, including the reaction forms, and short customized questionnaires to collect information from study participants. This system will be used by Duke for data management. All electronic linkages will fulfill regulations for protection of human participants and requirements to minimize the risk of breach of confidentiality. After initial set-up, the work load required for electronic data collection will be substantially reduced (description of REDCap resources below). Duke investigators have previously used the REDCap system for collection and analysis of large quantities of data. Participants will

be given the option to fill out their memory aid either directly in the REDCap system or on paper. All study-related documents containing protected health information, e.g. enrollment logs, case report forms, memory aids completed by study participants, will be maintained in secure research offices at Duke, which are accessible to research staff only.

7.4.1 Research Electronic Data Capture (REDCap)

Investigators within the NIH-funded Clinical and Translational Research Unit at Vanderbilt have developed REDCap (http://project-redcap.org/), to collect and manage data for diverse clinical and translational research studies. REDCap was designed around the concept of giving research teams an easy method to specify project needs and rapidly develop secure, web-based applications for collection, management and sharing of research data. REDCap accomplishes these key functions through use of a single study metadata table referenced by presentation-level operational modules. Based on this abstracted programming model, databases are developed in an efficient manner with little resource investment beyond the creation of a single data dictionary. The concept of metadata-driven application development is well established, and the critical factor for successful data collection lies in creating a simple workflow methodology allowing research teams to autonomously develop study-related metadata in an efficient manner.[19] Of particular interest for this project, a subcomponent of REDCap, the REDCap Survey is designed for studies where data are collected directly from the research participant. This will be used with the web-based reaction forms that will be completed by the study participants. Both products include secure institutional data hosting and include full audit-trails in compliance with HIPAA security requirements. The REDCap Consortium is comprised of 647 active institutional, including CCHMC. The REDCap currently supports 68,000 projects with over 89,000 users spanning numerous research focus areas across the consortium. The current project will use this software application for the design of electronic forms to collect information from study participants, to link the baseline data, sample collection date, and laboratory results in an automated database family, to perform data cleaning and data quality assurance efficiently, and to design an analytical dataset for the analysis of the project data.

Data will be entered directly into the REDCap database by members of the study team, from Duke, Cincinnati, and Boston. Study investigators will be responsible for assuring that all paper records are securely stored according to the requirements of their IRBs. The study investigators will be responsible for assuring the accuracy of the data entered from the paper forms into REDCap, as appropriate. Only the assigned identifiers will be used in REDCap. Therefore, personal health identifiers will not appear in the REDCap database.

In order to perform data cleaning and data quality assurance efficiently, numerous built-in filters and checks for consistency of the data including range and limit checks, branching logic and pull down menus to limit choices for categorical variables to a pre-specified list will be implemented and performed automatically to minimize data entry error. The data will be randomly sampled and checked against source records on a regular basis. The data and related analytical datasets will also be stored at the lead and contributing sites with secured password-protected computers. Coded data without personal identifiers will be made available to the CDC and transferred using a secure transfer method as described in Section 7.2.

7.4.2 Role of the CDC Investigators in the Project

This study is funded by a CDC contract with Duke University, Cincinnati Children's Hospital Medical Center and Boston University as Task Orders in the CISA Project Contract. CDC staff will collaborate with the sites to develop the protocol, conduct the study, ensure the study is aligned with US Department of Health and Human Services (CDC) public health priorities, and analyze the data and disseminate the results. CDC may receive access to coded data not containing any directly identifying information.

8 HUMAN PARTICIPANTS

8.1 Human Participants Involvement, Characteristics, and Design

Duke, Cincinnati, and Boston investigators will be responsible for submitting the protocol, informed consent, memory aids, recruitment materials and any written or verbally conveyed materials specific to this project to their institutional review boards. CDC staff will be responsible for submitting materials to the CDC for review and obtain reliance on Duke IRB.

To facilitate participant recruitment at the practices, we will request a waiver of consent and HIPAA authorization for ascertainment (identification, selection) and/or recruitment of potential participants while recording identifiable private health information (PHI) prior to obtaining the participant's consent. This information will be obtained from review of the electronic scheduling and medical record systems in the clinics in order to determine eligibility for study enrollment. We will review information only the minimum amount of information necessary to determine eligibility, i.e. date of birth, current pregnancy status, pregnancy history, medical and surgical history, ultrasounds pertaining to current pregnancy, and recent laboratory test results. The PHI collected prior to consent will be used to recruit and screen only. Use of PHI in this manner involves no more than minimal risk to participants and no information will leave the study sites.

Continuing reviews will be submitted to the IRBs in accordance with the new Common Rule.[20] Protocol deviations or concerns about study integrity will be reported promptly to the overseeing IRB in accordance with institutional requirements.

8.2 Sources of Material

Medical history and immunization history will be obtained from the medical record and from patient report. Demographic information will be obtained from the medical record and patient report. Participants will record solicited adverse reactogenicity events and any medical intervention sought on the day of and 7 days following vaccinations on the memory aid. Memory aid information will be reported to the study team during a telephone call or in the web-based REDCap system. The research staff will assess one or more of the following: weight, height, temperature, blood pressure, and pulse.

8.3 Potential Risks and Benefits

RIV4 and IIV4 are FDA licensed vaccines approved for use in pregnant women. IIV vaccines are standard clinical practice and recommended by the CDC and ACOG for women who are pregnant during the influenza season. Participants will be provided with the CDC Vaccine Information Statement (VIS) for IIV and RIV.

IIV and RIV risks include minor problems such as soreness, redness, swelling, or pain where the shot was given, hoarseness, sore, red or itchy eyes, cough, fever, aches,

headache, itching, fatigue, all of which usually occur within 1-2 days of vaccination and are self-limiting. Syncope (fainting) can occur in association with administration of injectable vaccines. More serious problems including a small increased risk of Guillain-Barré Syndrome estimated at 1 or 2 additional cases per million people vaccinated. This is much lower than the risk of severe complications from influenza infection, which can be prevented by IIV or RIV. (http://www.cdc.gov/vaccines/hcp/vis/vis-statements/flu.html).

Problems that could happen after any injected vaccine:

- People sometimes faint after a medical procedure, including vaccination. Sitting or lying down for about 15 minutes can help prevent fainting, and injuries caused by a fall. Participants should inform their doctor should they feel dizzy, or have vision changes or ringing in the ears.
- Some people get severe pain in the shoulder and have difficulty moving the arm where a shot was given. This happens very rarely.
- Any medication can cause a severe allergic reaction. Such reactions from a vaccine are very rare, estimated at about 1 in a million doses, and would happen within a few minutes to a few hours after the vaccination. As with any medicine, there is a very remote chance of a vaccine causing a serious injury or death.

Each maternal participant will be asked to have at least 2 blood samplings with the total volume not to exceed 30mL. An optional blood sampling of an additional 15ml may occur during delivery. Maternal participants may experience dizziness or fainting. There is no risk to the participant or their newborn for collection of cord blood, as the cord blood is drawn from the umbilical cord/placenta after the baby is not attached to it.

The Advisory Committee on Immunization Practices (ACIP) and the American College of Obstetricians & Gynecologists (ACOG) recommend all women who will be pregnant during influenza season receive influenza vaccine. A licensed, recommended, and age-appropriate IIV or RIV4 may be used.[21] However, as with any influenza vaccines we cannot assure or guarantee protection against influenza given the potential for mismatch between circulating influenza vaccine strains and those included in seasonal influenza vaccines and variation in individual immune responses.

There is the potential risk of loss of confidentiality about information obtained as part of this study.

8.4 Adequacy of Protection Against Risks

8.4.1 Protections against Risk

Participants will be counseled on possible side effects following vaccination and followed closely during the first 8 days post-vaccination for assessment of local and systemic reactogenicity, solicited and unsolicited adverse events (AEs), serious AEs (SAEs), concomitant medication use, and unscheduled medical care. All participants will be monitored in a sitting or lying position for at least 20 minutes following vaccinations to help prevent fainting, and injuries caused by a fall. Participants with a prior history of any precaution or contraindication to either IIV or RIV will be excluded from study enrollment.

Every effort possible will be made to keep information about participants confidential. Computerized participant information will be kept in password protected files on secured servers. Paper case report forms will be kept in locked files belonging to the study

personnel. Any publications resulting from this work will not contain any identifiable participant information.

8.4.2 ClinicalTrials.gov Requirements

The project is registered on ClinicalTrials.gov (NCT03969641). It is the responsibility of the lead site for creating, maintaining, and uploading pertinent information regarding the study as required by 45 CFR 46.116(h) to ClinicalTrials.gov. The lead site will post their IRB-approved informed consent within the study's record on ClinicalTrials.gov. Contributing sites will be responsible for providing the lead site with any changes to their site's information as applicable.

9.0 Human Participants

In obtaining and documenting informed consent, the Investigator and study team will comply with the applicable regulatory requirements, Good Clinical Practices, and ethical principles. The written informed consent form must be signed and dated by the study participant prior to initiation of any study activities.

9.1.1 Vulnerable Subjects Research

Vulnerable subjects

This study proposes to include pregnant women and neonates.

Pregnant women

RIV4 and IIV4 are FDA licensed vaccines and are recommended by the CDC and ACOG during pregnancy, however the risks for both vaccines are listed in section 8.3.

The specific procedures to be performed as part of this study are limited to minimal blood draw (< 35mL over a 4-week period), and other non-invasive procedures that are commonly performed during routine physical exams and are considered safe for pregnant women. These procedures do not pose greater than minimal risk to the fetus.

This study will involve only those women who have given their free and informed consent in accordance with 45 CFR 46.116 and has the prospect of direct benefit to both the mother and infant. Randomization of vaccine administration provides minimal risk to the participant and fetus as IIV vaccines are routinely given during pregnancy and do not go beyond the scope of standard of care. The small amount of blood that will be drawn during the duration of pregnancy (up to 30 ml) are considered safe and are not expected to cause any harm to the baby. No inducement, monetary or otherwise, will be offered to terminate a pregnancy. Individuals engaged in the research will have no part in any decisions as to the timing, method or procedures used to terminate a pregnancy, or in determining the viability of a neonate.

Infants

Identifiable private information will be collected about the infants at the time of delivery. Significant infant complications identified during the delivery visit will be followed up to 90 days of life.

Mothers will be informed about the infant data collection at the time they consent for the study, and thus, the consent form for pregnant women will also serve as the parental permission for including the infant as a participant after delivery.

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