CLINICAL STUDY PROTOCOL

Real-World Effectiveness of Perinatal RSV Immunoprophylaxis

IRB:

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Confidentiality Statement:

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization unless it is necessary to obtain informed consent from potential study participants.

Synopsis

Purpose Vaccination plays a critical role in reducing respiratory viral illnesses and in preventing ongoing disease transmission. Assessing the real-world post-licensure impact of vaccines to measure their effectiveness as used in clinical practice is an essential public health activity to monitor how patient, vaccination, and virus characteristics influence effectiveness. The purpose of this proposal is to assess the effectiveness of Respiratory Syncytial Virus (RSV) vaccines for the prevention of medically attended respiratory diseases in ambulatory settings.
Primary Objective To determine the real-world effectiveness of perinatal RSV immunoprophylaxis against medically attended infections in infants ≤60 months of age.
Secondary Objective -To quantify the extent to which viral genomic diversity impacts the effectiveness of immunoprophylaxis. -To define the impact of immunoprophylaxis on the immune trajectories of infants during medically attended acute RSV infection. -To uncover immune phenotypes of patients ≤60 months of age with RSV. -To examine the clinical epidemiology of RSV and testing practices in children ≤60 months of age during 2018-2028. -To develop and validate a Natural Language Processing (NLP) tool to extract relevant data from the narrative portion of electronic medical records (EHRs) to determine clinical characteristics for optimal RSV syndromic surveillance.

Study Design

This study will use a bidirectional test-negative case-control design to estimate the effectiveness of licensed RSV vaccines against medically attended acute respiratory illness (ARI). A patient with ARI will be defined as one who has defined as having an acute onset (<10 days) of at least one of the following symptoms: cough, coryza, labored breathing (including wheezing), apnea (or cyanosis), sore throat, or sepsis (defined as fever or hypothermia, shock or seriously ill without apparent cause).Patients will be identified with two strategies: a) case finding in clinical sites during the medical encounter, and b) next-day case-finding in electronic health records (EHR). All participants will be ≤12 months of age who sought care for acute respiratory illness (ARI) at the Yale New Haven Health System (YNHHS) hospital or primary, urgent, and emergency care centers. There will be no discrimination based on gender or race/ethnicity. Cases will be defined as individuals who had a clinical encounter for ARI symptoms and test positive for RSV. Controls will be individuals who test negative for the respective virus. Interviews, reviews of medical records, and immunization registry searches will be done similarly for cases and controls to provide information on patient characteristics, immunization history, potential confounders, and effect modifiers. Respiratory specimens will be collected from all participants for testing using standardized RT-PCR assays and genomic sequencing. In a subset of patients, acute and convalescent sera will be collected for investigations of immune responses to infection.

For our secondary aims, we will conduct two sub-studies. First, we will conduct viral genomic epidemiology studies. To this end, we will request remnant nasal swab samples from RSV confirmed YNHHS patients ≤12 months of age to perform genomic sequencing of the virus and investigate the impact of RSV variants on immune escape and vaccine breakthrough infections. Second, we will conduct clinical epidemiology studies. To this end, we will perform chart reviews of all pediatric patients ≤60 months of age who were tested inpatient or outpatient for RSV at Yale- New Haven Health System from 7/1/2018 and will continue forward until 7/1/2028. The patients' medical records will be abstracted and pertinent demographic, epidemiologic, clinical, laboratory, and radiographic data will be recorded. We will develop and validate a Natural Language Processing (NLP) tool that will allow us to extract data from the narrative portion of EHR's and conduct a rigorous, model-based investigation to identify sets of clinical characteristics (i.e., clinical syndromes) that are highly predictive of RSV infection and determine and quantify the impact of vaccine introduction.

Study Date Range and Duration

This will be a 5-year study. Enrollment of the case-control study will begin 10/1/2024 and will end 11/30/2028. Review of medical records will include data from 7/1/2018 to 7/1/2028.

Number of Study Sites

Patients will be screened for eligibility in inpatient and outpatient sites affiliated with the Yale New Haven Health System.

Primary Outcome Variables

The primary outcome measured is laboratory confirmed ARI infection (case/control status) by exposure (immunization history).

Secondary and Exploratory Outcome Variables (if applicable)

Secondary measures included estimated VE by clinical symptoms, number of vaccine doses received, and elapsed time from immunization.

Study Population Participants will include those who are ≤60 months of age and had a medically-attended ARI at one of the clinical recruitment sites. We will enroll males and females of all racial and ethnic groups meeting our inclusion criteria. For the genomic epidemiology studies, we will include only infants ≤12 months of age who tested positive for RSV and have residual clinical specimens.
Number of Participants For the case-control study, we aim to enroll 1250 RSV-positive and 2500 RSV-negative infants ≤12 months of age, and 500 RSV-positive and 1000 RSV-negative patients from 12-60 months of age for a total sample size of 5250. For the clinical and genomic epidemiology studies, it is difficult to anticipate the number of subjects that will be eligible, given that RSV epidemiology changes from year to year. In a typical respiratory season, YNHHS tests approximately 3,000 infants for RSV, with around 30%—approximately 1,000—testing positive.
Study Schedule For the case-control study, each patient will have 2-3 total contacts with investigators. Contact #1: For patients who participate in person, consent will be collected by the field team daily at participating sites. Patients who are seen remotely for symptoms or who are identified by EHR recruitment strategies will be contacted by telehealth for consent procedures. Contact #2: Follow-up surveys will be conducted on all enrolled participants 4-12 weeks after diagnosis. We will make every effort to coordinate encounters with already planned visits. If no routine follow-up visits are planned during our time frame, we will schedule a follow-up visit at the Church Street Research Unit (CSRU). Contact #3 may occur for hospitalized patients during their inpatient stay. No direct patient contact will take place for the clinical and genomic epidemiology analysis.

Protocol Revision History

Version Date	Summary of Substantial Changes
10/27/23	Congruency changes for NIH funding
7/30/2024	Congruency changes for NIH funding, expanded objectives, age of subjects, schedule and sample types.
12/10/2024	Added email recruitment of eligible participants.
1/29/2025	Requesting remnant RSV confirmed nasal swab samples of patients ≤12 months of age for viral genomic studies as part of our secondary objectives.

Statement of Compliance

This document is a protocol for a human research study. The purpose of this protocol is to ensure that this study is to be conducted according to the Common Rule at 45CFR46 (human subjects) and other applicable government regulations and Institutional research policies and procedures.

APPROVED BY THE YALE UNIVERSITY IRB 2/24/2025

Abbreviations

Abbreviation	Explanation
ARI	Acute Respiratory Infection
VE	Vaccine Effectiveness
RSV	Respiratory Syncytial Virus
CSRU	Church Street Research Unit
EHR	Electronic Health Records
YNHHS	Yale New Haven Health System
US	United States
NHPC	New Haven Primary Care Consortium
C NLP	Natural Language Processing
YNHH	Yale New Haven Hospital
ED	Emergency Department
YHC	Yale Health Center
YCH	Yale Children’s Hospital
Yale-PED	Yale pediatric Emergency Department

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1 Background/Literature Review

Acute respiratory infections (ARI) are one of the leading causes of death in the US and Respiratory syncytial virus (RSV) is one of the leading causes of morbidity and mortality in children. RSV is an RNA virus that infects the human respiratory system, causing illnesses ranging from mild upper respiratory tract symptoms to severe pneumonia. The inflammatory reactions inside the bronchiole lining cause the most severe manifestations of RSV, especially in infants. Globally, RSV is second only to malaria as the leading cause of infant death worldwide.[1] In the United States (US), RSV is associated with 1.5 million annual medical encounters in children less than five years and is the leading cause of hospitalization among infants under one year of age.[2] Not surprisingly, the most vulnerable subgroups of the population, such as those of lower socioeconomic status, consistently bear the burden of severe RSV.[3,4]

Vaccination is one of the greatest successes in modern medicine and is crucial for the control of respiratory viruses. Passive immunization with long-acting monoclonal antibodies (mAb) or maternal vaccines hold promise for reducing the burden of RSV in infants. Palivizumab was the first mAb approved by the Food and Drug Administration (FDA) for the prevention of lower respiratory tract disease caused by RSV. However, it has a short half-life (28 days) and needs to be given repeatedly throughout the RSV season to be effective. Thus, only infants at high risk are eligible for palivizumab, such as preterm infants, those with chronic lung illness, or those with impaired immune systems (<2% of infants). After years of slow progress, the RSV field is at a turning point, with multiple promising vaccines in the home stretch of clinical testing and a novel mAb that has already been authorized for use.[5] Nirsevimab (AstraZeneca and Sanofi) is an extended half-life mAb that is designed to be routinely administered as a single dose to all newborns, much like a vaccine. Given the safety and efficacy of nirsevimab in the phase 3 clinical trials (efficacy >75%),[6,7] it has already been authorized for use in the European Union and is expected to be authorized by the US FDA ahead of the 2023/2024 respiratory season.[8]

Vaccination of mothers during pregnancy can also provide passive immunity to infants. Currently, there are two notable RSV vaccines for pregnant women that aim to protect infants through transplacental transfer of antibodies (GlaxoSmithKline; NCT04605159 and Pfizer; NCT04424316). In December 2022, the FDA accepted a Biologics License Application for Pfizer's maternal RSV vaccine (RSVpreF). Given the vaccine's safety and

efficacy profile in phase 3 clinical trials (efficacy >82%),[9] it is also likely that this vaccine will be authorized for use during the 2023/24 respiratory season.[10]

However, the degree of protection a vaccine provides in real-world settings (i.e., its effectiveness) does not always equate to its efficacy in the highly controlled setting of a clinical trial. This gap between a vaccine's efficacy and its effectiveness is in large part due to the increased heterogeneity of the post-licensure target population, and the inconsistency of immunization practices in real-world settings. Immunization programs post-licensure target a far more heterogeneous group of people than can feasibly be studied in a clinical trial, many of whom may have underlying health conditions or other differences that can significantly influence the protective effect of the vaccine. Further, it is rarely feasible to replicate the strict protocols of a vaccine trial in day-to-day clinical practice. Thus, various other factors, including the storage of vaccines (e.g., ultra-cold storage) and adherence to the dosing schedules may contribute to the lower post-licensure benefits of vaccines.[11] Finally, temporal changes in the circulating viral strains may further impact effectiveness if strains with different properties emerge in the post-licensure period. It is, therefore, critical to continually monitor the effectiveness of vaccines as they are being used in real-world clinical practice to demonstrate their actual effectiveness in preventing disease. These data can provide evidence of gaps in our immunization programs that need to be addressed for optimal disease prevention and population health.

1.1 Prior Experience (if applicable)

Studies of vaccine effectiveness: Members of the research team have extensive expertise in studies to assess vaccine effectiveness. Dr. Niccolai, Dr. Oliveira, and Dr. Weinberger have conducted case-control studies to determine the effectiveness of vaccines for over 20 years, including studies on human papillomavirus (HPV),[12,13] varicella,[14] Lyme,[15] rotavirus,[16] and adult and pediatric COVID-19 vaccines,[17-21] human papillomavirus (HPV),[12,13] varicella,[14] and Lyme.[15] In the studies of varicella and COVID-19 vaccines, effectiveness was shown to decrease substantially over time; these findings contributed to the revised recommendations from the Advisory Committee on Immunization Practices to include a booster dose.[22,23] Our analyses of COVID-19 vaccines were among the first reports globally of the effectiveness of booster doses and of the effects of the vaccine on transmission, and they directly influenced decision-making in the US.

Yale Genomic Surveillance Initiative: Dr. Grubaugh and members of his laboratory have extensive experience with the laboratory techniques that will be used in this study.[24-28] He founded the Yale SARS-CoV-2 Genomic Surveillance Initiative, which has been tracking the evolution and epidemiology of SARS-CoV-2 since the start of the pandemic

2 Rationale/Significance

2.1 Rationale and Study Significance

The upcoming arrival of these novel immunoprophylactic strategies (Nirsevimab for newborns and the vaccines for pregnant mothers) provides unprecedented possibilities to confront the challenge of RSV. However, the degree of protection an immunization provides in real-world settings (i.e., its effectiveness) does not always equate to its efficacy in the highly controlled setting of a clinical trial. This gap between efficacy and effectiveness is in large part due to the increased heterogeneity of the post-licensure target population and the inconsistency of immunization practices in real-world settings. Immunization programs post- licensure target a far more heterogeneous group of people than can feasibly be studied in a clinical trial, many of whom may have underlying health conditions or other differences that can significantly influence the protective effect of the vaccine or mAb.[11] Developmentally programmed immune system responses with different normal responses as a function of age are another factor impacting RSV disease severity and responses to vaccines. Thus, post-licensure monitoring is crucial to ensure the benefits are being realized in the target populations.

Empirical estimates that quantify the real-world benefit of immunization are also important because they help build confidence and boost acceptance by patients. Vaccine hesitancy is often multifactorial; however, studies suggest that it is largely driven by a lack of trust and confidence.[30] The perception that vaccines are not effective has consistently been shown to be an important driver of hesitancy.[31] Mistrust in the vaccine development process is also an important driver of hesitance, especially for racial and ethnic minority groups who are often underrepresented in clinical trials.[32] Thus, scientifically rigorous, independent, and transparent studies that assess the real-world effectiveness of immunizations in the target population are critical.

There is also a need to improve capacity for identifying children with RSV. While laboratory-based surveillance provides excellent specificity, it is costly, not always available, and results can take a long time to return. Surveillance using an RSV syndromic case definition offers the potential to improve case-detection. A clinical syndrome is defined as a set of signs and symptoms that occur together more often than what would be expected by chance alone. Although a large body of work has been done to study the clinical manifestations of RSV, there are still no syndromic definitions that are suitable for the prediction of RSV infection.

2.2 Purpose of Study/Potential Impact

Empirical estimates that quantify the benefit of immunization on a yearly basis and within different relevant subgroups of the population can be a potent incentive to increase the strength and consistency of health care provider recommendations and the acceptance by patients. Additionally, we seek to understand developmental immune phenotypes associated with RSV responses to help inform future vaccinations and treatments. Lastly, at the completion of the EHR sub-aims, we will have an NLP tool that could be adapted in the future to efficiently identify patients with RSV based on their symptoms using routinely collected clinical data. These data may not only provide evidence for sustaining the uptake of these vaccines but may also fuel the development of strategies that increase their use so that they more fully realize their potential.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

This study presents minimal risks to the subjects as it only includes the collection of clinical data from EHRs and surveys, the collection of residual clinical specimens, and the collection of respiratory specimens by noninvasive means, and blood draws of small volumes.

Risk of phlebotomy and respiratory swabs/aspirates/induced sputum: The primary risks of phlebotomy include local discomfort, occasional bruising of the skin at the site of needle puncture, and rarely hematoma, infection, or fainting. The primary risk of a nasopharyngeal or oropharyngeal swab, respiratory aspirate and induced sputum is local discomfort. Rarely, there can be local bleeding from the nasal mucosa, which is controlled with local measures such as pressure or packing with gauze. Additionally, the swab can cause coughing, gagging, or infrequently vomiting.

Risks to privacy: Subjects will be asked to provide personal health information (PHI). There is a slight risk regarding the loss of confidentiality.

Protection Against Risks:

Risk of phlebotomy and respiratory swabs/aspirates/induced sputum: Investigators will prioritize the use of residual clinical specimens, when available, rather than collect fresh samples. When fresh samples need to be collected, the blood will be sampled through indwelling venous or arterial lines when in place. We will try to obtain the blood when other clinical labs are obtained. All blood draws will be performed by skilled practitioners. For children, parents will be present at the time of sampling. If study subjects express a desire to discontinue the protocol for any reason, the study procedures will be immediately terminated, and consent to participate may be rescinded at the subject's discretion. Phlebotomy will only be attempted in infants older than 1 month of age, and will be 1 tablespoon for infants 6-12 months of age and ½ a tablespoon for those 1-6 months of age. This is in line with United States NIH policy and is well below the recommended maximum of 3 ml/kg of blood per 24 hours (3.8% of TBV). For patients 12-60 months of age blood volumes will be drawn in compliance with National Institutes of Health (NIH) and Yale Guidelines (for pediatric patients: not exceeding 5 mL/kg in a single day or the lesser of 3 mL/kg or 50 mL over eight weeks. Respiratory aspirates will be collected as part of clinical care for select patients (such as those with endotracheal tubes).

Surveys: Based on the safety profile of the surveys, there is minimal risk of adverse events. Should any subjects experience adverse effects related to the intervention, these will be reported using a standard form to the Yale Human Research Protection Program.

Minimizing risk to study team: We will follow the YNHHS Infection Prevention guidelines and use appropriate Personal Protective Equipment when encountering subjects who are suspected of having RSV infection and following EHS guidelines while handling their specimens in BSL2 laboratories.

Minimizing risks of risks to privacy: All attempts will be made to keep this PHI confidential within the limits of the law. Only people who are involved in the conduct, oversight, monitoring, or auditing of this trial will be allowed access to the PHI that is collected. Only HIPAA-trained individuals will be conducting the study. Information will be kept in files on a secure system that requires a Yale net ID and password for access. Subject identifiers will be de-identified at the earliest reasonable time after we receive them. If information from this study is presented publicly or published in a medical journal, no research subject will be identified by name, picture, or any other personally identifying manner. For clinical and viral genomic epidemiology aims, no direct contact with participants is required, reducing

the risk of privacy breaches.

2.3.2 Potential Benefits

We do not anticipate that there will be a direct benefit to the individual participants in the study. The proposed studies, however, do offer the potential benefit of generalizable knowledge. The creation of a platform to monitor the effectiveness of RSV vaccines will improve confidence in the vaccines and will yield evidence to guide the development of new approaches to the prevention and control of RSV.

3 Study Purpose and Objectives

3.1 Hypothesis

The key questions this study is expected to answer are:

1) How effective are RSV vaccines in real-world settings?

2) To what extent is the effectiveness of vaccines affected by age, vaccination patterns, and virus type?

3) What are the immunologic and clinical drivers of vaccine failure?

4) What is the immune phenotype of children with RSV?

3.2 Primary Objective

The primary objectives of this study are to determine the real-world effectiveness of perinatal RSV immunoprophylaxis against medically attended infections in infants less than 12 months of age.

3.3 Secondary Objective (if applicable)

To quantify the extent to which viral genomic diversity impacts the effectiveness of immunoprophylaxis.

To define the impact of immunoprophylaxis on the immune trajectories of infants during medically attended acute RSV infection.

To uncover immune phenotypes of patients ≤60 months of age with RSV.

To examine the clinical epidemiology of RSV and testing practices in children ≤60 months of age during 2018-2028.

To develop and validate a Natural Language Processing (NLP) tool to extract relevant data from the narrative portion of electronic medical records (EHRs) to determine clinical characteristics for optimal RSV syndromic surveillance.

4 Study Design

4.1.1 General Design Description

We will conduct an epidemiologic test-negative case-control study, a design that is well-established as valid for studies of vaccine effectiveness. Cases will be defined as individuals who had a clinical encounter for ARI symptoms and test positive for RSV. Controls will be individuals who test negative for the respective virus. Interviews, reviews of medical records, and immunization registry searches will be done similarly for cases and controls to provide information on patient characteristics, immunization history, potential confounders, and effect modifiers. Respiratory specimens will be collected from all participants for testing using standardized RT-PCR assays and genomic sequencing. In a subset of patients, acute and convalescent sera will be collected for investigations of immune responses to infection.
Multivariate logistic regression will be used to estimate the vaccine's effectiveness, adjusting for potential confounders, and stratified by patient characteristics, vaccination patterns, and virus types/variants to determine salient influencing factors.

For our secondary aims, we will conduct two sub-studies. First, we will conduct viral genomic epidemiology studies. The main question we are trying to answer is whether specific mutations or groups of mutations, predict reduced vaccine or mAb effectiveness. To address this, we will request remnant nasal swab samples from RSV-confirmed YNHHS patients ≤12 months of age to perform genetic characterization of all RSV viruses and conduct formal sieve analyses to compare breakthrough infections with time-matched unimmunized infections, Second, we will conduct clinical epidemiology studies. To this end, we will perform a chart review of all pediatric patients ≤60 months of age who were tested inpatient or outpatient for RSV at Yale-New Haven Health System from 7/1/2018 and will continue forward until 7/1/2028. The patients' medical records will be abstracted and pertinent demographic, epidemiologic, clinical, laboratory, and radiographic data will be recorded. We will develop and validate a Natural Language Processing (NLP) tool that will allow us to extract data from the narrative portion of EHR's and conduct a rigorous, model-based investigation to identify sets of clinical characteristics (i.e., clinical syndromes) that are highly predictive of RSV infection and determine and quantify the impact of vaccine introduction.

4.1.2 Study Date Range and Duration

This will be a 5-year study. Enrollment of the case-control study will begin 10/1/2024 and will end 11/30/2028. Review of medical records will include data from 7/1/2018 to 7/1/2028.

4.1.3 Number of Study Sites

Patients will be screened for eligibility in inpatient and outpatient sites affiliated with the Yale New Haven Health System

4.2 Outcome Variables

4.2.1 Primary Outcome Variables

The primary outcome measured is laboratory confirmed ARI infection (case/control status).Respiratory samples will be obtained by study investigators on-site, or if samples have already been collected for clinical purposes, we will request residual specimens from the participating clinical virology laboratory. For the identification of RSV, all samples will be tested using FDA-authorized assays using standard clinical procedures, and positive samples will be stored for subsequent genomic sequencing.

4.2.2 Secondary and Exploratory Outcome Variables (if applicable)

The primary exposure of interest will be immunization status. Only written documentation of receipt of vaccines will be accepted as evidence of prior immunization as determined in either medical records or the state immunization information system. The vaccine effect will be captured using a series of categorical variables that represent the time since the most recent dose. Additional potential confounders will also be considered, such as the use of other preventative or therapeutic interventions, like masks, social distancing, and monoclonal antibodies.

4.3 Study Population

Eligible participants will include those who have a medically-attended ARI at one of the inpatient or ambulatory recruitment sites. To decrease potential selection biases that can arise from variability in physician testing practices, we enroll patients who meet the ARI case definition regardless of whether they were tested for RSV. Participants will include those who are ≤60 months of age. We will enroll males and females of all racial and ethnic groups meeting our inclusion criteria. However, we will restrict participation to residents of Connecticut so that immunizations can be verified in the state immunization registry.

4.3.1 Number of Participants

During the 5-year study period, we aim to enroll 1250 RSV-positive and 2500 RSV-negative infants ≤12 months of age, and 500 RSV-positive and 1000 RSV-negative from 12-60 months of age for a total sample size of 5250. For priority groups, such as hospitalized infants, all eligible patients will be contacted, whereas for clusters with large numbers, a simple random sample will be used until our yearly goals are met (250 cases and 500 controls).

4.3.2 Eligibility Criteria/Vulnerable Populations

In order to be eligible for inclusion in the case-control study, an individual must meet all of the following criteria:

- Male or female ≤60 months of age at the time of presentation for evaluation for an ARI
- Infants should weigh at least five pounds upon enrollment.
- Documentation of an ARI, which is defined as an acute onset (<10 days) illness that includes:
    - At least two of the following symptoms: fever (measured or subjective), chills, rigors, myalgia, headache, sore throat, nausea or vomiting, diarrhea, fatigue, congestion OR any one of the following: cough, shortness of breath, difficulty breathing, olfactory disorder, taste disorder, confusion, persistent chest pain, pale, gray, hypoxia, clinical or radiographic evidence of pneumonia or respiratory distress syndrome.
- Residents of Connecticut

Any individual who meets any of the following criteria will be excluded from participation in the case-control study:

- Illness duration of >10 days at the time of respiratory specimen collection, measured from the date of the first symptom of the current acute illness.
- Parent/guardian is not able to provide informed consent

We will also enroll a subgroup of healthy controls. To be eligible for inclusion in the study as a healthy control, an individual must meet any of the following criteria:

- Immunized against RSV ≤60 months of age
- Residents of Connecticut

- Match an enrolled case that was a vaccine failure by date of birth (±1 month), sex, and immunoprophylactic agent received (i.e., maternal vaccine vs mAb). Vaccine failures are cases who were immunized yet tested positive for RSV.

For our viral genomic sub-study, we will request remnant nasal swabs from YNHHS virology labs during the 5-year study period. This will include all RSV-confirmed patients aged 12 months or younger who did not opt out of research participation.

For our EHR aims, patients will be included if they received care at one of the Yale-New Haven Health System inpatient units or outpatient clinics between 7/1/2018 to 7/1/2028, were tested for RSV with a PCR or antigen test, were ≤60 months of age.

5 Study Methods/Procedures

5.1 Study Procedures

Recruitment plan: Point of care recruitment will be performed by the field team daily at participating sites. The field team will be comprised of trained staff who will review the roster of patients who receive care at the sites to identify subjects who fulfill the inclusion criteria. We will request a HIPAA waiver for screening/recruitment purposes and will contact eligible patients at the time of identification to verify eligibility criteria and invite them to participate in the study. Written informed consent will be obtained for all patients meeting enrollment criteria who are willing to participate in the study.

Patients who are seen remotely for symptoms or who are identified by EHR recruitment strategies will be contacted by telehealth for consent procedures. They will be sent an electronic message either through our EHR's patient portal (MyChart) or through the study email (peds.prove@yale.edu), inviting them to participate in the study. The JDAT team will send out MyChart invitations to eligible participants; however, since these invitations are scheduled to be sent only once every two weeks, this method might not be the most efficient for our study. To meet eligibility criteria, participants must be acutely ill (within 10 days of symptom onset), and to collect residual nasal swabs, the research team must enroll participants within 5 days of the swab being performed. As an alternative, for potential participants identified through EHR recruitment strategies who received medical care at the Yale New Haven Health System but could not be enrolled in person, we propose sending email invitations to participate in the study. To facilitate this, we will collaborate with JDAT to generate an EPIC report that includes all patients who had a medical encounter within the Yale New Haven Health System in the past 7 days and meet our study's eligibility criteria.

The research team will then review this report, identify potential participants, and send email invitations through peds.prove@yale.edu. Potential participants who respond to the initial electronic invitation with interest will be contacted within 48 hours of their encounter by telephone and given the option to participate in the study. When participants are enrolled remotely, we will request electronic signatures through electronic informed consent (E-consent through RedCap) that can be accessed through mobile devices. Subjects who have difficulty navigating or using electronic systems will be mailed a paper version of the consent form.

Recruitment will start in the 2024/25 RSV season and plan to continue enrollment for 5 consecutive seasons. We will leverage our partnership with the CT-EIP (RSV-NET and CT-Wiz) to determine the onset of the RSV season and the overall uptake of vaccine/mAb in our region. To optimize our resources, will initiate active surveillance after vaccine uptake among newborns is >20% and the mean percentage of specimens testing positive by PCR for RSV is ≥3% for 2 consecutive weeks. For priority groups, such as hospitalized infants, all eligible patients will be contacted, whereas for clusters with large numbers, a simple random sample will be used until our yearly goals are met (250 cases and 500 controls).

We will work with JDAT to build a study dashboard using a combination of billing codes and routinely collected clinical data such as demographics, vital signs, laboratory results, and free-text chief complaint or reason-for-visit data fields. This dashboard will allow us to both identify study-eligible patients in real-time and estimate weekly aggregate counts of ARI visits, all-cause patient visits (denominator), total patients tested, and percent positive lab results, by age group and key demographic variables. This informatics infrastructure will allow us to perform several other important study tasks, such as tracking our enrollment progress across demographic categories, and identifying problems related to study recruitment.

Interviews: Standardized questionnaire will be used to ascertain core measures (see Table 1). For in-person interviews, research coordinators will read questions to participants, and record the answers directly into a secure web-based data collection platform (RedCap). Participants will also be given an option to complete the survey using a computer-assisted survey interviewing (CASI) program on their own device at the time of their choosing. If there is no response by the participant to the request to complete the required surveys, the study team will (as needed) continue reaching out to participants via email, phone call, or text beyond this point.

Table 1. Proposed core measures to be ascertained

Category	**Variable**	**Method**
Sociodemographic	Age, gender, race, language, insurance, and address.	Survey, EHR

Medical History	Date of illness onset, symptoms and their severity, previous respiratory infections, medical history (ICD-10, CPT codes), healthcare utilization, medication history (antibodies, antivirals, immunosuppressants).	Survey, EHR
Immunization History	Dates, types, and names of all vaccines received. Name and address of vaccine providers.	Survey, EHR, Registries
Exposure History	Use of masks, adherence to social distancing, and exposure history (e.g., household, occupation, schools, long-term facilities, social gatherings).	Survey
Community-level Social Determinants Of Health	Area-based measures of race/ethnicity, poverty, income, vulnerability, and disadvantage.	US Census Data

*NIH PhenX toolkits, WHO's Global COVID-19 Clinical Platform, Social Vulnerability Index.

Medical record review: After identifying prior sources of care, members of the research team will review the medical records of each participant. Records from all offices at which the infant received care within the YNHHS, including those of subspecialists, will be ascertained to ascertain data about previous or subsequent visits (up to 2 months post-ARI) for symptoms that could be related to their index respiratory illness. The dates for the visits, diagnostic tests performed, and treatments received (ICD-10 and CPT codes) will be recorded. The presence of any other acute or chronic diseases also will be recorded. Trained staff will complete a standardized abstraction form by directly entering information into a computerized form. The core variables that we propose to capture during reviews of medical records are also shown in Table 1.

Respiratory specimen collection. Respiratory samples (nasal swabs) will be collected from all participants. If samples have already been collected for clinical purposes, we will request residual specimens from the participating clinical virology laboratory. Self-collected specimens (saliva or nasal swabs) will be used for participants who do not have residual clinical specimens and are enrolled via telehealth. For these patients, we will provide the parents/guardians with a sample-collection kit, and the study team will supervise self-collection using video conferencing. Specimens will be collected in media that neutralizes the virus yet stabilizes the RNA, which alleviates biosafety concerns related to sample collection and transportation. Respiratory specimens will be transported to Dr. Grubaugh's BSL-2+ laboratory at the YSPH for viral classification and sequencing. A subset of patients will have respiratory aspirates and/or

induced sputum collected, processed and stored in the Lucas lab for immunophenotyping.

Acute and convalescent blood: Blood samples will be collected for participants who provide consent. We will collect acute samples within 1 week of diagnosis and convalescent specimens 4-12 weeks after diagnosis. We will make every effort to coordinate blood samples with already planned visits and/or will collect residual/excess clinical specimens from their outpatient visits when available. If no routine follow-up visits are planned during our time frame, we will schedule a follow-up visit for a blood draw at the Church Street Research Unit (CSRU). The amount of blood drawn for research purposes will not exceed the lesser of 50 ml or 3 ml/kg in an 8-week period and collection will not occur more frequently that 2 times per week. Study team members will be trained in consenting and collecting samples as well as proper Personal Protective Equipment. In addition to in-person blood collection, we will provide parents with the option of using a home blood biospecimen collection kit (Tasso device). The Tasso device is an FDA-approved Class I product.

Follow-up assessments: We will also conduct follow-up surveys 4-12 weeks after the initial ARI using a similar approach. The follow-up surveys will aim to assess the infant's treatment and clinical outcomes, as well as parental outcomes, such as physical, emotional, and social health, as well as health-related quality of life measures. We will use instruments that have been extensively validated for proxy-report (PedsQL).[33]If participants do not have access to a computer or Internet, they will be given the option to fill out the questionnaire in person at the Yale medical campus.

Immunological markers: We hypothesize that maternal immunization and antibody-mediated passive immunity modulates immune priming and memory responses in infants who are infected with RSV. To test this hypothesis, we will collect acute and convalescent blood from a subset of infants (N=75), and recruit an additional 50 healthy community controls, and we will employ a single-cell and multi-omics approach to study the dynamics of the innate and adaptive immune responses during RSV infection and derive systems-level mechanistic models of effective and aberrant vaccine response. These findings might help guide management decisions involving precision vaccinology.

We will make every effort to coordinate blood samples with already planned visits and/or will collect residual/excess clinical specimens from their outpatient visits when available. Serum from immunized and unimmunized infants who were RSV positive will be collected during acute illness and longitudinally after recovery to be analyzed together with serum from healthy community controls who were also immunized. Community controls will be individually matched to enrolled vaccine failures (immunized and RSV+) by date of birth (±1

month), sex, and/or immunoprophylactic agent received (i.e., maternal vaccine vs mAb). We will obtain a list of potential controls from the YNHHS EHR and will use a random number generator to establish the order in which controls will be contacted until one control per case has been successfully enrolled. All blood samples will be processed at the Lucas Laboratory, labeled with bar codes and labels containing study ID, and kept frozen at -80 degrees Celsius or liquid nitrogen tank until ready for testing via immunologic assays. Handling, transport, and storage of the samples will be according to Infectious Disease requirements.

Clinical Epidemiology: For our EHR aims, we will collaborate with Yale's Joint Data Analytics Team (JDAT), to create a secure data warehouse that will receive abstract relevant clinical information and full-length clinical notes for all children who meet eligibility criteria and were tested for RSV within the YNHHS during our study period. We will begin by building a dataset as early as 7/1/2018 and will set up a system that continually prospectively extracts data going forward. We will use natural language processing (NLP) to extract relevant data from clinical notes. Once all desired entities are identified, coded, and labeled based on their context, we will build a program that generates a structured output that can then be analyzed. Variables that will be extracted from EHR include date of birth, insurance information, self-reported demographics (gender, race/ethnicity, preferred language, marital status, religion, city, state of residence, zip code), visit specific variables (date of visits/admission, visit diagnosis), ICD9 diagnoses, laboratory tests and imaging studies performed to aid in diagnosis (RSV PCR with cycle threshold, typing results, white blood cell count with differential, hemoglobin, platelet count, electrolyte, ALT, AST, total bilirubin, creatinine, c-reactive protein, sedimentation rate, ferritin, coagulation panel, procalcitonin, d-dimer, cytokine panel, troponin, brain natriuretic peptide, TEG mapping, EKG results, echocardiogram, chest X-ray findings, and computed tomography scan), management of diagnosis (what treatment was offered, what doses were used, how long where they treated, were there drug side effects, duration of fever, respiratory distress, oxygen requirement, ventilator days, duration of hospitalization, and outcome). Full length encounter notes. Immunization history, including dates of prior RSV immunizations.

Viral Genomic Epidemiology: For our viral genomic sub-study, no contact with human subjects by the PI or any research team member is involved. Instead, we will collaborate with YNHHS virology labs to collect clinical specimens that are leftover from routine diagnostic tests. Specifically, we will request remnant RSV-positive nasal swabs from all patients aged 12 months or younger who have not opted out of research participation. Viral RNA from all RSV-positive samples with PCR cycle threshold values below 35 will be sequenced using amplicon-based

protocols. The viral sequencing data will not be shared with the patient or used for clinical purposes. In addition to the residual specimens, we will collect a limited dataset from the EHR on these patients for the planned analysis. This limited dataset will include patient demographics (age, sex, race/ethnicity, state of residence), sample collection date, PCR assay or platform used, and the cycle threshold (CT) value from the PCR diagnostic test, which indicates the amount of virus present in the specimen. If applicable, we will collect dates of vaccination, maternal vaccination, previous positive RSV tests, and the outcome of the index infection (mild, moderate, or severe disease). After data collection is complete, individuals for these genomic analyses are assigned a unique ID, and any information not listed above (including all personal identifying information) will be removed from this analysis on any other device. Identifiers will be destroyed at the earliest possible stage, in compliance with institutional policies, so that the specimens cannot be linked or re-linked to identifiable human subjects. At no point will this information be linked back to personal identifiers.

Data Collection

For in-person interviews, research coordinators will record the answers directly into a secure web-based data collection platform. Participants will also be given an option to complete the survey using their own device and an online computer-assisted survey interviewing (CASI) program. For medical record reviews, trained staff will complete a standardized abstraction form by directly entering information into a computerized form. The biological samples will be stored in locked freezers. Information about the subject-donors will be maintained in a password-protected computer and password-protected data files. We will follow EHS requirements while processing of specimens from suspected RSV infections under BSL2 procedures. All clinical data, and results of tests on biological samples will be entered into an electronic RedCap database. The database is a HIPAA-compliant electronic data management system. The RedCap registry is fully integrated with the labs allowing linkage of biospecimens to the database. Subject identifiers will be de-identified at the earliest reasonable time after we receive it, meaning we will replace the identifying information with a code that does not directly identify each subject. The principal investigator will keep a link that identifies PHI to the coded information, and this link will be kept secure and available only to selected members of the research team. Only people who are involved in the conduct, oversight, monitoring, or auditing of this trial will be allowed access to the PHI that is collected.

5.1.1 Adverse Events Definition and Reporting

This study will include the collection of data from medical records, surveys, residual clinical specimens, and the collection of nasal swabs by noninvasive means, and blood draws of small volumes for research purposes. Thus, we would consider this protocol to be of Minimal Risk to patients.

The principal investigator is responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews every 6 months. During the review process, the principal investigator will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. The principal investigator and the Institutional Review Board (IRB) have the authority to stop or suspend the study or require modifications.

This protocol presents minimal risks to the subjects and Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs), including adverse events, are not anticipated. In the unlikely event that such events occur, Reportable Events (which are events that are serious or life-threatening and unanticipated (or anticipated but occurring with a greater frequency than expected) and possibly, probably, or definitely related) or Unanticipated Problems Involving Risks to Subjects or Others that may require a temporary or permanent interruption of study activities will be reported immediately (if possible), followed by a written report within 5 calendar days of the Principal Investigator becoming aware of the event to the IRB (using the appropriate forms from the website) and any appropriate funding and regulatory agencies. The investigator will apprise fellow investigators and study personnel of all UPIRSOs and adverse events that occur during the conduct of this research project through regular study meetings and via email as they are reviewed by the principal investigator.

5.2 Study Schedule

Each patient will have 2-3 total study visits. The first visit will be for the collection of informed consent, core survey, and biospecimens (nasal swabs and if participating in sub-study, acute blood). The first visit will take between 30-60 minutes. The second visit will entail the collection of follow-up surveys and for those participating in the immunologic sub-study, the collection of convalescent blood. A follow-up visit will be 4-12 weeks after enrollment and take between 15-30 minutes. We will make every effort to coordinate encounters with already planned visits. If no routine follow-up visits are planned during our time frame, we will schedule a follow-up visit at the Church Street Research Unit (CSRU). Contact #3 may occur for hospitalized patients during their inpatient stay.

5.3 Informed Consent

Written consent from parents will be required for participation in the case-control study as these patients will be identified prospectively. For the clinical epidemiologic and viral genomic studies, which involve chart reviews and secondary analysis of residual specimens, we will request a Waiver of HIPAA Authorization for the use of identifiable private information.

5.3.1. Screening (if applicable)

Eligibility for the case-control study will be determined based on the daily screening of candidate patients through the review of patient registries by research staff stationed on each participating ambulatory site. The research coordinator will screen these patients for eligibility based on the inclusion and exclusion criteria. To identify eligible patients using the EHR, we will utilize Joint Data Analytics Team (JDAT) to build dashboards to assist in identifying all patients seeking care in our sites who have not opted out of research and meet our case definition. We will contact eligible patients at the time of identification for recruitment and to obtain written informed consent. We are requesting a HIPAA Waiver of Authorization for screening purposes to allow us to review medical records to recruit subjects for the study. No direct contact with these subjects will be made other than as specified in the recruitment strategies.

5.3.2. Recruitment, Enrollment and Retention (if applicable)

If appropriate subjects are identified while still in the ambulatory site, investigators will approach the provider to refer subjects to study personnel when appropriate. For patients identified through EHR, an email or myChart message will be sent to the patient inviting them to participate in the study. After determining that a subject is eligible, the PI or designee will approach the patient or parent/guardian to offer participation in the study. The patient or parent/guardian will be informed about the objectives of the study and the potential risks and benefits of participation. They will receive a concise and focused presentation of key information about the study verbally and with a written consent form. The key information about the study will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate. After this, patients or parents/guardians will be asked to read and review the consent form. Once signed, a copy of the consent and assent form will be given to the subject for their records. If the patient or parent/guardian refuses to consent OR refuses permission for their child to participate, then all clinical management for the patient will be provided by the clinical staff in accordance with institutional practice and judgment.

When able, we will use electronic consents/assents that are identical to the paper version. All communications through our E-consent platform are encrypted and support HIPAA certification. Subjects who have difficulty navigating or using electronic systems (i.e., a lack of familiarity with electronic systems, poor eyesight, or impaired motor skills) will not be enrolled with E-consent. Instead, they will be given a paper version of the consent form and will be asked to send the signed consent form back to the research staff.

Study participation will be voluntary and confidential, and participants will be informed that none of their information will be shared with anyone outside the study team unless required by law, nor will their participation or decision not to participate affect their medical care in any way. Infants and children will be eligible for this study. Safeguards against coercion or undue influence will be in place, and the children's rights and welfare will be protected at all times.

For healthy controls, it will be the same procedure as those enrolled remotely. Namely, healthy controls that meet inclusion/exclusion criteria will be sent an electronic message through our EHR's patient portal (MyChart), inviting them to participate in the study. Healthy controls will match the vaccine failure by date of birth (±1 month), sex, and/or immunoprophylactic agent received (i.e., maternal vaccine vs mAb). A vaccine failure is a case that was previously immunized yet tested positive for RSV. Eligible healthy control subjects will be contacted until one control per vaccine failure has been successfully enrolled.

Gift Cards: Participants who complete the study protocol will be given gift cards for participation in different phases of the study: $50 for initial recruitment, and additional $25 for each blood collection.

Retention: All participants will be encouraged to participate by appropriate staff training in recruitment techniques. Various additional retention strategies will be employed, including the use of messaging strategies that are culturally tailored and that underscore the study's gain and impact on society, as well as maintaining meticulous recruitment and retention data. To enable follow-up, detailed contact information will be collected, and participants will be asked if they wish to be contacted by phone, text, regular mail, or electronic mail.

5.3.1 Study Visits (if applicable)

Visit 1 (30-60 minutes): At diagnosis of ARI (or routine visit for healthy controls)

- Consent
- Intake survey

- Collection of respiratory specimens (not for healthy controls)
- Collection of acute blood (if participating in sub-study, or healthy control)

Visit 2 (15-30 minutes): 4-12 weeks after diagnosis of ARI

- Follow-up surveys
- Collection of convalescent blood (if participating in sub-study)
- Collection of convalescent nasal swab/induced sputum (if participating in sub-study)

Visit 3 (15 minutes; for inpatients): 2-3 days after Visit 1.

- Collection of acute blood (if participating in sub-study, or healthy control)
- Collection of respiratory specimens (if participating in sub-study)

5.4 Statistical Method

5.4.1 Statistical Design

For case-control studies, the standard measure of association is the odds ratio, and the vaccine's protective effect can be estimated as 1-OR X100%. For our primary analysis, we will estimate the overall effectiveness of each immunoprophylactic strategy (mAb and maternal immunization) separately. Logistic regression models will be used for analysis, with the outcome being case/control status and immunization status as the main predictor, controlling for known confounders such as the time period (penalized spline for time), age, disease severity, breastfeeding, and race/ethnicity. To ensure rigor, we will perform extensive model validation and testing through the comparison of alternative modeling structures, uncertainty analyses, and internal consistency checks. For our primary analyses of the vaccine's effectiveness, we will use backward selection to identify covariates to include in the final model.

For our EHR aims, we will estimate the impact of introduction of the vaccine on the age-standardized rate (ASR) of monthly and annual disease by analyzing trends with an interrupted time-series model. This model fits the monthly outcomes of interest to an ordinary least-squares regression model including a linear trend, an indicator variable for the post-vaccine period, and Fourier terms (sine and cosine functions) to control for seasonality. For these models, we will use Newey-West standard errors and a first-order autoregressive function. For comparison, counterfactual outcomes will be calculated to predict the outcome of interest (RSV-related disease) during the post-vaccine period had the vaccine not been introduced. Counterfactuals will be predicted by carrying forward the pre-intervention trend estimates to the last month of observation while also controlling for the trends of other health care utilization. This will allow us to more effectively estimate the

changes in incidence of RSV that may have occurred as a consequence of the vaccine while accounting for pre-existing trends. Absolute reduction in the rates of each outcome will be

calculated by taking the incidence rate of each outcome from the first year of the study and subtracting the same rate from the last year of the study. The attributable reduction in RSV will be similarly calculated but rather than using incidence of the first year of observation, we will use the predicted incidence of the last counterfactual year. This will allow us to estimate the reduction of RSV at the end of the study accounting for the pre-existing trends. Lastly, to assess if the incidence of RSV infection and disease varies significantly by RSV vaccine coverage in this population, we will fit logistic regression change-point models that account for various degrees of vaccine coverage post-vaccine introduction.

5.4.2 Sample Size Considerations

The number of cases needed to detect a range of estimates of effectiveness with $\alpha<.05$ and 80% power. We calculated the sample sizes presented for different proportions of controls that might be immunized and for a 2:1 ratio of controls to cases using established formulas.[34] It is not known what proportion of patients will be immunized. However, we can model our sample size calculations based on our experience with the implementation of neonatal hepatitis B vaccine and uptake of influenza vaccine in pregnant women. In Connecticut, within a year of the CDC issuing the recommendation that all newborns receive their first dose of hepatitis B vaccine before hospital discharge, coverage of the vaccine was 55% by 3 days of life.[35] For the 2022/23 season, the coverage of influenza vaccine was 47.1% for all pregnant women, lowest (28.4%) for Black, and highest (65.2%) for Asians.

During the 5-year study period, we aim to enroll 1250 RSV-positive and 2500 RSV-negative infants ≤12 months of age, and 500 RSV-positive and 1000 RSV-negative 12-60 month old children for a total sample size of 5250. For priority groups, such as hospitalized infants, all eligible patients will be contacted, whereas for clusters with large numbers, a simple random sample will be used until our yearly goals are met (250 cases and 500 controls). Healthy community controls will only be enrolled if an enrolled case is identified as a vaccine failure. A maximum of 50 healthy controls will be enrolled during the study period.

5.4.3 Planned Analyses

VE will be estimated using multivariate logistic regression with RSV status as the outcome and

vaccination status as the main predictor with an estimated VE = (1 – adjusted odds ratio) x 100. The effect of immunization over time will be captured using a series of categorical variables that represent the time since the most recent dose. As an indicator of the level of exposure at different time points, we will also consider the number of positive tests performed at focal time throughout the study population. Additional analyses will also consider disease severity. If effect modification is observed by disease severity, or other participant characteristics (e.g., race, ethnicity, social determinants of health), the effectiveness for each will be determined separately if a sufficient sample size allows.

Additional analyses will be performed to address the secondary aims of the study. If effect modification is observed by participant characteristics (race, ethnicity, social determinants of health), vaccine regimens (number of doses, or products), or viral variants in circulation, the effectiveness for each will be determined separately if a sufficient sample size allows. Hierarchical modeling approaches will be used to allow robust estimates of variability in the vaccine's effectiveness by subgroup. Since it is possible (indeed, it is likely) that in some instances, the vaccine may modify the severity of disease rather than prevent it, cases will be stratified according to the clinical severity of their illnesses (mild, moderate, or severe) and the protective effect of the vaccine within each of these strata will be assessed using the methods described previously.

We will conduct several additional analyses to assess whether bias might have been introduced in the selection of the controls. First, since we do not expect the vaccine to induce an immune response during the first 7 days following its receipt, we will use this as a negative control period and create an indicator variable for vaccines given within this period. Any significant effect for this variable could indicate the presence of residual confounding.[19] We will also assess and compare the completeness of other immunizations. Receipt of other vaccines could be used as a variable in the multivariable analyses to assess whether adjusting for this potential marker for selection bias has an effect on the estimate of the vaccine's effectiveness. Conversely, if there is no significant difference between the cases and the controls in the proportion who had received other vaccines, it would suggest that selection bias from vaccine ascertainment is not likely to have been an important factor. From serum, we will look for differences in the proportion of innate immune cell populations (neutrophils, dendritic cells, monocytes, and natural killer cells) and adaptive immune cells (B cells and T cells) in infants with breakthrough infections compared to healthy matched controls and test-negative controls. Second, we will assess if maternal antibodies can impede the infant's immune response following exposure to the virus. To define the kinetics of the innate and adaptive immune response at the cellular level, we will quantify the frequency and phenotypic differences of peripheral blood immune cells during acute and

convalescent blood of RSV+ infants who were both immunized and unimmunized. We will use unpaired T- tests for between-groups comparisons and paired T-test to compare acute vs convalescent data. Third, we will examine for prenatal priming. To do this, we will select samples collected during the acute phase of illness of RSV-positive infants whose mothers were immunized or who received mAb, and samples of infants who were not immunized and compare the proportions of naïve T cells and memory cells, as well as RSV-specific immune responses on acute presentation.

EHR subaims: We will conduct a rigorous, model-based investigation to identify sets of clinical characteristics that are highly predictive of RSV infection. To determine which set of clinical predictors to use in the optimal case definition, we will use latent class analysis and compare the probabilities of various sets of clinical features. Last, we will explore the utility of using an integrative and adaptative case definition. That is, one that integrates multiple levels of data (i.e., individual, provider, and community) and that adapts to various age groups. We will use optimization algorithms to identify the ideal cutoffs for the dynamic variables and select the approach that maximizes accuracy. Next, using an ecological study design, trends in RSV testing patterns and disease will be assessed using EHR data within the Yale New-Haven Health System (YNHH). A database will be created with data extracted from the EHR electronically with support of Yale's Joint Data Analytics (JDAT) team. This database includes encounter specific data from inpatient and outpatient visits at YNHH clinics. Data will be collected for all patients age ≤60 months who are Connecticut residents, were tested for RSV, and received care at the YNHHS between 7/1/2018 to 7/1/2028, which includes 2 seasons of pre-COVID-19 data.

For our viral genomic analysis, the first question we aim to answer here is whether the effectiveness of the vaccine and mAb differs significantly between the major groups or clades. To do this, we will perform a case-only analysis in which we compare the immune status among cases infected with RSV group A versus B and assess the relative effectiveness of immunization. For this analysis, logistic regression will be used to control for the time of testing and other relevant confounders. The second question we intend to answer is whether the viral mutations that were previously linked with F-protein antibody neutralization resistance (N67I/N208Y in RSVA; N208S, N208D, K68N/N208S, and

K68N/N201S in RSV B), are overrepresented in the vaccine breakthrough group, relative to non-immunized RSV cases in the same respiratory season. The third question we will consider is whether the vaccines/mAb are more likely to prevent infections from RSV strains that are genetically closest to the strains used to derive the vaccine/mAb (i.e., "vaccine reference"). We will conduct pairwise genetic distance comparisons between breakthrough cases and "vaccine reference" and test for statistical significance using two-way ANOVA with Tukey's for multiple comparisons. We will then compare the Hamming distance between immunized and unimmunized cases using logistic regression models adjusting for the calendar time.

Analysis of Subject Characteristics (if applicable)

We will compare baseline characteristics of cases versus controls using chi-square test, conditional logistic regression, or the Wilcoxon signed-rank test (depending on the nature of the variable). To assess the possibility of selection bias, we will compare demographic variables of cases we enroll with those who decline to participate.

5.4.4 Interim Analysis (if applicable)

We will conduct analyses every year to assess the annual VE. Analyses of the clinical characteristics of patients with medically attended acute respiratory illness will be repeated at regular intervals to identify changes in the clinical presentation of RSV, as well as changes in proportions of patients with ARI due to the different viruses. An interim analysis will be conducted to estimate the number of patients infected with RSV other respiratory viruses, in the source population (over a given period of time) based on the number of positive enrollees in the study and an estimate of the number or proportion of study-eligible patients that were missed (i.e., not enrolled).

5.4.5 Handling of Missing Data

We will address missing data via modern imputation methods as appropriate for each analysis. For any situation in which missing data are substantial, we will conduct sensitivity analyses.

6 Trial Administration

6.1 Ethical Considerations: Informed Consent/Assent and HIPAA Authorization

Screening every individual that is seen during a clinical encounter can obstruct the clinical workflow and be overburdening for ineligible patients. Thus, we are requesting a HIPAA Waiver of Authorization for this study to allow the study coordinator and investigators to

review medical records at YNHHS as pre-screening for recruitment. No direct contact with these subjects will be made other than as specified in the recruitment strategies above. The PHI viewed and used during the pre-screening process for ineligible patients will never be reused or disclosed to any person or entity other than those listed in this study application.

We are also requesting a waiver for the clinical epidemiologic studies, which involve retrospective chart reviews and secondary analysis patients who were tested for RSV. Obtaining individual authorization for the clinical epidemiologic studies is impracticable due to the retrospective nature of the study and the necessity of analyzing a large, diverse cohort.

Additionally, we seek a waiver of consent for infants who test positive for RSV so that we may collect their residual respiratory swabs for viral genomic studies. This waiver is critical for several reasons. First, the research cannot be conducted without identifiable information, as linking viral genetic sequences to clinical outcomes and vaccination status is essential for accurately classifying breakthrough infections and performing time-matched comparisons. Second, requiring authorization would reduce statistical power and introduce selection bias by restricting the dataset to individuals who consent, thereby compromising the scientific validity of epidemiologic estimates and time-matched analyses. An underpowered and biased dataset undermines the very purpose of this research—to generate reliable, generalizable findings that can guide clinical and policy decisions. Third, it will be impracticable to conduct the genomic studies if the waiver is not granted. Many infants are tested at Yale but do not receive ongoing care within the system, making direct contact challenging. Further, virology labs store residual respiratory swabs for only 2–7 days before discarding them. In that short window, it is simply not feasible to reach every parent and obtain authorization before the samples are lost. Finally, granting this waiver would facilitate participation in the main case-control study by reducing subject burden. Many parents are willing to contribute to research but are hesitant to subject their child to additional swabbing. By accessing and storing residual RSV-positive samples from all Yale labs, we can reduce the burden on families who are willing to participate in the full study. This approach would allow us to reach out to potential participants after their initial swab, giving them time to consider enrollment without losing valuable specimens. If a patient opts out of research or declines participation all corresponding samples and data will be discarded.

For all other aspects of this protocol, including participation in the case-control study, an informed consent form approved by the Yale School of Medicine Human Investigation Committee will be used to explain the purpose of the study and the risks and benefits of study participation before the subject is entered into the study. A member of the investigative research team will approach subjects or authorized representatives/surrogates and discuss the information provided on the consent form in detail. Subjects and authorized representatives/surrogates will be given time to read the form and ask questions during the

process of consent. After they sign the consent form, they will be given a copy of the signed form and informed to contact the PI or study coordinator if they have further questions or concerns. Informed consent will be obtained from each subject or authorized representative/surrogate with appropriate signatures and dates on the informed consent documents prior to the performance of any protocol procedures (see Recruitment Procedure above). When an authorized representative/surrogate provides the initial consent, the subject will be asked to continue their participation when and if they regain the capacity to make decisions. Subjects refusing to continue participation in the study will be withdrawn from the study and their study samples and information will be discarded/destroyed if requested.

To reduce exposure to potentially infected paper, when able, we will request electronic signatures through electronic informed consent that can be accessed through mobile devices. Subjects who have difficulty navigating or using electronic systems because of, for example, a lack of familiarity with electronic systems, poor eyesight, or impaired motor skills, will not be enrolled with electronic consent. Instead they will be given a paper version of the consent form, and after signing the consent form, will be asked to send the complete signed consent form to the research staff.

The ability and capacity to consent/assent will be determined by the Principal Investigator or research team members by questioning during the informed consent process and by discussion with the inpatient medical staff. Potential subjects will be asked, “Could you explain to me what we are going to ask you to do in this study? This will help me be to be sure that you understand the research,” as well as, “What more would you like to know about this study?” If the subject is unable to understand, we will not continue to pursue consent.

Consent documents will be translated into Spanish versions for Spanish-speaking subjects. Spanish version consent documents will be submitted to the HIC for review and approval before administration. For eligible participants who speak Spanish, a translated consent form will be provided, and consenting procedures will proceed in Spanish by study personnel fluent in Spanish.

Study participation will be voluntary and confidential, and participants will be informed that none of their information will be shared with anyone outside the study team unless required by law, nor will their participation or decision not to participate affect their medical care in any way. Infants and children will be eligible for this study. Safeguards against coercion or undue influence will be in place, and the children’s rights and welfare will be protected at all times.

The results from assays that are FDA-authorized will be returned directly to participants if

they indicated on the consent form that s/he would like the results returned. For positive test results, participants will be advised to begin early isolation while they consult with their primary care provider for confirmatory testing and treatment. Tests that are exploratory in nature, such as the sequencing, will not be conducted in an actionable timeframe post sample collection. Therefore, these results will not be reported back to the individuals or their families but may be aggregated for deidentified publication of study results.

6.2 Institutional Review Board (IRB) Review

The protocol will be submitted to the IRB for review and approval. Approval of the protocol must be obtained before initiating any research activity. Any change to the protocol or study team will require an approved IRB amendment before implementation. A study closure report will be submitted to the IRB after all research activities have been completed.

6.3 Subject Confidentiality

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval. All research activities will be conducted in as private a setting as possible.

The study monitor, representatives of the Institutional Review Board (IRB), or regulatory agencies may inspect all documents and records required to be maintained by the investigator. The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or, if applicable, sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored in a RedCap folder. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used will be secured and password protected. At the end of the study, all study databases will be de-identified and archived in the Yale Secure Box folder.

6.4 Deviations/Unanticipated Problems

A protocol deviation is any noncompliance with the study protocol. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of

deviations, corrective actions are to be developed by the site and implemented promptly. The principal investigator will report the following types of events to the IRB: a) adverse events that are serious or life-threatening AND unanticipated (or anticipated but occurring with a greater frequency than expected) AND possibly, probably or definitely related to the drug/device/intervention; and b) other unanticipated problems involving risks to subjects or others. These adverse events or unanticipated problems involving risks to subjects or others will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website.

6.5 Data Quality Assurance

The use of computer-assisted data entry will be used to help minimize data entry errors. All variables will be examined for missing and out-of-range values by examining the frequency distributions of each variable. Consistency and logic checks will be conducted, and any errors will be corrected.

6.6 Study Records

Study records include regulatory documents, protocols, consent forms, subject medical records, surveys, and specimens.

6.7 Access to Source

Source data will be maintained per Medical Records policy in a password-protected, secure, Health Insurance Portability and Accountability Act (HIPAA) compliant, web-based electronic database with a built-in audit trail. Only Institutional Review Board (IRB) approved research team members who have current HIPAA and Collaborative Institutional Training Initiative (CITI) and human subjects protection training will be authorized to access records.

6.8 Data or Specimen Storage/Security

The samples contained in the repository will be coded and stored in a freezer in a locked laboratory. The link to the code will be stored in a separate password-protected file. Information about the subjects will be maintained in a password-protected computer and password-protected data files. The information resides on a server considered by ITS-Med to adhere to the HIPAA Security Rule.

6.9 Retention of Records

At the completion of the study, the link to personal information will be kept for 5 years, after which time the link will be destroyed and the data will become anonymous. Data destruction will occur according to Yale policy. The data will be kept in this anonymous form indefinitely. If a research subject withdraws permission for identifiable biologic material or data to be used for future research purposes, the data and specimens from

those subjects will be destroyed and not used to generate any further data.

6.10 Study Monitoring

The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency, which must be conducted at a minimum of every 6 months.

6.11 Study Modification

During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment. Either the principal investigator or the Yale Human Investigations Committee have the authority to stop or suspend the study or require modifications.

6.12 Study Completion

The study is expected to be completed on 11/30/2028. The PI will submit a study closure report to the IRB after all research activities have been completed.

6.13 Funding Source

NIH R01AI179874
The Hartwell Foundation
NIH 1K08AI177743

6.14 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with the appropriate conflict of interest review committee has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest. All investigators will follow the applicable Yale conflict of interest policies.

6.15 Publication Plan

The PI will be primarily responsible for publishing the results of this work. If information from this study is presented publicly or published in a medical journal, no research subject will be identified by name, picture, or any other personally identifying manner.

Appendices

Appendix #	Title	Section	Topic

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