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Real-world effectiveness of perinatal RSV immunoprophylaxis: protocol for a test-negative case–control study

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ABSTRACT

Introduction Respiratory syncytial virus (RSV) is a leading cause of hospitalisation in infants worldwide. New immunoprophylactic products, including long-acting monoclonal antibodies and maternal vaccines, have demonstrated high efficacy in prelicensure clinical trials. Understanding how these interventions perform outside controlled trials, and how viral evolution or host factors influence protection, is essential for sustaining confidence in RSV prevention programmes.

Methods and analysis We will conduct a 5-year, test-negative case–control study among infants ≤12 months of age who present with acute respiratory illness (ARI) within a large healthcare delivery network serving a demographically diverse population. Cases will be infants testing positive for RSV by PCR, and controls will be RSV-negative infants meeting the same ARI criteria. Data will be obtained from electronic health records, structured caregiver surveys and state immunization registries to ensure accurate classification of exposures and covariates. Vaccine effectiveness will be estimated using multivariable logistic regression controlling for potential confounding. RSV-positive specimens will undergo full-genome sequencing to identify variant lineages and potential immune-escape mutations. A subset of participants will provide acute and convalescent blood samples for single-cell immune profiling to define innate and adaptive responses associated with breakthrough infection.

Ethics and dissemination The study protocol has been approved by the Yale Human Investigation Committee (HIC #2000036550). Written informed consent will be obtained from all parents or legal guardians prior to participation. Study findings will be disseminated through peer-reviewed publications, scientific meetings and public repositories, with fully de-identified participant data to protect privacy and confidentiality. Viral genomic data will be shared in accordance with the National Institutes of Health Genomic Data Sharing Policy, and analytical code will be made publicly available to ensure reproducibility.

Trial registration number NCT06172660.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study uses a well-established test-negative case–control design with systematic testing of all eligible infants with acute respiratory illness, minimising bias from healthcare-seeking behaviour and reducing outcome misclassification due to variability in clinician testing practices.
⇒ Linkage of electronic health records, immunization registries and caregiver surveys enhances completeness and accuracy of exposure and confounder classification, reducing information bias.
⇒ Integration of viral genomic sequencing and immune profiling within the same surveillance framework is a key strength, enabling exploration of both real-world effectiveness and biological mechanisms of breakthrough infections.
⇒ The immune profiling substudy involves a smaller, select subset of participants and may not fully represent the broader study population.
⇒ As with all test-negative case–control studies, residual selection bias may affect the estimates of effectiveness if enrolment differs by immunization status.

INTRODUCTION

Respiratory syncytial virus (RSV) remains a major contributor to morbidity and mortality in children worldwide, and the leading cause of hospitalisation in US infants.[1 2] New immunoprophylactic agents have recently been introduced with demonstrated efficacy in preventing RSV-related lower respiratory tract infection (LRTI) in early life. Nirsevimab and clesrovimab, extended half-life monoclonal antibodies (mAbs) given as a single dose, showed >75% efficacy in phase three trials and have been licensed for routine use in multiple countries.[3–7] Maternal immunization with RSV prefusion F vaccine has also shown high efficacy in protecting infants against RSV via transplacental antibody transfer and is now recommended during pregnancy.[8 9] Despite the progress, important questions remain about how these

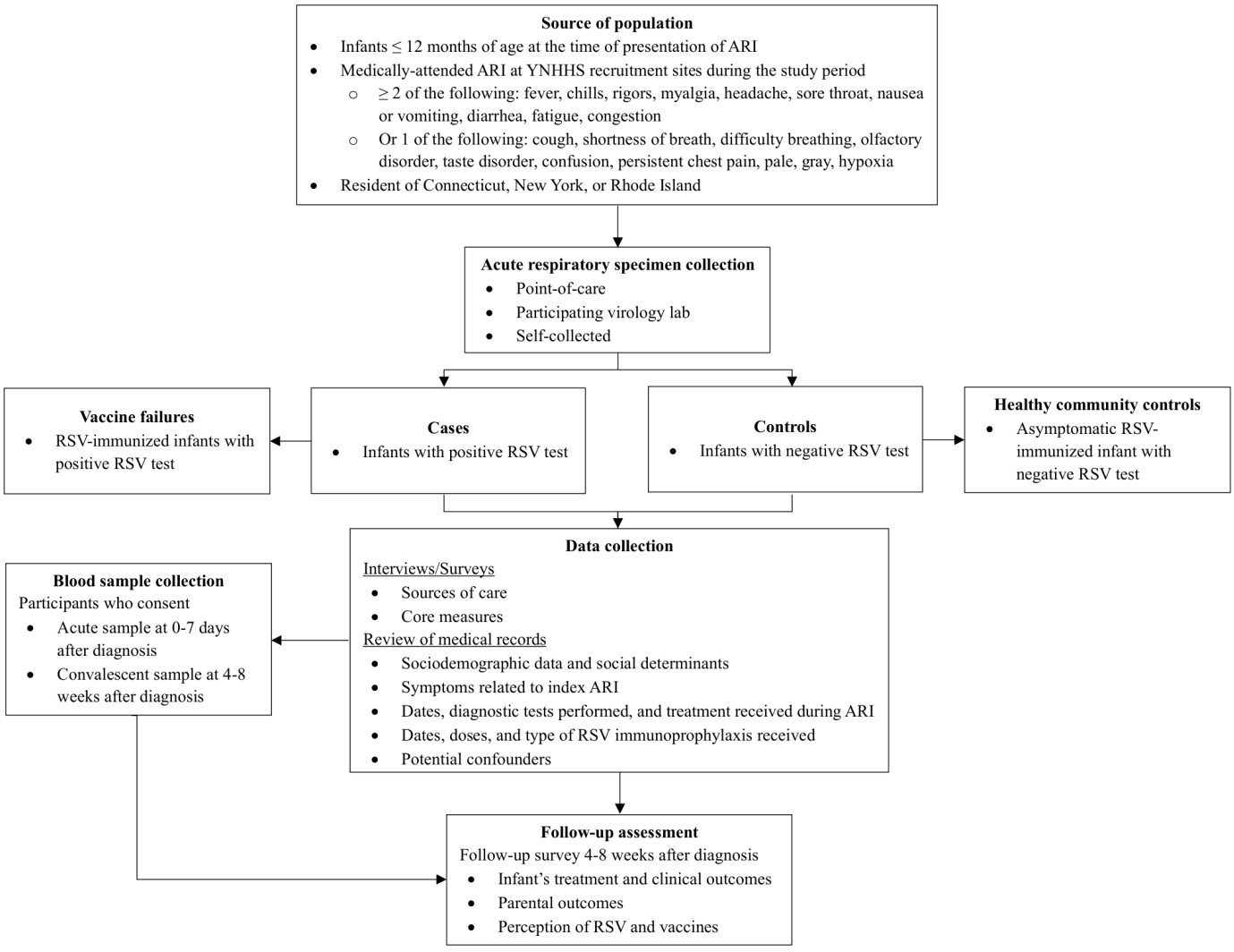

Figure 1 Study design and case–control definitions. ARI, acute respiratory infection; RSV, respiratory syncytial virus; YNHHS, Yale New Haven Health System.

new immunoprophylactic agents will perform outside clinical trials. The protection observed in prelicensure clinical trials (efficacy) does not always translate directly into postlicensure benefits (effectiveness). Effectiveness may be influenced by real-world factors such as timing of administration or host conditions that alter pharmacokinetics or immune responses.[10 11] Thus, postlicensure monitoring of these new immunoprophylactic agents is crucial to ensure the benefits are being realised in clinical practice.

Viral genetic diversity may also affect protection. Several studies have shown evidence that genomic diversity may be contributing to RSV's propensity to evade herd immunity.[12 13] The emergence of variants with changes in major surface proteins raises concerns that the virus could adapt to vaccine-mediated or mAb-mediated pressure,[14] which could potentially undermine the long-term success of the programme. Genomic surveillance will therefore be critical to detect such variants early and to assess the extent to which these new variants alter the protective effect of vaccines and mAbs.

There are also several open questions about the extent to which passive immunization will modulate the immune response of infants. Key aspects of early immune maturation are still being defined, including whether maternal vaccination antigens prime the fetal immune system and whether these antibodies might blunt subsequent immune memory. Safety must also be continuously monitored. Studies from the original formalin-inactivated RSV vaccine demonstrated that immune complex deposition and pathogenic T helper cell 2 responses were likely key drivers of vaccine-enhanced disease.[15] These events generated lasting concerns that slowed RSV vaccine development and could affect uptake postlicensure if not rigorously evaluated. More research is needed to define how prenatal vaccination and passive immunization shape innate and adaptive immune responses to RSV in early life.

Thus, postlicensure monitoring of the clinical, virological and immunological outcomes of infants is critical to maintaining trust in these immunoprophylactic agents and sustaining their uptake in the population. To address

these important questions, we propose a large-scale epidemiological study and an in-depth multiomics analysis with the following specific aims:

- Aim 1: To determine the real-world effectiveness of perinatal RSV immunoprophylaxis against medically attended infections in infants aged 12 months and under.
- Aim 2: To quantify the extent to which viral genomic diversity impacts the effectiveness of immunoprophylaxis.
- Aim 3: To define the impact of immunoprophylaxis on the immune trajectories of infants during medically attended acute RSV infection.

METHODS AND ANALYSIS

Overview

The overall framework of the proposed work is shown in figure 1. For Aim 1, we will conduct a large-scale test-negative case–control study, a design that is well-established as valid for studies of real-world vaccine effectiveness (VE). Cases will be defined as infants ≤12 months old who had a clinical encounter for acute respiratory illness (ARI) and tested positive for RSV using PCR. Controls will be individuals with ARI who test negative for RSV. Interviews, reviews of medical records and immunization registry searches will be conducted in the same manner for cases and controls to provide information on immunization history, clinical outcomes and effect modifiers. Logistic regression will be used to estimate the effectiveness of each immunoprophylactic strategy (maternal vaccine or mAb). Valid inference from the test-negative design depends on several assumptions, including accurate classification of infection, similar testing and enrolment of symptomatic patients regardless of immunization status, and controls drawn from the same underlying population as the cases.[16 17] To evaluate possible departures from these assumptions, we will compare enrolled versus non-enrolled eligible infants, assess testing and enrolment patterns by immunization status, and perform sensitivity analyses using alternative outcome and control definitions. For Aim 2, we will sequence all identified RSV viruses and conduct formal sieve analyses to compare breakthrough infections with time-matched unimmunized infections and examine the effects viral lineages have on the effectiveness of mAb and vaccines. For Aim 3, we will collect acute and convalescent blood from a subset of infected infants with and without prior immunization and will recruit an additional group of uninfected controls. We will then employ a single-cell multiomics approach to study the dynamics of the innate and adaptive immune responses during infection and explore potential molecular mechanisms that contribute to immunoprophylaxis failure.

Study Population and Eligibility Criteria

The study will be conducted within Yale New Haven Health System (YNHHS), Connecticut's largest healthcare delivery network. The YNHHS has an extensive network of hospitals, ambulatory clinics, urgent care centres and clinical laboratories which serve a diverse patient population representative of US national demographics.[18 19] All YNHHS clinical sites are unified by a single, integrated electronic health record (EHR) system, which facilitates case-finding and standardised data abstraction.

The study population will consist of infants aged 12 months and younger who present with an ARI to YNHHS-affiliated sites for care and whose guardian/parent can communicate in English, Spanish or Portuguese for consent and participation. To decrease potential selection biases that can arise from variability in physician testing practices, we will enrol patients who meet the ARI case definition (online supplemental appendix table 1) regardless of whether they were tested for RSV as part of routine clinical care. Respiratory specimens will be collected from all participants and screened for RSV using molecular assays. Cases will be defined as individuals who had both a clinical encounter for ARI symptoms and tested positive for RSV. Controls will be individuals who test negative for the virus. We will restrict participation to residents of Connecticut, New York and Rhode Island so that immunizations can be verified in the statewide immunization registries.

Recruitment

Eligible infants will be identified through two primary methods: (1) real-time screening of chief concerns and triage notes in the YNHH paediatric emergency department and inpatient units, and (2) next-day case-finding via EHR review for patients seen at affiliated sites. After confirming eligibility, trained research staff will approach the parents/guardians, explain the study's objectives and procedures (online supplemental appendix document S1), and obtain written informed consent. Parents/guardians will be provided gift cards for their involvement in the various phases of the study.

Data Collection

For this study, we aim to collect data from multiple sources, including EHRs, self-report (interviews and questionnaires) and public health data (immunization registries and population-based surveys). This multimodal approach will allow us to evaluate data congruence as well as implement corrective measures if problems are detected. To reduce the potential for misclassification bias due to missing data, we will use identical data collection procedures for both cases and controls.

During intake, parents or guardians will be asked to complete a standardised survey to capture potential confounders not routinely available in the EHR, including household characteristics, daycare attendance and maternal health (see table 1, online supplemental appendix 2, Instrument S1). Surveys will be administered either by research coordinators, who will read questions aloud and record responses into a secure web-based platform, or by participants using a computer-assisted self-interviewing programme. Follow-up surveys will be sent to

Table 1 Core measures to be ascertained

Category	Variables	Method
Sociodemographic	Age, gender, race, language, insurance, employment and household characteristics.	Interviews/surveys Medical record (EHR)
Medical history	Date of illness onset, symptoms and their severity, previous respiratory infections, medical history (ICD-10, CPT codes), healthcare utilisation, medication history (antibodies, antivirals, immunosuppressants).	Interviews/surveys Medical record (EHR)
Immunization history	Dates, types and names of all vaccines received. Name and address of vaccine providers. Adverse effects to immunization.	Interviews/surveys Medical record (EHR) Immunization registries
Exposure history	Exposure history (eg, household, daycare). Smoke. Prenatal maternal medical history (eg, comorbidities, prenatal care, immunizations, medications).	Interviews/surveys
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.
CPT, Current Procedural Terminology; EHR, electronic health record; ICD-10, International Classification of Diseases, 10th Revision; NIH, National Institutes of Health.

parents/guardians 4–8 weeks after the initial ARI-related encounter to assess the infant's treatment and clinical outcomes, as well as measures related to health-related quality of life (online supplemental appendix 2, Instrument S2). The follow-up survey will also include questions about the parent/guardian's knowledge and perception of RSV and vaccines. Survey instruments were developed using a combination of previously validated and custom measures. All custom items underwent cognitive testing and are provided in the online supplemental appendix 2.

Comprehensive medical record reviews will be conducted for all participants using a standardised abstraction form. We will abstract data from all encounters within the YNHHS, including subspecialty visits, to capture diagnoses, treatments and comorbidities for up to 2 months following the initial ARI. Immunization histories will be verified using the Connecticut Immunization Information System (CT-WiZ), New York State Immunization Information System (NYSIIS) and Rhode Island Child and Adult Immunization Registry (RICAIR/KIDSNET) to ensure accurate classification. The core variables that we aim to capture during the review of medical records are also outlined in table 1.

Viral detection and genomic sequencing

Specimens will be coded so that both the technicians and the investigators who perform and interpret the tests will be blind as to whether the subject has received the RSV mAb or maternal vaccine. Respiratory samples will be collected from all participants. If respiratory samples have already been collected for clinical purposes, we will request residual specimens from the participating clinical virology laboratory. These samples will be screened by reverse-transcription quantitative PCR (RT-qPCR) for human RNase P, to check for overall sample viability; only those with a cycle threshold (Ct) value ≤35 will be included in the study.

An on-site study investigator will collect a midnasal turbinate swab specimen from enrolled patients for whom a respiratory sample is not available from the clinical virology laboratory, or from those who consent to provide an additional respiratory sample. Using a minitip Copan FLOQ swab, the investigator will insert the swab into the nostril parallel to the palate about 1 cm and gently twist the swab for 15 s to ensure adequate contact with the nasal mucosa.[20] Immediately after collection, the swab will be placed into a tube containing viral transport media and kept at 4°C for up to 4 hours prior to transfer for storage in −80°C. Self-collected specimens will be used for participants enrolled via telehealth who have not already provided a clinical specimen. For these infants, we will provide the parents/guardians with a sample-collection kit, and the study team will supervise self-collection using video conferencing.

All on-site collected, self-collected and remnant specimens with a Ct ≤35 will be sent for genomic analysis at the Yale School of Public Health. Total nucleic acid will be isolated from all samples using the Monarch Mag Viral DNA/RNA Extraction Kit (New England Biolabs), omitting the use of carrier RNA to prevent downstream interference in sequencing. Isolated nucleic acid will be screened for RSV using RT-qPCR using previously described oligonucleotide sequences to assess viral genome quality.[21] All isolated samples, despite their Ct, will be prepared for targeted amplicon sequencing with the COVIDSeq RUO Test Kit (Illumina), using a custom PCR primer panel designed to amplify the RSV genome. All testing runs will include negative controls, added at each major step in the protocol, to monitor for mass contamination. Prepared DNA libraries will be sequenced at 2×150 bp, with 1 million reads per sample, on the NovaSeq X (Illumina) at the Yale Center for Genomic Analysis. Sequence reads will be aligned to established RSV-A and RSV-B reference genomes (GenBank). We will then use Nextstrain and maximum-likelihood phylogeny to characterise circulating viral lineages.

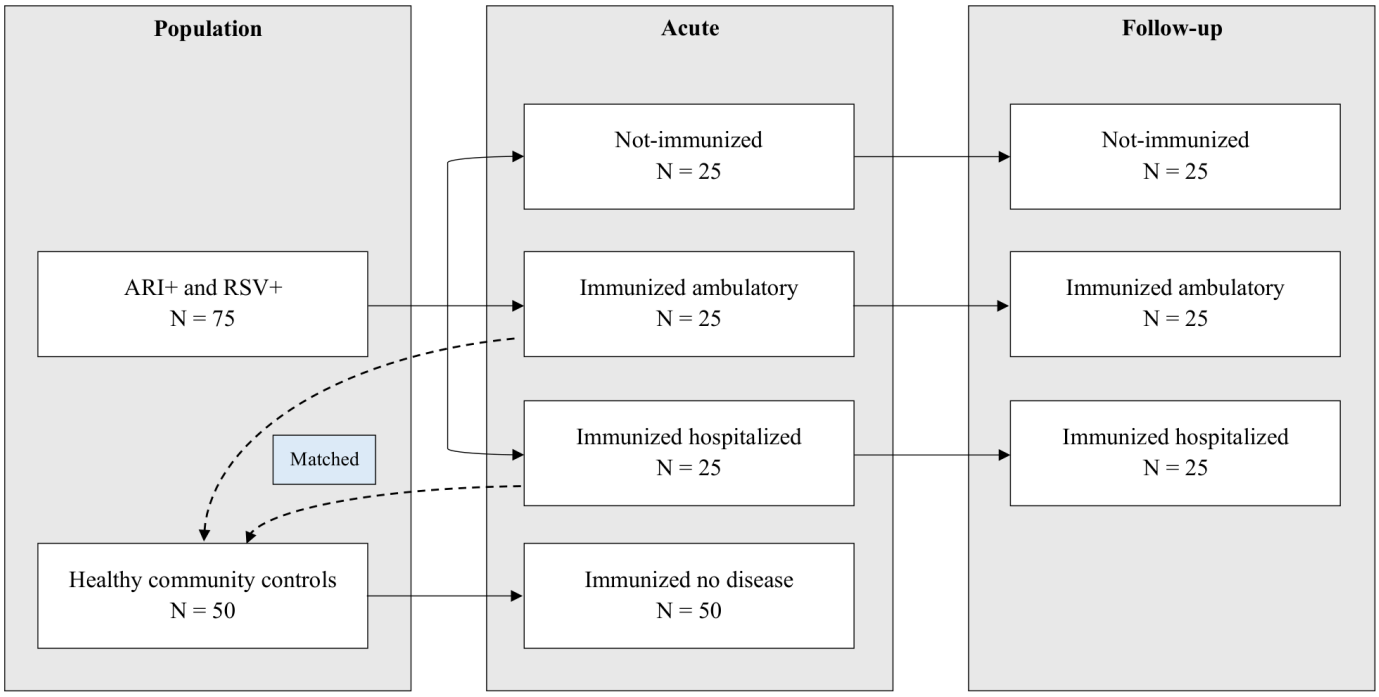

Figure 2 Sample for deep immune profiling. ARI, acute respiratory infection; RSV, respiratory syncytial virus.

Immune profiling

We will collect acute and convalescent blood from a subset of infants (n=75) identified in Aim 1 and recruit an additional 50 healthy community controls for deep immune profiling (figure 2). Community controls will be individually matched to enrolled vaccine failures (immunized and RSV+) by date of birth (±1 month), sex and immunoprophylactic agent received (ie, maternal vaccine vs mAb). Acute samples will be obtained within 1 week of ARI diagnosis, and convalescent samples 4–8 weeks post-diagnosis. To minimise burden, collection will be coordinated with routine clinical draws, and residual laboratory specimens will be used whenever possible. In the absence of an indwelling line, capillary blood will be obtained for both acute and convalescent samples using a Food and Drug Administration-approved microneedle device, as previously described.[22 23] Caregivers will be supervised remotely by study staff during the at-home collection and will be provided with prepaid materials for sample return (online supplemental appendix document S2).

We will use paired blood samples to characterise both innate and adaptive immune responses using high-dimensional single-cell profiling methods.[24] Circulating immune cell populations will be examined to define the composition and activation state of major lymphocyte and myeloid subsets. Antigen-specific T cell populations will be identified and characterised to assess the nature of the cellular immune response to RSV, including distinctions between naïve and memory subsets and functional responses following exposure. We will monitor for vaccine-enhanced disease by comparing immune responses in hospitalised vaccine breakthrough cases to those in unimmunized infants with and without RSV.

Statistical analysis

Descriptive analyses and baseline comparisons

All analyses will use a two-sided type I error rate of 5%. Baseline characteristics of cases and controls will be compared using χ^2 or Fisher's exact tests for categorical variables and Wilcoxon rank-sum tests for continuous variables. To assess participation bias, we will compare characteristics of enrolled versus non-enrolled individuals.

Primary analysis of overall vaccine effectiveness

For our primary aim, we will estimate the protective effect of RSV immunoprophylaxis using ORs from logistic regression models, with VE calculated as (1 – OR) × 100%. In these models, the outcome is the infant's RSV test status (case or control) and immunization status is the main predictor. For infant mAb prophylaxis, we will classify an infant as immunized only if the dose was administered ≥7 days before RSV testing to minimise inclusion of infections acquired before prophylaxis and to align with prior trials and postlicensure studies.[25] For maternal vaccination, we will define immunization as doses given ≥14 days before delivery to allow time for development and transplacental transfer of protective antibody to the infant.[26] Effectiveness will be estimated overall and for each strategy (maternal vaccine and mAb) separately using multivariable models that adjust for age, calendar time and other relevant confounders. We will use correlation matrices and variance inflation factors to assess correlation among candidate covariates, including individual-level and area-level social determinants of health, and limit models to non-redundant measures. If effect modification is observed by time from immunization, disease severity (ie, hospitalised vs not hospitalised),

or other participant characteristics (eg, social determinants of health), the effectiveness for each will be determined separately. Missing covariate data will be handled with multiple imputation.

Bayesian analysis of yearly vaccine effectiveness

For yearly VE estimates, where sample sizes may be limited, we will supplement frequentist analyses with Bayesian logistic regression models. These models will incorporate prespecified covariates and allow the effect of immunization to vary by epidemiological year. Year-specific estimates will be modelled hierarchically, with log-ORs for each year drawn from a common distribution, enabling partial pooling across years.

Informative priors for the overall effect will be derived from phase 3 trial efficacy estimates and early postlicensure data, with variance chosen to reflect uncertainty and to allow the data to update prior beliefs. Posterior distributions of yearly VE will be summarised as medians with 95% credible intervals, along with posterior probabilities that VE exceeds clinically relevant thresholds. This approach improves efficiency in years with small sample sizes, provides transparent integration of prior and current evidence, and allows a more robust characterisation of the yearly variation in effectiveness.

Impact of viral diversity on effectiveness

The second aim will evaluate whether the effectiveness of maternal vaccination or mAb prophylaxis varies across specific strains or genotypes of RSV. To do this, we will first conduct a case-only analysis and model viral group (eg, RSV-B vs RSV-A) as the outcome and immunization status as the predictor to estimate whether breakthrough infections differ by group (adjusting for infant age and calendar time). Next, we will investigate whether specific F-protein mutations previously linked with antibody neutralisation resistance (N67I/N208Y in RSVA; N208S, N208D, K68N/N208S and K68N/N201S in RSV B) are over-represented in breakthrough infections (ie, immunized cases). Last, we will test the hypothesis that immunoprophylaxis is less effective against viruses that are genetically more distant from the vaccine reference strain. We will calculate amino acid differences (Hamming distance) between the F-protein sequence of each RSV case and that of the reference vaccine strain (Netherlands RSV A and B). Distance will be modelled as a continuous predictor in logistic regression with immunization status as the exposure, adjusting for calendar time and other relevant confounders.

Analysis of acute and convalescent immune profiles

Acute and convalescent blood samples from a matched subset of participants (n=3–5 per group; figure 2) will undergo multiomic profiling using Olink proteomics and single-cell RNA sequencing. Integrated findings from these assays will guide the selection of markers incorporated into high-dimensional spectral flow cytometry panels used to characterise baseline and post-activation immune signatures across all samples. Spectral flow cytometry data will undergo standard quality control and normalisation procedures, followed by dimensionality reduction (t-distributed stochastic neighbor embedding or uniform manifold approximation and projection (UMAP)) and unsupervised clustering (eg, FlowSOM or Phenograph) to define immune cell subsets and activation states. Cluster frequencies and marker expression will be compared between acute and convalescent samples using paired t-tests or Wilcoxon signed-rank tests, and between clinical subgroups using unpaired t-tests or Wilcoxon rank-sum tests. Associations between immune features and clinical outcomes will be examined using multivariable regression models adjusted for relevant covariates such as age, viral status and time from symptom onset.

Sensitivity analysis

We will conduct several pre-specified sensitivity analyses and test alternative definitions of the core components of our study. First, we will test our outcome definition by re-calculating VE using a stricter set of clinical criteria for LRTI. We will then explore alternative definitions of controls, such as only including controls that tested positive for other respiratory viruses (eg, influenza). We will also vary exposure windows to account for potential misclassification. Last, we will use 'sham' exposures as a way to assess the potential effects of uncontrolled confounding, as we have previously described.[27 28] To ensure the robustness of our findings, we will address uncertainty from both measured and unmeasured confounders. First, to address uncertainty in model specification for measured covariates, we will use Bayesian model averaging (BMA). We will define the BMA model space as all combinations of non-collinear confounders, with immunization status included in every model and base inference on the distribution of the immunization coefficient (VE) across models. This method moves beyond relying on a single model by systematically evaluating a wide range of plausible models and generating a consensus VE estimate by averaging across them, weighted by each model's posterior probability. This framework shows the distribution of VE across all plausible specifications, providing a transparent assessment of the sensitivity of our conclusions to model choice.[29] Next, we will conduct a formal falsification analysis to quantify the robustness of our conclusions against potential unmeasured confounding. Specifically, we will calculate the E-value for our primary VE estimate. The E-value quantifies the minimum strength of association that a hypothetical unmeasured confounder would need to have with both immunization status and RSV disease to explain the observed protective effect.[30] To evaluate the plausibility of this E-value, the effect sizes of the strongest measured confounders identified via BMA will serve as an empirical reference point.

Sample size and statistical power

The number of cases and controls needed to detect a range of estimates of effectiveness with α <0.05 and 80% power

Table 2 Number of cases needed to have 80% power using 2:1 test-negative design

Proportion of controls immunized	Effectiveness of perinatal RSV immunoprophylaxis			
	40%	50%	60%	70%
20%	340	220	124	81
30%	246	154	87	56
40%	204	124	70	44
50%	186	108	61	38

RSV, respiratory syncytial virus.

is presented in table 2. During the 5-year study period, we aim to enrol a total sample size of 3750 infants with ARI. Assuming immunoprophylaxis uptake of approximately 30% in the control population and 20% of the patients are RSV-positive cases, this sample size will provide >80% power to detect a VE >50% in each individual respiratory season. Precision for subgroup analyses (eg, by immunization type, social determinants of health or disease severity) will depend on the distribution of these factors among enrolled cases and controls. If uptake or case counts are insufficient to provide stable yearly estimates, data may be combined across seasons with adjustment for calendar year.

Ethics and dissemination

All study procedures have been approved by the Yale School of Medicine Human Investigation Committee (HIC: 2000036550), and the protocol is registered at ClinicalTrials.gov (NCT06172660). Participation in this study will be entirely voluntary, and no individuals will be excluded based on race or ethnicity. Written informed consent will be obtained from all parents or legal guardians of eligible infants prior to enrolment. Research staff will be trained in ethical principles, study procedures and culturally sensitive communication. The privacy, rights and confidentiality of human subject participants in this study will be protected. Identifiable information will be accessible only to authorised personnel as required for study operations.

Study findings will be disseminated broadly through peer-reviewed publications, scientific meetings and lay-friendly summaries for the general public. All viral genomics data will be shared in accordance with the National Institutes of Health's Policy on Sharing Genomic Data. De-identified study data that are not designated as restricted use will be made available as public use data to the research community through the ClinicalTrials.gov repository. Methodological tools and analytical code developed for this project will be released publicly in an annotated format through our GitHub repository to maximise transparency and reproducibility.

Patient and public involvement

Parents of young children were involved in the development of our study surveys. Using cognitive 'think aloud' interviews, parent feedback was used to iteratively revise and improve the clarity and relevance of our data collection instruments. Patients or the public have not been engaged in the reporting, or dissemination strategies of our research.

DISCUSSION

This paper describes a comprehensive methodological framework for the postlicensure evaluation of perinatal RSV immunoprophylaxis. We have outlined a test-negative case–control study design that is rigorous and can bridge the gap between prelicensure efficacy and real-world effectiveness. The framework's primary innovation is the integration of viral genomics and host immunology. This multipronged approach moves beyond simply estimating effectiveness to provide crucial mechanistic insights into why immunoprophylaxis succeeds or fails.

The proposed methodology has several notable strengths, beginning with the test-negative design, which is particularly well suited for postlicensure vaccine evaluation.[31–34] By enrolling infants with ARI who test either positive or negative for RSV, this approach controls for healthcare-seeking behaviour and access, two major sources of bias in observational studies. Case–control studies also tend to provide more precise estimates of effectiveness for a given sample size, making this design both statistically efficient and practical for large-scale surveillance. This rigorous epidemiological approach is supported by multi-source data collection (EHRs, immunization registries, surveys) and systematic collection of respiratory specimens from all eligible infants with ARI, not only those tested clinically, which mitigates physician testing bias and reduces risk of misclassification. Finally, our statistical framework incorporates Bayesian models to stabilise yearly estimates and pre-specified sensitivity analyses to evaluate unmeasured confounding, adding robustness and transparency to the analytical approach.

Integrating viral genomics and immune profiling within a single surveillance framework is another key strength of this work. Traditional postlicensure studies estimate the magnitude of protection but rarely address its underlying mechanisms. By linking genomic and immunological data to clinical outcomes, this design allows us to distinguish between loss of protection driven by viral evolution, waning immunity or host susceptibility. This shift from purely epidemiological to mechanistic evaluation improves interpretability and establishes a model for assessing other emerging immunization strategies.

Despite its strengths, our approach has potential limitations. First, while the test-negative design minimises many biases, selection bias could still occur if enrolled participants differ systematically from those who decline. To address this, we will compare the demographic and clinical characteristics of enrolled versus non-enrolled eligible individuals to assess the potential magnitude and direction of this bias. Second, complete blinding is not feasible, as study staff will be aware of participants' case or

control status during data abstraction, creating a potential for information bias. We will minimise this by using a standardised protocol and identical, exhaustive data collection procedures for both cases and controls, and we will formally assess whether ascertainment differed between groups. Third, the deep immune profiling will be restricted to a small subset of participants, which may not be fully representative of the broader study population. Finally, generalisability may be limited in settings with different healthcare access, immunoprophylaxis uptake or testing practices. We will report key contextual factors and perform stratified analyses where feasible to support interpretation across settings.

The robust evidence generated from the proposed work will have significant implications for public health and policy. Accurate, real-world effectiveness estimates are critical for policymakers determining whether these new products warrant sustained inclusion in national immunization programmes. By monitoring for viral immune escape and investigating the immunological correlates of protection, this work will also provide crucial data to inform the development of next-generation RSV vaccines and mAbs. Furthermore, should the data demonstrate substantial protection, the resulting evidence can help strengthen provider recommendation to immunize and sustain public trust in the immunization programme.

In conclusion, this paper presents a rigorous approach for the postlicensure evaluation of new RSV immunoprophylaxis strategies. By integrating genomic sequencing and immune profiling directly into a well-established epidemiological surveillance workflow, this study design provides both population-level effectiveness estimates and the critical biological insights needed to understand why these interventions succeed or fail. The proposed work will ensure the generation of rigorous evidence to guide clinical practice, inform public health policy and sustain public trust in immunizations.

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Competing interests Potential conflict of interest: Dr LN has served as a scientific advisor for Merck and Moderna (both unrelated to this work). Dr DMW has been the principal investigator on grants from Pfizer and Merck to Yale University for work unrelated to this manuscript and has received consulting fees from Pfizer, Merck and GSK for work unrelated to this manuscript. Dr PA is the principal investigator on a grant from Merck that aims to increase parental confidence in newborn RSV immunoprophylaxis administration. All other authors declare no conflicts of interest.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

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